Evaluation of $CD8^+$ T cell responses towards conserved HIV-1 epitopes in naturally infected and vaccinated individuals

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Declaration of Originality

I hereby declare that all the work illustrated in this thesis signifies my novel work and was performed in the laboratories of International AIDS Vaccine Initiative, Department of Immunology, Imperial College of Science, Technology and Medicine, London and has not been submitted to any other institution. Furthermore, I have used no other source of information except those which are notified by citations.

Ms Ambreen Ashraf

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Abstract of thesis

Human Immunodeficiency Virus (HIV) infection is a global public health priority. An effective HIV-1 vaccine strategy must overcome the enormous genetic diversity generated by the virus while inducing potent, long lasting immune responses. In this thesis, virus-specific cellular immune responses directed towards HIV conserved regions were evaluated using a validated IFN-γ enzyme-linked immunospot (ELISpot) assay and two sets of peptide panels based upon HIV-1 vaccine candidates. One panel was based on the 14 of the most highly conserved (HIV_{consv} – 806 amino acids) regions of the HIV-1 proteome and was designed to provide extensive coverage of subtypes A, B, C and D. The second panel represents Gag, Reverse Transcriptase (RT), Integrase (INT), Nef and Envelope sequences derived from a consensus subtype A reference (GRIN/Env). The purpose of this study was to examine the extent of T cell responses to HIV-1 epitopes elicited at different stages of HIV-1 infection and in vaccinees. Individuals infected with HIV-1 subtypes (A, B, C and D) from the UK, Rwanda, Uganda, and Zambia representing a wide range of genetic backgrounds were tested using a HIV_{consv} peptide matrix and GRIN pools. CD8 responses were identified across Gag, RT and INT and there was no apparent subtype specific bias in terms of recognition of both peptide panels (HIV_{consv} and GRIN/Env). GRIN appeared to be more antigenic because GRIN incorporated both conserved and variable epitopes compared to HIV_{consv} panel that only comprised conserved peptide sequences. Furthermore, there was no association between IFN-γ T cell responses and control of viraemia in early infection between viraemic controllers (VC) and viraemic progressors (VP). However, VC appeared to be slightly more polyfunctional than VP. Viral inhibition assay (VIA) employed to assess the vaccine specific responses demonstrated that conserved regions of the HIV-1 proteome could result in an increased ability to inhibit a panel of HIV-1 isolates *in vitro*. Although detection of a $CD8^+$ T cell response against a particular epitope does not necessarily translate to protection, the conserved nature of HIV-1 epitopes suggests that these domains are immunogenic and might be important for viral function. Thus, these data support the inclusion of conserved sequences in any future vaccine candidate.

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List of Abbreviation

Acquired Immune Deficiency Syndrome	AIDS
Acute HIV infection	AHI
Ad35 Gag, RT, INT and Nef sequences	Ad35 GRIN
Ad35 gp140 Env	Ad35 Env
Adenovirus serotype 5	Ad5
AEQASQDVKNW	AW1
Amino acids	aa
Antibodies	Abs
Antibody-Dependent Cell-mediated Cytotoxicity	ADCC
Anti-Retroviral Therapy	ART
Apolipoprotein B mRNA-editing, enzyme-catalytic, polypeptide-like	APOBEC
Autoimmune Diagnostika	AID
Becton Dickinson	BD
Best defined epitopes	BDE
Bi-specific monoclonal antibodies	BSMAB
Brefeldin	BFA
Broadly neutralising antibodies	bNAbs
Buffy Coat	BC
Central memory T cells	TCM
Centre of the tree	COT
Chemokine receptor type 4	CXCR4
Chemokine receptor type 5	CCR5
Chronic Progressors	CP
Circulating Recombinants Forms	CRFs
Conservation Score	CS
Cut off	CO
Cytomegalovirus	CMV
Cytotoxic T lymphocyte	CTL
Dendritic cells	DC
Deoxyribonucleic acid	DNA
Dimethyl sulfoxide	DMSO
Effector memory T cells	TEM
Elite Controller	EC
Entropy Scores	ES
Envelope	Env
Enzyme Linked Immunosorbant Assay	ELISA
Enzyme-linked Immunosorbent Spot	ELISpot
Epstein Barr Virus	EBV
Estimated Days Post Infection	eDPI
Et cetera	etc
ETINEEAAW	EW9
FKRKGGIGGY	FY10
FKRKGGIGGY	FY10
Foetal Calf Serum	FCS
Fragment crystalisable	Fc

Gag, RT, INT and Nef / Envelope	GRIN/Env
Gut-associated lymphoid tissue	GALT
heat inactivated FCS	HIFCS
High Performance Liquid Chromatography	HPLC
HIV conserved	HIV_{consv}
HIV databases	HIV db
Human Immunodeficiency Virus	HIV
Human Immunology Laboratory	HIL
Human Leukocyte Antigen	HLA
Human T-lymphotropic viruse	HTLV
IAVI sponsored Clinical Trial	B001
Influenza virus, Epstein-Barr virus and cytomegalovirus	FEC
Integrase	INT
Intercellular Cytokine Staining	ICS
Interferon alpha	IFN-α
Interferon gamma	IFN-γ
Interleukin	IL
International AIDS Vaccine Initiative	IAVI
Inter-Quartile Range	IQR
ISPRTLNAW	IW9
KAFSPEVIPMF	KF11
Kaposi's sarcoma	KS
Killer immunoglobulin like receptors	KIR
Kilobases	Kb
KRWIILGLNK	KK10
KTAVQMAVF	KF9
Liquid nitrogen	LN_2
Long-Term Non-Progressors	LTNP
Lymph nodes	LNs
Lymphocytic Choriomeningitis Virus	LCMV
Lysosomal-Associated Membrane Protein	LAMP
Macrophage and Secreted inflammatory protein 1α	MIP-1α
Macrophage inflammatory protein 1β	MIP-1β
Major histocompatibility complex	MHC I
Matrix	MA
MHEDIISLW	MW9
Murine Leukemia Virus	MLV
Natural Killer	NK
Neutralising antibodies	NAbs
Non-Human Primates	NHP
Overlapping peptides	OLP
PBS-Tween	PBST
Peripheral blood mononuclear cells	PBMC
Phosphate-buffered saline	PBS
Phytohemagglutinin	PHA
Plasma Viral Load	pVL
Polymerase Chain Reaction	PCR
Polyvinyl-difluoride	PVDF

Potential T cell epitopes	PTE
Protease	PR
RDYVDRFYKTL	RL11
Regulated upon Activation, Normal T-cell Expressed	RANTES
Reverse transcriptase	RT
Revolutions per minute	RPM
Rhesus	rh
Ribonucleic acid	RNA
RKAKIIRDY	RY9
Roswell park memorial institute	RPMI
RPMI containg 10% FCS	R10
RPMI containing 20% FCS	R20
Simian immunodeficiency virus	SIV
Simian-Human Immunodeficiency Virus	SHIV
SLFNTVAL	SL9
Spot Forming Units	SFU
Standard deviations	s.d
Staphylococcal enterotoxin B	SEB
T cell receptor	TCR
TPQDLNTML	TL9
Transmembrane glycoprotein	gp160
Transporter associated with Antigen Processing	TAP
Tripartite motif-containing protein 5α	TRIM5α
Tris-borate-EDT buffer	TBE
TSTLQEQIAW	TW10
Tumor necrosis factor alpha	TNFα
United Kingdom	UK
Violet amine-reactive dye	VIVID
Viraemic Controllers	VC
Viraemic Progressors	VP
Virus Inhibition Assay	VIA
Virus particles	VP
Visit	V
Visit 1.0	V1.0
Visit 12	V12
Visit 24	V24
Week	W
Week 0	W0
Week 4	W4
Week 28	W28
Wild Type	WT
World Health Organization	WHO

Publication arising from this Thesis

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Presentation title:

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Chapter 1: Introduction

1.1 Discovery of Human Immunodeficiency Virus

The Human Immunodeficiency Virus (HIV) is one of the most extensively studied viruses. It was first detected in the early 1980s when a small number of men presented to clinics with either *Pneumocystis carinii* pneumonia (PCP) or a skin cancer known as Kaposi's sarcoma (KS); both rare opportunistic infections and usually seen in the immuno-compromised host (Friedman-Kien and Saltzman, 1990; Hymes et al., 1981). The disease was named Acquired Immune Deficiency Syndrome (AIDS) by the Centres for Disease Control and Prevention. In the early 1980s, two leading scientists Robert Gallo (USA) and Luc Montagnier (France) separately discovered that AIDS patients may have been infected by a new retrovirus (Barre-Sinoussi et al., 1983). Gallo's group had used peripheral blood mononuclear cells (PBMC) from an AIDS patient and isolated a virus similar in structure to human T-lymphotropic viruses (HTLVs) and named it HTLV-III (Gallo et al., 1988; Gallo et al., 1983). Simultaneously, Montagnier's group challenged Gallo's findings and demonstrated that p19 and p24 (both structural proteins) of a virus isolated from a lymphadenopathy patient were not antigenically similar to those of HTLV and named it as lymphadenopathy-associated virus (Barre-Sinoussi et al., 1983). In 2008 Montagnier and Barré-Sinoussi were awarded the Nobel Prize in Physiology or Medicine.

1.2 HIV

HIV is a member of the *Retroviridae* family (genus *Lentiviridae*) and is transmitted by blood transfusion, vertically (*in utero*, breast milk), parenterally (intravenous drug users, blood transfusion) and sexually (via the vaginal tract or rectal mucosal tissues) (Patel et al., 2014). HIV predominantly infects cells expressing the CD4 receptor, these include dendritic cells (DC), macrophages, and most importantly, $CD4^+$ T helper cells, the infection of which results in the dramatic depletion of $CD4^+$ T lymphocytes (Koup et al., 1994; Macatonia et al., 1992; Verani et al., 2005). Despite an early, robust immune response, infected individuals are not able to eliminate HIV and following an asymptomatic chronic phase, $CD4^+$ T cells are depleted leading to opportunistic infections, which leads to AIDS and death of the host (Duesberg, 1989; Paiardini and Muller-Trutwin, 2013; Tsao et al., 2016).

1.3 Epidemiology

Despite the introduction of Anti-Retroviral Therapy (ART) 25 years ago (De Clercq, 2010; Furman et al., 1986) and improvements in sexual health awareness, it is estimated that 39 million deaths have been attributable to HIV/AIDS since the early 1980s and there are currently 35.3 million people living with the disease worldwide. The treatment of infected individuals with ART has led to a substantial

reduction in AIDS related cases and in prolonging the life of infected individuals (UNAIDS, 2014) (Figure 1).

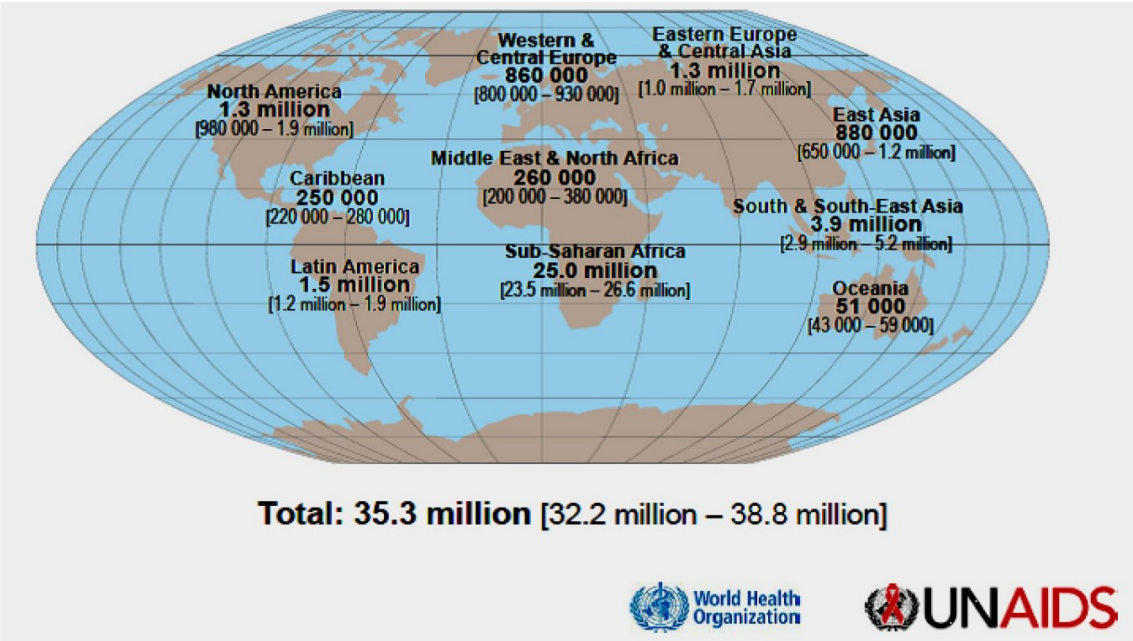


Figure 1. World Health Organisation (WHO) estimate showing worldwide prevalence of HIV (UNAIDS, 2014**)**

Moreover, according to the UNAIDS report numbers of new infections dropped by 13% globally between 2011-2013 perhaps due to the increasing awareness and in Sub-Saharan Africa cases dropped by 33% between 2005-2013. At present, South and South East Asian countries are the second most affected region with approximately 3.9 million people infected (Figure 1) (UNAIDS, 2014).

1.4 HIV testing and diagnosis

HIV testing is performed using number of methods typically either detecting HIV specific immunoglobulins G (IgG) in serum or plasma by enzyme linked immunosorbant assay (ELISA); by polymerase chain reaction (PCR) to detect HIV nucleic acid plasma viral load (pVL); by detecting p24 antigen in patient serum by Western Blot or in rare cases culturing HIV. It has been reported that risk of infection is usually high during acute infection compared to the chronic stage due to the high pVL (Piatak et al., 1993). Furthermore, it has also been shown in a study with macaques that infectious virions during the acute stage is 750 times more infectious than virions during the chronic stage (Ma et al., 2009). It is, however, quite challenging to diagnose HIV individuals prior to seroconversion thereby increasing the risk of HIV transmission during the acute phase of disease.

1.5 HIV-1 diversity and subtypes

The introduction of HIV into humans appears to have been as a result of several zoonotic events around the turn of the 20th century in West/Central Africa, which has given rise to at least four genetically diverse HIV clusters: HIV-1 Group M (main), HIV-1 Group O (outlier), HIV-1 Group N (non-M and non-O) and HIV-2 (Korber, 2000; Korber et al., 2001; Worobey et al., 2008). Perhaps as a result of the reduced virulence of the transmitted virus (in the case of HIV-2) or more favourable human and environmental conditions at the time of the early transmission(s), HIV-1 Group M has become established as the most prevalent viral group, ultimately giving rise to the current HIV/AIDS pandemic (UNAIDS, 2014). As a result of clonal geographical expansion during the early stages of the pandemic HIV-1 Group M can be further stratified into HIV-1 subtypes A to K (Figure 2). Subtype B is found mostly in North America and Europe, subtype C predominates in India and Southern Africa, while subtype E is prevalent in South-East Asia and subtypes A and D are limited largely to Central and East Africa. This nomenclature is further complicated by multiple infections resulting in circulating recombinants forms (CRFs) (Gaschen et al., 2002; Korber, 2000; Korber et al., 2001; Worobey et al., 2008).

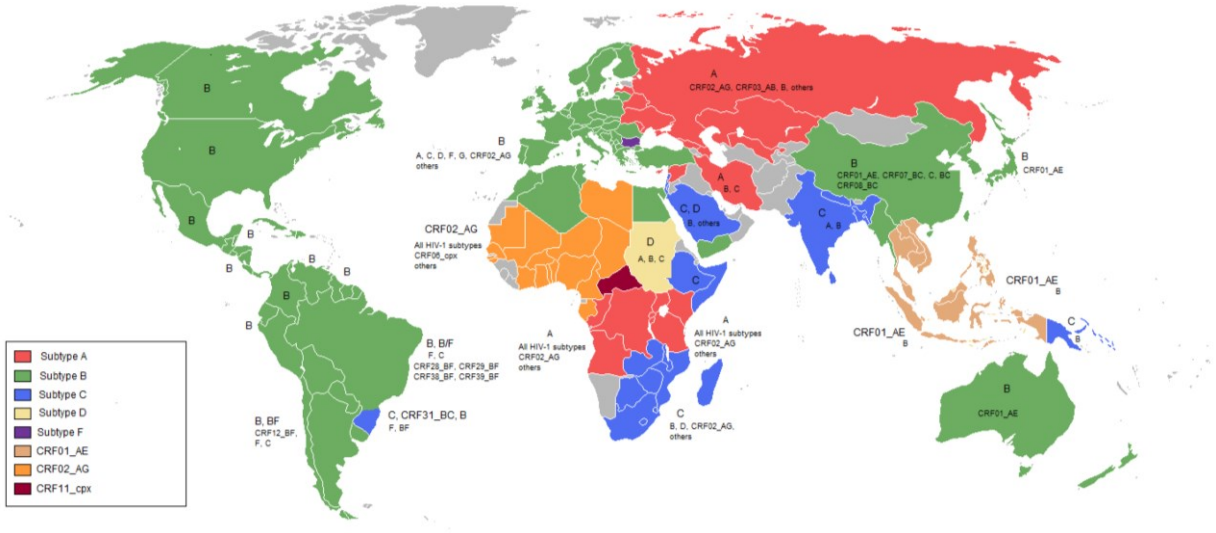


Figure 2. Illustration of diversity and global distribution of HIV-1 clades and Circulating recombinant forms (Santos and Soares, 2010)

Subtype C is prevalent in Sub-Saharan Africa, Eastern Africa and India. Subtype A is prevalent in Central African and Central Asia and clade B is predominant Europe and America.

The genetic sequences of HIV-1 circulating within a single host at a given time (or quasispecies) can differ by as much as 6% and within a transmission cluster by as much as 20% (Korber et al., 2001). By contrast, the seasonal influenza epidemics generally have less than 1-2% sequence variation (Figure 3) (Fischer et al., 2008; Gaschen et al., 2002; Korber, 2000). The extraordinary sequence

diversity of HIV-1 results from the error-prone viral reverse transcriptase essential for HIV-1 replication, as the viral Ribonucleic acid (RNA) genome is copied to a pre-integration Deoxyribonucleic acid (DNA) intermediate; the diploid RNA genomes' ability to recombine with itself and with the rapid viral turnover estimated at 10^{10} billion copies per day (Ho et al., 1995; Perelson et al., 1996).

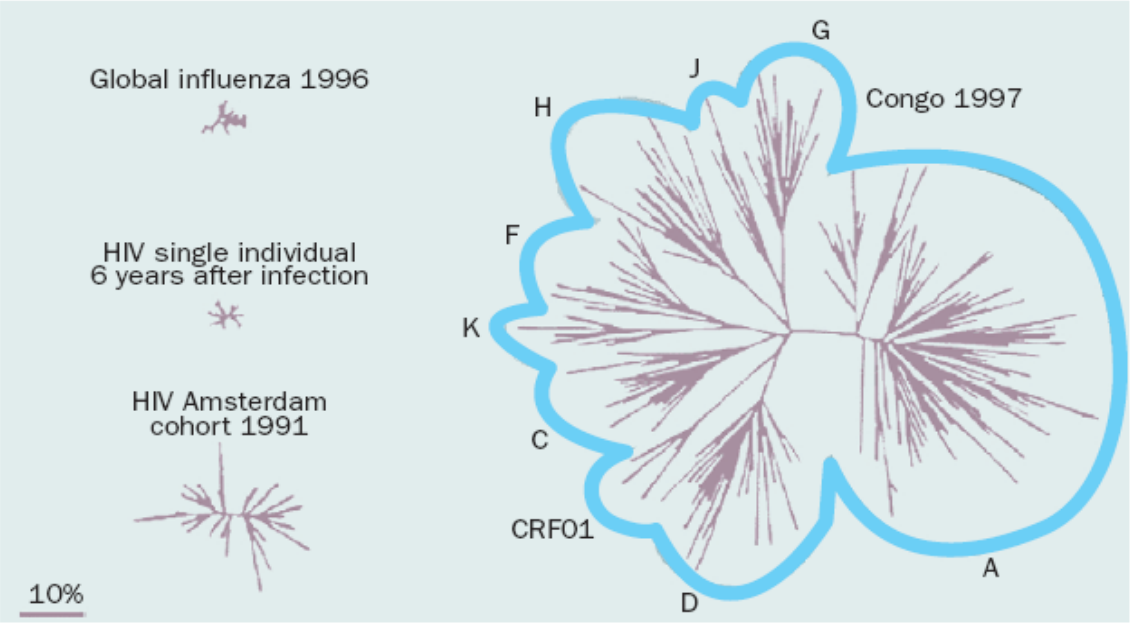


Figure 3. Comparison of the variability in HIV-1gp120 sequences with the variability HA1 domain of human influenza H3N2 (Garber et al., 2004)

1.6 HIV-1 genome and replication cycle

The HIV genome is a duplex single stranded RNA approximately 9.8 kilobases (Kb) in length; with its integrated DNA form referred to as the provirus. When compared to a typical retrovirus such as Murine Leukemia Virus (MLV) the HIV genome encodes nine proteins; structural proteins (Gag, Pol and Envelope (Env), regulatory proteins (Tat and Rev) and additional multiple accessory proteins including Vpu, Vpr, Vif and Nef (Frankel and Young, 1998). Gag is comprised of matrix (MA), capsid (CA) and the nucleocapsid (NC), Pol comprised of enzymes Protease (PR), Reverse transcriptase (RT) and Integrase (INT), and Envelope (Env) is comprised of Surface (SU) and Transmembrane glycoprotein (gp160) proteins (Figure 4) (Frankel and Young, 1998; Ganser-Pornillos et al., 2008; van der Putten et al., 1981).

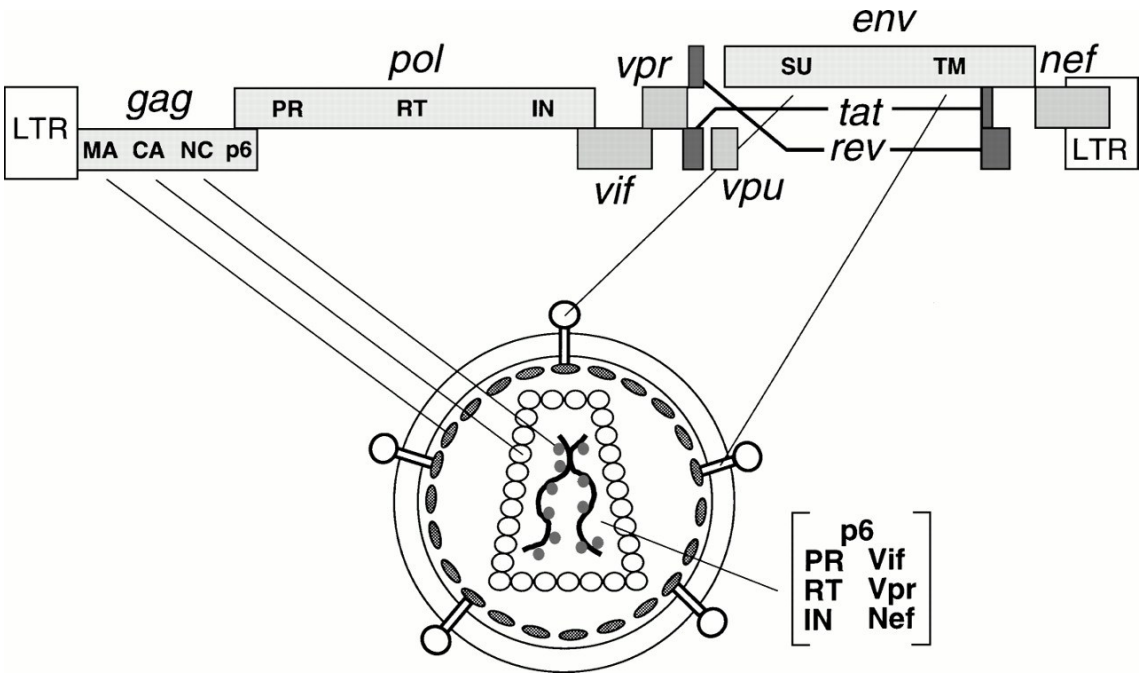


Figure 4. Structure of HIV-1 virion illustrating structural and accessory proteins

Illustration of HIV structural (*gag, pol* and *env*), regulatory (*tat* and *rev*) and accessory (*vpu*, *vpr*, *vif*, and *nef*) genes and proteins (Frankel and Young, 1998)

Cellular entry of HIV occurs when HIV gp120 binds to CD4 receptors on T helper cells or macrophages/DC resulting in conformational changes in the gp120 structure (Landau et al., 1988). This in turn facilitates binding of gp120 to either chemokine receptor type 5 (CCR5) or chemokine receptor type 4 (CXCR4) co-receptors (Deng et al., 1996), an interaction that allows union of the viral and cellular membrane mediated by gp41. Once fusion occurs, the nucleocapsid containing the viral RNA genome is released into the cytoplasm of the host cell (Frankel and Young, 1998), whereupon the RT enzyme converts the RNA genome into cDNA. This DNA provirus then integrates into the host genome, a process mediated by the INT enzyme (Cimarelli and Darlix, 2002; Frankel and Young, 1998; Freed, 2001). During the later stages of virus replication, structural proteins predominate with gp120 and gp41 (Env glycoproteins) being exported to the cell membrane, while complexes of Gag proteins and viral RNA associate with Env glycoproteins in the cell membrane, resulting in budding of mature virions from the host cell (Cimarelli and Darlix, 2002; Frankel and Young, 1998; Freed, 2001).

1.7 Immunopathogenesis of HIV-1 infection

The natural history of HIV infection has two phases, an acute phase in which the virus depletes $CD4^+$ T cells to some extent and a chronic phase where the immune system incompletely maintains and reconstitutes memory $CD4^+$ T cells, while constantly challenged by HIV (Moir et al., 2011; Pantaleo and Fauci, 1995). HIV-1 is transmitted across the mucosal barriers and infects a small population of

mucosal cells which are either $CD4^+/CXCR4^+$ or $CD4^+/CCR5^+$, with a low level of viral replication (Burgener et al., 2015). Fiebig et al described six stages of HIV early infection shown in Figure 5 and Table 1. Prior to the detection HIV RNA in plasma, there is an initial stage of infection referred to as the "eclipse phase" that usually lasts for ~10 days. At this point virus replicates in local tissues at the site of infection (Cohen et al., 2010; Keele and Estes, 2011).

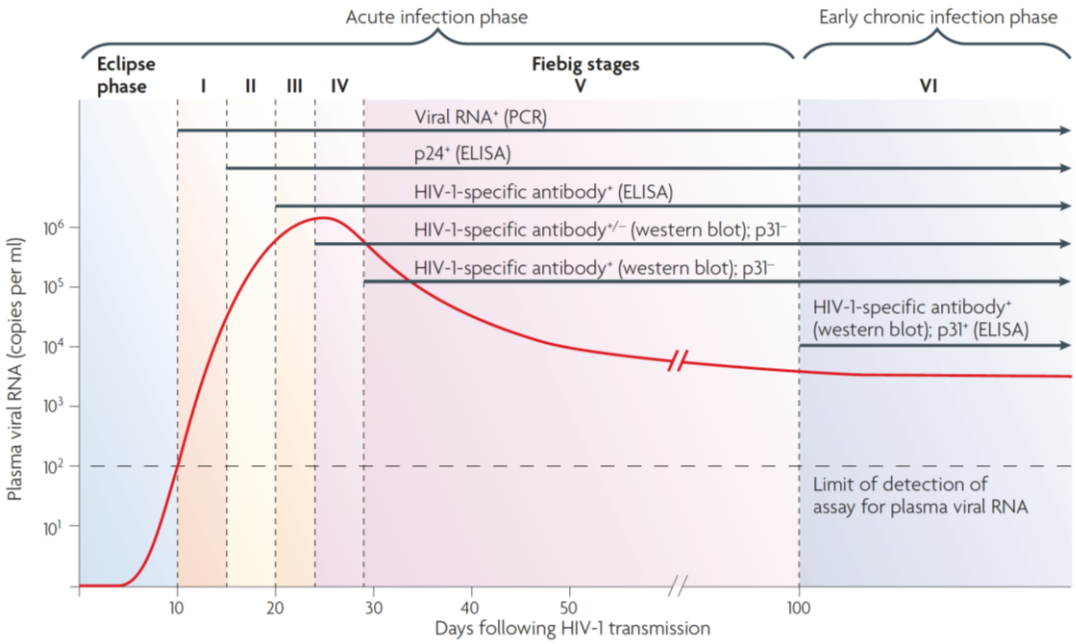


Figure 5. Stages of HIV infection showing different stages of infection

Kinetics of HIV infection during acute and chronic stages of the disease illustrating Fiebig stages I-VI. (Extracted from (McMichael et al., 2010)

Table 1. Fiebig stages of HIV-1 primary infection (Cohen et al., 2010)

		Duration, mean (range), days	
Stage	Defining finding and/or marker	Individual phase	Cumulative duration
Eclipse	…	10 (7–21)	10 (7–21)
I	vRNA positive	7 (5–10)	17 (13–28)
II	p24 antigen positive	5 (4–8)	22 (18–34)
III	ELISA positive	3 (2–5)	25 (22–37)
IV	Western blot positive or negative	6 (4–8)	31 (27–43)
V	Western blot positive, p31 antigen negative	70 (40–122)	101 (71–154)
VI	Western blot positive, p31 antigen positive	Open-ended	…

During the early stage of infection (~2 weeks after HIV infection) some HIV infected people usually experience a mononucleosis-like illness displaying non-specific symptoms including fever, myalgia, malaise, anorexia, photophobia, headaches, lymphadenopathy and rash (Cohen et al., 2010). Prior to

the generation of an effective immune response usually at the end of an eclipse phase, HIV-1 replication takes place at a very fast rate resulting in extremely high pVL and a spread of virus to draining lymphoid organs and gut-associated lymphoid tissue (GALT), where target $CD4^+$ T cells are present in abundance allowing efficient HIV replication. This results in depletion of $CD4^+$ T cells (Ho et al., 1995; Monteiro et al., 2011; Perelson et al., 1996) and most likely occurs due to the cytopathogenic effect of HIV infection and/or killing of bystander T cells (Brenchley et al., 2006; Walker et al., 1986). Animal studies with Rhesus (rh) macaques infected with Simian immunodeficiency virus (SIV) showed massive depletion in memory $CD4^+$/ $CCR5^+$ T cells in the gut associated lymphoid tissues (Douek et al., 2009; Li et al., 2005; Mattapallil et al., 2005; Picker and Watkins, 2005). Both HIV and SIV rapidly disseminate at this stage of infection resulting in the spread of HIV to the lymph nodes (LNs) (Pantaleo and Fauci, 1995; Pantaleo et al., 1993). Normally at this point HIV specific antibodies (Adachi et al., 1986) can be detected in the periphery (Cohen et al., 2010; Fiebig et al., 2003).

There is a progressive decline in viraemia concomitant with the emergence of an HIV-1 specific $CD8^+$ T cell response followed by a chronic phase of infection which may last for many years without symptoms (Koup et al., 1994; Pantaleo and Fauci, 1995) (Figure 5). However, the HIV-1 specific cytotoxic T lymphocyte (CTL) response is inadequate to eradicate the virus, and virus continues to replicate in the LNs (Cao and Walker, 2000; Pantaleo and Fauci, 1995; Pantaleo et al., 1993). It is observed that the level of pVL stabilises by six months post-acute phase and is referred to as the set point. Recent study has shown this rise and a fall in viraemia during primary infection has been correlated with the set point pVL, this association underlined the important role of the very initial interplay between the host and HIV-1 in defining the continuing disease status (Robb et al., 2016). During the chronic stage of HIV infection there is continuous viral replication usually results in chronic immune activation ($CD8^+$ $CD38^+$, HLA-DR^+) (Figure 6). The increased levels of activation markers HLA-DR^+, $CD8^+$ $CD38^+$ T lymphocytes are believed to be responsible for $CD4^+$ T cell depletion and progression to AIDS (Deeks et al., 2004; Smith et al., 2013). It is presumed that immune activation (Figure 6) may be predictive of the rate of CD4 decline in each infected individual but, the association between immune activation and loss of CD4 cells is not fully understood. (Paiardini and Muller-Trutwin, 2013; Smith et al., 2013). However, it is not clear in what way immune activation may be responsible for disease progression but, one possibility is the exhaustion of $CD4^+$ and $CD8^+$ T cells with antigen load and inability of the thymus to replenish new naïve T cell (Douek et al., 1998). Usually 10^{10} copies of HIV-1 are produced per day which results in a high mutation rate of HIV-1 and therefore, increases the possibility of immune escape (Karlsson Hedestam et al., 2008; Yeh et al., 2010). Typically, 5-20 years post infection, peripheral CD4 counts fall below 200 cells/µl

with the infected individuals becoming more susceptible to opportunistic infections, marking the beginning of AIDS and progression to death (Balter, 1997), if ART is not provided.

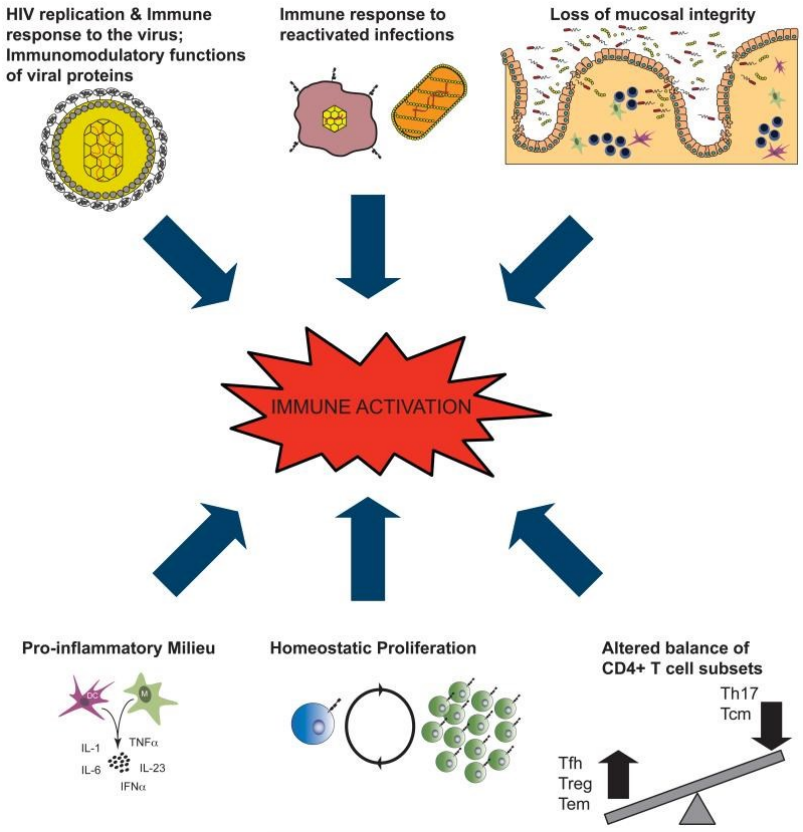


Figure 6. Possible factors contributing to HIV chronic phase immune activation

Schematic representation of molecular and cellular mechanisms contributing to chronic HIV immune activation generating an uncontrolled positive feedback (Extracted from (Paiardini and Muller-Trutwin, 2013)

A small proportion of individuals are apparently able to control HIV-1 replication (low or undetectable pVL) without ART, maintaining high CD4 counts without disease progression and are known as Long-Term Non-Progressors (LTNP). In some cases individuals infected for than a decade or more can remain in good health, with a steady CD4 count and pVL of <50 copies/ml (Kiepiela et al., 2007; Migueles et al., 2008; Migueles et al., 2000; Pantaleo and Fauci, 1995; Pantaleo et al., 1993). There is increasing evidence that protective alleles (B*13, B*27, B*57, B*58, B*81) facilitate escape mutations that may result in low viral fitness and also host factors (i.e. adaptive immunity, innate immunity, CCR5/CXCR mutations) may contribute to a lower viral replication and slower disease progression in LTNP (Crawford et al., 2009; Goulder et al., 1996; Goulder et al., 1997). Hence, individuals enriched in protective alleles (B*13, B*27, B*57, B*58, B*81) are the focus of much attention in the field of HIV-1 research to elucidate the possible immunological mechanisms

involved in the control of HIV-1 replication and the outcome of slower disease progression (Emu et al., 2008; Migueles and Connors, 2015; Tang et al., 2011).

1.8 Immune response to HIV-1

In mammals the first line of defence against pathogens is non-specific. Upon infection the innate immunity is activated and obstructs the entry of pathogens such as parasites, bacteria, and viruses. The elements of the innate immune system include; 1) physical barriers (skin, respiratory tract, gastrointestinal tract and body hair) 2) chemical factors (mucous, bile, gastric acid, enzymes in sweat, tears and saliva) and 3) general immune response (inflammation and non-specific cellular immune response) (Dempsey et al., 2003). Innate immunity is not specific to any particular invading pathogen compared to the adaptive immunity. The cells of the innate immunity (such as neutrophils, eosinophils and macrophages) recognise pathogens and become quickly activated to engulf and eradicate foreign organisms. The second line of defence is the adaptive immune response which is more specific and carried out by white blood cells known as lymphocytes. There are two types of such responses; antibody responses and cell-mediated immune responses. In antibody responses, B cells are activated to secrete antibodies and those antibodies bind to pathogens and inactivate them. In cell mediated immune responses, T cells directly kill a foreign antigen presented to them on the surface of the host cell (Dempsey et al., 2003). In the case of HIV-1 infection there is a complex interplay between HIV and the cells of the immune system (innate and adaptive), but, as mentioned previously, these responses are insufficient to eliminate the virus from infected individuals (Douek et al., 2009; Gandhi and Walker, 2002; McMichael and Phillips, 1997).

1.8.1 Innate Immunity

1.8.1.1 Soluble factors

It has been demonstrated that during acute infection proteins such as serum amyloid A and alpha1-antitrypsin can be detected in the plasma 5-7 days after HIV-1 acquisition (Kramer et al., 2010). The presence of acute phase proteins in plasma is one initial sign of HIV-1 infection. As pVL reaches to high level then there is an increase in up-regulation of cytokines and chemokines usually referred to as cytokine storm (Ruhwald and Ravn, 2007; Stacey et al., 2009). Analysis of plasma from donors during acute phase have shown elevation in the levels of cytokines for instance; Interleukin (IL)-10, IL-15, IL-18, Tumor necrosis factor alpha (TNF-α), Interferon alpha (IFN-α) and the up-regulation of many others pro-inflammatory chemokines and cytokines (Borrow, 2011; Stacey et al., 2009). However, the exact cellular mechanisms involved in the induction of these cytokines during the acute stage of infection is not fully defined. Studies have shown that IFN-α hinders virus replication by up-

regulating the expression of genes that obstruct with specific stages in the virus life cycle of HIV-1 (Borrow, 2011; Cheney and McKnight, 2010) and inhibits apoptosis of activated T cells (Rodriguez et al., 2006), but there is also a possibility that these cytokines may also be the reason behind harmful and unwanted immune activation and decline in CD4 (Chang and Altfeld, 2010).

1.8.1.2 Restriction Factors or Intrinsic factors

Retroviruses require integration of their genome into the hosts' to replicate successfully. However, HIV-1 infection may be controlled by intracellular proteins commonly known as "restriction factors" (Lu et al., 2013). A detailed understanding of the involvement of restriction factors in the control of HIV-1 replication would help researchers to understand the relationship between the host and the virus and could lead to better animal models of disease, improvements in ART, and may enable the use of viral vectors in gene therapy (Harris et al., 2012; Towers, 2007). So far, restriction factors such as tripartite motif-containing protein 5α (TRIM5α), APOBEC (apolipoprotein B mRNA-editing, enzyme-catalytic, polypeptide-like) and tetherin are known to be involved in control of HIV-1 (Harris et al., 2012; Pujol et al., 2016; Richardson et al., 2014). Both APOBEC and TRIM5α (Coren et al., 2015; Wang et al., 2012) promote HIV-1 control by interfering with the RT step of HIV-1 replication, whereas tetherin (Casartelli et al., 2010; Pujol et al., 2016) hinders HIV-1 infection by preventing the dissemination of mature virions after their release from infected cells. APOBEC3G, has been studied quite extensively and it has been established that it induce excessive G to A hyper mutations and highly likely to introduce multiple lethal stop codons within HIV-1 (Harris et al., 2012; Wang et al., 2012). Viral antagonists can overcome restriction factors using several different mechanisms, for example, the HIV-1 protein Vif antagonises APOBEC3G, hampering its antiviral activity. However, gene duplication of restriction factor increases the copy number of restriction factors against rapidly evolved virus thereby, restricting virus evolution and maintaining antiviral activity (Duggal and Emerman, 2012). On the other hand, TRIM5α cannot inhibit HIV replication but, blocks HIV-1 infection by targeting the HIV capsid after entry, leading to ineffective RT and virus capsid disassembly in CD4 cells (Coren et al., 2015; Richardson et al., 2014).

1.8.1.3 Natural Killer (NK) Cells

NK cells are a component of the innate immune response capable of killing both virally infected cells and tumours. During early stages of viral infection cytokines (IFNs, IL-12, IL-15 and IL-18) produced by infected cells or macrophages or dendritic cells activate NK cells (Brandstadter and Yang, 2011). However, the exact mechanisms involved in the activation of NK cells are not fully understood, but it has been reported that NK cells are regulated by inhibitory and activating receptors (Martin et al., 2007; Scully and Alter, 2016). Once activated, they mediate antiviral activity during the acute stage

of HIV-1 infection by up-regulating killer immunoglobulin like receptors (KIR) (Martin et al., 2007). Each KIR locus encodes either an inhibitory (KIR3DL1) or an activating receptor (KIR3DS1). These receptors are known to work together with Human leukocyte antigen (HLA) class I alleles and NK activity is usually up-regulated in the presence of protective class I alleles. The ligands for the inhibitory KIR3DL1 allotypes are HLA-B molecules whereas no ligands has been described for KIR3DS1. However, due to the sequence homology (97%) with KIR3DL1 allotypes there is a possibility that KIR3DS1 may recognise similar ligands (Martin et al., 2007). Martin et al have shown that several allelic combinations of NK cell receptor KIR3DL1 and HLA-B Bw4 significantly influence both disease progression and pVL level and most importantly, this protective effect mainly depends on the existence of HLA-B Bw4.

In addition to this, NKs are known to eradicate cells infected with HIV-1 through the Antibody-Dependent Cell-mediated Cytotoxicity (ADCC) pathway (Scully and Alter, 2016; Wren et al., 2013). The precise mechanism involved is not fully understood, but it has been reported that Fc receptors on NK cells generally bind to the Fragment crystallisable (Fc) domain of the antibodies which has bound to the HIV-1 proteins on the cell membrane of virally infected cell. This results in the lysis of infected cells by secreting perforin and granzymes (Klenerman et al., 2002; Stratov et al., 2008). NK cells are also known to block HIV-1 infection by releasing chemokines such as RANTES (Regulated upon Activation, Normal T-cell Expressed), Macrophage and Secreted inflammatory protein 1α (MIP-1α) and Macrophage inflammatory protein 1β (MIP-1β) and thus, interfere with HIV-1 interaction with CCR5 (Fauriat et al., 2010) . In addition to this, NK cells also secrete pro-inflammmatory cytokines such as IFN-γ, TNF-α and Granulocyte-Macrophage Colony Stimulating Factor and help in the induction of adaptive immune response to HIV-1 infection (Fauriat et al., 2010; Scully and Alter, 2016).

1.8.2 Adaptive Immunity

1.8.2.1 Humoral Immunity – B Lymphocytes

HIV-1 specific antibodies have been detected early in infection; however, these antibodies (Abs) are unable to control virus replication compared with other viral infections (Ahlers, 2014; Seaman et al., 2010). Neutralizing antibodies (NAbs) are accepted as the main correlate of protection for most licensed vaccines and thus, numerous efforts have been made to induce HIV-1 specific NAbs (Plotkin, 2010). Not only that, a small proportion (20-30%) of HIV-1 infected individuals produce neutralising antibodies against a number of different HIV-1 isolates usually referred to as broadly neutralising antibodies (Rubens et al., 2015; Sliepen and Sanders, 2015). Broadly NAbs mediate viral control by targeting HIV-1 Env glycoprotein, essential for HIV-1 to enter target cells (Hraber et al.,

2014; Sliepen and Sanders, 2015). These Abs which neutralise autologous virus often start to appear approximately 12 weeks post infection (Barouch and Korber, 2010). The majority of these Abs are generated against gp120 and have no effect on viraemia and do not exert selection pressure on envelope and unable to neutralise the virus (Karlsson Hedestam et al., 2008; Sather et al., 2009; Wyatt et al., 1998). Several broadly reactive human monoclonal antibodies (2G12, 4E10, 447-52D, 2F5, Z13, b12, PG9, PG16, PGT125-128, PGT130 PGT135-1, NIH45-46 and 3BNC117) cloned from B cells have ability to neutralise sensitive (Tier I), intermediate (Tier II) and relatively resistant strains (Tier III) but were not able to neutralise contemporary host virus (Horiya et al., 2014; Seaman et al., 2010; Shin, 2016; Zhou et al., 2007). The development of soluble native like Env trimers as immunogens could elicit broadly NAbs and consider to be a promising candidate for HIV-1 vaccine development. However, HIV vaccine candidate based on SOSIP trimer that mimics the native trimer still unable to elicit broadly NAbs (Haynes, 2015; Sliepen and Sanders, 2015).

1.8.2.2 Cell mediated immunity – T Lymphocytes

T cells are generally classified into two subgroups, CD4 and CD8 and both play vital roles to generate host immune responses upon infection. Moreover, T cell are also be able to identify whether the infectious material is derived from the cytosol (endogenous proteins) or vesicles (exogenous proteins) To produce an effective and robust immune response, T cell expresses T cell receptor which recognises peptides presented on major histocompatibility complex (MHC) by T cell receptor, which in humans are called HLA. (Morrison et al., 1986). HLA genes are located on chromosome 6 and are known to be the most polymorphic genes in humans (Parham et al., 1988; Shiina et al., 2009). Their diversity allows accommodation of a range of different peptides for presentation to T cells. There are two types of HLA molecules HLA class I and HLA class II. Human HLA class I molecules are glycoproteins generally categorised into two groups; classical (HLA-A, B and C) and non-classical (HLA-E, F and G). Of these, HLA-B has the highest degree of polymorphism among different group of people worldwide (Pereyra et al., 2010). HLA genes produce proteins that would form the major histocompatibility complex (MHC) (Neefjes et al., 2011). MHC I molecules are heterodimeric and composed of two polypeptide chains; α and β2-microglobulin (Heinrichs and Orr, 1990). The two alpha helices (α1& α2 – heavy chain) are highly polymorphic whereas the light chain β2-microglobulin is less polymorphic (Neefjes et al., 2011).

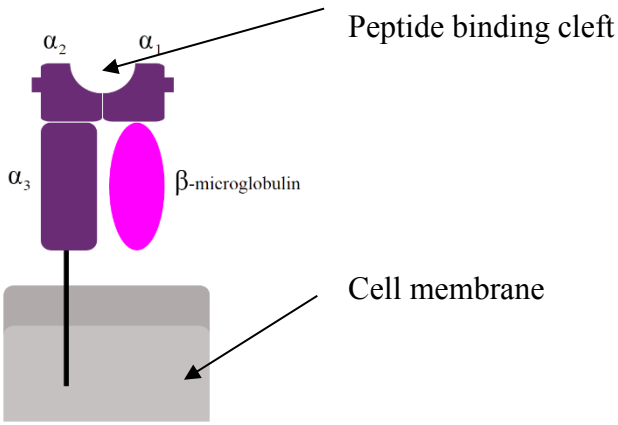


Figure 7. Schematic representation of MHC I

(Source: https://upload.wikimedia.org/wikipedia/commons/e/ee/MHC_Class_1.svg)

However, the third α3 domain is quite conserved and interacts with the CTL co-receptor (Hewitt, 2003). The peptide binding groove of MHC I is closed at both ends, composed of α1 and α2 domains and holds a short processed peptide of 8-11 aa. HLA class I molecules present processed peptides to $CD8^+$ T cells whereas HLA class II molecules present peptides to $CD4^+$ T cells (Neefjes et al., 2011). A peptide usually binds to six specialised pockets within the HLA groove designated as A-F. These pockets are essential for peptide binding specificity and pockets B and F accommodate peptide side chains of residues 2 and 9 (Sidney et al., 2008). Regardless of polymorphic nature of HLA, it is known that the centre part of the peptide binding groove of the HLA molecule is quite variable whereas the ends are more conserved (Bell et al., 2009; Young et al., 1995). The binding of the peptide to the HLA groove is dependent on both its length and the anchor residue sequence. A single amino acid change even at non-anchor residues can alter peptide binding (Bell et al., 2009; Lee, 2004; Young et al., 1995).

Recognition of foreign material by T cells requires antigen processing that takes place within an infected cell by proteolysis of endogenous proteins into small fragments (i.e. peptides) by the proteasome within the cytosol. This process is usually referred to as the "endogenous pathway", (Figure 8) (Hewitt, 2003; Morrison et al., 1986) whereas, the exogenous proteins are endocytosed and presented by MHC II and this process is referred to as "exogenous pathway". Moreover, it has been demonstrated that there is a link between endogenous and exogenous pathways such as exogenous antigen are presented with MHC I is known as cross presentation (Kurts et al., 2010; Neefjes and Ovaa, 2013).

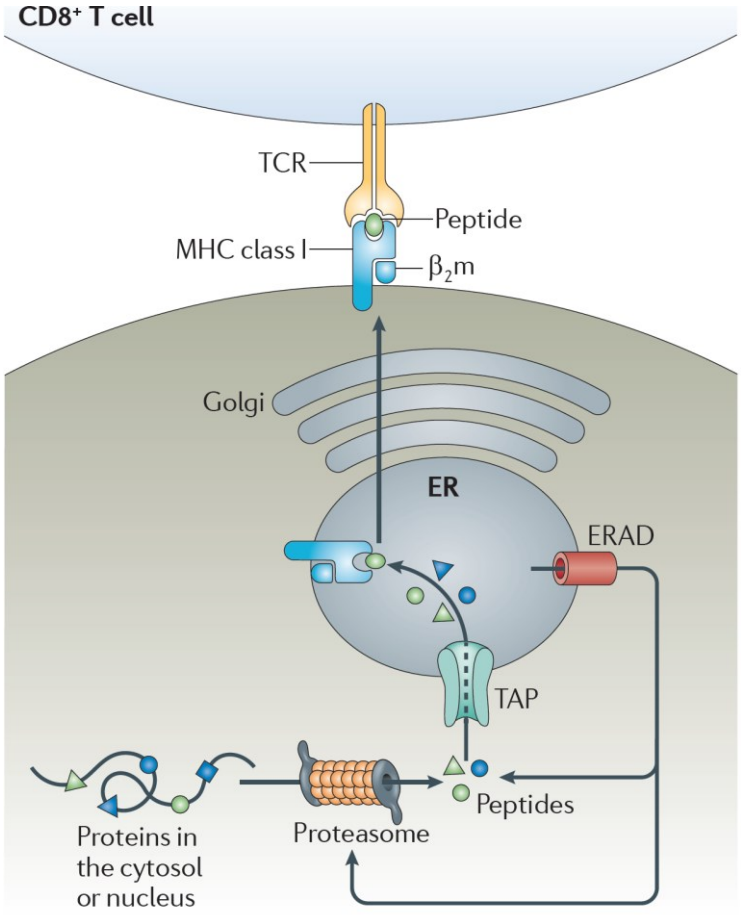


Figure 8. Illustration of the MHC class I antigen processing and presentation pathway
(Extracted from (Neefjes et al., 2011)

The processed peptides are translocated by TAP (Transporter associated with Antigen Processing) proteins into the endoplasmic reticulum (ER) lumen. Successful peptide binding requires folding of nascent MHC I molecules facilitated by ER inhabitant chaperones. The heavy chain and β2-microglobulin domain of MHC I bind to TAP to form a complex of chaperones calreticulin and ERP57. At this stage, Tapasin is required not only to associate nascent MHC I to TAP but, also to complete the assembly of MHC I and the delivery of peptides to the binding groove (Schmid et al., 2010; Verweij et al., 2015). Finally, the MHC class I molecule disconnects from TAP and the assembled peptide-MHC class I complex leaves the ER to be transported to the plasma membrane by means of the Golgi apparatus and may be recognized by the T cell receptor (TCR) on $CD8^+$ T cells (Figure 8). Moreover, peptides that are unable to bind to MHC I in the ER usually reappear in the cytosol for degradation. Exogenous antigens can also be presented by MHC class I molecules, however the exact mechanisms involved remains unclear (Hewitt, 2003; Neefjes et al., 2011; Schmid et al., 2010; Verweij et al., 2015).

1.8.2.3 $CD4^+$ T cell help and HIV-1

There is increasing evidence that supports the role of protective T cell responses against HIV-1; however, those correlates of protection still remain undefined (Adland et al., 2013; Batorsky et al., 2014; Chakraborty et al., 2014; Jin et al., 1999). Most researchers investigate $CD4^+$ and $CD8^+$ T cell responses in HIV-1 infection and focus less on other T cell subsets such as regulatory T cells and gamma delta etc (Seder et al., 2008). It is complex to dissect T cell responses to an invading pathogen; which reflects the essential role of T cells as they orchestrate not only the adaptive and humoral immune responses but, in some way also augment the innate immune response (Dempsey et al., 2003).

The role of $CD4^+$ T cells in HIV-1 anti-viral activity is unclear. It has been suggested that HIV-1 specific $CD4^+$ T cells may mediate antiviral activity against HIV if recruited early into lymphoid tissue but, conversely may enhance HIV-1 replication by providing targets for HIV-1 infection (Cheynier et al., 1998). Memory $CD4^+$ T cells are known to be the primary targets for HIV infection and replication. Consequently, these cells may remain infectious for a long period of time (Douek et al., 2002; Haqqani et al., 2015; Monteiro et al., 2011). It is therefore possible for HIV-1 to undergo numerous replication cycles by targeting memory $CD4^+$ T cells (Betts et al., 2001). This process results in a substantial loss of $CD4^+$ T helper cells mediated by CTL; however, the exact mechanisms involved in $CD4^+$ T cell depletion *in vivo* still remain elusive (Douek et al., 2002; Okoye and Picker, 2013). Importantly, IFN-γ producing $CD4^+$ T cells are detected at very low frequency throughout the course of infection but, these $CD4^+$ T cell responses have no or little effect on HIV-1 control (Betts et al., 2001; Seder et al., 2008). It has been reported that there is a gradual loss of $CD4^+$ T cells in most patients as large numbers of $CD4^+$ T cells are infected and eliminated per day (Ho et al., 1995; Pantaleo, 1999). Data generated from human studies infer that T helper cells are required for $CD8^+$ T cell cytotoxic activity and demonstrated that $CD4^+$ T cell depletion abrogates the immune control of HIV, even in the presence of CTL (Baker et al., 2009; McMichael et al., 2010). Loss of immune control in viral infection has been established in a mouse model of lymphocytic choriomeningitis virus (LCMV) infection. Virus-specific $CD8^+$ T cells in mice were unable to confer protection against LCMV in the absence of $CD4^+$ T cell help (Kalams and Walker, 1998; Rosenberg and Walker, 1998). Regardless of their reduced frequency, $CD4^+$ T cells are able to provide help to $CD8^+$ T cells to mount their first strong anti-viral immune response in acute infection (Streeck et al., 2014). In the later stage of disease, CTL activity is typically reduced due to the loss of $CD4^+$ T cell help and results in rapid disease progression to AIDS (McMichael and Phillips, 1997; Streeck et al., 2009b).

1.8.2.4 $CD8^+$ T cell responses

As mentioned earlier, the control of viral infection by CTL has been defined in the LCMV model. In the same way, HIV specific $CD8^+$ T cell responses are believed to be key players in controlling HIV infection but the mechanisms involved in this process remain unresolved. During acute infection there is an increase in viraemia, generally to $>10^7$ copies/ml, followed by a drop to a viral set point in chronic infection (Goulder and Watkins, 2008; Koup et al., 1994). This drop to viral set point possibly occurs as result of strong anti-viral activity *in vivo* when CTL recognise the processed viral peptides presented on surface of HIV infected cells in the context of HLA.

The principal mechanism by which CTL eliminate infected $CD4^+$ T cells is through the calcium-dependent release of granules containing cytolytic enzymes such as granzymes (A and B) and perforin. Upon release perforin subunits form transmembrane pores in the membrane of the target cell allowing delivery of granzymes into the target cells, which then undergoes apoptosis (Liu et al., 1995; Migueles et al., 2008; Sewell et al., 2000). Although calcium-independent cytotoxicity is also mediated by Fas-Fas ligand interactions (Sewell et al., 2000) the secretory granule (granzymes and perforin) facilitated pathway is the main mechanism by which lytic granules eliminate infected cells (Migueles et al., 2008). The secretory granules are released into the immunological synapse formed between CTL (effector cells) and infected $CD4^+$ T cells (target cells) (Voskoboinik et al., 2010).

$CD8^+$ cells are also able to upregulate anti-viral activity of immune cells by releasing cytokines such as IFN-γ, TNF-α and IL-2 (Almeida et al., 2009; Betts et al., 2001; Migueles et al., 2008; Migueles et al., 2000). In addition to this, direct control of viral replication may be provided by the release of chemokines such as RANTES, MIP1-α and MIP1-β which directly inhibit infection by hindering HIV entry to $CD4^+$ T cells by blocking the CCR5 co-receptor (Almeida et al., 2009; Betts et al., 2001). The drop in pVL during acute and chronic infection was demonstrated in the SIV model where depletion of $CD8^+$ T cells in rh macaques resulted in rapid SIV replication (Jin et al., 1999; Schmitz et al., 1999; Tsukamoto et al., 2007). Thus, there is an association between decline in viraemia and emergence of CTL responses against HIV, suggesting that $CD8^+$ T cells may be responsible for clearing virus in early infection (Goulder and Watkins, 2008; Koup et al., 1994).

There is increasing evidence that the quality of T cell responses in the acute phase of HIV infection is similar to that observed in acute stages of other viral infections such as influenza and Epstein barr virus (EBV), suggesting that early CTL responses in HIV infection are important in controlling early viral load (McMichael and Phillips, 1997; Migueles et al., 2008). Nevertheless, there are qualitative dissimilarities between HIV-1 specific $CD8^+$ T cell responses observed during chronic infections with HIV and those detected in other effectively controlled infections such as Cytomegalovirus (CMV)

and EBV. These dissimilarities are that HIV-1 specific $CD8^+$ T cells are not able to proliferate and secrete IL-2 or other combinations of cytokines effectively (Koup et al., 1994; Migueles et al., 2002b). However, in a minority of individuals termed LTNP, HIV-1 specific $CD8^+$ T cells retain their capacity for proliferation along with cytolytic potential when compared with progressors. LTNP have also been shown to a have higher proportion of polyfunctional cells (Betts et al., 2001; Ferrari et al., 2011; Migueles et al., 2008; Migueles et al., 2000). The CTLs characterised in LTNP were found to be polyfunctional (i.e. CTL capable of expressing multiple cytokines such as IFN-γ, IL-2, TNF-α), whilst mobilising CD107 (Lysosomal-associated membrane protein (LAMP) – a degranulation marker) along with chemokines such as RANTES, MIP1-α and MIP1-β (Almeida et al., 2009; Betts et al., 2001; Precopio et al., 2007). Yet, it is still not apparent which of these effector functions are responsible for clearing virus. However, these observations do propose that polyfunctional instead of monofunctional $CD8^+$ T cell responses may be a characteristic of HIV disease control throughout infection (Almeida et al., 2009; Baker et al., 2009; Migueles et al., 2002b).

Several studies have demonstrated an association between Gag specific $CD8^+$ T cell responses and lower pVL (Edwards et al., 2002; Masemola et al., 2004; Novitsky et al., 2003), while Env and Nef responses were not associated with a lower pVL (Edwards et al., 2002; Novitsky et al., 2003). One well-designed population study characterised dominant $CD8^+$ T cell responses in 578 HIV-1 infected South African individuals and demonstrated that CTL responses against Env were linked with increased viraemia, whereas broad Gag-specific responses were associated with decreasing viraemia (Kiepiela et al., 2007). This was further supported by data obtained from an animal study where pigtail macaques were immunised with HIV-Env and SIV-Gag and challenged with simian human immunodeficiency virus (SHIV)-mn229. There was no significant difference observed in pVL of Env responders and non-responders whereas the pVL of Gag responders was significantly lower than the Gag non-responders (Dale et al., 2004; Peut and Kent, 2007). Thus, CTL appear to be the key player in lowering the pVL to "set point" during initial infection and also controlling viral replication during the chronic phase (Gandhi and Walker, 2002; Koup et al., 1994).

1.8.2.5 T-cell memory

Immunological memory is defined as the ability of the immune system to deal with a pathogen more promptly and efficiently when it is re-encountered and thus, confer life-long protection against invading pathogens encountered previously (Farber et al., 2014). The robust generation of memory T cell responses can be divided into three phase; 1) the selection and expansion of naïve T cells; 2) extinction of the bulk of effector T cells and 3) where T cells that survived become memory T cells and persist long-term (Kaech et al., 2002; Lanzavecchia and Sallusto, 2000). It has been shown in a

mouse model that naïve $CD4^+$ or $CD8^+$ T cells once activated undergo rapid expansion and differentiation, upon antigen encounter, into effector T cells. It is believed that these effector T cells are the ancestors of long lived antigen specific memory T cells. These antigen specific T cells persist in the host at several sites and mount protective immune responses when exposed to pathogens seen previously (Farber et al., 2014; Wherry and Ahmed, 2004). Protective memory $CD8^+$ T cell responses have been shown in the LCMV mouse model (Wherry and Ahmed, 2004) similarly, protective memory CD4 responses were also shown against Mycobacterium tuberculosis (Khader et al., 2007), flu (Teijaro et al., 2010) and parasite infections (Anthony et al., 2006).

Moreover, two subgroups of memory CD4 and CD8 populations have been identified based on their phenotypic characteristics i.e. the existence or lack of effector function and the up-regulation of homing receptors (Farber et al., 2014). Central memory T (TCM) cells are identified as CCR7+, $CD45RA^-$ and generally release IL-2, however, they produce substantial quantities of IFN-γ and IL-4 once differentiated into effector cells (Farber et al., 2014; Kaech et al., 2002). The expression of CCR7 allows the relocation of TCM to secondary lymphoid tissues. Similarly, the second subgroup known as effector memory T cells (TEM) are identified as $CCR7^-$ $CD45RA^-$, usually known as terminally differentiated, and they upregulate cytokine production when they encounter antigen however, these cells proliferate very slowly and tend to have short longevity (Farber et al., 2014; Kaech et al., 2002).

During HIV infection frequencies of $CD4^+$ TCM decrease especially during the acute stage of disease, where they are rapidly depleted and the frequency drops to a very low level during the chronic stage of infection (Dai et al., 2009). In comparison, low frequency $CD8^+$ TCM do exist in the chronic phase of disease but, it is driven towards short-lived effector phenotype (Ladell et al., 2008). The precise mechanisms involved in the skewing of this $CD8^+$ TCM phenotype are unclear and could be due to a number of factors; no $CD4^+$ help, fast elimination of memory $CD8^+$ T cells due to rapid turnover (Ladell et al., 2008; Sachsenberg et al., 1998) and/or reduced CD127 (IL-7R) expression on $CD8^+$ T cells which may result in impaired IL-7 dependent homeostasis (Wherry and Ahmed, 2004).

1.8.2.6 Immune mediated control of HIV-1: HLA alleles diversity impact

Several lines of evidence illustrate that the host genetic background is critical for the control of any disease including HIV. For instance, certain HLA class I alleles are associated with better disease outcomes, whereas homozygous alleles may be linked with more rapid disease progression as fewer alleles provide a more limited variety of HLA molecules for antigen presentation to $CD8^+$ T cells compared to heterozygous alleles (Carrington, 1999; Leslie et al., 2004; Tang et al., 1999). Similar to

other infections, in HIV infection disease outcome may depend on the $CD8^+$ T cell responses induced against processed viral peptides presented to the TCR in association with HLA MHC-I alleles.

A few HLA-B alleles (B*07, B*13, B*15, B*27, B*44, B*57, B*58 and B*81) may be associated with slower HIV disease progression (Kiepiela et al., 2007; Kloverpris et al., 2014; Migueles and Connors, 2015; Migueles et al., 2000), while others such as B*3502 and B*3503 are associated with failure to control pVL (Gao et al., 2001; Tomiyama et al., 1997). One of the reasons given for this failure was that the P2 (anchor position 2) pockets of B*3501, B*3502 and B*3503 are the same, however, the P9 pockets of HLA- B*3502 and B*3503 can differ by as few as one amino acid that significantly affect peptide MHC recognition and results in rapid disease progression. No such P9 polymorphisms were observed with the B*3501 molecule (so peptides bind MHC effectively) and hence, it is associated with HIV control (Gao et al., 2001; Hill et al., 1992; Jin et al., 2002; Tomiyama et al., 1997).

Evidently, Gag specific $CD8^+$ T cell responses vary according to the HLA background of the infected individuals and patterns of immunodominance are affected by this; for example 39% of African patients recognised TPQDLNTML (TL9 [$Gag_{180-188}$]) compared to 4% of Caucasians (Geldmacher et al., 2009; Goulder et al., 2000). Several lines of evidence suggest that there is a correlation between certain HLA-B alleles (for example B*27, B*51, B*57 and B*58) and a better disease outcome when related to targeting immunodominant Gag epitopes during acute infection. These include: the $Gag_{162-172}$ epitope KAFSPEVIPMF (KF11 B*5701 restricted), $Gag_{180-188}$ epitope TL9 (B*81 and B*42 restricted), $Gag_{240-249}$ epitope TSTLQEQIGW (TW10 B*57 and B*5801 restricted) and $Gag_{263-272}$ epitope KRWIILGLNK (KK10 B*27 restricted) (Ammaranond et al., 2010; Brockman et al., 2007; Crawford et al., 2009; Goulder et al., 2000; Kawashima et al., 2009; Kiepiela et al., 2007; Novitsky et al., 2003). However, subtle differences in the allele supertype can result in drastic differences in prognosis, for example CTL responses restricted by B*5801 alleles have been correlated with lower viraemia whereas B*5802 was linked to a poor prognosis in a cohort of 1,000 ART naive South African individuals. B*5801 allele is only different from B*5802 by three aa and results in control of HIV (Miura et al., 2008b; Ngumbela et al., 2008). These minor differences in class I alleles not only affect the binding of peptides but, can have a major impact on outcome of HIV control (Goulder and Watkins, 2008; Kiepiela et al., 2004). B*0702 allele is prevalent in African population infected with subtype C and shown to be associated with favourable outcome compared to individuals infected with subtype B, targeting Gag epitopes (Kloverpris et al., 2014).

1.8.2.7 CTL escape, viral fitness and reversion in context of HLA

The concept of "escape" from immunodominant CTL responses was first described in the early 1990s by Zinkernagel and colleagues in transgenic mice where escape variants were detected soon after infection with LCMV (Pircher et al., 1990). Similarly, during the course of HIV infection the virus evolves and evades the host's immune response in both the acute and chronic stages of the disease, likely due to the pressures exerted by both arms of the immune system (McMichael et al., 2010). The pressure imposed by CTL has been well studied both in HIV infection and in the SIV macaque model and it has been reported that CTL variants/escape mutants usually arise within four weeks of infection and may be driven by the potency and the breadth of $CD8^+$ T cells responses (Boutwell et al., 2010; Goonetilleke et al., 2009; Kiepiela et al., 2007). Moreover, these escape variants may encompass polymorphisms in the flanking regions of epitopes rather than within the epitopes themselves which could affect peptide processing and presentation (Letvin et al., 1999; Martinez-Picado et al., 2006; Steers et al., 2014).

The selection pressure exerted by CTL may result in quasispecies that have polymorphisms which are detrimental to the replication efficiency of the virus and hence incur a fitness cost to the virus (Claiborne et al., 2015; Prince et al., 2012; Schneidewind et al., 2009; Troyer et al., 2009; Yue et al., 2015). In simple terms, viral fitness is defined as the ability of the virus to adapt to its environment and to replicate efficiently (Arnott et al., 2010). This is governed by factors such as the host immune response (CTL, NAbs, concentration of CCR4 and CXCR5, NK cells) and the capacity of the virus to replicate and mutate, with one external factor which may influence viral fitness being the use of ART (Goulder and Watkins, 2004; Nicastri et al., 2003; Nijhuis et al., 2001; Van Gulck et al., 2012). CTL escape mutations in the immunodominant B*57-restricted Gag epitope TW10, targeted in the acute phase have been linked with reduced virus fitness and are associated with effective control of disease in HLA-B*57 and HLA-B*5801 individuals (Goonetilleke et al., 2009; Rousseau et al., 2008; Schneidewind et al., 2009; Troyer et al., 2009). Viral replication may be restored by compensatory mutations (Leslie et al., 2004; Schneidewind et al., 2009). However, a TW10 escape mutant reverts back to wild type (WT) upon transmission to B*57 and B*58 negative individuals due to the lack of immune pressure exerted by the host's genetic background (Goonetilleke et al., 2009; Rousseau et al., 2008; Troyer et al., 2009). Another study described an escape variant (R264K anchor residue P2, abrogating peptide binding to the HLA groove) of immunodominant Gag epitope KK10 in B*27 positive subjects that was associated with significant defects in HIV-1 replication. However, this polymorphism was shown to be compensated by a rare upstream compensatory mutation S173A occurring late in infection which is linked to increased viraemia (Goulder and Watkins, 2004, 2008; Schneidewind et al., 2008; Schneidewind et al., 2007).

Escape mutations have also been studied in vertical transmission (Feeney et al., 2005; Schneidewind et al., 2009) where mother and child share the same HLA allele (both B*27) (Goulder et al., 2001). $CD8^+$ T cell epitopes can revert back to the WT when transmitted to a new host (different HLA). However, when an escape variant R264T together with L268M (B*27 restricted epitope KK10) from B*27 mothers was transmitted to B*27 infants it continued to persist in infants without reverting back to the WT and resulted in the failure of HIV-1 control in B*27 positive children (Goulder et al., 2001; Klenerman et al., 2002). In another study, escape mutations were reported in B57 epitope TW10 in B57/B58 positive children born to B57 negative mothers. Most of the children mounted potent response to the TW10 escape variant rather than the WT epitope, which is often not observed in HIV infected adults. These findings suggest that consequences of immune escape may vary in children and adults as their immune system is constantly developing during early years of life (Feeney et al., 2005). These findings appear to confirm that CTL escape mutations in protective epitopes are usually associated with loss of viral control. Therefore, responses against protective epitopes are an important basis for research and development for an HIV-1 vaccines.

Importantly, not all escape variants result in reduced viral fitness and previous studies examining mutations arising in the immunodominant Gag epitope SLYNTVATL (SL9) during the chronic phase of infection showed no effect on viral fitness and disease outcome in HLA-A2 individuals (Brander et al., 1999; Christie et al., 2009). Additional data confirm that escape mutants associated with CTL responses to Env gp120 also had no effect on virus fitness (Ball et al., 2003; Marozsan et al., 2005).

Nef mediated down regulation of HLA molecules (A and B), is one other potential mechanism by which HIV-1 can evade recognition by CTL (Balamurugan et al., 2013).Through decreasing the surface density of these class I molecules, Nef renders infected cells less visible to $CD8^+$ T-cells (Adland et al., 2013; Yaciuk et al., 2014). Nef acts on the cytoplasmic tails of HLA-A and B-molecules mediating their intracellular retention and results in down regulation of MHC-I (Rajapaksa et al., 2012). One potential reason proposed for the protective effects of Gag-specific CTL was the availability of Gag epitopes. It was shown in a study that Gag specific $CD8^+$ T cells were able to recognise infected cells within two hours post SIV infection in rh macaques, prior to Nef mediated down-regulation of MHC-1 (Sacha et al., 2007).

1.9 HIV-1 Vaccine development and strategies

The simplest way to control the HIV pandemic would be to develop a safe and globally effective vaccine. At present most infectious disease is controlled by vaccination, such as Hepatitis A, Hepatitis B, Human papillomavirus, Influenza, Polio, Rabies, Rotavirus, Tuberculosis and Yellow fever etc (Plotkin, 2010). Despite the efforts and resources dedicated to HIV-1 research it still remains

extremely challenging to develop an HIV-1 vaccine. Perhaps the most substantial stumbling block is the extraordinary sequence diversity of the virus. HIV-1 diversity is a major hurdle for vaccine design and it is critical to understand factors that determine HIV-1 evolution not only within an individual but, also globally (Carlson and Brumme, 2008; Shankarappa et al., 1999). The most desirable HIV-1 vaccine should be the one that could not only prevent transmission, but also induce sterilising immunity (Koup et al., 2011). An HIV vaccine that could produce potent immune responses may control viraemia, thereby delaying or even preventing AIDS. HIV-1 vaccine must elicit both cellular and humoral responses to block the infection whereas, both innate and cell mediated immune responses play a key role in the containment of the virus post HIV infection. Advances in DNA technology have enabled scientists to synthesise different structural components and proteins artificially which has led researchers to speculate that an effective HIV-1 vaccines may be produced in the near future (Rubens et al., 2015). So far, many HIV vaccine candidate have been developed and tested for their safety and immunogenicity and some tested in efficacy trails (Table 2) (Demberg and Robert-Guroff, 2012; Mann and Ndung'u, 2015).

