The Dissertation Committee for Jiwon Lee Certifies that this is the approved version of the following dissertation:

The molecular-level characterization of the serum antibody repertoire to influenza

Committee:

George Georgiou, Supervisor

Hal S. Alper

Andrew D. Ellington

Ning J. Jiang

Edward M. Marcotte

Jennifer A. Maynard

The molecular-level characterization of the serum antibody repertoire to influenza

by

Jiwon Lee, B.A.; M.E.Chem.E.

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Dedication

I dedicate this work to all who have made it possible for me to make it this far, especially my parents who are my inspirations and my guiding light.

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The molecular-level characterization of the serum antibody repertoire to influenza

Jiwon Lee, Ph.D.

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Supervisor: George Georgiou

Vaccination is the most effective means to protect populations against infectious viruses by eliciting a diverse repertoire of antibodies. For influenza, a rapidly mutating virus posing a constant threat of a pandemic, seasonal vaccination is considered the best prophylactics, but it has limited efficacy and requires annual vaccination. To develop more effective influenza vaccine strategies, a comprehensive understanding of serum antibodies elicited by vaccination is essential, yet it has been confounded by the complexity of the antibody response. In this work, we used the high-resolution proteomics analysis of immunoglobulin (Ig-seq) coupled with the high-throughput sequencing of B cell receptor transcripts (BCR-seq) to quantitatively delineate the serum antibody repertoire to influenza. In Chapter 2, we analyzed the sera collected from four young adults before and after receiving trivalent seasonal influenza vaccine, which contains hemagglutinins from two influenza A virus strains (H1 and H3) and one influenza B strain. The serum repertoire comprised between 40-147 clonotypes specific to each of the three monovalent components of the vaccine, with ~60% of the vaccine response consisting of antibodies that were already present in serum before vaccination. We also observed a surprisingly high fraction of serum antibodies recognizing both the H1 and H3 monovalent vaccines (H1 + H3 cross-reactive antibodies). For subsequent

analysis, in Chapter 3, we recombinantly expressed representative serum antibodies, and the H1 + H3 cross-reactive antibodies displayed a broad range of binding specificities to hemagglutinins from historic viral strains. We identified a group of antibodies recognizing the same conserved epitope in the hemagglutinin head domain that is only exposed in its monomeric form. These antibodies protected mice in challenging with H1N1 and H3N2 virus strains. In Chapter 4, we performed a longitudinal analysis of an individual's serological repertoire specific to pandemic A/California/04/2009 (CA09pdm) viral strain across 6 years. Through multiple exposures to CA09pdm from infection and vaccination, our analysis revealed that the immediate antibody response to CA09pdm differed for each instance of exposure, but the antibody repertoire returned back to the pre-exposure state. This observation was due to the persistent antibodies comprising about half of the serum antibodies, while the intermittent antibodies were elicited following exposures but decayed soon after. The subsequent analysis on their binding specificities revealed that the persistent antibodies were likely to be targeting the stem region of the hemagglutinin while the intermittent antibodies tended to be head-specific. Collectively, our data provide unprecedented insights on the serological responses to influenza with direct implications for engineering a future influenza vaccine endowed with higher and broader protective efficacy.

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Chapter 1: Introduction

The development of smallpox vaccination by Edward Jenner in 1796 remains one of the greatest medical achievements in recent history and has had a significant impact on the health of the general population. It also marked the beginning of the practice of deliberately mounting protective immunity against various infectious diseases. Today, millions of lives are saved each year as a result of vaccination, and once deadly diseases like smallpox, polio and tetanus are now either eradicated or well under control [1, 2]. However, unlike these successful examples of developed vaccination, influenza vaccines have been largely ineffective. Vaccination against influenza still leaves a large fraction of the population vulnerable, primarily failing to elicit robust and lasting protective responses against the virus [3]. Every year, influenza causes over 1 billion infections, 5 million cases of severe illness, and approximately half a million deaths globally. In the U.S. alone, the economic burden arising from annual influenza epidemics totals over $80 billion [4, 5]. Influenza is a persisting global threat, and the most pressing unmet need is to improve vaccination strategies to be more effective, with this fundamentally hinging on a better understanding of vaccine-mediated immunity in humans.

OVERVIEW OF INFLUENZA VIRUS

Types, Structures and Infection Cycle

Influenza is an enveloped, single-stranded RNA virus of the Orthomyxoridae family. Influenza was first isolated from humans in 1933 [6], and is classified into three genera: A, B and C. Influenza A virus causes moderate to severe illness for humans and

other mammals, consisting mostly of wild aquatic birds, which serves as the natural reservoir for the viruses. In contrast, influenza B affects almost exclusively humans with milder symptoms while influenza C virus is rarely reported in humans [7]. Transmission of the virus occurs through air with rapid replication in the human respiratory system, making the virus transmittable from coughing and sneezing [8].

On the surface of the influenza virion envelope, there are two major glycoproteins, hemagglutinin (HA) and neuraminidase (NA) (Figure 1.1) [9]. HA is a trimeric protein responsible for binding to target cell surfaces via sialic acid receptors and subsequent fusion with the host cell, while NA is involved in virus progeny release from infected cells [10, 11]. Currently, there are 18 different known influenza A virus HA subtypes (H1 through H18), which are categorized into group 1 (which includes H1, H2, H5, H6, H8, H9, H11, H12, H13, H16, H17 and H18) and group 2 (which includes H3, H4, H7, H10, H14 and H15) based on their antigenic differences and 11 distinct known NA subtypes (N1 through N11) identified in nature [12]. The differences in HA and NA determine the subtype of viral strains. For example, the H1N1 viral strain name indicates that it contains the H1 subtype of HA and the N1 subtype of NA. Of all the different HAs and NAs, only H1, H2 and H3, and N1 and N2 are commonly found in human infections [13]. For the influenza B virus, strains are grouped into two lineages, Victoria and Yamagata, based on the antigenic properties of the surface proteins [14]. Influenza A and B viruses carry eight pieces of segmented viral RNA that encode for eleven proteins required for infection and replication of the viruses, inside of the viral envelopes [15]. As shown in Figure 1.1, information regarding the viral type based on the genetic materials, geographical origin, strain number, year of isolation and virus subtype is used to determine the specific nomenclature for each identified viral strain [8].

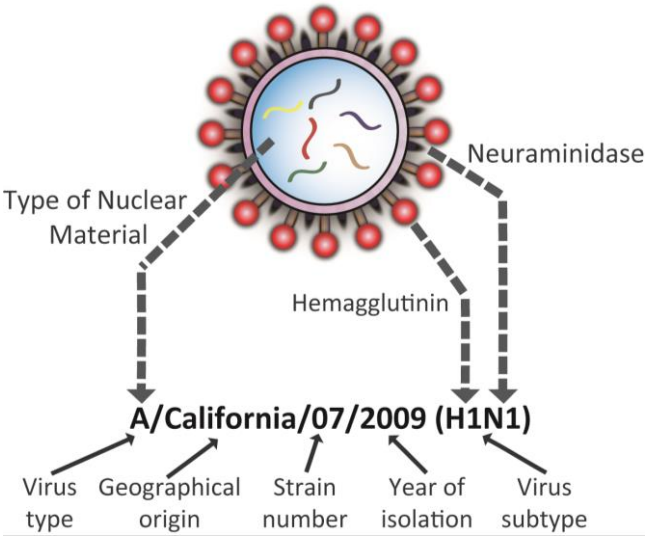

Figure 1.1: Structure and nomenclature of influenza virus. Hemagglutinin and neuraminidase are two major surface proteins that define virus subtypes, while the genetic materials inside of the viral envelope determine the virus type. Nomenclature for each viral strain includes information about virus type, location of virus origin, strain number and year of isolation.

In order for the virion to be infectious, HA needs to be cleaved by the host's trypsin-like protease [16]. Since these proteases are mainly produced in the respiratory tract in humans, viral replication is generally restricted to the cells in the respiratory tissue. The uncleaved HA is called HA0, and the cleaving of HA0 generates two subunits, HA1 and HA2, which remain bound to each other. This cleavage of HA0 also generates the fusion peptide, which consists of amino acids at the N-terminus of HA2. This fusion peptide is required for the viral infectivity. As shown in Figure 1.2, the head domain of HA is mainly formed by HA1, and it mediates the attachments to host cells through a pocket known as the receptor-binding site (RBS). Underneath the head domain is the stem domain, where the fusion peptide is located. Following the attachment of the virion to the cell surface through RBS, the virus is endocytosed and placed in endosomal

vesicles inside of the host cell. The pH inside of the endosomal vesicle then drops, which causes a conformational change of the HA from pre-fusion to post-fusion structures. This transformation projects the fusion peptide outwards, and it is inserted into the host's endosomal membrane during the infection cycle. This leads to the fusion of the viral envelope and the host cell's endosomal membrane [8, 17, 18].

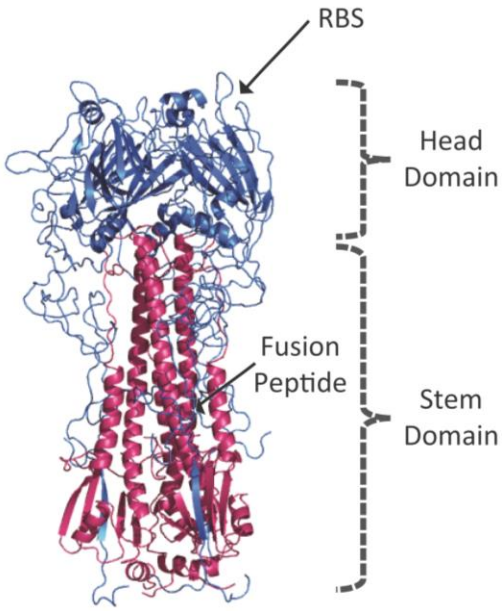

Figure 1.2: Structure of hemagglutinin. Hemagglutinin contains HA1 (blue) and HA2 (magenta) subunits that form head and stem domains. The receptor-binding site (RBS) mediates the attachment to host cells while the fusion peptide is essential for fusion between viral and host cells' membranes for subsequent infection.

Upon fusion, viral RNAs, along with other viral proteins including core proteins and RNA-dependent RNA polymerases, are released into the host cell's cytoplasm [15]. The core proteins transport the viral RNA to the cell nucleus, and the viral RNA serves as a template for the viral RNA-dependent RNA polymerase. Some of the newly

synthesized viral RNA leave the nucleus and are translated into HA and NA proteins, which are secreted to the cell surface. Other newly-translated viral proteins required for RNA replication are transported back into the nucleus. As HA and NA cluster on the surface of the cell and begin budding, viral RNA along with other viral proteins are packaged into a virion. Through the action of NA cleaving sialic acid from the surface of the cell, virus particles are released and the host cell then dies. The free virions then attach to other healthy host cells and repeat the infection cycle [8, 19].

Antigenic Drift and Shift

Influenza virus is equipped with the ability to rapidly evolve due to the low fidelity of the viral RNA polymerase. The RNA polymerase incorporates roughly one error every 10,000 nucleotides, accumulating point mutations on the surfaces of HA and to a lesser extent NA [14]. Considering the entire genome of the virus is roughly 14,000 bases long, the incorporation of mutations leads to rapid diversification of the virus, which aids in evading the host's immune system. This mechanism of escape is known as antigenic drift [8]. It is worth noting that not every amino acid residue is equally inclined to mutate. This is because the resulting mutations are constrained by the necessity for the virus to maintain its cell-binding capability and assembly into the infective viral structure. Accordingly, some regions are more conserved than others; in the case of HA, the stem region and the RBS tend to be conserved across different strains of virus while the residues around the RBS diverge substantially [20].

Antigenic shift, on the other hand, results from the shuffling of genomic RNA segments between different influenza A virus strains, particularly strains infecting different hosts (i.e. avian, swine, and human influenza viruses). When two viral strains

co-infect a single host cell, viral RNAs from each virus are copied in the host cell's nucleus (Figure 1.3). As the cell surface begins budding and genetic materials are packaged as described above, the reassortments of genetic material can occur where some of the RNA segments could have derived from either of the viruses. As a result, progeny virions carrying reassorted RNA are produced, and they are called reassortants. Unlike antigenic drift, which integrates limited variations, antigenic shift can result in extensive alterations of the virus. In contrast, because influenza B virus mainly infects humans only, it is difficult for influenza B viruses to undergo antigenic shift [8].

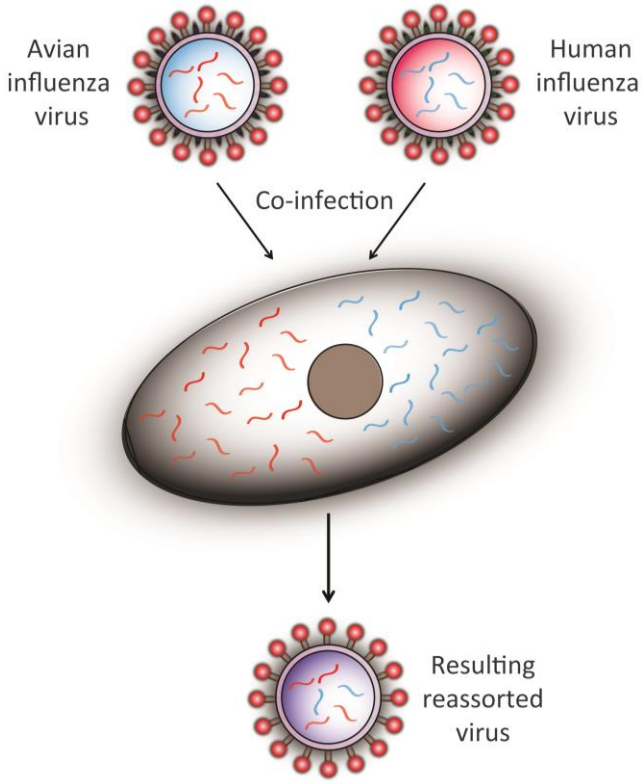

Figure 1.3: Influenza virus reassortment. When two different strains of influenza A viruses (avian and human influenza viruses in this case) co-infect a single host cell, reassortments of the genetic materials can occur, resulting in the generation of reassorted viruses, which could become a pandemic strain.

Significant transformation in viral properties resulting from antigenic shift can potently give rise to a pandemic influenza virus. The major difference in avian and human influenza viruses is their ability to recognize different sialic acids; most avian influenza viruses bind to sialic acid linked to galactose via an α-2,3 linkage, while human-infecting viruses have a preference for an α-2,6 linkage [21]. The majority of epithelial cells in the gut of birds have the α-2,3 linkage while human respiratory epithelial cells have the α-2,6 linkage. Due to this difference, avian influenza viruses do not transmit well in humans. However, in animals such as pigs, which have cells with both α-2,3 and α-2,6 linkages, both influenza viruses can co-infect a single cell, resulting a reassortant strain that is similar to avian influenza viruses but now has a capability to bind to the α-2,6 linkage and spread between humans. The divergent antigenic nature of the surface proteins of avian influenza viruses, which humans have not been exposed to before, could quickly lead to a pandemic especially when the reassortants are highly virulent.

Such shuffling of genetic materials has led to numerous pandemics throughout the history. The last deadly pandemic took place back in 1918 when a strain of virus jumped from birds to humans and caused an estimated 50-100 million deaths [4]. A more recent pandemic occurred in the spring of 2009 caused by A/California/07/2009pdm (CA09pdm) virus strain. While the lack of mortality associated with CA09pdm infection prevented a large number of deaths, an estimated 43-89 million people were still infected [4]. CA09pdm was capable of binding to both α-2,3 and α-2,6 linked sialic acids since it was a reassortant from human, avian, and swine influenza viruses [22]. These past incidents highlight the potential danger of influenza virus the need for controlling the infection.