Table 2. The HIV-1 vaccine clinical trials (Demberg and Robert-Guroff, 2012)

Clinical trial identifier	Status	Phase	Vaccine approach	Alternative study ID	Vaccine components	Comment/ references
NCT00002441	Complete	III	Subunit priming and boosting	AIDSVAX B/B, VAX 004	MN rgp120/HIV-1 and GNE8 rgp120/HIV-1	No protection; Flynn et al., 2005
NCT00006327	Complete	III	Subunit priming and boosting	AIDSVAX B/E, VAX 003	MN rgp120/HIV-1 and A244 rgp120/HIV-1	No protection; Pitisuttithum et al., 2006
NCT00223080	Active	III	Vector priming, subunit boosting	RV-144	ALVAC-HIV vCP1521 + AIDSVAX	31.2% efficacy; Rerks-Ngarm et al., 2009
NCT00095576	Terminated	II	Vector priming and boosting	STEP study	Trivalent MRKAd5 HIV-1 gag/pol/nef	No protection; Buchbinder et al., 2008
NCT00413725	Suspended	II	Vector priming and boosting	Phambili study	MRKAd5 HIV-1 gag/pol/nef	No protection; Gray et al., 2011
NCT00865566	Recruiting	II	DNA priming, vector boosting	HVTN 505	VRC-HIVDNA016-00-VP (DNA) + VRC-HIVADV014-00-VP (rAd5)	–
NCT00820846	Active	II	DNA priming, vector boosting	HVTN 205	pGA2/JS7 DNA + MVA /HIV clade B gag-pol-env	–
NCT01418235	Recruiting	I	DNA/vector/subunit	HVTN 086/SAAVI 103	DNA-C2 + MVA-C + gp140 in MF59	–
NCT00062530	Not yet recruiting	I	Recombinant bacteria	P01AI47490	Oral recombinant Salmonella typhi HIV-1 gp120	–
NCT01441193	Recruiting	I	Subunit priming and boosting	ISS P-002	HIV-1 Tat; delta-V2 Env	–
NCT01095224	Recruiting	I	Vector priming and boosting	HVTN 083	rAd35 Env A+rAd5 Env A or rAd5 Env B	–

Numerous HIV-1 vaccine candidates have been produced including: peptide vaccines, recombinant subunit proteins, live vectors, virus-like particle, DNA prime and recombinant vaccinia virus boost, however, live attenuated and heat killed HIV vaccine strategies are not used due to the risk of infection

(Table 2 and Table 3) (Demberg and Robert-Guroff, 2012; Karch and Burkhard, 2016). Not all but, many HIV-1 vaccines have been based on the premise of inducing T cell immunity to the virus that may possibly, while others focusing on eliciting Ab, decrease the level of acute viraemia and lower the set point pVL during the ensuing chronic stage of infection, consequently lowering the risk of transmission and disease progression (Letvin, 2005; Walker and Burton, 2008).

However, the candidates used in STEP (HVTN 502) and associated Phambili (HVTN 503) along with HVTN 505 trials were disappointing. These trials used an adenovirus serotype 5 (Ad5) vector expressing HIV-1 Gag, Pol and Nef genes designed to elicit T cell responses (Altfeld and Goulder, 2011; Buchbinder et al., 2008). Results were disappointing as there was a slight increase in infection rates in those vaccinated individuals compared to placebos, which was associated with the presence of vector specific-immunity (Buchbinder et al., 2008). One of the possible reasons for the failure of the vaccine to protect may have resulted from the narrow breadth of insert-specific $CD8^+$ T cell responses generated, necessitating the need for a vaccine candidate that could induce more robust $CD8^+$ T cell responses (Hertz et al., 2013; Li et al., 2011).

Table 3. HIV-1 vaccine design strategies and progress (Mann and Ndung'u, 2015)

Design strategy	Expected outcome	*In vitro* evaluation	Animal trials	Human trials
1. Mimicking native trimer: remove non-functional Env from VLP	bNAb	Recognised by NAb but not non-NAb	-	-
2. Mimicking native trimer: soluble SOSIP-modified Env trimer	bNAb	Recognised by bNAb but not non-NAb Resembles Env trimer by electron microscopy	-	-
3. Stabilised bNAb epitope: epitope-scaffolds	bNAb	Bound to bNAb	Ones tested did not elicit NAb	-
4. Stabilised bNAb epitope: targeting germline and driving maturation	bNAb	Potently activated germline and mature VRC01 B cells	-	-
5. Stabilised bNAb epitope: fragment immunogen	bNAb	Ab induced in rabbits neutralised tier I, II and III viruses	Induced b12 bNAb in rabbits	-
6. Mosaic immunogens	T cell responses to diverse strains, reduce escape	Processed and expressed by human T cells	Increased breadth and depth of T cell responses Reduced per exposure probability of infection by ≈ 90%	-
7. Conserved element immunogens	T cell responses to diverse strains, reduce escape/ attenuate virus	T cell responses elicited in humans inhibited viruses	Highly immunogenic	High magnitude and breadth of T cell responses 100% vaccinees
8. Escape-cornering immunogens (computational model)	Reduce escape/ attenuate virus	Fitness testing of mutants supported model predictions	-	-
9. Immunogens using CMV vectors	Persistent T cell responses to act early	-	50% monkeys clear SIV infection early Persistent, unusually broad T cell responses	-

Another HIV-1 vaccine trial RV144 combining two vaccines ALVAC HIV vaccine (prime) and AIDSVAX B/E vaccine (boost) constituted gp120 surface protein. The results showed only 31.2%

efficacy against HIV-1 and were considered to be as "modest". (Haynes et al., 2012; Rerks-Ngarm et al., 2009). Furthermore, transmitted viruses found in infected vaccinees may have undergone a vaccine-induced sieve effect that effectively enriched for viruses that would escape vaccine induced responses when compared to viruses transmitted in individuals receiving placebo. This can provide useful information about how viruses evaded vaccine elicited immune responses (Edlefsen et al., 2015).

Hope for T-cell based vaccines comes from pre-clinical studies using SHIV hybrids as both the immunogen and challenge virus in rh macaques which have demonstrated induction of CTL which limit pVL and protects from disease progression (protective immunity), but does not protect from infection (sterilizing immunity) (Amara and Robinson, 2002). An effective HIV-1 vaccine possibly would be required to induce both humoral and cellular arms of the adaptive immune response (Miedema, 2008). Recently the use of a CMV vector incorporating the SIV Gag protein was shown to control pVL and ultimately cleared SIV challenge in 50% of rh macaques. While virus could not be isolated from the controlling 50% of monkeys, this was attributed to the vectors ability to induce presentation of non-canonical peptides either through MHC-E or the majority of the CD8 responses were MHC II restricted, thus broadening antigen-specific T-cell responses (Hansen et al., 2016; Pietra et al., 2010). Another study has shown that expression of HLA-E specific inhibitory NK receptors (NKG2A/CD94) were able to lyse HIV infected cells efficiently, even though NKG2A/CD94 interact poorly with HLA-E. Thus, suggesting that HLA-E restricted NKG2A/CD94^{+} T cells may be responsible for eradicating HIV infected cells (Davis et al., 2016). As HLA-E is not very polymorphic, a vaccine incorporating MHC-E restricted CTL epitopes may induce broad immune responses in most vaccinees, possibly providing for efficacy in all individuals regardless of MHC-I genotype (Hansen et al., 2016; Pietra et al., 2010).

The failure to produce an effective prophylactic vaccine using monovalent consensus-sequence based vaccine inserts has stemmed from their inability to overcome virus sequence diversity. This has led researchers to re-consider the insert design using "centralised sequences" based on the ancestral, or centre of the tree (COT) sequences (Fischer et al., 2008; Nickle et al., 2007), selection of solely conserved regions of the virus (Abdul-Jawad et al., 2016; Letourneau et al., 2007; Liu et al., 2009a; Rolland et al., 2007b; Yang et al., 2015; Yang et al., 2011), subtype specific consensus (Keefer et al., 2012; Kopycinski et al., 2014) and mosaic immunogens (Fischer et al., 2007) as future candidates for vaccine inserts to enable the induction of broad, cross-reactive T cell responses in an effort to deal effectively with enormous virus diversity (Fischer et al., 2008; Gaschen et al., 2002).

COT sequences are derived using a computational algorithm that reduces the distances between the branches and the centre of the tree (Fischer et al., 2008; Nickle et al., 2007). Thus, a vaccine insert sequence based on this approach reduced the genetic difference between a vaccine candidate and circulatory quasispecies in the population (Barouch and Korber, 2010; Nickle et al., 2007; Santra et al., 2008). The COT vaccine candidates have been shown to be immunogenic both in animal models (Kothe, 2006; Weaver et al., 2006) and in phase I clinical trials (Goonetilleke et al., 2006), but COT immunogens may not cover the full breadth (82% coverage for Gag and 62% for Nef) of all potential T cell epitopes (Nickle et al., 2007; Santra et al., 2008).

Alternatively, computationally created polyvalent *Mosaic* immunogens typically induce cross reactive T cell responses against most circulating forms of virus (Fischer et al., 2008; Fischer et al., 2007; Ndhlovu et al., 2011). Mosaic immunogens are based on natural sequences of HIV-1 proteins, maintain natural processing of antigen and have been shown to provide improved T cell epitope coverage compared to the COT, conserved and consensus candidates (Barouch et al., 2010; Corey and McElrath, 2010; Santra et al., 2010). Studies performed in rh macaques have demonstrated the increased breadth (median of 7.75 and 5.5 with mosaic and consensus peptides, respectively) of responses elicited by mosaic immunogens compared to the consensus immunogens in immunised animals (Barouch et al., 2010; Fischer et al., 2007; Santra et al., 2008). Mosaic immunogens provided better coverage compared to the COT when the same set of data was analysed with two algorithms suggesting that mosaic immunogens could cover most circulating strains of the virus and could deal with virus diversity more effectively than COT (Korber et al., 2009a). Mosaic immunogens incorporate both variable and conserved regions of the HIV-1 proteome and may induce responses to variable regions, which may not be effective or protective (Fischer et al., 2007; Letourneau et al., 2007).

There is increasing evidence that T cell based vaccines can generate strong immune responses towards highly conserved epitopes and may confer protection against numerous strains of viruses/pathogens (i.e. HIV and influenza) (Berkhoff et al., 2006; Borthwick et al., 2014; Brown and Kelso, 2009; Jin and Chen, 2014; Kreijtz et al., 2011; Letourneau et al., 2007). Despite the diversity of the HIV-1 genome, conserved epitopes have been identified which escape slowly, likely because of poor immune responses directed against them or these regions are structurally and/or functionally critical for viral fitness (i.e. HIV replication) (Crawford et al., 2007; Davenport et al., 2008; Leslie et al., 2004; Yang, 2009). CTL escape mutations conferring a fitness cost to the virus have been shown to be beneficial to the host in terms of the pathogenesis of HIV, by reducing pVL (Boutwell et al., 2010; Goepfert et al., 2008; Liu et al., 2009b; Yang et al., 2010). It is believed that a HIV-1 vaccine candidate incorporating conserved sequences could induce robust $CD4^+$ and $CD8^+$ T cell responses

to eliminate virus and provide a good coverage of circulating quasispecies in the population as these HIV-1 sequences are known to be extremely conserved among different clades. (Korber et al., 2009a; Liu et al., 2009a; Rosario et al., 2010; Yang, 2009). It has been demonstrated that T cell responses to conserved regions of HIV-1 are associated with lower viraemia and are elicited during the disease course in naturally infected individuals (Liu et al., 2009a; Yang et al., 2011). As conserved regions of the HIV-1 genome are likely to be of functional importance, using such sequences in a vaccine candidate may induce escape variants with a reduced fitness (Letourneau et al., 2007; Liu et al., 2009a). Studies have shown that a vaccine insert based on the 14 most highly conserved (HIV_{consv}) regions of HIV-1 proteome designed to provide cross clade coverage of subtype A, B, C and D contains an abundance of CTL epitopes and may confer cross reactive protection against HIV infection (Letourneau et al., 2007; Liu et al., 2009a; Rosario et al., 2010; Yang, 2009; Yang et al., 2010). However, there are caveats to this strategy; the highly conserved nature of these HIV_{consv} domains might not be immunogenic across all HLA backgrounds and the virus may not be under sufficient selection pressure to escape the immune response which usually incurs fitness cost and may be beneficial to the host due to the reduced replicative capacity (Korber et al., 2009a; Schneidewind et al., 2008; Schneidewind et al., 2007).

Another vaccine candidate has been produced based on full length HIV-1 subtype A sequences (AY253305 & AF457081) or A encoding recombinant strains (Dowling et al., 2002). This International AIDS Vaccine Initiative (IAVI) vaccine candidate used a recombinant adenovirus serotype 35 (Ad35) vector expressing whole Gag, RT, INT and Nef sequences (abbreviated as Ad35 GRIN) and the second vector expressing gp140 Env was designated as Ad35 Env. The IAVI vaccine candidate referred as Ad35 GRIN/Env was used in two independent Phase I clinical trials (NCT00851383, 2009; NCT01264445, 2010) to evaluate the safety and immunogenicity of GRIN/Env. The secondary goal was to assess the humoral and cellular immune responses of the vaccines and to investigate potential Env immunodominance.

1.9.1 Use of viruses as vaccine vectors

Viruses when used as vaccine vectors enter host cells and use their cellular machinery to produce virus proteins (Rollier et al., 2011), making viral vectors attractive candidates to deliver vaccines. Viral vectors seem to be an extremely useful approach to deliver vaccine antigens and are known to induce strong Ab and T cell responses (Saxena et al., 2013). Table 4 shows a list of viral vectors and their advantages and issues. CTL responses are usually required for clearing intracellular pathogens and in controlling cancerous tumours, which is generally not possible with protein-based vaccines (Liu, 2010; Rollier et al., 2011). However, the biggest disadvantage of viral vectors is the pre-existing

immunity to vector parent viruses. Pre-existing immunity/seroprevalence to herpes simplex virus-1 and Ad5 in an American population was reported to be as high as 45% and 70%, respectively (Nwanegbo et al., 2004; Pichla-Gollon et al., 2009). Pre-existing immunity to viral vectors may hamper the transduction of target cells, which decreases the quantity of vaccine antigen that can be generated and reduces the ensuing adaptive immune responses (Pichla-Gollon et al., 2009; Saxena et al., 2013). One potential explanation for the failure of the STEP trial was pre-existing immunity to Ad5 (Buchbinder et al., 2008). Merck vaccine recombinant Ad5 expressing HIV-1 Gag/Pol/Nef did not prevent HIV-1 infection and may have even increased the risk of HIV-1 infection, post-immunisation in uncircumcised men with pre-existing immunity to Ad5 (Buchbinder et al., 2008; Cheng et al., 2012; Priddy et al., 2008). In contrast, Ad35 seroprevalence was shown to be much lower than Ad5 seroprevalence in the populations at risk of HIV-1 infection (Kostense et al., 2004; Nwanegbo et al., 2004). 60% of HIV positive individuals from the Netherlands and 90% sub-Saharan African were shown to have pre-existing immunity to Ad5 compared to 7% and 20% Ad35 seroprevalence respectively, and similar frequencies were seen in non HIV healthy donors (Nwanegbo et al., 2004). These findings support further development and deployment of Ad35 as a viral vector for potential HIV and other disease vaccination (Kostense et al., 2004; Nwanegbo et al., 2004).

Table 4. List of viral vectors to deliver vaccine immunogen (Liu, 2010)

Vector	Potential Advantages	Issues/Concerns
Nucleic Acids		
Plasmid DNA	Licensed veterinary vaccines	Low human potency without heterologous boost
	Relatively generic construction and production	Use of delivery device or technology will increase complexity and cost
	Potent prime in animal studies	
RNA	Considered to have fewer safety regulatory issues than other vectors, e.g., no integration, no issue of autoimmunity	Instability; likely require formulation and/or delivery technology
	Facile production with high purity	
Viruses		
Poxviruses	Licensed veterinary vaccine vector	Possible issue of pre-existing immunity for vaccinia (smallpox) vaccinees
	MVA known clinical safety	Probably will need prime, increasing complexity and cost
	Room for large gene insert	
	Various strains available	
Adenovirus	Historic oral adenovirus vaccine	Pre-existing immunity to certain human strains may not preclude use as vectors, but results of HIV STEP trial need to be understood re: safety for high-titered vaccinees relative to subsequent risk of HIV infection if exposed
	Strong immune responses	Human Ad strains oncogenic in animals
	Many strains available	
Adeno-associated virus	Apparent efficacy as vector in small gene therapy trial for eye disease[a]	Disputed risk of integration
	Safety shown in small gene-Rx trials	Limited gene insert capacity
Alphaviruses	Virus particles or DNA replicons	Modest gene capacity
	High expression	
	No risk of integration because RNA particle can target DCs	
Herpes virus	Broad tropism including DCs	Neurotropism may be safety issue
		Possible pre-existing immunity
Measles virus	Mucosal delivery possible	Possible pre-existing immunity
	RNA virus (no integration issue)	
Vesicular stomatitis virus	Broad tropism including DCs	Neurotropism[b] may be safety issue
	High expression	
Bacteria		
Salmonella/Shigella	Oral delivery	Concern about heterologous gene if on plasmid
	Infect M cells (gut antigen uptake cells)	Potential stability of antigen gene if on plasmid
BCG	Extensive safety of BCG vaccine	Bacterial (versus mammalian) posttranslational modifications
Listeria	Efficient mechanisms for CTL induction	Concerns re: vector virulence in immunocompromised

1.10 Specific Aims and hypothesis

It is hypothesised that the magnitude and/or breadth of $CD8^+$ T cell response(s) directed against highly conserved regions of HIV-1 detected by ex vivo Enzyme-linked immunosorbent spot (ELISpot), may correlate with reduced pathogenesis and slower disease progression (i.e. higher CD4 counts and lower pVL) *in vivo*. The main aim of this thesis was to examine HIV-1 specific $CD8^+$ T cell responses in naturally infected individuals and vaccinees. In particular the goal was to evaluate if pre-existing HIV-1 specific T cell responses preferentially targeted conserved or variable regions of the HIV-1 proteome.

To accomplish these objectives this thesis describes work whose purpose was:

- Development and application of an IFN-γ ELISpot assay using HIV-1 conserved peptides to accurately detect T cell responses.
- Apply the validated IFN-γ ELISpot assay using both HIV_{consv} (Letourneau et al., 2007) matrices and GRIN/Env peptide pools to evaluate the nature of the epitope-specific T cell responses induced during HIV-1 infection in individuals infected with different HIV-1 subtypes from different populations**.** To examine if cell mediated responses generated by subjects infected with different subtypes may lead to specific profiles of $CD8^+$ T cell responses towards conserved regions.
- To understand whether cellular responses against HIV_{consv} and non-conserved (GRIN/Env) sequences during different stages (viraemic controller and progressors) of infection in a cross-clade cohort are associated with their disease prognosis.
- To elucidate if HIV-1 specific T cell responses in vaccinees immunised with GRIN/Env immunogen were targeted towards conserved or variable epitopes in healthy volunteers. Furthermore, to resolve whether the targeting of particular epitopes by vaccinees relates to *in vitro* control using the IAVI virus inhibition assay (VIA) as a proxy, and further try to examine if T cell responses in natural infection are comparable in vaccinees.

Chapter 2: Materials and Methods

2.1 Study Cohorts

2.1.1 UK HIV infected cohort

Sodium heparin treated blood samples (50-60ml) taken from chronically infected HIV-1 positive subjects were obtained to isolate Peripheral blood mononuclear cells (PBMC). The study participants were mostly men (94%) and homosexual (89%). The median age of participants was 46 years. Subjects (n=21) were recruited at outpatient clinics of the Chelsea and Westminster hospital with informed consent from Hammersmith, Queen's Charlotte and Chelsea Research ethics committee. The majority (94%) of the individuals were receiving HAART. Bloods were processed within six hours of collection and PBMCs and plasma were frozen down in liquid nitrogen (LN_2) (-180˚C) and -80˚C, respectively.

2.1.2 African Cohort

More than 400 HIV-1 seroconverters were recruited from Kenya, Rwanda, Uganda, South Africa, and Zambia in longitudinal studies supported by IAVI to follow the immunologic, virologic, and clinical parameters in newly infected volunteers. Subjects were followed for five years, taking samples monthly for the first three months, then every three months for the first 24 months and every six months until the study was completed. The estimated days post infection (eDPI) was calculated as the midpoint between the last negative and first positive antibody test results, 14 days prior to a positive p24 antigen test and negative antibody test, or 10 days prior to a positive PCR test and negative antibody test.

The study participants consisted of both men and women. All recruited volunteers were asked to sign an informed consent form for this study. The longitudinal samples were obtained from HIV positive subjects at three time points visit 1 (V1.0), visit 12 (V12) and visit 24 (V24) months after infection. Blood samples were processed and PBMC isolated at each site and shipped to the human immunology laboratory (HIL), London, UK in vapour phase LN_2. Upon arrival samples were stored in LN_2. This cohort was used to measure the cell mediated immune response in naturally infected individuals.

2.1.3 HIV Negative Cohort

HIV negative buffy coats (BC) were obtained from IAVI laboratories in East (Uganda and Kenya) and South Africa (Johannesburg). Samples were tested for HIV-1 antibodies and other infectious agents at source before being shipped to the HIL. Anticoagulant sodium heparin treated blood samples

(50-60ml) were processed within six hours and PBMC and plasma was frozen in LN_2 (-180˚C) and -80˚C, respectively, before being received at HIL.

2.1.4 B001 (IAVI Sponsored Clinical Trial) Vaccinees

IAVI conducted a phase I clinical trial (ClinicalTrials.gov NCT00851383) to assess the safety and immunogenicity of a novel IAVI vaccine candidate (Keefer et al., 2012; Kopycinski et al., 2014). The trial was conducted at the University of Rochester, New York State, USA where 56 healthy subjects were enrolled. The study participants included both men and women. All recruited volunteers were asked to sign an informed consent form. Participants were at low risk of HIV infection and all the volunteers underwent an HIV screening test. Sexually active men and women used appropriate contraception until at least four months post second immunisation. Participants were excluded from the study if they were positive for Ad35 or HIV antibodies, exhibited high-risk behaviour for HIV infection or had a sexually transmitted disease, were pregnant, had any clinically significant acute or chronic medical condition and in receipt of live attenuated vaccine. Bloods were drawn at multiple time points and isolated PBMC were shipped to HIL in vapour phase LN_2.

2.2 Plasma Viral Load (pVL) and Lymphocyte Count

2.2.1 UK Cohort

pVL and CD4 counts were determined for all HIV infected patients. The pVL assay was performed at Chelsea and Westminster Hospital by PCR using the Bayer HIV-1 RNA 3.0 branched DNA assay (Bayer, UK). The pVL was performed by the staff at the Clinical Immunology Department, Chelsea and Westminster Hospital. The lower limit of detection was 50 copies/ml whereas the upper limit of detection was $5x10^5$ copies/ml. CD4 counts were also determined at Chelsea and Westminster Hospital using whole blood staining with monoclonal antibodies against CD3, CD4, CD8, CD45, CD56 and CD19.

2.2.2 African Cohort

pVL and CD4 counts were determined by PCR using Roche Amplicor Monitor v1.5 (Roche Diagnostics, Indianapolis, USA) or Abbott Real Time HIV-1 v1.0 (Abbott Molecular Diagnostics, Mississauga, Canada) as described previously (Amornkul et al., 2013).

2.3 Media for cell culture

Cell culture media constituted of Roswell park memorial institute (RPMI)-1640 (R10) (Sigma Aldrich, UK), supplemented with 10% heat inactivated Foetal Calf Serum (HIFCS) (Sigma Aldrich,

USA), penicillin (100 IU)/streptomycin (100µg/ml) (Sigma Aldrich, USA), 10mM Hepes (Sigma Aldrich, UK), 1mM Sodium pyruvate (Sigma Aldrich, UK) and 2mM L-glutamine (Sigma Aldrich, UK). R20 was supplemented with 20% HIFCS. R10 and R20 were stored at 2-8°C for up to a month before use.

2.4 Peptides pools and matrices

2.4.1 HIV conserved (HIV_{consv}) peptides pools and matrices

The peptides used in this study were based on a construct encoding fourteen of the most highly conserved regions of HIV (referred to as HIV_{consv}) (Figure 9 and Table 5) and designed by Dr Tomas Hanke (Oxford, UK) (Letourneau et al., 2007).

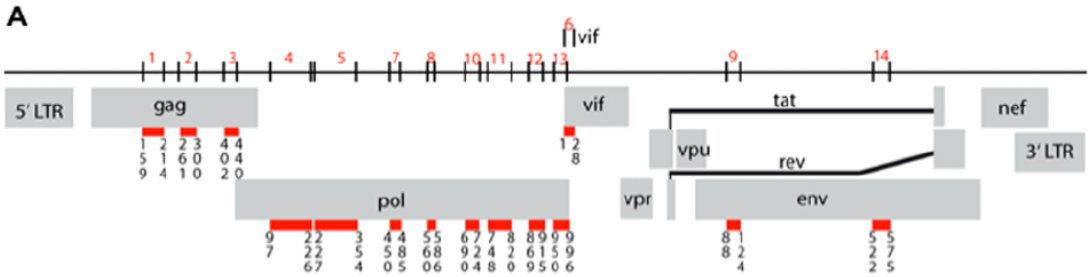


Figure 9. Fourteen of the most highly conserved regions of the HIV-1 used to synthesis HIV_{consv} peptides (Letourneau et al., 2007)

Table 5. Summary of the 14 most conserved fragments used for HIV_{consv} immunogen

Fragment	Protein	Clade	HIV_{consv} (aa)
1	Gag	C	1-56
2	Gag	D	57-96
3	Gag	A	97-135
4	Pol	B	136-265
5	Pol	C	266-393
6	Vif	D	394-421
7	Pol	A	422-457
8	Pol	B	458-484
9	Env	C	485-521
10	Pol	D	522-556
11	Pol	A	557-629
12	Pol	B	630-676
13	Pol	C	677-723
14	Env	D	724-777
15	Non-Human epitopes		778-806
Note.	Amino acid (aa)		

Based on the HIV conserved proteome 199 high performance liquid chromatography (HPLC) purified HIV_{consv} peptides (15 mers overlapping by 11 amino acids) were custom ordered and manufactured by ANASPEC (San Jose, CA, USA). *Deconvolute This!* software version 1.0 (Roederer, 2003) (kindly provided by Mario Roederer, Vaccine Research Centre, NIH, USA) was used to generate pools of overlapping peptides (OLP). This software examines all of the possible peptide pool configurations and also determines each responding positive peptide in a pool (deconvolution). Using these optimal configurations peptide pools were prepared accordingly (Figure 10). The predefined matrix used in this study had a combination of 80 different peptide pools (80 pools of 10 peptides) prepared from the 199 HIV_{consv} peptides. Peptides were reconstituted in sterile Dimethyl sulfoxide (DMSO) (Sigma Aldrich, UK) to 50mg/ml and stored at -80˚C. DMSO reconstituted peptides were diluted in sterile Phosphate buffered saline (PBS) to prepare 80 pools (10 peptides/pool) of 300µg/ml per peptide (Figure 10).

As explained above peptide matrices were generated using Deconvolute this! (Figure 10). Sterile 96 well deep well plates (Sigma Aldrich, UK) were used to further dilute the 80 HIV_{consv} pools to achieve a concentration of 3µg/ml per peptide in R10. The final concentration of HIV_{consv} pools in the assay was 2µg/ml. Mock (0.45% DMSO), CMV and Phytohemagglutin (PHA) were used as assay controls. Pre-made peptide plates were kept at -80˚C. Each peptide in this assay had a coverage of four in the predefined matrix used in this study, meaning that each peptide was represented in a unique combination of four different pools, for example HIV_{consv} peptide 23 was added to pools 3, 24, 41 and 64 along with nine other peptides in each case (Figure 10).

1	1	2	3	4	5	6	7	8	9	10
2	11	12	13	14	15	16	17	18	19	20
3	21	22	23	24	25	26	27	28	29	30
4	31	32	33	34	35	36	37	38	39	40
5	41	42	43	44	45	46	47	48	49	50
6	51	52	53	54	55	56	57	58	59	60
7	61	62	63	64	65	66	67	68	69	70
8	71	72	73	74	75	76	77	78	79	80
9	81	82	83	84	85	86	87	88	89	90
10	91	92	93	94	95	96	97	98	99	100
11	101	102	103	104	105	106	107	108	109	110
12	111	112	113	114	115	116	117	118	119	120
13	121	122	123	124	125	126	127	128	129	130
14	131	132	133	134	135	136	137	138	139	140
15	141	142	143	144	145	146	147	148	149	150
16	151	152	153	154	155	156	157	158	159	160
17	161	162	163	164	165	166	167	168	169	170
18	171	172	173	174	175	176	177	178	179	180
19	181	182	183	184	185	186	187	188	189	190
20	191	192	193	194	195	196	197	198	199	200
21	1	11	21	31	41	51	61	71	81	91
22	92	101	111	121	131	141	151	161	171	181
23	2	12	22	32	42	52	62	72	182	191
24	3	13	23	33	43	53	63	73	82	192
25	4	14	24	34	44	54	64	74	83	193
26	5	15	25	35	45	55	65	75	84	194
27	6	16	26	36	46	56	66	76	85	195
28	7	17	27	37	47	57	67	77	86	196
29	8	18	28	38	48	58	68	78	87	197
30	9	19	29	39	49	59	69	79	88	198
31	10	20	30	40	50	60	70	80	89	199
32	90	93	102	112	122	132	142	152	162	200
33	94	103	113	123	133	143	153	163	172	183
34	95	104	114	124	134	144	154	164	173	184
35	96	105	115	125	135	145	155	165	174	185
36	97	106	116	126	136	146	156	166	175	186
37	98	107	117	127	137	147	157	167	176	187
38	99	108	118	128	138	148	158	168	177	188
39	100	109	119	129	139	149	159	169	178	189
40	110	120	130	140	150	160	170	179	180	190
41	1	12	23	34	45	56	67	78	88	92
42	93	101	113	124	135	146	157	168	178	182
43	3	11	24	35	46	57	68	79	183	191
44	2	14	21	36	47	55	69	80	87	192
45	5	13	22	31	48	59	66	77	89	193
46	4	16	27	32	41	53	70	90	94	194
47	7	15	28	33	42	51	64	95	102	195
48	6	18	25	39	43	52	61	74	96	196
49	9	17	26	40	44	62	71	82	97	197
50	8	20	37	54	63	72	81	98	103	198
51	19	38	65	73	83	91	104	111	122	199
52	10	29	58	75	85	99	105	114	121	200
53	30	49	76	84	100	106	112	123	131	144
54	50	86	107	116	128	145	151	162	172	184
55	60	108	117	126	132	141	153	164	179	185
56	109	115	127	133	142	154	161	177	186	190
57	110	118	119	125	134	143	152	166	171	187
58	120	129	130	136	147	155	163	173	181	188
59	137	138	148	149	156	160	165	167	180	189
60	139	140	150	158	159	169	170	174	175	176
61	1	13	24	32	47	58	65	76	93	103
62	4	104	112	121	133	145	156	168	176	191
63	5	11	26	34	42	60	86	94	101	192
64	2	15	23	37	41	68	85	96	106	193
65	3	12	21	38	44	59	95	105	113	194
66	8	14	22	35	43	67	71	90	99	195
67	9	16	28	31	45	54	73	100	107	196
68	6	19	27	33	50	55	72	92	108	197
69	7	18	30	36	53	62	75	83	109	198
70	17	25	46	51	63	78	110	114	123	199
71	20	39	48	56	64	82	91	115	124	200
72	10	49	52	66	81	97	102	111	125	137
73	29	40	57	61	138	141	152	163	174	182
74	69	74	84	98	116	122	134	148	155	183
75	70	77	87	117	129	131	142	158	165	184
76	79	89	118	127	136	144	151	169	180	185
77	80	88	119	128	132	135	147	160	161	186
78	120	126	139	143	146	154	162	177	187	189
79	140	149	153	157	164	166	171	173	188	190
80	130	150	159	167	170	172	175	178	179	181

Figure 10. Deconvolute this! software generated 80 pools of 10 peptides per pool using 199 HIV_{consv} peptides.

Each peptide had a coverage of four shown in coloured cells. Two peptides (23: 3, 24, 41, 64 and 150:15, 40, 60 and 80) are highlighted. The first column (highlighted in green) is the identity number of peptide pools (80 pools) generated.

2.4.2 Ad35 GRIN/Env (Adeno-virus 35 encoding Gag, RT, INT, Nef and Envelope) matrices and pools

In total there were six whole antigen peptide pools representing Gag (125 peptides), RT (111 peptides), INT (102 peptides), Nef (49 peptides), Env1 (79 peptides) and Env2 (79 peptides) used to detect responses in HIV positive volunteers and also to screen vaccinees for IFN-γ responses. Peptides were synthesised by ANASPEC, Inc (San Jose, CA, USA) and all the peptides were HPLC purified (15 mers overlapping by 11 amino acids). These pools were used for initial screening of responses to refine subsequent mapping for B001 clinical trial. The PBMC were seeded at a concentration of 200,000 cells/well. The final concentration of peptide pools in the assay was 2µg/ml and the assay controls were the same as above.

Ad35 GRIN/Env peptide matrices were similarly generated using Deconvolute this! as explained earlier. Each matrix pool consisted of 10 OLP (15 mers overlapping by 11 amino acid) and used a configuration of three to generate peptide plates. Plates were made with R10 at the concentration of 3µg/ml for each antigen i.e. Gag, RT, INT, Nef and Env and were stored at -80˚C.

2.5 Isolation of PBMC

25 ml of blood was layered on to 20ml of sterile histopaque (Sigma Aldrich, UK) in a 50ml centrifuge tube (Sarstedt, Germany). Tubes were centrifuged (Eppendorf 5810R, Germany) at 400g for 40 minutes at room temperature without brake. For all subsequent centrifugations the brake was applied. Plasma was removed and centrifuged at 1800g for 20 minutes to pellet debris or platelets and plasma aliquots were stored at -80˚C. PBMC were removed from the histopaque interface and washed with 40ml Hank's balanced salt solution without Ca^{2+} or Mg^{2+} (HBSS) (Sigma Aldrich, UK) at 500g for 10 minutes. PBMC were washed again with HBSS at 400g for 10 minutes. The supernatant was discarded and cells were washed twice with R10 (Sigma Aldrich, UK). PBMC were counted using Vi-cell counter (Beckman Coulter, USA) and resuspended in freezing media at $1x10^7$ PBMC/ml. Cryovials were transferred to a cooled (2-8˚C) stratacooler (Stratagene, USA) designed to freeze down slowly overnight at 1˚C per minute by placement in a -80˚C freezer and stored in LN_2 for long term storage. Freezing media was generally prepared on the day of isolation and contained 10% DMSO (Sigma Aldrich, UK) and 90% HIFCS.

2.5.1 Thawing of PBMC

Cryopreserved PBMC were removed from the LN_2 and immediately placed in a water bath (Fischer Scientific, UK) at 37 ˚C. PBMC vials were kept in the water bath until a small ice crystal remained. PBMC were transferred to 50ml falcon tube (Starlab, Germany) containing 9ml of R10. Tubes were

centrifuged (Eppendorf 5810R, Germany) at 400g for 10 minutes at room temperature. The PBMC pellet was re-suspended in 1ml R10 for counting.

2.5.2 Cell Counting

Cell counts and viability was determined using Vi-cell counter (Beckman Coulter, USA), which measures viability by trypan blue exclusion. 50µl of cell suspension was added to 550µl of 1x PBS (Sigma Aldrich, UK). The total cell number was calculated by multiplying the viable cell count with the amount of R10 used to re-suspend the cell pellet. PBMC were not used to perform an assay if viability was lower than 80% (Boaz et al., 2009).

2.6 IFN-γ Enzyme-Linked ImmunoSpot (ELISpot)

IFN-γ ELISpot assay was used to detect vaccine or virus specific T cell responses using a validated assay (Boaz et al., 2009).

2.6.1 Blocking of pre-coated IFN-γ Plates and Thawing of PBMC

ELISpot plates purchased from MABTECH (Sweden) were pre-coated with a monoclonal antibody specific for human IFN-γ. Pre-coated 96 well polyvinyl-difluoride (PVDF) membrane IFN-γ ELISpot plates (MABTECH, Sweden) were washed three times with 200µl/well of sterile Dulbecco's PBS without calcium or magnesium (Sigma Aldrich, UK) and blocked with 200µl/well of R10. Plates were transferred to an incubator (Binder cb series, Germany) set at 37±1^{o}C, 5% CO_2 for minimum of two hours or overnight.

2.6.2 Stimulation with peptide pools

Previously thawed and rested overnight (37±1^{o}C, 5% CO_2) PBMC in R20 were washed with 10ml of R10. Viable cell counts were determined using the Beckman Coulter, UK. Overall the cell viability (African Cohort) was good [median 91.2% (IQR 85.4 - 93.6%)]. Cells were diluted in R10 medium at 2x10^{6}cells/ml. The blocking medium was tipped off and stimuli and controls added. The negative control for the assay was 0.45% DMSO diluted in R10. The positive controls for the ELISpot assay were Cytomegalovirus (CMV pp65; 2µg/ml-final concentration) and PHA (5µg/ml-final concentration). 100µl of all the diluted peptides (HIV_{consv} and GRIN/Env pools) and assay controls were added to the ELISpot plate. If cell count was low then the coverage of three was used for HIV_{consv} matrix (last 20 pools were not included in the experiment set up). Without touching any wells, 50µl of cells (1x10^{5}) were added to each well. Plates were incubated in an incubator maintained at 37±1^{o}C with 5% CO_2 for 16-24 hours. However, when clinical trial samples were tested, then PBMC cells were diluted in R10 at 4x10^{6}cells/ml and seeded at the concentration of 2x10^{5} cells/well.

2.6.3 Development of ELISpot plates

The next day, wash buffer was made by mixing one sachet of powdered PBS-Tween (PBST) (Sigma Aldrich, UK) in one litre of deionised water. ELISpot plates were washed six times with 200µl 0.05% PBST (Sigma Aldrich, UK) with an automatic plate washer (Titertek, USA) and blotted dry on lint free paper after washings in all subsequent steps in this procedure. 100µl of 1µg/ml biotinylated 7-B6-1 mouse-anti human IFN-γ antibody (MABTECH, Sweden) diluted in 0.5% bovine serum albumin (Sigma Aldrich, UK) and PBS was added to each well. Plates were incubated at room temperature for two hours. Plates were washed (6 x 200µl of 0.05% PBST) using the plate washer and dried as before. ABC peroxidase (VECTOR laboratories, USA) complex was prepared by adding one drop A and one drop B per 10ml 0.05% PBST. 100µl of ABC avidin-biotin peroxidase complex (VECTOR laboratories, USA) solution diluted in 0.05% PBST was added to each well and plates were incubated for an hour at room temperature. Plates were washed with 3x200µl of 0.05% PBST followed by further three washes with 200µl PBS. 100µl of 3-Amino-9-ethylcarbazole (AEC) substrate (Sigma Aldrich, UK) solution was added to each well and the plates were incubated at room temperature for four minutes. The reaction was stopped under gently running tap water without any delay. Plates were left in the dark to dry before reading. Plates were analysed by using an Autoimmune Diagnostika (AID) reader (Autoimmune Diagnostika GmbH, Strasbourg, Germany) to count spots per well and the results were expressed as spot forming units (SFU) per million PBMC.

2.7 Enumeration of ELISpot responses to whole antigen pools

IFN-γ ELISpot responses of ≥50 SFU/10^6 (five times the background) were defined as positive. Positive responses were used to deconvolute responding peptides with the aid of Deconvolute This! Software (Roederer, 2003). The ELISpot assay was classified as "failed" and data disqualified from analysis if the mock wells had ≥50 SFU/10^6 and/or if PHA response was ≤50 SFU/10^6. On the basis of the ELISpot data following stimulation from whole antigen pools, those participants who mounted IFN-γ ELISpot responses of ≥100 SFU/10^6 (background subtracted) were selected for B001 epitope mapping.

2.8 DNA Extraction

DNA was extracted from PBMC pellets ($5x10^5$-$1x10^6$ cells) using a Qiagen DNA extraction kit (Qiagen, Germany) as per manufacturer's instructions. All the wash buffers were provided in the kit. In brief, cells were washed twice with 1xPBS (Sigma Aldrich, UK) and re-suspended in 200µl of 1xPBS. Next, 20µl of Qiagen proteinase K was added to the cell suspension and the mixture was mixed thoroughly by inverting tubes a few times. Cells were lysed by adding 200µl of AL buffer and

tubes incubated in a water bath at 56°C for 10 minutes. 200µl absolute ethanol (VWR, UK) was added to the lysate and the cell suspension mixture was transferred to a QIAamp Mini spin column. QIAamp Mini spin columns were washed with 500µl buffer AW1 and AW2, respectively. Columns were spun at 8000 revolutions per minute (RPM) for one minute. DNA was eluted with 50µl elution buffer in a clean tube after incubating for five minutes at room temperature. To enhance the yield a second elution step was carried out with the eluted DNA. A NanoDrop 1000 spectophotometer (Thermo-Scientific, USA) was used to quantify DNA.

2.8.1 PCR Polymerase chain reaction (PCR)

Gag/INT genes were PCR amplified from the genomic DNA for proviral DNA sequencing. The nested PCR primers are detailed in (Table 6).

Table 6. Primers used for nested PCR and to sequence Gag and Integrase genes

Primer ID	Sequence	Position[c]
Nested PCR Primers		
Gag Outer (F)	5' AAATCTCTAGCAGTGGCGCCCGAA 3'	623–646
Gag Outer (R)	5' GTTGACAGGTGTAGGTCCTAC 3'	2481–2501
Gag Inner (F)[a]	5' TCTCGACGCAGGACTCGGCTTG 3'	684–705
Gag Inner (R)[a]	5' CTCCAATTCCYCCTATCATTTTTGGTTTCC 3'	2377–2406
Pol Outer (F)	5' CAGCATGYCAGGGAGTRGGRGGACC 3'	1832–1856
Pol Outer (R)	5'GGYTCTTGRTAAATTTGATATGTCCATTG 3'	3555–3583
INT Outer (F)	5' GCCATTGACAGAAGARAAAATAAAAGCATT 3'	2621–2650
INT Outer (R)	5' GGRGCACAATAATGTATRGGAATTGG 3'	6858–6883
INT Inner (F)[a]	5' GDTATGAACTCCATCCTGAYAAATGGAC 3'	3241–3268
INT Inner (R)[a]	5' CCACACAGGTACCCCATARTADAC 3'	6336–6359
Sequencing Primers		
Gag Seq (F)	5' CAGYCAAAATTAYCCTATAGTGCA 3'	1173–1196
Gag Seq (R)	5' CCTTCYTTGCCACARTTGAAACAY 3'	1962–1985
Gag Seq (R&C)[b]	5' RTGTTTCAAYTGTGGCAARGAAGG 3'	1962–1985
Gag Seq 2nd (F)	5' AATCCACCTATCCCAGTRGGAG 3'	1546–1567
INT Seq (F)	5' GATGGVATAGATAARGCYCAAGA 3'	4236–4258
INT Seq (R)	5' YCAGGRTCTAYTTGTGTGCTAT 3'	5321–5342

[a]Used also as sequencing primers
[b]Reverse and compliment (R&C) of Gag seq (F) primer
[c]Primer positions based on HXB2 strain (K03455)
IUB codes R (A, C), D (G, A, T), Y (C, T) and V (G, A, C)

PCR had previously been optimised in another study (Data not shown) . In the first round of PCR, 5µl DNA was used in a 50µl reaction containing 20pmol of primers either Gag outer (F)/Gag outer (R) pair for HIV gag, pol outer (F)/pol outer (R) pair for HIV *pol* and INT outer (F)/INT outer (R) for HIV INT, 10X PCR buffer (5µl), 1.5mM $MgCl_2$ or $MgSO_4$, 20mM dNTPs, 1.25 units of either Hi-fidelity (Invitrogen, USA) or Platinum *Taq* (Invitrogen, USA) and sterile DNase/RNase free water (Gibco, UK). The PCR conditions for HIV *gag* amplification consisted of 1 cycle of 94°C for 2 minutes (min), 40 cycles of 94°C for 20 seconds, 53°C for 30 seconds, 72°C for 2 min and 1 cycle of 72°C for 7 min (Kousiappa et al., 2009a; Kousiappa et al., 2009b). The amplification conditions for the *pol* gene were the same as the *gag* except that the hybridisation temperature was 52°C. Two negative and one positive control was used per PCR. The negative controls included a reaction with

sterile water in place of the template DNA, a reaction with HIV negative DNA and the positive control included HIV-1 subtype-A reference clone (SE8131) (NIBSC, UK). In the second round of nested PCR, 3µl of the first round products were used in a 50µl reaction. The amplification conditions were the same as the first round except the hybridisation temperatures were 56°C for 2.0 min and 20 seconds for gag and 52°C for 2.0 min for *pol* as described previously (Kousiappa et al., 2009a; Kousiappa et al., 2009b). PCR products underwent 1% agrose (Sigma Aldrich, USA) gel electrophoresis for one hour at 120 volts in Tris-borate-EDT buffer (TBE) (Sigma Aldrich, USA). Separated DNA fragments were visualised using a long-wavelength Ultra violet (UV) trans-illuminator (UVP, USA) and DNA products were identified by comparing the fragments with 1Kb+ DNA ladder (Invitrogen, USA). PCR products were purified using a QIAquick PCR Purification Kit (Qiagen, Germany) as per manufacturer's instructions. NanoDrop 1000 spectophotometer (Thermo-Scientific, USA) was used to quantify purified PCR product.

2.8.2 Subtypes by Viral Sequencing

2.8.2.1 In house Sequencing

The UK cohort (n=15) and some of the African patients (n=9) were sequenced in-house. Purified PCR product (200ng) containing 3.2pmol of each primer was sequenced by Medical Research Council (MRC) sequencing facility (Imperial College, London). Sequencing primers are listed in Table 6. UK cohort subtype data was also obtained from Chelsea and Westminster hospital clinics. The subtyping was performed by Tibotec-Virco Virology BVBA, Beerse, Belgium, by amplifying Pol.

2.8.2.2 African Cohort Subtype

Remaining African cohort subtype data is described by (Amornkul et al., 2013). In short, sequences were generated by amplifying 1.7kb *pol* gene using five primers and the ABI Big Dye terminator kit (version 3.1, Applied Biosystems, Foster City, CA). Data was analysed with the REGA HIV-1 Subtyping tool (http://hivdb.stanford.edu/) (Amornkul et al., 2013).

2.9 Human leukocyte antigen (HLA) genotyping

2.9.1 UK Cohort and Vaccinees

To determine the potential T cell epitopes, HLA class I alleles (A, B and C) were typed at high resolution (4 digits) by IMGM® laboratories (Martinsriedm, Germany) genotyping service. HLA typing was performed by PCR and SBT (sequence-based typing) technology using generic and group-specific high throughput sequencing (Sanger sequencing).

2.9.2 African Cohort

HLA data on the African cohort was obtained from IAVI partners. HLA genotyping at three HLA class I loci (HLA-A, HLA-B and HLA-C) were typed to 4-digit resolution using PCR technique and sequence based typing, as has been described previously (Tang et al., 2011).

2.10 T cell function and phenotype analysis using flow cytometry

A nine-colour flow cytometry panel was designed to characterise the phenotype of antigen-specific T-cell responses. The LSRII cytometer (Beckton Dickenson (BD), UK) was used to acquire stimulated, stained samples (for configuration see Figure 11). The panel was designed to perform comprehensive functional analysis of antigen specific $CD8^+$ T cell responses mounted during acute HIV-1 infection. All the antibodies were pre-titrated by Dr J. Kopycinski to determine the optimal volume required for staining.

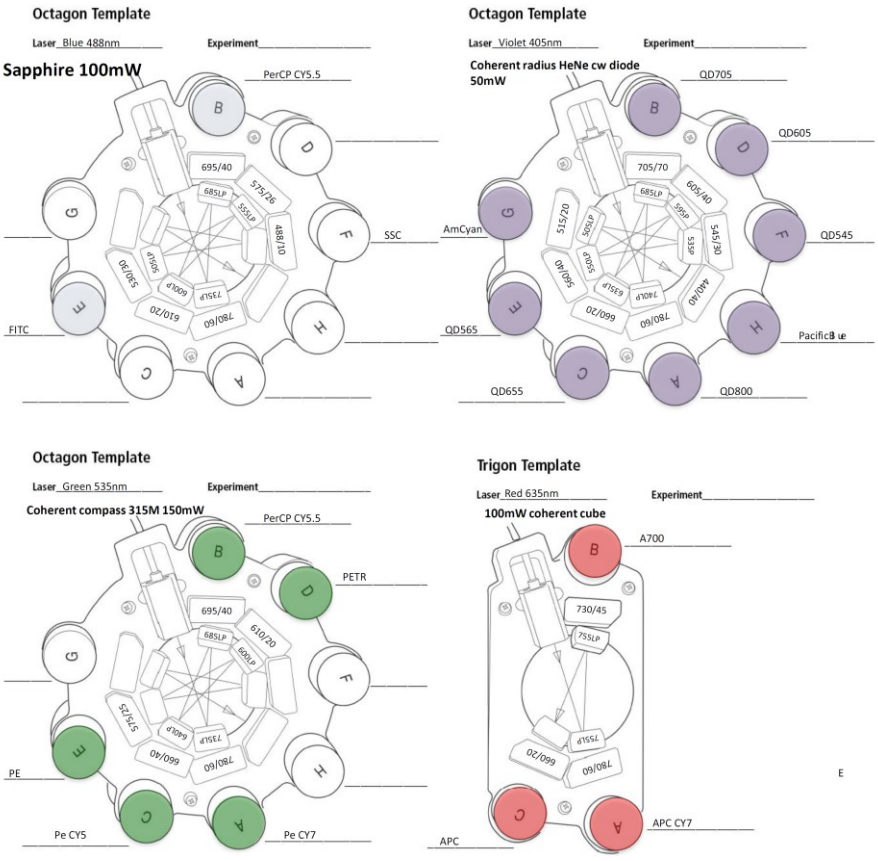


Figure 11. LSRII Flow cytometer IAVI's custom design technical configuration

Table 7 lists all the antibodies, clones and the volumes used to prepare master mix for surface and intercellular cytokine staining (ICS). All stimulations were performed in 100µl final volume. 0.75-1x10^6 PBMCs were used to perform staining, with a minimum of 10,000 $CD8^+$ events acquired. All stimulations for ICS were performed in R10 in a round bottom 96 well plates (Becton, Dickinson,

UK) with individual peptides (final concentration of peptide 2µg/ml). The positive control for the assay was staphylococcal enterotoxin B (SEB-2µg/ml) (Sigma Aldrich, UK) and negative control was 0.45% DMSO. The negative and positive controls were performed for all the assays.

Table 7. Antibody clones and volumes used for 9 colour panel to assess functional and phenotypic analysis of $CD8^+$ antigen specific T cell responses in acute HIV-1 infection.

Antibody	Fluorochrome	Clone	Volume Used (50µl/test)	Vendor
Surface Stain				
CD4	605 QDot	S3.5	0.5	Invitrogen
CD8	565 QDot	3B5	2.5	Invitrogen
CD14	Pacific Blue	M5E2	0.625	BD Biosciences
CD19	Pacific Blue	SJ25-C1	0.25	Invitrogen
ICS				
CD3	655 QDot	S4.1	0.5	Invitrogen
IFN-γ	PECy7	HS.B3	0.375	BD Biosciences
TNF-α	Alexa700	MAB11	0.5	BD Biosciences
IL-2	APC	MQ1-17H12	2	BD Biosciences
Perforin	PE	B-D48	2.5	Diaclone
CD107[a]	PECy5	H4A3	2.5	BD Biosciences
Live Dead[b]	Violet Amine		100µl of 1:1600	Invitrogen
NOTE.	[a]Added during stimulation [b]Live dead stain was performed pre surface and per ICS staining			

2.10.1 Reagents Preparation

2.10.1.1 Brefeldin (BFA)

Under sterile conditions, 5mg of Brefeldin (BFA) (Sigma Aldrich, UK) was reconstituted with 250µl of DMSO to achieve a concentration of 20mg/ml. BFA was aliquoted in 5µl aliquots and stored at -20°C.

2.10.1.2 BFA/Golgi stop cocktail

On the day of experiment setup, an aliquot of BFA (20mg/ml) was removed from -20°C and thawed at room temperature (RT). BFA/Golgi stop was prepared by adding 380µl PBS and 14µl Golgi stop (BD Biosciences, UK) to a vial of BFA.

2.10.1.3 1x Perm wash buffer

50ml of water was removed from a 500 ml bottle of sterile water (Sigma Aldrich, UK) and replaced with 50ml of 10x perm wash buffer (BD Biosciences, UK) and the buffer stored at 4°C.

2.11 ICS experiment setup

2.11.1 PBMC Stimulation

Overnight rested PBMC were washed once with R10 and resuspended in 1ml of R10 for counting. Cells were counted as explained in section 2.5.2. Cell concentration was adjusted to achieve $1x10^7$ cells/ml. 100µl of cell suspension was added to all reactions (peptides and controls) in a 96 well round bottom plate. Next, 2.5µl CD107a PECy5 (BD Biosciences, UK), and 5µl BFA/Golgi stop cocktail was added per well. Plates were incubated for six hours at 37°C / 5% CO_2. CD107a is a marker for $CD8^+$ T cell degranulation usually up-regulated following stimulation. Plates were kept at 4°C overnight.

2.11.2 Violet amine-reactive dye (VIVID) live/dead staining

On the following day, VIVID (Invitrogen, UK) was reconstituted with 50µl DMSO and was stored at -20°C for subsequent use. Reconstituted VIVID was diluted at 1:1600 in 1x PBS. Plates were spun at 400g for 4 minutes and supernatant was removed by flicking in one fluid movement. Plates were vortexed to resuspend cell pellets. Cells were washed with 200µl of PBS and stained with 100µl of diluted VIVID for 20 minutes in the dark. VIVID stained cells were washed twice with 200µl of PBS and cell pellets were resuspended by vortexing.

2.11.3 Surface Staining

Following VIVID staining, 50µl surface stain (Table 7) prepared in 1x PBS was added to each reaction and mixed well by pipetting. Samples were incubated in the dark for 20 minutes at room temperature. Samples were washed twice with 200µl of PBS and centrifuged at 400g for 4 minutes, supernatant discarded and cell pellet resuspended by vortexing. Cells were fixed by adding 100µl of cold Cytofix-cytoperm (BD Biosciences, USA) to each well and incubated at 4°C for 20 minutes. Samples were washed twice with pre-diluted 1x perm wash buffer (BD Biosciences, UK) at 400g for 4 minutes, supernatant discarded and cell pellet resuspended by vortexing.

2.11.4 Intracellular cytokine staining

50µl of intracellular stain mastermix (Table 7) prepared in 1x Perm wash buffer (BD Biosciences, USA) was added to each reaction and incubated at room temperature for 20 min. Samples were washed twice with pre-diluted 1x perm wash buffer at 400g for 4 minutes, supernatant discarded and cell pellet resuspended in 200µl of 1x perm wash buffer. Samples were stored at 4°C and were acquired on the same day.

2.11.5 Compensation Controls

Compensation is a crucial part of multicolour flow cytometry experiments, as it corrects for spectral overlap or spillover among different fluorochromes. Thus, it is necessary to setup compensation controls for each experiment to analyse the complex data properly. Moreover, if the compensation is incorrect data cannot be interpreted accurately. Both anti-rat and anti-mouse Igκ CompBeads (BD Biosciences, UK), usually bind to any κ light chain bearing immunoglobulin. All the antibodies used in the assay were mouse apart from IL-2 APC (rat). Prior to use, CompBeads were vortexed thoroughly and one drop of CompBeads (anti-rat, anti-mouse, or ArC™ Amine Reactive beads) was added to polystyrene FACS tubes (Scientific Laboratory, UK). 1µl of each antibody was added to a FACS tube containing Compbeads. One drop of ArC™ Amine Reactive beads (Thermo Fisher Scientific, UK) were stained with 1µl VIVID. For the negative control one drop of anti-rat, anti-mouse or ArC™ Amine Reactive beads were added to a FACS tube and left unstained. Tubes were incubated in the dark for 20 minutes at room temperature. Following incubation, beads were resuspended in 200µl of 1x Perm Wash buffer (BD Biosciences, UK). Beads were normally stained in parallel with samples and kept at 4°C until acquisition using BD DIVA software.

2.11.6 Analysis of Flow Data

Data was analysed using FlowJo Version 9.9.4 (Treestar, Oregon, USA) the gating strategy used is shown in Figure 12. The criteria for a positive response to any antigen was defined as $CD3^+/CD8^+$ cytokine (i.e. IFN-γ, IL-2, TNF-α) producing T cells minus, the background (mock). HIV-1 specific T cell responses were considered positive if the percentage of HIV specific cells was twice the background of mock and > 0.05% minus the background (Cellerai et al., 2011; Ferrari et al., 2011). ICS data were further analysed and plotted by using SPICE and PESTLE (version 5.1) kindly provided by Dr M. Roederer Vaccine Research Centre, NIH, Bethesda, MD, USA (Roederer et al., 2011).

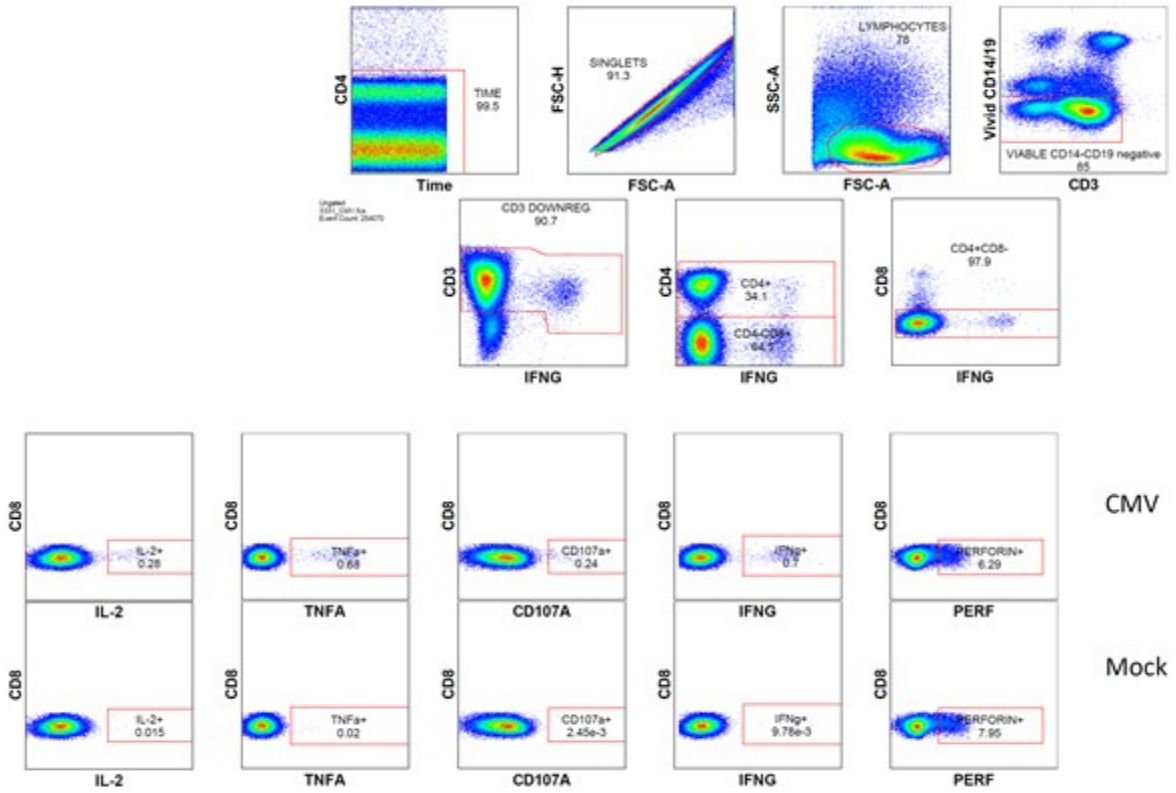


Figure 12. Gating strategy used with nine colour panel to analyse T cell responses

Above figure demonstrates the gating strategy used to analyse functional phenotype of antigen specific T cell responses detected in the African cohort. In this example, PBMC were gated for time, singlets and lymphocytes. Correct gating strategy was determined using fluorescence minus one (FMO) controls during panel validation/optimisation. The lymphocyte gate was further analysed for Live/Dead stain to determine viable cells. $CD3^+/CD8^+$ cells were further analysed from viable cells for polyfunctional responses. $CD8^+$ T cell responses were plotted against IL-2, TNFA, CD107A, IFN-γ and perforin to determine the frequency of HIV specific T cell responses.

2.12 Viral Inhibition Assay (VIA)

VIA experiments were performed by Dr Peter Hayes and he has kindly given his consent to use these data for the purpose of this thesis. VIA measures the *in vitro* ability of $CD8^+$ T cells to directly inhibit the replication of HIV-1 isolates in autologous $CD4^+$ T cells (Spentzou et al., 2010). This assay can be applied to both the study of $CD8^+$ T cell function in HIV-1 infected individuals, or to evaluate the ability of vaccine induced $CD8^+$ T cells to function (Spentzou et al., 2010). Bi-specific monoclonal antibodies (BSMAB) work when one arm of the antibody binds to CD3 and the other to either CD8 or CD4, instead of both arms of the antibody binding to the same antigen. CD3/8 BSMAB and CD3/4 BSMAB result in the expansion of $CD4^+$ and $CD8^+$ T cells, respectively, with purity of >90% of $CD4^+$ or $CD8^+$ T cell populations (Spentzou et al., 2010).

2.12.1 Target ($CD4^+$) and effector ($CD8^+$) cells

Target and effector cells were generated using BSMAB kindly provided by Johnson Wong, Harvard Medical School, Boston, USA. B001 Vaccinees' PBMC were thawed and $1.5x10^6$ viable PBMC were cultured in R10 supplemented with IL-2 (50 units/ml) (R10/IL-2) (Roche, Diagnostics, UK) and BSMAB CD3/8 (final concentration 0.5 µg/ml) in a T75 flask (VWR, UK) for the generation of target cells (CD4). Similarly, for CD8, BSMAB CD3/8 was replaced with CD3/4 under the same culture conditions as used for CD4. Media volumes were doubled with R10/IL-2 on days 3 and 6. After seven days, target $CD4^+$ T cells were centrifuged at 400g for 10 minutes. Supernatant was discarded and cell pellets resuspended in R10 and counted. Cells were infected with virus isolates of different clades at a multiplicity of infection (MOI) of 0.01 for four hours at 37°C / 5% CO_2. CD4 T Cells were seeded in 48 well culture plates (VWR, UK) after washing once with R10 at 250g for 10 minutes. For each donor, 0.5 million $CD4^+$ targets infected with HIV-1 were plated either alone or with 0.5 million $CD8^+$ T cells at 1:1 ratio in 1ml R10/IL-2 at 37°C / 5% CO_2 and cultured for 13 days in total. Supernatant (500µl) was removed on day 3, 6, 9 and 13 from each well and stored at -80°C to detect p24 production by ELISA (Perkin Elmer, UK). CD8 inhibited viral replication was measured as the Log_{10} reduction in p24 content of CD4 and $CD8^+$ co-cultures compared with $CD4^+$ cultures grown in the absence of $CD8^+$. An illustration of the VIA method is shown in Figure 13.

The cut off (CO) value to determine positive VIA responses was defined by statisticians at EMMES Corporation (Rockville, Maryland). CO value was based on VIA performed with HIV positive donors and was defined as any response >1.51 Log_{10} (Spentzou et al., 2010).

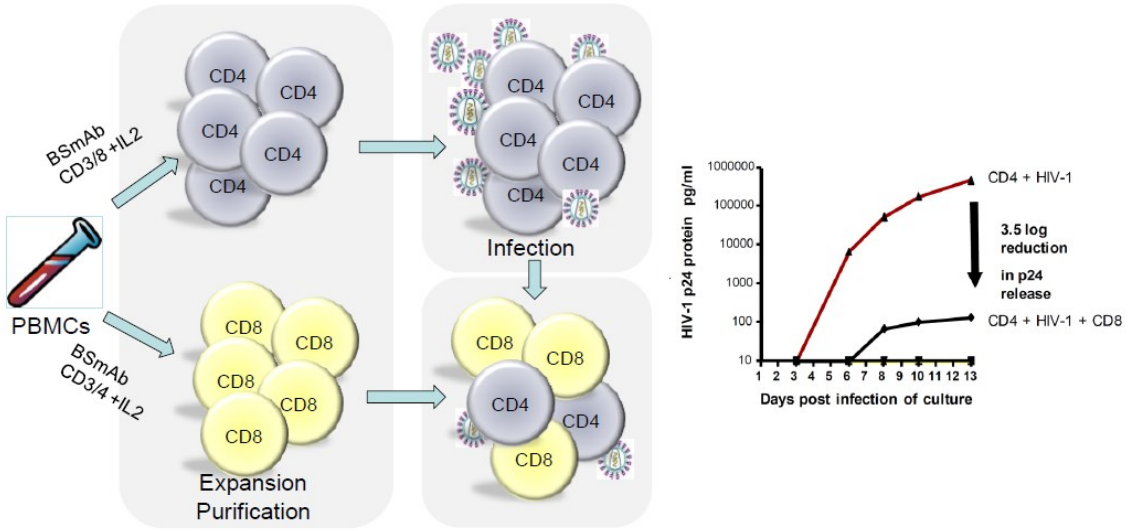


Figure kindly generated and provided by Dr Peter Hayes (IAVI, HIL, London, UK)

Figure 13. Graphical representation of VIA displaying assay procedure

2.13 Entropy Scores (ES)

A Shannon entropy score can be used to measure HIV-1 sequence diversity (http://www.hiv.lanl.gov/content/sequence/ENTROPY/entropy.html). The entropy score was calculated for each amino acid position in the protein alignment. It measures both the frequency and the range of different aa present at each position (Bansal et al., 2005; Yusim et al., 2002). For this study Shannon entropy scores were derived from pre aligned clade A (n=16), B (n=35), C (n=21), D (n=11) and AE/AG (n=19) HIV-1 protein sequences obtained from HIV Sequence db (https://www.hiv.lanl.gov/content/sequence/HIV/mainpage.html). Thus, an ES was calculated as an average of Shannon Entropy of amino acid positions encompassed in the epitope (Figure 14). Entropy is thus described as a degree of epitope variation such as, the higher the ES the more variable the epitope. In this study if the epitope ES was <0.2 then the epitope was nominated as "conserved" and >0.2 was described as "variable".

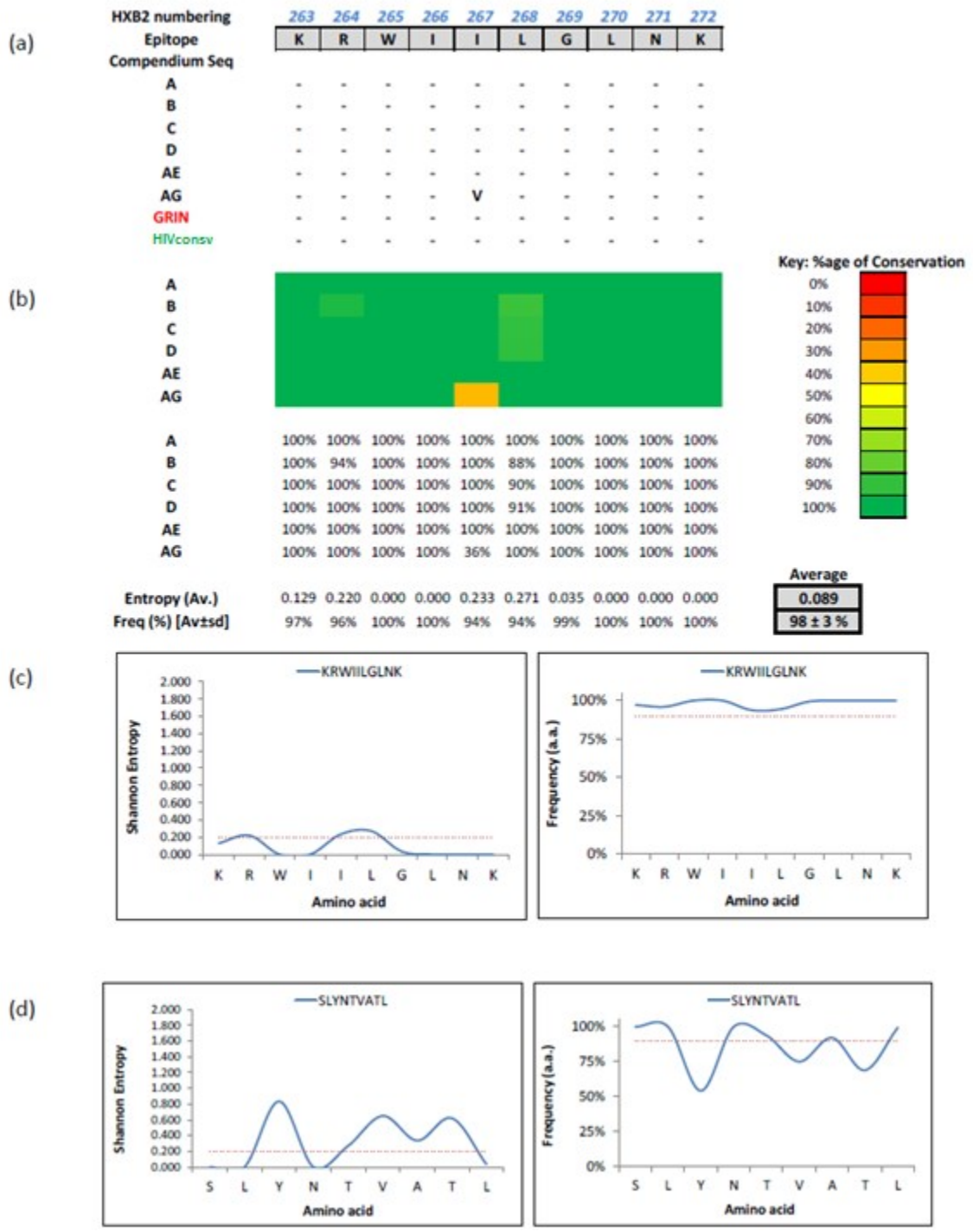


Figure 14. Example of Shannon Entropy score

An example of an Excel calculator to measure Shannon entropy scores (a) displays the conservation of KK10 epitope in the population. The coloured key represents the frequency of amino acid residues at each position (b) represents the frequency of amino acid [A (n=16), B (n=35), C (n=21), D (n=11) and AE/AG (n=19)] residues present at each position (c&d) illustrates the graphic representation of Shannon entropy and frequency of amino acid residues of highly conserved (c) and variable (d) epitope KRWIILGLNK (KK10) and SL9 (SLFNTVAL), respectively.

2.14 Conservation score (CS) and VIA

An in house Excel calculator was generated to determine epitope conservation score for the panel of viruses used in the VIA. This was accomplished by aligning GRIN/Env sequence and seven viral isolates (Clade A, B, C, D) sequences in Vector NTI (Invitrogen Corporation, Carlsbad, USA). The alignment was then imported to Excel to determine CS (Figure 15). The queried GRIN/Env epitope sequence was compared with each viral sequence to determine the frequency of amino acid present at each position within all virus isolates. The epitope CS was measured by calculating the percentage of amino acid difference at each position for each viral isolate for instance; U455 KK10 epitope contained a single mutation K263R. Hence, U455 KK10 epitope CS was 0.1 (amino acid difference/length of epitope 1/10=0.1) (Figure 15). Thus, the epitopes were described as "conserved" when the CS was zero and if the CS > zero then the epitope was defined as "variable".