Therapeutics and Vaccines for Influenza

There are several therapeutic options available for influenza infection. Antiviral therapeutics, zanamivir (Relenza) and oseltamivir (Tamiflu) are major drugs currently available on the market [23, 24]. These are NA-inhibiting small molecules rationally designed based on the structure of NA. However, because of their mode of action, which relies on preventing the release of progeny from infected cells, they have a limited window of therapeutic efficacy. If these inhibitors are not taken shortly after the onset of flu symptoms, they will not be sufficient to stop the infection. Moreover, there have been recent reports of emerging drug-resistant viral strains as well [25]. In addition to the antiviral drugs, several promising monoclonal antibodies (mAb) against influenza are currently under clinical trials [26]. These antibodies provide protection against a wide range of influenza virus subtypes, including the ones that have caused pandemics, mostly through targeting conserved regions of the HA stem.

Alternatively, influenza vaccination offers a prophylactic means to control the disease. The history of influenza vaccine dates back to 1938, where a vaccine developed by Jonas Salk and Thomas Francis was administered monovalently against influenza A virus of the H1N1 subtype [27]. In 1942, a bivalent vaccine was formulated after the discovery of influenza B virus, and it included an influenza A H1N1 strain and an influenza B strain. The first trivalent vaccine containing two strains of influenza A virus (H1N1 and H3N2 subtypes), and a strain of influenza B was introduced in 1978, and in 2012, a quadrivalent vaccine was approved in the United States that added another influenza B strain to ensure the inclusion of a strain from both the Victoria and Yamagata lineages [28].

Currently, there is one major licensed influenza vaccine available - inactivated influenza vaccines (IIV). Live attenuated influenza vaccine (LAIV) was once used widely,

but in 2016, the Centers for Disease Control and Prevention (CDC) recommended to stop administering it due to its inefficacy [29]. IIV is prepared mainly by propagating influenza viruses using an egg-based process, although there is one approved IIV made from a cell-based process [30]. From the virus-containing fluid, viruses are inactivated, and HA from viruses are isolated to produce IIVs [31]. Alternatively, recombinant influenza vaccine (RIV) is also available, where HA is recombinantly purified, but is not as widely used as IIV [32].

Because of the seasonal change of influenza virus in circulation resulting from antigenic drift and shift, flu vaccine is the only immunization that is annually updated. This is a critical drawback of the vaccine. In 1952, the World Health Organization (WHO) influenza surveillance system was established to constantly monitor influenza strains in nature. Each year, based on the epidemiology of influenza, WHO decides which strains are most likely to affect the populations and recommend them to be included in the list of vaccines being manufactured by commercial suppliers [8]. However, there have been several instances where the predictions mismatched the strains that spread in the population that particular year, which greatly limited the vaccine efficacy [33, 34]. Nonetheless, a protective antibody response elicited by vaccination holds the most promise for protecting the population against influenza infection, especially pandemics.

HUMORAL IMMUNE SYSTEM

The goal of influenza vaccination is to provide a lifelong protective immunity against a wide range of influenza virus strains. Over 200 years have passed since the first development of smallpox vaccines, but the current influenza vaccines work on essentially the same principles demonstrated by Jenner. This principle encompasses the activation of

humoral immunity through the administration of antigenic material derived from the pathogen, prompting the body to produce antibodies to mount defense against the infection. Humoral immunity relies on diverse repertoires of the membrane-bound form of antibodies, called B cell receptors (BCR) on the surface of B cells, and soluble immunoglobulin (Ig) molecules secreted by a subset of B cells [35].

Antibody Isotypes, Structure and Functions

The basic structure of antibodies consists of two identical heavy chains and two identical light chains connected by disulfide bonds, with both heavy and light chains contain variable (V) and constant (C) domains [36] (Figure 1.4). In humans, there are 5 different isotypes of antibodies (IgM, IgA, IgG, IgD and IgE), classified based on the different C regions of the heavy chains, with IgG and IgA being the most abundant isotypes in circulation and at mucosal sites, respectively. The heavy chain of IgG is comprised of a V region (V_H), and 3 C regions (C_{H1}, C_{H2} and C_{H3}). Light chains, on the other hand, have two types of C domains, λ and κ [35], and in humans, about 65% of antibodies contain the κ C domain. V_H, V_L, C_{H1}, and C_L domains of an antibody molecule form an antigen-binding fragment (Fab), which, as its name indicates, mediates binding to the antibody's target antigen. The rest of the C region of the heavy chain is known as the crystallizable fragment (Fc), and this Fc domain is important in mediating various immune functions by acting through binding to specific receptors, known as Fc receptors (FcRs).

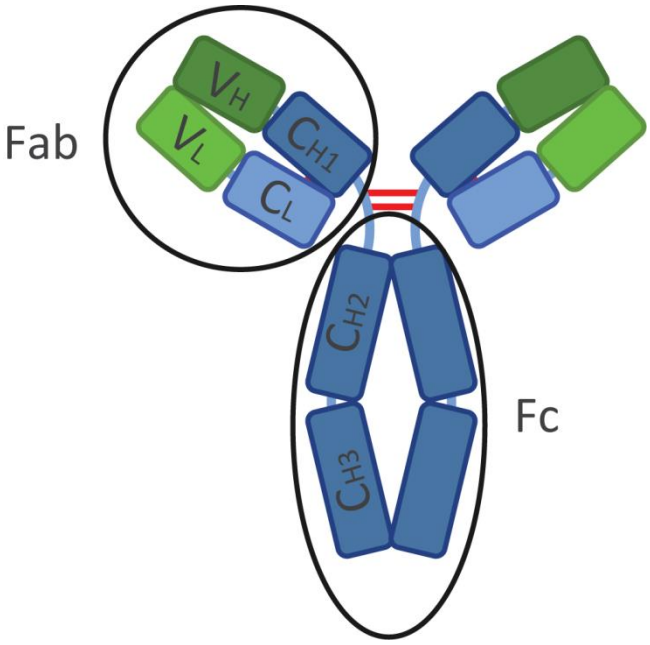

Figure 1.4: Diagram of an immunoglobulin G (IgG) molecule. The heavy chain molecule consists of variable (V_H) and constant (C_{H1}, C_{H2} and C_{H3}) domains, and the light chain molecule is comprised of variable (V_L) and constant (C_L) domains. Red lines indicate disulfide bonds connecting two heavy chains. Fab, antigen-binding fragment; Fc, crystallizable fragment.

The specificity of an antibody molecule toward its target antigen is mainly determined by the complementarity determining regions (CDRs), which are highly variable regions in the V domain that form loops [37, 38]. A combination of 3 CDRs from both the heavy and light chain (CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2 and CDR-L3) forms the antigen-binding site of an antibody molecule [39]. This binding site is also known as a paratope, and it targets an epitope on its target antigen. In particular, CDR-H3 and CDR-L3 hypervariable regions contain the most diversity in terms of length and amino acid composition. Variations in these regions are fundamental to the generation of highly diverse antibody molecules that can bind to variety of different antigens [40-42].

Soluble Ig molecules in circulation provide protection against viral infections through several mechanisms. First, they can prevent pathogens from infecting cells by blocking parts of the surface of pathogens, and this process is known as neutralization. In the case of influenza, antibodies that target the RBS of HA can neutralize the virus by inhibiting the binding of HA to sialic acid receptors. Additionally, antibodies that bind tightly to the fusion peptide, or other nearby regions in the stem of HA, can limit the conformational change of HA that is required for infection, thereby nullifying the infectivity of the virus. These epitopes on HA that lead to neutralization of the virus are called neutralizing epitopes, and antibodies targeting them are referred to as neutralizing antibodies [43].

Antibodies can also trigger other immune responses to disable pathogens. Even when antibodies target non-neutralizing epitopes on pathogens, they can coat the surface of the pathogen by binding via their Fab domains. The resulting cluster of Fc domains surrounding the pathogen stimulate effector functions by recruiting immune cells that express FcRs. Different isotypes of antibodies, IgA, IgG and IgE, have different FcRs specific for each. Upon binding to the Fc clustered on the surface of pathogens, effector cells are activated which leads to variety of mechanisms resulting in the elimination of pathogens [44]: phagocytosis of pathogens can occur via phagocytes, via a process known as antibody-dependent cell-mediated phagocytosis (ADCP); natural killer cells can release cytokines and cytotoxic molecules through a mechanism called antibody-dependent cell-mediated cytotoxicity (ADCC); and mast cells and neutrophils can also degranulate to destroy pathogens. Because the different isotypes of antibodies have their own cognate FcRs, effector functions can be modulated with a great control. Furthermore, pathogens can be cleared through another process called the complement pathway [44]. In addition to binding to FcRs, the Fc domain of IgG can also bind to the first

complement protein in the pathway, C1q, which triggers a cascade of other complement protein activation. As a result, pathogens can be destroyed through opsonization via phagocytes, as well as lysed through a membrane-attacking complex formed by complement pathway components [45]. Through these different mechanisms of action, a conglomerate of antibodies specific to pathogens, also referred to as an antibody repertoire, moderates infections.

B Cell Development and Antibody Diversification

Antibody diversity is the foundation for the immune system to mount protective humoral immune responses against a variety of pathogens encountered during a lifetime, with diversification of the antibody repertoire occurring in B cells. The initial development of B cells takes place within the bone marrow, originating from hematopoietic stem cells (HSC). These cells differentiate into pro-B cells, which can become, among others, a pre-B cell [35].

During these stages of the B cell development, B cells first generate diversity through the process of recombining gene segments, which is known as V(D)J recombination [46]. Genes for heavy and light chains of antibodies are encoded on three loci of the genome. The immunoglobulin heavy gene locus is located on chromosome 14, and the immunoglobulin light kappa chain and lambda chain loci are located on chromosomes 2 and 22, respectively [47-49]. In the heavy chain locus, there are multiple copies of variable (V), diversity (D), and joining (J) gene segments while each light chain locus contains V and J gene segments only. As illustrated in Figure 1.5, the heavy chain locus contains several V, D, and J gene segments, as well as multiple C genes. In HSC, before becoming early pro-B cells, one copy of a D gene and one copy of J gene are

recombined (DJ) by removing the D and J gene segments located between the two. Then recombination of V with DJ occurs in late pro-B cells, where effectively one copy of V, D, and J are recombined while the other gene segments are deleted from the genome.

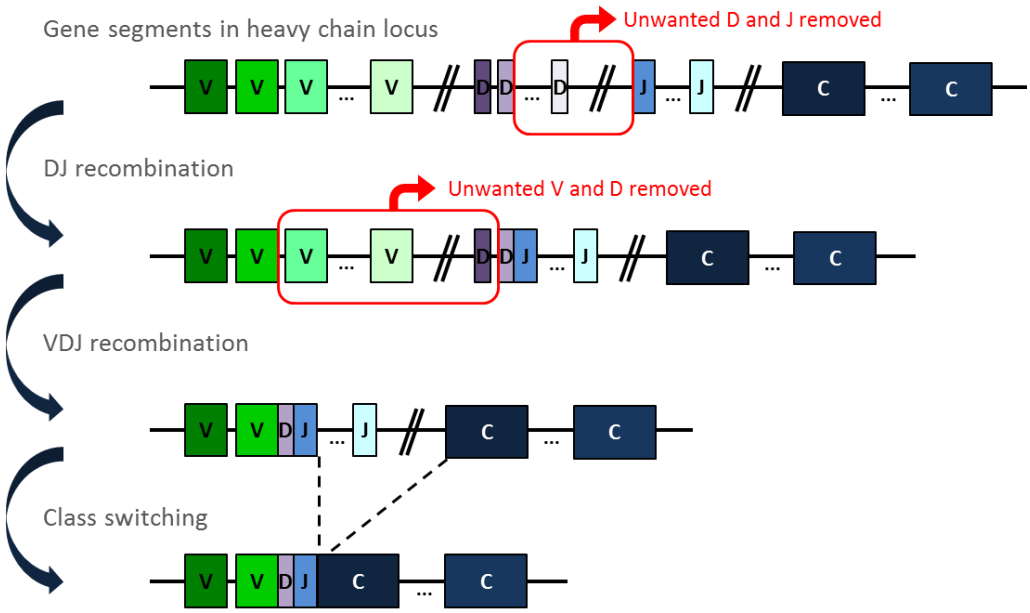

Figure 1.5: VDJ recombination of heavy chain locus.

Following the VDJ recombination of the heavy chain, pro-B cells become pre-B cells by expressing pre-B cell receptor (pre-BCR) consisting of the rearranged heavy chains with μ constant region assembled with surrogate light chains [49]. A surrogate light chain consists of lambda 5 and VpreB subunits, which resemble constant and variable regions of a light chain molecule, respectively. The purpose of the surrogate light chain is to check whether the rearranged heavy chains are productive. These surrogate light chains are replaced by light chains once they undergo VJ recombination, and when the assembly of productive heavy and light chains genes is achieved in B cells,

they become immature B cell. The junction of the recombined V(D)J gene segments forms CDR-3 regions, which explains the aforementioned high degree of diversity found in them. Combined with the junctional diversity resulting from imprecise recombinations, such as palindromic (P) and non-templated (N) nucleotide addition and deletion by terminal deoxynucleotidyl transferase (TdT) [50], and D gene's ability to use three reading frames [51], a theoretical diversity of antibodies reaches over 10^{12} [52]. At this stage of the development, B cells are checked for auto-reactivity, and B cells expressing BCR that bind to self-antigens are either corrected through a process known as receptor editing or die through apoptosis [53]. Cells then further differentiate and express IgD on the surface to become what is known as mature naïve B cells and leave the bone marrow and enter peripheral circulation.

Mature naïve B cells become activated upon encountering cognate antigen, but BCR on mature naïve B cells tend to have low affinities for its target (Figure 1.6). With the necessary signals, and help from T follicular helper (Tfh) cells with antigen-presenting cells (APCs) such as dendritic cells (DCs) [54], these B cells proceed to enter germinal centers (GC). In the case of influenza infection, DCs that exit the respiratory tract upon infection with influenza virus are the main source of antigen, which then travel to lymph nodes or the spleen [55, 56]. In the GCs, B cells rapidly proliferate through a process known as B cell clonal expansion [57, 58]. At this stage of B cell development, the multiplicity of antibody specificity is further increased through an activation-induced (cytidine) deaminase (AID)-catalyzed mechanism called somatic hypermutation (SHM), which introduces point mutations in V regions [59]. Following clonal expansion in the GCs, the affinity of BCRs to the antigen will change through SHM, and B cells with BCRs that have increased affinities will be able to outcompete those B cells that have decreased antigen-binding potency or acquired autoreactivity. They survive through

signaling from follicular DCs (FDCs) and undergo additional expansion; this mechanism is called affinity maturation [60, 61]. Class switching recombination then takes place and BCRs of either IgG, IgA, or IgE isotype are generated [35].