(a)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15			
cf., epitope	K	R	W	I	I	L	G	L	N	K						n	N	Score
GRIN	.	.	.	.	.	.	.	.	.	.						0	10	0.00
U455	R	.	.	.	.	.	.	.	.	.						1	10	0.10
IIIB	.	.	.	.	.	.	.	.	.	.						0	10	0.00
ELI	.	.	.	.	.	V	.	.	.	.						1	10	0.10
CH106	.	.	.	.	.	.	.	.	.	.						0	10	0.00
CH077	.	.	.	.	.	.	.	.	.	.						0	10	0.00
ZA97012	.	.	.	.	.	M	.	.	.	.						1	10	0.10
247Fv2	.	.	.	.	.	.	.	.	.	.						0	10	0.00
%age Variation (Epitope)	14%	0%	0%	0%	0%	29%	0%	0%	0%	0%								

(b)

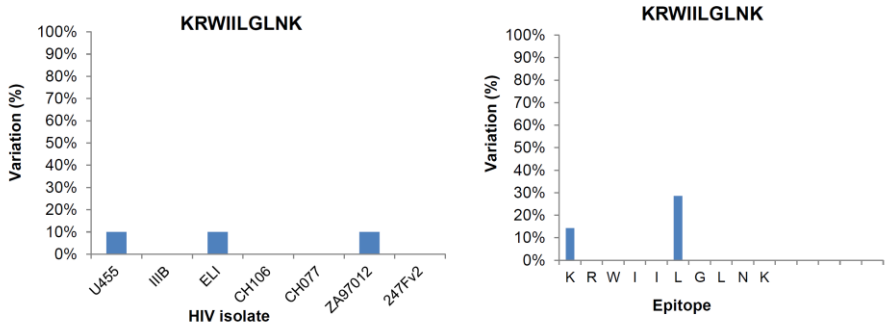


Figure 15. Illustration of Conservation Scores calculator using Microsoft Excel

Example of an in house generated Excel calculator displaying how conservation scores were calculated. (a) Displays the conservation of KK10 within all virus isolates used in VIA and the percentage of variation of amino acid residues present at each position. Conservation scores (n/N n= amino acid residues different from responding epitope, N= number of amino acid residue in an epitope) were calculated for each responding epitope (b) Graphic representation of KK10 variation within all seven isolates used in VIA and the variation in KK10 epitope at each position.

2.15 Bio-informatics

2.15.1 Sequencing and Phylogenetic Analysis

DNA sequences were analysed using Vector NTI version 11.0 (Invitrogen, USA) and MEGA 4.0 (http://www.megasoftware.net/). A neighbour joining tree was created with PhyML V3.0 (Guindon and Gascuel, 2003) and was edited with FigTree V1.12 (http://tree.bio.ed.ac.uk) using subject's sequences and reference strains (A, A1, A2, AE, AG, B, C, and D).

2.15.2 Deconvolute this! software

The Deconvolute this! programme (courtesy of Mario Roederer) was used to deconvolute responding peptides by using the optimal configuration of peptide pools. Deconvolute this software predicts individual peptide responses from pools of OLPs (Roederer, 2003).

2.15.3 HIV databases (HIV db)

HIV db was used to identify the potential T cell epitopes (http://www.hiv.lanl.gov/content/sequence/ELF/epitope_analyzer.html). The sequences of the reference strains were obtained from http://www.hiv.lanl.gov/content/sequence/BASIC_BLAST/basic_blast.html for phylogenetic analyses. Furthermore, HIV db was also used to determine Shannon entropy score http://www.hiv.lanl.gov/content/sequence/ENTROPY/entropy.html of responding epitopes, to define if the epitope was conserved or variable.

2.15.4 Statistical and graphical Analysis

Descriptive statistics applied in this thesis are ranges, interquartile ranges (IQRs), averages, medians, and standard deviation. Graphs were generated either with Microsoft Excel or Graph Pad prism version 7.0 (GraphPad Software, La Jolla, California, USA). Statistical analysis was performed with Graph Pad prism version 7.0. Mann-Whitney U test was used for the comparison of two unpaired groups (continuous variables); Fisher's exact test was employed for categorical variables; the Wilcoxon signed-rank test was used for the comparison of paired samples; One way ANOVA Kruskal-Wallis test was used for the comparison of three or more unpaired observations. The McNemar test was used to establish if there was a difference between two matched groups (dependent variable) and the Kappa Statistic was applied to measure agreement between categorical variables. A non-parametric test nptrend was used to determine the trend across ordered groups. For ICS data, statistical significance was performed using a Student's T test using SPICE (version 5.1) (Roederer et al., 2011). A p value of <0.05 was considered statistically significant.

Chapter 3: Validation of IFN-γ ELISpot assay using novel HIV_{consv} matrix and GRIN/Env pools

3.1 Introduction

T cells play an important role in the fight against a variety of viral infections and cancer (Kamphorst et al., 2015; Ndhlovu et al., 2015a). Numerous methods have been established in the field of vaccine research which allow the detection of *ex-vivo* antigen specific T cell responses without the use of *in vitro* expansion (Letsch and Scheibenbogen, 2003). Several well-characterised functional T cell assays have been employed in clinical trials to measure vaccine efficacy such as ELISpot, ICS, proliferation assays (thymidine and Carboxyfluorescein succinimidyl ester (CFSE)) and methods to measure cytotoxicity, VIA and regulatory T cell suppression, all based on several different readouts. However, functional assays such as the ELISpot assay and the ICS are considered the primary assays to quantify antigen specific T cell secretion of cytokines on a single cell basis (Karlsson et al., 2003; Letsch and Scheibenbogen, 2003; Smith et al., 2009).

The ELISpot assay is an efficient and highly sensitive method used to enumerate the number of PBMC producing cytokines (i.e. IFN-γ, IL-2, granzyme) upon stimulation by a whole range of different antigens consisting of individual peptides/peptide pools, proteins, mitogens and/or super-antigens (Smith et al., 2009). The ELISpot assay only detects activated/memory T cells and determines the cytokine secretion at the single cell level, the results demonstrate frequencies of responding T cells. It is highly sensitive and easy to perform and allows *ex-vivo* enumeration of peptide specific T cells. Thus, it an eminently useful technique to detect T cell responses (Carvalho et al., 2001). The basic principle of the ELISpot assay is very much similar to ELISA, which detects cytokine secretion by cells upon recognition of cognate antigens used for stimulation. These cytokines are then captured by cytokine specific immobilised Ab present on the membrane of an ELISpot plate and subsequently visualized by a chromogenic reaction following incubation with a substrate broken down by an enzyme linked to a secondary Ab (Figure 16). The ELISpot assay is not restricted to the release of cytokines and it has also been shown effective at measuring the secretion of other effector molecules such as perforin, granzyme B or even antibodies produced by B-cells (Anthony and Lehmann, 2003). The IFN-γ ELISpot assay is typically performed either with freshly isolated or cryopreserved PBMC and on average cultured for 16-24 hours. An assay performed in this manner is informative usually concerning T cell differentiation (detects memory T cells) and clonal size (Anthony and Lehmann, 2003; Keating et al., 2005). PBMC are plated either in the presence or absence (mock wells i.e. background response) of test antigens (Anthony and Lehmann, 2003).

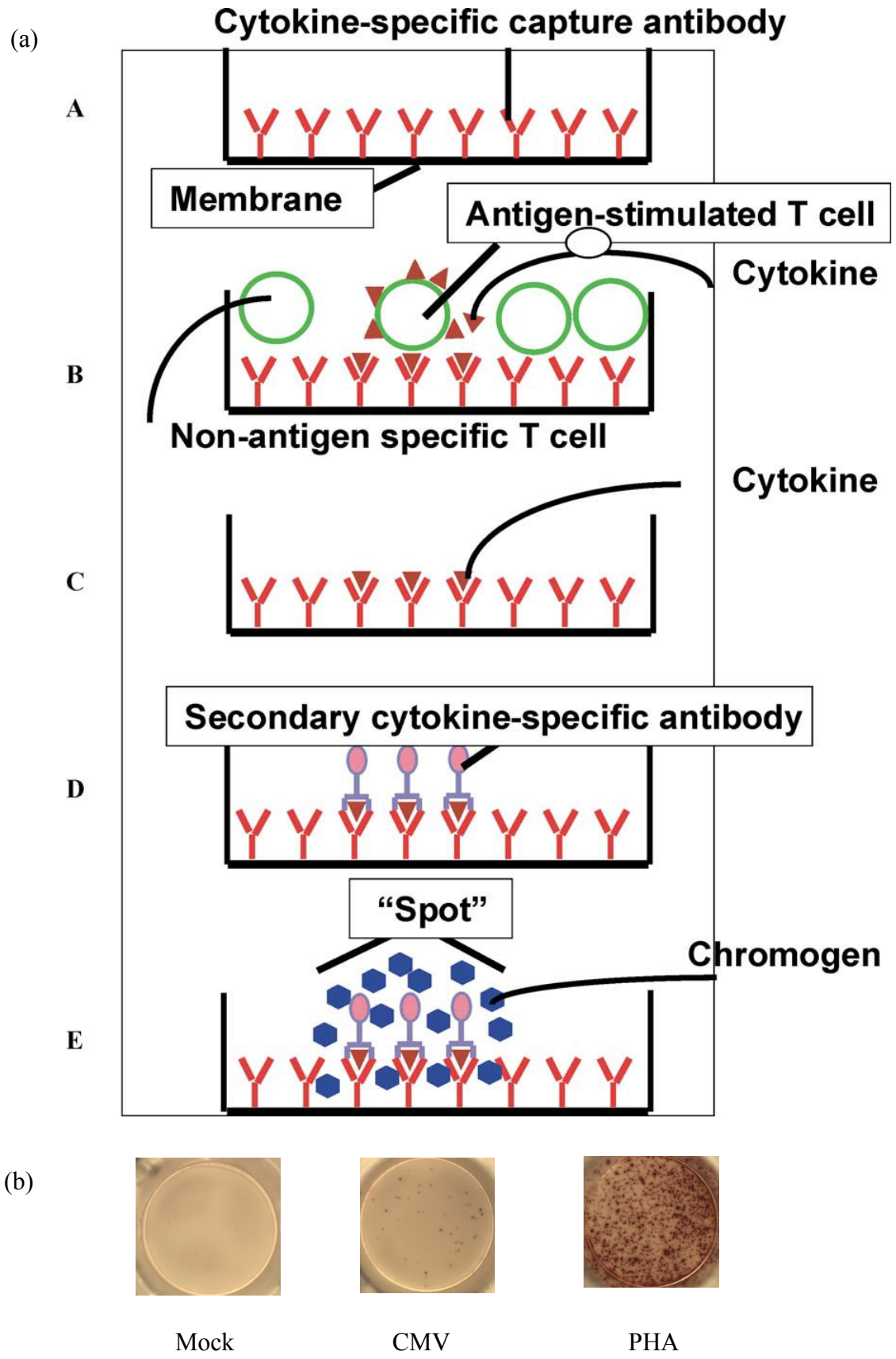


Figure 16. (a) Basic diagram of ELISpot assay procedure (Anthony and Lehmann, 2003) **(b) Example of enumeration of IFN- γ producing cells stimulated with Mock, CMV and PHA**

During incubation with stimuli, antigen specific stimulation of PBMCs results in the secretion of cytokine or other effector molecules which are then captured by monoclonal Ab coated on the surface of the ELISpot plate (Figure 16) (Letsch and Scheibenbogen, 2003; Smith et al., 2009). Hence, each antigen specific cell leaves a residue of its secretory action at the single cell level. Both the size and the intensity of the spot is an indicator of amount of cytokine secreted per cell (Helms et al., 2000; Hesse et al., 2001; Karulin et al., 2000).

One major advantage of ELISpot assay is that it is one of the very few immunological assays that measure immune responses using frozen PBMCs without apparently losing any significant immune activity (Boaz et al., 2009; Kreher et al., 2003), an important factor when dealing with longitudinal studies or clinical trials where blood samples are taken at regular intervals to monitor immune responses or vaccine efficacy. Furthermore, only a few million PBMC are generally required to test many different assay parameters. The high throughput and ease of use of this assay makes it the most practical way to map T-cell epitopes across multiple pools comprising hundreds of OLP (Fu et al., 2007; Karlsson et al., 2003; Kreher et al., 2003; Samri et al., 2006).

However, when employing any immunological assay for a study or clinical trial, all required assays have to be validated. Assay validation is a common practice to allow comparison and establish equivalence between different research laboratories. There are guidelines that have to be followed especially when highly technical procedures are involved (Burd, 2010; Mwau et al., 2002). Validation processes establish that an assay in use meets the following criteria:

- Accuracy (*Gives the value comparable to reference value*)
- Specificity (*Ability of the assay to measure what it should measure*)
- Reproducibility (*Ability of the assay to produce the same results when repeated*)
- Sensitivity (*Upper and lower limits of detection/cut off (CO*)
- Precision (*How reproducible test results are when an assay is repeated with multiple aliquots of the same sample*)
- Robustness (*Ability of the assay to stay unaffected under certain minor variations e.g. concentrations, pH, temperature, batch number of reagents and equipment*) (Burd, 2010; Mwau et al., 2002)

Thus, assay validation provides confidence in the consistency of the assay whenever and wherever it is employed and therefore at times it is referred to as the process that provides concrete evidence that the test assay does what it is required to do i.e. is fit for purpose.

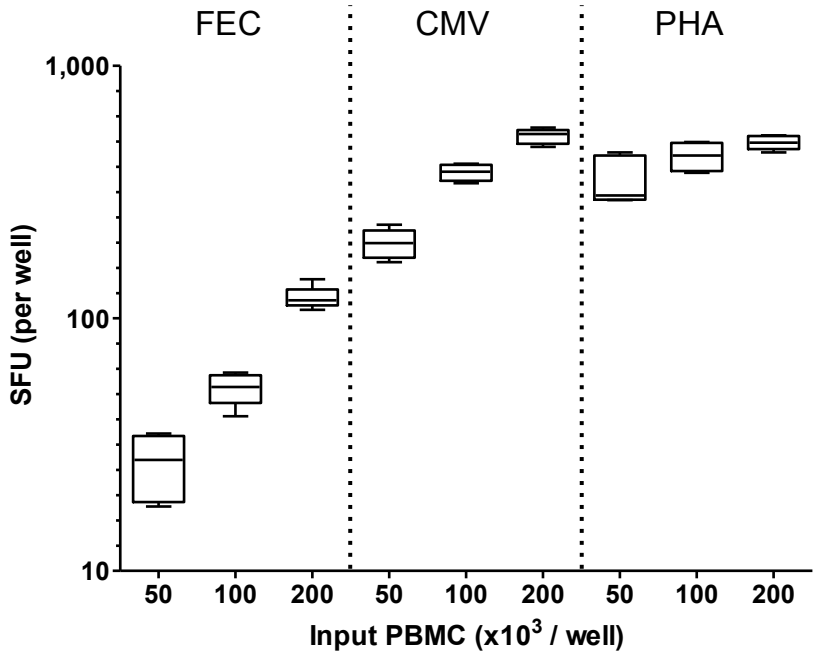
This chapter therefore focuses on validating the IFN-γ ELISpot assay for use in epitope matrix mapping with peptide pools derived from the inserts of two HIV vaccine candidates: HIV_{consv} and GRIN/Env.

3.2 Results

3.2.1 Effect of cell density on IFN-γ responses

To determine the correct number of cells required to perform the ELISpot assay without loss of sensitivity using the HIV_{consv} matrix pools, five seronegative BC were tested with Mock, FEC peptides (Influenza virus, Epstein–Barr virus and Cytomegalovirus), CMV(pp65) and PHA at three different cell concentrations i.e. $5x10^4$, $1x10^5$and $2x10^5$ per well in triplicates in two different experiments. These data showed that significant stepwise increase in SFU for both FEC ($p<0.05$; nptrend) and CMV ($p<0.05$) responses were in direct linear relationship with the number of PBMC plated, while increasing cell densities for PHA ($p<0.05$) stimulation appeared to have little additional effect (Figure 17). However, when the raw data was normalised per million PBMC after subtracting the background response (mock) then there was no significant differences observed for FEC ($p>0.05$), while both CMV ($p<0.05$) and PHA ($p<0.05$) responses suggest that increased cell number actually induced an over whelming number of confluent spots and thus, could not be differentiated or counted.

(a)



(b)

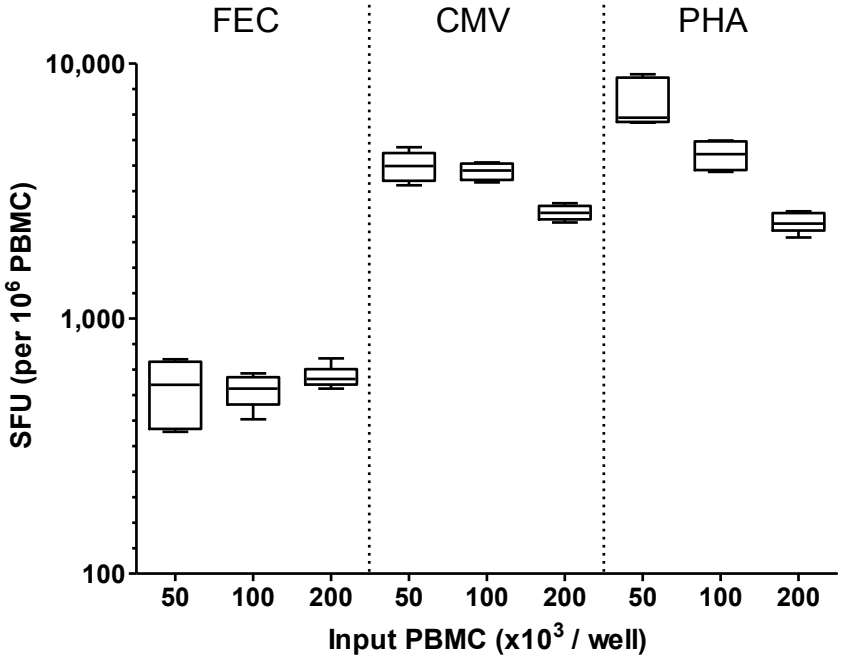


Figure 17. The effect of cell density/well on ELISpot assay performance

Three different cell densities ($5x10^4$, $1x10^5$, $2x10^5$) were plated in triplicate using HIV negative PBMC (n=5) to measure IFN-γ responses against FEC, CMV and PHA. Results are displayed here after background subtraction. Box and whisker display median, upper and lower interquartile range (IQR) and a range of the IFN-γ responses.

3.2.2 IFN-γ ELISpot assay Cut off and validation

Nineteen African healthy donors, 21 UK and 32 African HIV-1 positive individuals were used to measure T cell responses against HIV_{consv} pools and assay controls (Mock, CMV and PHA). SFU data generated from African seronegative PBMC against HIV_{consv} pools (n=1,520 wells across the plates) and mock values of both the UK (n=288) and African cohort (n=224) (both HIV-1 positive) were used to establish a CO to score positive responses to HIV antigens (Table 8). The majority of seronegative individuals induced no IFN-γ responses (<5 SFU/10^5 PBMC) to HIV_{consv} pools. However, occasionally, non-specific signals were observed with seronegative PBMC due to a number of potential factors including debris in the well, broken PVDF membrane and an AID reader artefact (Table 8).

Table 8. Assessment of background levels of SFU using mock wells of PBMC from HIV positive and negative donors to define assay cut-offs

	SFU (per 10^5 PBMC)			
	HIV positive Mock[a]		HIV negative PBMC [b]	
	UK (n=21)	African (n=32)	Unadjusted (n=19)	Background subtracted (n=19)
Number of wells	288	224	1,520	1,520
Minimum	0	0	0	0
Maximum	7	12	41	40.3
Median (IQR)	0 (0 – 1)	0 (0 – 1)	0 (0 – 1)	0 (0 – 0.5)
Average + 3 s.d.	3.6	5.2	6.2	5.7
Cut-off and % false positive wells				
≥5 SFU	2 (0.7%)	5 (2.2%)	29 (1.9%)	17 (1.1%)
≥10 SFU	0 (0%)	1 (0.4%)	4 (0.3%)	4 (0.3%)
≥15 SFU	0 (0%)	0 (0%)	2 (0.1%)	2 (0.1%)

NOTE. [a] Based on mock values derived from UK and African HIV-1 positive PBMC, 4-6 mock wells/assay
[b] Based on mock values derived from African HIV-1 negative PBMC, 4-6 mock wells/assay
IQR inter-quartile range, s.d. standard deviation from the mean; SFU spot-forming units

Compared to the UK individuals African PBMC generated slightly higher background responses (p<0.05; Mann Whitney U test). Based on the average plus three times standard deviations (s.d) of background subtracted negative PBMC, UK and African mock data (Table 8) a CO of 5 SFU/1x10^5 cells seemed to be applicable for this study (Table 8). Furthermore, a CO of 5 SFU/1x10^5 demonstrated a false positive of rate of 1.1% (Table 8), which was lower than other international studies. Raising the CO to either 10 SFU/10^5 or 15 SFU/10^5 lowered the false positive rate to 0.3% and 0.1%, respectively, but this increased CO would potentially miss low magnitude specific responses (Table 8). Hence, a CO of 5 SFU/10^5 (or 50 SFU/10^6) cells was implemented as the assay threshold to investigate IFN-γ ELISpot responses using the novel HIV_{consv} pools.

3.2.3 Reproducibility of IFN-γ ELISpot responses using novel HIV_{consv} pools

HIV positive PBMC from a UK cohort (n=18) were used to examine the reproducibility (i.e. assay variability) of IFN-γ ELISpot responses to HIV_{consv} pools across three independent tests. Overall reproducibility of SFU to individual pools by each responding individual was found to be good (Figure 18) with some weak responses being less reproducible.

These differences were more obvious following deconvolution (Table 9); for example peptide 93 for subject I220 was deconvoluted in one out of three tests but failed to deconvolute in the other two tests due to the low signal (<50 SFU/10^6 PBMC) generated by one or more HIV_{consv} pools. In this study, peptides could only be deconvoluted if all four HIV_{consv} pools generated a signal of ≥50 SFU/10^6. However, when the signal generated by each responding pool was strong as observed with B600, each responding peptide was deconvoluted successfully in all three tests (Table 9).

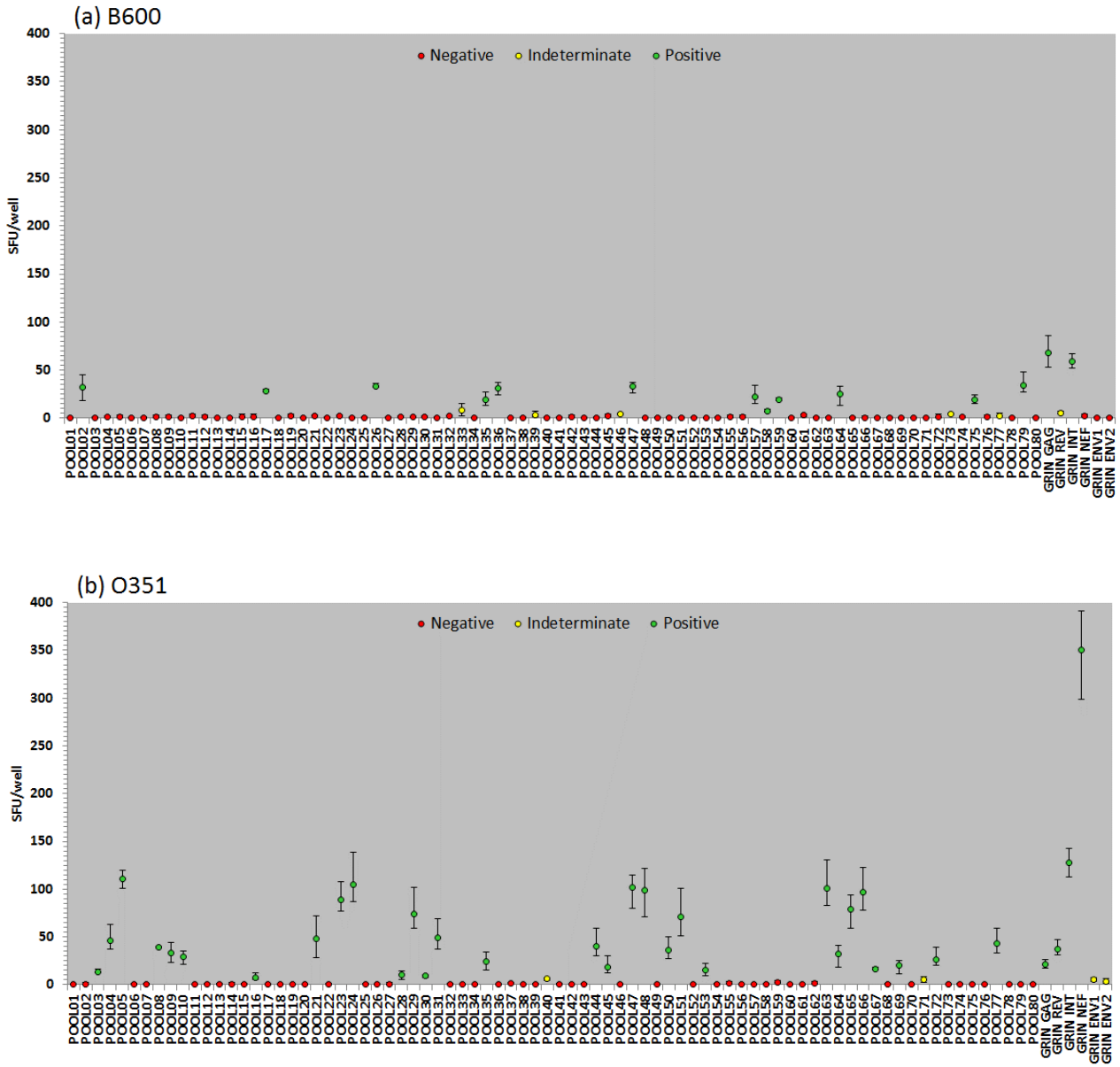


Figure 18. Example of reproducible and non-reproducible responses elicited against HIV_{consv} and GRIN/Env pools

Example of two individuals ([a] B600 and [b] O351) illustrating reproducible (Green) and non-reproducible (Yellow) IFN-γ responses against HIV_{consv} and GRIN/Env pools in three independent tests. Y-axis represents raw SFU/1x10^5 PBMC were plotted against 80 HIV_{consv} and GRIN/Env pools along x-axis. Top error bar is the maximum response (SFU) and bottom error bar is the minimum response (SFU) elicited in one of the three tests. Reproducible responses (Green) were above the assay CO 5SFU/1x10^5, whereas non-reproducible responses did not always meet the assay CO 5SFU/1x10^5.

Table 9. Assessment of deconvoluted peptides response generated in three different experiments

			SFU (per 10^6 PBMC) for indicated experiment		
Patient ID	Peptide[a]	Pool[b]	Experiment 1	Experiment 2	Experiment 3
I220	P93	10	83	126	<50
		32	<50	96	123
		42	113	66	<50
		61	73	66	<50
B600	P15	2	173	450	336
		26	353	320	316
		47	363	260	356
		64	293	130	326

NOTE. [a] Deconvoluted responding peptides
[b] Pools numbers containing responding peptides

IFN-γ responses following stimulation with HIV_{consv} pools were defined as reproducible if a peptide was deconvoluted in all three tests (e.g. B600, peptide 15, Table 9), or non-reproducible if a peptide was deconvoluted in only one or two of the three tests (e.g. I220, peptide 93, Table 9).

The average SFU for each deconvoluted peptide in each test was determined and assigned to the appropriate dataset (reproducible [3x] or non-reproducible [1x or 2x] (Table 10). For example, for volunteer I220 the peptide 93-specific SFU in test one in which peptide 93 failed to deconvolute were 83, 113, 43 and 73 (average 78) were assigned as non-reproducible whereas, B600 peptide 15 was deconvoluted in all three tests and was assigned as reproducible. In all, the 30 peptides deconvoluted from 12/18 HIV_{consv} responders (UK donors only) gave 60 reproducible (median [IQR], 208 [159 – 260 SFU/10^6]) and 30 non-reproducible (80 [53 – 102 SFU/10^6]) data points (p<0.05; Mann Whitney U test). A second assay threshold is proposed wherein responses ≥150 SFU/10^6 are designated as being strong. Responses between 50 and 150 SFU are classified as weak, as the majority (97%) of the responses in that range were non-reproducible; however, it did comprise 20% of lower but still reproducible responses and 1.1% of negative (or false positive responses) (Table 10 and Figure 19).

Table 10. Reproducibility of deconvoluted HIV_{consv} peptides

	Effective deconvolution [a]	
	3x	1x or 2x
Data point (n)	60	30
Min	74	9
Max	377	189
Median (IQR)	208 (159 – 260)	80 (53 – 102)
Average + 3 s.d	463	190
≥150 SFU, n (%)	48 (80%)	1 (3%)

NOTE. [a] Number of tests wherein specific peptides were effectively deconvoluted (1x, 2x or 3x)
IQR inter-quartile range, s.d. standard deviation from the mean, SFU spot-forming units

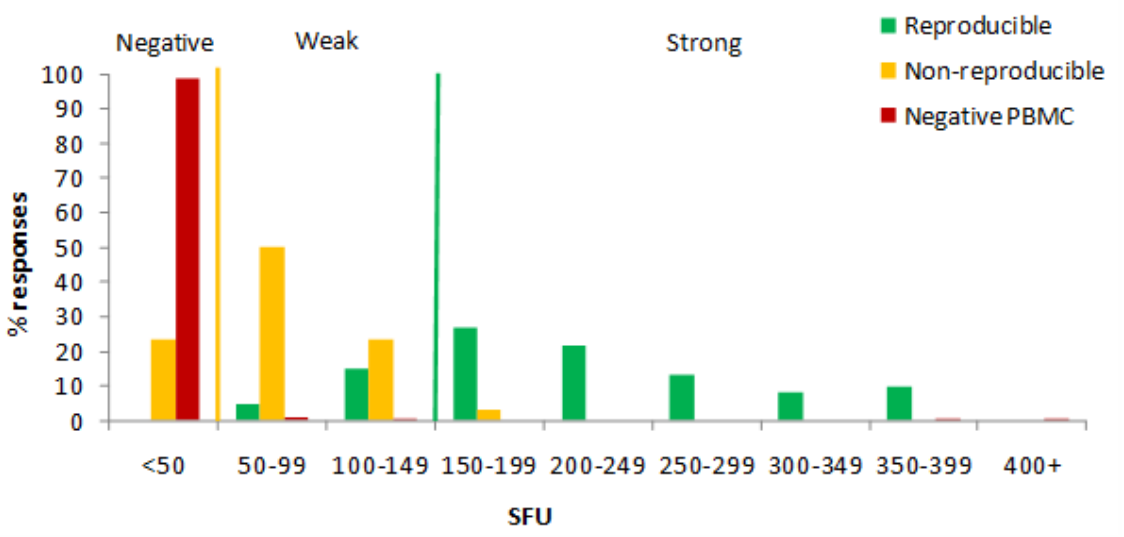


Figure 19. IFN-γ ELISpot assay COs to define negative and positive responses

Percentage of IFN-γ responses against HIV_{consv} pools that were defined as negative (red), weak/non-reproducible (yellow) and strong/reproducible (green) based on data generated in three independent tests. Majority of responses against HIV_{consv} pools were negative. Responses <50 SFU were defined as negative, between 50 and 150 SFU were nominated as weak wherein responses ≥150 SFU were defined as strong.

3.3 Discussion

Virus-specific $CD8^+$ T-cells has been shown to be crucially important in establishing viral set point and the maintenance of control in elite controllers (Crawford et al., 2009; Mendoza et al., 2012; Ndhlovu et al., 2015a; Shasha, 2013). As a consequence the characterisation of such responses both induced through natural infection and by way of vaccination should be investigated to define whether targeting any specific region of the HIV-1 proteome will be beneficial. It is therefore crucial to asses/evaluate both the occurrence and the function of HIV-1 specific CTL responses (Blankson, 2010). To determine the frequencies of HIV-1 specific T-cells in PBMC, an ELISpot assay was employed in this study to quantify IFN-γ producing T cells upon *ex vivo* stimulation with HIV-1 antigens. This was to characterise the exact regions within those antigens that are recognised by infected or vaccinated individuals. To do this bloods were drawn from HIV-1 positive and negative donors to isolate PBMCs, cells were stimulated with HIV-1 pools for 16-24 hours, the resulting spots were enumerated by AID reader and responses were deconvoluted.

In the current study, IFN- γ ELISpot assay was validated for immunogens derived from two vaccine inserts, HIV_{consv} matrices and GRIN/Env pools. Because this was the first time the ELISpot assay had been employed to map T-cell epitopes in our laboratory, a number of factors needed to be considered during assay validations such as cell density, assay CO, assay reproducibility and specificity, as described in previous studies (Burd, 2010; Mwau et al., 2002; Samri et al., 2006).

Two experiments were conducted with Mock, FEC, CMV and PHA to determine the appropriate cell seeding density to resolve the minimum number of cells required to map T cell epitopes using HIV_{consv} matrix including assay controls. This was vital to determine the correct cell input to perform an assay without loss of IFN-γ responses and also to minimise cell usage, while maximising results. In the present study, all the assays were performed in triplicate using given HIV-1 negative African donors and antigens (FEC, CMV and PHA). There was a direct linear relationship observed between the increased cell density and the SFU data. However, after normalisation per million PBMC, as expected there were no significant differences observed with increased cell density with FEC pool. However, both CMV and PHA responses were significantly subsided as the cell number was increased (Smith et al., 2009). This could be due to the large number of spots per well, therefore it was vital to determine the correct cell density required for ELISpot assay to circumvent this problem. Karlsson *et al* have shown that it is difficult to establish the correct estimate of the count (SFU) if there are >1000 spots/well due to the inherent limit of assay detection (Karlsson et al., 2003). Similarly, other studies have reported that low cell numbers seemed to be associated with decreased assay sensitivity, highlighting the need to optimise the cell density required for the assay (Jin et al., 2013; Karlsson et

al., 2003; Smith et al., 2009). However, this problem could be resolved once the correct cell input was determined. Thus, in this study, optimum cell input of $1x10^5$ cells/well was determined to map T cell epitopes, as using lower cell number $5x10^4$ cells/well could result in missing out low frequency responses, but not high magnitude responses, which is important when measuring vaccine elicited responses.

One of the critical components of the validation process was to define a CO criterion to distinguish between positive responses and background responses. All median background (mock) and seronegative responses to HIV_{consv} pools were negligible. Based on the average plus three standard deviation of background subtracted negative PBMC, UK and African mock data a CO was implemented for this study which is similar or lower than other studies (Fu et al., 2007; Samri et al., 2006). Previously, the threshold for positive responses was based on using HIV negative and positive donors and tested against HIV antigens (Fu et al., 2007; Samri et al., 2006). Similarly, here assay threshold for positive responses was defined by analysing non-specific HIV-1 T cell responses from seronegative donors. These findings suggest that raising the CO decreased the frequency of false-positive results. Moreover, a threshold determined in this study is the most common CO value used to verify HIV specific IFN-γ responses in other studies (Boaz et al., 2009; Dinges et al., 2010; Frahm et al., 2004; Fu et al., 2007; Samri et al., 2006). Rarely, non-specific responses were detected due to technical issues. However, these random artefacts are not expected to interfere with ELISpot assay data using the HIV_{consv} peptide matrix due to the requirement for multiplicity of deconvoluted peptides.

Furthermore, PBMC from the UK cohort was used to determine the assay reproducibility and these data showed that overall IFN-γ ELISpot assay was quite reproducible among three different tests. However, weak responses were less reproducible and this was clearer after deconvolution of peptide. In the current study, IFN-γ responses to HIV_{consv} pools were only considered as reproducible if a peptide was deconvoluted in all three tests. Nonetheless, peptides were deconvoluted repeatedly where IFN-γ responses to HIV_{consv} pools were strong. Thus, those responses were considered as reproducible and strong. Based on this investigation a second assay CO criterion was assigned in order to distinguish between weak and strong responses.

To summarise, ELISpot assay was validated to quantify antigen specific $CD8^+$ T cell responses in natural infection. By focusing on robust, specific and reproducible responses to peptides it is likely that the populations recognising these antigens are the most likely to mediate any potential protective effects (Mothe et al., 2015; Ndhlovu et al., 2015a; Pantaleo and Koup, 2004).

Chapter 4: Comprehensive evaluation of HIV-1 specific cytotoxic T cell responses in HIV-1 infected individuals in a mixed subtype population

4.1 Introduction

Due to its tremendous diversity, HIV-1 can be sub-grouped into a number of different clades (A-G) (Korber, 1998, 2000). There is also a geographical bias in subtype distribution such that A, C and D subtypes predominate in sub-Saharan Africa but, the majority of the HIV infected people in the United States and Europe are infected with subtype B (Bennett et al., 2008; Spira, 2003). Not only that, recombinant strains are becoming common and 55 CRFs have been reported globally (Korber et al., 2009a; Lau and Wong, 2013; McKinnon et al., 2005). As $CD8^+$ T cells play a vital role in controlling HIV infection (Hancock et al., 2015; Jin et al., 1999; Schmitz et al., 1999), numerous HIV-1 vaccine candidates have been designed to induce $CD8^+$ T cell responses (Haynes, 2015; Keefer et al., 2012; Letourneau et al., 2007; Mudd et al., 2012). However, clade specific polymorphisms in CD8 epitopes have been reported (Walker and Korber, 2001). Thus, a vaccine based on a single virus strain may not be effective against multiple clades of HIV-1 (Ahmed et al., 2016; Bennett et al., 2008; Hraber et al., 2014). It is, therefore, important to develop HIV-1 vaccine candidates based on epitopes targeted in natural infection as those epitopes may be capable of recognising different viruses. Thus, inducing effective cross clade $CD8^+$ T cell responses (McMichael et al., 2002).

$CD8^+$ T cell responses towards specific targets in one HIV-1 isolate have been shown to protect against those present in multiple subtypes/quasispecies (Betts et al., 1997; Currier et al., 2002; Geels et al., 2005; Watanabe et al., 2013) and one study showed that an HIV-1 vaccine candidate based on subtype B could induce cross clade $CD8^+$ T cell responses (Ferrari et al., 1997). Although CTL responses to subtype B viruses have been investigated thoroughly, relatively less is known about the cross-clade cell mediated immune response to non-subtype B viruses and CRFs in the population (Cao et al., 1997; Keating et al., 2002; Lau and Wong, 2013). Further characterisation of such responses is therefore of the utmost importance for HIV vaccine design (Watanabe et al., 2013). However, the selection of HIV-1 antigens required to detect cell mediated immune responses in naturally infected individuals is limited (Hertz et al., 2013; Keating et al., 2002). A few standardised peptide reagents have been developed using consensus, autologous, potential T cell epitopes, mosaic polyvalent antigen and COT sequences to measure the magnitude and breadth of the T-cell response (Altfeld et al., 2003b; Fischer et al., 2007; Frahm et al., 2008; Kunwar et al., 2013; Li et al., 2006). As conserved regions of the HIV-1 proteome are common to many HIV-1 clades, an antigen based on conserved sequences could be used to detect HIV-1 specific $CD8^+$ T cell responses in a wide range of naturally infected individuals (Borthwick et al., 2014; Cao et al., 1997; Korber et al., 2009a;

Letourneau et al., 2007; Ondondo et al., 2016). Potential HIV-1 vaccine candidates should also consider global HLA class I diversity to ensure insert sequences can be recognised in all populations (Chen et al., 2012; Geels et al., 2005; Ondondo et al., 2016).

The objective here was to assess the recognition of T cell responses against two novel sets of overlapping peptides in individuals (UK and sub-Saharan African), naturally infected with HIV-1 subtypes (A, B, C and D). Individuals were at different stages of their disease course and of different genetic back-grounds. The IFN-γ ELISpot assay was employed to detect cell mediated immune responses using two different antigens. The standard IFN-γ ELISpot is a reference method for the *ex-vivo* monitoring of antigen specific T cell responses, this method was adapted to use peptide matrices of GRIN/Env and HIV_{consv} insert to evaluate CD8 responses against clade A (GRIN/Env) and highly conserved (HIV_{consv}) sequences. Data were examined to determine whether the magnitude and breadth of responses to HIV_{consv} pools and subtype-specific GRIN/Env pools are comparable. Hypothesising that cross clade responses are more likely to occur to elements common in the majority of clades, these findings may further indicate which peptide pools would make a better tool to detect cell-mediated responses in populations infected with different HIV-1 clades. Such information may improve our understanding of the nature of T cell responses generated during the disease course and information regarding cross clade responses might help to design vaccine candidates to overcome HIV-1 global diversity.

4.2 Results

4.2.1 Study participants

74 HIV-1 infected individuals were tested in the IFN-γ ELISpot assay, including 18 from UK (Chelsea and Westminster Hospital, London, UK) and 56 from East (Kenya, Rwanda and Uganda) and Southern Africa (Zambia). Participant demographics are shown in Table 11.

Table 11. Demographics of the study cohorts.

	Participants Data from	
Characteristic	East and Southern Africa[g] (n=56)	United Kingdom (n=18)
Age[a], median (IQR)[b]	32 (27-39)	46 (41-54)
Sex, % Females	36	6
CD4 Count, median (IQR) cells/µl[c]	450 (377-617)	502 (353-757)
Log_{10} pVL, median (IQR) copies/ml[d]	4.5 (3.5-5.2)	3.7 (1.6-4.3)
ART %[e]	0	94
AIDS related Death %[f]	9	0
Subtype, N (%)		
A	20 (36%)	0 (0%)
B	0 (0%)	15 (83%)
C	27 (48%)	0 (0%)
D	9 (16%)	0 (0%)
CRFs	0 (0%)	3 (17%)

NOTE. [a]Age at Entry not available for Rwanda (1/12), Uganda (5/16) and Zambia (2/27)

[b]Interquartile range (IQR 1-3), [c]CD4 count not available Zambian (1/27) and United Kingdom (UK) (3/18)

[d]pVL not available Uganda (1/16) and UK (6/18), [e]anti retroviral therapy (ART) information not available UK (1/18)

[f] Death between month 15-month 70

[g] 4% from Kenya, 21% from Rwanda, 29% from Uganda and 46% from Zambia

CRFs Circulating Recombinant Forms

4.2.2 Infecting HIV-1 subtype

Subtyping data were available for both African and UK participants. African participants were subtype A, C and D according to the data obtained from IAVI African sites whereas, UK participants were all subtype B although the clinic was unable to provide data on three UK participants (I220, O351 and A550). UK samples (n=18) and a small number of African samples (n=9) were sequenced as part of this study to confirm their subtype. A phylogenetic analysis of the UK and African cohort samples was undertaken by PCR amplification of a fragment of the *gag* gene ($Gag_{171-323}$ amino acid; 461bp) (Figure 20). Reference sequences were obtained from the HIV db to create a phylogenetic tree allowing identification of the virus subtypes in both cohorts (Figure 20). UK individuals were predominantly a subtype B; however, one individual was subtype AE (I220) and two were of subtype AG (O351, A550). UK participants infected with CRFs (AE and AG) were excluded from further data analysis in this study. Three UK individuals (B650, W425, and S400) were repeatedly PCR negative and therefore subtype indeterminable by the Gag PCR method. African individuals subtyped at HIL were either infected with HIV-1 subtype A (C193000, C270006 and C270012) or subtype C (C175003, C235021, C235023, C175011, C305105 and C235034) and the subtype results were identical to those generated by IAVI African sites by amplifying the Pol gene.

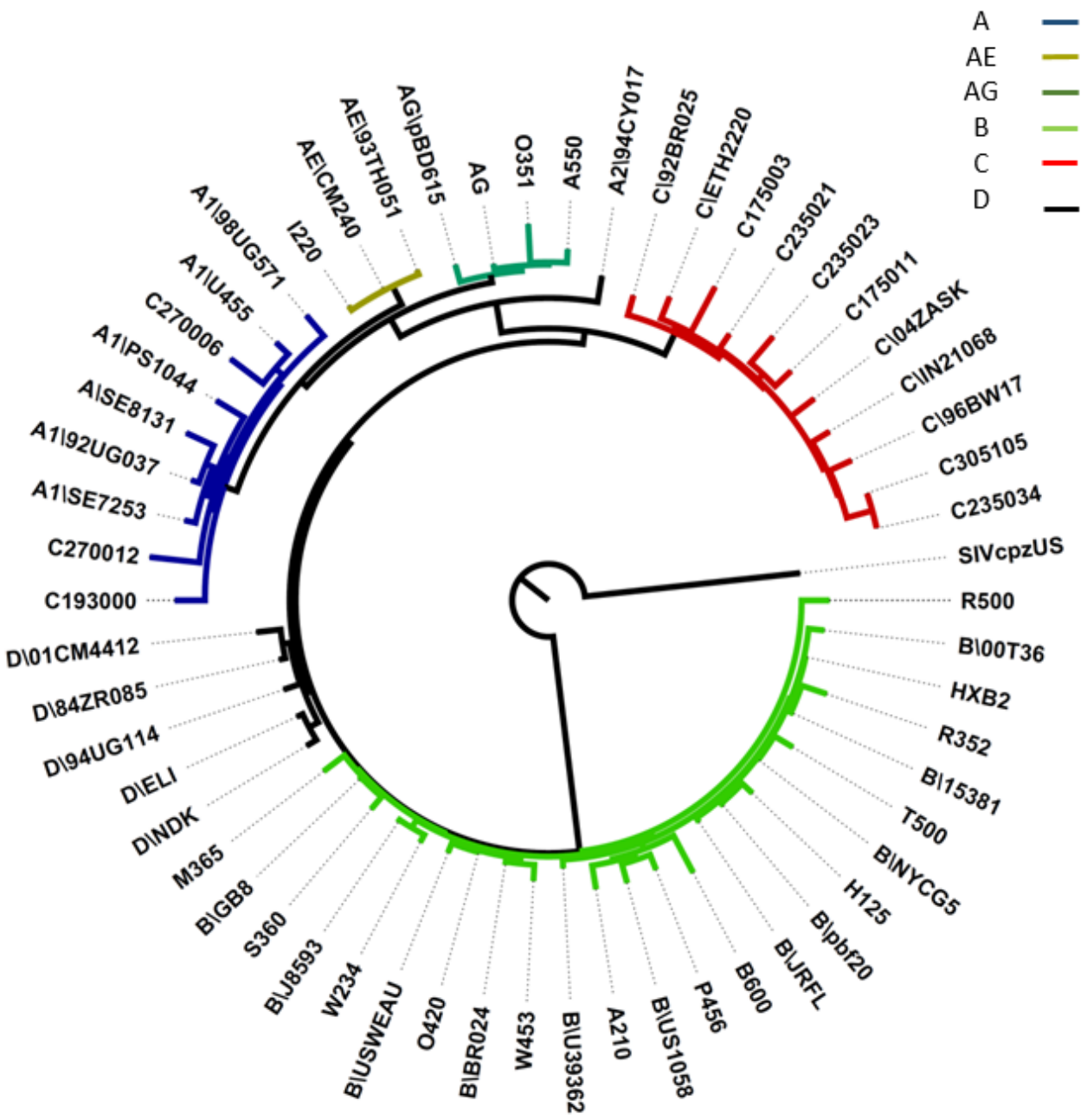


Figure 20. Classification of the viral Subtype in study participants

Phylogenetic analysis of proviral DNA sequences of the *gag* gene ($Gag_{171-323}$ amino acid) showing a neighbour joining tree confirming subtype(s) of both the UK and African cohort in relation to the reference sequences from the Los Alamos National Lab HIV db. Reference sequences are shown with subtype followed by a back slash and the clone identity, for example C\92BR025. Accession numbers of reference sequences used to generate phylogenetic tree: A\SE8131 (AF107771), A1\98UG571 (AF484509), A1\U455 (M62320), A1\PS1044 (DQ676872), A1\92UG037 (AB253429), A2\94CY017 (AF286237), AE\93TH051 (AB220944), AE\CM240 (U54771), AG\pBD615 (L39106), B\GB8 (AJ271445), B\J8593 (GQ371400), B\USWEAU1 (U21135), B\BR024 (FJ645388), B\39362 (U39362), B\US1058 (AY331295), B\JRFL (U63632), B\pbf20 (AY835766), B\NYCG5 (M38431), B\15381 (DQ853462), B\00T36 (AY423387), C\96BW17 (AF110980), C\IN21068 (AF067155), C\04ZASK (AY772699), C\ETH2220 (U46016), C\92BR025 (U52953), D\01CM4412 (AY371157), D\84ZR085 (U88822), D\94UG114 (U88824), D\ELI (K03454) and D\NDK (M27323).

4.2.2.1 Sequence homology of HIV_{consv} and GRIN with subtype A, B, C and D reference sequences

GRIN and HIV_{consv} sequences were compared with HIV-1 reference sequences (A, B, C and D) to estimate (Gag, Pol, Nef and Env fragments) homology between any of the four different subtypes. For the HIV_{consv} panel, all reference sequences were truncated to fit HIV_{consv} Gag (138 aa fragment), Pol (510 aa fragment) and Env (105 aa fragment). Similarly for the GRIN panel, all reference sequences were trimmed to align with GRIN Gag (488 aa fragment), Pol (848 aa fragment), Nef (120 aa fragment) and Env (634 aa fragment). Phylogenetic tree analysis showed HIV_{consv} Gag and RT did not align with any another subtype and were outliers/out-grouped whereas, HIV_{consv} Env was grouped with subtype B Env (Figure 21). However, GRIN Gag, Pol, Nef and Env was aligned with subtype A as GRIN/Env was designed using subtype A sequences (Figure 22).

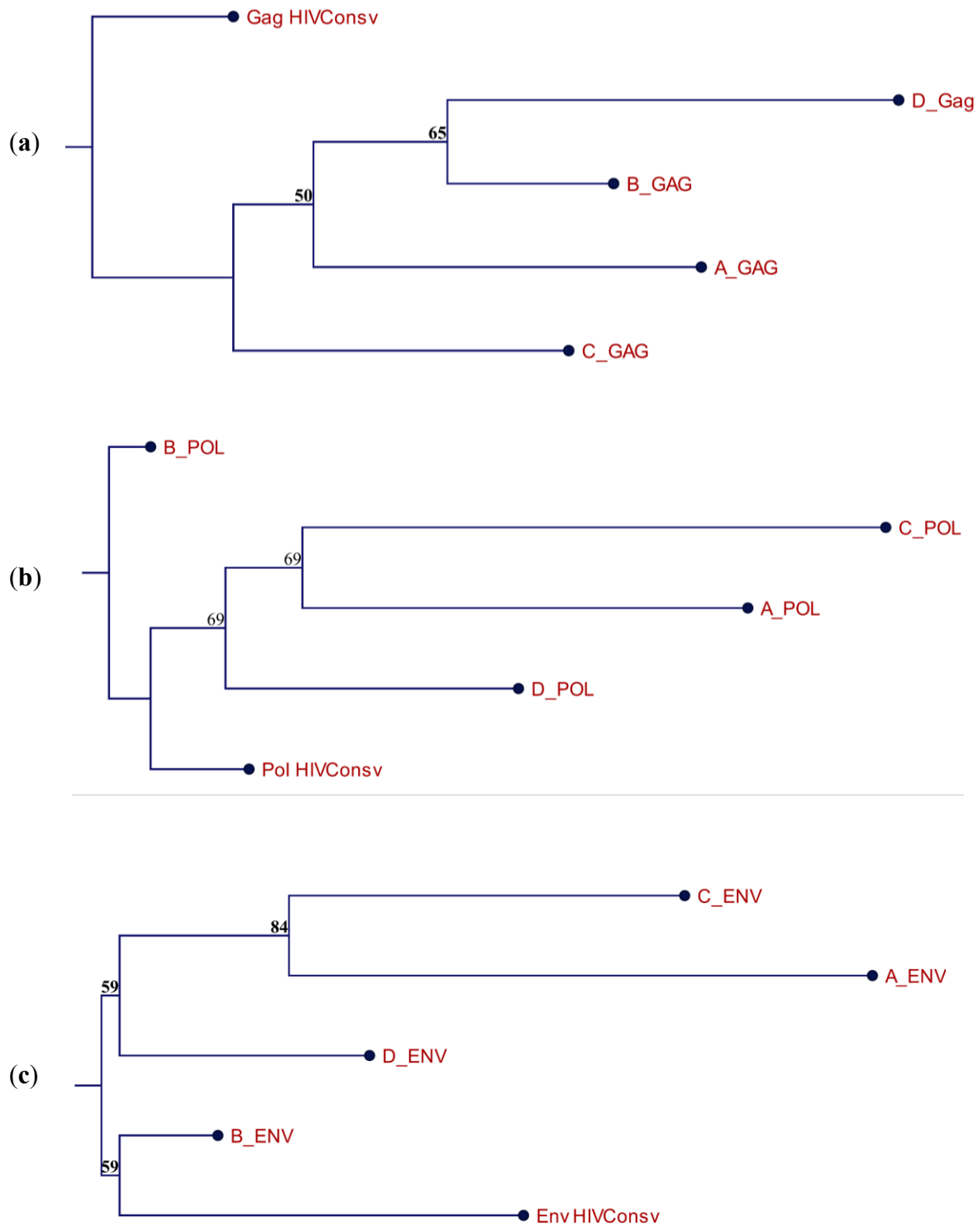


Figure 21. Sequence homology between HIV_{consv} and four HIV-1 subtypes

A neighbour joining tree illustrating sequence homology between HIV_{consv} (a) Gag (138 aa fragment), (b) Pol (510 aa fragment) and (c) Env (105 aa fragment) using reference sequences from the Los Alamos National Lab HIV db. Bootstrap values of >80% are considered to be statistically significant. Accession numbers of reference sequences used to generate phylogenetic tree: A1\92UG037 (AB253429), B\HXB2 (K03455), C\04ZSK146 (AY772699 & U46016) and D\ELI (K03454)

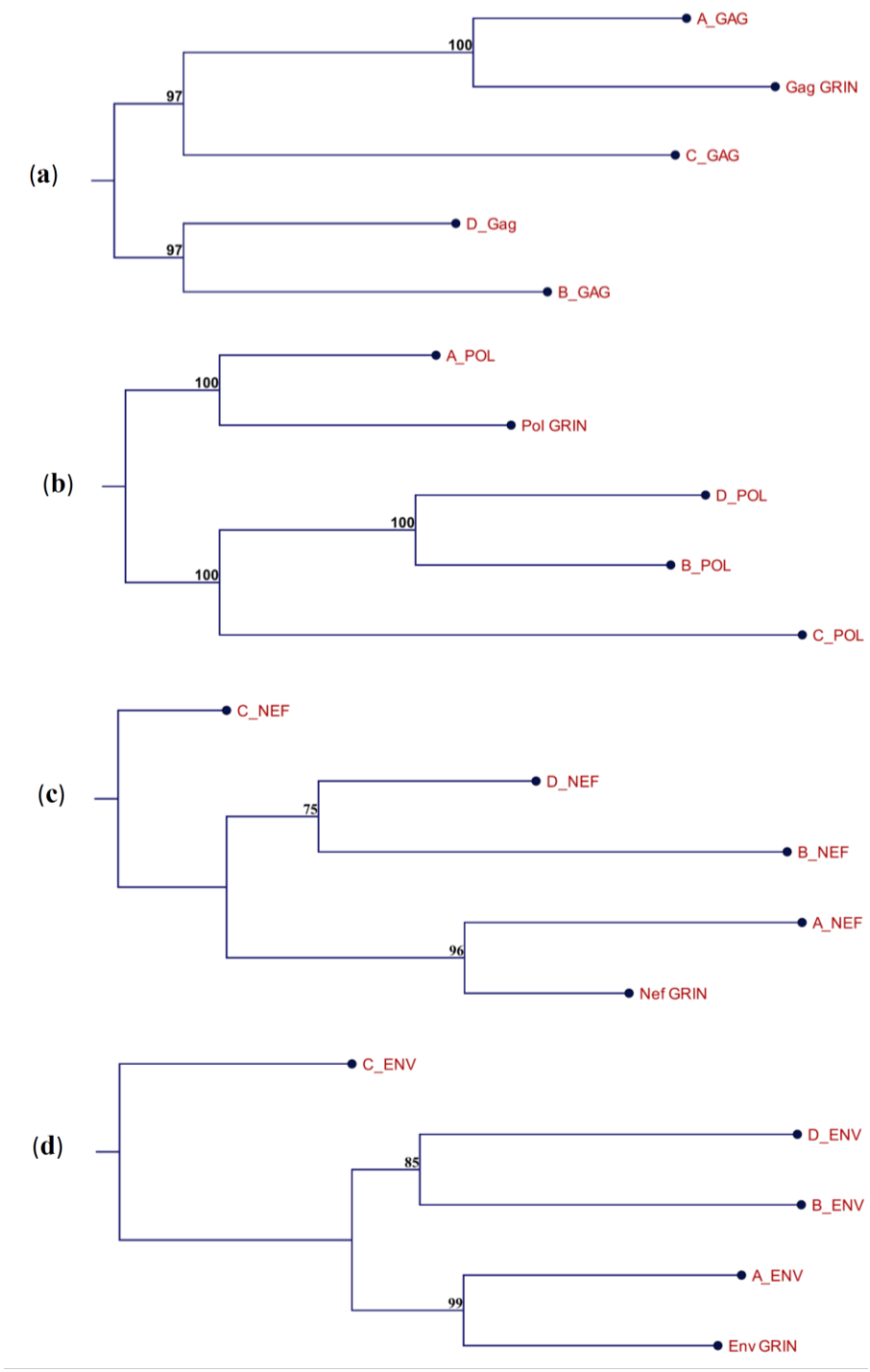


Figure 22. Sequence homology between GRIN and HIV-1 subtypes

A neighbour joining tree illustrating sequence homology between GRIN (a) Gag (488 aa fragment), (b) Pol (848 aa fragment), (c) Nef (120 aa fragment) and (d) Env (634 aa fragment) using reference sequences from the Los Alamos National Lab HIV db. Bootstrap values of >80% are considered to be statistically significant. Accession numbers of reference sequences used to generate phylogenetic tree: A1\92UG037 (AB253429), B\HXB2 (K03455), C\04ZSK146 (AY772699 & U46016) and D\ELI (K03454).

4.2.3 Proportion of responders to the HIV-1_{consv} peptide matrix and GRIN/Env pools

Three different patterns of ELISpot responses were observed with the two panels of peptides used in this study: (a) no response (b) responses against GRIN pools only and (c) responses elicited against both panels of peptides (Figure 23). Most (96%) individuals elicited a response against CMV peptide in the ELISpot assay and there were no differences in responses to CMV by individuals infected with different HIV-1 subtypes (A-D) (p>0.05; 2-tailed Fisher's exact test). As expected, all individuals generated IFN-γ following mitogen (PHA) activation and the levels produced by individuals infected with different HIV-1 subtypes was median 4, 310 (IQR, 2, 966 – 5,123) SFU/10^6 PBMC.

Most individuals responded to GRIN pools such as GRIN-Gag (subtype A (85%), subtype B (93%), subtype C (93%), subtype D (100%)), GRIN-RT ((subtype A (60%), subtype B (33%), subtype C (59%), subtype D (67%)), GRIN-INT (subtype A (45%), subtype B (40%), subtype C (74%), subtype D (78%)), GRIN-Nef (subtype A (60%), subtype B (33%), subtype C (81%) and subtype D (67%)) and GRIN-Env (subtype A (10%), subtype B (67%), subtype C (41%), subtype D (44%)) (Figure 24). Furthermore, the proportion of individuals infected with either one of the four major HIV-1 clades generated a response to at least one peptide within the HIV-1_{consv} matrix for subtype A was; Gag (20%), RT (25%) and INT (25%), for subtype B Gag (30%), RT (20%) and INT (40%), for subtype C Gag (33%), RT (26%) and INT (41%) and for subtype D Gag (11%), RT (22%) and INT (33%) (Figure 24). Overall, no difference was observed between the proportion of responders for each clade and for each responding HIV-1_{consv} region such as Gag, RT and INT (all p>0.05). No HIV_{consv} Env responses were detected in subtype A and B individuals whereas, a few subtype C and (15%) subtype D (22%) individuals generated a response against HIV_{consv} Env matrices (Figure 24).

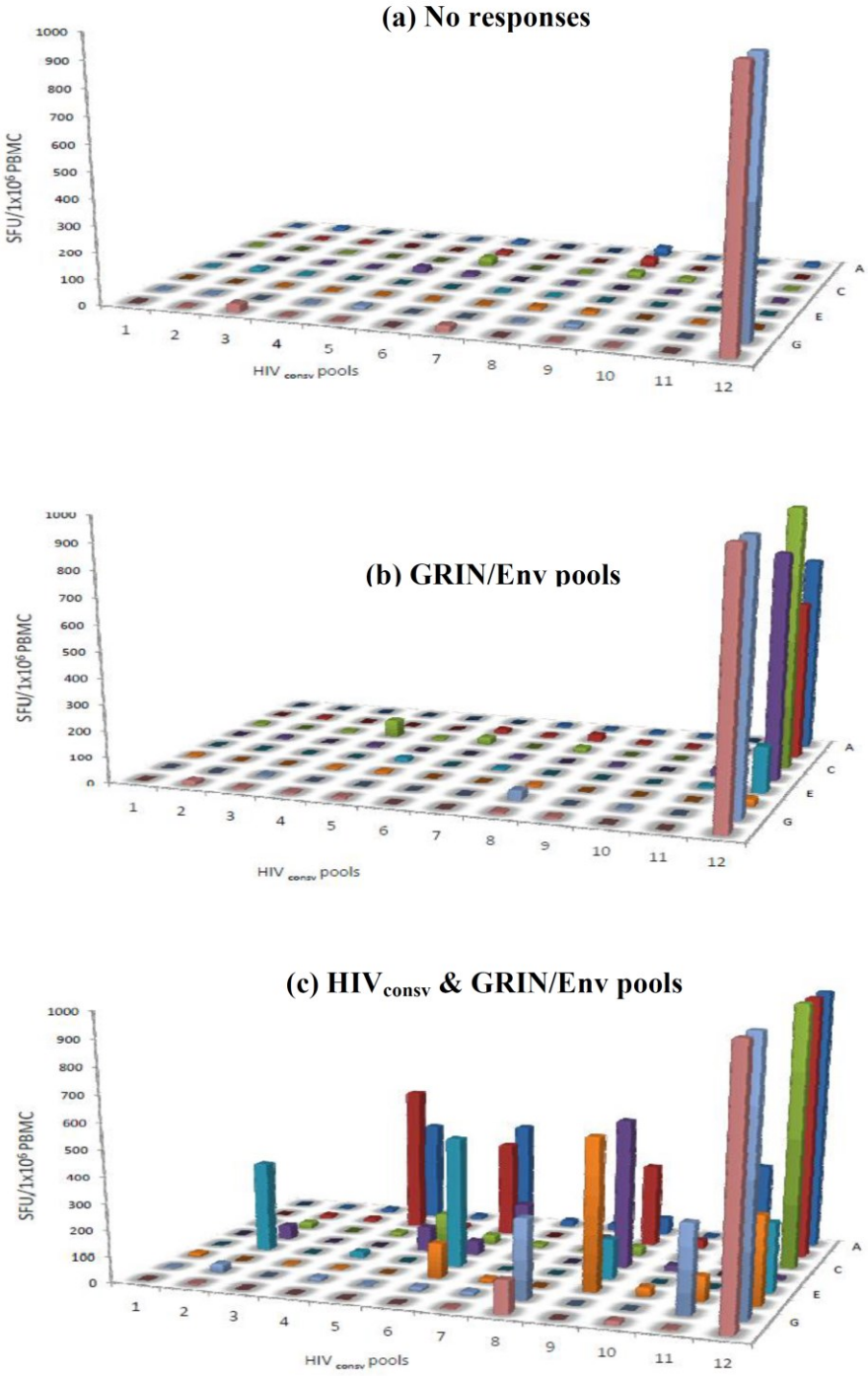


Figure 23. IFN-γ ELISpot response to two different antigens

Representative examples of IFN-γ ELISpot responses elicited against 80 HIV_{consv} pools in three different infected individuals, GRIN/Env pools, mock, CMV and PHA, after background subtraction and normalised to SFU/1x10^6. Column #1 represents mock wells only, column #2-11 (1-80) HIV_{consv} pools, column #12 (wells A-F) GRIN/Env pools, well 12G CMV(pp65) and well 12H PHA, respectively. (a) No responses (subtype not available) apart from PHA and CMV indicated that PBMC were non responsive to HIV_{consv} peptides (b) Responses to HIV GRIN/Env pools (subtype A1), CMV and PHA, but no response against HIV_{consv} pools (c) IFN-γ ELISpot responses to HIV_{consv} pools, GRIN/Env pools (subtype A1) and both to CMV and PHA.

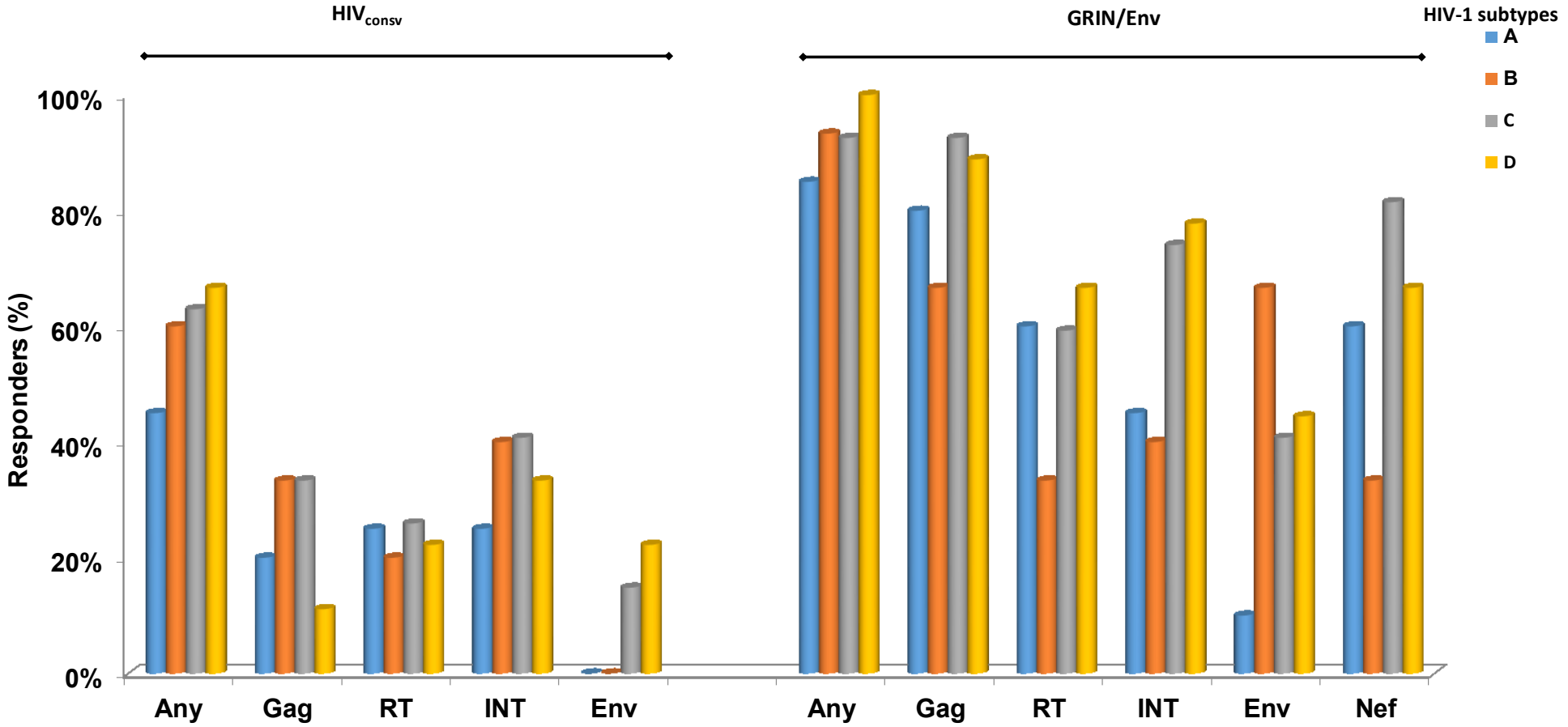


Figure 24. Proportion of responders during different stages of infection

IFN-γ ELISpot responses to HIV$_{consv}$ matrix and GRIN pools demonstrating that most individuals induced responses (≥50 SFU/10^6) against different HIV-1 antigens regardless of their subtype. X-axis represents HIV-1 antigens used in the ELISpot assay and Y-axis is the percentage of responders to HIV$_{consv}$ and GRIN pools. The proportion of individuals who exhibited IFN-γ responses to HIV$_{consv}$ pools was lower than the GRIN pools.

Comparatively, fewer individuals (41/71, 58%) had a response to at least one peptide within the HIV-1_{consv} matrix than to the number of individuals (65/71, 92%) responding to at least one of the GRIN/Env peptide pools (p<0.05) (Figure 24). There was no clade specific bias in the recognition of GRIN Gag, RT and INT pools and HIV_{consv} matrices (all p>0.05). However, subtype A GRIN-Env were lower when compared with other subtypes responders (p<0.05). As Nef has not been included in HIV_{consv} matrix, Nef responses will be excluded from any further analysis. Similarly, Env is poorly represented by HIV_{consv} matrix and comparing HIV_{consv} Env responses to GRIN/Env would not be atrue representation of the data. Thus, Env responses will also be excluded from any further analysis.

4.2.4 Comparison of HIV_{consv} pool responses with GRIN/Env pool responses

Pairwise Euclidean distances were used to assess ELISpot responses to HIV_{consv} matrix and GRIN pools which were clustered using the neighbour-joining algorithm (Figure 25). There were no actual HIV_{consv} Gag, RT and INT pools performed with the participants used in the study thus, HIV_{consv} pool data (Gag, RT and INT) were assimilated through the summation of SFU of all responding deconvoluted peptides for comparison with GRIN pools. This evaluation demonstrated that subtype background did not dictate the ability of these individuals to respond to HIV_{consv} matrix and GRIN pools; that is, there was no apparent subtype specific clustering of these IFN-γ ELISpot responses (Figure 25).

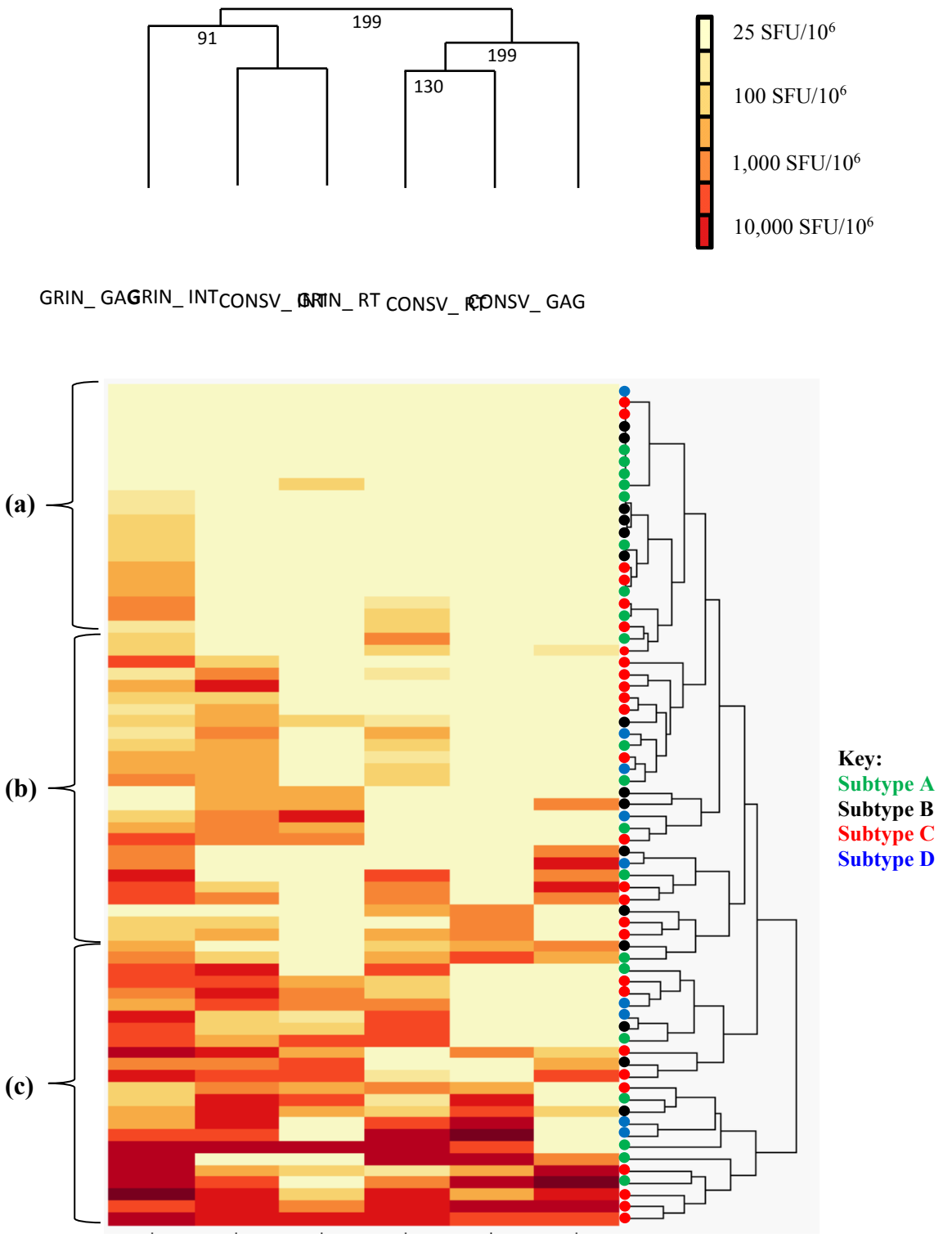


Figure 25. Heatmap demonstrating GRIN and HIV$_{consv}$ pools responses

Dendrogram comparing HIV$_{consv}$ and GRIN/Env pool responses separated into three different clusters based on their magnitude and breadth: (a) weak or low breadth responses to both panels, (b) responses predominantly to GRIN pools and (c) strong magnitude and breadth to both panels. Top dendrogram shows separation of HIV$_{consv}$ peptide matrix and GRIN pools. ELISpot responses (right dendrogram) highlights subtype of individual, colour coded according to the key. There is no apparent subtype specific clustering in terms of IFN-γ ELISpot responses. Bootstrap values of >80% are considered to be statistically significant.