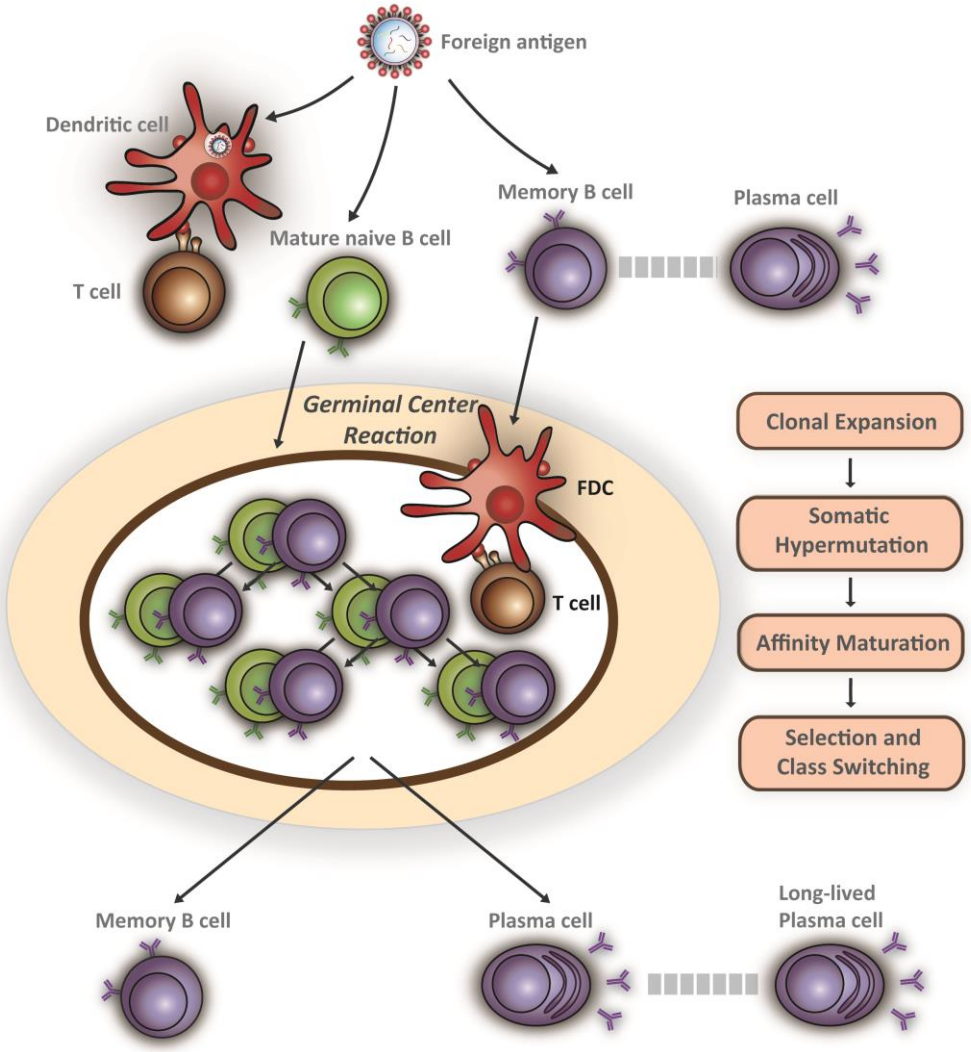

Figure 1.6: Development of antigen-specific B cells and antibody diversification During the primary response (green), with the help from T follicular helper cells (T cells), uptake and presentation of foreign antigen by dendritic cells leads to germinal center (GC) reaction for mature naïve B cells where clonal expansion, somatic hypermutation, affinity maturation, selection, and class switching occurs. Upon leaving the GC, some differentiate into memory B cells (mBCs) while the others differentiate into plasma cells (PCs) that secrete soluble antibodies. Some of these PCs become long-lived plasma cells. Upon repeated exposure to the antigen, a secondary response (purple) follows where mBCs can either re-enter the GC or differentiate into PCs. FDC, follicular dendritic cell.

Ultimately, these B cells can terminally differentiate into plasmablasts (PBs) or plasma cells (PCs) that secrete soluble Ig molecules, which will bind to pathogens and trigger the mechanisms of protection described above, or differentiate into memory B cells (mBCs). Upon repeated exposure to the same antigen, these mBCs serve as a mediator for a rapid recall response and quickly differentiate into PBs and PCs that generate a burst of Ig molecules [54, 62, 63]. A small fraction of PCs can also hone in the bone marrow and become long-lived, bone marrow plasma cells (BM-PCs) [64]. These BM-PCs are antibody-secreting cells responsible for maintaining antibody levels in the periphery. Once secreted into circulation, or in serum, IgG molecules have a half-life of about 3 weeks. Without the persistence of the cognate antigen to continuously activate B cells to secrete more antibodies, serum antibody levels begin to decay as short-lived PBs and PCs die. However, the BM-PCs in the bone marrow continue to secrete antibodies, achieving steady-state serum antibody levels [65, 66]. It is postulated that BM-PCs can survive in the bone marrow for a very long time, possibly throughout an entire lifetime [65]. Thus, immunological memory is rendered by the diverse repertoires of antibodies maintained by the mBCs and BM-PCs. These antibodies secreted by BM-PCs provide the immediate, first line of protection against pathogens before the reactivation of mBCs [64]. With the different compartmentalization of antibody repertoires, there is a great level of control mediating the humoral responses.

TOOLS FOR ANALYZING ANTIBODY RESPONSES

An indication of an effective vaccination is the generation of diversified antibodies that can confer protection against various pathogens. To assess the elicitation of such antibodies, several serologic assays relying on the bulk serum antibodies have

been developed. These assays use the aggregate of antibodies in serum without an understanding of their identities. For influenza, the gold standard of evaluating vaccine responses encompasses the use of total anti-influenza antibody binding, hemagglutinin-inhibition (HAI), and neutralizing titers. These assays provide quantitative information on the total antibodies with binding specificity to the antigens, the level of antibodies that can prevent its attachment of the virus to cells, and the level of antibodies capable of neutralizing the infectivity of the virus, respectively. Each assay uses different dilution of serum, which contains circulating antibodies. Currently, HAI titers of a 1:40 dilution is considered to be the main correlate of protection, which stems from clinical tests illustrating such levels achieved following vaccination being associated with protection in 50-70% of the cases [66-68]. Vaccine manufacturers and the U.S. Food and Drug Administration (FDA) also primarily use these bulk analyses for the determination of vaccine efficacy. However, these methods are not precise enough to understand antibody responses elicited by complex viruses like influenza. Such understanding requires a much detailed interrogation and identification of the constituent monoclonal antibodies that comprise the bulk antibody response and the subsequent characterization of these antibodies. Through the development of novel technologies enabling such studies, our understanding of antibody response has increased tremendously in recent years.

Single Cell Analysis

Because Ig molecules are encoded by B cells, information on the antibody repertoire is encoded in B cells. Thus, analyzing B cells has served as a substitute to dissect the humoral response. The recombinant expression of monoclonal antibodies comprising bulk antibody properties can be used for functional characterization as well as

for the identification of epitopes on antigens to better understand the mechanisms of protective immunity. With peripheral blood B cells being the primary source due to sample availability, PCs and PBs, as well as mBCs following vaccination or infection have been extensively analyzed through two major methods developed for the examining single B cells.

The first breakthrough was the development of a technique for immortalizing B cells [69]. This technique relies on isolating B cell subsets of interest and immortalizing those B cells with Epstein Barr Virus (EBV). The EBV-immortalized B cells are then allowed continuously secrete Ig molecules expressed by each cell inside of individual well, and the culture supernatants can be directly used for binding and functional assays. Because single cells are immobilized in each well, the supernatants contain monoclonal antibodies. This technique bypasses the need for cloning and purifying each monoclonal antibody; instead, it enables a very rapid functional screening of antibodies being secreted by EBV-immobilized B cells. After isolating antibodies with interesting features, their sequences are determined through classical Sanger sequencing of transcripts encoding the antibodies. In 2011, Corti *et al.* combined the immortalization technique with influenza neutralization assays to screen for antibodies secreted by B cells that have neutralization capabilities against a broad range of viral strains [70]. One of their isolated bNAbs, called FI6, showed its remarkable ability to neutralize all of the influenza A virus strains through the targeting of a conserved epitope in the stem of HA.

Another technique involves isolating single PBs, PCs, or mBCs and recovering antibody sequences through single-cell polymerase chain reaction (PCR) for subsequent cloning, expression, and functional characterization of monoclonal antibodies [71, 72]. After sorting single cells into individual wells, transcripts from the cells are used to amplify and subsequently clone the encoded antibodies. These antibodies are then

recombinantly purified and further characterized. A notable study by Wrammert and colleagues employed such technique to generate 86 recombinant mAbs from PCs isolated after influenza vaccination to determine that 71% of PCs at 7 days post-vaccination were specific to influenza [72]. A further breakdown of the specificities indicated that 20% of the antibodies could bind and neutralize the virus, while others only bound to HA (20%), other surface proteins (12%), a denatured form of the surface proteins (10%), or a non-influenza component of the vaccine (9%). In a similar study involving sorted PBs from an adult one week after 2007-2008 TIV vaccination, Whittle *et al.* identified and characterized an antibody, named CH65, with CDR-H3 loop that binds to the RBS pocket of the HA by mimicking sialic acid [73]. This antibody garnered great interest due to its wide range of neutralization capability against H1 strains (31 out of 36) by targeting the conserved receptor-binding region.