For subtype A, C and D a difference was observed in the recognition of GRIN-Gag by these individuals ($p<0.05$; 2-tailed Fisher's exact). For subtype B ($p>0.05$) there was no difference in the recognition of Gag by both panels. No differences were observed in the recognition of RT region of both panels by all subtypes (all $p>0.05$). However, GRIN-INT was somewhat better recognised by subtype C individuals ($p<0.05$), but this apparent frequency was not significant ($p>0.05$) for subtypes A, B and D. Consequently, there was no simple subtype discrimination or clustering in terms of recognition of the different regions by these subtypes (A, B, C and D).

There was an apparent antigenic clustering of RT and INT responses elicited by both panels and some disparities were apparent between Gag responses induced against the two panels (Figure 25). To corroborate this, 2x2 contingency tables were created for Gag, RT and INT regions for all subtypes. McNemar analysis and Kappa (κ) values were used to identify whether or not discordance was in favour of one peptide set being better recognised than the other (Table 12). Concordance between HIV_{consv} and GRIN responses for subtype A individuals against Gag was poor and was fair against RT and INT. For subtype B apart from maybe Gag the rest exhibited good concordance (Table 12). Similarly, concordance between HIV_{consv} and GRIN responses for subtype C individuals against Gag was poor and only slightly better against RT and INT. For subtype D, fairly poor concordance was observed for Gag/RT/INT (Table 12).

Table 12. Contingency table showing the concordance or discordance between HIV_{consv} and GRIN pools

		GRIN/HIV_{consv}				Kappa (κ)	McNemar's Test
Clade	Region	+/+	+/-	-/+	-/-		
A	Gag	4	12	0	4	0.12	<0.05
	RT	5	7	0	8	0.37	<0.05
	INT	4	5	1	10	0.37	NS
B	Gag	4	6	1	4	0.16	NS
	RT	3	2	0	10	0.66	NS
	INT	6	0	0	9	1.00	NS
C	Gag	9	16	0	2	<0	<0.05
	RT	6	10	2	9	0.17	<0.05
	INT	10	10	0	7	0.34	<0.05
D	Gag	1	7	0	1	<0	<0.05
	RT	2	4	0	3	0.25	NS
	INT	3	4	0	2	0.25	NS

NOTE. < 0.05 Significant, NS Non-Significant
Interpretation of Kappa; Poor (<0), Slight (0.01 - 0.20), Fair (0.21 - 0.40), Moderate (0.41 - 0.60), Substantial (0.61 - 0.80) and Almost Perfect (0.81 - 1.00)

4.2.5 Evaluation of magnitude and breadth of ELISpot responses

More than half of the individuals (39/71) infected with either subtype A, B, C, D elicited IFN-γ responses to HIV_{consv} Gag, RT and INT (Figure 26). The total magnitude of HIV_{consv} responses between subtypes was similar (all $p>0.05$; Mann Whitney U test). Similarly, no difference was observed in the recognition of the number of HIV_{consv} peptides between each subtype (all $p>0.05$). Furthermore, most of the individuals (87%) elicited IFN-γ responses against GRIN Gag, RT and INT (Figure 27). Some differences were observed in the total magnitude of subtype A and B ($p<0.05$), B and C ($p<0.05$) and B and D ($p<0.05$) whereas, comparison between the total magnitude of other subtypes were similar (all $p>0.05$). However, as expected when the total magnitude (n=71) of HIV_{consv} and GRIN pools were assessed regardless of the subtype then the total GRIN responses ($p<0.05$) were higher than total HIV_{consv} responses for all regions (Gag, RT and INT).

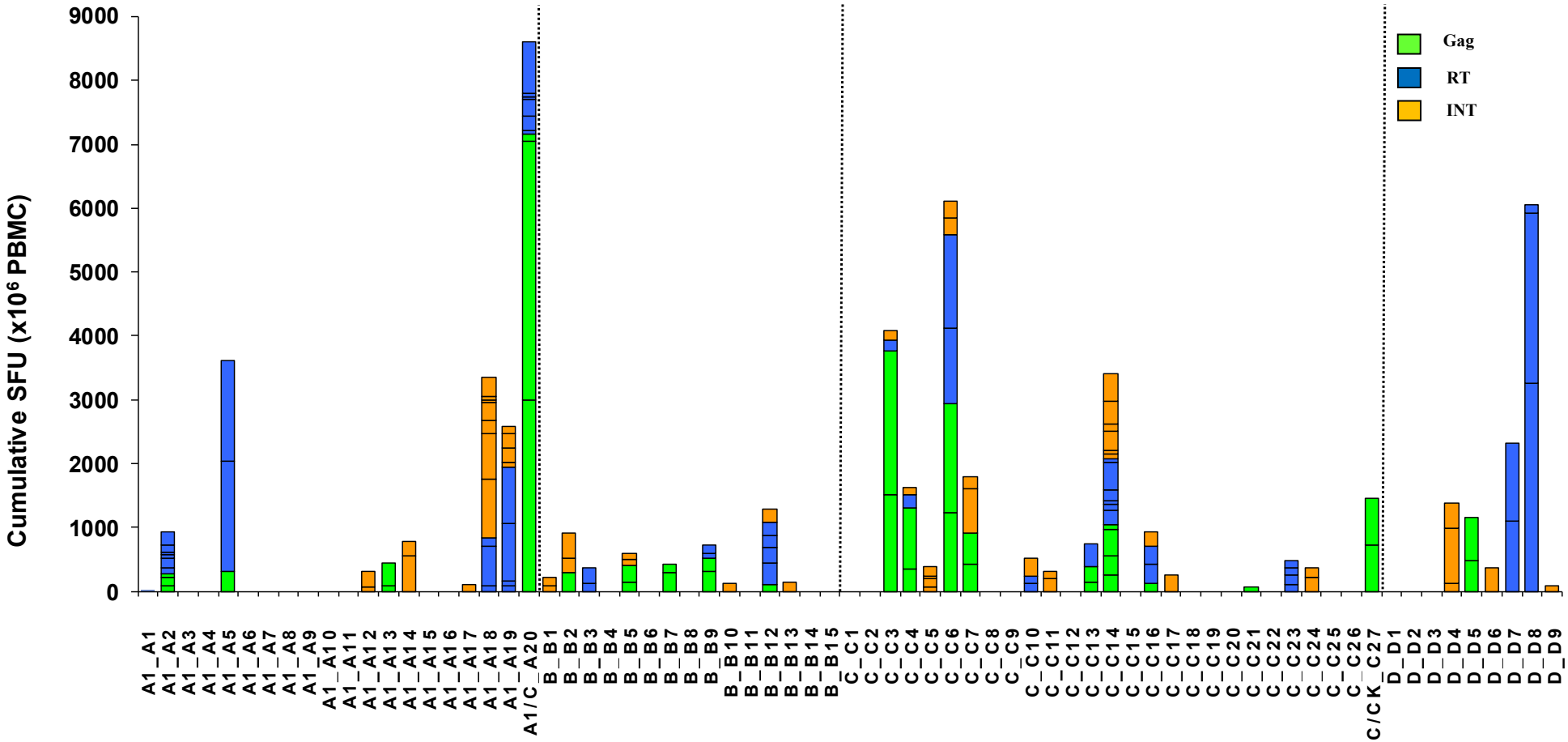


Figure 26. Magnitude and breadth of responses to HIV$_{consv}$matrices in HIV-1 infected individuals

Deconvoluted HIV$_{consv}$ peptides representing the magnitude and breadth of responding regions among 71 HIV-1 infected participants. Each stacked bar represents the number of responding HIV$_{consv}$ peptides (i.e. breadth), magnitude of each peptide and the totality of each individual's response.

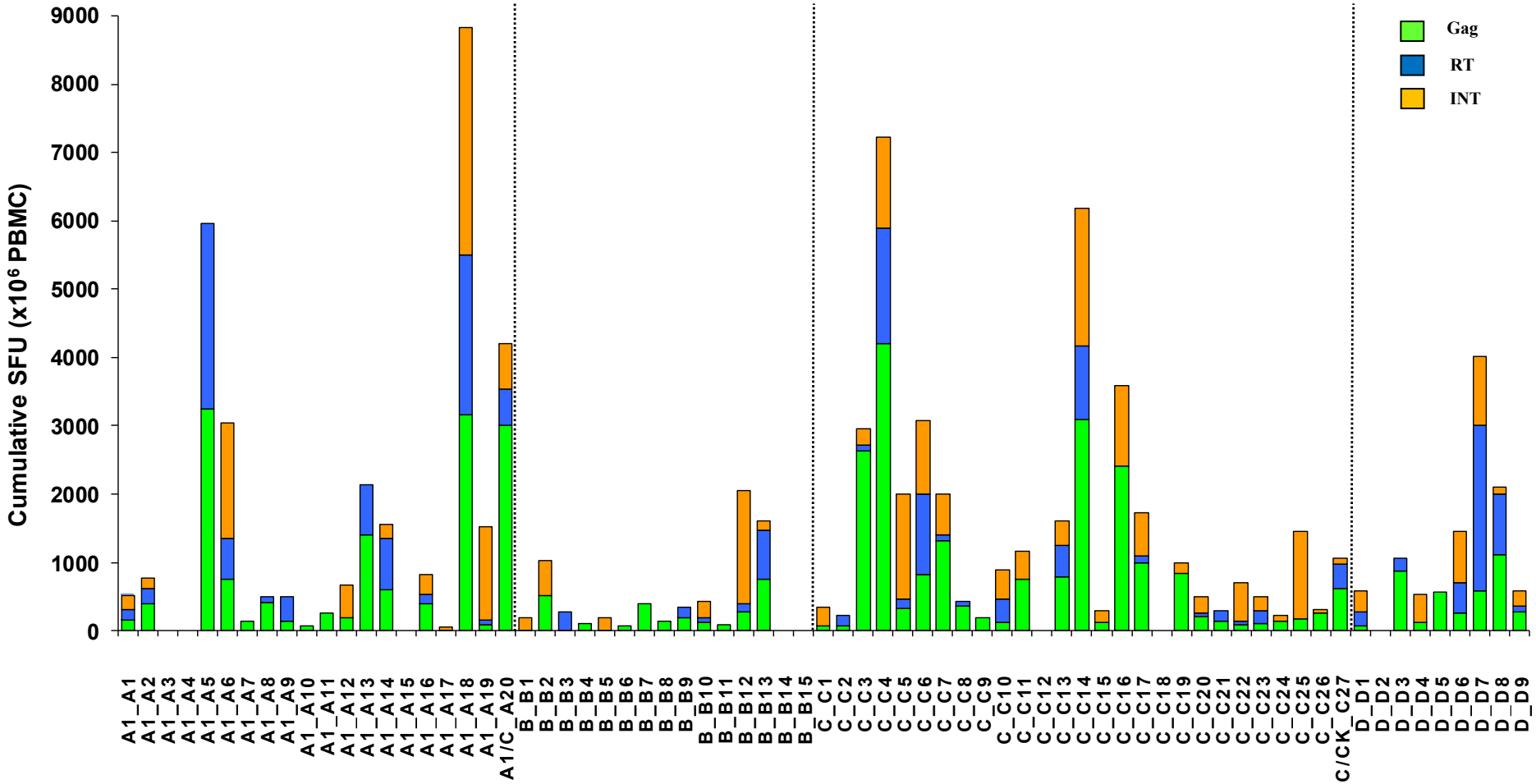


Figure 27. ELISpot responses to GRIN pools in HIV-1 infected individuals

Representative of the magnitude and breadth of responding GRIN regions (Gag, RT and INT) among 71 HIV-1 infected participants. Each stacked bar represents the number of responding GRIN pools (i.e. breadth), magnitude of each responding pool and the totality of each individual's response.

4.3 Preliminary comparison and delineation of HIV_{consv} and GRIN HLA-restricted epitopes

Due to the large number of cells (> $20x10^6$) required to map T cell responses with both HIV_{consv} and GRIN matrices, it was not possible to map all the participants' T cell responses with both panels due to the sample availability. Hence, only small number of participants (18/71, 25%) T cell responses were deconvoluted using both HIV_{consv} and GRIN matrices to determine if the responding epitopes were conserved or variable. Of the 7/18 HIV_{consv} responders, for which some degree of HIV_{consv} Gag, RT and INT antigenicity was apparent, 16 single or overlapping peptide responses were recognised. Similarly, in 15/18 GRIN responders for which some degree of GRIN Gag, RT and INT antigenicity was apparent, a total of 75 single or overlapping peptide responses were recognised. HLA matched epitopes were searched in the HIV db and were scored as conserved (Entropy ≤0.2) or variable (Entropy ≥0.2). In some cases responding epitopes could not be identified in the HIV db based on patient's HLA. Three kinds of responding epitope (Table 13) within OLP were identified; 1) the responding epitope was "identical" between GRIN and HIV_{consv}, 2) responding epitope differs by one or two amino acid substitutions and referred to as "similar" (Asparagine (N) was replaced by Glycine (G) or 3) peptide sequence "varied" by different adjacent residues on either side of the epitopes (Table 13).

Table 13. Comparison of GRIN and HIV_{consv} epitopes

		Responding single or OLP and epitope[a]	
Classification	Region	GRIN	HIV_{consv}
Identical	Gag	VIEEKAFSPEVIPMFSALS	MEEKAFSPEVIPMFT
Similar	RT	DVGDAYFSVPLDE**N**FRKY	VGDAYFSVPLDE**G**FRKYTA
Varied	Gag	GATPQDLN**V**MLNIVGGHQA	LSEGATPQDLN**T**MLNTVGG
Note.	[a]Underlined sequence is the responding epitope		

Ten HIV_{consv} and 41 GRIN epitopes were identified in the HIV db (Table 14) and were categorised into weak and strong IFN-γ responses. Of those, 27/41 and 14/41 GRIN epitopes were classified as weak and strong responses, respectively. In comparison, 1/10 and 9/10 HIV_{consv} epitopes were classified as weak and strong responses, respectively. Further analysis showed that 18/27 (67%) weak GRIN responding sequences were either not incorporated or partly truncated (for example; GRIN INT epitope LPPIV**AKEI** was represented as truncated HIV_{consv} INT epitope -----**AKEI**) when compared with HIV_{consv} antigen (Table 14). Only 9/27 weak GRIN responding sequences were represented by HIV_{consv} panels and the responding HIV_{consv} epitopes were either identical or similar (Table 14), but HIV_{consv} IFN-γ ELISpot responses were not observed against analogues of GRIN

epitopes. Similarly, strong GRIN responses (14/41) demonstrated the same pattern of representation in HIV_{consv} panels as described above for GRIN weak responses. This can be potentially explained by the weak magnitude of these GRIN response (67%). These responses are usually non-reproducible when tested with the HIV_{consv} matrix (See section 3.2.3).

Table 14. Epitopes identified in HIV db based on their HLA

		Epitopes division based on ELISpot Data[a]	
		Weak Responses	Strong Responses
Classification		n/N	n/N
GRIN epitopes (N=41)		27/41	14/41
Not represented in HIV_{consv}		18/27	6/14
Represented in HIV_{consv}		9/27	8/14
Identical to HIV_{consv}	Conserved[b]	5/9 (56%)	5/8 (63%)
	Variable[c]	1/9 (11%)	0/8 (0%)
Similar to HIV_{consv}	Conserved[b]	3/9 (33%)	2/8(25%)
	Variable[c]	0/9 (0%)	1/8 (25%)
HIV_{consv} epitopes (N=10)		1/10	9/10
Not represented in GRIN		0	1/9
Represented in GRIN		1/10(10%)	8/9 (89%)
Identical to GRIN	Conserved[b]	0/10 (0%)	3/9 (33%)
	Variable[c]	0/10 (0%)	0/9 (0%)
Similar to GRIN	Conserved[b]	0/10 (0%)	3/9 (33%)
	Variable[c]	1/10	2/9 (22%)

NOTE. [a]Weak ELISpot response (50-120 SFU/10^6), [b]Strong ELISpot response (>120 SFU/10^6)
[b]Conserved entropy score (<0.2), [c]Variable entropy score (>0.2)

In addition, 9/10 (90%) HIV_{consv} and 8/14 (57%) GRIN epitopes were represented by both panels and one HIV_{consv} epitope was not represented by GRIN (Table 14). Of those, three epitopes were recognised by both panels, two epitopes were identical (FKRKGGIGGY restricted by B*1503 and KTAVQMAVF restricted by B*5801) and one was similar (HIV_{consv} TPQDLNTML (TL9) and GRIN TPQDLNVML (TL9) restricted by B*8101).

4.3.1 Comparison of proviral DNA sequence to the HIV_{consv} panel

The proviral DNA from sixteen Gag and eight INT responders was partially sequenced 24 months post infection (Figure 28 and Figure 29**)**. The available sequence data from this study and the HIV db suggests that these regions (HIV_{consv} Gag and INT) are highly conserved across A, AE, AG, B, C and D subtypes (Figure 28 and Figure 29). Most of the sequences were highly conserved across different subtypes in the HIV db (Figure 28 and Figure 29). The HIV_{consv} Gag starts at HXB2 position Gag_{159} and ends in Gag_{440}, and INT starts at HXB2 position Pol_{776} and ends at Pol_{996} (Figure 28 and Figure 29). Responses were spread across the whole of these regions, but largely fell into a few clusters that were recognised by different individuals infected with subtypes, while other regions were apparently non-antigenic. These data demonstrated that proviral HIV sequences of most responders are coincident with sequence homology to the peptides used. Indeed, 77% of Gag and 75% of INT responding regions were identical to the HIV_{consv} peptides.

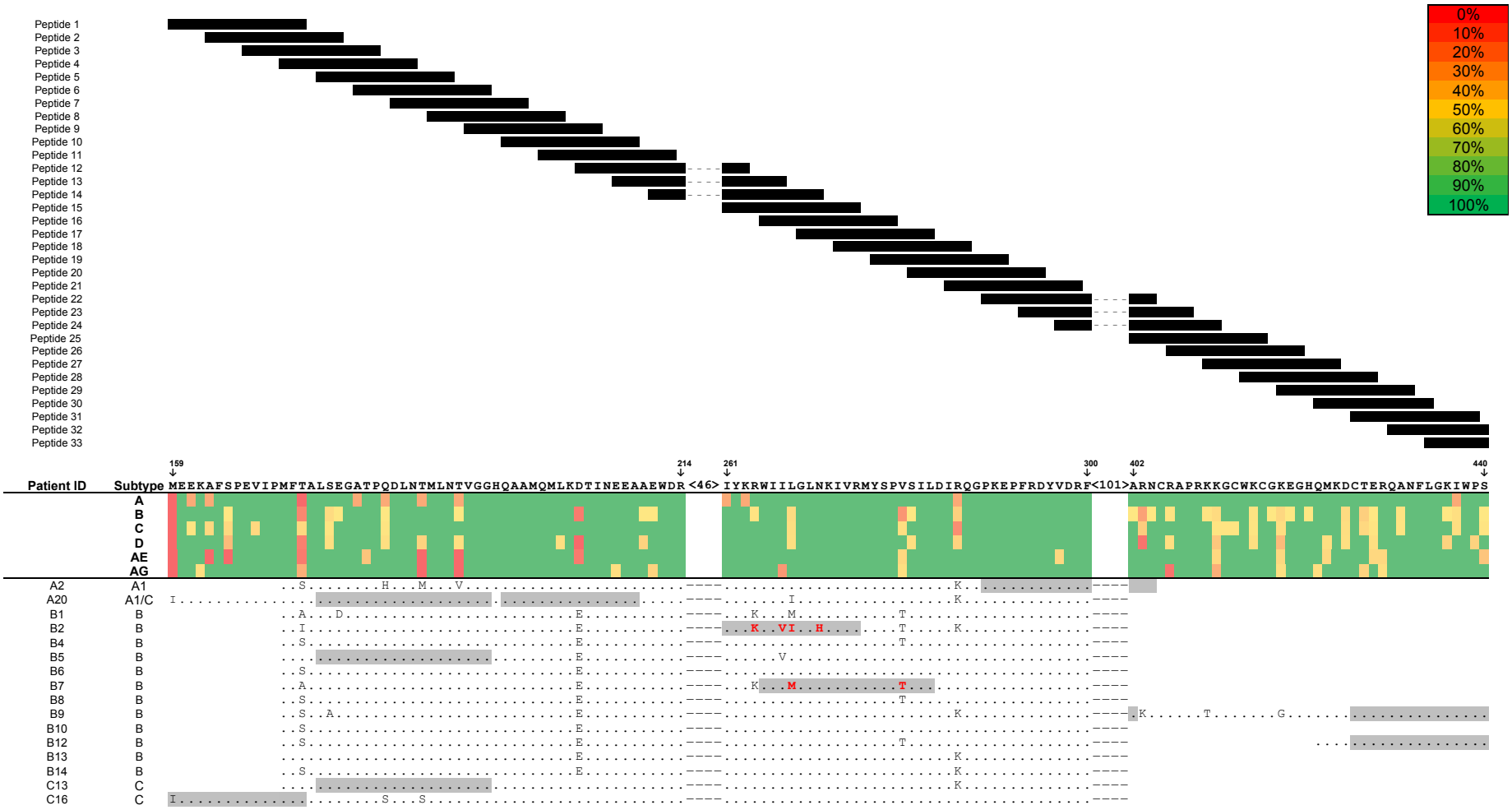


Figure 28. Comparison of patients Gag proviral DNA sequence with HIV_{consv} insert

Sequence homology of patient Gag sequences compared to the HIV_{consv} template sequence and the OLPs (black rectangles). All the numbering is based on HXB2 and the gaps in HIV_{consv} panel are shown. Grey boxes highlight the responding peptides showing, if any, polymorphisms in red text. Key represents percentage homology with HIV db reference sequence A (n=2), AE (n=7), AG (n=11), B (n=34), C (n=21) and D (n=7).

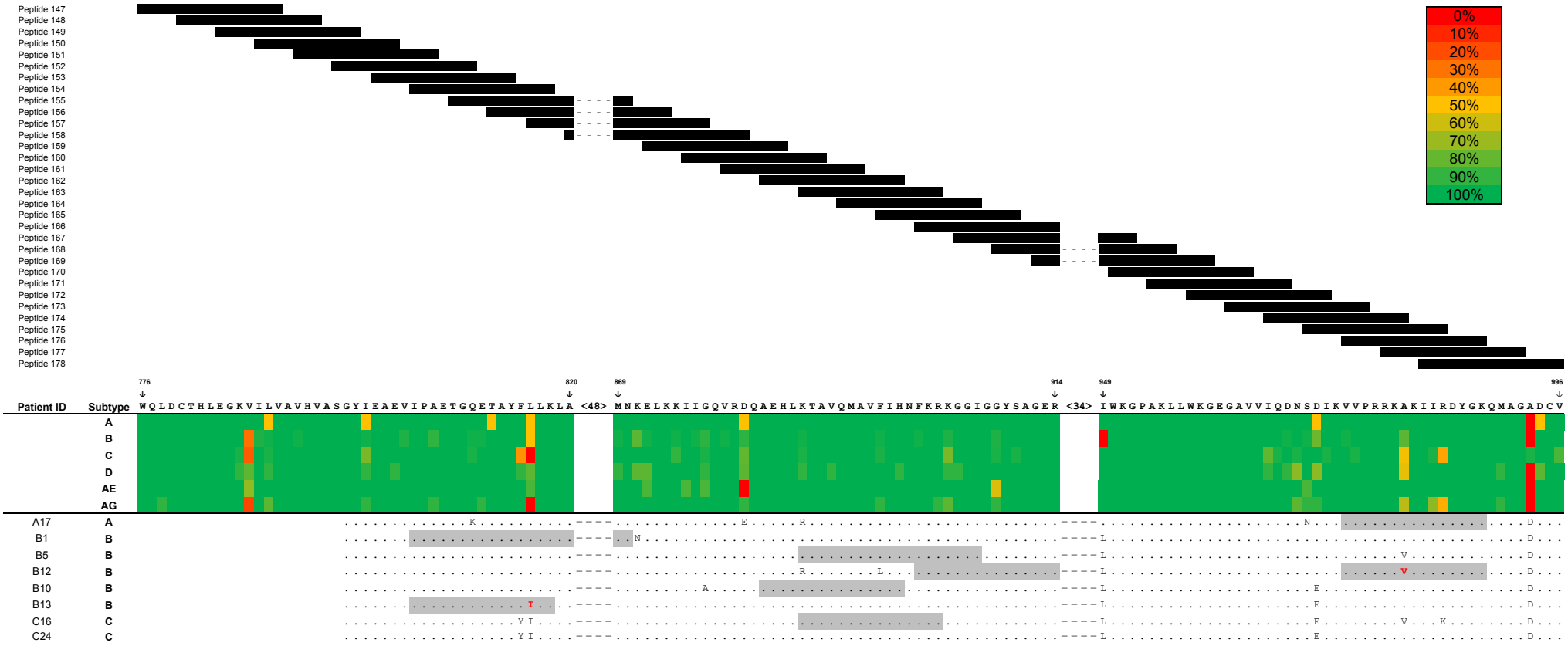


Figure 29. Comparison of patients INT proviral DNA sequence with HIV_{consv} insert

Sequence homology of patient INT sequences compared to the HIV_{consv} template sequence and the OLPs (black rectangles). All the numbering is based on HXB2 and the gaps in HIV_{consv} panel are shown. Grey boxes highlight the responding peptides showing, if any, polymorphisms in red text. Key represents percentage homology with HIV db reference sequence A (n=2), AE (n=7), AG (n=11), B (n=34), C (n=21) and D (n=7).

4.4 Discussion

To date, an effective HIV-1 vaccine which can overcome the problem of HIV-1 diversity remains elusive. Currently, many successful vaccines for other diseases are used globally and one such example is *Streptococcus pneumonia* (Shapiro et al., 1991). The issue of pathogen diversity, in the case of *Streptococcus pneumonia*, was resolved by designing a multivalent vaccine based on the predominant circulating strains of the invading pathogen. It was possible to overcome diversity of the pneumococcus as this pathogen evolves slowly within its subtypes (Shapiro et al., 1991). This approach could not be used for influenza virus as it is highly variable in its intraspecies genetic diversity. To deal with influenza diversity every year, a new vaccine has to be generated to match the circulating virus strains which emerge during seasonal influenza outbreaks (Jin and Chen, 2014). However, the genetic diversity of HIV-1 is greater compared to influenza virus such that the global genetic diversity of influenza in any given year is comparable to the genetic diversity of HIV in a single individual infected with HIV. This often results in immunological escape and evolution of and variability in CD8 epitopes (Chakraborty et al., 2014; Korber et al., 2001). There is increasing evidence that CTLs recognise variants of the same epitope(s) and have been shown to be cross reactive against different clades of HIV-1 (Cao et al., 2000; Coplan et al., 2005; Geels et al., 2005; Keating et al., 2002; McKinnon et al., 2005). So even though CTL responses in HIV infection may control disease progression, it still remains challenging to identify a T cell based vaccine immunogen that could provide sterilising immunity against different strains of HIV-1 circulating in the population. Indeed, a potential HIV-1 vaccine candidate must have the ability to induce potent cell mediated immune responses most likely against all HIV-1 clades (McMichael et al., 2002). Therefore, this would make it possible to test the immunogenicity of an HIV-1 vaccine based on one specific clade in other geographical regions where other clades exist in the population (Cao et al., 2003).

In this study, two HIV-1 antigens were investigated to determine how broadly recognised they were in individuals infected with different HIV-1 clades (A, B, C and D). Data generated in the present study demonstrate that responses against GRIN and HIV_{consv} Gag, RT and INT are quite common in infected individuals. These included individuals from different countries (UK, Rwanda, Uganda, Kenya and Zambia), infected with different subtypes (A, B, C and D), and with an extensive variety of genetic backgrounds (HLA A1, A2, A3, A11, A23, A26, A30, A68, A74, B7, B8, B15, B18, B27, B35, B42, B45, B51, B53, B57, B58, B81, Cw02, Cw03, Cw04, Cw06, Cw07, Cw08 Cw16, Cw17 and Cw18 and more).

The majority of responses were directed against HIV_{consv} and GRIN Gag and Pol (RT and INT) regions and there was no apparent subtype discrimination. This was in line with other studies

reporting that Gag and Pol regions are the most common targets for CTL responses (Ahmed et al., 2016; Mothe et al., 2015; Radebe et al., 2015).

In general, GRIN pools were frequently recognised and this was evident for Gag, RT and INT responses generated by individuals tested in this study. However, it was not possible to identify what proportion of these responses was directed towards conserved or the more variable epitopes for all the subjects due to lack of sample availability. Nevertheless, these data do suggest that the sequence template of GRIN may inherently offer a broader matrix to define CD8 responses in a wider range of HIV-infected individuals than the HIV_{consv} matrix. This could be because GRIN is based on the full genome and incorporates more potential T cell epitopes (Keefer et al., 2012; Kopycinski et al., 2014). Several other matrices have also been used to measure cross reactive T responses against COT, multiple antigen cocktails and Mosaic antigens (Fischer et al., 2007; Korber et al., 2009b; Rolland et al., 2007a).

Furthermore, most of the subtype B individuals which were receiving ART mounted T cell responses against HIV_{consv} Gag, RT and INT, inferring that these individuals retained their memory CTL responses. It was presumed that perhaps these individuals may not be able to maintain T cell memory due to the lack of antigen stimulation (Casazza et al., 2001). Recently studies have also shown a low level of CTL activity in individuals on ART, however these $CD8^+$ T cell responses were sufficient to control viral replication and viral rebound even after treatment interruption (Conway and Perelson, 2015; Smith et al., 2016).

It was apparent using available sequencing data from this study and in the HIV db that HIV_{consv} regions are highly conserved across Gag and INT (no RT sequences were available from these individuals). These data indicate that infected individuals were able to respond to conserved regions and preserved sequence of conserved epitopes. Although only a small number of samples could be sequenced, their identity with the peptide sequences within the HIV_{consv} matrix and the retention of identical responding peptides from the ELISpot data suggests that these sequences remained unchanged during the course of the infection. However, it may also suggest that the responses measured here to conserved epitopes may not be exerting sufficient selection pressure on the virus to escape.

A small number of participants' were tested with both matrices. It was not possible to delineate all participant's GRIN responses to an epitope level as this required a large number of PBMC. The responding GRIN epitopes were both conserved and variable, spread throughout Gag and Pol proteins and varied from participants infected with HIV-1 clade A, C or D. A higher number of GRIN epitopes were recognised by these individuals compared to HIV_{consv} epitopes. The data possibly explains why

HIV_{consv} appears to be less antigenic than GRIN as HIV_{consv} only incorporated low number of conserved epitopes compared to GRIN. A large number of GRIN responses were low magnitude and generally such responses had poor reproducibility (See section 3.2.3). Of those, a number of GRIN responses were mounted towards epitopes not represented by the HIV_{consv} panel and vice versa. Similarly, some of the high magnitude (reproducible) responses were also missed as those sequences were not represented by either panel. However, when a sequence was represented by both GRIN and HIV_{consv} antigen, both peptide sets did not always elicit the same ELISpot responses. There may be a number of factors associated with this lack of recognition, such as the consequence of aa substitution at anchor residues or the length of the peptide was too long to fit MHC-I snugly or that the flanking aa surrounding the responding epitopes within OLP may affect the processing of the peptide resulting in reduced affinity that might lead to inadequate biological activity (Culshaw et al., 2012; Marcilla et al., 2016; Neefjes and Ovaa, 2013; Ranasinghe et al., 2011; Steers et al., 2014).

The chance of detecting $CD8^+$ T cell responses in natural infection would be better when using both antigens rather than employing one of them alone. These data showed that cross clade responses were detectable to conserved regions of HIV-1 genome, common in the majority of the clades. However, there was no subtype bias to detect CD8 responses using these antigens. GRIN appeared to be recognised more commonly; as GRIN incorporates near full genome whereas HIV_{consv} is based on only 806 aa. These findings suggest that an effective HIV-1 vaccine could be one that induces responses to multiple epitopes, resulting in broader recognition of HIV-1 circulating in a population. In short, both HIV_{consv} and GRIN antigen detected cell mediated responses against conserved and non-conserved regions of HIV-1 and similar responses could also be induced by a vaccine comprised of either GRIN or HIV_{consv} immunogen and should be tested in clinical trials. Importantly, this further provides tools for assessing the potential of candidate vaccines to induce responses to HIV_{consv} /non-conserved epitopes.

There were a few caveats of this study that should be noted. There is a possibility that autologous peptides or potential T cell epitope (PTE) peptides could enhance the ability to detect $CD8^+$ T cell responses in HIV-1 infected individuals over the peptide sets used in this study (Altfeld et al., 2003b; Kunwar et al., 2013; Li et al., 2011; Malhotra et al., 2007). Moreover, due to the low sample number per subtype this study lacked power to show any differences between recognition of the two study panels by different groups of people infected with different subtypes of virus.

Chapter 5: Evaluation of immunologic and viral markers of disease progression in HIV-1 viraemic controllers, viraemic progressors and chronic progressors

5.1 Introduction

The appearance of HIV specific $CD8^+$ T cells during the acute stage of infection plays a critical role in determining the clinical outcome of the disease. The drop in peak pVL in acute HIV infection (AHI) usually overlaps with the emergence of HIV specific $CD8^+$ T cells responses which may be responsible for early virus containment (Borrow et al., 1994; Koup et al., 1994). These initial immune responses may contribute in controlling viraemia, but nevertheless whether these early responses determine the set point VL still remains undetermined (Ndhlovu et al., 2015a). It was shown in an animal model of disease control that depletion of $CD8^+$ T cells during the acute phase led to a substantial increase in pVL, which dropped to a lower level when CD8 populations were reintroduced (Jin et al., 2013; Schmitz et al., 1999). Most importantly, if it is possible to predict progression rates during the early days of AHI based on set point pVL then this may allow more effective prospects for treatment of HIV infected individuals and prevention of disease progression (Cohen et al., 2010; Lyles et al., 2000).

In the absence of ART, a small number of HIV infected individuals are able to control their pVL to <50 copies/ml, these individuals are identified as elite controllers (EC) (Cortes et al., 2015; Okulicz et al., 2009). However, those individuals who are capable of maintaining their pVL ≤2000 copies/ml are classified as viraemic controllers (VC) (Cortes et al., 2015; Okulicz et al., 2009; Tansiri et al., 2015). The vast majority of HIV infected people are unable to control their pVL (>10000 copies/ml) and are referred to as non-controllers (Cortes et al., 2015; Tansiri et al., 2015). Thus, it is important to understand the mechanisms underlying HIV control and pathogenesis which may identify correlates of immune control. The exact role of HIV specific $CD8^+$ T cell responses in control of HIV replication in EC is not completely understood as these responses are variable from individual to individual (Freel et al., 2012; Kuang et al., 2014; Radebe et al., 2015; Streeck et al., 2014).

There are a number of elements which may contribute to HIV pathogenesis such as the host's genetic background, immunological and/or viral factors (Cortes et al., 2015; Kuang et al., 2014; Prince et al., 2012; Singh and Spector, 2009). Protective alleles such as HLA B*57 and B*27 in EC are usually associated with reduced viral replication and slower disease progression (Kloverpris et al., 2012; Mendoza et al., 2012; Migueles et al., 2015; Pereyra et al., 2010). However, not all EC harbour such protective alleles and several HIV-1 infected subjects encoding these alleles failed to control viraemia to low levels (Cortes et al., 2015; Emu et al., 2008; Migueles et al., 2000; Pereyra et al., 2010). This

could be due to the replicative capacity of the transmitted (founder) virus shown to be associated with CD4 decline and immune dysfunction in acute infection in B57/B58 individuals (Claiborne et al., 2015). Moreover, studies have shown that polyfunctional T cell responses and the production of granzyme and perforin in EC maybe associated with control of viraemia in EC (Hersperger et al., 2010; Migueles et al., 2002a; Ndhlovu et al., 2012; Riou et al., 2014).

There is a huge gap in our understanding of the basis of virus control in groups of individuals who can control HIV-1 efficiently. Indeed, insufficient information is available regarding initial events post HIV-1 infection that lead to the clinical outcome in these individuals. The mechanisms underpinning the initial cell mediated immune responses in controlling HIV-1 pathogenesis are still not fully understood. Data generated during the early stages of HIV-1 infection may shed light on factors affecting the clinical outcomes of patients and may predict morbidity and mortality in individuals.

Work presented in this chapter focuses on a group of individuals defined as viraemic controllers (VC), viraemic progressors (VP) and chronic progressors (CP). VC and VP are groups of HIV infected individuals identified through a population-wide HIV screening as described earlier (See section 2.1.2). VC are defined as individuals maintaining pVL at or ≤2000 copies/ml, whereas VP are defined as individuals maintaining pVL at or ≥100,000 copies/ml and CD4 count ≤350/µl. However, the CP are defined as individuals who do not fit the definition of VC and VP. The purpose was to investigate any immunological differences in the T-cell responses between VC and VP and attempt to associate these responses with disease prognosis. $CD8^+$ T cell responses of VC and VP were compared with CP to examine if there are any differences in T cell responses between these three groups. These data may indicate that $CD8^+$ T cell responses to conserved regions may have an association with lower pVL and therefore may inform vaccine design.

5.2 Results

5.2.1 Study Participants

A total of 15 VC (viraemic controllers), 14 VP (viraemic progressors) and 19 CP (chronic progressors) were studied, all of whom were ART naïve and included both males and females. Study subjects were from Kenya, Rwanda, Uganda and Zambia and included individuals infected with subtype A, C and D (Table 15).

Table 15. Demographics of the study participants

	Study groups		
	VC (n=15)	VP (n=14)	CP (n=19)
Characteristic			
Age[a], median (IQR)[b]	30 (27-39)	28 (25-33)	35 (31-46)
Sex, % Females	53	29	26
ART %[c]	0	0	0
AIDS related Death %[d]	0	14	10
Subtype, N (%)			
A	8 (53)	4 (29)	7 (37)
C	2 (13)	6 (42)	11 (58)
D	4 (27)	4 (29)	1 (5)

NOTE. [a]Age at Entry, not available for CP (5/19)
[b]Interquartile range (IQR 1-3), [c]Anti retroviral therapy
[d] Death between month 15-month 70

The times of V1.0 (baseline) sample collection varied between all three groups ($p<0.05$; Kruskal Wallis test). The V12 and V24 eDPI were approximately 12 months and 24 months post infection, respectively (Table 16). CD4 counts for VC were maintained at a high level with no difference at V1, V12 and V24 ($p>0.05$), but the pVL varied significantly between three time points ($p<0.05$) (Figure 30 and Table 16). VC pVL was high at V1.0 (median 3.3; IQR (2.6-3.6) Log_{10} copies/ml) and dropped to lower levels at V12 (2.5 (1.7-3.0) Log_{10} copies/ml) ($p<0.05$; Wilcoxon Signed rank test) whereas, no difference was observed in CD4 counts ($p>0.05$) between two time points (Table 16). In contrast, VP CD4 counts ($p<0.05$; Kruskal Wallis test) and pVL ($p<0.05$) varied significantly at V1.0, V12 and V24. VP CD4 counts were high at V1.0 (391 (299-446) and dropped at V12 (249 (229-332) ($p<0.05$; Wilcoxon Signed rank test) while pVL remained high over the period of 24 months but, fluctuated between three points ($p>0.05$) (Figure 30 and Table 16). Furthermore for CP, no significant difference was observed among all three points both for CD4 counts and pVL (both $p<0.05$; Kruskal Wallis test (Figure 30 and Table 16).

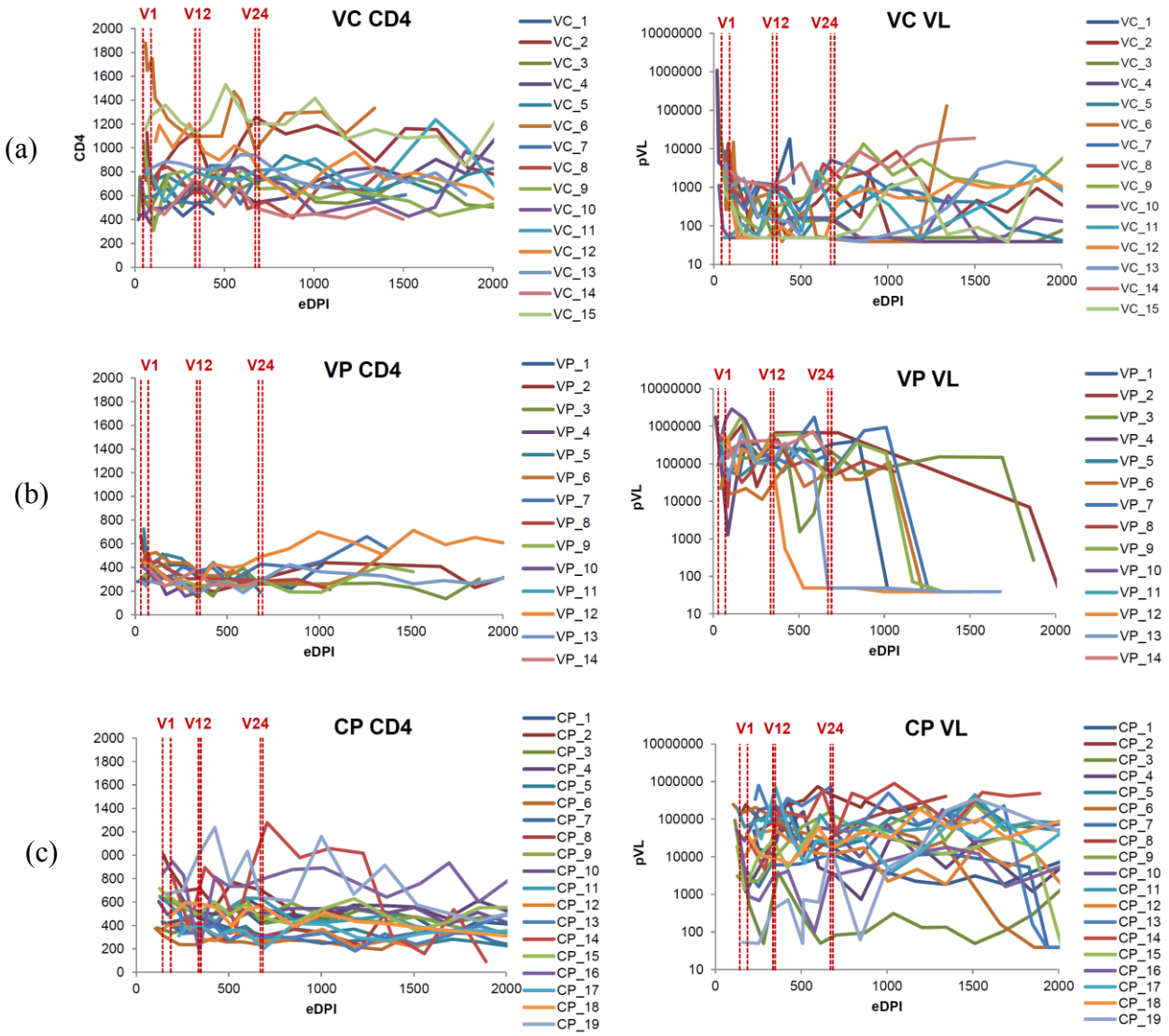


Figure 30. Illustration of pVL and CD4 count of study cohorts

pVL and CD4 count of VC, VP and CP during 1st five years of infection. Each coloured line represents the pVL/CD4 of each individual (a) pVL and CD4 counts plotted for 15 VC (b) pVL and CD4 counts plotted for 14 VP (c) pVL and CD4 counts plotted for 19 CP against estimated eDPI. Two dotted red line indicate upper and lower 95% confidence interval around the mean of their V1, V12 and V24. VP_1, VP_2, VP_3, VP_6, VP_9, VP_12 and VP_13 started ART post V12.

Table 16. Description and characteristics of three study groups

Subjects		V1.0	V12	V24	*p* Kruskal wallis
VC (n=15)	eDPI[a], median (IQR)[b]	59 (52-71)	341 (335-356)	674 (672-688)	
	Log_{10} pVL copies/ml, median (IQR)	3.3 (2.6-3.6)	2.5 (1.7-3.0)	2.2 (1.8-3.0)	<0.05
	CD4 counts cells/µl[c], median (IQR)	751 (549-1014)	714 (638-825)	743 (605-876)	>0.05
	HIV_{consv} Responders[d]	6/15 (40%)	6/8 (75%)		
	GRIN Responders[e]	13/15 (87%)	8/8 (100%)		
	Tested for polyfunctional responses	8/15			
VP (n=14)	eDPI, median (IQR)	44 (33-52)	338 (336-346)	675 (672-683)	
	Log_{10} pVL copies/ml, median (IQR)	5.5 (4.9-5.6)	5.3 (5.1-5.5)	4.7 (4.7-5.3)	<0.05
	CD4 counts, median (IQR)	391 (299-446)	249 (229-332)	287 (277-364)	<0.05
	HIV_{consv} Responders	4/14 (29%)	4/4 (100%)		
	GRIN Responders	11/14 (79%)	4/4 (100%)		
	Tested for polyfunctional responses	6/14			
CP (n=19)	eDPI, median (IQR)	148 (129-206)	338 (336-350)	673 (672-685)	
	Log_{10} pVL copies/ml, median (IQR)	4.6 (4.2-5.0)	4.7 (3.9-5.1)	4.2 (4.1-4.7)	>0.05
	CD4 counts, median (IQR)	603 (394-690)	461 (385-512)	451 (303-597)	>0.05
	HIV_{consv} Responders		12/19 (63%)	14/19 (74%)	
	GRIN Responders		17/19 (89.5%)	18/19 (94.7%)	
	Tested for polyfunctional responses				
NOTE.	[a]Estimated days post infection, [b]Interquartile range (IQR 1-3) and [c]CD4 count not available for CP (1/19), [d]HIVconsv responders=ELISpot responses (≥50SFU/10^6) to conserved pools, [e]GRIN responders=ELISpot responses (≥50SFU/10^6) to GRIN pools V1.0 (~ 30 days), V12 (~365 days) and V24 (~730 days)				

5.2.2 Proportion of responders to the HIV-1_{consv} peptide matrix and GRIN pools

In this study, 15 VC and 14 VP from visit 1.0 (V1.0) were initially tested with HIV_{consv} sub-matrices and GRIN pools by employing IFN-γ ELISpot assay (Table 16). No individuals were found to respond to the HIV_{consv} sub matrices alone. At V1.0 40% of VC and 29% of VP generated a response to at least one peptide within the HIV_{consv} sub-matrices; Gag (13%), RT (6%) and INT (33%) for VC and Gag (7%), RT (7%) and INT (14%) for VP (Figure 31a). Overall, no difference (all $p>0.05$; 2-tailed Fisher's exact test) was observed between the proportion of VC and VP responses against HIV_{consv} region; Gag, RT and INT. All individuals (VC and VP) elicited a response against CMV peptide in the ELISpot assay (median 942 [IQR, 373–2,326] SFU/10^6 PBMC). As expected, all individuals generated IFN-γ following mitogen (PHA) activation (median 4,437 [IQR, 3,588 – 5,142] SFU per 10^6 PBMC). On the whole, fewer VC (6/15; 40%) and VP (4/14; 29%) had a response to at least one peptide within the HIV_{consv} matrix compared to VC (13/15; 87%) and VP (11/14; 79%) responding to at least one of the GRIN peptide pools ($p<0.05$; 2-tailed Fisher's exact test) (Figure 31a). No difference (all $p>0.05$) was observed between the proportion of VC and VP responding to GRIN regions Gag, RT and INT. However, when the proportion of HIV_{consv} responders (VC and VP) were compared to GRIN pools then there was a difference between number of responders to Gag and RT regions ($p<0.05$; 2-tailed Fisher's exact test) but, not to the INT ($p>0.05$) for both groups. VC (n=8) and VP (n=4) T cell responses were compared with the third group CP (n=19) at a later time point (V12) (Figure 31b). VC (75%), VP (100%) and CP (63%) responded to different regions of HIV_{consv} matrix. VC, VP and CP at V12, responded to at least one peptide within the HIV_{consv} matrix; Gag (13%), RT (50%) and INT (50%) in VC, Gag (25%), RT (0%) and INT (75%) in VP and in CP Gag (26%), RT (36%) and INT (42%). These data show that the proportion of responders (VC and VP) at V12 appeared to be higher than at V1, but this did not reach statistically significant difference ($p>0.05$). All three groups also exhibiting responses to at least one peptide within GRIN pools - VC (100%), VP (100%) and CP (89%). No difference ($p>0.05$) was observed between the number of responders between the three groups. Furthermore, no difference ($p>0.05$; Kruskal-Wallis test) was observed between number of responders (all three groups) for both HIV_{consv} and GRIN antigenic regions. All individuals also exhibited responses against CMV peptides and PHA (Figure 31b).

In addition, CP (n=19) were tested for T cell responses at 24 months post infection (Figure 31c) to examine if T cell responses changed over time. The proportion of CP eliciting IFN-γ responses at V24 was slightly increased (Figure 31c) Gag (42%), RT (58%) and INT (47%) compared to V12 (Figure 31b).

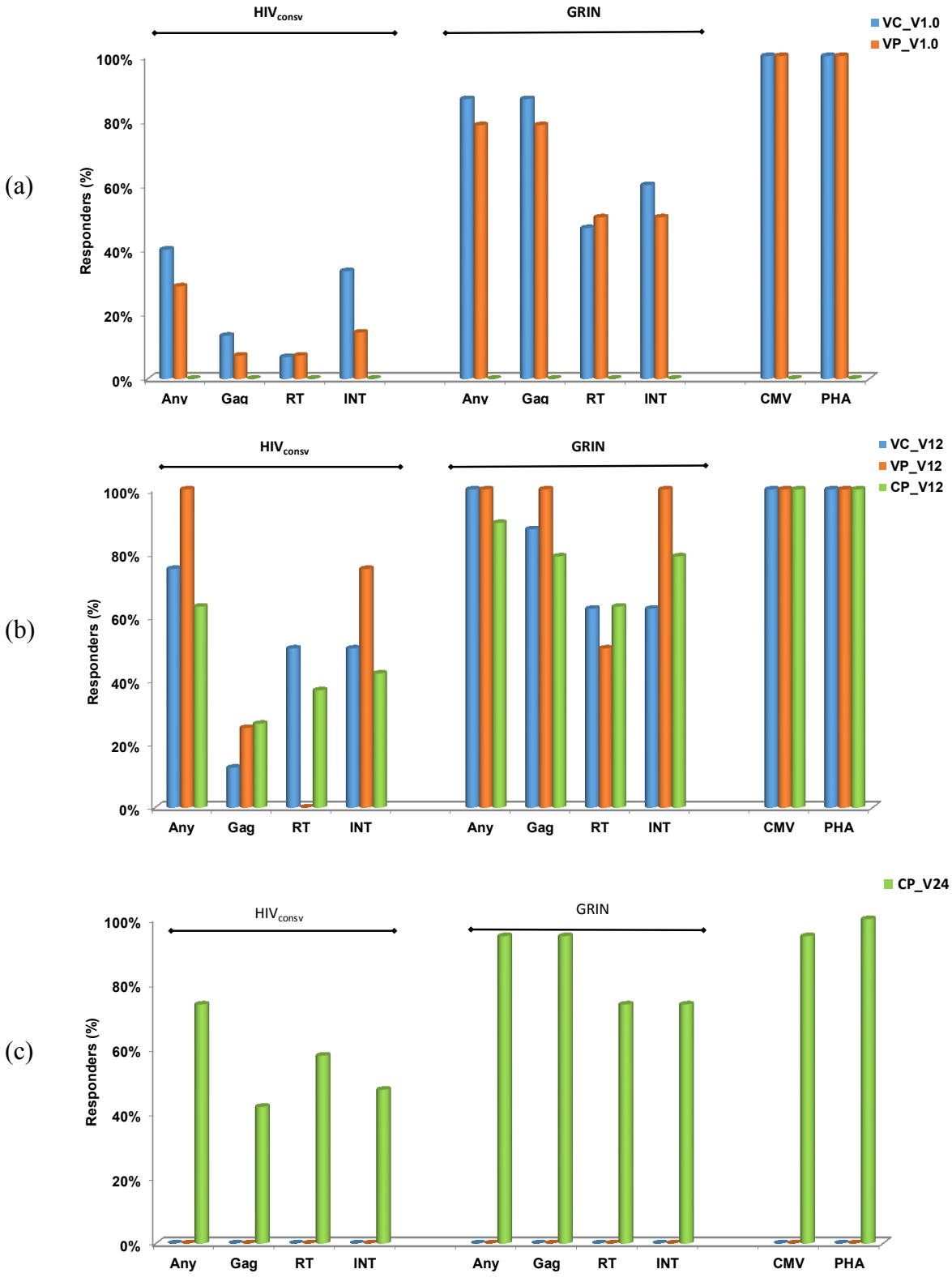


Figure 31. Proportion of responders to HIV-1 antigens at V1.0, V12 and V24

IFN-γ ELISpot responses to HIV_{consv} matrix and GRIN pools demonstrating number of individuals with responses against the two HIV-1 antigens at V1.0, V12 and V24. Blue, red and green bar represents VC (n=15), VP (n=14) and CP (n=19), respectively. (a) the proportion of IFN-γ ELISpot responders; VC and VP at V1.0 (b) VC, VP and CP at V12 and (c) CP at V24 .

Overall, there was no difference (all $p>0.05$; 2-tailed Fisher's exact test) between the proportion of responders at CP_V12 and CP_V24 for both HIV_{consv} matrix and GRIN pools for all regions. Almost all CP (V12 & V24) exhibited responses to CMV peptide and PHA at both time points, apart from one individual who did not elicit a response against CMV at V24 (Figure 31c).

5.2.3 Evaluation of the magnitude and breadth of ELISpot responses in three different groups of volunteers (VC, VP and CP) at two time points

VC and VP were tested for IFN-γ ELISpot responses at V1.0 and at V12 (Figure 32 a & b). Those who responded (VC & VP at V1.0 and V12) exhibited IFN-γ responses to HIV_{consv} Gag, RT and INT (Figure 32 a & b) and the total magnitude of HIV_{consv} responses at V1.0 and V12 was similar ($p>0.05$; Wilcoxon signed rank test). For both VC (6/8, 75%) and VP (4/4, 100%) there appeared to be greater breadth at V12 (Figure 32 a & b) as demonstrated by the median (IQR) number of peptides deconvoluted which at V1.0 was 0 (0-2) and 0 (0-1) compared to V12, 3 (1-3) and 2 (2-3) for VC and VP, respectively.

Similarly, both VC and VP exhibited IFN-γ responses against GRIN Gag, RT and INT pools at V1.0 and V12. There was no difference in the magnitude of GRIN Gag responses ($p>0.05$; Wilcoxon signed rank test) between V1.0 and V12 both for VC and VP. However, there was a difference between V1.0 and V12 GRIN RT and INT responses for VC, also there was a difference between GRIN RT responses ($p<0.05$) for VP, but not for INT responses ($p>0.05$) (Data not illustrated just descriptive).

To further corroborate whether T cell responses changed with time, CP were tested for IFN-γ ELISpot responses at V12 and V24 (Figure 32c). As observed with VC and VP the responding peptides covered all three antigenic regions; Gag, RT and INT. One individual (CP_6) lost their T cell response at V24 whereas, two subjects (CP_7 and CP_15) gained responses at V24. Even though the magnitude and breadth of T cell responses seemed to be lower at V12 than at V24, these responses were not significantly different ($p>0.05$ (Figure 32c). Likewise, the median (IQR) number of peptides deconvoluted at V12 2 (0-4) and at V24 median 3 (1-4), was not significantly different ($p>0.05$). In the same way, most of the CP exhibited IFN-γ responses against GRIN Gag, RT and INT pools and there was no difference in the magnitude of GRIN Gag, RT and INT responses at V12 and V24 ($p>0.05$) for all the participants.

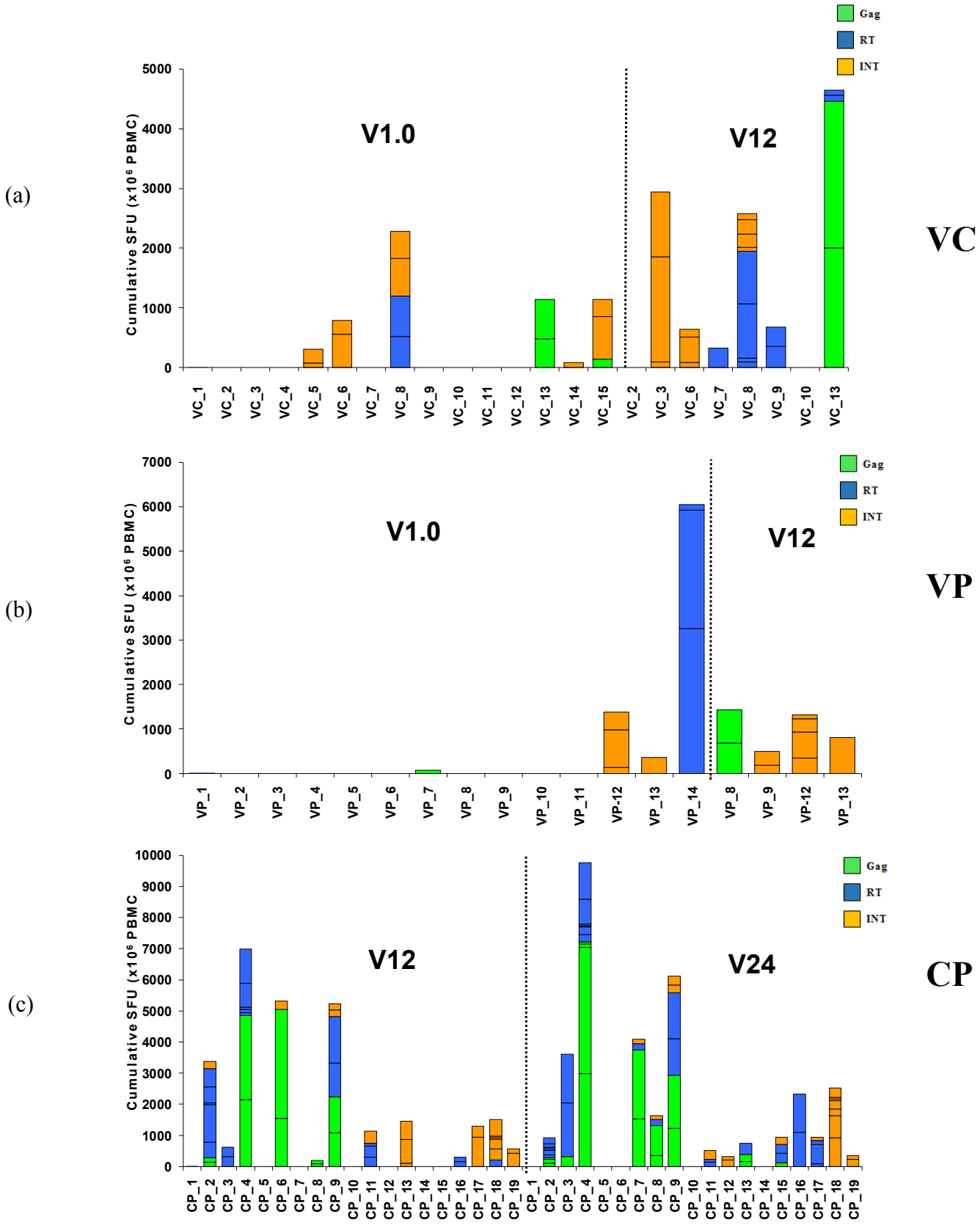


Figure 32. Illustration of magnitude and breadth of responses against HIV_{consv}

Deconvoluted HIV_{consv} peptides representing the magnitude and breadth of responding regions (a) VC (n=15), (b) VP (n=14) and (c) CP (n=19) were tested at V1.0 V12 and V24. Each stacked bar represents the number of responding HIV_{consv} peptides (i.e. breadth), magnitude of each peptide and the totality of each individual's response.

5.2.4 Assessment of VC and VP CD8$^+$ T cell responses to HIV$_{consv}$ and GRIN sub-matrices

The majority of individuals tested exhibited ELISpot responses against GRIN pools (Figure 31). PBMC from a subset of VC (n=9) and VP (n=9) collected at V1.0 were compared using both HIV$_{consv}$ and GRIN matrices to identify responding epitopes (Figure 33). Even though higher numbers of VC (89%) responded to GRIN matrices than VP (67%), no difference (all $p>0.05$; 2-tailed Fisher's exact test) was observed between the proportion of responders (VC and VP) for each responding GRIN region Gag, RT and INT (Figure 33a). Although the proportion of responders to HIV$_{consv}$ (VC (44%) and VP (33%)) was not similar to GRIN (VC (89%) and VP (67%)), no significant difference ($p>0.05$) was observed between the proportion of GRIN and HIV$_{consv}$ responders at this early time point. HIV$_{consv}$ INT and GRIN Gag were the most commonly recognised regions at V1.0 with these two study groups (Figure 33 b & c). These data showed some differences between GRIN and HIV$_{consv}$ responses in terms of breadth and magnitude. The median number of GRIN peptide responses was 7 (IQR 4-7) for VC and 1 (0-3) for VP, whereas the median number of HIV$_{consv}$ peptides was 0 (0-2) and 0 (0-1) for VC and VP, respectively. Thus, there was a difference ($p<0.05$; Wilcoxon signed rank test) between the number of GRIN and HIV$_{consv}$ responding peptides for VC, but not for VP ($p<0.05$). In addition to this, there was no difference in the magnitude of responses between the two groups against both sub-matrices ($p>0.05$).

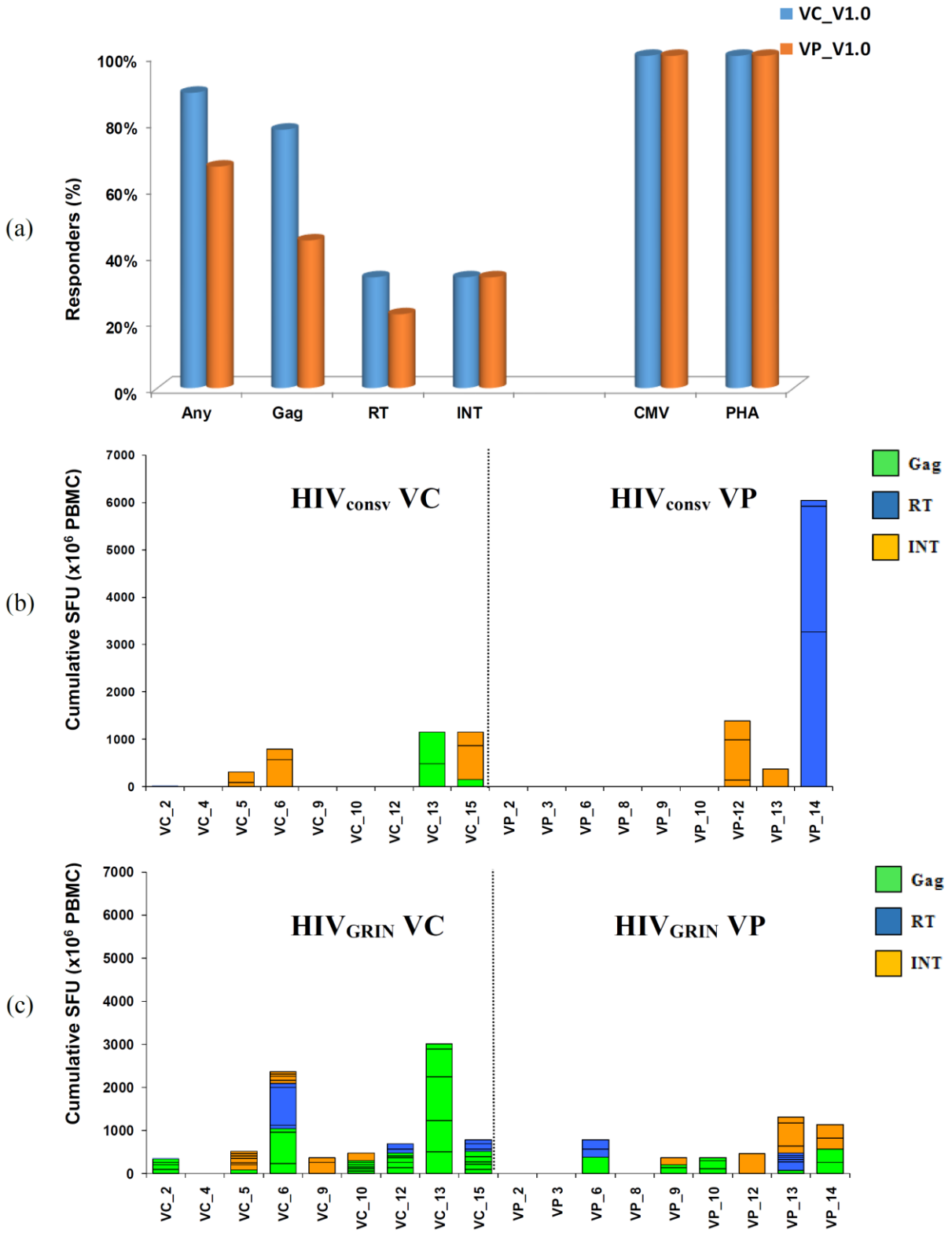


Figure 33. VC and VP responses to GRIN matrices at V1.0

IFN-γ ELISpot responses to GRIN matrices (a) Proportion of responders represented by blue (VC n=9) and red (VP n=9) bars at V1.0 to different GRIN regions (b) Responses to HIV_{consv} regions at V1.0 illustrated by stack bars showing the number of responding peptides and the total magnitude (c) Responses to GRIN sub-matrices at V1.0; each stack bar represents the number of different peptides deconvoluted and the totality of their magnitude.

5.2.5 T cell epitopes identified in the HIV data base in acute and chronic HIV-1 infection using HIV_{consv} peptide pools

HIV_{consv} antigen is based on the 14 most conserved regions of HIV-1 (Letourneau et al., 2007) and HIV_{consv} Gag starts at HXB2 position $Gag_{159-440}$, RT starts at $Pol_{194-586}$ and INT starts at HXB2 position $Pol_{776-996}$. HIV_{consv} responses were spread across the whole of these regions, but largely fell into a few clusters that were recognised by individuals infected with different subtypes, while other regions were apparently non-antigenic. Where an HLA matched epitope was found in the HIV db it was considered as a "probable/best defined epitopes (BDE)" whereas an epitope that was not HLA matched but potential to bind to an HLA allele it was described as "possible/putative" (Table 17). Overall, responding epitopes for each region were classified for this study as either conserved (entropy score <0.2) or variable (entropy score >0.2) calculated using an entropy calculator (Section 2.13).

Table 17. Optimally defined and putative HIV_{consv} epitopes identified in HIV data base restricted by HLA alleles.

					Best defined epitopes		Potential epitope		Conserved or Variable
VID[a]	Visit	Protein	Sub-Protein	Peptide	Epitope	HLA-restriction	Epitope	HLA-restriction	
VC_3	V12	$Pol_{900-909}$	INT	165-166	FKRKGGIGGY	B*1503			Conserved
VC_3	V12	$Pol_{978-986}$	INT	176-177	RKAKIIRDY	B*1503			Variable
VC_5	V1.0	$Pol_{975-983}$	INT	175-177	VPRRKVKII	B*4201			Conserved
VC_6	V1.0	$Pol_{978-986}$	INT	176-177	RKAKIIRDY	B*1503			Variable
VC_6	V12	$Pol_{900-909}$	INT	165	FKRKGGIGGY	B*1503			Conserved
VC_6	V12	$Pol_{978-986}$	INT	176-177	RKAKIIRDY	B*1503			Variable
VC_7	V12	$Pol_{297-305}$	RT	88			IRYQYNVLP	B*7301	Conserved
VC_8	V1.0	$Pol_{283-290}$	RT	80-81	TAFTIPSI	B*5101			Conserved
VC_8	V1.0	$Pol_{978-986}$	INT	176-177	RKAKIIRDY	B*1503			Variable
VC_8	V12	$Pol_{104-112}$	Protease	35			GGIGGFIKV	B*5101	Conserved
VC_8	V12	$Pol_{283-290}$	RT	80-81	TAFTIPSI	B*5101			Conserved
VC_8	V12	$Pol_{978-986}$	INT	176-177	RKAKIIRDY	B*1503			Variable
VC_9	V12	Pol_{158_167}	RT	49-50	SPIETVPVKL	B*8101			Conserved
VC_13	V1.0	$Gag_{147-155}$	p24	5-6	TPQDLNTM	B*8101			Conserved
VC_13	V12	$Gag_{147-155}$	p24	5-6	TPQDLNTM	B*8101			Conserved
VC_13	V12	Pol_{158_167}	RT	49-50	SPIETVPVKL	B*8101			Conserved
VC_14	V1.0	$Pol_{811-819}$	INT	155	ETAYFILKL	A*6802			Conserved
VC_15	V1.0	$Gag_{265-279}$	p24	16	None[b]				

VC_15	V1.0	$Pol_{981-990}$	INT	177-178	KIIKDYGKQM	B*4201			Conserved
VP_7	V1.0	$Gag_{269-277}$	p24	16			GLNKIVRMY	B*4403	Conserved
VP_8	V12	$Gag_{277-285}$	p24	18-19	YSPVSILDI	Cw*0102			Conserved
VP_9	V12	$Pol_{900-909}$	INT	165-166	FKRKGGIGGY	B*1503			Conserved
VP_12	V1.0	$Pol_{900-909}$	INT	166	FKRKGGIGGY	B*1503			Conserved
VP_12	V1.0	$Pol_{978-986}$	INT	176-177	RKAKIIRDY	B*1503			Conserved
VP_12	V12	$Pol_{900-909}$	INT	165-166	FKRKGGIGGY	B*1503			Conserved
VP_12	V12	$Pol_{978-986}$	INT	176-177	RKAKIIRDY	B*1503			Conserved
VP_13	V1.0	$Pol_{888-896}$	INT	163	KTAVQMAVF	B*5801			Conserved
VP_13	V12	$Pol_{888-896}$	INT	163	KTAVQMAVF	B*5801			Conserved
CP_2	V12	$Pol_{292-301}$	RT	82-83	NETPGIRYQY	B*18			Conserved
CP_2	V12	$Pol_{330-338}$	RT	91-93	NPEIVIYQY	B*18			Variable
CP_2	V12	$Pol_{811-819}$	INT	155	ETAYFILKL	A*6802			Conserved
CP_2	V24	$Gag_{433-440}$	p2p7p1p6	31-32	FLGKIWPS	A*0201			Conserved
CP_2	V24	$Pol_{292-301}$	RT	82-83	NETPGIRYQY	B*18			Conserved
CP_2	V24	$Pol_{330-338}$	RT	91-93	NPEIVIYQY	B*18			Variable
CP_2	V24	$Pol_{811-819}$	INT	155	ETAYFILKL	A*6802			Conserved
CP_3	V12	$Pol_{271-279}$	RT	77-78	FSVPLDEGF	B*5703			Variable
CP_3	V24	$Gag_{161-173}$	p24	1	KAFSPEVIPMF	B*5703			Conserved
CP_3	V24	$Pol_{271-279}$	RT	77-78	FSVPLDEGF	B*5703			Variable
CP_4	V12	$Gag_{180-187}$	p24	5-6	TPQDLNTM	B*8101			Conserved
CP_4	V12	Pol_{158_167}	RT	49-50	SPIETVPVKL	B*8101			Conserved

CP_4	V12	$Pol_{263-273}$	RT	76	VLDVGDAYFSV	A*0201			Conserved
CP_4	V12	$Pol_{268-275}$	RT	76	DAYFSVPL	B*5101			Conserved
CP_4	V12	$Pol_{283-290}$	RT	80-81	TAFTIPSI	B*5101			Conserved
CP_4	V24	$Gag_{180-187}$	p24	5-6	TPQDLNTM	B*8101			Conserved
CP_4	V24	$Gag_{197-205}$	p24	10			AMQMLKDTI	B*5101	Conserved
CP_4	V24	$Gag_{433-440}$	p2p7p1p6	32	FLGKIWPS	A*0201			Conserved
CP_4	V24	Pol_{158_167}	RT	49-50	SPIETVPVKL	B*8101			Conserved
CP_4	V24	$Pol_{246-260}$	RT	72	None[b]				
CP_4	V24	$Pol_{268-275}$	RT	76	DAYFSVPL	B*5101			Conserved
CP_4	V24	$Pol_{283-290}$	RT	80-81	TAFTIPSI	B*5101			Conserved
CP_7	V12	$Pol_{811-819}$	INT	155	ETAYFILKL	A*6802			Conserved
CP_7	V24	$Gag_{147-155}$	p24	5-6	TPQDLNTML	B*0702			Conserved
CP_7	V24	$Pol_{311-319}$	RT	88	SPAIFQSSM	B*0702			Conserved
CP_7	V24	$Pol_{811-819}$	INT	155	ETAYFILKL	A*6802			Conserved
CP_8	V12	$Gag_{161-173}$	p24	1-2	KAFSPEVIPMF	B*5703			Conserved
CP_8	V24	$Gag_{161-173}$	p24	1-2	KAFSPEVIPMF	B*5703			Conserved
CP_8	V24	$Pol_{271-279}$	RT	77	FSVPLDEGF	B*5703			Conserved
CP_8	V24	$Pol_{781-789}$	INT	147	THLEGKVIL	B*1510			Conserved
CP_9	V12	$Gag_{167-175}$	p24	2-3	EVIPMFTAL	A*2601			Conserved
CP_9	V12	$Pol_{311-319}$	RT	87-88	SPAIFQSSM	B*0702			Conserved
CP_9	V12	$Pol_{900-909}$	INT	165-166	FKRKGGIGGY	B*1503			Conserved
CP_9	V24	$Gag_{167-175}$	p24	2-3	EVIPMFTAL	A*2601			Conserved

CP_9	V24	$Pol_{311-319}$	RT	87-88	SPAIFQSSM	B*0702			Conserved
CP_9	V24	$Pol_{900-909}$	INT	165-166	FKRKGGIGGY	B*1503			Conserved
CP_11	V12	$Pol_{330-338}$	RT	91-93			NPEIVIYQY	B*5301	Conserved
CP_11	V12	$Pol_{966-980}$	INT	174	None[b]				
CP_11	V24	$Pol_{330-338}$	RT	92-93			NPEIVIYQY	B*5301	Conserved
CP_11	V24	$Pol_{966-980}$	INT	174	None[b]				
CP_12	V12	$Pol_{900-909}$	INT	166	FKRKGGIGGY	B*1503			Conserved
CP_12	V12	$Pol_{978-986}$	INT	176-177	RKAKIIRDY	B*1503			Conserved
CP_12	V24	$Pol_{900-909}$	INT	166	FKRKGGIGGY	B*1503			Conserved
CP_12	V24	$Pol_{978-986}$	INT	176	RKAKIIRDY	B*1503			Conserved
CP_13	V24	$Gag_{183-191}$	p24	5-6	DLNTMLNTV	B*1402			Variable
CP_13	V24	$Pol_{173-181}$	RT	55	GPKVKQWPL	B*0801			Conserved
CP_15	V24	$Gag_{190-168}$	p24	1			EEKAFSPEV	B*0801	Conserved
CP_15	V24	$Pol_{325-333}$	RT	91-92			PFRAQNPEI	B*0801	Variable
CP_15	V24	$Pol_{888-902}$	INT	163	None[b]				
CP_16	V12	$Pol_{562-571}$	RT	115-116	QATWIPEWEF	B*5702			Conserved
CP_16	V24	$Pol_{562-571}$	RT	115-116	QATWIPEWEF	B*5702			Conserved
CP_17	V12	$Pol_{978-986}$	INT	176-177	RKAKIIRDY	B*1503			Variable
CP_17	V24	$Pol_{978-986}$	INT	177	RKAKIIRDY	B*1503			Variable
CP_18	V12	$Pol_{147-155}$	Protease	47	TQIGCTLNF	B*1503			Conserved
CP_18	V12	$Pol_{888-906}$	INT	163-164	None[b]				
CP_18	V12	$Pol_{900-909}$	INT	166	FKRKGGIGGY	B*1503			Conserved

CP_18	V12	$Pol_{962-976}$	INT	173	None		
CP_18	V12	$Pol_{978-986}$	INT	176	RKAKIIRDY	B*1503	Variable
CP_18	V24	$Pol_{147-155}$	Protease	47	TQIGCTLNF	B*1503	Conserved
CP_18	V24	$Pol_{900-909}$	INT	163-166	FKRKGGIGGY	B*1503	Conserved
CP_18	V24	$Pol_{962-980}$	INT	173-174	None[b]		
CP_18	V24	$Pol_{978-986}$	INT	176	RKAKIIRDY	B*1503	Variable
CP_19	V24	$Pol_{978-986}$	INT	176-177	RKAKIIRDY	B*1503	Variable
CP_19	V24	$Pol_{978-986}$	INT	176-177	RKAKIIRDY	B*1503	Variable

NOTE. [a]VID Volunteer Identity, [b]Epitopes not identified in HIV db

These data indicate that HIV-1 infected individuals either responded to the same or different epitopes during the course of disease when tested at different visits (V1 and V12 or V12 and V24). Responding epitopes are presented in Table 17. VC and CP had responses to both conserved and variable epitopes (based on the entropy scores) of HIV_{consv} antigen, whereas VP had responses to conserved epitopes only (Figure 34). There was a slight apparent increase in the number of epitopes at the later time point compared with the early time point for all three groups, but this difference was not significant ($p>0.05$; Fisher exact test). For all three groups responding epitopes were to Gag, RT and INT.

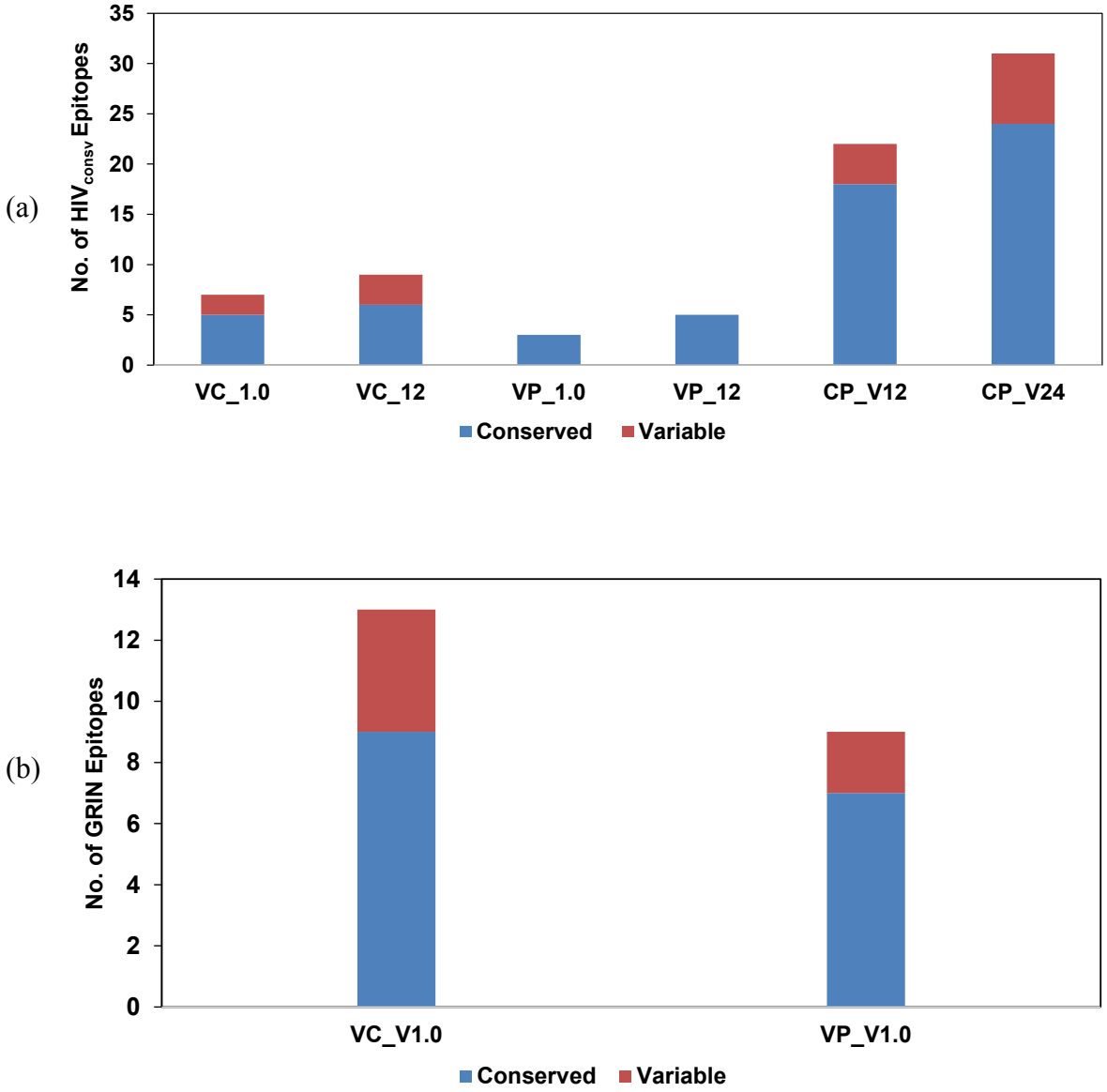


Figure 34. Number of conserved and variable best defined epitopes in HIV_{consv} and GRIN antigen

Stack bar representing the total number of epitopes elicited by VC, VP and CP during the course of infection against HIV_{consv} regions. Each stack bar shows conserved (blue bar) and variable (red bar) epitopes and the total number of epitopes identified against (a) HIV_{consv} and (b) GRIN antigen. X-axis shows different study groups (VC, VP and CP) followed by underscore and the visit number. Y-axis represents the number of different BDE.

5.2.5.1 Gag Epitopes

Of the 12 individuals (VC, VP and CP) for which some degree of Gag antigenicity was apparent, a total of nine different p24 epitopes were recognised within five antigenic clusters encompassing peptides 1-3 ($Gag_{159-180}$), 5-6 ($Gag_{175-193}$), 10 ($Gag_{195-214}$), 15-19 ($Gag_{261-285}$) and 31-32 ($Gag_{433-440}$). Of those, six epitopes were best defined and three were putative (Table 17).

Four individuals responded to p24 epitopes within $Gag_{159-180}$, CP_3 (V24), CP_8 (V12 & V24) and CP_9 (V12 and V24) responded to epitope KAFSPEVIPMF (KF11) restricted through B*5703. One individual CP_15 possibly responded to epitope EEKAFSPEV restricted through B*0801 (Table 17).

Four individuals responded to epitopes within $Gag_{180-187}$. Individuals, VC_13 (V1.0 & V12), and CP_4 and CP_7 (both V12 & V24) responded to the immunodominant p24 epitope TPQDLNTML (TL9) restricted through B*8101, commonly recognised by African subjects (Goulder et al., 2000), while individual CP_13 (V24) responded to p24 epitope DLNTMLNTV restricted by HLA B*1402 (Table 17).

One individual CP_4 (V24) possibly responded to p24 epitope within $Gag_{197-205}$ AMQMLKDTI restricted through B*5101.

Two individuals responded to epitopes within $Gag_{433-440}$. Individuals CP_2 (V24) and CP_4 (V24) both responded to p2p7p1p6 epitope FLGKIWPS restricted through A*0201 (Table 17).

5.2.5.2 Pol (Protease, RT and INT) Epitopes

(a) Protease: Of the two individuals (VC and CP) for which some degree of Pol antigenicity was apparent, a total of two different protease epitopes were recognised within two antigenic clusters encompassing peptides 35 ($Pol_{104-112}$) and 47 ($Pol_{147-155}$). One individual VC_8 (V12) possibly responded to epitope GGIGGFIKV (B*5101) and the other individual CP_18 (V12 & V24) responded to epitope TQIGCTLNF (B*1503) (Table 17).

(b) RT: Of the 14 individuals (VC and CP) for which some degree of Pol antigenicity was apparent, a total of 10 different RT epitopes were recognised within four antigenic clusters encompassing peptides 49-55 ($Pol_{158-181}$), 76-81 ($Pol_{263-290}$), 82-93 ($Pol_{292-338}$) and 115-116 ($Pol_{562-571}$). Four individuals responded to epitopes within $Pol_{158-188}$. One individual CP_13 (V24) responded to epitope GPKVKQWPL (B*0801) and three individuals VC_9 (V12), VC_13 (V12) and CP_4 (V12 & V24) responded to epitope SPIETVPVKL (B*8101) (Table 17).

Four individuals responded to epitopes within $Pol_{271-290}$. One individual CP_4 (V12) responded to epitope VLDVGDAYFSV restricted through A*0201 and also responded to another epitope DAYFSVPL (V12 & V24), within the same peptide at V12, restricted through B*5101. Two individuals CP_3 (V12 & V24) and CP_8 (V24) responded to epitope FSVPLDEGF restricted through B*5703. VC_8 (V1 & V12) and CP_4 (V12 & V24) responded to epitope TAFTIPSI restricted through the same HLA allele B*5101 (Table 17).

Six individuals responded to epitopes within $Pol_{292-338}$. One individual CP_2 (V12 & V24) responded to epitopes NETPGIRYQY and NPEIVIYQY both restricted through B*18 allele whereas, two individuals CP_7 (V24) and CP_9 (V12 & V24) both responded to an epitope SPAIFQSSM restricted through the same allele B*0702. One individual possibly responded to an epitope IRYQYNVLP restricted through B*7301whereas, individuals CP_11 (V12 & V24) and CP_15 (V24) possibly responded to an epitope NPEIVIYQY (B*5301) and PFRAQNPEI (B*0801), respectively.

CP_16 (V12 & V24) responded to an epitope QATWIPEWEF restricted through B*5702 within $Pol_{562-571}$ (Table 17).

(c) INT: Of the 14 individuals (VC, VP and CP) for which some degree of Pol antigenicity was apparent, a total of 7 different INT epitopes were recognised within four antigenic clusters encompassing peptides 147 ($Pol_{781-789}$), 155 ($Pol_{811-819}$), 163-166 ($Pol_{888-906}$) and 173-178 ($Pol_{962-990}$) (Table 17).

CP_8 (V24) responded to an epitope THLEGKVIL restricted through B*1510 within $Pol_{781-789}$.

Three individuals, VC_14 (V1.0), CP_2 (V12 & V24) and CP_7 (V12 and V24) responded to an epitope ETAYFILKL restricted through A*6802 within $Pol_{811-819}$.

Eight individuals responded to epitopes within $Pol_{888-906}$. VP_13 (V1.0 & V12) responded to an epitope KTAVQMAVF restricted through B*5801. VP_3 (V12), VC_6 (V12), VP_9 (V12), VP_12 (V1.0 & V12), CP_9 (V12 & V24), CP_12 (V12 & V24) and CP_18 (V12 & V24), all responded to an epitope FY10 restricted through the same HLA allele B*1503 (Table 17).

Ten individuals responded to epitopes within $Pol_{962-990}$. CP_5 (V1.0) responded to an epitope VPRRKVKII restricted through B*4201. VC_3 (V12), VC_6 (V1.0 & V12), VC_8 (V1.0 & V12), VP_12 (V1.0 & V12), CP_12 (V12 & V24), CP_17 (V12 & V24), CP_18 (V12 & V24)

and CP_19 (V12 & V24) all responded to an epitope RKAKIIRDY restricted through B*1503. One individual VC_15 (V1.0) responded to an epitope KIIKDYGKQM restricted through B*4201 (Table 17).

5.2.6 T cell epitopes identified in the HIV db in acute HIV-1 infection using whole GRIN matrices

VC (n=9) and VP (n=9) were tested with GRIN peptides and these individuals had ELISpot responses to both conserved and variable epitopes (Table 18 and Figure 34). Both VC and VP responded to a similar number of GRIN epitopes (p>0.05; 2-tailed Fisher exact test). In total for VC, 46% and 23 % of Gag responses were to conserved and variable BDE, respectively. Furthermore, 8% of VC RT responses and 23% INT responses were to variable and conserved BDE, respectively. Comparatively, VP Gag BDE were to both conserved (46%) and variable (23%) regions, whereas no RT (0%) BDE were identified in the HIV db, while INT (44%) responses were only to conserved BDE.

5.2.6.1 Gag

Of the 11 individuals (VC and VP) for which some degree of Gag antigenicity was apparent, a total of 14 epitopes were recognised within three antigenic clusters encompassing peptide(s) 4-6 (Gag_{20-36}), 37-50 ($Gag_{147-212}$) and 60-78 ($Gag_{240-320}$). Of those, 11 epitopes were best defined and three were putative (Table 18).

Three individuals responded to p17 epitopes within Gag_{20-36}. VC_6 (V1.0) and VC_13 (V1.0) responded to an epitope RLRPGGKKK restricted through the same allele A*0301, whereas one individual VC_9 (V1.0) responded to an epitope HYMLKHIVW restricted through A*2301 (Table 18).

Four individuals responded to p24 epitopes within $Gag_{147-212}$. VC_12 (V1.0) responded to epitopes ISPRTLNAW and KF11 both restricted through the same allele B*5703. VC_2 (V1.0) responded to epitopes KF11 and ETINEEAAEW restricted through B*5703 and B*58, respectively. VC_13 (V1.0) responded to an epitope TL9 restricted through B*8101, while VC_5 (V1.0) possibly responded to an epitope IVGGHQAAM restricted through A*3001 (Table 18).

Six individuals responded to p24 epitopes within $Gag_{240-320}$. VC_2 (V1.0) responded to epitope TW10 restricted through B*5703, while VC_12 (V1.0) responded to epitope IILGLNKIVR restricted through A*03. VC_15 (V1.0) possibly responded to an epitope GLNKIVRMY restricted through A*3001, while VP_6 (V1.0) and VP_10 (V1.0) potentially responded to an epitope RDYVDRFYKTL restricted through B*18 and B*1510, respectively. VP_14 (V1.0) responded to an

epitope YVDRFFKTL restricted through B*1510. VP_10 (V1.0) also responded to an epitope DVKGWMTET restricted through B*3501, while VP_13 possibly responded to an epitope QATQDVKNW restricted through B*5801 (Table 18).

5.2.6.2 Pol (RT and INT)

RT

Of the four individuals for which some degree of Pol antigenicity was apparent, a total of seven RT epitopes were recognised within three antigenic clusters encompassing peptide(s) 29-31 ($Pol_{274-279}$), 58-69 ($Pol_{381-432}$) and 95 ($Pol_{530-538}$). Of those, one epitope was best defined and five were putative (Table 18).

Two individuals responded to epitopes within ($Pol_{274-279}$). VC_6 (V1.0) and VC_15 (V1.0) possibly responded to an epitope PLDENFRKY restricted through A*0301, while VC_12 (V1.0) potentially responded to an epitope FSVPLDEGF restricted through B*5703.

Two individuals responded to epitopes within ($Pol_{381-432}$). VC_6 (V1.0) possibly responded to epitopes QIYPGIKVR and PFLWMGYEL restricted through A*0301 and A*3001, respectively, while VC_15 (V1.0) possibly further responded to an epitope YPGIKVRQL restricted through B*4201.

One individual VP_13 (V1.0) possibly responded to an epitope IAMESIVIW restricted through B*5801 within $Pol_{530-538}$ (Table 18).

INT

Of the seven individuals for which some degree of Pol antigenicity was apparent, a total of eight different INT epitopes were recognised within four antigenic clusters encompassing peptide(s) 19 ($Pol_{669-677}$), 36-56 ($Pol_{743-826}$), 60-78 ($Pol_{838-927}$) and 95 ($Pol_{975-983}$). Of those, 5 epitopes were best defined and three were putative (Table 18).

One individual VC_5 (V1.0) possibly responded to an epitope ESELVNQII restricted through B*1516 within $Pol_{669-677}$ (Table 18).

Four individuals responded to epitopes within $Pol_{743-826}$. Two individuals VC_5 (1.0) and VC_9 (V1.0) responded to an epitope LPPIVAKEI restricted through B*4201 and B*5101, respectively. VC_6 (V1.0) responded to an epitope ILKLAGRWPVK restricted through A*0301, while VP_6 (V1.0) responded to an epitope THLEGKIIL restricted through B*15.

Four individuals responded to epitopes within $Pol_{838-927}$. VC_5 (V1.0) possibly responded to an epitope GSNFTSAAV restricted through B*1516. Two individuals VP_9 (V1.0) and VP_12 (V1.0) responded to an epitope FKRKGGIGGY restricted through B*1503, while VP_13 (V1.0) responded to an epitope KTAVQMAVF restricted through B*5801 (Table 18).

Table 18. Optimally defined and putative GRIN epitopes identified in HIV db restricted by HLA alleles

					Best defined epitopes		Potential epitope		Conserved or Variable
VID[a]	Visit	Protein	Sub-Protein	Peptide	Epitope	HLA-restriction	Epitope	HLA-restriction	
VC-2	V1.0	Gag_{161_173}	p24	40-41	KAFSPEVIPMF	B*5703			Conserved
VC-2	V1.0	$Gag_{203-212}$	p24	50	ETINEEAAEW	B*58			Conserved
VC-2	V1.0	$Gag_{240-249}$	p24	60	TSTLQEQIAW	B*5703			Variable
VC_5	V1.0	$Gag_{190-198}$	p24	48			IVGGHQAAM	A*3001	Conserved
VC_5	V1.0	$Pol_{669-677}$	RT	19			ESELVNQII	B*1516	Conserved
VC_5	V1.0	$Pol_{743-751}$	INT	36-37	LPPIVAKEI	B*4201			Conserved
VC_5	V1.0	$Pol_{833-841}$	INT	60			GSNFTSAAV	B*1516	Variable
VC_5	V1.0	$Pol_{897-991}$	INT	76	None[b]		None		
VC_5	V1.0	$Pol_{913-927}$	INT	78	None		None		
VC_6	V1.0	Gag_{20-28}	p17	4-5	RLRPGGKKK	A*0301			Variable
VC_6	V1.0	$Gag_{210-225}$	p24	53	None[b]		None		
VC_6	V1.0	$Pol_{274-282}$	RT	31			PLDENFRKY	A*0301	Variable
VC_6	V1.0	$Pol_{424-432}$	RT	68-69			QIYPGIKVR	A*0301	Variable
VC_6	V1.0	$Pol_{816-826}$	INT	55-56	ILKLAGRWPVK	A*0301			Conserved
VC_9	V1.0	$Pol_{743-751}$	INT	36-37	LPPIVAKEI	B*5101			Conserved
VC_10	V1.0	$Pol_{833-847}$	INT	60	None[b]		None		
VC_12	V1.0	$Gag_{147-155}$	p24	37	ISPRTLNAW	B*5703			Conserved
VC_12	V1.0	Gag_{161_173}	p24	40-41	KAFSPEVIPMF	B*5703			Conserved
VC_12	V1.0	$Gag_{266-275}$	p24	66	IILGLNKIVR	A*03			Conserved
VC_12	V1.0	$Pol_{271-279}$	RT	29-30	FSVPLDEGF	B*5703			Variable

VID[a]	Visit	Protein	Sub-Protein	Peptide	Best defined epitopes		Potential epitope		Conserved or Variable
					Epitope	HLA-restriction	Epitope	HLA-restriction	
VC_13	V1.0	Gag_{20-28}	p17	4-5	RLRPGGKKK	A*0301			Variable
VC_13	V1.0	$Gag_{180-187}$	p24	44-46	TPQDLNTML	B*8101			Conserved
VC_15	V1.0	$Gag_{122-136}$	p17	31	None[b]				
VC_15	V1.0	$Gag_{142-156}$	p24	36	None[b]				
VC_15	V1.0	$Gag_{269-277}$	p24	66-67			GLNKIVRMY	A*3001	Conserved
VC_15	V1.0	$Pol_{381-389}$	RT	58			PFLWMGYEL	A*3001	Conserved
VC_15	V1.0	$Pol_{426-434}$	RT	68-69			YPGIKVRQL	B*4201	Variable
VP_6	V1.0	$Gag_{294-304}$	p24	74	RDYVDRFYKTL	B*18			Conserved
VP_6	V1.0	$Pol_{781-789}$	INT	46-47	THLEGKIIL	B*15			Conserved
VP_9	V1.0	Gag_{28-36}	p17	5-6	HYMLKHIVW	A*2301			Variable
VP_9	V1.0	$Pol_{900-909}$	INT	76	FKRKGGIGGY	B*1503			Conserved
VP_10	V1.0	$Gag_{294-304}$	p24	73-74	RDYVDRFYKTL	B*1510			Conserved
VP_10	V1.0	$Gag_{312-320}$	p24	78	DVKGWMTET	B*3501			Variable
VP_12	V1.0	$Pol_{900-909}$	INT	76	FKRKGGIGGY	B*1503			Conserved
VP_13	V1.0	$Gag_{308-316}$	p24	76			QATQDVKNW	B*5801	Variable
VP_13	V1.0	$Pol_{530-538}$	RT	95			IAMESIVIW	B*5801	Variable
VP_13	V1.0	$Pol_{888-896}$	INT	73-74	KTAVQMAVF	B*5801			Conserved
VP_13	V1.0	$Pol_{975-983}$	INT	95			VPRRKAKIL	B*3501	Conserved
VP_14	V1.0	$Gag_{296-304}$	p24	73-74	YVDRFFKTL	B*1510			Conserved

NOTE. [a]VID Volunteer Identity, [b]Epitopes not identified in HIV db

5.3 Evaluation of polyfunctional responses of HIV-1 specific $CD8^+$ T cells

To further investigate if there were potential functional differences between HIV-1 specific $CD8^+$ T cell responses at an early time point in VC (n=8) and VP (n=6), individuals with IFN-γ ELISpot responses of >100 SFU/10^6 cells were characterised by intra-cellular cytokine staining analysis to examine cytokine profiles. $CD8^+$ T cells are capable of a number of functions such as cytolysis and up-regulation of cytokines and chemokines. Thus, it was important to measure multiple functions to understand the quality of T cell responses. PBMC were stimulated with optimally mapped HIV_{consv} and GRIN peptides and were examined by intra-cellular cytokine staining (ICS) to measure polyfunctional responses.

Both VC and VP were found to express similar levels of IFN-γ, TNF-α, IL-2, perforin, and CD107a following peptide stimulation (Figure 35). After background subtraction, the geometric mean percentage of HIV-1 peptide specific $CD8^+$ T-cells generating CD107a was 0.45% (0.35-0.58) and 0.59% (0.39-0.88) in VC and VP respectively (p>0.05; Student's T test). The geometric mean values in VC and VP for IFN-γ were 0.4% (0.3-0.53) and 0.59% (0.45-0.78) (p>0.05). For IL-2 geometric mean values were 0.073% (0.057-0.095) and 0.071% (0.059-0.085) (p<0.05); and for TNF-α 0.17% (0.13-0.22) and 0.24% (0.17-0.33) (p>0.05). No difference was observed between the two study cohorts in the percentage of peptide specific $CD8^+$ T cells generating IFN-γ, TNF-α and CD107a, whereas a higher percentage of IL-2 (p<0.05) and perforin (p<0.05) producing $CD8^+$ T cells were seen in VC than in VP (Figure 35).

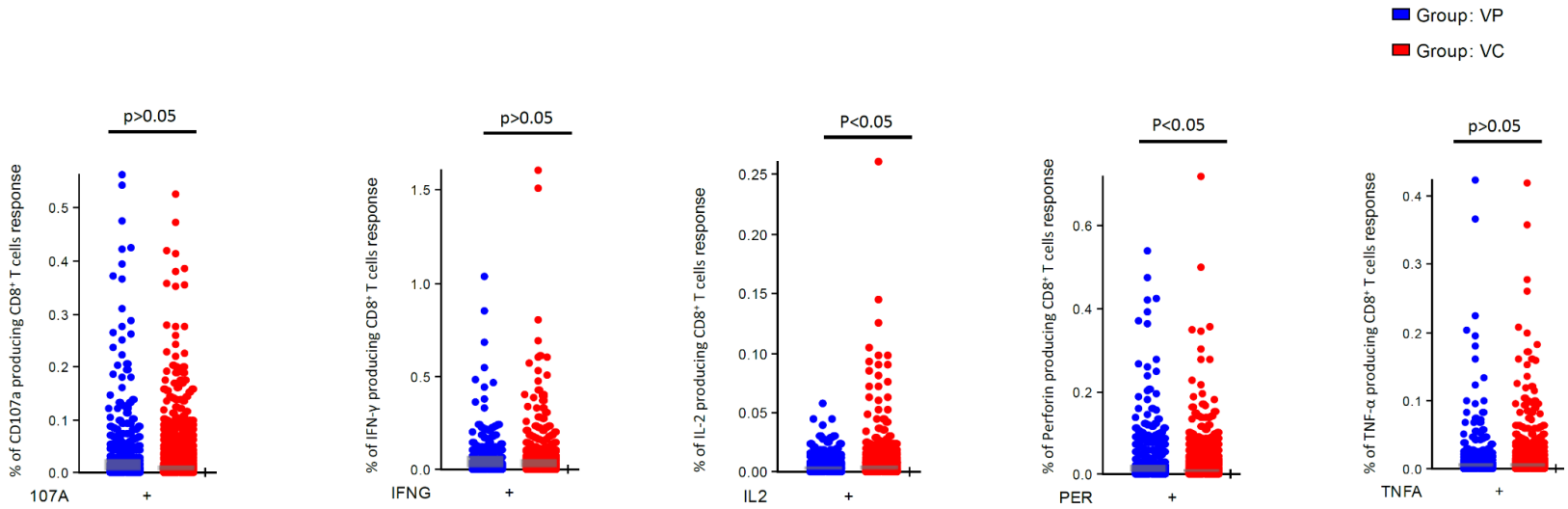


Figure 35. Cytokine profile of CD8$^+$ T cells mediating different function

The percentage of CD8$^+$ T cell response producing CD107A, IFN-γ, IL-2, Perforin (Per) and TNF-α was assessed in 8 VC and 6VP and is shown as single function. Medians are shown as grey bars and differences between two groups were evaluated by Student's T Test marked by *p* value.

A Boolean gating approach generated 32 different possible response patterns by consolidating five different functional responses (IFN-γ, TNF-α, IL-2, Perforin, and CD107a). The data is represented as the percentage of total HIV-1 specific $CD8^+$ T cell response (Figure 36). As shown in Figure 36 HIV-1 specific $CD8^+$ T cells secrete more than one cytokine simultaneously and polyfunctional $CD8^+$ T cell populations were observed in both study cohorts. There were quadruple and triple positive populations observed in VC and VP (Figure 36). Significant differences were seen (although not for all combinations) in cells secreting more than one cytokine in VC with cells simultaneously expressing CD107a, IFN-γ, IL-2 and TNF-α in VC and VP ($p<0.05$) and also cells expressing CD107a, IFN-γ, perforin and TNF-α ($p<0.05$). Similarly, some differences were also observed in triple positive populations in VC and VP CD107a, IFN-γ and perforin ($p<0.05$), CD107a, IL-2 and TNF-α ($p<0.05$), CD107a, Perforin and TNF-α ($p<0.05$), IFN-γ, IL-2 and perforin ($p<0.05$) and IFN-γ, IL-2 and TNF-α ($p<0.05$). Furthermore, differences were seen in double [(1) IFN-γ/$IL\text{-}2^{+/+}$, (2) IFN-γ/$TNF\text{-}\alpha^{+/+}$) and single positive population (CD107a, IL-2 and TNF-α) between the two study cohorts ($p<0.05$), respectively.

However, all the other functions were not significantly different between the two study cohorts.

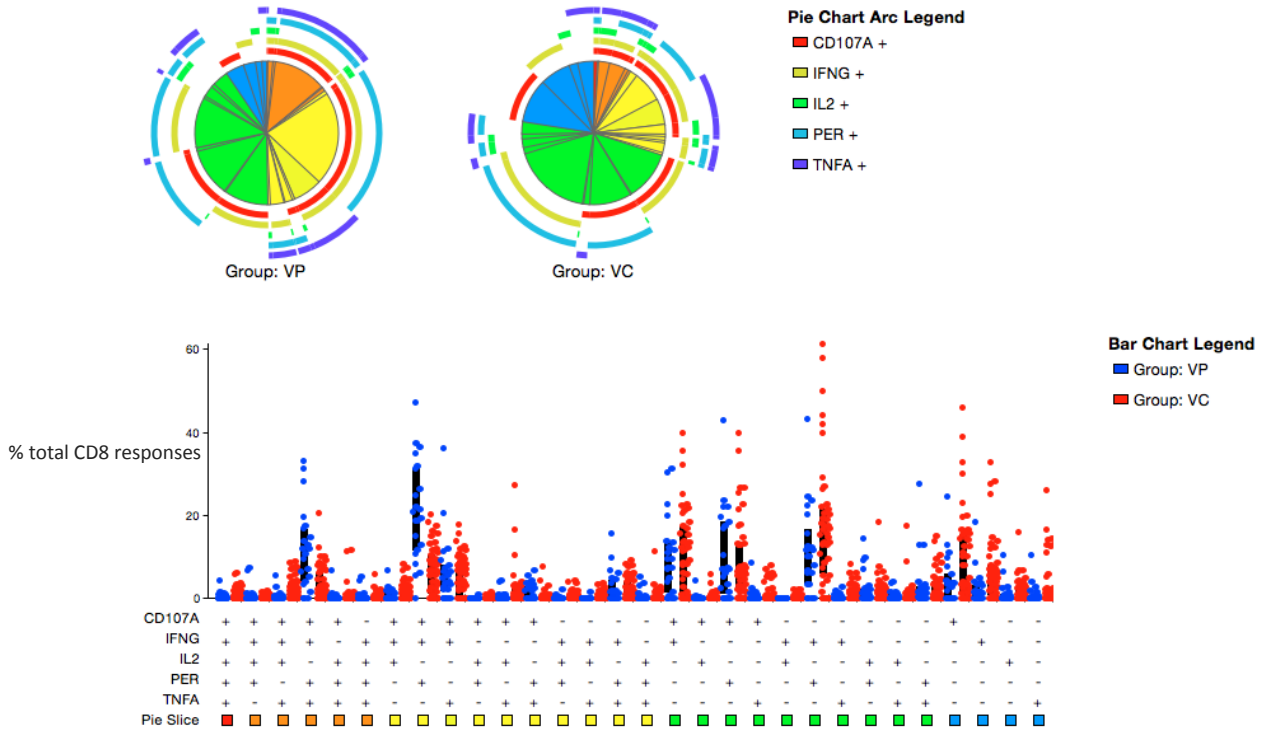


Figure 36. Comparison of polyfunctional profiles of VC vs VP

HIV-1 specific $CD8^+$ T cell responses against responding peptides determined by mapping. The x-axis shows number of different responses marked by a + or - for the presence or absence of CD107A, IFN-γ, IL-2, Perforin (Per) and TNF-α. The percentage of total $CD8^+$ T cell response contributed by two groups of participants and the median and IQR are shown by black bar. Responses are grouped and positive functions are identified by different colours shown in the pie charts. The arc surrounding each pie chart characterises each function as shown in the arc legend and depict cytokine make up within pie slice. Each pie slice represents the number of different functions ranging from 1-5.

5.4 Discussion

It is vital to understand the mechanisms by which elite controllers (EC) control HIV-1 infection, as this potentially provides a means to improve treatment in individuals who are less able to control their viraemia and to inform the design of prophylactic and/or therapeutic vaccines. However, there are a number of different factors associated with control of HIV-1 in EC such as host genetic background, immune response and the virus itself (Cortes et al., 2015). Early immune responses appear to influence HIV disease progression and therefore a comprehensive analysis of HIV-1 specific $CD8^+$ T cell responses in primary HIV-1 infection could inform vaccine design (Ananworanich et al., 2016; Freel et al., 2012; McMichael et al., 2010). It is therefore crucial to understand which HIV-1 specific CD8 T cell responses are associated with the control of viraemia during early infection. Thus, in the current study HIV-1 specific $CD8^+$ T cell responses were investigated during early and late HIV-1 infection using two antigen panels (HIV_{consv} and GRIN).

In this study, three groups of participants (viraemic controller (VC), viraemic progressors (VP) and chronic progressors (CP)) were tested for IFN-γ ELISpot responses at two time points, VC and VP at V1.0, V12 and CP at V12 and V24. In line with previous studies VC were defined as participants maintaining their pVL at low levels, VP were individuals with high pVL and CP were subjects with intermediate levels of pVL (Cortes et al., 2015; Mendoza et al., 2012; Tansiri et al., 2015).

Data generated herein demonstrated that responses against HIV_{consv} Gag, RT and INT regions were common in infected individuals. All three groups of individuals recognised at least one of the HLA Class I-restricted epitopes within the HIV_{consv} matrix. These included individuals from different countries (Rwanda, Uganda and Zambia) infected with different subtypes (A, C and D) and an extensive variety of genetic backgrounds (HLA A1, A2, A3, A23, A26, A30, A68, A74, B7, B8, B15, B18, B35, B42, B45, B51, B53, B57, B58, B81, Cw02, Cw03, Cw04, Cw06, Cw07, Cw08 Cw16, Cw17 and Cw18 and more). The majority of responses were directed against the HIV_{consv} Gag and Pol for all three study groups. During primary HIV-1 infection $CD8^+$ T cell responses to HIV_{consv} regions were somewhat lower (limited breadth) compared to the chronic infection. Data generated in this study was comparable to other studies demonstrated that the magnitude and breadth of $CD8^+$ T cell responses increase over time, but without any further reduction in the viral set point (Radebe et al., 2015; Streeck et al., 2014; Streeck and Nixon, 2010; Turnbull et al., 2009). There may be a number of factors contributing to the T cell responses at later time points such as responses to conserved regions being a function of time, antigen load, exposure to different viral sequences during the course of the infection, peptide/MHC complex and the peptide affinity for class I (Bihl et al., 2006; Ndhlovu et al., 2012).

Responses to GRIN pools (based on subtype A) were consistently more frequent than the HIV_{consv} matrix, such that the majority of individuals generated a response to one or more of the GRIN pools. As GRIN is based on the near full genome of HIV-1 it may inherently offer a broader antigenic matrix to define $CD8^+$ T cell responses in a wider range of HIV-infected individuals than the HIV_{consv} matrix based only on a small proportion (806 amino acid) of the HIV-1 proteome (Letourneau et al., 2007). Not all GRIN responses were able to be mapped to their optimal individual epitopes, so it is unclear at this time what proportion of all non-mapped responses are directed towards conserved or the more variable epitopes. A small number of individuals were mapped with GRIN sub-matrices, and this demonstrated that most of the individuals (VC and VP) exhibited IFN-γ responses to GRIN sub-matrices compared to HIV_{consv} sub-matrices. VC GRIN responses appeared to be of higher breadth and magnitude compared to VP, but this was not statistically significant. A large number of GRIN epitopes were not represented in HIV_{consv} panel and this may account for discrepancies seen between GRIN and HIV_{consv} responders. This further suggests that perhaps responses exhibited against GRIN epitopes may be associated with control of viraemia in VC.

Indeed, there was no association between pVL and $CD8^+$ T cell responses and to conserved regions at V1.0 which is in contrast with a recent study (Ndhlovu et al., 2015a). Measurable $CD8^+$ T cell responses to HIV_{consv} panel did not coincide with decreasing viraemia in most cases for VC. Most of the individuals (VC and VP) did not exhibit $CD8^+$ T cell response to HIV_{consv} matrices, as conserved regions are subdominant in early infection whereas responses to variable regions are immunodominant and therefore were not detected by HIV_{consv} matrices. Thus, it is important to define factors that influence effective $CD8^+$ T cell responses in early infection. The majority of responding VC targeted responses to Pol, suggesting that Pol-specific responses may be better correlated with viral control. However, previous studies have shown that responses to Gag are correlated with reduction in viraemia, but not responses to Pol, Env or accessory proteins (Edlefsen et al., 2013; Gounder et al., 2015; Kiepiela et al., 2007; Novitsky et al., 2003; Radebe et al., 2015; Streeck et al., 2014). It has been demonstrated in previous studies that initial $CD8^+$ T responses measured by IFN-γ ELISpot were low/weak at early infection (Goonetilleke et al., 2009; Liu et al., 2013; Ndhlovu et al., 2015a; Ndhlovu et al., 2012; Radebe et al., 2015), but this does not mean that strong $CD8^+$ T cells responses cannot be detected in AHI. This suggests that control of viraemia in VC may be associated with other factors such as innate immune responses, transmitted virus with low replication capacity and perhaps immune escape from conserved sites may cause large fitness cost to the virus (Batorsky et al., 2014; Goepfert et al., 2008; Martinez-Picado et al., 2006; Nagaraja et al., 2016; Schneidewind et al., 2007) which could be beneficial to the host. Similarly, low proportions of HIV-1 specific $CD8^+$ T cell responses were also observed in VP which suggests that these individuals may be infected with

high replicative capacity viruses driving immune activation which may be a contributing factor to constantly high pVL and impaired CTL function in VP (Claiborne et al., 2015; Mudd and Lederman, 2014; Turnbull et al., 2009; Zhang et al., 2003). A recent study has reported high viral replication capacity of the transmitted virus was responsible for immune dysfunction and disease trajectory in acute infection. It was demonstrated that viral replication capacity in acute infection was associated with decline in CD4 counts which could not be reversed even though infected individuals harboured protective alleles such as B*57 and B*5801 (Claiborne et al., 2015).

It is possible that lack of $CD8^+$ T cell response to HIV_{consv} regions in VC and VP could also be due to escape mutations in the CTL epitopes. However, viral sequencing data from a limited number of patients (Section 4.3.1) demonstrated that HIV_{cosnv} regions were preserved over time. Lack of responses has also been observed for protective conserved epitopes such as KK10 and TW10 and was associated with transmitted escape mutations (Streeck and Nixon, 2010). The absence/lack of IFN-γ production during early infection was in line with previous studies, reporting impaired IFN-γ production in early infection (Ndhlovu et al., 2015a; Ndhlovu et al., 2012; Radebe et al., 2011; Streeck et al., 2009b; Trautmann et al., 2012). Nevertheless, more potent CTL lytic ability has been shown in primary than chronic HIV-1 infection (Freel et al., 2012; Goonetilleke et al., 2009; Trautmann et al., 2012) and in other contained infection (Horton et al., 2004). As minimal HIV-1 specific $CD8^+$ T cells responses were detectable by *ex vivo* ELISpot an alternative approach such as cultured ELISpot could have been employed at the early time point. Thus, the evaluation of HIV-1 specific $CD8^+$ T cells by measuring IFN-γ may underestimate the frequency of antigen specific CD8 T cells responses (Ndhlovu et al., 2012; Radebe et al., 2015). Even though the standard IFN-γ ELISpot assay failed to display any association of $CD8^+$ T cell responses between VC and VP at an early time point as shown in a recent study (Tansiri et al., 2015), robust IFN-γ responses were detected in CP at V12/V24 towards HIV_{consv} regions and appeared to be greater in breadth and magnitude compared to VC and VP (V12). Previous studies have reported that persistent viral replication is required to maintain immune memory. It was demonstrated that during ART, once the antigen stimulation was removed, a drop in HIV specific CTL frequency was observed (Gray et al., 1999; Ortiz et al., 1999; Wodarz et al., 2000). Thus, stable pVL in CP perhaps maintained immune memory which may be associated with cytotoxic ability of T cells as shown in other contained chronic infections such as CMV and EBV (Horton et al., 2004). Another possible explanation could be that the immune system may have seen more viral variants during the course of disease which may have been represented by HIV_{consv} pools.

Based on their HLA type, 24 probable (optimal defined) and 4 possible (putative) HIV_{consv} epitopes were identified in the HIV db for all three groups (VC, VP and CP) at V1.0, V12 and V24,

respectively. In some cases no epitopes were identified in the HIV db. According to the ES the majority of the HIV_{consv} epitopes were categorised as conserved however, a small number of epitopes ES was >0.2 and were categorised as variable in this study (Section 2.13). Epitope(s) responded at an earlier time point were also responded at a later time point, this potentially suggests that the responding epitopes did not escape during the course of infection. Further breakdown of these data identified Gag immunodominant epitopes, such as TL9 (B*8101 and B*0702 restricted) and KF11 (B*5703), that have been previously associated with disease control in infected individuals (Ammaranond et al., 2010; Goulder et al., 2000; Payne et al., 2014). In the present study, TL9 was also recognised by three subjects. Thus, confirming previous findings that TL9 is restricted by a B*8101 and B*0702 (Geldmacher et al., 2009; Goulder et al., 2000). Though, in this study responses to TL9 epitope had no effect in reducing or clearing pVL in chronic infection, regardless of the high magnitude of IFN-γ responses to TL9. Two CP elicited IFN-γ responses to B*5703 conserved epitope KF11 and these responses may be associated with reducing pVL in one of the participants. However, harbouring protective alleles such as B*27 and B*57 does not necessarily control HIV-1 replication (Cortes et al., 2015; Emu et al., 2008). Fifteen (B*1503) subjects exhibited IFN-γ responses to INT epitopes, seven to FY10 and eight subjects to RKAKIIRDY, respectively. In this study, HLA-B*1503 restricted immune responses were both ineffective and common in clade C infected individuals, as reported previously (Frahm et al., 2005; Kiepiela et al., 2007). Indeed, B*1503 restricted $CD8^+$T cell responses are not associated with reduction in pVL and hence, not protective (Frahm et al., 2005).

The majority of the HIV_{consv} RT epitopes were observed in CP (Table 3). Epitopes recognised were DAYFSVPL (B*5101), FSVPLDGEGF (B*5703), SPAIFQSSM (B*0702), TAFTIPIS (B*5101) and QATWIPEWEF (B*5702). Despite an enrichment of protective alleles in specific CP, these individuals did not have favourable outcomes, in agreement with other studies (Cortes et al., 2015; Kloverpris et al., 2012; Migueles et al., 2015; Tang et al., 2011).

17 probable and 11 possible GRIN epitopes were identified in the HIV db for VC and VP at V1.0 and in some cases no epitopes were identification for responses. The majority of the responding GRIN epitopes were conserved and a minority were variable. Volunteers from both VC and VP groups responded to GRIN Gag epitopes TL9 (B*8101), KF11 (B*5703) and FKRKGGIGGY (B*1503). One VC responded to p24 immunodominant epitope TW10 (B*5703) associated with control (Kloverpris et al., 2012; Streeck et al., 2014), however, TW10 was not represented in the HIV_{consv} sequence. The majority of GRIN responders did not produce responses to the homologous HIV_{consv} sequences, even though these sequences were incorporated in GRIN insert. This lack of HIV_{consv} sequence recognition has been discussed previously (See Section 4.3). Overall, as GRIN was based on the near full HIV-1 genome there appeared to be more responses (conserved and variable) than

observed with HIV_{consv}. HIV_{consv} is only comprised of conserved regions (806 aa) of the HIV-1 genome whereas GRIN is based on near full genome. Indeed, autologous peptides would possibly be a better approach to determine the immune responses exhibiting against circulatory virus in natural infection (Altfeld et al., 2003b; Doroudchi et al., 2012)

In this study, no significant difference was seen between the breadth and magnitude of responses in two study groups at V1.0. Consequently, polychromatic ICS analysis was employed to evaluate the functional differences of virus-specific $CD8^+$ T cells between these two groups, as used in other studies (Lichterfeld, 2004; Migueles et al., 2002b; Owen et al., 2010; Riou et al., 2014). High frequency polyfunctional HIV-1 specific $CD8^+$ T cells producing IFN-γ, TNF-α, IL-2 has been associated with reduced pVL and slower disease progression in natural infection (Cellerai et al., 2011; Freel et al., 2012) and has been shown to have an impact on viral set point in early infection (Riou et al., 2014). Here, VC displayed higher levels of IL-2 and perforin compared to VP, following peptide stimulation, suggesting that $CD8^+$ T cell proliferation and perforin expression play an important role in decreasing viraemia. Perhaps the higher level of IL-2 and perforin expression in VC resulted in potent CTL activity and thus, this effector function distinguish these VC from VP and may account for their differing pVLs (Hersperger et al., 2010; Migueles et al., 2002b). $CD8^+$ T cell proliferation and cytotoxic capacity is usually correlated with IFN-γ/IL-2 up-regulation and perforin expression (Cellerai et al., 2011; Hersperger et al., 2010; Migueles et al., 2002a; Younes, 2003). In contrast with previous studies, performed on *ex vivo* antigen specific $CD8^+$ T cells, expression of perforin in CD8s of EC, VC and CP was found to be either low or absent and no noticeable dissimilarities were found in perforin expression among all the study groups (Appay et al., 2000; Migueles et al., 2002b; Zhang et al., 2003). Hersperger et al. have further showed that the expression of CD107a/perforin$^{+/+}$ $CD8^+$ T cells was significantly higher in EC than in non-controllers (Hersperger et al., 2010). Data from this study corroborated that VC co-expressed higher levels of CD107a/perforin$^{+/+}$ than VP. Thus, perforin expression may be associated with reducing pVL in VC. Perforin kills infected cells directly and is considered to be the most powerful mediator of $CD8^+$T cell exhibiting cytotoxicity (Migueles et al., 2002a; Migueles et al., 2008).

Besides differences in cytotoxic abilities of $CD8^+$ T cells, VC appeared to be slightly more polyfunctional than VP as they were able to degranulate and produce multiple cytokines (IFN-γ, TNF-α and IL-2) simultaneously, but $CD8^+$ T cells producing all five functions were rarely observed (Ferrando-Martinez et al., 2012; Riou et al., 2014; Riou et al., 2012). Some clear differences in polyfunctional HIV-1 specific $CD8^+$ T cell responses were observed in both VC and VP. It is not certain if these low level polyfunctional responses have any association with control of viraemia seen here. In acute HIV-1 infection virus replicates rapidly and results in CD4 T helper cells depletion.

Lack of CD4 help prevents the establishment of CD8 memory which may resulted in the weak $CD8^+$ T cell activity observed here (Claiborne et al., 2015; Douek et al., 2002; Kemball et al., 2007; Mudd and Lederman, 2014; Owen et al., 2010; Sun et al., 2004; Turnbull et al., 2009).

To summarise, both VC and VP showed similar patterns of T cell responses to HIV_{consv} sequences in primary infection, whereas responses in chronic infection were more frequent. It was hypothesised that VC may have potent CD8 responses at an early time point compared to VP, which may explain their disease pathogenesis/prognosis as responses to conserved regions could cause fitness cost to the virus. As conserved regions were targeted by VC and VP during different stages of disease suggests that these regions remain conserved for a long time and those regions can be used as a potential vaccine candidate. However, no such differences were seen in cell mediated immune responses between VC and VP in this study. Furthermore, responses to GRIN pools (based on near full genome) were frequently detected among all three groups. This suggests that GRIN pools appeared to be a better tool in order to determine T cell responses in natural infection, because responding pools incorporated both conserved and variable sequences therefore, more PTE to be recognised. In addition to this, when limited number of GRIN responses were deconvoluted then responding GRIN epitopes were mostly conserved based on their ES and only conserved epitopes were able to cross recognise the peptides in the GRIN OLPs. In this study, as conserved epitopes were detected in natural infection during the first two years of disease targeting conserved sequences in a vaccine could be beneficial, as shown in a recent immunogenicity study (Abdul-Jawad et al., 2016; Ahmed et al., 2016). Finally, unavoidable limitations of small sample sizes and PBMC numbers limited the significant differences seen between the three study groups. However, where possible, data were compared between groups and time points; it is clear that anecdotal differences do exist between study groups. Thus, there is need for a longitudinal study to evaluate T cell responses at multiple time points to further investigate if T cell responses correlate with disease status.

Chapter 6: Assessment of CD8$^+$ T cell responses in healthy uninfected volunteers immunised with HIV GRIN/Env vaccine candidate

6.1 Introduction

Although an effective HIV vaccine has not been developed, there have been major advances in the creation of novel inserts, vectors, vaccine strategies and adjuvants (Barouch et al., 2013b; Kunwar et al., 2013; Plotkin, 2009; Santra et al., 2010). One of the major hurdles in developing an effective HIV-1 vaccine is the inability to identify correlates of protection of natural immunity to HIV (Haynes et al., 2012; Kim et al., 2015; Koup et al., 2011; Plotkin, 2009; Van Gulck et al., 2012). Even though non-human primate (NHP) studies have helped researchers to better understand HIV-1 infection, the data produced by animal studies cannot be directly compared to humans to identify immune responses required to control HIV-1 (Evans and Silvestri, 2013; Henning et al., 2015; Sui et al., 2013).

Several groups are working towards T cell based HIV-1 vaccine immunogens (Barouch et al., 2013a; Barouch et al., 2013b; Borthwick et al., 2014; Fischer et al., 2007; Hammer et al., 2013; Korber et al., 2009a; Letourneau et al., 2007; Liu et al., 2009b; Rolland et al., 2007b). One such immunogen was Merck Ad5 HIV-1 Gag/Pol/Nef construct tested in the STEP trial (also referred to as HVTN 502 or Merck V520-023) (Buchbinder et al., 2008; Priddy et al., 2008; Sekaly, 2008). The majority (75%) of vaccinees exhibited CD8$^+$ T cell responses which were not very broad, but were polyfunctional (Buchbinder et al., 2008; Huang et al., 2014; Li et al., 2011; Priddy et al., 2008). Unfortunately, the Merck vaccine candidate increased the risk of HIV-1 acquisition in individuals with pre-existing immunity to Ad5 (baseline titre $\leq$200) both in vaccinees and placebo groups (Buchbinder et al., 2008). Another HIV-1 vaccine efficacy trial RV144 followed a prime-boost strategy was designed to induced binding antibodies. Prime was ALVAC-HIV (canary pox: vCP1521) encoding Env-Gag-Pro (gp41 from subtype B) and boost with AIDSVAX® B/E gp120 (Kim et al., 2015). The RV144 trial showed some (31.2%) protection against infection apparently mediated by HIV specific Abs to the second variable (V2) loop of gp120 and IgG mediated ADCC, associated with decreased transmission risk, but had no effect on disease course (Haynes, 2015; Haynes et al., 2012; Kim et al., 2015). Even though the results of the STEP and RV144 trials were a disappointment in the field of HIV-1 vaccines, researchers have gained tremendous knowledge (i.e. vector-based immunity, vaccine mediated increased risk of HIV acquisition, magnitude and breadth of T cell responses, polyfunctionality) from the STEP study and are moving forward optimistically (Buchbinder et al., 2008; Haynes, 2015; Klein et al., 2013; Ondondo et al., 2016; Rubens et al., 2015; Sekaly, 2008; Tongo and Burgers, 2014).

This chapter focuses on one potential HIV-1 vaccine candidate used in a Phase I randomised, double-blinded, dose-escalation, placebo-controlled HIV-1 clinical trial (NCT00851383). The vaccine insert was based on full length HIV-1 subtype A sequences (AY253305 & AF457081) (Dowling et al., 2002; Keefer et al., 2012) and referred to as Ad35 GRIN/Env. Most of the IAVI conducted clinical trial B001 subjects (80%) recognised four to five GRIN/Env pools and these responses were shown to be polyfunctional (Keefer et al., 2012).

The focus of this section was to determine whether; (1) vaccine elicited responses were broad (i.e. responses were generated against different regions of HIV-1 genome or concentrated mainly on one part of the genome), (2) if responding epitopes were conserved or variable (3) or whether responding epitopes were able to control virus isolates *in vitro*. The Merck STEP trial failed to show any correlation between vaccine induced IFN-γ producing $CD8^+$ T cells and viral clearance (Altfeld and Goulder, 2011; Barouch and Korber, 2010; Huang et al., 2014). Hence, relying solely on IFN-γ ELISpot responses to assess vaccine immunogenicity may be insufficient in informing about vaccine efficacy.

This chapter's aim was to systematically map T cell responses in healthy volunteers post second (week 24 onwards) immunisation with the Ad35 GRIN/Env immunogen to determine whether vaccine specific $CD8^+$T cell epitopes responses were focused against conserved or non-conserved epitopes and whether the inhibition of a broad cross clade panel of viruses used in the VIA has any association with targeting of such regions. VIA enables the evaluation of the anti-viral activity of $CD8^+$ T cells *in vitro* and may act as a proxy of a potential protection (Ahmed et al., 2016; Spentzou et al., 2010).

6.1.1 Trial Design

B001 is an IAVI sponsored double blinded dose-escalation phase I clinical trial (ClinicalTrials.gov NCT00851383) to evaluate the safety and immunogenicity of a potential HIV-1vaccine candidate, referred to as Ad35 GRIN/Env. The vaccine was prepared by mixing non replication competent Ad35 GRIN and Ad35 Env in the same vial (ratio of 1:1) and was administered intramuscularly at month 0 and month 6 as a single injection. Placebo (n=16) was a colourless injection consisting of 1 mM MgCl2, Tween80 54 mg/L, 1M Saccharose, 150mM NaCl, 10mm Tris/HCl, in water for injection, final pH 8.5. In total there were four dosing groups from low (A, n=10), mid (B, n=10) to high (C, n=10) and each group received an increasing dose from 10^9, 10^{10}, 10^{11} virus particles (VP) of each of the Ad35 GRIN and Ad35 Env vectors. The fourth group (D, n=10) only received 10^{10} VP of Ad35 GRIN and it is equivalent to the mid group but, without Ad35 Env constructs. Bloods were drawn to isolate PBMCs at multiple time points for ELISpot assay and VIA (Figure 37).

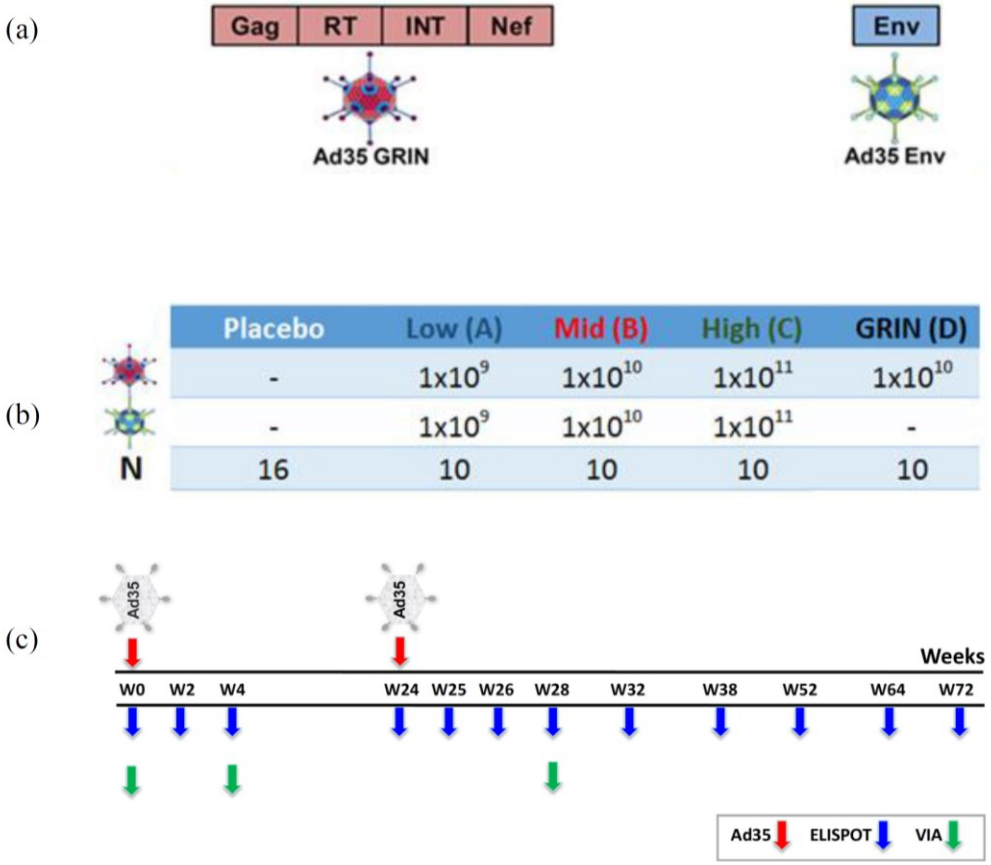


Figure 37. Immunisation and sampling schedule of Ad35 GRIN/Env vaccine

(a) Illustration of Ad35 GRIN (pink) and Ad35 Env (green) vaccine. (b) Schematic representation of Ad35 GRIN/Env Vaccine regimen showing group A-C received Ad35 GRIN/Env whereas group D received Ad35 GRIN only. (c) Ad35 GRIN/Env vaccine candidate was administered intramuscularly at week (W) W0 (month 0) and W24 (month 6), shown in red. Bloods were drawn at multiple time points for PBMCs isolation for ELISpot assay highlighted in blue. PBMCs were further tested in VIA at W0, W4 and W28 (shown in Green).

6.2 Results

6.2.1 Demographics

HIV negative healthy volunteers were recruited into the B001 study. The majority of participants were white and consisted of both males and females. Overall, there was a broad variety of HLA alleles (Table 19).

Table 19. Demographic characteristics of the cohort

Demographic		Overall (n=56)
Age (median [range] years)		27 (18-48)
Sex (n, %)	Male	34 (61%)
	Female	22 (39%)
Race (n, %)	White	47 (84%)
	Black	5 (9%)
	Asian	2 (3.5%)
	American Indian	2 (3.5%)
HLA represented	HLA-A	A1 (28%)
		A2 (36%)
		A3 (16%)
		Others (<10%)
	HLA-B	B7 (15%)
		B15 (12%)
		B44 (12%)
		Others (<10%)
	HLA-C	Cw*03 (16%)
		Cw*04 (20%)
		Cw*07 (28%)
		Others (<10%)

6.2.2 Proportion of responders to Ad35 GRIN/Env peptide pools

The IFN-γ ELISpot assay was used to evaluate cellular immune responses elicited in volunteers at each visit and the percentage of responders (IFN-γ ELISpot ≥50 SFU/10^{6}) for each study arm is shown in Table 20. IFN-γ ELISpot responses to Ad35 GRIN/Env pools four weeks post first (W4) and second vaccination (W28) were detected in all four vaccinated groups (Low, Mid, High and GRIN) (Figure 38) whereas responses in placebo volunteers were negative. The magnitude of the ELISpot responses among all four groups was stratified as weak or strong (Figure 38) based on the SFU/10^{6} PBMC generated against any antigen.

Table 20. Percentage of responders post Ad35 GRIN/Env (Group A-C) and Ad35 GRIN (Group D) vaccination at W4 and W28.

Groups	Number of Responders Post 1st Vaccination (W4)	Number of Responders Post 2nd Vaccination (W28)
Low (A)	5/10 (50%)	6/8 (75%)
Mid (B)	5/9 (56%)	7/7 (100%)
High (C)	7/10 (70%)	7/8 (88%)
GRIN (D)	9/10 (90%)	6/7 (86%)
Placebo	0/16 (0%)	0/16 (0/16)

For mid group (B) 5/9 (56%) individuals responded at W4 after being immunised with $1x10^{10}$ VP and 7/7 (100%) responded at W28 and there was no increase in the number of responders between two time points ($p>0.05$; 2-tailed Fisher's exact test). This was also observed for the other GRIN/Env dosing groups, suggesting there was no difference between the proportion of responders post second vaccination for groups A-C ($p>0.05$) and group D ($p>0.05$) (Table 20). Overall, no difference was observed between the proportion of responders at W4 and W28 ($p>0.05$). Similarly, there did not appear to be a difference in the proportion of responders with increasing vaccine dose (Low, Mid and High) at W4 ($p>0.05$) and W28 ($p>0.05$).

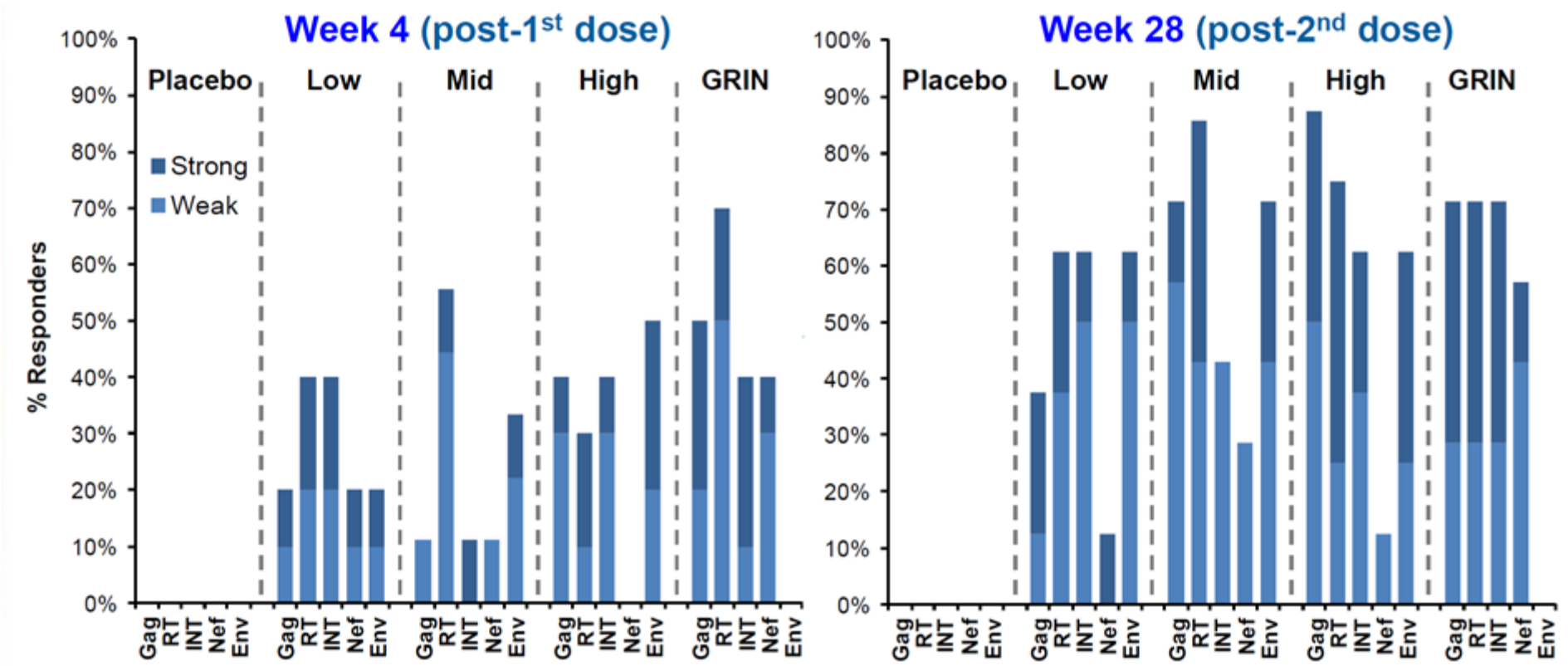


Figure 38. Number (%) of responders to Ad35 GRIN/Env peptide pools

IFN-γ ELISpot responses to Ad35 GRIN/Env pools showing percentage of responders in placebo, low, mid, high and GRIN only group post first (W4) and post second (W28) vaccination. Each stack bar shows proportion of strong (>150 SFU/10^6-dark blue) and weak (50-150 SFU/10^6-light blue) responders at W4 and W28.

6.2.3 Evaluation of vaccine specific $CD8^+$ T cell responses using Ad35 GRIN/Env matrices

Mapping was only performed using PBMC from the 2nd vaccination time point. Volunteers (25/56) who mounted T cell responses of ≥100 SFU/10^6 PBMC after background subtraction to any antigen in each group were selected to assess their T cell responses at an epitope level. More than half of the volunteers 18/25 (72%) were mapped using 52 different GRIN/Env matrix pools post 2nd vaccination while others (7/25) could not be tested for ELISpot responses post 2nd immunisation due to lack of PBMC availability. All 18 volunteers received both doses of vaccine and the time points (week number) chosen to map T cell responses are shown in Table 21.

Table 21. List of volunteers and the time point selected post 2nd vaccination to map T cell responses to Ad35 GRIN/Env immunogen

Volunteers ID	Group	Week Post 2nd Immunisation
6005	Low (A)	W26
6009	Low (A)	W32
6017	Low (A)	W25
6036	Mid (B)	W32
6040	Mid (B)	W26
6048	Mid (B)	W25
6053	Mid (B)	W25
6072	Mid (B)	W28
6070	High (C)	W25
6076	High (C)	W28
6078	High (C)	W25
6081	High (C)	W28
6085	GRIN (D)	W25
6120	GRIN (D)	W25
6135	GRIN (D)	W25
6136	GRIN (D)	W25
6139	GRIN (D)	W28
6140	GRIN (D)	W32

12/18 (Group A-C) received Ad35 GRIN and Ad35 Env whereas, 6/18(Group D) received Ad35 GRIN only immunogen. The median week/time point used post 2nd vaccination was 26 (IQR 25-28) and the median number of weeks passed 2nd vaccination was 2 (1-4). Of those mapped, 8/18 (44%) induced responses to Gag, 11/18 (61%) to RT, 7/18 (39%) to INT, 2/18 (11%) to Nef and 9/12 (75%) to Env among all four groups (Table 22).

Table 22. Number of peptides deconvoluted post 2nd immunisation using Ad35 GRIN/Env and Ad35 GRIN matrices

Antigen	n/N %[a]	Total number of unique regions[b]	Median IQR (1-3) peptides
Gag	8/18 (44)	9	1 (1-2)
RT	11/18 (61)	20	2 (1-3)
INT	7/18 (39)	10	1 (1-2)
Nef	2/18 (11)	3	1.5 (1-2)
Env	9/12 (75)	21	2 (1-3)
Total		63	3 (2-5)

NOTE. [a]Percentage of responders=Number of responders (n) divided by total number of vaccinees mapped (N). Gag, RT, INT and Nef (N=18) whereas Env (N=12)
[b]Overlapping peptides encompassing the same sequence or single

In total, 117 different OLP were recognised and of those 33/117 (28%) responding OLPs could not be deconvoluted. This is because there were more than one possible responding peptide in a pool however, those pools were still counted as a response. However, 72% of the peptide responses were successfully deconvoluted which was either comprised of two OLP encompassing the same sequence or a single responding peptide. IFN-γ responses were detected among all four groups and 63 different unique sequences were identified (Table 22) among 18 vaccinees. Figure 39 displays T cell responses elicited by each volunteer in a dose escalation manner and the vaccine regime each group received, with 11/18 (61%) vaccinees responding to more than one HIV-1 region. Furthermore, the majority of participants responded to ≥ 1 peptide (Table 22 and Figure 39) and the median number of peptides recognised was 3 (IQR 2-5) among all four groups. In addition, 9/12 (75%) participants (Groups A-C) responded to Env compared to other antigens. However, there was no significant difference between the proportion of Env responders and all the other antigens (p>0.05; 2-tailed Fisher's exact test) apart from Nef (p<0.05).

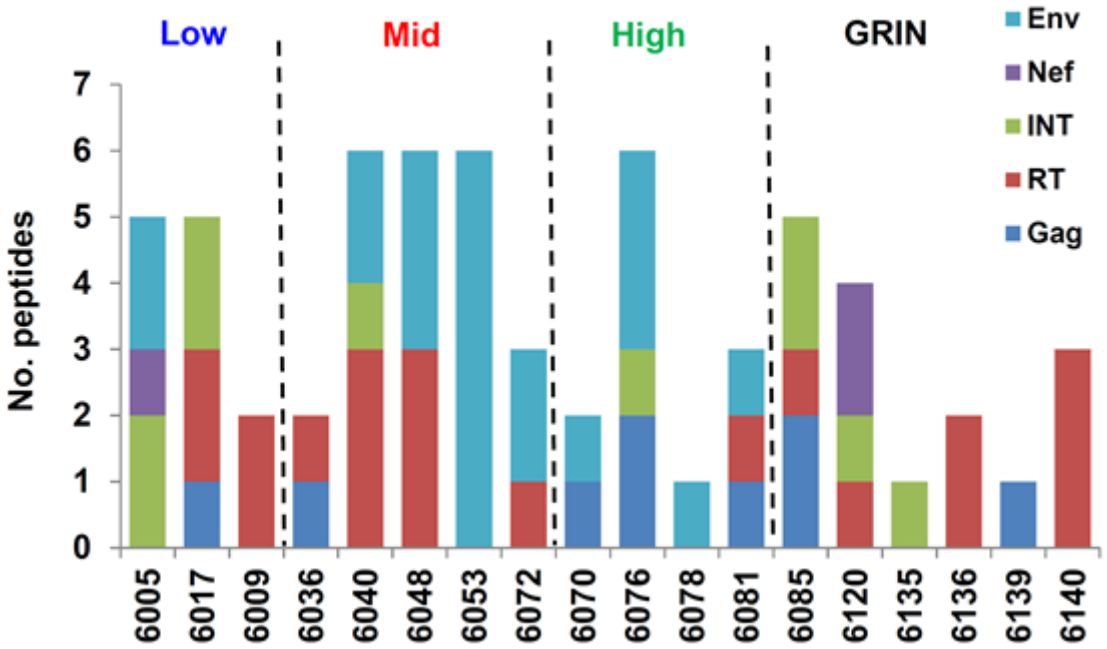


Figure 39. Breadth of IFN-γ ELISpot responses post 2nd vaccination

ELISpot data showing the breadth of pool specific responses for all the participants among all four groups (Low, Mid, High and GRIN) post 2nd vaccination in a dose escalation manner showing the number of unique region/peptides recognised per antigen for each vaccinee. Unique regions are either two overlapping peptides encompassing the same sequence or single responding peptides.