Additionally, single cell analysis has led to identification of numerous potent and broadly neutralizing antibodies against influenza virus [74-83]. Some of these antibodies are currently being developed as therapeutic mAbs. Characterization of mAbs from these studies has elucidated the neutralizing epitopes that these antibodies target which are conserved across multiple strains of influenza virus. These studies also helped identify conserved epitopes in the stem and shown how mAbs targeting the stem region have the potential to neutralize a wide range of strains, which led to the inception of using stem-only HA as a universal vaccine [76, 84-86]. However, the main limitation encompassed by these approaches is the low-throughput nature of those analyses since such techniques capture only a small fraction of the antibody repertoire, making it difficult to accomplish a broader characterization of the antibody response.

High-throughput Sequencing Analysis

More recently, the rapid advancement of next generation sequencing (NGS) technology with lowering cost and increasing throughput has enabled in-depth characterization of B cells through the sequencing of millions of B cells simultaneously by relying on the generation of cDNA from mRNA. Initial studies have focused on sequencing heavy chain sequences since previous studies have shown that heavy chains largely determine the antigen specificity [41]. Such development has led to the utilization of NGS to study the antibody responses following influenza vaccination and infection [87-91].

In a study involving the new pandemic 2009 H1N1 vaccine, Li and colleagues determined that the vaccination with the pandemic strain elicited many antibodies that were cross-reactive between different H1N1 strains. They hypothesized that the influenza response is mainly driven by recall responses mediated by mBCs, and that the significantly altered HA head domain of novel H1N1 pandemic strain led to the stimulation of stem-specific mBCs generated from previous exposure [91]. Moreover, a couple of studies from Quake's group uncovered that most of vaccine response is established by the recall response, through the discovery of identical sequences after two different annual vaccines in the same individual [88], and that the repertoire is highly clonal in the elderly with a high number of accumulated mutations [89]. These studies have shown the effect that pre-existing antibodies can have on a subsequent exposure to influenza virus, a notion first observed over a half-century before using aggregate serology [92].

Still, high-throughput sequencing of BCR repertoire has a limitation associated with expressing and characterizing monoclonal antibodies due to the lack of information on the endogenous light chain paired with sequenced heavy chains. The problem arises

from the fact that the heavy chain and light chain of an Ig molecule comes from two distinct mRNA molecules, and that this information is lost during the NGS of heavy chains. To resolve this issue, several additional technologies have been developed that enable high-throughput identification of native V_H:V_L pairing [93-95]. In all, B cell repertoire studies have provided us with significant insights about the immune response to influenza.

Proteomics-based Technology for Antibody Repertoire Analysis

Despite all the recent advancements in technology, there still is a sizable gap in our understanding of the monoclonal antibodies comprising the serum antibody repertoire in circulation at the protein level. This is because peripheral blood B cells represent only a very small fraction of the total antibody producing cells in the body [96]. Additionally, B cells and circulating Ig molecules have great difference in their temporal persistence (Figure 1.7). For instance, pathogen-specific B cells peak in abundance around 7 days after exposure but quickly decay in the periphery, while pathogen-specific Ig molecules peak in abundance around 3 weeks after the exposure and decay with a slower rate until a steady-a state level is reached. Because of this difference, it is particularly difficult to understand the protein-level serological abundance and temporal dynamics of the antibody identified through the interrogation of the B cell compartment. Due to the technical complexities arising from the high frequency of homologous sequences obscuring the direct identification of the monoclonal antibodies through proteomics, however, such direct analysis of serum has not been attempted until recently.

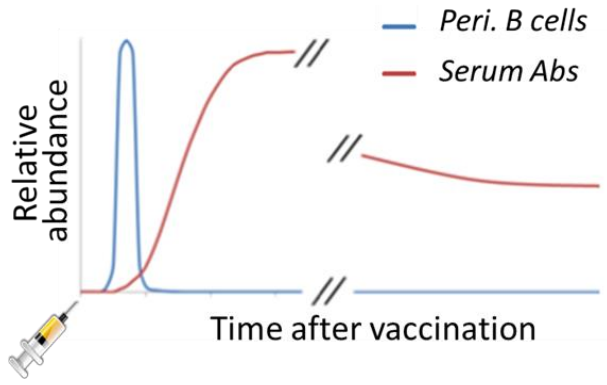

Figure 1.7: Temporal dynamics of antigen-specific peripheral B cells and serum antibodies following vaccination. While peripheral B cells peak in abundance around day 7 and quickly decay, serum antibodies peak around 3 weeks after vaccination and slowly decay until reaching a steady-state level.

In recent years, the development of bottom-up proteomics technology using shotgun liquid chromatography tandem mass spectrometry (LC-MS/MS) have led to the deconvolution of the antibody repertoire at the protein-level in various studies [97-101]. These techniques involve using antigen-immobilized resins to isolate antigen-specific Ig molecules from the serum samples collected after vaccination via affinity chromatography. These antibodies are trypsinized, which generates CDR-H3 peptides, and analyzed through mass spectrometry. For peptide identification, custom databases are constructed for each donor by NGS of day 7 peripheral B cells. Individual databases are used to interpret the MS/MS spectra by searching the spectra against the given database of sequences. Such a method was used for antibody discovery studies where antigen-specific antibodies from rabbits, mice, and humans were isolated [97-101]. In addition to antibody discovery, one of the studies applied the technology to analyze the repertoire of antibodies elicited by tetanus vaccine boosters from two volunteers and determined the temporal dynamics of serological tetanus-specific antibodies [98]. The study compared

between peripheral B cell repertoire and the steady-state molecular antibody repertoire, and showed that less than 5% of sequences from antigen-specific PCs identified at day 7 after vaccination encoded for antibodies that were actually present at steady-state (9 months after the booster) in serum. This finding is significant since it highlighted the potential misinterpretation of the antibody repertoire following influenza vaccination that could arise from solely relying on the analysis of transcripts from B cells. It is apparent that there is the need for the repertoire analysis at the protein-level to decipher which antibodies are actually present and involved in providing protection, in particular, for determining which antibodies are maintained to mediate long-term protection.

SUMMARY

The holy grail of influenza vaccine research is to develop a 'universal' vaccine that can elicit long-term protective antibodies to combat a variety of different strains, negating the need for annual influenza vaccination. Such development may require immunogen engineering [84, 86], adjuvant studies to improve immune responses [102], or a better understanding of how innate immunity to enhance vaccine response [103]. Nevertheless, a comprehensive understanding of the vaccine response is the cornerstone of this goal.

Recent studies interrogating B cells have led to the discovery of therapeutically promising bNAbs; yet, whether the bNAbs encoded by peripheral PCs and mBCs are present at physiologically relevant abundances as Ig molecules in serum and can contribute to protection remains unanswered. Previous studies showing the apparent discordance between the B cell compartments and the serum antibody compartment

strongly argue that the determination of the serological repertoire is critical for a comprehensive understanding of the response to influenza vaccination or infection.

In this dissertation, we utilize a proteomics-based analysis of the antibody repertoire to characterize how seasonal vaccination changes the serum antibody repertoire in healthy young adults, to better understand the functions and biochemical properties of abundant monoclonal antibodies, and to illustrate the changes in the serum response in one individual following 6 years of repeated exposure to the same viral strain.