6.2.4 Responding epitopes identified in the HIV data base

Putative CD8 T cell epitopes were identified using vaccinees' specific HLA alleles in the HIV db (Table 23). 3/18 participants (6005, 6017 and 6140) HLA data were not available and therefore the responding epitopes could not be established. Identified epitopes are based on the responding pools deconvoluted (72%). Some of the potential T cell epitopes determined are based on anchor residues (Table 23). Overall, responding epitopes for each region were both conserved (entropy score <0.2) and variable (entropy score >0.2). The hierarchy of conservation of epitopes was RT> INT> Gag> Env> Nef (Figure 40). The magnitude of responses towards conserved epitopes was not significantly different to those of the variable epitopes (p>0.05; 2-tailed Fisher's exact test).

Table 23. Responding best define and putative epitopes identified in the HIV db based on HLA allele restriction of vaccinees

Group	VID	HLA-A	HLA-A	HLA-B	HLA-B	HLA-C	HLA-C	Antigen	Epitopes	Conserved or Variable[b]	HLA allele
A	6009	A*0101	A*0202	B*4001	B*4901	Cw*0304	Cw*0701	RT	IETVPVKL	Conserved	B*4001
								RT	IEELRQHLL	Variable	B*4001
B	6036	A*0101	A*0301	B*2705	B*5701	Cw*0102	Cw*0602	Gag	KRWIILGLNK	Conserved	B*2705
								Gag	WIILGLNKI[a]	Conserved	Cw*0602
								RT	QIYPGIKVR	Variable	A*0301
								RT	YAGIKVKQL[a]	Variable	Cw*0602
B	6040	A*0201	A*2609	B*0702	B*5801	Cw*0702	Cw*0718	RT	SPAIFQSSM	Conserved	B*0702
								RT	GWKGSPAIF[a]	Conserved	Cw*0702
								RT	IVLPEKDSW	Variable	B*5801
								RT	IAMESIVIW	Variable	B*5801
								INT	IPYNPQSQGVV	Conserved	B*0702
B	6048	A*0201	A*0201	B*1302	B*3801	Cw*0602	Cw*1203	RT	LTEEAELEL[a]	Conserved	B*3801/Cw*0602[c]
								RT	GQDQWTYQI[a]	Variable	B*3801
								RT	QDQWTYQIY[a]	Variable	Cw*0602
								RT	VIWGKTPKF[a]	Conserved	B*3801
								RT	WGKTPKFKL[a]	Variable	Cw*0602
								Env	FNMWKNNMV[a]	Variable	Cw*0602
								Env	QMHEDIISL	Variable	A*0201
								Env	IISLWDQSL	Variable	A*0201
								Env	MHEDIISLW	Variable	B*3801
								Env	MHTDIISLWDQSLKP	Variable	B*3801
								Env	TLPCRIKQI[a]	Variable	Cw*0602
								Env	QIIRMWQRV[a]	Variable	Cw*0602
B	6053	A*0201	A*2601	B*0702	B*3801	Cw*0702	Cw*1203	Env	LWVTVYYGV	Conserved	A*0201
								Env	FCASDAKAY[a]	Variable	Cw*0702
								Env	NIWATHACV	Conserved	A*0201
								Env	DPNPQEIPL[a]	Variable	B*0702/B*3801/Cw*0702[c]
								Env	IISLWDQSL	Variable	A*0201
								Env	MHEDIISLW	Variable	B*3801
								Env	MHTDIISLWDQSLKP	Variable	B*3801
								Env	QVYSLFYRL[a]	Variable	B*0702

(*continued on next page*)

Group	VID	HLA-A	HLA-A	HLA-B	HLA-B	HLA-C	HLA-C	Antigen	Epitopes	Conserved or Variable[b]	HLA allele
B	6072	A*0301	A*1101	B*0801	B*1302	Cw*0602	Cw*0701	RT	QIYAGIKVK	Variable	A*0301/A*1101[c]
								Env	CIRPNNNTR[a]	Variable	A*0301
								Env	IRPNNNTRK[a]	Variable	A*1101
								Env	PNNNTRKSY[a]	Variable	Cw*0602
								Env	PCRIKQIIR[a]	Variable	B*0801
								Env	TITLPCRIK[a]	Variable	A*0301
C	6070	A*6801	A*6801	B*3503	B*3503	Cw*0401	Cw*0401	Gag	TPQDLNVML	Conserved	B*3503
								Env	NAKNIIVQF[a]	Variable	Cw*0401
C	6076	A*3303	A*6601	B*1510	B*7801	Cw*0304	Cw*1601	Gag	NPSLLETTE[a]	Variable	B*7801
								INT	IPAETGQET[a]	Conserved	B*7801
								Env	RAIEAQQQL	Variable	B*1510/Cw*0304[c]
C	6078	A*0202	A*7401	B*1801	B*5802	Cw*0501	Cw*0602	Env	RIGPGQTFY[a]	Variable	Cw*0602
C	6081	A*0301	A*2402	B*1402	B*3502	Cw*0401	Cw*0802	Gag	RFALNPSLL[a]	Variable	A*2402/Cw*0401[c]
								RT	QIYPGIKVR	Variable	A*0301
								Env	ERYLRDQQL	Variable	B*1402
								Env	RYLRDQQLLGI	Variable	A*2402
								Env	RYLRDQQL	Variable	A*2402
D	6085	A*0201	A*0201	B*4402	B*4402	Cw*0501	Cw*0501	Gag	SLFNTVATL	Variable	A*0201
								Gag	AEQASQDVKNW	Variable	B*4402
								RT	RAHLLSWGF[a]	Variable	Cw*0501
								INT	QEEHERYHSNW	Variable	B*4402
D	6120	A*2402	A*2902	B*4501	B*5501	Cw*0303	Cw*0602	RT	LPQGWKGSP[a]	Conserved	B*5501
								RT	QGWKGSPAI[a]	Conserved	Cw*0602
								INT	IPYNPQSQG[a]	Conserved	B*5501
								INT	PYNPQSQGV[a]	Conserved	Cw*0602
								Nef	EEEVGFPVRPQ	Variable	B*4501
								Nef	VGFPVRPQV[a]	Conserved	Cw*0602
								Nef	GAFDLSHFL	Variable	Cw*0602
D	6135	A*0201	A*2402	B*0702	B*5101	Cw*0102	Cw*0702	INT	LPPIVAKEI	Conserved	B*5101
D	6136	A*0201	A*6801	B*4001	B*4402	Cw*0304	Cw*0501	Gag	KELYPLTSL	Variable	B*4001
								RT	IETVPVKL	Conserved	B*4001
								RT	IEELRQHLL	Variable	B*4001
D	6139	A*1101	A*2902	B*2705	B*4403	Cw*0202	Cw*1601	Gag	KRWIILGLNK	Conserved	B*2705

NOTE. [a]Potential/putative epitopes based on anchor residues
[b]Conserved and Variable epitopes are defined on the basis of Shannon Entropy score
[c]Epitope restricted through more than one HLA allele in HIV db
VID Volunteer Identity

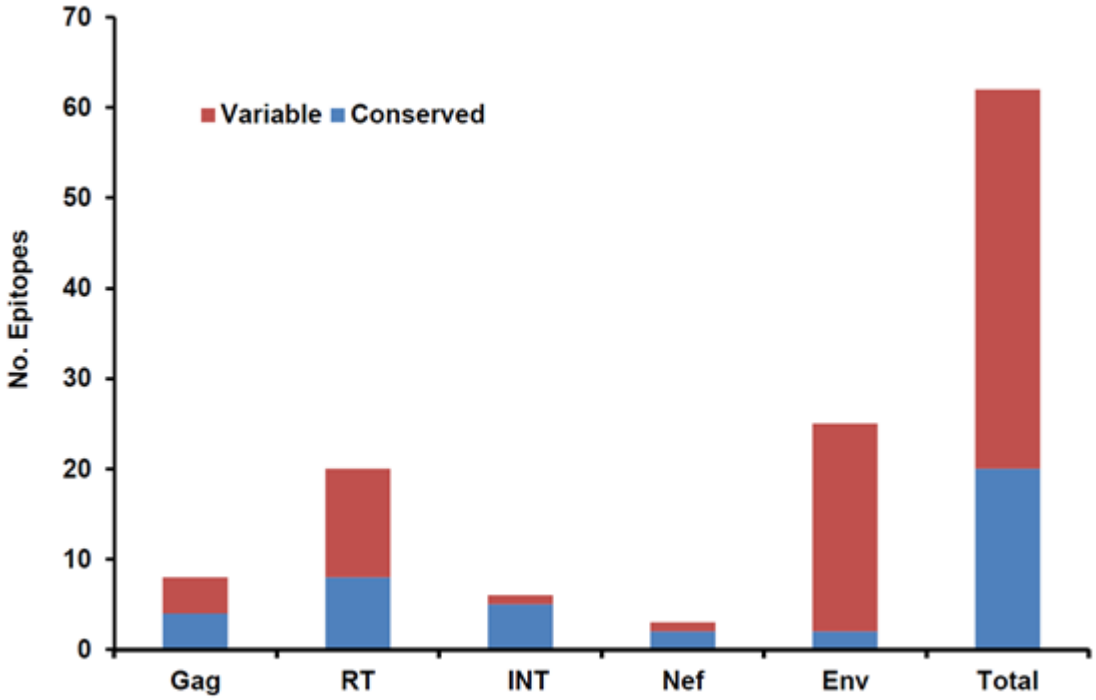


Figure 40. Proportion of conserved and variable epitopes

Number of conserved (<0.2) and variable (>0.2) epitopes targeted per region Gag, RT, INT, Nef and Env. Blue bar represents conserved and red bar represents variable epitopes based on Shannon entropy score.

Four out of eight Gag responders mounted IFN-γ ELISpot responses against the p17 region. Of those, 3/8 p17 Gag responders (p17) responded to peptide DRFALNPSLLETTEG encompassing variable epitopes RFALNPSLL (A*2402/Cw*0401) and NPSLLETTE (B*7801). In addition, 2/8 (p17) participants responded to peptide EIKSLFNTVATLYCV incorporating variable epitope SLFNTVATL (SL9) and one individual expressed the HLA A*0201 allele associated with SL9. In contrast, a few participants induced responses to conserved sequences of p24; YKRWIILGLNKIVRM (2/8), ALSEGATPQDLNVML (1/7), containing KRWIILGLNK (KK10) and TPQDLNVML (TL9) epitopes presented by B*2705 and B*3503 alleles respectively (Table 23).

In contrast, 41 peptides were deconvoluted for Pol region and this included both RT and INT responses. 52% of the responses were mounted towards a conserved region of Pol and the two sequences targeted within RT were SQGSPISPIETVPVTLKPGM (2/12) and YNVLPQGWKGSPAIFQSSMMTKIL (2/12) encompassing epitopes IETVPVKL, LPQGWKGSP and SPAIFQSSM associated with vaccinees' HLA alleles B*4001, B*5501 and B*0702,

respectively. Most potent conserved responses were against INT sequence MASDFNLPPIVAKEIVASC (1/12) and EFGIPYNPQSQGVVA (1/12) encompassing epitopes LPPIVAKEI and IPYNPQSQG associated with vaccinees' HLA B*5101and B*5501 alleles. Moreover, the most immunogenic regions recognised within RT were HRTKIEELRAHLLSWGFTT (3/12) and WASQIYAGIKVKQLC (3/12) sequences. These individuals targeted variable epitopes IEELRQHLL (B*4001), RAHLLSWGF (Cw*0501) and QIYPGIKVR (A*0301, A*3010/A*1101) and also expressed alleles known be associated with those epitopes (Table 23).

Only 2/18 individuals responded to variable Nef known epitopes EEEEVGFPVRPQ and GAFDLSHFL and expressed alleles B*4501 and Cw*0602, known to be associated with those epitopes. One recipient, whose HLA alleles were not determined, responded to DSRLALKHRAQELHP sequence, most likely targeted an epitope ALKHRAYEL known to be restricted by HLA A*0201, A*0202 and A2. Hence, it is not possible to elucidate the exact epitope for this individual due to missing HLA data (Table 23).

These data indicate that while the Env component of the vaccine appeared to be immunogenic the majority of the Env (92%) epitopes were variable with a very few conserved (8%) responses (Table 23 and Figure 40). Furthermore, individuals immunised with Ad35 GRIN/Env induced broad Env specific $CD8^+$ T cell responses (Table 23). However, most of the responding epitopes (92%) were variable based on their entropy score (>0.20). The two main regions targeted were PCRIKQIIRMWQRVG (3/6) and MHEDIISLWDQSLKP (3/6), encompassing epitopes TLPCRIKQI and QIIRMWQRV, known to be restricted by that individual's HLA Cw*0602 and B*0801, respectively, determined on the basis of anchor residues. For sequence MHEDIISLWDQSLKP three possible epitopes IISLWDQSL/MHEDIISLW and MHTDIISLWDQSLKP were elucidated restricted by vaccinee's HLA A*0201 and B*3801, respectively (Table 23).

Taken together these data indicate that the Ad35 GRIN/Env vaccine regime induced CD8 responses restricted through an array of HLA alleles for example A*02, A*03, A*24, A*30, B*07, B*27, B*38, B*44, B*51 Cw*06 (Table 23), suggesting certain epitopes ought to be incorporated or targeted as future HIV vaccine candidates.

6.2.5 Viral Inhibition assay (VIA)

All participants were tested in the VIA to determine the ability of Ad35 GRIN/Env specific $CD8^+$ T cells to inhibit viral replication *in vitro*, where bi-specifically expanded $CD4^+$ T cells were infected with a panel of seven different viruses encompassing clades A, B, C and D (Table 24). The Cut Off

value to identify positive responses was defined by IAVI statisticians EMMES Corporation (Rockville, Maryland) and it was ≥1.5 Log_{10} inhibition based on 95% confidence interval.

Table 24. Panel of viruses used in B001 clinical trial to determine vaccine specific CD8 T cell mediated viral inhibition

Virus	Type	Clade	Region
U455	Lab adapted	A	Uganda
IIIB	Lab adapted	B	US
CH077	*IMC	B	US
CH106	*IMC	B	US
ZA97012	Primary	C	South Africa
247Fv2	*IMC	C	Zambia
ELI	Lab adapted	D	DR Congo

Note. *Infectious Molecular Clone (IMC)

49/56 individuals were tested in VIA post second vaccination (W28). Of those, U455 virus isolate VIA data are not available for two individuals (6005 and 6009) and we were also unable to generate VIA data for a participant (6140) was not generated with any of the virus isolates, likely due to technical failure (Dr Peter Hayes, personal communication). In Group A (Low dose) 5/13 (38%) volunteers inhibited U455, 4/13 (31%) inhibited CH077, 2/13 (15%) inhibited CH106 and IIIB, respectively, and only 1/13 donor (8%) inhibited ZA97012. However, isolates such as ELI and 247Fv2 were not inhibited by group A participants (Figure 41).

Comparatively, all isolates were inhibited by specific group B (low) volunteers to some degree, 10/12 (83%) donors inhibited U455, 8/12 (66%) inhibited IIIB, CH077 and 247Fv2, respectively, 5/12 (42%) inhibited CH106, 3/12 (25%) inhibited both ELI and ZA97012. Similarly, 8/13 (61%) group C (high) donors inhibited U455, 6/13 (46%) inhibited CH077 and 247Fv2, respectively, 5/13 (38%) inhibited CH106 and 4/13 (31%) inhibited isolate IIIB, whereas isolates such as ELI and ZA97012 were not inhibited by group C vaccinees. In addition, 8/11 (73%) group D (GRIN only group) donors inhibited U455, 4/11 (36%) inhibited IIIB, 2/11 (18%) inhibited CH077 and 247Fv2, 1/11 (9%) inhibited CH106 and ZA97012, but ELI was not restricted by group D donors (Figure 41). As a result, the low dose group had lower and narrower VIA results; median 0.8 and IQR (0.5-1.3), the mid dose group had stronger and broader VIA responses compared to all other groups, median 1.6 and IQR (1.1-1.2). Moreover, high (1.0 (0.6-1.8)) and GRIN (1.0 (0.6-1.4)) groups had somewhat high magnitude VIA responses, but overall were of smaller breadth compared to the mid group (Figure 41). Comparatively, U455 was restricted by a majority of individuals (31/47) but, to different degrees, whereas ELI (3/49), IIIB (18/49), CH077 (20/49), CH106 (13/49), 247Fv2 (16/49) and ZA97012

(5/49) had VIA responses of smaller breadth and magnitude. Evidently (Figure 41), there is a difference between vaccine groups. The low group have low VIA responses and the mid, high and GRIN groups have higher responses, suggesting that vaccine groups are different in their VIA responses ($p<0.05$; Kruskal–Wallis test). Thus, there is a relationship between VIA responses and vaccine dosing suggesting that VIA responses are dose dependent (Figure 41).

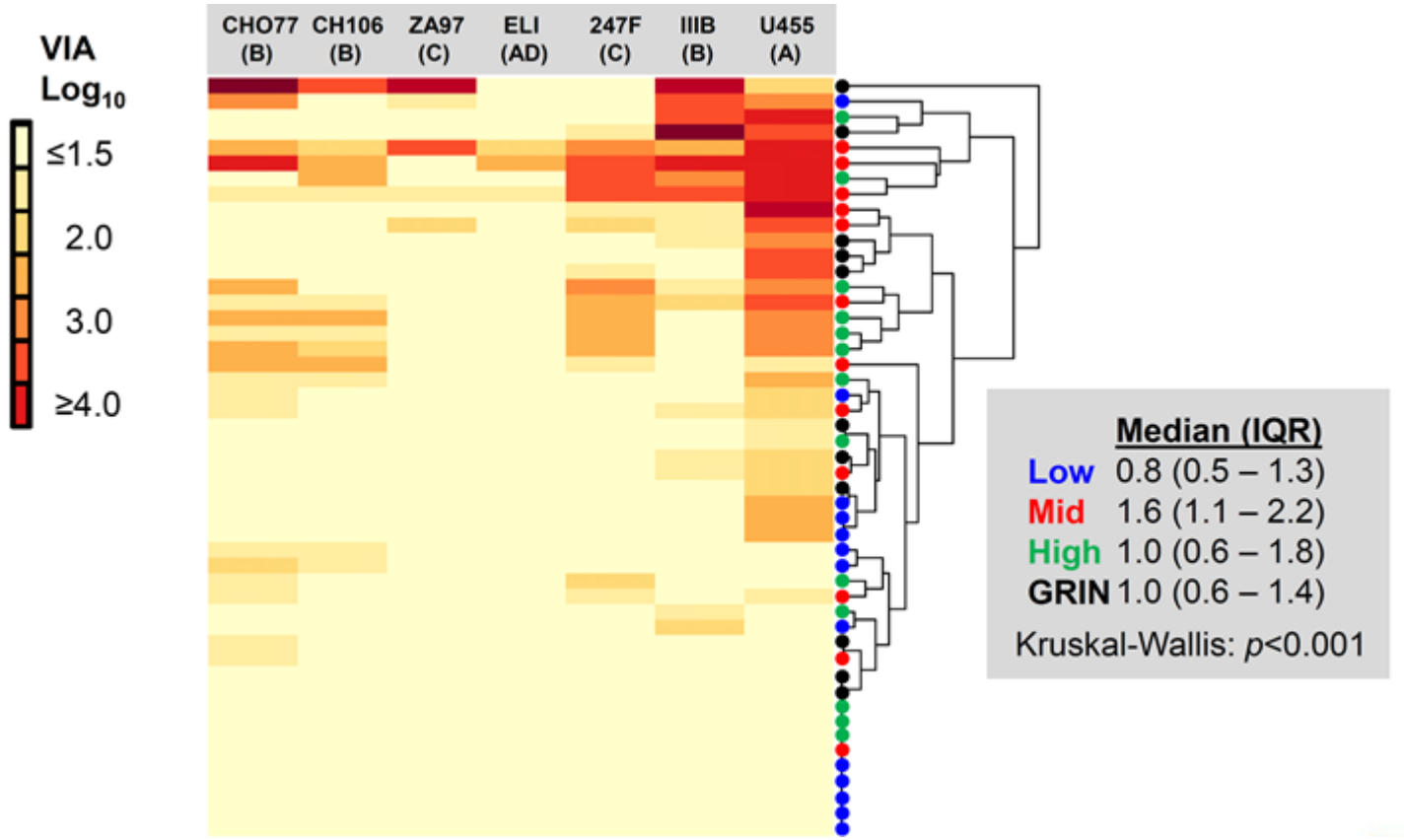


Figure 41. Heatmap showing the degree of virus inhibition of seven different viruses by vaccinees

The heatmap was created using clustal analysis and a neighbour joining algorithm displaying VIA responses for each vaccinee against a set of viruses. Each dot on the dendrogram to the right represents an individual vaccinee; low (blue dot-group A), mid (red dot-group B), high (green dot-group C) and GRIN only (black dot- group D) group. VIA colour scheme is based on a log scale and responses >1.5 log10 are defined to be positive. Darker the colour higher was the inhibition. Viral isolates and subtypes are specified at the top of heatmap. The data was kindly provided by Dr Peter Hayes.

6.2.6 Sequence conservation between VIA isolates and GRIN Immunogen

The above data appear to show an association between virus inhibition and vaccine dosing in the VIA. Data were further analysed to examine whether the sequence of the responding $CD8^+$ epitopes were conserved within VIA viral isolates and if there was an association between the responding conserved epitopes and the degree of viral inhibition. Table 25 shows percentage of comparative sequence homology between vaccine insert and viral isolates used in the VIA. As GRIN/Env was based on subtype A, U455 has the highest percentage of sequence homology to GRIN-Gag-Pol-Env (Table 25) (Kopycinski et al., 2014). Conservation score (CS) (Section 2.14) of zero was established as highly conserved and greater than zero was computed as variable. Immunodominant responses (usually defined as peptide generating the highest $SFU/10^6$ or the most commonly recognised epitopes) here are described as the most common T cell response to GRIN/Env peptides.

Table 25. Percentage sequence homology between GRIN/Env and VIA isolates

Virus	Accession No.	Clade	Gag	Pol	Nef	Env
U455	M62320	A	87.3	94.2	75.9	82.4
IIIB	K03455	B	81.4	91.3	77.2	73.0
CH77	FJ496000	B	82.1	90.2	74.5	72.8
CH106	JN944942	B	81.8	90.4	78.0	72.5
247FV2	FJ496200	C	83.5	90.7	78.3	74.3
97ZA012	AF286227	C	83.6	91.2	76.8	73.6
ELI	A07108	D	83.6	90.8	77.7	75.0

These data suggest that vaccine induced responses to HIV conserved epitopes tend to control viral replication significantly better in *vitro* compared to the responses mounted towards variable epitopes ($p<0.05$; Mann Whitney U test) (Figure 42a). These data indicate that the degree of inhibition (median =1.67 Log_{10}) was higher when the response was mounted towards conserved epitopes than towards the variable epitopes (median=1.21 Log_{10}). Furthermore, when VIA responses were distributed into three categories such as negative (0.1-1.4 Log_{10}), low (1.5-3.0 Log_{10}) and high (≥3.0 Log_{10}) then the highest degree of inhibition was in direct relationship with the lowest conservation score of the responding epitope/sequence (Figure 42b). This difference in inhibition and targeting conserved epitopes was clearly significant between low and the high VIA responders ($p<0.05$and also between negative and high VIA responders ($p<0.05$), however, it was not significant between negative and low VIA responders ($p>0.05$) (Figure 42b).

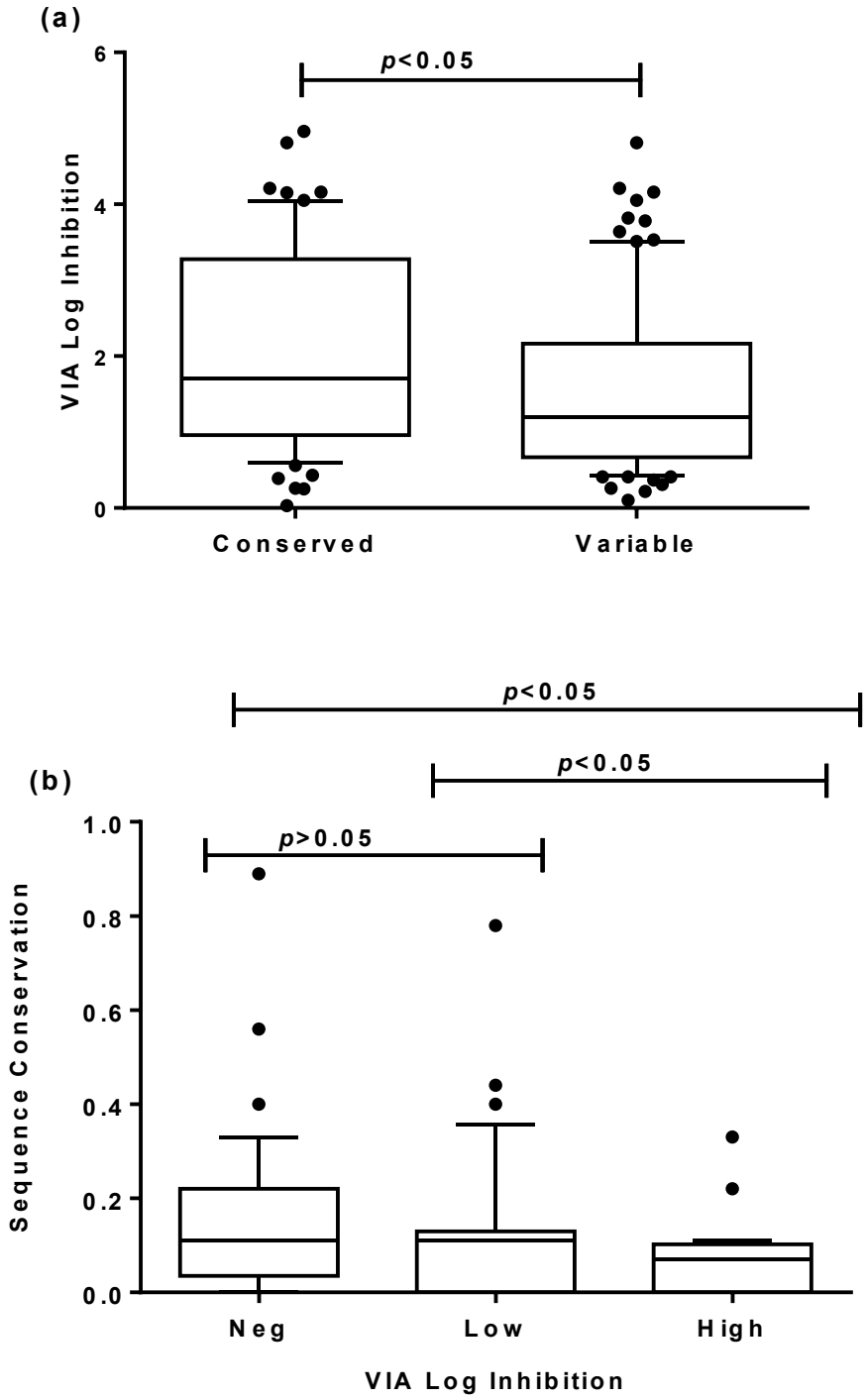


Figure 42. Sequence conservation between isolates used in VIA and GRIN immunogen associate with ELISpot responses

Box and whisker plot comparing the (a) ability to control viral replication when conserved regions were targeted. Virus inhibition was significantly higher when the targeted epitopes were conserved. (b) Illustrates that the degree of viral inhibition was associated with targeting of conserved epitopes. These data show that this difference was significant between negative (0.1-1.4 Log_{10}), low (1.5-3.0 Log_{10}) and high ($\geq$ 3.0 Log_{10}) VIA responders.

6.3 Discussion

The B001 study was designed to assess the safety and immunogenicity for T cell responses elicited by Ad35 GRIN/Env HIV-1 vaccine candidate. All recipients received two doses of their assigned vaccine per group and the vaccination regimen was tolerated by all recipients. The trial showed that Ad35 GRIN/Env vaccine was safe and immunogenic and induced IFN-γ ELISpot responses in Ad35 sero-negative healthy volunteers at different dose levels. Vaccine induced IFN-γ responses were detected as early as W2 by ELISpot assay using Ad35 GRIN/Env whole pools. After two immunisations no significance difference was observed between the numbers of responders post 1st immunisation and post 2nd immunisation among all four groups (Keefer et al., 2012). Not only that, increasing the vaccine dose did not appear to increase the number of ELISpot responders. This, however, may be due to the small sample size. As a primary objective magnitude and breadth of vaccine specific CD8^{+} T cell responses were assessed in vaccinees. This was performed by a comprehensive mapping of CD8^{+} T cell responses in 18 vaccinees using Ad35 GRIN/Env matrices. Vaccine specific ELISpot responses appeared to be of larger breadth for GRIN/Env groups than the GRIN only group but, this did not reach statistical significance. This apparent non-significant difference in breadth is due to Env specific T cell responses, as 3/4 groups (A-C) were immunised with Ad35 GRIN/Env whereas group D was immunised with GRIN only and therefore, had no Env specific responses. Another contributing factor could be the greater number of recipients in GRIN/Env groups compared to the GRIN only group. In addition to this, responding epitopes were either conserved or variable for each immunogenic region (Gag, RT, INT and Nef) whereas, Env epitopes were predominantly variable. Even though the Env component of the vaccine appears to be more immunogenic compared to the other regions (Env>RT>INT>Gag>Nef), this did not reach to the statistical significance apart from Nef. It has been demonstrated that Ad35 GRIN/Env vaccine induced polyfunctional responses to Gag and Pol but not to Env, suggesting that Env specific responses could be mediated by CD4 (Keefer et al., 2012). Overall, a median of 2.5 (IQR 1-5) epitope specific immune responses were observed, in comparison to the STEP trial where the median number of vaccine specific T cell epitopes was 2 (Altfeld and Goulder, 2011; Huang et al., 2014).

Based on vaccinees' HLA types, both probable (optimal/best defined) and possible (putative) epitopes were identified in the HIV db, but in some cases no epitopes were identified. These individuals responded to both conserved and variable epitopes and of those more than half of the responding epitopes were variable. However, in a very few cases some of the putative epitopes were associated with more than one allele in the HIV db so it was not possible to associate the responding epitope with its restricting HLA allele. Analysis of epitopes in the context of HLA alleles has

identified protective alleles such as B07, B13, B27, B57, B58 and B44 (Altfeld and Goulder, 2011; Migueles and Connors, 2015). Studies have shown that HIV-1 infected individuals expressing these alleles have better control of HIV-1 viral replication and undergo slower disease progression by targeting conserved Gag epitopes (Altfeld et al., 2003a; Ammaranond et al., 2010; Borthwick et al., 2014; Goulder and Watkins, 2008). In the current study, individuals expressing protective alleles such as B27 and B44 targeted both highly conserved Gag epitopes such as KK10 and less conserved epitopes such as AEQASQDVKNW (AW11), respectively. Another recipient targeted highly conserved Gag epitope TL9 known to be associated with protective alleles such as B*0702, B*8101 and B*4201 (Ammaranond et al., 2010; Geels et al., 2005; Goulder et al., 2000; Kloverpris et al., 2014; Schneidewind et al., 2008; Tang et al., 2011). This individual did not express any of the known protective alleles associated with TL9. However, these data indicate that TL9 may be associated with B*3503/Cw*0401 alleles in addition to other alleles (B*0702, B*4201 and B*8101) previously reported in the HIV db (Altfeld and Goulder, 2011; Matthews et al., 2012; Streeck et al., 2009b). Protective Ad35 GRIN/Env specific responses, as defined by broad VIA response, seem to be mediated through $CD8^+$ T cell responses circumscribed by specific HLA alleles. These would most likely target conserved regions of the virus proteome and may result in selection pressure and a fitness cost to the virus and a better disease outcome (Batorsky et al., 2014; Brockman et al., 2007; Leslie et al., 2004; Prince et al., 2012; Yue et al., 2015).

To examine $CD8^+$ T cell responses further, VIA was employed as a functional T cell assay, to establish if responding recipients were able to inhibit a set of viruses of different clades (A-D). CD8 T cells from the majority of the participants were able to inhibit more than one virus in VIA. There were significant differences between vaccinee groups in the number of viruses inhibited whereas, almost all mid group vaccinees inhibited multiple viruses. Furthermore, when VIA responses were analysed in the context of responding epitopes then there seemed to be a trend in inhibition of the viruses tested in the VIA with U455 inhibited by the majority of subjects and then in the order of IIIB, CH077, 247Fv2, CH106, ZA97012 and ELI. The frequency of inhibition detected with U455 (clade A) may be due to the higher sequence homology of U455 with Ad35 GRIN/Env vaccine insert based on clade A. Interestingly, III B (clade B virus – less sequence homology and deleted Nef) was inhibited better than ELI (clade D), regardless of the high sequence homology of ELI with Ad35 GRIN/Env insert. One possible explanation is that the IIIB isolate had defective Nef and this may have prevented CTL escape and MHC-I down regulation; however, this was not investigated here (Kopycinski et al., 2014; Mahiti et al., 2016; Rajapaksa et al., 2012; Toyoda et al., 2015). These data suggest that the sequence conservation between the vaccine insert and the virus may not entirely account for the virus control seen with B001 vaccinees. Most of the individuals inhibited >1 virus

isolate and a higher degree of inhibition was observed when the targeted epitopes were highly conserved. In contrast a, the lower degree of inhibition or no inhibition was observed when the responding epitopes were not highly conserved. The improved control of virus replication was detected in two (Low group B) recipients who mounted VIA responses of larger breadth and magnitude. Both vaccinees mounted CD8 response to gp120 epitope MHEDIISLW (MW9), which was highly conserved within all virus isolates tested apart from CH077. In contrast, Env specific $CD8^+$ T cell responses in natural infection are linked with disease progression and may not be desirable (Kiepiela et al., 2007). A previous study has suggested that Env specific antiviral activity could be beneficial to the host (Chen et al., 2011) and perhaps Env specific $CD8^+$ T cell responses could be incorporated in CTL based HIV-1 vaccine candidates (Keefer et al., 2012). Furthermore, both subjects responded through highly conserved B*27 epitope KK10 (Gag p24) and exhibited VIA responses of higher breadth. KK10 was highly conserved within all virus isolates apart from U455 (K263R), ELI (L268V) and ZA97012 (L268M). Isolate ZA97012 carrying L268M was not inhibited by either of these vaccinees and previous studies have reported that L268M is an early CTL escape mutant and may impair binding of peptides to MHC or disrupt T cell receptor recognition (Schneidewind et al., 2008; Schneidewind et al., 2007; Steers et al., 2014). In contrast, regardless of 100% sequence homology with KK10, CH0106, CH077 and 247FV2 were not inhibited by one of the subjects. This absence of inhibition may be associated with lack of KK10 presentation on the cell surface, generally governed by epitope abundance, which depends on proteasomal cleavage of proteins. Moreover, changes in the epitope flanking region can also influence epitope generation and can effect antigen processing and presentation by MHC. If proteasome activity is abrogated then it may influence CTL responses and would result in loss of viral control (Steers et al., 2014; Tenzer et al., 2009).

In summary, the use of epitope mapping and VIA demonstrated the immunogenicity of the HIV-1 vaccine insert. VIA data demonstrated that it was possible to induce potent CTL responses to inhibit multiple viruses in vitro. These data suggest there was an association between T cell responses to conserved epitopes within the panel of viruses used and the degree of inhibition in the VIA (Ahmed et al., 2016; Hancock et al., 2015; Kopycinski et al., 2014). Whether this association emulates a role for CTL in vaccine induced protective responses against HIV infection/disease cannot be gathered from this study alone. Even though the mechanism of protection for a successful vaccine may not be due solely to a $CD8^+$ T cell response *per se*, such responses may be a key component with other immune mechanisms in the control of HIV-1 infection and replication. Hence, it is critical to establish if such effective $CD8^+$ T cell responses are important when designing future HIV vaccine candidates.

Chapter 7: Concluding remarks and future work

Despite much effort, the HIV-1 pandemic is a global problem and in 2014 alone, 2.1 million new cases were reported worldwide (UNAIDS, 2014). Thirty five years after the discovery of HIV, effective prophylactic or therapeutic vaccines remain elusive. Vaccines are considered to be one of the major achievements in public health as they can globally prevent mortality and morbidity in human populations (Karch and Burkhard, 2016). The first successful vaccine that showed protection from a viral disease (smallpox) was developed from observations by Edward Jenner in the late 18th century (Smith, 2011). Since then numerous successful vaccines against both bacterial and viral infections have been developed and most of these pathogens such as polio virus, papillomavirus, Influenza, rubella, *Mycobacterium tuberculosis*, *Salmonella enterica* (Typhi) and *Vibrio cholera* infect the mucosa (Karch and Burkhard, 2016; Plotkin, 2014). Two types of immunity can be induced by vaccines: (i) protective immunity: usually protects from illness after exposure to a pathogen (for example Bacillus Calmette-Guérin vaccine against tuberculosis, MMR measles and mumps) and (ii) sterilising immunity which generally prevents infection by a pathogen (Hepatitis A/B, Rabies, HIV and Influenza) (Plotkin, 2014; Walker and Burton, 2008). A few live attenuated virus vaccines (measles, Influenza intranasal and varicella) have been shown to induce both T cells and neutralising antibodies and confer protection against infection (Belyakov and Ahlers, 2009), while others, such as the Human Papillomavirus vaccine, Hepatitis B, Rabies, and Rotavirus are thought to mediate protection solely through the induction of neutralising antibodies (Offit, 2012; Sasagawa et al., 2012; Schiller and Lowy, 2010).

HIV-1 vaccine development is more challenging than other viral vaccines largely as a result of the tremendous HIV-1 viral sequence diversity, immune dysfunction of monocytes, dissemination in dendritic cells results in viral pathogenesis, immune evasion, integration of the HIV-1 genetic material into the host genome and resultant formation of latently infected “reservoirs” (Coleman and Wu, 2009; Haqqani et al., 2015; Haynes, 2015; Hraber et al., 2014; Li et al., 2005; Ritchie et al., 2014; Virgin and Walker, 2010). The most important reasons behind lack of HIV-1 vaccine is because HIV evades and the host is unable to produce an effective immune response and thus, there is no correlate of immune protection. Furthermore, HIV sequence diversity is a major obstacle to produce an HIV vaccine that will be effective globally. There are still large gaps in our knowledge and the failure of a CTL based vaccine (Ad5-STEP trial and Vaccine Research Centre DNA/Ad5) for HIV-1 was disappointing (Buchbinder et al., 2008; Hayes et al., 2016; Huang et al., 2014) suggesting that an effective HIV-1 vaccine should induce both the humoral and cell mediated facets of the immune response (Stephenson and Barouch, 2013). In this thesis, two novel HIV-1 insert immunogens,

HIV_{consv} and GRIN, were investigated as potential vaccine candidates (Keefer et al., 2012; Letourneau et al., 2007), by assessing $CD8^+$ T cell responses to these proteins in HIV-1 infected subjects and uninfected subjects vaccinated in an IAVI-sponsored clinical trial with an Ad35 vector containing the GRIN/Env sequence.

Highly conserved regions of HIV may be immunogenically subdominant to variable regions and perhaps factors such as, expression of viral proteins, antigen processing and presentation and interactions between epitope and HLA might play some role in the immunodominance of variable epitopes (Li et al., 2011; Liu et al., 2009b; Rolland et al., 2011). The HIV_{consv} vaccine candidate encoded in Modified Vaccinia Ankara (MVA) was shown to be immunogenic in HLA-A*0201 humanized mice and HIV_{consv} peptide pools were shown to be antigenic *ex vivo*, demonstrated by short term culture of PBMC from naturally infected individuals with peptides (Letourneau et al., 2007). A recent HIV_{consv} trial demonstrated that vaccine specific $CD8^+$ T cells were polyfunctional, capable of proliferation and able to inhibit replication of several cross clade HIV-1 isolates *in vitro* (Ahmed et al., 2016; Borthwick et al., 2014), such attributes are considered to be desirable for effective HIV-1 vaccine induced T cell responses. Similarly, the GRIN immunogen was also shown to be immunogenic in a clinical trial (ClinicalTrials.gov NCT00851383). GRIN induced CTL responses were shown to be polyfunctional and inhibited multiple clades of HIV-1 *in vitro* (Keefer et al., 2012; Kopycinski et al., 2014). This project sought to extend these findings by performing a comprehensive antigenic evaluation of conserved epitope recognition and consolidate these data with HLA type and clinical parameters in order to define the extent, and perhaps limitations, of such recognition. Thus, data generated in this thesis will inform the development of vaccine candidates targeting conserved regions of HIV-1.

Quantification of HIV-1 specific $CD8^+$ T cell responses in vaccinees and infected individuals remains challenging. Previous studies have shown the ELISpot assay can be used not only to quantify T cell responses in infected individuals but, can also be used to measure the immunogenicity of prophylactic and therapeutic vaccine candidates for different diseases (Anthony and Lehmann, 2003; Han et al., 2016; Jin et al., 2013; Keefer et al., 2012; Mwau et al., 2002; Smith et al., 2001; Streeck et al., 2009a). In this thesis, IFN-γ ELISpot was validated using HIV_{consv} matrices and GRIN peptide pools to evaluate HIV-1 specific T cell responses in PBMC. The assay was shown to be highly robust, reproducible, sensitive and specific (Boaz et al., 2009; Burd, 2010; Mwau et al., 2002; Zhang et al., 2009). It was essential to define an assay positivity or cut off which was in line or in some cases lower than other studies (See Chapter 3) (Boaz et al., 2009; Dinges et al., 2010; Frahm et al., 2004; Fu et al., 2007; Samri et al., 2006). Data generated in this study also corroborate other studies describing

ELISpot assay as a valuable tool to quantify both memory $CD4^+$ and $CD8^+$ T cell responses in natural infection and vaccinees (Calarota and Baldanti, 2013; Han et al., 2016).

For HIV vaccine development it is also crucial to understand HIV-1 specific immune responses elicited in populations infected with different HIV-1 subtypes and from diverse genetic backgrounds. T cell responses in HIV-1 infected individuals were studied as a proxy for evaluating T cell cross reactivity to potential HIV-1 vaccine candidates and 71 individuals from Kenya, Uganda, Rwanda, Zambia and UK infected with different HIV-1 subtypes were tested to address this issue (See Chapter 4).

Previous studies have demonstrated that HIV-1 infected subjects can produce cross-reactive T cell responses against different clades of HIV-1 (Cao et al., 2000; Coplan et al., 2005; Ferrari et al., 1997; Giorgi et al., 2014; Gudmundsdotter et al., 2008; McKinnon et al., 2005; Watanabe et al., 2013). Similarly in this study, cross-reactivity was observed when two different peptide pools were tested using individuals infected with HIV-1 subtypes A, B, C or D. The majority of the targeted regions were in Gag, RT and INT. Overall, broad T cell cross reactivity was observed regardless of subjects disease state or use of ART. These findings showed that half of infected individuals tested recognised at least one of the HLA Class I-restricted epitopes within the HIV_{consv} panel compared to almost all recognising at least one epitope in GRIN (See Chapter 4). Overall, GRIN peptide pools were better recognised, as GRIN was based on a HIV-1 near complete (excluding Tat, Rev, Vif and Vpu) virus proteome and incorporated both conserved and variable regions whereas the HIV_{consv} insert was based just on conserved regions of the HIV-1 genome (Letourneau et al., 2007). GRIN pool responses could be the result of both conserved and variable epitopes and variable regions are also known to contain epitope rich regions in the HIV-1 proteome (Kunwar et al., 2013). However, based on the limited number of samples used to map GRIN responses, the majority of targeted GRIN epitopes were within the conserved regions of HIV-1 proteome, suggesting that conserved regions were constantly being targeted in infected subjects. Moreover, only conserved epitopes (which were present in both inserts) were able to cross recognise the peptides in the GRIN OLPs. This study exhibits effective $CD8^+$ T-cell recognition of viral infected cells via HIV-1 OLPs and cross-recognition of conserved regions by the $CD8^+$ T cells (See Chapter 5). This is consistent with another study showing that a full-length HIV-1 immunogen produced potent CTL responses towards conserved regions and those responses were comparable with an immunogen based only on conserved regions (Stephenson et al., 2012). Although the HIV_{consv} insert covers only a small percentage (8%) of the HIV-1 proteome, more than half of individuals infected with different clades responded to HIV_{consv} peptide pools, suggesting that HIV_{consv} regions are antigenic and are often recognised during different stages of the infection (Ahmed et al., 2016; Hancock et al., 2015; Mothe et al., 2015) with no obvious discrimination

between subtypes. It was presumed that individuals infected with subtype A may recognise GRIN pools better than the other subtypes as GRIN was based on subtype A sequence. However, no such difference was observed in the frequency of recognition of GRIN by subjects infected with different subtypes. Furthermore, no significant difference was observed between GRIN and HIV_{consv} recognition by subjects infected with different subtypes. This non-significant difference observed could be due to low sample number and perhaps the outcome may differ if the sample number was increased. Thus, future work should consider testing larger cohort infected with different subtypes and use a number of different peptides sets (consensus peptide sets based on A,B,C and D, mosaic, COT, HIV_{consv}, GRIN) to assess cross clade T cell responses to determine if there is any subtype bias towards different antigens.

In the absence of ART the majority of infected individuals fail to control virus replication and die within two years of the beginning of AIDS (Poorolajal et al., 2016). However, there are a groups of individuals able to control HIV without ART. Exploring the immunological characteristics underpinning this control would generate crucial information which can be used to understand HIV disease pathogenesis and inform rational vaccine insert design. From a larger cohort, a group of individuals were identified with significantly different disease outcomes (viraemic controllers (VC), viraemic progressors (VP) and chronic progressors (CP)) based on their pVL and CD4 counts (See Chapter 5). Longitudinal analysis of pVL and CD4 counts defined the control of viraemia in VC, but not in VP and virologic control was not achievable during the chronic stage of disease as shown in another study (Okulicz et al., 2009). For both VC and VP, T cell responses were measured within a median of 59 estimated days post infection. $CD8^+$ responses elicited towards HIV_{consv} sequences by the majority of VC and VP during primary infection were less frequent and lower in magnitude than previous studies (Borthwick et al., 2014; Ondondo et al., 2016) and did not correlate with the disease outcome. Epitope mapping data suggests that focusing responses towards conserved epitopes in acutely infected individuals had little impact on virus set point and CD4 counts. The lower immune responses observed in this study could be due to a number of factors such as host genetic background (HLA), sampling time post infection, small number of study subjects and the use of different peptide pools (Kunwar et al., 2013; Ondondo et al., 2016). This is in line with previous studies reporting that both ICS and ELISpot responses were poorly elicited in primary infection (Goonetilleke et al., 2009; Ondondo et al., 2016; Radebe et al., 2015; Trautmann et al., 2012). Even though GRIN pools appeared to be more antigenic but, this did not reach statistical significance when VC and VP T cell responses were compared at V1.0. Furthermore, no statistical difference was observed in HIV_{consv} IFN-γ ELISpot responses between VC and VP at two different time points (V1.0 and V12). Regardless of absent or low IFN-γ responses to HIV_{consv} sequences, VC were able to contain HIV-1 replication for

more than 24 months post infection. Moreover, small percentage of VC harbour B*57/B*58 allele but, B*57/B*58 responses were absent in these individuals and one of the potential reason could be lack of B*57/B*58 epitopes in HIV_{consv} insert. Neither the magnitude nor the breadth of antigen specific T cell responses demonstrated any correlation with reduced pVL (Addo et al., 2003; Betts et al., 2001). It is possible that the limitations of IFN-γ ELISpot as readout may contribute to the lack of association between $CD8^+$ T cell responses and pVL in early infection (Radebe et al., 2015; Streeck et al., 2014). Previous studies have shown that $CD8^+$ T cell responses were not detectable in acutely infected individuals and EC by the *ex-vivo* ELISpot, but were with a cultured ELISpot approach (Ndhlovu et al., 2012; Radebe et al., 2015). This suggests that low frequency central memory responses can be missed with *ex-vivo* ELISpot, which may be informative in HIV vaccine design. (Burgers et al., 2009; Ndhlovu et al., 2012; Radebe et al., 2015). Thus, future experiments could be performed with cultured ELISpot to detect HIV-1 specific T cell responses in acute infection to determine if VC and VP immunological status matches with virologic outcome. It is also possible that peptide matrices used in this study may have underestimated the IFN-γ responses when using peptides based on a particular sequence (Radebe et al., 2011). Other groups have reported that autologous peptides were recognised better than the non-autologous peptides and may enhance the ability to detect T cell responses i.e. magnitude, breadth and phenotype (Addo et al., 2003; Altfeld et al., 2003b; Doroudchi et al., 2012; Radebe et al., 2011). To quantify *ex-vivo* $CD8^+$ T cell responses to conserved regions more accurately, future mapping experiments could be performed with autologous peptides or peptides incorporating global potential T cell epitopes (PTE) (Kunwar et al., 2013).

There is increasing evidence that $CD8^+$ responses exert selection pressure on HIV-1 to escape (Borrow et al., 1997; Boutwell et al., 2010; Leslie et al., 2004; Troyer et al., 2009; Yue et al., 2015) and vary conserved regions which may result in a fitness cost to the virus which could be beneficial to the host (Batorsky et al., 2014; Martinez-Picado et al., 2006; Ondondo et al., 2016; Rolland et al., 2013). In this study controllers may have responded to conserved regions early in infection resulting in reduced pVL, but these responses were not detected by ELISpot assay or the virus may be less fit with reduced replication capacity resulting in lower viraemia and reducing the amount of antigen available to prime for measureable responses in PBMC (Lobritz et al., 2011; Miura et al., 2008a; Yue et al., 2015). Similarly, the majority of VP also did not elicit $CD8^+$ T cell responses to HIV_{consv} regions at an early time point. This leads the hypothesis that transmitted virus may come from individuals harbouring protective alleles (B*27, B*57, B*58) and carried escape variants to new recipients. In HLA mismatched recipients (non B*27, B*57/B*58), virus may revert back to the wild type and the hosts inability to mount potent CTL responses against wild type virus leads to higher pVL (Crawford

et al., 2009; Duda et al., 2009; Fryer et al., 2010; Nagaraja et al., 2016). Thus, CTL escape has been identified as a major hurdle in the maintenance of protective immunity against HIV-1 (Ndhlovu et al., 2015a). These findings further corroborate data from another study demonstrating that anti-viral T cell responses measured by IFN-γ secretion at an early time point were narrowly directed and of low magnitude but, are more readily detectable during chronic infection. This suggests immunogenic epitopes were not often targeted during primary infection (Radebe et al., 2011; Streeck et al., 2009b; Turnbull et al., 2009). Perhaps due to the continuous antigenic exposure, the majority of the tested VC, VP and CP had more frequently detectable HIV-1 specific $CD8^+$ T cell responses at later time points (V12 and V24) (Addo et al., 2003). However, as shown in other studies, measurable CTL responses did not have any effect on pVL in VP (V1.0 & V12) and CP (V12 & V24) suggesting impaired functional CTL responses (Addo et al., 2003; Betts et al., 2001; Cao et al., 2003) in both early and late phases of the infection. Alternatively, perhaps targeted epitopes were not beneficial to the host due to the lack of protective alleles (Elahi et al., 2011; Lopez et al., 2011). Despite the most commonly recognised (immunodominant) HIV_{consv} region being Pol (INT and RT) in this study, responses to Gag have been frequently associated with reductions in viraemia, but not responses to Pol, Env or accessory proteins (Kiepiela et al., 2007; Radebe et al., 2015; Streeck et al., 2014; Zuniga et al., 2006). In contrast, studies have associated Pol responses with better disease outcome both in EC and in chronic infection towards the HLA-B*5101 restricted RT epitope TAFTIPSI (Kuse et al., 2014; Motozono et al., 2014). Previous studies have employed VIA to demonstrate strong $CD8^+$ T cell mediated control of viral replication in the absence of ELISpot responses and demonstrated that controllers have shown greater suppressive activity than non-controllers *in vivo* (Ndhlovu et al., 2012; Ndhlovu et al., 2015b). Thus, future experiments should be performed employing VIA to investigate inhibitory capacity of low frequency memory $CD8^+$ T cell responses. Furthermore, T cell responses to HIV_{consv} epitopes were maintained at later time points for all three groups suggesting that HIV_{consv} viral sequences were preserved. Limited number of sequencing data generated in this study showed that virus sequence was preserved 24 months post infection. Therefore, in the future viral sequencing data should be generated for all the subjects and the responding epitopes at multiple time points should be compared to determine the extent of epitope conservation. Matched ELISpot data should also be generated using all three groups (VC, VP and CP) and at least three different time points (V1.0, V6.0 and V12) with both peptide sets.

ELISpot data in this study failed to show any difference in $CD8^+$ T cell responses between VC and VP upon antigen stimulation whereas, CP demonstrated broader ELISpot responses. A small number of participants with high ELISpot responses were tested by ICS to determine whether there was a functional component to control in these individuals. PBMC from these individuals were stimulated

with mapped peptides and the data were analysed by comparing VC and VP as two groups (See Chapter 5). CTL are known to kill cells infected with HIV-1 by releasing perforin and granzymes from lytic granules and this process is usually referred to as degranulation (Duvall et al., 2008; Hersperger et al., 2010). Here, VC expressed higher levels of perforin than VP suggesting that VC may have better cytotoxic potential and ability to lyse infected cells than VP, which may have a direct impact on decreasing pVL in VC. Several groups have shown a strong association between better clinical outcome and polyfunctional T cell responses. Furthermore, VC were able to degranulate and produce multiple cytokines (IFN-γ, TNF-α, and IL-2) upon antigen stimulation and some of the polyfunctional responses were statistically different from VP (Almeida et al., 2009; Betts et al., 2006; Hersperger et al., 2010; Owen et al., 2010). Due to the limited sample availability it was not possible to identify the characteristics of early $CD8^+$ T cell responses among all participants that could be associated with their disease outcome. Studies have shown that viral control is associated with central memory and intermediate memory T cell phenotypes with those with high viral loads having higher numbers of effector memory T cells (Burgers et al., 2009; Ndhlovu et al., 2012; Ndhlovu et al., 2015b). Thus, future work should investigate the role of these two memory T cell subpopulations in the two study groups and their role in disease outcome.

Previous studies have used different approaches to define conserved epitopes. The most commonly used methods to define conservation score (CS) of HIV epitopes in the field were based on (1) Shannon entropy (Bansal et al., 2005; Fontaine Costa et al., 2010), (2) Epitope prevalence scores (Li et al., 2011) and (3) conseq (Berezin et al., 2004). In this study epitopes were defined as conserved (<0.2) and variable (>0.2) based on their entropy score (Altfeld et al., 2003b; Hancock et al., 2015; Yusim et al., 2002) (See section 2.13). Most of the targeted HIV_{consv} and GRIN epitopes in HIV infected subjects were highly conserved whereas others were categorised as variable (Entropy score (ES) >0.2). Higher numbers of GRIN antigenic regions were recognised and there was no subtype bias and some of the subtype A infected subjects lacked GRIN responses (See Chapter 4). Comparison of GRIN and HIV_{consv} responses showed that even though HIV_{consv} sequences were part of the GRIN insert, the majority of deconvoluted GRIN epitopes were not the same as HIV_{consv} epitopes (See Chapter 4). Perhaps the simple explanation behind these observations is that the sequences of responding peptides/epitopes were not homologous in both antigens and differences in flanking regions and peptide sequence could affect epitope presentation and recognition (Neefjes et al., 2011; Ranasinghe et al., 2011; Steers et al., 2014). In this study HIV_{consv}-INT and GRIN-Gag sequences were frequently recognised among the three groups of participants. The most commonly targeted HIV_{consv} region was INT. INT epitopes recognised during acute and chronic infection were RKAKIIRDY (RY9) and FKRKGGIGGY (FY10) respectively, both restricted by allele B*1503.

However, responses to GRIN FY10 were hardly detected and to GRIN RY9 were absent due to sequence variations in two antigens. A previous study has shown that HLA B*1503 restricted CTL responses are common in clade C infected individuals but, are not associated with disease control in non-clade B infected individuals (Frahm et al., 2005). In contrast, two VC elicited strong $CD8^+$ T cell responses to FY10 (B*1503) and RY9 (B*1503) and maintained low pVL 12 months post infection, suggesting responses to these regions could be protective. Gag immunodominant epitope TL9 is usually restricted by HLA B7 superfamily (B*0702, B*3910, B*4201/02 B*8101) is commonly recognised in individuals from sub-Saharan Africa (subtype C) (Goulder et al., 2000; Ntale et al., 2012). Escape mutations have been associated with certain alleles incurring fitness costs to the virus usually results in a better disease outcome. In the present study, TL9 was restricted by B*0702 and B*8101 alleles and control was seen in a VC expressing B*8101 but not in others. Late escape in CD8 epitopes such as TL9 could result in a disease progression (Geldmacher et al., 2009; Goulder et al., 1997; Ntale et al., 2012; Wright et al., 2010). In addition, one VC responded to conserved Gag epitope TW10 restricted by B*5703, was only detected by GRIN Gag pools, has been associated with low pVL. This decline in viraemia could be due to an escape mutation at residue T242N which may be responsible for low replication capacity (Martinez-Picado et al., 2006; Wright et al., 2010). It was not possible to detect responses to TW10 using HIV_{consv} pools as this epitope was not incorporated in this insert and failure to target protective epitopes restricted by B57/58 alleles could result in low CD4 counts and high viraemia (Chopera et al., 2011).

Comparatively, the majority of the responding GRIN epitopes in the IAVI conducted GRIN immunogen clinical trial (B001) were variable whereas the majority of the responding GRIN epitopes in natural infection were conserved. Previous study has also raised questions regarding the immunodominace of variable epitopes in natural infection and it is believed immunodominance could be due to how proteins are processed and presented and the interactions between TCR and MHC-I (Liu et al., 2009b). Both GRIN and HIV_{consv} Env and GRIN Nef responses were not analysed in natural infection in this study due to the poor representation of Env and absence of Nef in the HIV_{consv} insert. GRIN Gag and Pol regions were recognised both in natural infection and by vaccinees. However, in HIV infected individuals GRIN Gag conserved sequences were more commonly recognised compared to GRIN vaccinees, as those frequently targeted Pol. These data also show that in natural infection GRIN Gag conserved epitopes KF11 (B*5703), TL9 (B*8101, B*4201, B*0702), ETINEEAAW (EW9-B*58), TW10-B*5703, ISPRTLNAW (IW9-B*5703), RDYVDRFYKTL (RL11-B*18 and B*1510) and INT conserved epitopes FY10 (B*1503) and KTAVQMAVF (KF9-B*5801) were frequently targeted. Responses to KF11, TW10 andTL9 have been previously associated with immune control (Altfeld et al., 2003a; Balamurugan et al., 2013; Kloverpris et al.,

2015; Miura et al., 2008b; Ntale et al., 2012; Payne et al., 2014). Even though some of the B001 participants harbour the same HLA alleles as infected individuals, none of these epitopes were recognised by vaccinees. The assumption was that vaccinees with protective alleles (B*57, B*58 and B*42) and immunised with two doses of Ad35 GRIN may induce potent memory responses against conserved epitopes recognised in natural infection. However, the majority of the GRIN conserved epitopes targeted in natural infection were not targeted by vaccinees. These data demonstrated that GRIN responses elicited in HIV positive individuals infected with different subtypes (A, C and D) were not the same as GRIN responses induced by GRIN vaccinees encoding the same alleles, measured by IFN-γ ELISpot assay using pools of peptide matrices. Thus, GRIN as an antigen, elicited conserved responses in HIV infected individuals which could be beneficial but the majority of these conserved responses were absent in HLA matched vaccinees, suggesting that GRIN was not very immunogenic in targeting conserved epitopes in immunised volunteers (See Chapter 6). However, no further tests were done to confirm responses to epitopes targeted in this study. Thus, future work could be done to identify and characterise antigen specific T cells using tetramer staining (staining T cells with a peptide MHC complex) where possible (Altman et al., 2011).

To summarise, this study was designed to provide a comprehensive assessment of HIV-1 specific T cell responses, detectable amongst HIV positive individuals and vaccinees. A small number of individuals based on their disease status were selected from a large group in a cohort infected with different subtypes. HIV-1 specific $CD8^+$ T cell responses to both study panels (HIV_{consv} and GRIN) were shown to be detectable in all three groups infected with HIV-1, but to a varying degree. These responses spanned across Gag, RT and INT of the HIV-1 proteome and were more frequently detected with GRIN pools based on the full genome containing both conserved and variable epitopes. Although sample size was low, there was no indication that infecting subtypes had an impact on modulating their $CD8^+$ T cell responses towards both panels. Furthermore, there was no evidence to support the hypothesis that pre-existing immunity towards HIV_{consv} sequences in early infection were prerequisite/responsible to define disease pathogenesis/status. In addition to this, vaccinees immunised with GRIN immunogen demonstrated the role of $CD8^+$ T cell responses (in VIA) targeting conserved regions of HIV proteome in cross clade inhibition of a panel of viruses *in vitro*. These data suggest that conserved regions were recognised both in vaccinees and in natural infection and targeting those regions could be beneficial for vaccine development. Furthermore, the HIV_{consv} antigen was shown to be immunogenic (Borthwick et al., 2014; Letourneau et al., 2007) and has recently been employed in an oligovalent mosaic design demonstrating increased breadth and depth of T cell responses (Abdul-Jawad et al., 2016). No doubt an effective HIV-1 vaccine should be one that could induce both the humoral and adaptive arms of the immune system.

Bibliography

Abdul-Jawad, S., Ondondo, B., van Hateren, A., Gardner, A., Elliott, T., Korber, B., Hanke, T., 2016. Increased Valency of Conserved-mosaic Vaccines Enhances the Breadth and Depth of Epitope Recognition. Molecular therapy : the journal of the American Society of Gene Therapy 24, 375-384.

Adachi, A., Gendelman, H.E., Koenig, S., Folks, T., Willey, R., Rabson, A., Martin, M.A., 1986. Production of acquired immunodeficiency syndrome-associated retrovirus in human and nonhuman cells transfected with an infectious molecular clone. Journal of virology 59, 284-291.

Addo, M.M., Yu, X.G., Rathod, A., Cohen, D., Eldridge, R.L., Strick, D., Johnston, M.N., Corcoran, C., Wurcel, A.G., Fitzpatrick, C.A., Feeney, M.E., Rodriguez, W.R., Basgoz, N., Draenert, R., Stone, D.R., Brander, C., Goulder, P.J., Rosenberg, E.S., Altfeld, M., Walker, B.D., 2003. Comprehensive epitope analysis of human immunodeficiency virus type 1 (HIV-1)-specific T-cell responses directed against the entire expressed HIV-1 genome demonstrate broadly directed responses, but no correlation to viral load. Journal of virology 77, 2081-2092.

Adland, E., Carlson, J.M., Paioni, P., Kloverpris, H., Shapiro, R., Ogwu, A., Riddell, L., Luzzi, G., Chen, F., Balachandran, T., Heckerman, D., Stryhn, A., Edwards, A., Ndung'u, T., Walker, B.D., Buus, S., Goulder, P., Matthews, P.C., 2013. Nef-specific CD8+ T cell responses contribute to HIV-1 immune control. PloS one 8, e73117.

Ahlers, J.D., 2014. All eyes on the next generation of HIV vaccines: strategies for inducing a broadly neutralizing antibody response. Discov Med 17, 187-199.

Ahmed, T., Borthwick, N.J., Gilmour, J., Hayes, P., Dorrell, L., Hanke, T., 2016. Control of HIV-1 replication in vitro by vaccine-induced human CD8(+) T cells through conserved subdominant Pol epitopes. Vaccine 34, 1215-1224.

Almeida, J.R., Sauce, D., Price, D.A., Papagno, L., Shin, S.Y., Moris, A., Larsen, M., Pancino, G., Douek, D.C., Autran, B., Saez-Cirion, A., Appay, V., 2009. Antigen sensitivity is a major determinant of CD8+ T-cell polyfunctionality and HIV-suppressive activity. Blood 113, 6351-6360.

Altfeld, M., Addo, M.M., Rosenberg, E.S., Hecht, F.M., Lee, P.K., Vogel, M., Yu, X.G., Draenert, R., Johnston, M.N., Strick, D., Allen, T.M., Feeney, M.E., Kahn, J.O., Sekaly, R.P., Levy, J.A., Rockstroh, J.K., Goulder, P.J., Walker, B.D., 2003a. Influence of HLA-B57 on clinical presentation and viral control during acute HIV-1 infection. AIDS (London, England) 17, 2581-2591.

Altfeld, M., Addo, M.M., Shankarappa, R., Lee, P.K., Allen, T.M., Yu, X.G., Rathod, A., Harlow, J., O'Sullivan, K., Johnston, M.N., Goulder, P.J., Mullins, J.I., Rosenberg, E.S., Brander, C., Korber, B., Walker, B.D., 2003b. Enhanced detection of human immunodeficiency virus type 1-specific T-cell responses to highly variable regions by using peptides based on autologous virus sequences. Journal of virology 77, 7330-7340.

Altfeld, M., Goulder, P.J., 2011. The STEP Study Provides a Hint That Vaccine Induction of the Right CD8+ T Cell Responses Can Facilitate Immune Control of HIV. Journal of Infectious Diseases 203, 753-755.

Altman, J.D., Moss, P.A., Goulder, P.J., Barouch, D.H., McHeyzer-Williams, M.G., Bell, J.I., McMichael, A.J., Davis, M.M., 2011. Phenotypic analysis of antigen-specific T lymphocytes. Science. 1996. 274: 94-96. Journal of immunology (Baltimore, Md. : 1950) 187, 7-9.

Amara, R.R., Robinson, H.L., 2002. A new generation of HIV vaccines. Trends Mol Med 8, 489-495.

Ammaranond, P., van Bockel, D.J., Petoumenos, K., McMurchie, M., Finlayson, R., Middleton, M.G., Davenport, M.P., Venturi, V., Suzuki, K., Gelgor, L., Kaldor, J.M., Cooper, D.A., Kelleher, A.D., 2010. HIV Immune Escape at an Immunodominant Epitope in HLA-B*27-Positive Individuals Predicts Viral Load Outcome. The Journal of Immunology 186, 479-488.

Amornkul, P.N., Karita, E., Kamali, A., Rida, W.N., Sanders, E.J., Lakhi, S., Price, M.A., Kilembe, W., Cormier, E., Anzala, O., Latka, M.H., Bekker, L.G., Allen, S.A., Gilmour, J., Fast, P.E., 2013. Disease progression by infecting HIV-1 subtype in a seroconverter cohort in sub-Saharan Africa. AIDS (London, England) 27, 2775-2786.

Ananworanich, J., Sacdalan, C.P., Pinyakorn, S., Chomont, N., de Souza, M., Luekasemsuk, T., Schuetz, A., Krebs, S.J., Dewar, R., Jagodzinski, L., Ubolyam, S., Trichavaroj, R., Tovanabutra, S., Spudich, S., Valcour, V., Sereti, I., Michael, N., Robb, M., Phanuphak, P., Kim, J.H., Phanuphak, N., 2016. Virological and immunological characteristics of HIV-infected individuals at the earliest stage of infection. Journal of virus eradication 2, 43-48.

Anthony, D.D., Lehmann, P.V., 2003. T-cell epitope mapping using the ELISPOT approach. Methods (San Diego, Calif.) 29, 260-269.

Anthony, R.M., Urban, J.F., Alem, F., Hamed, H.A., Rozo, C.T., Boucher, J.L., Van Rooijen, N., Gause, W.C., 2006. Memory TH2 cells induce alternatively activated macrophages to mediate protection against nematode parasites. Nat Med 12, 955-960.

Appay, V., Nixon, D.F., Donahoe, S.M., Gillespie, G.M., Dong, T., King, A., Ogg, G.S., Spiegel, H.M., Conlon, C., Spina, C.A., Havlir, D.V., Richman, D.D., Waters, A., Easterbrook, P., McMichael, A.J., Rowland-Jones, S.L., 2000. HIV-specific CD8(+) T cells produce antiviral cytokines but are impaired in cytolytic function. The Journal of experimental medicine 192, 63-75.

Arnott, A., Jardine, D., Wilson, K., Gorry, P.R., Merlin, K., Grey, P., Law, M.G., Dax, E.M., Kelleher, A.D., Smith, D.E., McPhee, D.A., 2010. High viral fitness during acute HIV-1 infection. PloS one 5.

Baker, B.M., Block, B.L., Rothchild, A.C., Walker, B.D., 2009. Elite control of HIV infection: implications for vaccine design. Expert opinion on biological therapy 9, 55-69.

Balamurugan, A., Ali, A., Boucau, J., Le Gall, S., Ng, H.L., Yang, O.O., 2013. HIV-1 gag cytotoxic T lymphocyte epitopes vary in presentation kinetics relative to HLA class I downregulation. Journal of virology 87, 8726-8734.

Ball, S.C., Abraha, A., Collins, K.R., Marozsan, A.J., Baird, H., Quinones-Mateu, M.E., Penn-Nicholson, A., Murray, M., Richard, N., Lobritz, M., Zimmerman, P.A., Kawamura, T., Blauvelt, A., Arts, E.J., 2003. Comparing the ex vivo fitness of CCR5-tropic human immunodeficiency virus type 1 isolates of subtypes B and C. Journal of virology 77, 1021-1038.

Balter, M., 1997. How does HIV overcome the body's T cell bodyguards? Science (New York, N.Y.) 278, 1399-1400.

Bansal, A., Gough, E., Sabbaj, S., Ritter, D., Yusim, K., Sfakianos, G., Aldrovandi, G., Kaslow, R.A., Wilson, C.M., Mulligan, M.J., Kilby, J.M., Goepfert, P.A., 2005. CD8 T-cell responses in early HIV-1 infection are skewed towards high entropy peptides. AIDS (London, England) 19, 241-250.

Barouch, D.H., Korber, B., 2010. HIV-1 Vaccine Development After STEP. Annual Review of Medicine 61, 153-167.

Barouch, D.H., Liu, J., Peter, L., Abbink, P., Iampietro, M.J., Cheung, A., Alter, G., Chung, A., Dugast, A.S., Frahm, N., McElrath, M.J., Wenschuh, H., Reimer, U., Seaman, M.S., Pau, M.G., Weijtens, M., Goudsmit, J., Walsh, S.R., Dolin, R., Baden, L.R., 2013a. Characterization of humoral and cellular immune responses elicited by a recombinant adenovirus serotype 26 HIV-1 Env vaccine in healthy adults (IPCAVD 001). The Journal of infectious diseases 207, 248-256.

Barouch, D.H., O'Brien, K.L., Simmons, N.L., King, S.L., Abbink, P., Maxfield, L.F., Sun, Y.-H., La Porte, A., Riggs, A.M., Lynch, D.M., Clark, S.L., Backus, K., Perry, J.R., Seaman, M.S., Carville, A., Mansfield, K.G., Szinger, J.J., Fischer, W., Muldoon, M., Korber, B., 2010. Mosaic HIV-1 vaccines expand the breadth and depth of cellular immune responses in rhesus monkeys. Nature Medicine 16, 319-323.

Barouch, D.H., Stephenson, K.E., Borducchi, E.N., Smith, K., Stanley, K., McNally, A.G., Liu, J., Abbink, P., Maxfield, L.F., Seaman, M.S., Dugast, A.S., Alter, G., Ferguson, M., Li, W., Earl, P.L., Moss, B., Giorgi, E.E., Szinger, J.J., Eller, L.A., Billings, E.A., Rao, M., Tovanabutra, S., Sanders-Buell, E., Weijtens, M., Pau, M.G., Schuitemaker, H., Robb, M.L., Kim, J.H., Korber, B.T., Michael, N.L., 2013b. Protective efficacy of a global HIV-1 mosaic vaccine against heterologous SHIV challenges in rhesus monkeys. Cell 155, 531-539.

Barre-Sinoussi, F., Chermann, J.C., Rey, F., Nugeyre, M.T., Chamaret, S., Gruest, J., Dauguet, C., Axler-Blin, C., Vezinet-Brun, F., Rouzioux, C., Rozenbaum, W., Montagnier, L., 1983. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science (New York, N.Y.) 220, 868-871.

Batorsky, R., Sergeev, R.A., Rouzine, I.M., 2014. The Route of HIV Escape from Immune Response Targeting Multiple Sites Is Determined by the Cost-Benefit Tradeoff of Escape Mutations. PLoS computational biology 10, e1003878.

Bell, M.J., Burrows, J.M., Brennan, R., Miles, J.J., Tellam, J., McCluskey, J., Rossjohn, J., Khanna, R., Burrows, S.R., 2009. The peptide length specificity of some HLA class I alleles is very broad and includes peptides of up to 25 amino acids in length. Molecular immunology 46, 1911-1917.

Belyakov, I.M., Ahlers, J.D., 2009. What Role Does the Route of Immunization Play in the Generation of Protective Immunity against Mucosal Pathogens? The Journal of Immunology 183, 6883-6892.

Bennett, M.S., Ng, H.L., Ali, A., Yang, O.O., 2008. Cross-clade detection of HIV-1-specific cytotoxic T lymphocytes does not reflect cross-clade antiviral activity. The Journal of infectious diseases 197, 390-397.

Berezin, C., Glaser, F., Rosenberg, J., Paz, I., Pupko, T., Fariselli, P., Casadio, R., Ben-Tal, N., 2004. ConSeq: the identification of functionally and structurally important residues in protein sequences. Bioinformatics (Oxford, England) 20, 1322-1324.

Berkhoff, E.G., de Wit, E., Geelhoed-Mieras, M.M., Boon, A.C., Symons, J., Fouchier, R.A., Osterhaus, A.D., Rimmelzwaan, G.F., 2006. Fitness costs limit escape from cytotoxic T lymphocytes by influenza A viruses. Vaccine 24, 6594-6596.

Betts, M.R., Ambrozak, D.R., Douek, D.C., Bonhoeffer, S., Brenchley, J.M., Casazza, J.P., Koup, R.A., Picker, L.J., 2001. Analysis of Total Human Immunodeficiency Virus (HIV)-Specific CD4+

and CD8+ T-Cell Responses: Relationship to Viral Load in Untreated HIV Infection. Journal of virology 75, 11983-11991.

Betts, M.R., Krowka, J., Santamaria, C., Balsamo, K., Gao, F., Mulundu, G., Luo, C., N'Gandu, N., Sheppard, H., Hahn, B.H., Allen, S., Frelinger, J.A., 1997. Cross-clade human immunodeficiency virus (HIV)-specific cytotoxic T-lymphocyte responses in HIV-infected Zambians. J Virol 71, 8908-8911.

Betts, M.R., Nason, M.C., West, S.M., De Rosa, S.C., Migueles, S.A., Abraham, J., Lederman, M.M., Benito, J.M., Goepfert, P.A., Connors, M., Roederer, M., Koup, R.A., 2006. HIV nonprogressors preferentially maintain highly functional HIV-specific CD8+ T cells. Blood 107, 4781-4789.

Bihl, F., Frahm, N., Di Giammarino, L., Sidney, J., John, M., Yusim, K., Woodberry, T., Sango, K., Hewitt, H.S., Henry, L., Linde, C.H., Chisholm, J.V., 3rd, Zaman, T.M., Pae, E., Mallal, S., Walker, B.D., Sette, A., Korber, B.T., Heckerman, D., Brander, C., 2006. Impact of HLA-B alleles, epitope binding affinity, functional avidity, and viral coinfection on the immunodominance of virus-specific CTL responses. Journal of immunology (Baltimore, Md. : 1950) 176, 4094-4101.

Blankson, J.N., 2010. Effector mechanisms in HIV-1 infected elite controllers: Highly active immune responses? Antiviral research 85, 295-302.

Boaz, M.J., Hayes, P., Tarragona, T., Seamons, L., Cooper, A., Birungi, J., Kitandwe, P., Semaganda, A., Kaleebu, P., Stevens, G., Anzala, O., Farah, B., Ogola, S., Indangasi, J., Mhlanga, P., Van Eeden, M., Thakar, M., Pujari, A., Mishra, S., Goonetilleke, N., Moore, S., Mahmoud, A., Sathyamoorthy, P., Mahalingam, J., Narayanan, P.R., Ramanathan, V.D., Cox, J.H., Dally, L., Gill, D.K., Gilmour, J., 2009. Concordant proficiency in measurement of T-cell immunity in human immunodeficiency virus vaccine clinical trials by peripheral blood mononuclear cell and enzyme-linked immunospot assays in laboratories from three continents. Clin Vaccine Immunol 16, 147-155.

Borrow, P., 2011. Innate immunity in acute HIV-1 infection. Current opinion in HIV and AIDS 6, 353-363.

Borrow, P., Lewicki, H., Hahn, B.H., Shaw, G.M., Oldstone, M.B., 1994. Virus-specific CD8+ cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 infection. Journal of virology 68, 6103-6110.

Borrow, P., Lewicki, H., Wei, X., Horwitz, M.S., Peffer, N., Meyers, H., Nelson, J.A., Gairin, J.E., Hahn, B.H., Oldstone, M.B., Shaw, G.M., 1997. Antiviral pressure exerted by HIV-1-specific cytotoxic T lymphocytes (CTLs) during primary infection demonstrated by rapid selection of CTL escape virus. Nat Med 3, 205-211.

Borthwick, N., Ahmed, T., Ondondo, B., Hayes, P., Rose, A., Ebrahimsa, U., Hayton, E.J., Black, A., Bridgeman, A., Rosario, M., Hill, A.V., Berrie, E., Moyle, S., Frahm, N., Cox, J., Colloca, S., Nicosia, A., Gilmour, J., McMichael, A.J., Dorrell, L., Hanke, T., 2014. Vaccine-elicited human T cells recognizing conserved protein regions inhibit HIV-1. Molecular therapy : the journal of the American Society of Gene Therapy 22, 464-475.

Boutwell, Christian L., Rolland, Morgane M., Herbeck, Joshua T., Mullins, James I., Allen, Todd M., 2010. Viral Evolution and Escape during Acute HIV-1 Infection. The Journal of infectious diseases 202, S309-S314.

Brander, C., Goulder, P.J., Luzuriaga, K., Yang, O.O., Hartman, K.E., Jones, N.G., Walker, B.D., Kalams, S.A., 1999. Persistent HIV-1-specific CTL clonal expansion despite high viral burden post in utero HIV-1 infection. Journal of immunology (Baltimore, Md. : 1950) 162, 4796-4800.

Brandstadter, J.D., Yang, Y., 2011. Natural killer cell responses to viral infection. Journal of innate immunity 3, 274-279.

Brenchley, J.M., Ruff, L.E., Casazza, J.P., Koup, R.A., Price, D.A., Douek, D.C., 2006. Preferential infection shortens the life span of human immunodeficiency virus-specific CD4+ T cells in vivo. Journal of virology 80, 6801-6809.

Brockman, M.A., Schneidewind, A., Lahaie, M., Schmidt, A., Miura, T., DeSouza, I., Ryvkin, F., Derdeyn, C.A., Allen, S., Hunter, E., Mulenga, J., Goepfert, P.A., Walker, B.D., Allen, T.M., 2007. Escape and Compensation from Early HLA-B57-Mediated Cytotoxic T-Lymphocyte Pressure on Human Immunodeficiency Virus Type 1 Gag Alter Capsid Interactions with Cyclophilin A. Journal of virology 81, 12608-12618.

Brown, L.E., Kelso, A., 2009. Prospects for an influenza vaccine that induces cross-protective cytotoxic T lymphocytes. Immunology and cell biology 87, 300-308.

Buchbinder, S.P., Mehrotra, D.V., Duerr, A., Fitzgerald, D.W., Mogg, R., Li, D., Gilbert, P.B., Lama, J.R., Marmor, M., Del Rio, C., McElrath, M.J., Casimiro, D.R., Gottesdiener, K.M., Chodakewitz, J.A., Corey, L., Robertson, M.N., 2008. Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. Lancet 372, 1881-1893.

Burd, E.M., 2010. Validation of laboratory-developed molecular assays for infectious diseases. Clin Microbiol Rev 23, 550-576.

Burgener, A., McGowan, I., Klatt, N.R., 2015. HIV and mucosal barrier interactions: consequences for transmission and pathogenesis. Current opinion in immunology 36, 22-30.

Burgers, W.A., Riou, C., Mlotshwa, M., Maenetje, P., de Assis Rosa, D., Brenchley, J., Mlisana, K., Douek, D.C., Koup, R., Roederer, M., de Bruyn, G., Karim, S.A., Williamson, C., Gray, C.M., 2009. Association of HIV-specific and total CD8+ T memory phenotypes in subtype C HIV-1 infection with viral set point. Journal of immunology (Baltimore, Md. : 1950) 182, 4751-4761.

Calarota, S.A., Baldanti, F., 2013. Enumeration and characterization of human memory T cells by enzyme-linked immunospot assays. Clinical & developmental immunology 2013, 637649.

Cao, H., Kaleebu, P., Hom, D., Flores, J., Agrawal, D., Jones, N., Serwanga, J., Okello, M., Walker, C., Sheppard, H., El-Habib, R., Klein, M., Mbidde, E., Mugyenyi, P., Walker, B., Ellner, J., Mugerwa, R., 2003. Immunogenicity of a recombinant human immunodeficiency virus (HIV)-canarypox vaccine in HIV-seronegative Ugandan volunteers: results of the HIV Network for Prevention Trials 007 Vaccine Study. The Journal of infectious diseases 187, 887-895.

Cao, H., Kanki, P., Sankale, J.L., Dieng-Sarr, A., Mazzara, G.P., Kalams, S.A., Korber, B., Mboup, S., Walker, B.D., 1997. Cytotoxic T-lymphocyte cross-reactivity among different human immunodeficiency virus type 1 clades: implications for vaccine development. J Virol 71, 8615-8623.

Cao, H., Mani, I., Vincent, R., Mugerwa, R., Mugyenyi, P., Kanki, P., Ellner, J., Walker, B.D., 2000. Cellular immunity to human immunodeficiency virus type 1 (HIV-1) clades: relevance to HIV-1 vaccine trials in Uganda. The Journal of infectious diseases 182, 1350-1356.

Cao, H., Walker, B.D., 2000. Immunopathogenesis of HIV-1 infection. Clin Dermatol 18, 401-410.

Carlson, J.M., Brumme, Z.L., 2008. HIV evolution in response to HLA-restricted CTL selection pressures: a population-based perspective. Microbes and Infection 10, 455-461.

Carrington, M., 1999. HLA and HIV-1: Heterozygote Advantage and B*35-Cw*04 Disadvantage. Science (New York, N.Y.) 283, 1748-1752.

Carvalho, L.H., Hafalla, J.C., Zavala, F., 2001. ELISPOT assay to measure antigen-specific murine CD8(+) T cell responses. Journal of immunological methods 252, 207-218.

Casartelli, N., Sourisseau, M., Feldmann, J., Guivel-Benhassine, F., Mallet, A., Marcelin, A.G., Guatelli, J., Schwartz, O., 2010. Tetherin restricts productive HIV-1 cell-to-cell transmission. PLoS pathogens 6, e1000955.

Casazza, J.P., Betts, M.R., Picker, L.J., Koup, R.A., 2001. Decay kinetics of human immunodeficiency virus-specific CD8+ T cells in peripheral blood after initiation of highly active antiretroviral therapy. Journal of virology 75, 6508-6516.

Cellerai, C., Harari, A., Stauss, H., Yerly, S., Geretti, A.M., Carroll, A., Yee, T., Ainsworth, J., Williams, I., Sweeney, J., Freedman, A., Johnson, M., Pantaleo, G., Kinloch-de Loes, S., 2011. Early and prolonged antiretroviral therapy is associated with an HIV-1-specific T-cell profile comparable to that of long-term non-progressors. PloS one 6, e18164.

Chakraborty, S., Rahman, T., Chakravorty, R., 2014. Characterization of the Protective HIV-1 CTL Epitopes and the Corresponding HLA Class I Alleles: A Step towards Designing CTL Based HIV-1 Vaccine. Adv Virol 2014, 321974.

Chang, J.J., Altfeld, M., 2010. Innate immune activation in primary HIV-1 infection. The Journal of infectious diseases 202 Suppl 2, S297-301.

Chen, D.Y., Balamurugan, A., Ng, H.L., Yang, O.O., 2011. Antiviral activity of human immunodeficiency virus type 1 Gag-specific cytotoxic T lymphocyte targeting is not necessarily intrinsically superior to envelope targeting. Journal of virology 85, 2474-2478.

Chen, H., Ndhlovu, Z.M., Liu, D., Porter, L.C., Fang, J.W., Darko, S., Brockman, M.A., Miura, T., Brumme, Z.L., Schneidewind, A., Piechocka-Trocha, A., Cesa, K.T., Sela, J., Cung, T.D., Toth, I., Pereyra, F., Yu, X.G., Douek, D.C., Kaufmann, D.E., Allen, T.M., Walker, B.D., 2012. TCR clonotypes modulate the protective effect of HLA class I molecules in HIV-1 infection. Nature immunology 13, 691-700.

Cheney, K.M., McKnight, A., 2010. Interferon-alpha mediates restriction of human immunodeficiency virus type-1 replication in primary human macrophages at an early stage of replication. PloS one 5, e13521.

Cheng, C., Wang, L., Gall, J.G., Nason, M., Schwartz, R.M., McElrath, M.J., DeRosa, S.C., Hural, J., Corey, L., Buchbinder, S.P., Nabel, G.J., 2012. Decreased pre-existing Ad5 capsid and Ad35 neutralizing antibodies increase HIV-1 infection risk in the Step trial independent of vaccination. PloS one 7, e33969.

Cheynier, R., Gratton, S., Halloran, M., Stahmer, I., Letvin, N.L., Wain-Hobson, S., 1998. Antigenic stimulation by BCG vaccine as an in vivo driving force for SIV replication and dissemination. Nat Med 4, 421-427.

Chopera, D.R., Mlotshwa, M., Woodman, Z., Mlisana, K., de Assis Rosa, D., Martin, D.P., Abdool Karim, S., Gray, C.M., Williamson, C., 2011. Virological and immunological factors associated with HIV-1 differential disease progression in HLA-B 58:01-positive individuals. Journal of virology 85, 7070-7080.

Christie, N.M., Willer, D.O., Lobritz, M.A., Chan, J.K., Arts, E.J., Ostrowski, M.A., Cochrane, A., Luscher, M.A., MacDonald, K.S., 2009. Viral fitness implications of variation within an immunodominant CD8+ T-cell epitope of HIV-1. Virology 388, 137-146.

Cimarelli, A., Darlix, J.L., 2002. Assembling the human immunodeficiency virus type 1. Cellular and molecular life sciences : CMLS 59, 1166-1184.

Claiborne, D.T., Prince, J.L., Scully, E., Macharia, G., Micci, L., Lawson, B., Kopycinski, J., Deymier, M.J., Vanderford, T.H., Nganou-Makamdop, K., Ende, Z., Brooks, K., Tang, J., Yu, T., Lakhi, S., Kilembe, W., Silvestri, G., Douek, D., Goepfert, P.A., Price, M.A., Allen, S.A., Paiardini, M., Altfeld, M., Gilmour, J., Hunter, E., 2015. Replicative fitness of transmitted HIV-1 drives acute immune activation, proviral load in memory CD4+ T cells, and disease progression. Proceedings of the National Academy of Sciences of the United States of America 112, E1480-1489.

Cohen, M.S., Gay, C.L., Busch, M.P., Hecht, F.M., 2010. The detection of acute HIV infection. The Journal of infectious diseases 202 Suppl 2, S270-277.

Coleman, C.M., Wu, L., 2009. HIV interactions with monocytes and dendritic cells: viral latency and reservoirs. Retrovirology 6, 51.

Conway, J.M., Perelson, A.S., 2015. Post-treatment control of HIV infection. Proceedings of the National Academy of Sciences of the United States of America 112, 5467-5472.

Coplan, P.M., Gupta, S.B., Dubey, S.A., Pitisuttithum, P., Nikas, A., Mbewe, B., Vardas, E., Schechter, M., Kallas, E.G., Freed, D.C., Fu, T.M., Mast, C.T., Puthavathana, P., Kublin, J., Brown Collins, K., Chisi, J., Pendame, R., Thaler, S.J., Gray, G., McIntyre, J., Straus, W.L., Condra, J.H., Mehrotra, D.V., Guess, H.A., Emini, E.A., Shiver, J.W., 2005. Cross-reactivity of anti-HIV-1 T cell immune responses among the major HIV-1 clades in HIV-1-positive individuals from 4 continents. The Journal of infectious diseases 191, 1427-1434.

Coren, L.V., Trivett, M.T., Jain, S., Ayala, V.I., Del Prete, G.Q., Ohlen, C., Ott, D.E., 2015. Potent restriction of HIV-1 and SIVmac239 replication by African green monkey TRIM5alpha. Retrovirology 12, 11.

Corey, L., McElrath, M.J., 2010. HIV vaccines: mosaic approach to virus diversity. Nat Med 16, 268-270.

Cortes, F.H., Passaes, C.P., Bello, G., Teixeira, S.L., Vorsatz, C., Babic, D., Sharkey, M., Grinsztejn, B., Veloso, V., Stevenson, M., Morgado, M.G., 2015. HIV controllers with different viral load cutoff levels have distinct virologic and immunologic profiles. Journal of acquired immune deficiency syndromes (1999) 68, 377-385.

Crawford, H., Lumm, W., Leslie, A., Schaefer, M., Boeras, D., Prado, J.G., Tang, J., Farmer, P., Ndung'u, T., Lakhi, S., Gilmour, J., Goepfert, P., Walker, B.D., Kaslow, R., Mulenga, J., Allen, S., Goulder, P.J.R., Hunter, E., 2009. Evolution of HLA-B*5703 HIV-1 escape mutations in HLA-B*5703-positive individuals and their transmission recipients. Journal of Experimental Medicine.

Crawford, H., Prado, J.G., Leslie, A., Hue, S., Honeyborne, I., Reddy, S., van der Stok, M., Mncube, Z., Brander, C., Rousseau, C., Mullins, J.I., Kaslow, R., Goepfert, P., Allen, S., Hunter, E., Mulenga, J., Kiepiela, P., Walker, B.D., Goulder, P.J.R., 2007. Compensatory Mutation Partially Restores Fitness and Delays Reversion of Escape Mutation within the Immunodominant HLA-B*5703-Restricted Gag Epitope in Chronic Human Immunodeficiency Virus Type 1 Infection. Journal of virology 81, 8346-8351.

Culshaw, A., Dong, T., Rowland-Jones, S., 2012. A two amino acid shift in position leads to a substantial difference in the pattern of processing of two HIV-1 epitopes. Journal of acquired immune deficiency syndromes (1999).

Currier, J.R., deSouza, M., Chanbancherd, P., Bernstein, W., Birx, D.L., Cox, J.H., 2002. Comprehensive screening for human immunodeficiency virus type 1 subtype-specific CD8 cytotoxic T lymphocytes and definition of degenerate epitopes restricted by HLA-A0207 and -C(W)0304 alleles. Journal of virology 76, 4971-4986.

Dai, J., Agosto, L.M., Baytop, C., Yu, J.J., Pace, M.J., Liszewski, M.K., O'Doherty, U., 2009. Human immunodeficiency virus integrates directly into naive resting CD4+ T cells but enters naive cells less efficiently than memory cells. Journal of virology 83, 4528-4537.

Dale, C.J., De Rose, R., Stratov, I., Chea, S., Montefiori, D.C., Thomson, S., Ramshaw, I.A., Coupar, B.E., Boyle, D.B., Law, M., Kent, S.J., 2004. Efficacy of DNA and fowlpox virus priming/boosting vaccines for simian/human immunodeficiency virus. Journal of virology 78, 13819-13828.

Davenport, M.P., Loh, L., Petravic, J., Kent, S.J., 2008. Rates of HIV immune escape and reversion: implications for vaccination. Trends in microbiology 16, 561-566.

Davis, Z.B., Cogswell, A., Scott, H., Mertsching, A., Boucau, J., Wambua, D., Le Gall, S., Planelles, V., Campbell, K.S., Barker, E., 2016. A Conserved HIV-1-Derived Peptide Presented by HLA-E Renders Infected T-cells Highly Susceptible to Attack by NKG2A/CD94-Bearing Natural Killer Cells. PLoS pathogens 12, e1005421.

De Clercq, E., 2010. Antiretroviral drugs. Current Opinion in Pharmacology 10, 507-515.

Deeks, S.G., Kitchen, C.M., Liu, L., Guo, H., Gascon, R., Narvaez, A.B., Hunt, P., Martin, J.N., Kahn, J.O., Levy, J., McGrath, M.S., Hecht, F.M., 2004. Immune activation set point during early HIV infection predicts subsequent CD4+ T-cell changes independent of viral load. Blood 104, 942-947.

Demberg, T., Robert-Guroff, M., 2012. Controlling the HIV/AIDS epidemic: current status and global challenges. Frontiers in immunology 3, 250.

Dempsey, P.W., Vaidya, S.A., Cheng, G., 2003. The art of war: Innate and adaptive immune responses. Cellular and molecular life sciences : CMLS 60, 2604-2621.

Deng, H., Liu, R., Ellmeier, W., Choe, S., Unutmaz, D., Burkhart, M., Di Marzio, P., Marmon, S., Sutton, R.E., Hill, C.M., Davis, C.B., Peiper, S.C., Schall, T.J., Littman, D.R., Landau, N.R., 1996. Identification of a major co-receptor for primary isolates of HIV-1. Nature 381, 661-666.

Dinges, W.L., Richardt, J., Friedrich, D., Jalbert, E., Liu, Y., Stevens, C.E., Maenza, J., Collier, A.C., Geraghty, D.E., Smith, J., Moodie, Z., Mullins, J.I., McElrath, M.J., Horton, H., 2010. Virus-Specific CD8+ T-Cell Responses Better Define HIV Disease Progression than HLA Genotype. Journal of virology 84, 4461-4468.

Doroudchi, M., Yegorov, O., Baumgartner, T., Kernaleguen, A.E., Breton, G., Ndongala, M.L., Boulassel, M.R., Routy, J.P., Bernard, N.F., Sekaly, R.P., Yassine-Diab, B., 2012. Autologous HIV-1 clade-B Nef peptides elicit increased frequency, breadth and function of CD8+ T-cells compared to consensus peptides. PloS one 7, e49562.

Douek, D.C., Brenchley, J.M., Betts, M.R., Ambrozak, D.R., Hill, B.J., Okamoto, Y., Casazza, J.P., Kuruppu, J., Kunstman, K., Wolinsky, S., Grossman, Z., Dybul, M., Oxenius, A., Price, D.A., Connors, M., Koup, R.A., 2002. HIV preferentially infects HIV-specific CD4+ T cells. Nature 417, 95-98.

Douek, D.C., McFarland, R.D., Keiser, P.H., Gage, E.A., Massey, J.M., Haynes, B.F., Polis, M.A., Haase, A.T., Feinberg, M.B., Sullivan, J.L., Jamieson, B.D., Zack, J.A., Picker, L.J., Koup, R.A., 1998. Changes in thymic function with age and during the treatment of HIV infection. Nature 396, 690-695.

Douek, D.C., Roederer, M., Koup, R.A., 2009. Emerging Concepts in the Immunopathogenesis of AIDS*. Annual Review of Medicine 60, 471-484.

Dowling, W.E., Kim, B., Mason, C.J., Wasunna, K.M., Alam, U., Elson, L., Birx, D.L., Robb, M.L., McCutchan, F.E., Carr, J.K., 2002. Forty-one near full-length HIV-1 sequences from Kenya reveal an epidemic of subtype A and A-containing recombinants. AIDS (London, England) 16, 1809-1820.

Duda, A., Lee-Turner, L., Fox, J., Robinson, N., Dustan, S., Kaye, S., Fryer, H., Carrington, M., McClure, M., McLean, A.R., Fidler, S., Weber, J., Phillips, R.E., Frater, A.J., 2009. HLA-associated clinical progression correlates with epitope reversion rates in early human immunodeficiency virus infection. Journal of virology 83, 1228-1239.

Duesberg, P.H., 1989. Human immunodeficiency virus and acquired immunodeficiency syndrome: correlation but not causation. Proceedings of the National Academy of Sciences of the United States of America 86, 755-764.

Duggal, N.K., Emerman, M., 2012. Evolutionary conflicts between viruses and restriction factors shape immunity. Nature reviews. Immunology 12, 687-695.

Duvall, M.G., Precopio, M.L., Ambrozak, D.A., Jaye, A., McMichael, A.J., Whittle, H.C., Roederer, M., Rowland-Jones, S.L., Koup, R.A., 2008. Polyfunctional T cell responses are a hallmark of HIV-2 infection. European journal of immunology 38, 350-363.

Edlefsen, P.T., Gilbert, P.B., Rolland, M., 2013. Sieve analysis in HIV-1 vaccine efficacy trials. Current opinion in HIV and AIDS 8, 432-436.

Edlefsen, P.T., Rolland, M., Hertz, T., Tovanabutra, S., Gartland, A.J., deCamp, A.C., Magaret, C.A., Ahmed, H., Gottardo, R., Juraska, M., McCoy, C., Larsen, B.B., Sanders-Buell, E., Carrico, C., Menis, S., Kijak, G.H., Bose, M., Arroyo, M.A., O'Connell, R.J., Nitayaphan, S., Pitisuttithum, P., Kaewkungwal, J., Rerks-Ngarm, S., Robb, M.L., Kirys, T., Georgiev, I.S., Kwong, P.D., Scheffler, K., Pond, S.L., Carlson, J.M., Michael, N.L., Schief, W.R., Mullins, J.I., Kim, J.H., Gilbert, P.B., 2015. Comprehensive sieve analysis of breakthrough HIV-1 sequences in the RV144 vaccine efficacy trial. PLoS computational biology 11, e1003973.

Edwards, B.H., Bansal, A., Sabbaj, S., Bakari, J., Mulligan, M.J., Goepfert, P.A., 2002. Magnitude of functional CD8+ T-cell responses to the gag protein of human immunodeficiency virus type 1 correlates inversely with viral load in plasma. Journal of virology 76, 2298-2305.

Elahi, S., Dinges, W.L., Lejarcegui, N., Laing, K.J., Collier, A.C., Koelle, D.M., McElrath, M.J., Horton, H., 2011. Protective HIV-specific CD8+ T cells evade Treg cell suppression. Nat Med 17, 989-995.

Emu, B., Sinclair, E., Hatano, H., Ferre, A., Shacklett, B., Martin, J.N., McCune, J.M., Deeks, S.G., 2008. HLA class I-restricted T-cell responses may contribute to the control of human immunodeficiency virus infection, but such responses are not always necessary for long-term virus control. Journal of virology 82, 5398-5407.

Evans, D.T., Silvestri, G., 2013. Nonhuman primate models in AIDS research. Current opinion in HIV and AIDS 8, 255-261.

Farber, D.L., Yudanin, N.A., Restifo, N.P., 2014. Human memory T cells: generation, compartmentalization and homeostasis. Nature reviews. Immunology 14, 24-35.

Fauriat, C., Long, E.O., Ljunggren, H.G., Bryceson, Y.T., 2010. Regulation of human NK-cell cytokine and chemokine production by target cell recognition. Blood 115, 2167-2176.

Feeney, M.E., Tang, Y., Pfafferott, K., Roosevelt, K.A., Draenert, R., Trocha, A., Yu, X.G., Verrill, C., Allen, T., Moore, C., Mallal, S., Burchett, S., McIntosh, K., Pelton, S.I., St John, M.A., Hazra, R., Klenerman, P., Altfeld, M., Walker, B.D., Goulder, P.J., 2005. HIV-1 viral escape in infancy followed by emergence of a variant-specific CTL response. Journal of immunology (Baltimore, Md. : 1950) 174, 7524-7530.

Ferrando-Martinez, S., Casazza, J.P., Leal, M., Machmach, K., Munoz-Fernandez, M.A., Viciana, P., Koup, R.A., Ruiz-Mateos, E., 2012. Differential Gag-specific polyfunctional T cell maturation patterns in HIV-1 elite controllers. Journal of virology 86, 3667-3674.

Ferrari, G., Humphrey, W., McElrath, M.J., Excler, J.L., Duliege, A.M., Clements, M.L., Corey, L.C., Bolognesi, D.P., Weinhold, K.J., 1997. Clade B-based HIV-1 vaccines elicit cross-clade cytotoxic T lymphocyte reactivities in uninfected volunteers. Proceedings of the National Academy of Sciences of the United States of America 94, 1396-1401.

Ferrari, G., Korber, B., Goonetilleke, N., Liu, M.K., Turnbull, E.L., Salazar-Gonzalez, J.F., Hawkins, N., Self, S., Watson, S., Betts, M.R., Gay, C., McGhee, K., Pellegrino, P., Williams, I., Tomaras, G.D., Haynes, B.F., Gray, C.M., Borrow, P., Roederer, M., McMichael, A.J., Weinhold, K.J., 2011. Relationship between functional profile of HIV-1 specific CD8 T cells and epitope variability with the selection of escape mutants in acute HIV-1 infection. PLoS pathogens 7, e1001273.

Fiebig, E.W., Wright, D.J., Rawal, B.D., Garrett, P.E., Schumacher, R.T., Peddada, L., Heldebrant, C., Smith, R., Conrad, A., Kleinman, S.H., Busch, M.P., 2003. Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection. AIDS (London, England) 17, 1871-1879.

Fischer, W., Liao, H.X., Haynes, B.F., Letvin, N.L., Korber, B., 2008. Coping with viral diversity in HIV vaccine design: a response to Nickle et al. PLoS computational biology 4, e15; author reply e25.

Fischer, W., Perkins, S., Theiler, J., Bhattacharya, T., Yusim, K., Funkhouser, R., Kuiken, C., Haynes, B., Letvin, N.L., Walker, B.D., Hahn, B.H., Korber, B.T., 2007. Polyvalent vaccines for optimal coverage of potential T-cell epitopes in global HIV-1 variants. Nat Med 13, 100-106.

Fontaine Costa, A.I., Rao, X., Lechenadec, E., van Baarle, D., Kesmir, C., 2010. HLA-B molecules target more conserved regions of the HIV-1 proteome. AIDS (London, England) 24, 211-215.

Frahm, N., Kiepiela, P., Adams, S., Linde, C.H., Hewitt, H.S., Sango, K., Feeney, M.E., Addo, M.M., Lichterfeld, M., Lahaie, M.P., Pae, E., Wurcel, A.G., Roach, T., St John, M.A., Altfeld, M., Marincola, F.M., Moore, C., Mallal, S., Carrington, M., Heckerman, D., Allen, T.M., Mullins, J.I., Korber, B.T., Goulder, P.J.R., Walker, B.D., Brander, C., 2005. Control of human immunodeficiency virus replication by cytotoxic T lymphocytes targeting subdominant epitopes. Nature immunology 7, 173-178.

Frahm, N., Korber, B.T., Adams, C.M., Szinger, J.J., Draenert, R., Addo, M.M., Feeney, M.E., Yusim, K., Sango, K., Brown, N.V., SenGupta, D., Piechocka-Trocha, A., Simonis, T., Marincola, F.M., Wurcel, A.G., Stone, D.R., Russell, C.J., Adolf, P., Cohen, D., Roach, T., StJohn, A., Khatri, A., Davis, K., Mullins, J., Goulder, P.J.R., Walker, B.D., Brander, C., 2004. Consistent Cytotoxic-T-Lymphocyte Targeting of Immunodominant Regions in Human Immunodeficiency Virus across Multiple Ethnicities. Journal of virology 78, 2187-2200.

Frahm, N., Nickle, D.C., Linde, C.H., Cohen, D.E., Zuniga, R., Lucchetti, A., Roach, T., Walker, B.D., Allen, T.M., Korber, B.T., Mullins, J.I., Brander, C., 2008. Increased detection of HIV-specific T cell responses by combination of central sequences with comparable immunogenicity. AIDS (London, England) 22, 447-456.

Frankel, A.D., Young, J.A., 1998. HIV-1: fifteen proteins and an RNA. Annu Rev Biochem 67, 1-25.

Freed, E.O., 2001. HIV-1 replication. Somat Cell Mol Genet 26, 13-33.

Freel, S.A., Picking, R.A., Ferrari, G., Ding, H., Ochsenbauer, C., Kappes, J.C., Kirchherr, J.L., Soderberg, K.A., Weinhold, K.J., Cunningham, C.K., Denny, T.N., Crump, J.A., Cohen, M.S., McMichael, A.J., Haynes, B.F., Tomaras, G.D., 2012. Initial HIV-1 antigen-specific CD8+ T cells in acute HIV-1 infection inhibit transmitted/founder virus replication. Journal of virology 86, 6835-6846.

Friedman-Kien, A.E., Saltzman, B.R., 1990. Clinical manifestations of classical, endemic African, and epidemic AIDS-associated Kaposi's sarcoma. J Am Acad Dermatol 22, 1237-1250.

Fryer, H.R., Frater, J., Duda, A., Roberts, M.G., Phillips, R.E., McLean, A.R., 2010. Modelling the evolution and spread of HIV immune escape mutants. PLoS pathogens 6, e1001196.

Fu, T.-M., Dubey, S.A., Mehrotra, D.V., Freed, D.C., Trigona, W.L., Adams-Muhler, L., Clair, J.H., Evans, T.G., Steigbigel, R., Jacobson, J.M., Goepfert, P.A., Mulligan, M.J., Kalams, S.A., Rinaldo, C., Zhu, L., Cox, K.S., Guan, L., Long, R., Persaud, N., Caulfield, M.J., Sadoff, J.C., Emini, E.A., Thaler, S., Shiver, J.W., 2007. Evaluation of Cellular Immune Responses in Subjects Chronically Infected with HIV Type 1. AIDS research and human retroviruses 23, 67-76.

Furman, P.A., Fyfe, J.A., St Clair, M.H., Weinhold, K., Rideout, J.L., Freeman, G.A., Lehrman, S.N., Bolognesi, D.P., Broder, S., Mitsuya, H., et al., 1986. Phosphorylation of 3'-azido-3'-deoxythymidine and selective interaction of the 5'-triphosphate with human immunodeficiency virus reverse transcriptase. Proceedings of the National Academy of Sciences of the United States of America 83, 8333-8337.

Gallo, R., Wong-Staal, F., Montagnier, L., Haseltine, W.A., Yoshida, M., 1988. HIV/HTLV gene nomenclature. Nature 333, 504.

Gallo, R.C., Sarin, P.S., Gelmann, E.P., Robert-Guroff, M., Richardson, E., Kalyanaraman, V.S., Mann, D., Sidhu, G.D., Stahl, R.E., Zolla-Pazner, S., Leibowitch, J., Popovic, M., 1983. Isolation of

human T-cell leukemia virus in acquired immune deficiency syndrome (AIDS). Science (New York, N.Y.) 220, 865-867.

Gandhi, R.T., Walker, B.D., 2002. Immunologic control of HIV-1. Annu Rev Med 53, 149-172.

Ganser-Pornillos, B.K., Yeager, M., Sundquist, W.I., 2008. The structural biology of HIV assembly. Current Opinion in Structural Biology 18, 203-217.

Gao, X., Nelson, G.W., Karacki, P., Martin, M.P., Phair, J., Kaslow, R., Goedert, J.J., Buchbinder, S., Hoots, K., Vlahov, D., O'Brien, S.J., Carrington, M., 2001. Effect of a single amino acid change in MHC class I molecules on the rate of progression to AIDS. The New England journal of medicine 344, 1668-1675.

Garber, D.A., Silvestri, G., Feinberg, M.B., 2004. Prospects for an AIDS vaccine: three big questions, no easy answers. The Lancet Infectious Diseases 4, 397-413.

Gaschen, B., Taylor, J., Yusim, K., Foley, B., Gao, F., Lang, D., Novitsky, V., Haynes, B., Hahn, B.H., Bhattacharya, T., Korber, B., 2002. Diversity considerations in HIV-1 vaccine selection. Science (New York, N.Y.) 296, 2354-2360.

Geels, M.J., Dubey, S.A., Anderson, K., Baan, E., Bakker, M., Pollakis, G., Paxton, W.A., Shiver, J.W., Goudsmit, J., 2005. Broad Cross-Clade T-Cell Responses to Gag in Individuals Infected with Human Immunodeficiency Virus Type 1 Non-B Clades (A to G): Importance of HLA Anchor Residue Conservation. Journal of virology 79, 11247-11258.

Geldmacher, C., Metzler, I.S., Tovanabutra, S., Asher, T.E., Gostick, E., Ambrozak, D.R., Petrovas, C., Schuetz, A., Ngwenyama, N., Kijak, G., Maboko, L., Hoelscher, M., McCutchan, F., Price, D.A., Douek, D.C., Koup, R.A., 2009. Minor viral and host genetic polymorphisms can dramatically impact the biologic outcome of an epitope-specific CD8 T-cell response. Blood 114, 1553-1562.

Giorgi, E.E., Balachandran, H., Muldoon, M., Letvin, N.L., Haynes, B.F., Korber, B.T., Santra, S., 2014. Cross-reactive potential of human T-lymphocyte responses in HIV-1 infection. Vaccine 32, 3995-4000.

Goepfert, P.A., Lumm, W., Farmer, P., Matthews, P., Prendergast, A., Carlson, J.M., Derdeyn, C.A., Tang, J., Kaslow, R.A., Bansal, A., Yusim, K., Heckerman, D., Mulenga, J., Allen, S., Goulder, P.J.R., Hunter, E., 2008. Transmission of HIV-1 Gag immune escape mutations is associated with reduced viral load in linked recipients. Journal of Experimental Medicine 205, 1009-1017.

Goonetilleke, N., Liu, M.K.P., Salazar-Gonzalez, J.F., Ferrari, G., Giorgi, E., Ganusov, V.V., Keele, B.F., Learn, G.H., Turnbull, E.L., Salazar, M.G., Weinhold, K.J., Moore, S., Letvin, N., Haynes, B.F., Cohen, M.S., Hraber, P., Bhattacharya, T., Borrow, P., Perelson, A.S., Hahn, B.H., Shaw, G.M., Korber, B.T., McMichael, A.J., 2009. The first T cell response to transmitted/founder virus contributes to the control of acute viremia in HIV-1 infection. Journal of Experimental Medicine 206, 1253-1272.

Goonetilleke, N., Moore, S., Dally, L., Winstone, N., Cebere, I., Mahmoud, A., Pinheiro, S., Gillespie, G., Brown, D., Loach, V., Roberts, J., Guimaraes-Walker, A., Hayes, P., Loughran, K., Smith, C., De Bont, J., Verlinde, C., Vooijs, D., Schmidt, C., Boaz, M., Gilmour, J., Fast, P., Dorrell, L., Hanke, T., McMichael, A.J., 2006. Induction of Multifunctional Human Immunodeficiency Virus Type 1 (HIV-1)-Specific T Cells Capable of Proliferation in Healthy Subjects by Using a Prime-Boost Regimen of DNA- and Modified Vaccinia Virus Ankara-Vectored Vaccines Expressing HIV-1 Gag Coupled to CD8+ T-Cell Epitopes. Journal of virology 80, 4717-4728.

Goulder, P.J., Brander, C., Annamalai, K., Mngqundaniso, N., Govender, U., Tang, Y., He, S., Hartman, K.E., O'Callaghan, C.A., Ogg, G.S., Altfeld, M.A., Rosenberg, E.S., Cao, H., Kalams, S.A., Hammond, M., Bunce, M., Pelton, S.I., Burchett, S.A., McIntosh, K., Coovadia, H.M., Walker, B.D., 2000. Differential narrow focusing of immunodominant human immunodeficiency virus gag-specific cytotoxic T-lymphocyte responses in infected African and caucasoid adults and children. Journal of virology 74, 5679-5690.

Goulder, P.J., Brander, C., Tang, Y., Tremblay, C., Colbert, R.A., Addo, M.M., Rosenberg, E.S., Nguyen, T., Allen, R., Trocha, A., Altfeld, M., He, S., Bunce, M., Funkhouser, R., Pelton, S.I., Burchett, S.K., McIntosh, K., Korber, B.T., Walker, B.D., 2001. Evolution and transmission of stable CTL escape mutations in HIV infection. Nature 412, 334-338.

Goulder, P.J., Bunce, M., Krausa, P., McIntyre, K., Crowley, S., Morgan, B., Edwards, A., Giangrande, P., Phillips, R.E., McMichael, A.J., 1996. Novel, cross-restricted, conserved, and immunodominant cytotoxic T lymphocyte epitopes in slow progressors in HIV type 1 infection. AIDS research and human retroviruses 12, 1691-1698.

Goulder, P.J., Phillips, R.E., Colbert, R.A., McAdam, S., Ogg, G., Nowak, M.A., Giangrande, P., Luzzi, G., Morgan, B., Edwards, A., McMichael, A.J., Rowland-Jones, S., 1997. Late escape from an immunodominant cytotoxic T-lymphocyte response associated with progression to AIDS. Nat Med 3, 212-217.

Goulder, P.J.R., Watkins, D.I., 2004. HIV and SIV CTL escape: implications for vaccine design. Nature Reviews Immunology 4, 630-640.

Goulder, P.J.R., Watkins, D.I., 2008. Impact of MHC class I diversity on immune control of immunodeficiency virus replication. Nature Reviews Immunology 8, 619-630.

Gounder, K., Padayachi, N., Mann, J.K., Radebe, M., Mokgoro, M., van der Stok, M., Mkhize, L., Mncube, Z., Jaggernath, M., Reddy, T., Walker, B.D., Ndung'u, T., 2015. High frequency of transmitted HIV-1 Gag HLA class I-driven immune escape variants but minimal immune selection over the first year of clade C infection. PloS one 10, e0119886.

Gray, C.M., Lawrence, J., Schapiro, J.M., Altman, J.D., Winters, M.A., Crompton, M., Loi, M., Kundu, S.K., Davis, M.M., Merigan, T.C., 1999. Frequency of class I HLA-restricted anti-HIV CD8+ T cells in individuals receiving highly active antiretroviral therapy (HAART). Journal of immunology (Baltimore, Md. : 1950) 162, 1780-1788.

Gudmundsdotter, L., Bernasconi, D., Hejdeman, B., Sandstrom, E., Alaeus, A., Lidman, K., Ensoli, B., Wahren, B., Buttò, S., 2008. Cross-clade immune responses to Gag p24 in patients infected with different HIV-1 subtypes and correlation with HLA class I and II alleles. Vaccine 26, 5182-5187.

Guindon, S., Gascuel, O., 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 52, 696-704.

Hammer, S.M., Sobieszczyk, M.E., Janes, H., Karuna, S.T., Mulligan, M.J., Grove, D., Koblin, B.A., Buchbinder, S.P., Keefer, M.C., Tomaras, G.D., Frahm, N., Hural, J., Anude, C., Graham, B.S., Enama, M.E., Adams, E., DeJesus, E., Novak, R.M., Frank, I., Bentley, C., Ramirez, S., Fu, R., Koup, R.A., Mascola, J.R., Nabel, G.J., Montefiori, D.C., Kublin, J., McElrath, M.J., Corey, L., Gilbert, P.B., 2013. Efficacy trial of a DNA/rAd5 HIV-1 preventive vaccine. The New England journal of medicine 369, 2083-2092.

Han, W.G., Emmelot, M.E., Jaadar, H., Ten Hulscher, H.I., van Els, C.A., Kaaijk, P., 2016. Development of an IFNgamma ELISPOT for the analysis of the human T cell response against mumps virus. Journal of immunological methods 431, 52-59.

Hancock, G., Yang, H., Yorke, E., Wainwright, E., Bourne, V., Frisbee, A., Payne, T.L., Berrong, M., Ferrari, G., Chopera, D., Hanke, T., Mothe, B., Brander, C., McElrath, M.J., McMichael, A., Goonetilleke, N., Tomaras, G.D., Frahm, N., Dorrell, L., 2015. Identification of Effective Subdominant Anti-HIV-1 CD8+ T Cells Within Entire Post-infection and Post-vaccination Immune Responses. PLoS pathogens 11, e1004658.

Hansen, S.G., Wu, H.L., Burwitz, B.J., Hughes, C.M., Hammond, K.B., Ventura, A.B., Reed, J.S., Gilbride, R.M., Ainslie, E., Morrow, D.W., Ford, J.C., Selseth, A.N., Pathak, R., Malouli, D., Legasse, A.W., Axthelm, M.K., Nelson, J.A., Gillespie, G.M., Walters, L.C., Brackenridge, S., Sharpe, H.R., Lopez, C.A., Fruh, K., Korber, B.T., McMichael, A.J., Gnanakaran, S., Sacha, J.B., Picker, L.J., 2016. Broadly targeted CD8+ T cell responses restricted by major histocompatibility complex E. Science (New York, N.Y.).

Haqqani, A.A., Marek, S.L., Kumar, J., Davenport, M., Wang, H., Tilton, J.C., 2015. Central memory CD4+ T cells are preferential targets of double infection by HIV-1. Virology Journal 12.

Harris, R.S., Hultquist, J.F., Evans, D.T., 2012. The restriction factors of human immunodeficiency virus. The Journal of biological chemistry 287, 40875-40883.

Hayes, P.J., Cox, J.H., Coleman, A.R., Fernandez, N., Bergin, P.J., Kopycinski, J.T., Nitayaphan, S., Pitisuttihum, P., de Souza, M., Duerr, A., Morgan, C., Gilmour, J.W., 2016. Adenovirus-based HIV-1 vaccine candidates tested in efficacy trials elicit CD8+ T cells with limited breadth of HIV-1 inhibition. AIDS (London, England) 30, 1703-1712.

Haynes, B.F., 2015. New approaches to HIV vaccine development. Current opinion in immunology 35, 39-47.

Haynes, B.F., Gilbert, P.B., McElrath, M.J., Zolla-Pazner, S., Tomaras, G.D., Alam, S.M., Evans, D.T., Montefiori, D.C., Karnasuta, C., Sutthent, R., Liao, H.X., DeVico, A.L., Lewis, G.K., Williams, C., Pinter, A., Fong, Y., Janes, H., DeCamp, A., Huang, Y., Rao, M., Billings, E., Karasavvas, N., Robb, M.L., Ngauy, V., de Souza, M.S., Paris, R., Ferrari, G., Bailer, R.T., Soderberg, K.A., Andrews, C., Berman, P.W., Frahm, N., De Rosa, S.C., Alpert, M.D., Yates, N.L., Shen, X., Koup, R.A., Pitisuttithum, P., Kaewkungwal, J., Nitayaphan, S., Rerks-Ngarm, S., Michael, N.L., Kim, J.H., 2012. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. The New England journal of medicine 366, 1275-1286.

Heinrichs, H., Orr, H.T., 1990. HLA non-A,B,C class I genes: their structure and expression. Immunologic research 9, 265-274.

Helms, T., Boehm, B.O., Asaad, R.J., Trezza, R.P., Lehmann, P.V., Tary-Lehmann, M., 2000. Direct visualization of cytokine-producing recall antigen-specific CD4 memory T cells in healthy individuals and HIV patients. Journal of immunology (Baltimore, Md. : 1950) 164, 3723-3732.

Henning, T.R., McNicholl, J.M., Vishwanathan, S.A., Kersh, E.N., 2015. Macaque models of enhanced susceptibility to HIV. Virology Journal 12, 90.

Hersperger, A.R., Pereyra, F., Nason, M., Demers, K., Sheth, P., Shin, L.Y., Kovacs, C.M., Rodriguez, B., Sieg, S.F., Teixeira-Johnson, L., Gudonis, D., Goepfert, P.A., Lederman, M.M., Frank,

I., Makedonas, G., Kaul, R., Walker, B.D., Betts, M.R., 2010. Perforin expression directly ex vivo by HIV-specific CD8 T-cells is a correlate of HIV elite control. PLoS pathogens 6, e1000917.

Hertz, T., Ahmed, H., Friedrich, D.P., Casimiro, D.R., Self, S.G., Corey, L., McElrath, M.J., Buchbinder, S., Horton, H., Frahm, N., Robertson, M.N., Graham, B.S., Gilbert, P., 2013. HIV-1 Vaccine-Induced T-Cell Reponses Cluster in Epitope Hotspots that Differ from Those Induced in Natural Infection with HIV-1. PLoS pathogens 9.

Hesse, M.D., Karulin, A.Y., Boehm, B.O., Lehmann, P.V., Tary-Lehmann, M., 2001. A T cell clone's avidity is a function of its activation state. Journal of immunology (Baltimore, Md. : 1950) 167, 1353-1361.

Hewitt, E.W., 2003. The MHC class I antigen presentation pathway: strategies for viral immune evasion. Immunology 110, 163-169.

Hill, A.V., Elvin, J., Willis, A.C., Aidoo, M., Allsopp, C.E., Gotch, F.M., Gao, X.M., Takiguchi, M., Greenwood, B.M., Townsend, A.R., et al., 1992. Molecular analysis of the association of HLA-B53 and resistance to severe malaria. Nature 360, 434-439.

Ho, D.D., Neumann, A.U., Perelson, A.S., Chen, W., Leonard, J.M., Markowitz, M., 1995. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. Nature 373, 123-126.

Horiya, S., MacPherson, I.S., Krauss, I.J., 2014. Recent strategies targeting HIV glycans in vaccine design. Nature chemical biology 10, 990-999.

Horton, H., Russell, N., Moore, E., Frank, I., Baydo, R., Havenar-Daughton, C., Lee, D., Deers, M., Hudgens, M., Weinhold, K., McElrath, M.J., 2004. Correlation between interferon- gamma secretion and cytotoxicity, in virus-specific memory T cells. The Journal of infectious diseases 190, 1692-1696.

Hraber, P., Korber, B.T., Lapedes, A.S., Bailer, R.T., Seaman, M.S., Gao, H., Greene, K.M., McCutchan, F., Williamson, C., Kim, J.H., Tovanabutra, S., Hahn, B.H., Swanstrom, R., Thomson, M.M., Gao, F., Harris, L., Giorgi, E., Hengartner, N., Bhattacharya, T., Mascola, J.R., Montefiori, D.C., 2014. Impact of clade, geography, and age of the epidemic on HIV-1 neutralization by antibodies. Journal of virology 88, 12623-12643.

Huang, Y., Duerr, A., Frahm, N., Zhang, L., Moodie, Z., De Rosa, S., McElrath, M.J., Gilbert, P.B., 2014. Immune-Correlates Analysis of an HIV-1 Vaccine Efficacy Trial Reveals an Association of Nonspecific Interferon-gamma Secretion with Increased HIV-1 Infection Risk: A Cohort-Based Modeling Study. PloS one 9, e108631.

Hymes, K.B., Cheung, T., Greene, J.B., Prose, N.S., Marcus, A., Ballard, H., William, D.C., Laubenstein, L.J., 1981. Kaposi's sarcoma in homosexual men-a report of eight cases. Lancet 2, 598-600.

Jin, C., Roen, D.R., Lehmann, P.V., Kellermann, G.H., 2013. An Enhanced ELISPOT Assay for Sensitive Detection of Antigen-Specific T Cell Responses to Borrelia burgdorferi. Cells 2, 607-620.

Jin, H., Chen, Z., 2014. Production of live attenuated influenza vaccines against seasonal and potential pandemic influenza viruses. Current opinion in virology 6, 34-39.

Jin, X., Bauer, D.E., Tuttleton, S.E., Lewin, S., Gettie, A., Blanchard, J., Irwin, C.E., Safrit, J.T., Mittler, J., Weinberger, L., Kostrikis, L.G., Zhang, L., Perelson, A.S., Ho, D.D., 1999. Dramatic rise in plasma viremia after CD8(+) T cell depletion in simian immunodeficiency virus-infected macaques. The Journal of experimental medicine 189, 991-998.

Jin, X., Gao, X., Ramanathan, M., Deschenes, G.R., Nelson, G.W., O'Brien, S.J., Goedert, J.J., Ho, D.D., O'Brien, T.R., Carrington, M., 2002. Human Immunodeficiency Virus Type 1 (HIV-1)-Specific CD8+-T-Cell Responses for Groups of HIV-1-Infected Individuals with Different HLA-B*35 Genotypes. Journal of virology 76, 12603-12610.

Kaech, S.M., Wherry, E.J., Ahmed, R., 2002. Effector and memory T-cell differentiation: implications for vaccine development. Nature reviews. Immunology 2, 251-262.

Kalams, S.A., Walker, B.D., 1998. The critical need for CD4 help in maintaining effective cytotoxic T lymphocyte responses. The Journal of experimental medicine 188, 2199-2204.

Kamphorst, A.O., Araki, K., Ahmed, R., 2015. Beyond adjuvants: immunomodulation strategies to enhance T cell immunity. Vaccine 33 Suppl 2, B21-28.

Karch, C.P., Burkhard, P., 2016. Vaccine technologies: From whole organisms to rationally designed protein assemblies. Biochemical pharmacology.

Karlsson, A.C., Martin, J.N., Younger, S.R., Bredt, B.M., Epling, L., Ronquillo, R., Varma, A., Deeks, S.G., McCune, J.M., Nixon, D.F., Sinclair, E., 2003. Comparison of the ELISPOT and cytokine flow cytometry assays for the enumeration of antigen-specific T cells. Journal of immunological methods 283, 141-153.

Karlsson Hedestam, G.B., Fouchier, R.A., Phogat, S., Burton, D.R., Sodroski, J., Wyatt, R.T., 2008. The challenges of eliciting neutralizing antibodies to HIV-1 and to influenza virus. Nature reviews. Microbiology 6, 143-155.

Karulin, A.Y., Hesse, M.D., Tary-Lehmann, M., Lehmann, P.V., 2000. Single-cytokine-producing CD4 memory cells predominate in type 1 and type 2 immunity. Journal of immunology (Baltimore, Md. : 1950) 164, 1862-1872.

Kawashima, Y., Pfafferott, K., Frater, J., Matthews, P., Payne, R., Addo, M., Gatanaga, H., Fujiwara, M., Hachiya, A., Koizumi, H., Kuse, N., Oka, S., Duda, A., Prendergast, A., Crawford, H., Leslie, A., Brumme, Z., Brumme, C., Allen, T., Brander, C., Kaslow, R., Tang, J., Hunter, E., Allen, S., Mulenga, J., Branch, S., Roach, T., John, M., Mallal, S., Ogwu, A., Shapiro, R., Prado, J.G., Fidler, S., Weber, J., Pybus, O.G., Klenerman, P., Ndung'u, T., Phillips, R., Heckerman, D., Harrigan, P.R., Walker, B.D., Takiguchi, M., Goulder, P., 2009. Adaptation of HIV-1 to human leukocyte antigen class I. Nature 458, 641-645.

Keating, S.M., Bejon, P., Berthoud, T., Vuola, J.M., Todryk, S., Webster, D.P., Dunachie, S.J., Moorthy, V.S., McConkey, S.J., Gilbert, S.C., Hill, A.V., 2005. Durable human memory T cells quantifiable by cultured enzyme-linked immunospot assays are induced by heterologous prime boost immunization and correlate with protection against malaria. Journal of immunology (Baltimore, Md. : 1950) 175, 5675-5680.

Keating, S.M., Bollinger, R.C., Quinn, T.C., Jackson, J.B., Carruth, L.M., 2002. Cross-clade T lymphocyte-mediated immunity to HIV type 1: implications for vaccine design and immunodetection assays. AIDS research and human retroviruses 18, 1067-1079.

Keefer, M.C., Gilmour, J., Hayes, P., Gill, D., Kopycinski, J., Cheeseman, H., Cashin-Cox, M., Naarding, M., Clark, L., Fernandez, N., Bunce, C.A., Hay, C.M., Welsh, S., Komaroff, W., Hachaambwa, L., Tarragona-Fiol, T., Sayeed, E., Zachariah, D., Ackland, J., Loughran, K., Barin, B., Cormier, E., Cox, J.H., Fast, P., Excler, J.L., 2012. A phase I double blind, placebo-controlled,

randomized study of a multigenic HIV-1 adenovirus subtype 35 vector vaccine in healthy uninfected adults. PloS one 7, e41936.

Keele, B.F., Estes, J.D., 2011. Barriers to mucosal transmission of immunodeficiency viruses. Blood 118, 839-846.

Kemball, C.C., Pack, C.D., Guay, H.M., Li, Z.N., Steinhauer, D.A., Szomolanyi-Tsuda, E., Lukacher, A.E., 2007. The antiviral CD8+ T cell response is differentially dependent on CD4+ T cell help over the course of persistent infection. Journal of immunology (Baltimore, Md. : 1950) 179, 1113-1121.

Khader, S.A., Bell, G.K., Pearl, J.E., Fountain, J.J., Rangel-Moreno, J., Cilley, G.E., Shen, F., Eaton, S.M., Gaffen, S.L., Swain, S.L., Locksley, R.M., Haynes, L., Randall, T.D., Cooper, A.M., 2007. IL-23 and IL-17 in the establishment of protective pulmonary CD4+ T cell responses after vaccination and during Mycobacterium tuberculosis challenge. Nature immunology 8, 369-377.

Kiepiela, P., Leslie, A.J., Honeyborne, I., Ramduth, D., Thobakgale, C., Chetty, S., Rathnavalu, P., Moore, C., Pfafferott, K.J., Hilton, L., Zimbwa, P., Moore, S., Allen, T., Brander, C., Addo, M.M., Altfeld, M., James, I., Mallal, S., Bunce, M., Barber, L.D., Szinger, J., Day, C., Klenerman, P., Mullins, J., Korber, B., Coovadia, H.M., Walker, B.D., Goulder, P.J., 2004. Dominant influence of HLA-B in mediating the potential co-evolution of HIV and HLA. Nature 432, 769-775.

Kiepiela, P., Ngumbela, K., Thobakgale, C., Ramduth, D., Honeyborne, I., Moodley, E., Reddy, S., de Pierres, C., Mncube, Z., Mkhwanazi, N., Bishop, K., van der Stok, M., Nair, K., Khan, N., Crawford, H., Payne, R., Leslie, A., Prado, J., Prendergast, A., Frater, J., McCarthy, N., Brander, C., Learn, G.H., Nickle, D., Rousseau, C., Coovadia, H., Mullins, J.I., Heckerman, D., Walker, B.D., Goulder, P., 2007. CD8+ T-cell responses to different HIV proteins have discordant associations with viral load. Nat Med 13, 46-53.

Kim, J.H., Excler, J.L., Michael, N.L., 2015. Lessons from the RV144 Thai phase III HIV-1 vaccine trial and the search for correlates of protection. Annu Rev Med 66, 423-437.

Klein, F., Mouquet, H., Dosenovic, P., Scheid, J.F., Scharf, L., Nussenzweig, M.C., 2013. Antibodies in HIV-1 vaccine development and therapy. Science (New York, N.Y.) 341, 1199-1204.

Klenerman, P., Wu, Y., Phillips, R., 2002. HIV: current opinion in escapology. Current opinion in microbiology 5, 408-413.

Kloverpris, H.N., Adland, E., Koyanagi, M., Stryhn, A., Harndahl, M., Matthews, P.C., Shapiro, R., Walker, B.D., Ndung'u, T., Brander, C., Takiguchi, M., Buus, S., Goulder, P., 2014. HIV subtype influences HLA-B*07:02-associated HIV disease outcome. AIDS research and human retroviruses 30, 468-475.

Kloverpris, H.N., Cole, D.K., Fuller, A., Carlson, J., Beck, K., Schauenburg, A.J., Rizkallah, P.J., Buus, S., Sewell, A.K., Goulder, P., 2015. A molecular switch in immunodominant HIV-1-specific CD8 T-cell epitopes shapes differential HLA-restricted escape. Retrovirology 12, 20.

Kloverpris, H.N., Stryhn, A., Harndahl, M., van der Stok, M., Payne, R.P., Matthews, P.C., Chen, F., Riddell, L., Walker, B.D., Ndung'u, T., Buus, S., Goulder, P., 2012. HLA-B*57 Micropolymorphism Shapes HLA Allele-Specific Epitope Immunogenicity, Selection Pressure, and HIV Immune Control. Journal of virology 86, 919-929.

Kopycinski, J., Hayes, P., Ashraf, A., Cheeseman, H., Lala, F., Czyzewska-Khan, J., Spentzou, A., Gill, D.K., Keefer, M.C., Excler, J.L., Fast, P., Cox, J., Gilmour, J., 2014. Broad HIV epitope

specificity and viral inhibition induced by multigenic HIV-1 adenovirus subtype 35 vector vaccine in healthy uninfected adults. PloS one 9, e90378.

Korber, B., 1998. Limitations of a Molecular Clock Applied to Considerations of the Origin of HIV-1. Science (New York, N.Y.) 280, 1868-1871.

Korber, B., 2000. Timing the Ancestor of the HIV-1 Pandemic Strains. Science (New York, N.Y.) 288, 1789-1796.

Korber, B., Gaschen, B., Yusim, K., Thakallapally, R., Kesmir, C., Detours, V., 2001. Evolutionary and immunological implications of contemporary HIV-1 variation. Br Med Bull 58, 19-42.

Korber, B.T., Letvin, N.L., Haynes, B.F., 2009a. T-Cell Vaccine Strategies for Human Immunodeficiency Virus, the Virus with a Thousand Faces. Journal of virology 83, 8300-8314.

Korber, B.T., Letvin, N.L., Haynes, B.F., 2009b. T-Cell Vaccine Strategies for Human Immunodeficiency Virus, the Virus with a Thousand Faces ▽. Journal of virology 83, 8300-8314.

Kostense, S., Koudstaal, W., Sprangers, M., Weverling, G.J., Penders, G., Helmus, N., Vogels, R., Bakker, M., Berkhout, B., Havenga, M., Goudsmit, J., 2004. Adenovirus types 5 and 35 seroprevalence in AIDS risk groups supports type 35 as a vaccine vector. AIDS (London, England) 18, 1213-1216.

Kothe, D., 2006. Ancestral and consensus envelope immunogens for HIV-1 subtype C. Virology 352, 438-449.

Koup, R.A., Graham, B.S., Douek, D.C., 2011. The quest for a T cell-based immune correlate of protection against HIV: a story of trials and errors. Nature reviews. Immunology 11, 65-70.

Koup, R.A., Safrit, J.T., Cao, Y., Andrews, C.A., McLeod, G., Borkowsky, W., Farthing, C., Ho, D.D., 1994. Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. Journal of virology 68, 4650-4655.

Kousiappa, I., van de Vijver, D.A., Demetriades, I., Kostrikis, L.G., 2009a. Genetic analysis of HIV type 1 strains from newly infected untreated patients in cyprus: high genetic diversity and low prevalence of drug resistance. AIDS research and human retroviruses 25, 23-35.

Kousiappa, I., Van De Vijver, D.A., Kostrikis, L.G., 2009b. Near full-length genetic analysis of HIV sequences derived from Cyprus: evidence of a highly polyphyletic and evolving infection. AIDS research and human retroviruses 25, 727-740.

Kramer, H.B., Lavender, K.J., Qin, L., Stacey, A.R., Liu, M.K., di Gleria, K., Simmons, A., Gasper-Smith, N., Haynes, B.F., McMichael, A.J., Borrow, P., Kessler, B.M., 2010. Elevation of intact and proteolytic fragments of acute phase proteins constitutes the earliest systemic antiviral response in HIV-1 infection. PLoS pathogens 6, e1000893.

Kreher, C.R., Dittrich, M.T., Guerkov, R., Boehm, B.O., Tary-Lehmann, M., 2003. CD4+ and CD8+ cells in cryopreserved human PBMC maintain full functionality in cytokine ELISPOT assays. Journal of immunological methods 278, 79-93.

Kreijtz, J.H., Fouchier, R.A., Rimmelzwaan, G.F., 2011. Immune responses to influenza virus infection. Virus research 162, 19-30.

Kuang, X.T., Li, X., Anmole, G., Mwimanzi, P., Shahid, A., Le, A.Q., Chong, L., Qian, H., Miura, T., Markle, T., Baraki, B., Connick, E., Daar, E.S., Jessen, H., Kelleher, A.D., Little, S., Markowitz, M., Pereyra, F., Rosenberg, E.S., Walker, B.D., Ueno, T., Brumme, Z.L., Brockman, M.A., 2014. Impaired Nef function is associated with early control of HIV-1 viremia. Journal of virology 88, 10200-10213.

Kunwar, P., Hawkins, N., Dinges, W.L., Liu, Y., Gabriel, E.E., Swan, D.A., Stevens, C.E., Maenza, J., Collier, A.C., Mullins, J.I., Hertz, T., Yu, X., Horton, H., 2013. Superior control of HIV-1 replication by CD8+ T cells targeting conserved epitopes: implications for HIV vaccine design. PloS one 8, e64405.

Kurts, C., Robinson, B.W.S., Knolle, P.A., 2010. Cross-priming in health and disease. Nature Reviews Immunology 10, 403-414.

Kuse, N., Akahoshi, T., Gatanaga, H., Ueno, T., Oka, S., Takiguchi, M., 2014. Selection of TI8-8V mutant associated with long-term control of HIV-1 by cross-reactive HLA-B*51:01-restricted cytotoxic T cells. Journal of immunology (Baltimore, Md. : 1950) 193, 4814-4822.

Ladell, K., Hellerstein, M.K., Cesar, D., Busch, R., Boban, D., McCune, J.M., 2008. Central memory CD8+ T cells appear to have a shorter lifespan and reduced abundance as a function of HIV disease progression. Journal of immunology (Baltimore, Md. : 1950) 180, 7907-7918.

Landau, N.R., Warton, M., Littman, D.R., 1988. The envelope glycoprotein of the human immunodeficiency virus binds to the immunoglobulin-like domain of CD4. Nature 334, 159-162.

Lanzavecchia, A., Sallusto, F., 2000. Dynamics of T lymphocyte responses: intermediates, effectors, and memory cells. Science (New York, N.Y.) 290, 92-97.

Lau, K.A., Wong, J.J., 2013. Current Trends of HIV Recombination Worldwide. Infectious disease reports 5, e4.

Lee, J.K., 2004. T Cell Cross-Reactivity and Conformational Changes during TCR Engagement. Journal of Experimental Medicine 200, 1455-1466.

Leslie, A.J., Pfafferott, K.J., Chetty, P., Draenert, R., Addo, M.M., Feeney, M., Tang, Y., Holmes, E.C., Allen, T., Prado, J.G., Altfeld, M., Brander, C., Dixon, C., Ramduth, D., Jeena, P., Thomas, S.A., John, A.S., Roach, T.A., Kupfer, B., Luzzi, G., Edwards, A., Taylor, G., Lyall, H., Tudor-Williams, G., Novelli, V., Martinez-Picado, J., Kiepiela, P., Walker, B.D., Goulder, P.J.R., 2004. HIV evolution: CTL escape mutation and reversion after transmission. Nature Medicine 10, 282-289.

Letourneau, S., Im, E.J., Mashishi, T., Brereton, C., Bridgeman, A., Yang, H., Dorrell, L., Dong, T., Korber, B., McMichael, A.J., Hanke, T., 2007. Design and pre-clinical evaluation of a universal HIV-1 vaccine. PloS one 2, e984.

Letsch, A., Scheibenbogen, C., 2003. Quantification and characterization of specific T-cells by antigen-specific cytokine production using ELISPOT assay or intracellular cytokine staining. Methods (San Diego, Calif.) 31, 143-149.

Letvin, N.L., 2005. Progress toward an HIV vaccine. Annu Rev Med 56, 213-223.

Letvin, N.L., Schmitz, J.E., Jordan, H.L., Seth, A., Hirsch, V.M., Reimann, K.A., Kuroda, M.J., 1999. Cytotoxic T lymphocytes specific for the simian immunodeficiency virus. Immunological reviews 170, 127-134.

Li, F., Finnefrock, A.C., Dubey, S.A., Korber, B.T., Szinger, J., Cole, S., McElrath, M.J., Shiver, J.W., Casimiro, D.R., Corey, L., Self, S.G., 2011. Mapping HIV-1 Vaccine Induced T-Cell Responses: Bias towards Less-Conserved Regions and Potential Impact on Vaccine Efficacy in the Step Study. PloS one 6, e20479.

Li, F., Malhotra, U., Gilbert, P.B., Hawkins, N.R., Duerr, A.C., McElrath, J.M., Corey, L., Self, S.G., 2006. Peptide selection for human immunodeficiency virus type 1 CTL-based vaccine evaluation. Vaccine 24, 6893-6904.

Li, Q., Duan, L., Estes, J.D., Ma, Z.M., Rourke, T., Wang, Y., Reilly, C., Carlis, J., Miller, C.J., Haase, A.T., 2005. Peak SIV replication in resting memory CD4+ T cells depletes gut lamina propria CD4+ T cells. Nature 434, 1148-1152.

Lichterfeld, M., 2004. Loss of HIV-1-specific CD8+ T Cell Proliferation after Acute HIV-1 Infection and Restoration by Vaccine-induced HIV-1-specific CD4+ T Cells. Journal of Experimental Medicine 200, 701-712.

Liu, M.A., 2010. Immunologic basis of vaccine vectors. Immunity 33, 504-515.

Liu, M.K., Hawkins, N., Ritchie, A.J., Ganusov, V.V., Whale, V., Brackenridge, S., Li, H., Pavlicek, J.W., Cai, F., Rose-Abrahams, M., Treurnicht, F., Hraber, P., Riou, C., Gray, C., Ferrari, G., Tanner, R., Ping, L.H., Anderson, J.A., Swanstrom, R., B, C.C., Cohen, M., Karim, S.S.A., Haynes, B., Borrow, P., Perelson, A.S., Shaw, G.M., Hahn, B.H., Williamson, C., Korber, B.T., Gao, F., Self, S., McMichael, A., Goonetilleke, N., 2013. Vertical T cell immunodominance and epitope entropy determine HIV-1 escape. The Journal of Clinical Investigation 123, 380-393.

Liu, Y., McNevin, J., Rolland, M., Zhao, H., Deng, W., Maenza, J., Stevens, C.E., Collier, A.C., McElrath, M.J., Mullins, J.I., 2009a. Conserved HIV-1 epitopes continuously elicit subdominant cytotoxic T-lymphocyte responses. J Infect Dis 200, 1825-1833.

Liu, Y., McNevin, J., Rolland, M., Zhao, H., Deng, W., Maenza, J., Stevens, Claire E., Collier, Ann C., McElrath, M.J., Mullins, James I., 2009b. Conserved HIV-1 Epitopes Continuously Elicit Subdominant Cytotoxic T-Lymphocyte Responses. The Journal of infectious diseases 200, 1825-1833.

Lobritz, M.A., Lassen, K.G., Arts, E.J., 2011. HIV-1 replicative fitness in elite controllers. Current opinion in HIV and AIDS 6, 214-220.

Lopez, M., Peris, A., Soriano, V., Lozano, S., Vicario, J.L., Rallon, N.I., Restrepo, C., Benito, J.M., 2011. The expansion ability but not the quality of HIV-specific CD8(+) T cells is associated with protective human leucocyte antigen class I alleles in long-term non-progressors. Immunology 134, 305-313.

Lu, L., Yu, F., Du, L.Y., Xu, W., Jiang, S.B., 2013. Tactics used by HIV-1 to evade host innate, adaptive, and intrinsic immunities. Chinese medical journal 126, 2374-2379.

Lyles, R.H., Munoz, A., Yamashita, T.E., Bazmi, H., Detels, R., Rinaldo, C.R., Margolick, J.B., Phair, J.P., Mellors, J.W., 2000. Natural history of human immunodeficiency virus type 1 viremia after seroconversion and proximal to AIDS in a large cohort of homosexual men. Multicenter AIDS Cohort Study. The Journal of infectious diseases 181, 872-880.

Ma, Z.M., Stone, M., Piatak, M., Jr., Schweighardt, B., Haigwood, N.L., Montefiori, D., Lifson, J.D., Busch, M.P., Miller, C.J., 2009. High specific infectivity of plasma virus from the pre-ramp-up and ramp-up stages of acute simian immunodeficiency virus infection. Journal of virology 83, 3288-3297.

Macatonia, S.E., Gompels, M., Pinching, A.J., Patterson, S., Knight, S.C., 1992. Antigen-presentation by macrophages but not by dendritic cells in human immunodeficiency virus (HIV) infection. Immunology 75, 576-581.

Mahiti, M., Toyoda, M., Jia, X., Kuang, X.T., Mwimanzi, F., Mwimanzi, P., Walker, B.D., Xiong, Y., Brumme, Z.L., Brockman, M.A., Ueno, T., 2016. Relative Resistance of HLA-B to Downregulation by Naturally Occurring HIV-1 Nef Sequences. mBio 7, e01516-01515.

Malhotra, U., Li, F., Nolin, J., Allison, M., Zhao, H., Mullins, J.I., Self, S., McElrath, M.J., 2007. Enhanced detection of human immunodeficiency virus type 1 (HIV-1) Nef-specific T cells recognizing multiple variants in early HIV-1 infection. Journal of virology 81, 5225-5237.

Mann, J.K., Ndung'u, T., 2015. HIV-1 vaccine immunogen design strategies. Virology Journal 12, 3.

Marcilla, M., Alvarez, I., Ramos-Fernandez, A., Lombardia, M., Paradela, A., Albar, J.P., 2016. Comparative Analysis of the Endogenous Peptidomes Displayed by HLA-B*27 and Mamu-B*08: Two MHC Class I Alleles Associated with Elite Control of HIV/SIV Infection. Journal of proteome research.

Marozsan, A.J., Moore, D.M., Lobritz, M.A., Fraundorf, E., Abraha, A., Reeves, J.D., Arts, E.J., 2005. Differences in the Fitness of Two Diverse Wild-Type Human Immunodeficiency Virus Type 1 Isolates Are Related to the Efficiency of Cell Binding and Entry. Journal of virology 79, 7121-7134.

Martin, M.P., Qi, Y., Gao, X., Yamada, E., Martin, J.N., Pereyra, F., Colombo, S., Brown, E.E., Shupert, W.L., Phair, J., Goedert, J.J., Buchbinder, S., Kirk, G.D., Telenti, A., Connors, M., O'Brien, S.J., Walker, B.D., Parham, P., Deeks, S.G., McVicar, D.W., Carrington, M., 2007. Innate partnership of HLA-B and KIR3DL1 subtypes against HIV-1. Nat Genet 39, 733-740.

Martinez-Picado, J., Prado, J.G., Fry, E.E., Pfafferott, K., Leslie, A., Chetty, S., Thobakgale, C., Honeyborne, I., Crawford, H., Matthews, P., Pillay, T., Rousseau, C., Mullins, J.I., Brander, C., Walker, B.D., Stuart, D.I., Kiepiela, P., Goulder, P., 2006. Fitness cost of escape mutations in p24 Gag in association with control of human immunodeficiency virus type 1. Journal of virology 80, 3617-3623.