Chapter 2: Quantitative, Molecular-Level Analysis of the Serum Antibody Repertoire in Young Adults Before and After Seasonal Influenza Vaccination

This chapter is reproduced with some modifications from its initial publication:

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INTRODUCTION

Influenza infections cause over 5 million cases of severe illness resulting in approximately half a million deaths globally every year and constantly pose a threat of

another pandemic [4, 5]. The influenza hemagglutinin (HA) surface glycoprotein is the main antigenic determinant of the influenza virus, and neutralizing antibodies that bind to HA are known to confer protective immunity [85, 104]. Influenza A and influenza B are mainly responsible for the infection, and they are antigenically distinct from one another. There are two major groups, group 1 and group 2, belonging to influenza A, and there are two main lineages, Victoria and Yamagata lineages, in influenza B. Seasonal trivalent inactivated influenza vaccine (IIV3) currently in use contains HA of virus strains from each group of influenza A (H1N1 and H3N2) and one of the two lineages in influenza B (either the Yamagata or Victoria lineage). Presently, the IIV3 is gradually being replaced by quadrivalent inactivated influenza vaccine (IIV4) that includes HA from both Yamagata and Victoria lineage strains. Whether the IIV4 offers increased protection over IIV3 is actively debated [105, 106].

Every year, epidemiologically-identified circulating virus strains constitute the seasonal influenza vaccine, making it the only vaccine that is continually updated through surveillance systems. Despite the effort, however, it has been estimated that IIV3 confers protection in only about 60% of young adults and efficacy can be substantially lower for at-risk groups such as infants and the elderly [3, 107]. For this reason, improved immunogen designs aimed at increasing effectiveness are at the forefront of the quest for the next generation influenza vaccine [84-86], and it underscores the need to have better understanding of the antibody response elicited by the vaccination.

Over the past few years, cloning and characterization of antibodies and high-throughput sequencing of transcripts encoding heavy chain variable (V_H) regions from peripheral blood B cells has enhanced our understanding of antibody-mediated protection to influenza [70, 72, 73, 78, 81, 88, 108]. Nevertheless, it is antibodies circulating in serum, not immunoglobulin receptors on B cells, that directly mediate protection against

viral infection. For that reason, bulk serological metrics including ELISA and neutralization titers to viral strains have also been used to understand the response to vaccination or infection. However, neither investigation of peripheral B cells nor bulk serological assays provide information regarding the sequence, relative concentrations, temporal dynamics and functions of the individual monoclonal antibodies that comprise the polyclonal anti-influenza serum repertoire. In this chapter, the serum antibody repertoire is directly investigated at a molecular level through high-resolution liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) to determine the extent to which seasonal influenza vaccination either boosts levels of pre-existing serum antibodies or elicits new antibodies, how clonal diversity of the serum repertoire is affected by immunization and how it relates to the overall ELISA titer, the influenza-binding breadth of the antibody response, and the differences between vaccine-boosted and vaccine-elicited antibody responses.

MATERIALS AND METHODS

Study Volunteers, Peripheral Blood Mononuclear Cell (PBMC) Isolation and RNA Extraction

All study protocols were approved by the Institutional Review Boards at Stanford University. Informed consent was obtained from participants. Blood was taken before (day 0) and after (days 7, 28 and 180) vaccination. Volunteers were four young adults (22–32 yrs) in generally good health and vaccinated with one dose of IIV3 (FluZone; Sanofi-Pasteur); 60 ml peripheral blood were taken from individuals and heparinized. PBMCs were extracted from 10 ml blood using a Ficoll-Gradient and frozen in 10% (v/v) DMSO/40% (v/v) FBS according to the Protocols of the Stanford Human Immune Monitoring Center (HIMC). After thawing, total RNA was extracted from 5 million PBMCs using the Qiagen AllPrep Kit.

Library Preparation for High-throughput Sequencing of V_H-encoding Genes

500 ng of total RNA was used as input for library preparation. Reverse-transcription was performed according to the manufacturer's instructions using SuperScript III Enzyme (Life Technologies) and primers for all five isotypes containing eight random nucleotides and partial Illumina adapters containing Illumina barcodes. Second-strand synthesis was done using Phusion Polymerase (NEB), and primers containing eight random nucleotides and partial Illumina adaptor sequences that bind either framework region (FR) 1 or 3 of all variable (V) segments with a maximum of one mismatch (98 °C for 2 min, 52 °C for 2 min, and 72 °C for 10 min). Double-stranded cDNA was purified two times using Ampure XP beads at a ratio of 1:1. Double-stranded cDNA was amplified with Platinum Hifi Polymerase (Life Technologies) with two

primers completing Illumina adaptor sequences (amplification conditions: 95 °C for 2 min; 27 cycles of 95 °C for 30 s, 65 °C for 30 s and 68 °C for 2 min; and 68 °C for 7 min). Final sequencing libraries were generated by purifying the PCR product using Ampure XP beads at a ratio of 1:1, and they were sequenced using the Illumina MiSeq platform.

Reagents

Individual IIV1s comprising the 2011–2012 FluZone IIV3 (H1N1 A/California/07/2009 X-179A, H3N2 A/Victoria/210/2009 X-187 and B/Brisbane/60/2008), as well as the influenza B component of the 2012–2013 FluZone IIV3 (B/Texas/6/2011), were obtained from Sanofi-Pasteur. A/California/7/2009-X181 rHA was provided by Dr. Stephen Harrison (Harvard Medical School).

Purification of Total IgG from Serum and Subsequent Digestion into F(ab')2

For each serum sample analyzed in this study, 30 ml of diluted serum (1:2 dilution in PBS) was passed through a 4-ml Protein G Plus agarose (Pierce) affinity column in gravity mode. Serum flowthrough was collected and passed through the column three times. The column was washed with 15 column volumes (cv) of PBS before elution with 5 cv of 100 mM glycine-HCl, pH 2.7. The eluate, containing total IgG from serum, was immediately neutralized with 2 ml of 1 M Tris-HCl, pH 8.0, and dialyzed into 20 mM sodium acetate, pH 4.5, overnight at 4 °C. Purified IgG was digested into F(ab′)2 with 1 ml of immobilized pepsin resin (Pierce) per 40 mg of IgG in 20 mM sodium acetate, pH 4.5, for 4 h on an inverter at 37 °C. Pepsin resin was spun down at 2,000*g* to halt digestion and washed with PBS three times. Supernatants from the digestion reactions and washes were combined and pH-adjusted to 7.

Antigen Enrichment of F(ab')2 and Mass Spectrometry Sample Preparation

Respective IIV1s or rHA were immobilized on *N*-hydroxysuccinimide (NHS)-activated agarose resins (Pierce) by overnight rotation at 4 °C. The coupled agarose resins were washed with PBS, and unreacted NHS groups were blocked with 1 M ethanolamine, pH 8.3, for 30 min at room temperature (RT). The beads were further washed with PBS and packed into a 2-ml chromatography column (Clontech). The column was prewashed with 5 cv of 2 M urea and equilibrated with 10 cv of PBS. The prewashing step with urea was to remove HA that was noncovalently bound to the resin following immobilization, because we initially noticed that a large amount of HAs detaching from the resins interfered with the subsequent analysis. Exhaustive controls with elution and flowthrough samples from affinity chromatography were performed to ensure that the prewashing step with 2 M urea does not impede complete capture of F(ab′)2 molecules that recognize IIV1.

For each sample, the F(ab′)2 sample was divided into equal volumes, with each fraction being applied to the individual antigen affinity columns in gravity mode. Flowthrough was collected and reapplied to the column three times, and the column was washed with 10 cv of PBS and 10 cv of diluted PBS (1:2 in ddH_2O). Antigen-enriched F(ab′)2 was eluted with 60 mM HCl, pH 1.7, in 0.5-ml fractions and neutralized with NaOH/Tris. Flowthrough and elution fractions were assayed by indirect ELISA with the corresponding IIV1. To ensure that our enrichment steps were not biasing isolation for high-affinity antibodies, the depletion of ELISA signal in each flowthrough sample was checked. Elution fractions showing an ELISA signal were pooled and concentrated under vacuum to a volume of ~0.1 ml and desalted into ddH_2O using a 0.5-ml Zeba spin column (Pierce). The desalted eluate was further concentrated to 50 µl under vacuum.