Masemola, A.M., Mashishi, T.N., Khoury, G., Bredell, H., Paximadis, M., Mathebula, T., Barkhan, D., Puren, A., Vardas, E., Colvin, M., Zijenah, L., Katzenstein, D., Musonda, R., Allen, S., Kumwenda, N., Taha, T., Gray, G., McIntyre, J., Karim, S.A., Sheppard, H.W., Gray, C.M., 2004. Novel and promiscuous CTL epitopes in conserved regions of Gag targeted by individuals with early subtype C HIV type 1 infection from southern Africa. Journal of immunology (Baltimore, Md. : 1950) 173, 4607-4617.

Mattapallil, J.J., Douek, D.C., Hill, B., Nishimura, Y., Martin, M., Roederer, M., 2005. Massive infection and loss of memory CD4+ T cells in multiple tissues during acute SIV infection. Nature 434, 1093-1097.

Matthews, P.C., Koyanagi, M., Kloverpris, H.N., Harndahl, M., Stryhn, A., Akahoshi, T., Gatanaga, H., Oka, S., Juarez Molina, C., Valenzuela Ponce, H., Avila Rios, S., Cole, D., Carlson, J., Payne, R.P., Ogwu, A., Bere, A., Ndung'u, T., Gounder, K., Chen, F., Riddell, L., Luzzi, G., Shapiro, R., Brander, C., Walker, B., Sewell, A.K., Reyes Teran, G., Heckerman, D., Hunter, E., Buus, S.,

Takiguchi, M., Goulder, P.J., 2012. Differential clade-specific HLA-B*3501 association with HIV-1 disease outcome is linked to immunogenicity of a single Gag epitope. Journal of virology 86, 12643-12654.

McKinnon, L.R., Ball, T.B., Kimani, J., Wachihi, C., Matu, L., Luo, M., Embree, J., Fowke, K.R., Plummer, F.A., 2005. Cross-clade CD8(+) T-cell responses with a preference for the predominant circulating clade. Journal of acquired immune deficiency syndromes (1999) 40, 245-249.

McMichael, A., Mwau, M., Hanke, T., 2002. HIV T cell vaccines, the importance of clades. Vaccine 20, 1918-1921.

McMichael, A.J., Borrow, P., Tomaras, G.D., Goonetilleke, N., Haynes, B.F., 2010. The immune response during acute HIV-1 infection: clues for vaccine development. Nature reviews. Immunology 10, 11-23.

McMichael, A.J., Phillips, R.E., 1997. Escape of human immunodeficiency virus from immune control. Annual review of immunology 15, 271-296.

Mendoza, D., Royce, C., Ruff, L.E., Ambrozak, D.R., Quigley, M.F., Dang, T., Venturi, V., Price, D.A., Douek, D.C., Migueles, S.A., Connors, M., 2012. HLA B*5701-positive long-term nonprogressors/elite controllers are not distinguished from progressors by the clonal composition of HIV-specific CD8+ T cells. Journal of virology 86, 4014-4018.

Miedema, F., 2008. A brief history of HIV vaccine research: stepping back to the drawing board? AIDS (London, England) 22, 1699-1703.

Migueles, S.A., Connors, M., 2015. Success and failure of the cellular immune response against HIV-1. Nature immunology 16, 563-570.

Migueles, S.A., Laborico, A.C., Shupert, W.L., Sabbaghian, M.S., Rabin, R., Hallahan, C.W., Baarle, D.V., Kostense, S., Miedema, F., McLaughlin, M., Ehler, L., Metcalf, J., Liu, S., Connors, M., 2002a. HIV-specific CD8+ T cell proliferation is coupled to perforin expression and is maintained in nonprogressors. Nature immunology 3, 1061-1068.

Migueles, S.A., Laborico, A.C., Shupert, W.L., Sabbaghian, M.S., Rabin, R., Hallahan, C.W., Van Baarle, D., Kostense, S., Miedema, F., McLaughlin, M., Ehler, L., Metcalf, J., Liu, S., Connors, M., 2002b. HIV-specific CD8+ T cell proliferation is coupled to perforin expression and is maintained in nonprogressors. Nature immunology 3, 1061-1068.

Migueles, S.A., Mendoza, D., Zimmerman, M.G., Martins, K.M., Toulmin, S.A., Kelly, E.P., Peterson, B.A., Johnson, S.A., Galson, E., Poropatich, K.O., Patamawenu, A., Imamichi, H., Ober, A., Rehm, C.A., Jones, S., Hallahan, C.W., Follmann, D.A., Connors, M., 2015. CD8(+) T-cell Cytotoxic Capacity Associated with Human Immunodeficiency Virus-1 Control Can Be Mediated through Various Epitopes and Human Leukocyte Antigen Types. EBioMedicine 2, 46-58.

Migueles, S.A., Osborne, C.M., Royce, C., Compton, A.A., Joshi, R.P., Weeks, K.A., Rood, J.E., Berkley, A.M., Sacha, J.B., Cogliano-Shutta, N.A., Lloyd, M., Roby, G., Kwan, R., McLaughlin, M., Stallings, S., Rehm, C., O'Shea, M.A., Mican, J., Packard, B.Z., Komoriya, A., Palmer, S., Wiegand, A.P., Maldarelli, F., Coffin, J.M., Mellors, J.W., Hallahan, C.W., Follman, D.A., Connors, M., 2008. Lytic Granule Loading of CD8+ T Cells Is Required for HIV-Infected Cell Elimination Associated with Immune Control. Immunity 29, 1009-1021.

Migueles, S.A., Sabbaghian, M.S., Shupert, W.L., Bettinotti, M.P., Marincola, F.M., Martino, L., Hallahan, C.W., Selig, S.M., Schwartz, D., Sullivan, J., Connors, M., 2000. HLA B*5701 is highly associated with restriction of virus replication in a subgroup of HIV-infected long term nonprogressors. Proceedings of the National Academy of Sciences of the United States of America 97, 2709-2714.

Miura, T., Brockman, M.A., Brumme, Z.L., Brumme, C.J., Pereyra, F., Trocha, A., Block, B.L., Schneidewind, A., Allen, T.M., Heckerman, D., Walker, B.D., 2008a. HLA-Associated Alterations in Replication Capacity of Chimeric NL4-3 Viruses Carrying gag-protease from Elite Controllers of Human Immunodeficiency Virus Type 1. Journal of virology 83, 140-149.

Miura, T., Brockman, M.A., Schneidewind, A., Lobritz, M., Pereyra, F., Rathod, A., Block, B.L., Brumme, Z.L., Brumme, C.J., Baker, B., Rothchild, A.C., Li, B., Trocha, A., Cutrell, E., Frahm, N., Brander, C., Toth, I., Arts, E.J., Allen, T.M., Walker, B.D., 2008b. HLA-B57/B*5801 Human Immunodeficiency Virus Type 1 Elite Controllers Select for Rare Gag Variants Associated with Reduced Viral Replication Capacity and Strong Cytotoxic T-Lymphotye Recognition. Journal of virology 83, 2743-2755.

Moir, S., Chun, T.-W., Fauci, A.S., 2011. Pathogenic Mechanisms of HIV Disease*. Annual Review of Pathology: Mechanisms of Disease 6, 223-248.

Monteiro, P., Gosselin, A., Wacleche, V.S., El-Far, M., Said, E.A., Kared, H., Grandvaux, N., Boulassel, M.R., Routy, J.P., Ancuta, P., 2011. Memory CCR6+CD4+ T cells are preferential targets for productive HIV type 1 infection regardless of their expression of integrin beta7. Journal of immunology (Baltimore, Md. : 1950) 186, 4618-4630.

Morrison, L.A., Lukacher, A.E., Braciale, V.L., Fan, D.P., Braciale, T.J., 1986. Differences in antigen presentation to MHC class I-and class II-restricted influenza virus-specific cytolytic T lymphocyte clones. The Journal of experimental medicine 163, 903-921.

Mothe, B., Hu, X., Llano, A., Rosati, M., Olvera, A., Kulkarni, V., Valentin, A., Alicea, C., Pilkington, G.R., Sardesai, N.Y., Rocafort, M., Crespo, M., Carrillo, J., Marco, A., Mullins, J.I., Dorrell, L., Hanke, T., Clotet, B., Pavlakis, G.N., Felber, B.K., Brander, C., 2015. A human immune data-informed vaccine concept elicits strong and broad T-cell specificities associated with HIV-1 control in mice and macaques. Journal of translational medicine 13, 60.

Motozono, C., Kuse, N., Sun, X., Rizkallah, P.J., Fuller, A., Oka, S., Cole, D.K., Sewell, A.K., Takiguchi, M., 2014. Molecular basis of a dominant T cell response to an HIV reverse transcriptase 8-mer epitope presented by the protective allele HLA-B*51:01. Journal of immunology (Baltimore, Md. : 1950) 192, 3428-3434.

Mudd, J.C., Lederman, M.M., 2014. CD8 T cell persistence in treated HIV infection. Current opinion in HIV and AIDS 9, 500-505.

Mudd, P.A., Martins, M.A., Ericsen, A.J., Tully, D.C., Power, K.A., Bean, A.T., Piaskowski, S.M., Duan, L., Seese, A., Gladden, A.D., Weisgrau, K.L., Furlott, J.R., Kim, Y.I., Veloso de Santana, M.G., Rakasz, E., Capuano, S., 3rd, Wilson, N.A., Bonaldo, M.C., Galler, R., Allison, D.B., Piatak, M., Jr., Haase, A.T., Lifson, J.D., Allen, T.M., Watkins, D.I., 2012. Vaccine-induced CD8+ T cells control AIDS virus replication. Nature 491, 129-133.

Mwau, M., McMichael, A.J., Hanke, T., 2002. Design and validation of an enzyme-linked immunospot assay for use in clinical trials of candidate HIV vaccines. AIDS research and human retroviruses 18, 611-618.

Nagaraja, P., Alexander, H.K., Bonhoeffer, S., Dixit, N.M., 2016. Influence of recombination on acquisition and reversion of immune escape and compensatory mutations in HIV-1. Epidemics 14, 11-25.

NCT00851383, 2009. A Phase I Trial to Evaluate Safety and Immunogenicity of Ad35-GRIN/ENV HIV Vaccine in Healthy Adult Volunteers (http://clinicaltrials.gov/ct2/show/NCT00851383).

NCT01264445, 2010. A Trial to Evaluate the Safety and Immunogenicity of an Adjuvanted GSK Investigational HIV Vaccine Administered With Ad35-GRIN Investigational Vaccine (http://clinicaltrials.gov/ct2/show/NCT01264445?term=NCT01264445&rank=1).

Ndhlovu, Z.M., Kamya, P., Mewalal, N., Kloverpris, H.N., Nkosi, T., Pretorius, K., Laher, F., Ogunshola, F., Chopera, D., Shekhar, K., Ghebremichael, M., Ismail, N., Moodley, A., Malik, A., Leslie, A., Goulder, P.J., Buus, S., Chakraborty, A., Dong, K., Ndung'u, T., Walker, B.D., 2015a. Magnitude and Kinetics of CD8+ T Cell Activation during Hyperacute HIV Infection Impact Viral Set Point. Immunity 43, 591-604.

Ndhlovu, Z.M., Piechocka-Trocha, A., Vine, S., McMullen, A., Koofhethile, K.C., Goulder, P.J.R., Ndung'u, T., Barouch, D.H., Walker, B.D., 2011. Mosaic HIV-1 Gag Antigens Can Be Processed and Presented to Human HIV-Specific CD8+ T Cells. The Journal of Immunology 186, 6914-6924.

Ndhlovu, Z.M., Proudfoot, J., Cesa, K., Alvino, D.M., McMullen, A., Vine, S., Stampouloglou, E., Piechocka-Trocha, A., Walker, B.D., Pereyra, F., 2012. Elite controllers with low to absent effector CD8+ T cell responses maintain highly functional, broadly directed central memory responses. Journal of virology.

Ndhlovu, Z.M., Stampouloglou, E., Cesa, K., Mavrothalassitis, O., Alvino, D.M., Li, J.Z., Wilton, S., Karel, D., Piechocka-Trocha, A., Chen, H., Pereyra, F., Walker, B.D., 2015b. The Breadth of Expandable Memory CD8+ T Cells Inversely Correlates with Residual Viral Loads in HIV Elite Controllers. Journal of virology 89, 10735-10747.

Neefjes, J., Jongsma, M.L., Paul, P., Bakke, O., 2011. Towards a systems understanding of MHC class I and MHC class II antigen presentation. Nature reviews. Immunology.

Neefjes, J., Ovaa, H., 2013. A peptide's perspective on antigen presentation to the immune system. Nature chemical biology 9, 769-775.

Ngumbela, K.C., Day, C.L., Mncube, Z., Nair, K., Ramduth, D., Thobakgale, C., Moodley, E., Reddy, S., de Pierres, C., Mkhwanazi, N., Bishop, K., van der Stok, M., Ismail, N., Honeyborne, I., Crawford, H., Kavanagh, D.G., Rousseau, C., Nickle, D., Mullins, J., Heckerman, D., Korber, B., Coovadia, H., Kiepiela, P., Goulder, P.J., Walker, B.D., 2008. Targeting of a CD8 T cell env epitope presented by HLA-B*5802 is associated with markers of HIV disease progression and lack of selection pressure. AIDS research and human retroviruses 24, 72-82.

Nicastri, E., Sarmati, L., d'Ettorre, G., Palmisano, L., Parisi, S.G., Uccella, I., Rianda, A., Concia, E., Vullo, V., Vella, S., Andreoni, M., 2003. Replication capacity, biological phenotype, and drug resistance of HIV strains isolated from patients failing antiretroviral therapy. Journal of medical virology 69, 1-6.

Nickle, D.C., Rolland, M., Jensen, M.A., Pond, S.L., Deng, W., Seligman, M., Heckerman, D., Mullins, J.I., Jojic, N., 2007. Coping with viral diversity in HIV vaccine design. PLoS computational biology 3, e75.

Nijhuis, M., Deeks, S., Boucher, C., 2001. Implications of antiretroviral resistance on viral fitness. Current opinion in infectious diseases 14, 23-28.

Novitsky, V., Gilbert, P., Peter, T., McLane, M.F., Gaolekwe, S., Rybak, N., Thior, I., Ndung'u, T., Marlink, R., Lee, T.H., Essex, M., 2003. Association between virus-specific T-cell responses and plasma viral load in human immunodeficiency virus type 1 subtype C infection. Journal of virology 77, 882-890.

Ntale, R.S., Chopera, D.R., Ngandu, N.K., Assis de Rosa, D., Zembe, L., Gamieldien, H., Mlotshwa, M., Werner, L., Woodman, Z., Mlisana, K., Abdool Karim, S., Gray, C.M., Williamson, C., 2012. Temporal association of HLA-B*81:01- and HLA-B*39:10-mediated HIV-1 p24 sequence evolution with disease progression. Journal of virology 86, 12013-12024.

Nwanegbo, E., Vardas, E., Gao, W., Whittle, H., Sun, H., Rowe, D., Robbins, P.D., Gambotto, A., 2004. Prevalence of neutralizing antibodies to adenoviral serotypes 5 and 35 in the adult populations of The Gambia, South Africa, and the United States. Clin Diagn Lab Immunol 11, 351-357.

Offit, P.O., 2012. Vaccines, 6th ed, pp. 17-36.

Okoye, A.A., Picker, L.J., 2013. CD4(+) T-cell depletion in HIV infection: mechanisms of immunological failure. Immunological reviews 254, 54-64.

Okulicz, J.F., Marconi, V.C., Landrum, M.L., Wegner, S., Weintrob, A., Ganesan, A., Hale, B., Crum-Cianflone, N., Delmar, J., Barthel, V., Quinnan, G., Agan, B.K., Dolan, M.J., 2009. Clinical outcomes of elite controllers, viremic controllers, and long-term nonprogressors in the US Department of Defense HIV natural history study. The Journal of infectious diseases 200, 1714-1723.

Ondondo, B., Murakoshi, H., Clutton, G., Abdul-Jawad, S., Wee, E.G., Gatanaga, H., Oka, S., McMichael, A.J., Takiguchi, M., Korber, B., Hanke, T., 2016. Novel Conserved-region T-cell Mosaic Vaccine With High Global HIV-1 Coverage Is Recognized by Protective Responses in Untreated Infection. Molecular therapy : the journal of the American Society of Gene Therapy.

Ortiz, G.M., Nixon, D.F., Trkola, A., Binley, J., Jin, X., Bonhoeffer, S., Kuebler, P.J., Donahoe, S.M., Demoitie, M.A., Kakimoto, W.M., Ketas, T., Clas, B., Heymann, J.J., Zhang, L., Cao, Y., Hurley, A., Moore, J.P., Ho, D.D., Markowitz, M., 1999. HIV-1-specific immune responses in subjects who temporarily contain virus replication after discontinuation of highly active antiretroviral therapy. The Journal of Clinical Investigation 104, R13-18.

Owen, R.E., Heitman, J.W., Hirschkorn, D.F., Lanteri, M.C., Biswas, H.H., Martin, J.N., Krone, M.R., Deeks, S.G., Norris, P.J., 2010. HIV+ elite controllers have low HIV-specific T-cell activation yet maintain strong, polyfunctional T-cell responses. AIDS (London, England) 24, 1095-1105.

Paiardini, M., Muller-Trutwin, M., 2013. HIV-associated chronic immune activation. Immunological reviews 254, 78-101.

Pantaleo, G., 1999. Unraveling the strands of HIV's web. Nat Med 5, 27-28.

Pantaleo, G., Fauci, A.S., 1995. New concepts in the immunopathogenesis of HIV infection. Annual review of immunology 13, 487-512.

Pantaleo, G., Graziosi, C., Fauci, A.S., 1993. New concepts in the immunopathogenesis of human immunodeficiency virus infection. The New England journal of medicine 328, 327-335.

Pantaleo, G., Koup, R.A., 2004. Correlates of immune protection in HIV-1 infection: what we know, what we don't know, what we should know. Nat Med 10, 806-810.

Parham, P., Lomen, C.E., Lawlor, D.A., Ways, J.P., Holmes, N., Coppin, H.L., Salter, R.D., Wan, A.M., Ennis, P.D., 1988. Nature of polymorphism in HLA-A, -B, and -C molecules. Proceedings of the National Academy of Sciences of the United States of America 85, 4005-4009.

Patel, P., Borkowf, C.B., Brooks, J.T., Lasry, A., Lansky, A., Mermin, J., 2014. Estimating per-act HIV transmission risk: a systematic review. AIDS (London, England) 28, 1509-1519.

Payne, R.P., Branch, S., Kloverpris, H., Matthews, P.C., Koofhethile, C.K., Strong, T., Adland, E., Leitman, E., Frater, J., Ndung'u, T., Hunter, E., Haubrich, R., Mothe, B., Edwards, A., Riddell, L., Chen, F., Harrigan, P.R., Brumme, Z.L., Mallal, S., John, M., Jooste, J.P., Shapiro, R., Deeks, S.G., Walker, B.D., Brander, C., Landis, C., Carlson, J.M., Prado, J.G., Goulder, P.J., 2014. Differential escape patterns within the dominant HLA-B*57:03-restricted HIV Gag epitope reflect distinct clade-specific functional constraints. Journal of virology 88, 4668-4678.

Perelson, A.S., Neumann, A.U., Markowitz, M., Leonard, J.M., Ho, D.D., 1996. HIV-1 dynamics in vivo: virion clearance rate, infected cell life-span, and viral generation time. Science (New York, N.Y.) 271, 1582-1586.

Pereyra, F., Jia, X., McLaren, P.J., Telenti, A., de Bakker, P.I., Walker, B.D., Ripke, S., Brumme, C.J., Pulit, S.L., Carrington, M., Kadie, C.M., Carlson, J.M., Heckerman, D., Graham, R.R., Plenge, R.M., Deeks, S.G., Gianniny, L., Crawford, G., Sullivan, J., Gonzalez, E., Davies, L., Camargo, A., Moore, J.M., Beattie, N., Gupta, S., Crenshaw, A., Burtt, N.P., Guiducci, C., Gupta, N., Gao, X., Qi, Y., Yuki, Y., Piechocka-Trocha, A., Cutrell, E., Rosenberg, R., Moss, K.L., Lemay, P., O'Leary, J., Schaefer, T., Verma, P., Toth, I., Block, B., Baker, B., Rothchild, A., Lian, J., Proudfoot, J., Alvino, D.M., Vine, S., Addo, M.M., Allen, T.M., Altfeld, M., Henn, M.R., Le Gall, S., Streeck, H., Haas, D.W., Kuritzkes, D.R., Robbins, G.K., Shafer, R.W., Gulick, R.M., Shikuma, C.M., Haubrich, R., Riddler, S., Sax, P.E., Daar, E.S., Ribaudo, H.J., Agan, B., Agarwal, S., Ahern, R.L., Allen, B.L., Altidor, S., Altschuler, E.L., Ambardar, S., Anastos, K., Anderson, B., Anderson, V., Andrady, U., Antoniskis, D., Bangsberg, D., Barbaro, D., Barrie, W., Bartczak, J., Barton, S., Basden, P., Basgoz, N., Bazner, S., Bellos, N.C., Benson, A.M., Berger, J., Bernard, N.F., Bernard, A.M., Birch, C., Bodner, S.J., Bolan, R.K., Boudreaux, E.T., Bradley, M., Braun, J.F., Brndjar, J.E., Brown, S.J., Brown, K., Brown, S.T., Burack, J., Bush, L.M., Cafaro, V., Campbell, O., Campbell, J., Carlson, R.H., Carmichael, J.K., Casey, K.K., Cavacuiti, C., Celestin, G., Chambers, S.T., Chez, N., Chirch, L.M., Cimoch, P.J., Cohen, D., Cohn, L.E., Conway, B., Cooper, D.A., Cornelson, B., Cox, D.T., Cristofano, M.V., Cuchural, G., Jr., Czartoski, J.L., Dahman, J.M., Daly, J.S., Davis, B.T., Davis, K., Davod, S.M., DeJesus, E., Dietz, C.A., Dunham, E., Dunn, M.E., Ellerin, T.B., Eron, J.J., Fangman, J.J., Farel, C.E., Ferlazzo, H., Fidler, S., Fleenor-Ford, A., Frankel, R., Freedberg, K.A., French, N.K., Fuchs, J.D., Fuller, J.D., Gaberman, J., Gallant, J.E., Gandhi, R.T., Garcia, E., Garmon, D., Gathe, J.C., Jr., Gaultier, C.R., Gebre, W., Gilman, F.D., Gilson, I., Goepfert, P.A., Gottlieb, M.S., Goulston, C., Groger, R.K., Gurley, T.D., Haber, S., Hardwicke, R., Hardy, W.D., Harrigan, P.R., Hawkins, T.N., Heath, S., Hecht, F.M., Henry, W.K., Hladek, M., Hoffman, R.P., Horton, J.M., Hsu, R.K., Huhn, G.D., Hunt, P., Hupert, M.J., Illeman, M.L., Jaeger, H., Jellinger, R.M., John, M., Johnson, J.A., Johnson, K.L., Johnson, H., Johnson, K., Joly, J., Jordan, W.C., Kauffman, C.A., Khanlou, H., Killian, R.K., Kim, A.Y., Kim, D.D., Kinder, C.A., Kirchner, J.T., Kogelman, L., Kojic, E.M., Korthuis, P.T., Kurisu, W., Kwon, D.S., LaMar, M., Lampiris, H., Lanzafame, M., Lederman, M.M., Lee, D.M., Lee, J.M., Lee, M.J., Lee, E.T., Lemoine, J., Levy, J.A., Llibre, J.M., Liguori, M.A., Little, S.J., Liu, A.Y., Lopez, A.J., Loutfy, M.R., Loy, D., Mohammed, D.Y., Man, A., Mansour, M.K., Marconi, V.C., Markowitz, M., Marques, R., Martin, J.N., Martin, H.L., Jr., Mayer, K.H., McElrath, M.J., McGhee, T.A., McGovern, B.H., McGowan, K., McIntyre, D., McLeod, G.X., Menezes, P.,

Mesa, G., Metroka, C.E., Meyer-Olson, D., Miller, A.O., Montgomery, K., Mounzer, K.C., Nagami, E.H., Nagin, I., Nahass, R.G., Nelson, M.O., Nielsen, C., Norene, D.L., O'Connor, D.H., Ojikutu, B.O., Okulicz, J., Oladehin, O.O., Oldfield, E.C., 3rd, Olender, S.A., Ostrowski, M., Owen, W.F., Jr., Pae, E., Parsonnet, J., Pavlatos, A.M., Perlmutter, A.M., Pierce, M.N., Pincus, J.M., Pisani, L., Price, L.J., Proia, L., Prokesch, R.C., Pujet, H.C., Ramgopal, M., Rathod, A., Rausch, M., Ravishankar, J., Rhame, F.S., Richards, C.S., Richman, D.D., Rodes, B., Rodriguez, M., Rose, R.C., 3rd, Rosenberg, E.S., Rosenthal, D., Ross, P.E., Rubin, D.S., Rumbaugh, E., Saenz, L., Salvaggio, M.R., Sanchez, W.C., Sanjana, V.M., Santiago, S., Schmidt, W., Schuitemaker, H., Sestak, P.M., Shalit, P., Shay, W., Shirvani, V.N., Silebi, V.I., Sizemore, J.M., Jr., Skolnik, P.R., Sokol-Anderson, M., Sosman, J.M., Stabile, P., Stapleton, J.T., Starrett, S., Stein, F., Stellbrink, H.J., Sterman, F.L., Stone, V.E., Stone, D.R., Tambussi, G., Taplitz, R.A., Tedaldi, E.M., Theisen, W., Torres, R., Tosiello, L., Tremblay, C., Tribble, M.A., Trinh, P.D., Tsao, A., Ueda, P., Vaccaro, A., Valadas, E., Vanig, T.J., Vecino, I., Vega, V.M., Veikley, W., Wade, B.H., Walworth, C., Wanidworanun, C., Ward, D.J., Warner, D.A., Weber, R.D., Webster, D., Weis, S., Wheeler, D.A., White, D.J., Wilkins, E., Winston, A., Wlodaver, C.G., van't Wout, A., Wright, D.P., Yang, O.O., Yurdin, D.L., Zabukovic, B.W., Zachary, K.C., Zeeman, B., Zhao, M., 2010. The major genetic determinants of HIV-1 control affect HLA class I peptide presentation. Science (New York, N.Y.) 330, 1551-1557.

Peut, V., Kent, S.J., 2007. Utility of human immunodeficiency virus type 1 envelope as a T-cell immunogen. Journal of virology 81, 13125-13134.

Piatak, M., Jr., Saag, M.S., Yang, L.C., Clark, S.J., Kappes, J.C., Luk, K.C., Hahn, B.H., Shaw, G.M., Lifson, J.D., 1993. High levels of HIV-1 in plasma during all stages of infection determined by competitive PCR. Science (New York, N.Y.) 259, 1749-1754.

Pichla-Gollon, S.L., Lin, S.W., Hensley, S.E., Lasaro, M.O., Herkenhoff-Haut, L., Drinker, M., Tatsis, N., Gao, G.P., Wilson, J.M., Ertl, H.C., Bergelson, J.M., 2009. Effect of preexisting immunity on an adenovirus vaccine vector: in vitro neutralization assays fail to predict inhibition by antiviral antibody in vivo. Journal of virology 83, 5567-5573.

Picker, L.J., Watkins, D.I., 2005. HIV pathogenesis: the first cut is the deepest. Nature immunology 6, 430-432.

Pietra, G., Romagnani, C., Manzini, C., Moretta, L., Mingari, M.C., 2010. The emerging role of HLA-E-restricted CD8+ T lymphocytes in the adaptive immune response to pathogens and tumors. Journal of biomedicine & biotechnology 2010, 907092.

Pircher, H., Moskophidis, D., Rohrer, U., Burki, K., Hengartner, H., Zinkernagel, R.M., 1990. Viral escape by selection of cytotoxic T cell-resistant virus variants in vivo. Nature 346, 629-633.

Plotkin, S., 2014. History of vaccination. Proceedings of the National Academy of Sciences of the United States of America 111, 12283-12287.

Plotkin, S.A., 2009. Vaccines: the Fourth Century. Clinical and Vaccine Immunology 16, 1709-1719.

Plotkin, S.A., 2010. Correlates of Protection Induced by Vaccination. Clinical and Vaccine Immunology 17, 1055-1065.

Poorolajal, J., Hooshmand, E., Mahjub, H., Esmailnasab, N., Jenabi, E., 2016. Survival rate of AIDS disease and mortality in HIV-infected patients: a meta-analysis. Public health.

Precopio, M.L., Betts, M.R., Parrino, J., Price, D.A., Gostick, E., Ambrozak, D.R., Asher, T.E., Douek, D.C., Harari, A., Pantaleo, G., Bailer, R., Graham, B.S., Roederer, M., Koup, R.A., 2007.

Immunization with vaccinia virus induces polyfunctional and phenotypically distinctive CD8+ T cell responses. Journal of Experimental Medicine 204, 1405-1416.

Priddy, F.H., Brown, D., Kublin, J., Monahan, K., Wright, D.P., Lalezari, J., Santiago, S., Marmor, M., Lally, M., Novak, R.M., Brown, S.J., Kulkarni, P., Dubey, S.A., Kierstead, L.S., Casimiro, D.R., Mogg, R., DiNubile, M.J., Shiver, J.W., Leavitt, R.Y., Robertson, M.N., Mehrotra, D.V., Quirk, E., 2008. Safety and immunogenicity of a replication-incompetent adenovirus type 5 HIV-1 clade B gag/pol/nef vaccine in healthy adults. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 46, 1769-1781.

Prince, J.L., Claiborne, D.T., Carlson, J.M., Schaefer, M., Yu, T., Lahki, S., Prentice, H.A., Yue, L., Vishwanathan, S.A., Kilembe, W., Goepfert, P., Price, M.A., Gilmour, J., Mulenga, J., Farmer, P., Derdeyn, C.A., Tang, J., Heckerman, D., Kaslow, R.A., Allen, S.A., Hunter, E., 2012. Role of transmitted Gag CTL polymorphisms in defining replicative capacity and early HIV-1 pathogenesis. PLoS pathogens 8, e1003041.

Pujol, F.M., Laketa, V., Schmidt, F., Mukenhirn, M., Muller, B., Boulant, S., Grimm, D., Keppler, O.T., Fackler, O.T., 2016. HIV-1 Vpu Antagonizes CD317/tetherin by Adaptor protein-1-Mediated Exclusion from Virus Assembly Sites. Journal of virology.

Radebe, M., Gounder, K., Mokgoro, M., Ndhlovu, Z.M., Mncube, Z., Mkhize, L., van der Stok, M., Jaggernath, M., Walker, B.D., Ndung'u, T., 2015. Broad and persistent Gag-specific CD8+ T-cell responses are associated with viral control but rarely drive viral escape during primary HIV-1 infection. AIDS (London, England) 29, 23-33.

Radebe, M., Nair, K., Chonco, F., Bishop, K., Wright, J.K., van der Stok, M., Bassett, I.V., Mncube, Z., Altfeld, M., Walker, B.D., Ndung'u, T., 2011. Limited Immunogenicity of HIV CD8+ T-Cell Epitopes in Acute Clade C Virus Infection. The Journal of infectious diseases 204, 768-776.

Rajapaksa, U.S., Li, D., Peng, Y.C., McMichael, A.J., Dong, T., Xu, X.N., 2012. HLA-B may be more protective against HIV-1 than HLA-A because it resists negative regulatory factor (Nef) mediated down-regulation. Proceedings of the National Academy of Sciences of the United States of America 109, 13353-13358.

Ranasinghe, S.R., Kramer, H.B., Wright, C., Kessler, B.M., di Gleria, K., Zhang, Y., Gillespie, G.M., Blais, M.E., Culshaw, A., Pichulik, T., Simmons, A., Rowland-Jones, S.L., McMichael, A.J., Dong, T., 2011. The antiviral efficacy of HIV-specific CD8 T-cells to a conserved epitope is heavily dependent on the infecting HIV-1 isolate. PLoS pathogens 7, e1001341.

Rerks-Ngarm, S., Pitisuttithum, P., Nitayaphan, S., Kaewkungwal, J., Chiu, J., Paris, R., Premsri, N., Namwat, C., de Souza, M., Adams, E., Benenson, M., Gurunathan, S., Tartaglia, J., McNeil, J.G., Francis, D.P., Stablein, D., Birx, D.L., Chunsuttiwat, S., Khamboonruang, C., Thongcharoen, P., Robb, M.L., Michael, N.L., Kunasol, P., Kim, J.H., 2009. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. The New England journal of medicine 361, 2209-2220.

Richardson, M.W., Guo, L., Xin, F., Yang, X., Riley, J.L., 2014. Stabilized human TRIM5alpha protects human T cells from HIV-1 infection. Molecular therapy : the journal of the American Society of Gene Therapy 22, 1084-1095.

Riou, C., Burgers, W.A., Mlisana, K., Koup, R.A., Roederer, M., Abdool Karim, S.S., Williamson, C., Gray, C.M., 2014. Differential impact of magnitude, polyfunctional capacity, and specificity of HIV-specific CD8+ T cell responses on HIV set point. Journal of virology 88, 1819-1824.

Riou, C., Ganusov, V.V., Campion, S., Mlotshwa, M., Liu, M.K., Whale, V.E., Goonetilleke, N., Borrow, P., Ferrari, G., Betts, M.R., Haynes, B.F., McMichael, A.J., Gray, C.M., 2012. Distinct kinetics of Gag-specific CD4+ and CD8+ T cell responses during acute HIV-1 infection. Journal of immunology (Baltimore, Md. : 1950) 188, 2198-2206.

Ritchie, A.J., Cai, F., Smith, N.M., Chen, S., Song, H., Brackenridge, S., Abdool Karim, S.S., Korber, B.T., McMichael, A.J., Gao, F., Goonetilleke, N., 2014. Recombination-mediated escape from primary CD8+ T cells in acute HIV-1 infection. Retrovirology 11, 69.

Robb, M.L., Eller, L.A., Kibuuka, H., Rono, K., Maganga, L., Nitayaphan, S., Kroon, E., Sawe, F.K., Sinei, S., Sriplienchan, S., Jagodzinski, L.L., Malia, J., Manak, M., de Souza, M.S., Tovanabutra, S., Sanders-Buell, E., Rolland, M., Dorsey-Spitz, J., Eller, M.A., Milazzo, M., Li, Q., Lewandowski, A., Wu, H., Swann, E., O'Connell, R.J., Peel, S., Dawson, P., Kim, J.H., Michael, N.L., 2016. Prospective Study of Acute HIV-1 Infection in Adults in East Africa and Thailand. The New England journal of medicine.

Rodriguez, B., Lederman, M.M., Jiang, W., Bazdar, D.A., Garate, K., Harding, C.V., Sieg, S.F., 2006. Interferon-alpha differentially rescues CD4 and CD8 T cells from apoptosis in HIV infection. AIDS (London, England) 20, 1379-1389.

Roederer, M., 2003. Optimized determination of T cell epitope responses. Journal of immunological methods 274, 221-228.

Roederer, M., Nozzi, J.L., Nason, M.C., 2011. SPICE: exploration and analysis of post-cytometric complex multivariate datasets. Cytometry. Part A : the journal of the International Society for Analytical Cytology 79, 167-174.

Rolland, M., Frahm, N., Nickle, D.C., Jojic, N., Deng, W., Allen, T.M., Brander, C., Heckerman, D.E., Mullins, J.I., 2011. Increased breadth and depth of cytotoxic T lymphocytes responses against HIV-1-B Nef by inclusion of epitope variant sequences. PloS one 6, e17969.

Rolland, M., Jensen, M.A., Nickle, D.C., Yan, J., Learn, G.H., Heath, L., Weiner, D., Mullins, J.I., 2007a. Reconstruction and Function of Ancestral Center-of-Tree Human Immunodeficiency Virus Type 1 Proteins▽. Journal of virology 81, 8507-8514.

Rolland, M., Manocheewa, S., Swain, J.V., Lanxon-Cookson, E.C., Kim, M., Westfall, D.H., Larsen, B.B., Gilbert, P.B., Mullins, J.I., 2013. HIV-1 Conserved-Element Vaccines: Relationship between Sequence Conservation and Replicative Capacity. Journal of virology 87, 5461-5467.

Rolland, M., Nickle, D.C., Mullins, J.I., 2007b. HIV-1 group M conserved elements vaccine. PLoS pathogens 3, e157.

Rollier, C.S., Reyes-Sandoval, A., Cottingham, M.G., Ewer, K., Hill, A.V., 2011. Viral vectors as vaccine platforms: deployment in sight. Current opinion in immunology 23, 377-382.

Rosario, M., Bridgeman, A., Quakkelaar, E.D., Quigley, M.F., Hill, B.J., Knudsen, M.L., Ammendola, V., Ljungberg, K., Borthwick, N., Im, E.-J., McMichael, A.J., Drijfhout, J.W., Greenaway, H.Y., Venturi, V., Douek, D.C., Colloca, S., Liljeström, P., Nicosia, A., Price, D.A., Melief, C.J.M., Hanke, T., 2010. Long peptides induce polyfunctional T cells against conserved regions of HIV-1 with superior breadth to single-gene vaccines in macaques. European journal of immunology 40, 1973-1984.

Rosenberg, E.S., Walker, B.D., 1998. HIV type 1-specific helper T cells: a critical host defense. AIDS research and human retroviruses 14 Suppl 2, S143-147.

Rousseau, C.M., Daniels, M.G., Carlson, J.M., Kadie, C., Crawford, H., Prendergast, A., Matthews, P., Payne, R., Rolland, M., Raugi, D.N., Maust, B.S., Learn, G.H., Nickle, D.C., Coovadia, H., Ndung'u, T., Frahm, N., Brander, C., Walker, B.D., Goulder, P.J.R., Bhattacharya, T., Heckerman, D.E., Korber, B.T., Mullins, J.I., 2008. HLA Class I-Driven Evolution of Human Immunodeficiency Virus Type 1 Subtype C Proteome: Immune Escape and Viral Load. Journal of virology 82, 6434-6446.

Rubens, M., Ramamoorthy, V., Saxena, A., Shehadeh, N., Appunni, S., 2015. HIV Vaccine: Recent Advances, Current Roadblocks, and Future Directions. J Immunol Res 2015, 560347.

Ruhwald, M., Ravn, P., 2007. Immune reconstitution syndrome in tuberculosis and HIV-co-infected patients: Th1 explosion or cytokine storm? AIDS (London, England) 21, 882-884.

Sacha, J.B., Chung, C., Rakasz, E.G., Spencer, S.P., Jonas, A.K., Bean, A.T., Lee, W., Burwitz, B.J., Stephany, J.J., Loffredo, J.T., Allison, D.B., Adnan, S., Hoji, A., Wilson, N.A., Friedrich, T.C., Lifson, J.D., Yang, O.O., Watkins, D.I., 2007. Gag-specific CD8+ T lymphocytes recognize infected cells before AIDS-virus integration and viral protein expression. Journal of immunology (Baltimore, Md. : 1950) 178, 2746-2754.

Sachsenberg, N., Perelson, A.S., Yerly, S., Schockmel, G.A., Leduc, D., Hirschel, B., Perrin, L., 1998. Turnover of CD4+ and CD8+ T lymphocytes in HIV-1 infection as measured by Ki-67 antigen. The Journal of experimental medicine 187, 1295-1303.

Samri, A., Durier, C., Urrutia, A., Sanchez, I., Gahery-Segard, H., Imbart, S., Sinet, M., Tartour, E., Aboulker, J.P., Autran, B., Venet, A., 2006. Evaluation of the interlaboratory concordance in quantification of human immunodeficiency virus-specific T cells with a gamma interferon enzyme-linked immunospot assay. Clin Vaccine Immunol 13, 684-697.

Santos, A.F., Soares, M.A., 2010. HIV Genetic Diversity and Drug Resistance. Viruses 2, 503-531.

Santra, S., Korber, B.T., Muldoon, M., Barouch, D.H., Nabel, G.J., Gao, F., Hahn, B.H., Haynes, B.F., Letvin, N.L., 2008. A centralized gene-based HIV-1 vaccine elicits broad cross-clade cellular immune responses in rhesus monkeys. Proceedings of the National Academy of Sciences 105, 10489-10494.

Santra, S., Liao, H.-X., Zhang, R., Muldoon, M., Watson, S., Fischer, W., Theiler, J., Szinger, J., Balachandran, H., Buzby, A., Quinn, D., Parks, R.J., Tsao, C.-Y., Carville, A., Mansfield, K.G., Pavlakis, G.N., Felber, B.K., Haynes, B.F., Korber, B.T., Letvin, N.L., 2010. Mosaic vaccines elicit CD8+ T lymphocyte responses that confer enhanced immune coverage of diverse HIV strains in monkeys. Nature Medicine 16, 324-328.

Sasagawa, T., Takagi, H., Makinoda, S., 2012. Immune responses against human papillomavirus (HPV) infection and evasion of host defense in cervical cancer. Journal of infection and chemotherapy : official journal of the Japan Society of Chemotherapy 18, 807-815.

Sather, D.N., Armann, J., Ching, L.K., Mavrantoni, A., Sellhorn, G., Caldwell, Z., Yu, X., Wood, B., Self, S., Kalams, S., Stamatatos, L., 2009. Factors associated with the development of cross-reactive neutralizing antibodies during human immunodeficiency virus type 1 infection. Journal of virology 83, 757-769.

Saxena, M., Van, T.T., Baird, F.J., Coloe, P.J., Smooker, P.M., 2013. Pre-existing immunity against vaccine vectors--friend or foe? Microbiology 159, 1-11.

Schiller, J.T., Lowy, D.R., 2010. Vaccines to prevent infections by oncoviruses. Annu Rev Microbiol 64, 23-41.

Schmid, B.V., Kęsmir, C., de Boer, R.J., 2010. Quantifying how MHC polymorphism prevents pathogens from adapting to the antigen presentation pathway. Epidemics 2, 99-108.

Schmitz, J.E., Kuroda, M.J., Santra, S., Sasseville, V.G., Simon, M.A., Lifton, M.A., Racz, P., Tenner-Racz, K., Dalesandro, M., Scallon, B.J., Ghrayeb, J., Forman, M.A., Montefiori, D.C., Rieber, E.P., Letvin, N.L., Reimann, K.A., 1999. Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. Science (New York, N.Y.) 283, 857-860.

Schneidewind, A., Brockman, M.A., Sidney, J., Wang, Y.E., Chen, H., Suscovich, T.J., Li, B., Adam, R.I., Allgaier, R.L., Mothe, B.R., Kuntzen, T., Oniangue-Ndza, C., Trocha, A., Yu, X.G., Brander, C., Sette, A., Walker, B.D., Allen, T.M., 2008. Structural and Functional Constraints Limit Options for Cytotoxic T-Lymphocyte Escape in the Immunodominant HLA-B27-Restricted Epitope in Human Immunodeficiency Virus Type 1 Capsid. Journal of virology 82, 5594-5605.

Schneidewind, A., Brockman, M.A., Yang, R., Adam, R.I., Li, B., Le Gall, S., Rinaldo, C.R., Craggs, S.L., Allgaier, R.L., Power, K.A., Kuntzen, T., Tung, C.S., LaBute, M.X., Mueller, S.M., Harrer, T., McMichael, A.J., Goulder, P.J.R., Aiken, C., Brander, C., Kelleher, A.D., Allen, T.M., 2007. Escape from the Dominant HLA-B27-Restricted Cytotoxic T-Lymphocyte Response in Gag Is Associated with a Dramatic Reduction in Human Immunodeficiency Virus Type 1 Replication. Journal of virology 81, 12382-12393.

Schneidewind, A., Tang, Y., Brockman, M.A., Ryland, E.G., Dunkley-Thompson, J., Steel-Duncan, J.C., St. John, M.A., Conrad, J.A., Kalams, S.A., Noel, F., Allen, T.M., Christie, C.D., Feeney, M.E., 2009. Maternal Transmission of Human Immunodeficiency Virus Escape Mutations Subverts HLA-B57 Immunodominance but Facilitates Viral Control in the Haploidentical Infant. Journal of virology 83, 8616-8627.

Scully, E., Alter, G., 2016. NK Cells in HIV Disease. Current HIV/AIDS reports 13, 85-94.

Seaman, M.S., Janes, H., Hawkins, N., Grandpre, L.E., Devoy, C., Giri, A., Coffey, R.T., Harris, L., Wood, B., Daniels, M.G., Bhattacharya, T., Lapedes, A., Polonis, V.R., McCutchan, F.E., Gilbert, P.B., Self, S.G., Korber, B.T., Montefiori, D.C., Mascola, J.R., 2010. Tiered categorization of a diverse panel of HIV-1 Env pseudoviruses for assessment of neutralizing antibodies. Journal of virology 84, 1439-1452.

Seder, R.A., Darrah, P.A., Roederer, M., 2008. T-cell quality in memory and protection: implications for vaccine design. Nature Reviews Immunology 8, 247-258.

Sekaly, R.P., 2008. The failed HIV Merck vaccine study: a step back or a launching point for future vaccine development? Journal of Experimental Medicine 205, 7-12.

Sewell, A.K., Price, D.A., Oxenius, A., Kelleher, A.D., Phillips, R.E., 2000. Cytotoxic T lymphocyte responses to human immunodeficiency virus: control and escape. Stem cells (Dayton, Ohio) 18, 230-244.

Shankarappa, R., Margolick, J.B., Gange, S.J., Rodrigo, A.G., Upchurch, D., Farzadegan, H., Gupta, P., Rinaldo, C.R., Learn, G.H., He, X., Huang, X.L., Mullins, J.I., 1999. Consistent viral evolutionary

changes associated with the progression of human immunodeficiency virus type 1 infection. Journal of virology 73, 10489-10502.

Shapiro, E.D., Berg, A.T., Austrian, R., Schroeder, D., Parcells, V., Margolis, A., Adair, R.K., Clemens, J.D., 1991. The protective efficacy of polyvalent pneumococcal polysaccharide vaccine. The New England journal of medicine 325, 1453-1460.

Shasha, D., 2013. [Elite controllers and the mechanisms behind spontaneous control of HIV]. Harefuah 152, 481-485, 498.

Shiina, T., Hosomichi, K., Inoko, H., Kulski, J.K., 2009. The HLA genomic loci map: expression, interaction, diversity and disease. J Hum Genet 54, 15-39.

Shin, S.Y., 2016. Recent update in HIV vaccine development. Clinical and experimental vaccine research 5, 6-11.

Sidney, J., Peters, B., Frahm, N., Brander, C., Sette, A., 2008. HLA class I supertypes: a revised and updated classification. BMC Immunol 9, 1.

Singh, K.K., Spector, S.A., 2009. Host genetic determinants of human immunodeficiency virus infection and disease progression in children. Pediatric research 65, 55r-63r.

Sliepen, K., Sanders, R.W., 2015. HIV-1 envelope glycoprotein immunogens to induce broadly neutralizing antibodies. Expert review of vaccines.

Smith, J.G., Liu, X., Kaufhold, R.M., Clair, J., Caulfield, M.J., 2001. Development and validation of a gamma interferon ELISPOT assay for quantitation of cellular immune responses to varicella-zoster virus. Clin Diagn Lab Immunol 8, 871-879.

Smith, K.A., 2011. Edward jenner and the small pox vaccine. Frontiers in immunology 2, 21.

Smith, K.N., Mailliard, R.B., Piazza, P.A., Fischer, W., Korber, B.T., Fecek, R.J., Ratner, D., Gupta, P., Mullins, J.I., Rinaldo, C.R., 2016. Effective Cytotoxic T Lymphocyte Targeting of Persistent HIV-1 during Antiretroviral Therapy Requires Priming of Naive CD8+ T Cells. mBio 7.

Smith, M.Z., Bastidas, S., Karrer, U., Oxenius, A., 2013. Impact of antigen specificity on CD4+ T cell activation in chronic HIV-1 infection. BMC infectious diseases 13, 100.

Smith, S.G., Joosten, S.A., Verscheure, V., Pathan, A.A., McShane, H., Ottenhoff, T.H., Dockrell, H.M., Mascart, F., 2009. Identification of major factors influencing ELISpot-based monitoring of cellular responses to antigens from Mycobacterium tuberculosis. PloS one 4, e7972.

Spentzou, A., Bergin, P., Gill, D., Cheeseman, H., Ashraf, A., Kaltsidis, H., Cashin-Cox, M., Anjarwalla, I., Steel, A., Higgs, C., Pozniak, A., Piechocka-Trocha, A., Wong, J., Anzala, O., Karita, E., Dally, L., Gotch, F., Walker, B., Gilmour, J., Hayes, P., 2010. Viral inhibition assay: a CD8 T cell neutralization assay for use in clinical trials of HIV-1 vaccine candidates. The Journal of infectious diseases 201, 720-729.

Spira, S., 2003. Impact of clade diversity on HIV-1 virulence, antiretroviral drug sensitivity and drug resistance. Journal of Antimicrobial Chemotherapy 51, 229-240.

Stacey, A.R., Norris, P.J., Qin, L., Haygreen, E.A., Taylor, E., Heitman, J., Lebedeva, M., DeCamp, A., Li, D., Grove, D., Self, S.G., Borrow, P., 2009. Induction of a striking systemic cytokine cascade prior to peak viremia in acute human immunodeficiency virus type 1 infection, in contrast to more

modest and delayed responses in acute hepatitis B and C virus infections. Journal of virology 83, 3719-3733.

Steers, N.J., Currier, J.R., Jobe, O., Tovanabutra, S., Ratto-Kim, S., Marovich, M.A., Kim, J.H., Michael, N.L., Alving, C.R., Rao, M., 2014. Designing the epitope flanking regions for optimal generation of CTL epitopes. Vaccine 32, 3509-3516.

Stephenson, K.E., Barouch, D.H., 2013. A global approach to HIV-1 vaccine development. Immunological reviews 254, 295-304.

Stephenson, K.E., SanMiguel, A., Simmons, N.L., Smith, K., Lewis, M.G., Szinger, J.J., Korber, B., Barouch, D.H., 2012. Full-length HIV-1 immunogens induce greater magnitude and comparable breadth of T lymphocyte responses to conserved HIV-1 regions compared with conserved-region-only HIV-1 immunogens in rhesus monkeys. Journal of virology 86, 11434-11440.

Stratov, I., Chung, A., Kent, S.J., 2008. Robust NK cell-mediated human immunodeficiency virus (HIV)-specific antibody-dependent responses in HIV-infected subjects. Journal of virology 82, 5450-5459.

Streeck, H., Frahm, N., Walker, B.D., 2009a. The role of IFN-gamma Elispot assay in HIV vaccine research. Nat Protoc 4, 461-469.

Streeck, H., Jolin, J.S., Qi, Y., Yassine-Diab, B., Johnson, R.C., Kwon, D.S., Addo, M.M., Brumme, C., Routy, J.P., Little, S., Jessen, H.K., Kelleher, A.D., Hecht, F.M., Sekaly, R.P., Rosenberg, E.S., Walker, B.D., Carrington, M., Altfeld, M., 2009b. Human Immunodeficiency Virus Type 1-Specific CD8+ T-Cell Responses during Primary Infection Are Major Determinants of the Viral Set Point and Loss of CD4+ T Cells. Journal of virology 83, 7641-7648.

Streeck, H., Lu, R., Beckwith, N., Milazzo, M., Liu, M., Routy, J.P., Little, S., Jessen, H., Kelleher, A.D., Hecht, F., Sekaly, R.P., Alter, G., Heckerman, D., Carrington, M., Rosenberg, E.S., Altfeld, M., 2014. Emergence of individual HIV-specific CD8 T cell responses during primary HIV-1 infection can determine long-term disease outcome. Journal of virology 88, 12793-12801.

Streeck, H., Nixon, D.F., 2010. T cell immunity in acute HIV-1 infection. The Journal of infectious diseases 202 Suppl 2, S302-308.

Sui, Y., Gordon, S., Franchini, G., Berzofsky, J.A., 2013. Nonhuman primate models for HIV/AIDS vaccine development. Current protocols in immunology / edited by John E. Coligan ... [et al.] 102, Unit 12 14.

Sun, J.C., Williams, M.A., Bevan, M.J., 2004. CD4+ T cells are required for the maintenance, not programming, of memory CD8+ T cells after acute infection. Nature immunology 5, 927-933.

Tang, J., Cormier, E., Gilmour, J., Price, M.A., Prentice, H.A., Song, W., Kamali, A., Karita, E., Lakhi, S., Sanders, E.J., Anzala, O., Amornkul, P.N., Allen, S., Hunter, E., Kaslow, R.A., 2011. Human leukocyte antigen variants B*44 and B*57 are consistently favorable during two distinct phases of primary HIV-1 infection in sub-Saharan Africans with several viral subtypes. Journal of virology 85, 8894-8902.

Tang, J., Costello, C., Keet, I.P., Rivers, C., Leblanc, S., Karita, E., Allen, S., Kaslow, R.A., 1999. HLA class I homozygosity accelerates disease progression in human immunodeficiency virus type 1 infection. AIDS research and human retroviruses 15, 317-324.

Tansiri, Y., Rowland-Jones, S.L., Ananworanich, J., Hansasuta, P., 2015. Clinical outcome of HIV viraemic controllers and noncontrollers with normal CD4 counts is exclusively determined by antigen-specific CD8+ T-cell-mediated HIV suppression. PloS one 10, e0118871.

Teijaro, J.R., Verhoeven, D., Page, C.A., Turner, D., Farber, D.L., 2010. Memory CD4 T Cells Direct Protective Responses to Influenza Virus in the Lungs through Helper-Independent Mechanisms▽. Journal of virology 84, 9217-9226.

Tenzer, S., Wee, E., Burgevin, A., Stewart-Jones, G., Friis, L., Lamberth, K., Chang, C.H., Harndahl, M., Weimershaus, M., Gerstoft, J., Akkad, N., Klenerman, P., Fugger, L., Jones, E.Y., McMichael, A.J., Buus, S., Schild, H., van Endert, P., Iversen, A.K., 2009. Antigen processing influences HIV-specific cytotoxic T lymphocyte immunodominance. Nature immunology 10, 636-646.

Tomiyama, H., Miwa, K., Shiga, H., Moore, Y.I., Oka, S., Iwamoto, A., Kaneko, Y., Takiguchi, M., 1997. Evidence of presentation of multiple HIV-1 cytotoxic T lymphocyte epitopes by HLA-B*3501 molecules that are associated with the accelerated progression of AIDS. Journal of immunology (Baltimore, Md. : 1950) 158, 5026-5034.

Tongo, M., Burgers, W.A., 2014. Challenges in the design of a T cell vaccine in the context of HIV-1 diversity. Viruses 6, 3968-3990.

Towers, G.J., 2007. The control of viral infection by tripartite motif proteins and cyclophilin A. Retrovirology 4, 40.

Toyoda, M., Ogata, Y., Mahiti, M., Maeda, Y., Kuang, X.T., Miura, T., Jessen, H., Walker, B.D., Brockman, M.A., Brumme, Z.L., Ueno, T., 2015. Differential Ability of Primary HIV-1 Nef Isolates To Downregulate HIV-1 Entry Receptors. Journal of virology 89, 9639-9652.

Trautmann, L., Mbitikon-Kobo, F.M., Goulet, J.P., Peretz, Y., Shi, Y., Van Grevenynghe, J., Procopio, F.A., Boulassel, M.R., Routy, J.P., Chomont, N., Haddad, E.K., Sekaly, R.P., 2012. Profound metabolic, functional, and cytolytic differences characterize HIV-specific CD8 T cells in primary and chronic HIV infection. Blood 120, 3466-3477.

Troyer, R.M., McNevin, J., Liu, Y., Zhang, S.C., Krizan, R.W., Abraha, A., Tebit, D.M., Zhao, H., Avila, S., Lobritz, M.A., McElrath, M.J., Le Gall, S., Mullins, J.I., Arts, E.J., 2009. Variable fitness impact of HIV-1 escape mutations to cytotoxic T lymphocyte (CTL) response. PLoS pathogens 5, e1000365.

Tsao, L.C., Guo, H., Jeffrey, J., Hoxie, J.A., Su, L., 2016. CCR5 interaction with HIV-1 Env contributes to Env-induced depletion of CD4 T cells in vitro and in vivo. Retrovirology 13, 22.

Tsukamoto, T., Yuasa, M., Yamamoto, H., Kawada, M., Takeda, A., Igarashi, H., Matano, T., 2007. Induction of CD8+ Cells Able To Suppress CCR5-Tropic Simian Immunodeficiency Virus SIVmac239 Replication by Controlled Infection of CXCR4-Tropic Simian-Human Immunodeficiency Virus in Vaccinated Rhesus Macaques. Journal of virology 81, 11640-11649.

Turnbull, E.L., Wong, M., Wang, S., Wei, X., Jones, N.A., Conrod, K.E., Aldam, D., Turner, J., Pellegrino, P., Keele, B.F., Williams, I., Shaw, G.M., Borrow, P., 2009. Kinetics of expansion of epitope-specific T cell responses during primary HIV-1 infection. Journal of immunology (Baltimore, Md. : 1950) 182, 7131-7145.

UNAIDS, 2014. UNAIDS report on global HIV/AIDS epidemic.

van der Putten, H., Quint, W., van Raaij, J., Maandag, E.R., Verma, I.M., Berns, A., 1981. M-MuLV-induced leukemogenesis: integration and structure of recombinant proviruses in tumors. Cell 24, 729-739.

Van Gulck, E., Bracke, L., Heyndrickx, L., Coppens, S., Atkinson, D., Merlin, C., Pasternak, A., Florence, E., Vanham, G., 2012. Immune and Viral Correlates of "Secondary Viral Control" after Treatment Interruption in Chronically HIV-1 Infected Patients. PloS one 7, e37792.

Verani, A., Gras, G., Pancino, G., 2005. Macrophages and HIV-1: dangerous liaisons. Molecular immunology 42, 195-212.

Verweij, M.C., Horst, D., Griffin, B.D., Luteijn, R.D., Davison, A.J., Ressing, M.E., Wiertz, E.J., 2015. Viral inhibition of the transporter associated with antigen processing (TAP): a striking example of functional convergent evolution. PLoS pathogens 11, e1004743.

Virgin, H.W., Walker, B.D., 2010. Immunology and the elusive AIDS vaccine. Nature 464, 224-231.

Voskoboinik, I., Dunstone, M.A., Baran, K., Whisstock, J.C., Trapani, J.A., 2010. Perforin: structure, function, and role in human immunopathology. Immunological reviews 235, 35-54.

Walker, B.D., Burton, D.R., 2008. Toward an AIDS vaccine. Science (New York, N.Y.) 320, 760-764.

Walker, B.D., Korber, B.T., 2001. Immune control of HIV: the obstacles of HLA and viral diversity. Nature immunology 2, 473-475.

Walker, C.M., Moody, D.J., Stites, D.P., Levy, J.A., 1986. CD8+ lymphocytes can control HIV infection in vitro by suppressing virus replication. Science (New York, N.Y.) 234, 1563-1566.

Wang, X., Ao, Z., Chen, L., Kobinger, G., Peng, J., Yao, X., 2012. The cellular antiviral protein APOBEC3G interacts with HIV-1 reverse transcriptase and inhibits its function during viral replication. Journal of virology 86, 3777-3786.

Watanabe, K., Murakoshi, H., Tamura, Y., Koyanagi, M., Chikata, T., Gatanaga, H., Oka, S., Takiguchi, M., 2013. Identification of cross-clade CTL epitopes in HIV-1 clade A/E-infected individuals by using the clade B overlapping peptides. Microbes and infection / Institut Pasteur 15, 874-886.

Weaver, E.A., Lu, Z., Camacho, Z.T., Moukdar, F., Liao, H.X., Ma, B.J., Muldoon, M., Theiler, J., Nabel, G.J., Letvin, N.L., Korber, B.T., Hahn, B.H., Haynes, B.F., Gao, F., 2006. Cross-Subtype T-Cell Immune Responses Induced by a Human Immunodeficiency Virus Type 1 Group M Consensus Env Immunogen. Journal of virology 80, 6745-6756.

Wherry, E.J., Ahmed, R., 2004. Memory CD8 T-cell differentiation during viral infection. Journal of virology 78, 5535-5545.

Wodarz, D., Page, K.M., Arnaout, R.A., Thomsen, A.R., Lifson, J.D., Nowak, M.A., 2000. A new theory of cytotoxic T-lymphocyte memory: implications for HIV treatment. Philosophical transactions of the Royal Society of London. Series B, Biological sciences 355, 329-343.

Worobey, M., Gemmel, M., Teuwen, D.E., Haselkorn, T., Kunstman, K., Bunce, M., Muyembe, J.-J., Kabongo, J.-M.M., Kalengayi, R.M., Van Marck, E., Gilbert, M.T.P., Wolinsky, S.M., 2008. Direct evidence of extensive diversity of HIV-1 in Kinshasa by 1960. Nature 455, 661-664.

Wren, L.H., Stratov, I., Kent, S.J., Parsons, M.S., 2013. Obstacles to ideal anti-HIV antibody-dependent cellular cytotoxicity responses. Vaccine 31, 5506-5517.

Wright, J.K., Brumme, Z.L., Carlson, J.M., Heckerman, D., Kadie, C.M., Brumme, C.J., Wang, B., Losina, E., Miura, T., Chonco, F., van der Stok, M., Mncube, Z., Bishop, K., Goulder, P.J.R., Walker, B.D., Brockman, M.A., Ndung'u, T., 2010. Gag-Protease-Mediated Replication Capacity in HIV-1 Subtype C Chronic Infection: Associations with HLA Type and Clinical Parameters. Journal of virology 84, 10820-10831.

Wyatt, R., Kwong, P.D., Desjardins, E., Sweet, R.W., Robinson, J., Hendrickson, W.A., Sodroski, J.G., 1998. The antigenic structure of the HIV gp120 envelope glycoprotein. Nature 393, 705-711.

Yaciuk, J.C., Skaley, M., Bardet, W., Schafer, F., Mojsilovic, D., Cate, S., Stewart, C.J., McMurtrey, C., Jackson, K.W., Buchli, R., Olvera, A., Cedeno, S., Plana, M., Mothe, B., Brander, C., West, J.T., Hildebrand, W.H., 2014. Direct Interrogation of Viral Peptides Presented by the Class I HLA of HIV-Infected T Cells. Journal of virology 88, 12992-13004.

Yang, O.O., 2009. Candidate vaccine sequences to represent intra- and inter-clade HIV-1 variation. PloS one 4, e7388.

Yang, O.O., Ali, A., Kasahara, N., Faure-Kumar, E., Bae, J.Y., Picker, L.J., Park, H., 2015. Short conserved sequences of HIV-1 are highly immunogenic and shift immunodominance. Journal of virology 89, 1195-1204.

Yang, O.O., Daar, E.S., Ng, H.L., Shih, R., Jamieson, B.D., 2011. Increasing CTL Targeting of Conserved Sequences During Early HIV-1 Infection Is Correlated to Decreasing Viremia. AIDS research and human retroviruses 27, 391-398.

Yang, Y., Huang, J., Toth, I., Lichterfeld, M., Yu, X.G., 2010. Mutational escape in HIV-1 CTL epitopes leads to increased binding to inhibitory myelomonocytic MHC class I receptors. PloS one 5, e15084.

Yeh, W.W., Rahman, I., Hraber, P., Coffey, R.T., Nevidomskyte, D., Giri, A., Asmal, M., Miljkovic, S., Daniels, M., Whitney, J.B., Keele, B.F., Hahn, B.H., Korber, B.T., Shaw, G.M., Seaman, M.S., Letvin, N.L., 2010. Autologous Neutralizing Antibodies to the Transmitted/Founder Viruses Emerge Late after Simian Immunodeficiency Virus SIVmac251 Infection of Rhesus Monkeys. Journal of virology 84, 6018-6032.

Younes, S.A., 2003. HIV-1 Viremia Prevents the Establishment of Interleukin 2-producing HIV-specific Memory CD4+ T Cells Endowed with Proliferative Capacity. Journal of Experimental Medicine 198, 1909-1922.

Young, A.C., Nathenson, S.G., Sacchettini, J.C., 1995. Structural studies of class I major histocompatibility complex proteins: insights into antigen presentation. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 9, 26-36.

Yue, L., Pfafferott, K.J., Baalwa, J., Conrod, K., Dong, C.C., Chui, C., Rong, R., Claiborne, D.T., Prince, J.L., Tang, J., Ribeiro, R.M., Cormier, E., Hahn, B.H., Perelson, A.S., Shaw, G.M., Karita, E., Gilmour, J., Goepfert, P., Derdeyn, C.A., Allen, S.A., Borrow, P., Hunter, E., 2015. Transmitted virus fitness and host T cell responses collectively define divergent infection outcomes in two HIV-1 recipients. PLoS pathogens 11, e1004565.

Yusim, K., Kesmir, C., Gaschen, B., Addo, M.M., Altfeld, M., Brunak, S., Chigaev, A., Detours, V., Korber, B.T., 2002. Clustering patterns of cytotoxic T-lymphocyte epitopes in human immunodeficiency virus type 1 (HIV-1) proteins reveal imprints of immune evasion on HIV-1 global variation. Journal of virology 76, 8757-8768.

Zhang, D., Shankar, P., Xu, Z., Harnisch, B., Chen, G., Lange, C., Lee, S.J., Valdez, H., Lederman, M.M., Lieberman, J., 2003. Most antiviral CD8 T cells during chronic viral infection do not express high levels of perforin and are not directly cytotoxic. Blood 101, 226-235.

Zhang, W., Caspell, R., Karulin, A.Y., Ahmad, M., Haicheur, N., Abdelsalam, A., Johannesen, K., Vignard, V., Dudzik, P., Georgakopoulou, K., Mihaylova, A., Silina, K., Aptsiauri, N., Adams, V., Lehmann, P.V., McArdle, S., 2009. ELISPOT assays provide reproducible results among different laboratories for T-cell immune monitoring—even in hands of ELISPOT-inexperienced investigators. Journal of Immunotoxicology 6, 227-234.

Zhou, T., Xu, L., Dey, B., Hessell, A.J., Van Ryk, D., Xiang, S.-H., Yang, X., Zhang, M.-Y., Zwick, M.B., Arthos, J., Burton, D.R., Dimitrov, D.S., Sodroski, J., Wyatt, R., Nabel, G.J., Kwong, P.D., 2007. Structural definition of a conserved neutralization epitope on HIV-1 gp120. Nature 445, 732-737.

Zuniga, R., Lucchetti, A., Galvan, P., Sanchez, S., Sanchez, C., Hernandez, A., Sanchez, H., Frahm, N., Linde, C.H., Hewitt, H.S., Hildebrand, W., Altfeld, M., Allen, T.M., Walker, B.D., Korber, B.T., Leitner, T., Sanchez, J., Brander, C., 2006. Relative dominance of Gag p24-specific cytotoxic T lymphocytes is associated with human immunodeficiency virus control. Journal of virology 80, 3122-3125.

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Figure 2. Illustration of diversity and global distribution of HIV-1 clades and Circulating recombinant forms (Santos and Soares, 2010) on Page 21. Copyright permission as follows:

Figure 3. Comparison of the variability in HIV-1gp120 sequences with the variability HA1 domain **of human influenza H3N2 (Garber et al., 2004)** on Page 22. Copyright permission as follows:

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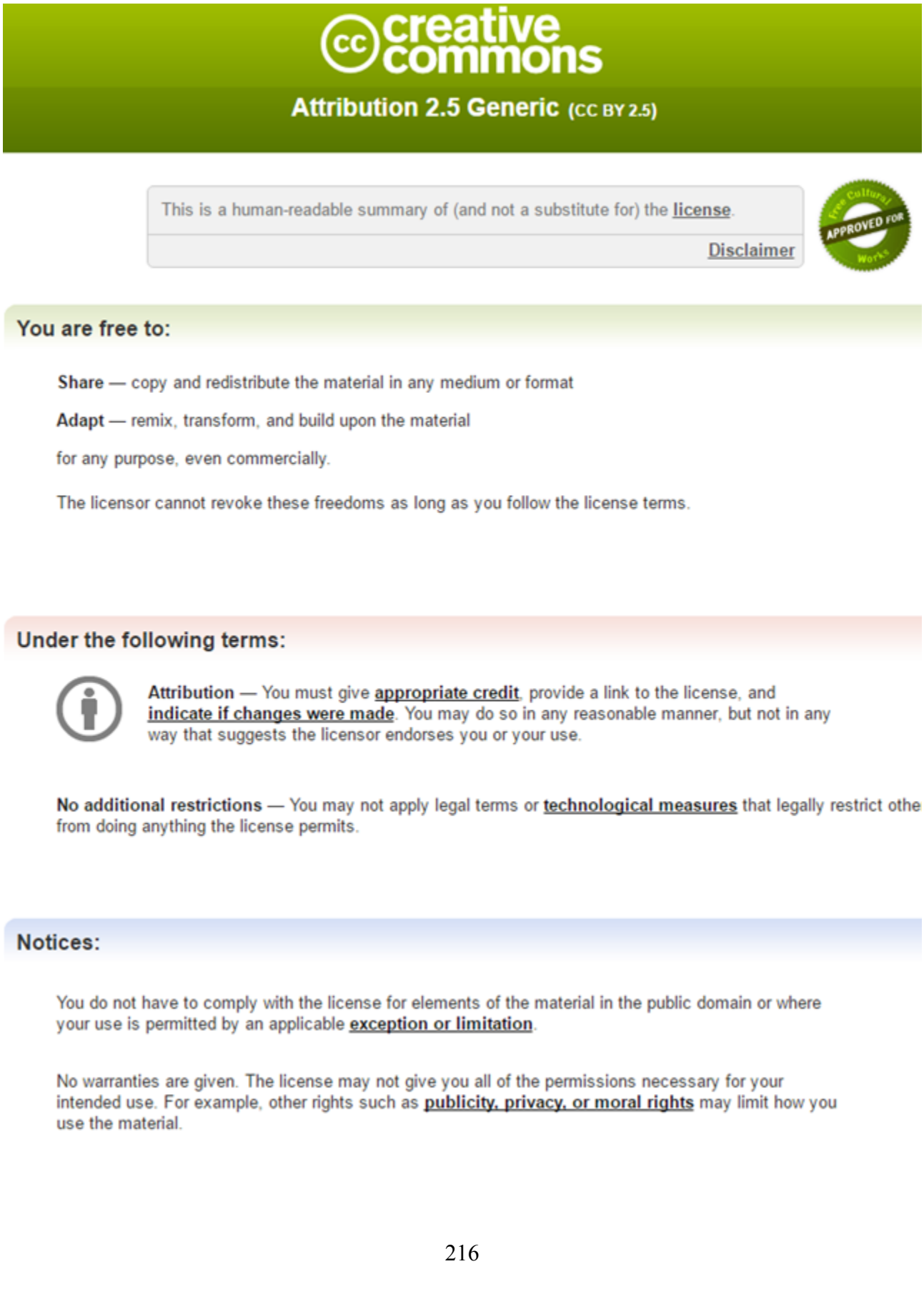

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Figure 8. Illustration of the MHC class I antigen processing and presentation pathway (Extracted from (Neefjes et al., 2011) on Page 32.Copyright permission as follows:

Figure 9. Fourteen of the most highly conserved regions of the HIV-1 used to synthesis HIVconsv peptides (Letourneau et al., 2007) on Page 49.

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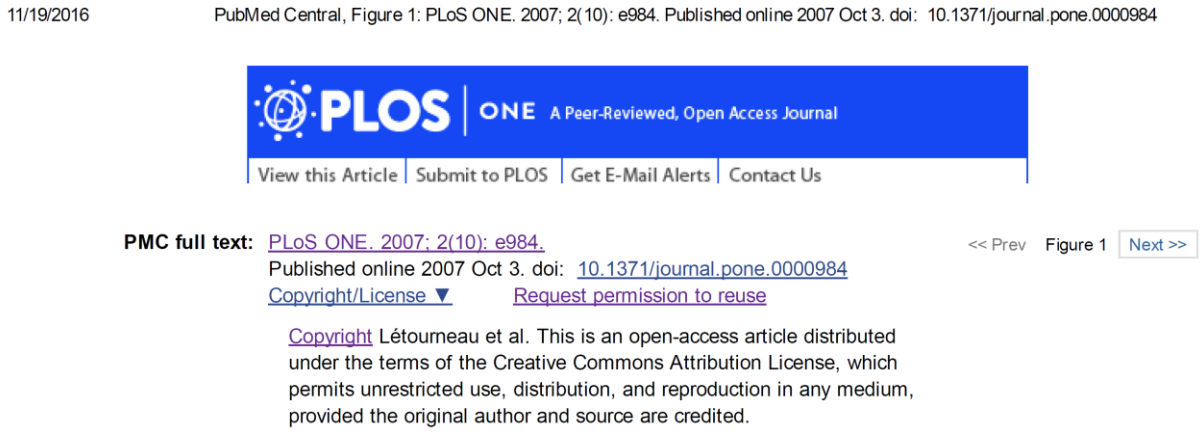

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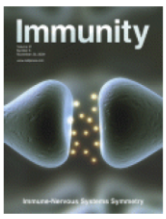

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