For each enrichment, elution and flowthrough samples were denatured in 50% (vol/vol) 2,2,2-trifluoroethanol (TFE), 50 mM ammonium bicarbonate and 10 mM dithiothreitol (DTT) at 60 °C for 1 h, then alkylated by incubation with 32 mM iodoacetamide (Sigma) for 1 h at RT in the dark. Alkylation was quenched by the addition of 20 mM DTT. Samples were diluted tenfold with 50 mM ammonium bicarbonate and digested with trypsin (1:30 trypsin:protein) for 16 h at 37 °C. Formic acid was added to 1% (vol/vol) to quench the digestion, and the sample volume was reduced to ~100 µl under vacuum. Peptides were then bound to a C18 Hypersep SpinTip (Thermo Scientific), washed three times with 0.1% formic acid and eluted with 60% acetonitrile, 0.1% formic acid. C18 eluate was dried under vacuum-centrifugation and resuspended in 50 µl in 5% acetonitrile, 0.1% formic acid.

LC-MS/MS Analysis

Samples were analyzed by liquid chromatography–tandem mass spectrometry on a Dionex Ultimate 3000 RSLCnano uHPLC system (Thermo Scientific) coupled to an LTQ Orbitrap Velos Pro mass spectrometer (Thermo Scientific). Peptides were first loaded onto an Acclaim PepMap RSLC NanoTrap column (Dionex; Thermo Scientific) before separation on a 75-µm × 15-cm Acclaim PepMap RSLC C18 column (Dionex; Thermo Scientific) using a 5–40% (vol/vol) acetonitrile gradient over 250 min at 300 nl/min. Eluting peptides were injected directly into the mass spectrometer using a nano-electrospray source. The LTQ Orbitrap Velos Pro was operated in data-dependent mode with parent ion scans (MS1) collected at 60,000 resolutions. Mono-isotopic precursor selection and charge-state screening were enabled. Ions with a charge ≥+2 were selected for collision-induced dissociation fragmentation spectrum acquisition (MS2) in the ion trap, with a maximum of 20 MS2 scans per MS1. Dynamic exclusion was active with a

45-s exclusion time for ions selected more than twice in a 30-s window. Each sample was run three times to generate technical replicate data sets.

MS/MS Data Analysis

Donor-specific protein sequence databases were constructed using the donor's V_H sequences with ≥2 reads, concatenated to a database of background proteins comprising non-donor-derived V_L sequences (HD1) [98], a consensus human protein database (Ensembl 73, longest sequence per gene) and a list of common protein contaminants (MaxQuant). Donor-specific spectra were searched against the corresponding donor-specific database using SEQUEST (Proteome Discoverer 1.4; Thermo Scientific). Searches considered fully tryptic peptides only, allowing up to two missed cleavages. A precursor mass tolerance of 5 p.p.m. and a fragment-mass tolerance of 0.5 Da were used. Modifications of carbamidomethyl cysteine (static) and oxidized methionine (dynamic) were selected. High-confidence peptide-spectrum matches (PSMs) were filtered at a false discovery rate of <1% as calculated by Percolator (*q*-value < 0.01, Proteome Discoverer 1.4; Thermo Scientific).

Isoleucine/leucine sequence variants were collapsed into single peptide groups. For each scan, PSMs were ranked first by posterior error probability (PEP), then *q*-value, and finally XCorr. Only unambiguous top-ranked PSMs were kept; scans with multiple top-ranked PSMs (equivalent PEP, *q*-value and XCorr) were designated ambiguous identifications and removed. Observed precursor masses were recalibrated according to the previous method [109], and the average mass deviation (AMD) for each peptide was calculated as described [97] using data from elutions only. Peptides with an AMD >1.5 p.p.m. were removed. Additionally, only peptides identified in ≥2 replicate injections for at least one elution sample were kept as high-confidence identifications.

Peptide abundance was calculated from the extracted-ion chromatogram (XIC) peak area, as described [98], using peak area values generated by the Precursor Ions Area Detector node in Proteome Discoverer. For each peptide, a total XIC area was calculated as the sum of all unique XIC areas of associated precursor ions, and the average XIC area across replicate injections was calculated for each sample. For each antigen data set, the eluate and flowthrough abundances were compared and peptides with ≥10-fold higher signal in the elution sample were considered to be antigen specific. Similarly, peptide abundances in elution samples were compared across antigens; peptides with ≥10-fold higher signal for a single antigen were considered specific (i.e., H1 or H3 specific), and peptides with <10-fold difference for two or more antigens were considered cross-reactive (i.e., H1 + H3 cross-reactive). In addition, in our H1 + H3 cross-reactive serological repertoire analysis, to avoid overestimating, we removed peptides that appeared in the eluates of all three IIV1 enrichments (which could potentially be binding to other constituents in the vaccine); on average, they accounted for <10% (by abundance) of the serological repertoire.

Clonotype Indexing and Peptide-to-peptide Mapping

V_H-encoding sequences were grouped into clonotypes based on single-linkage hierarchical clustering as described [98]. Cluster membership required ≥90% identity across the CDR-H3 amino sequence as measured by edit distance. High-confidence peptides identified by MS/MS analysis were mapped to clonotype clusters, and peptides uniquely mapping to a single clonotype were considered ‘informative’. The abundance of each antibody clonotype was calculated by summing the XIC areas of the informative peptides mapping to ≥4 amino acids of the CDR-H3 region.

Quantitating Abundance of Individual Antibody Clonotypes

For each serological repertoire, the relative abundance of a clonotype was calculated by dividing the XIC area for a particular clonotype by the sum of the XIC areas for all clonotypes. Fraction of serological repertoire refers to this relative abundance based on XIC. The amount for a particular clonotype was determined by multiplying its fraction of serological repertoire by the corresponding serum sample's binding titer. To generate heat maps, we accounted for the different total binding titers toward each IIV1 by normalizing amount using the day 28 titer as a reference, calling this 'relative amount'. For example, the amount for each clonotype divided by the serum titer of the day 28 sample refers to relative amount.

Enzyme-Linked Immunosorbent Assays (ELISAs)

EC_{50} values based on ELISA were used to determine the binding titers of IIV1-specific antibodies in the serum samples or apparent affinities of the recombinant monoclonal antibodies. First, costar 96-well ELISA plates (Corning) were coated overnight at 4 °C with 4 μg/ml IIV1 or recombinant HAs, and washed and blocked with 2% milk in PBS for 2 h at RT. After blocking, serially diluted recombinant antibodies or serum samples were bound to the plates for 1 h, followed by incubation with 1:5,000-diluted goat anti-human-IgG Fc horseradish peroxidase (HRP)-conjugated secondary antibodies (Jackson ImmunoResearch; 109-035-008) for 1 h. For detection, 50 μl TMB-ultra substrate (Thermo Scientific) was added before quenching with 50 μl 1 M H_2SO_4. Absorbance was measured at 450 nm using a Tecan M200 plate reader. Data were analyzed and fitted for EC_{50} using a four-parameter logistic nonlinear regression model in the GraphPad Prism software. All ELISA assays were performed in triplicate.

Hemagglutination Inhibition Assay

The HAI assay was used to assess abilities of functional antibodies specific to the HA to inhibit agglutination of turkey erythrocytes. The protocol was adapted from the CDC laboratory-based influenza surveillance manual [110]. To inactivate non-specific inhibitors, sera were treated with Receptor Destroying Enzyme (RDE; Denka Seiken, Co., Japan) prior to being tested [111]. Briefly, three parts RDE was added to one part sera and incubated overnight at 37 °C. RDE was inactivated by incubation at 56 °C for ~30 min. RDE-treated sera were two-fold serially diluted in v-bottom microtiter plates. An equal volume of each influenza virus strains (A/California/07/2009, A/Perth/16/2009 and B/Brisbane/60/2008), adjusted to approximately 8 HAU/50 µl, was added to each well. The plates were covered and incubated at RT for 20 min followed by the addition of 1% turkey erythrocytes (RBC; Lampire Biologicals, Pipersville, PA, USA) in PBS. Red blood cells were stored at 4 °C and used within 72 h of preparation. The plates were mixed by agitation, covered, and the RBCs were allowed to settle for 1 hr at room temperature. The HAI titer was determined by the reciprocal dilution of the last well that contained non-agglutinated RBC. Positive and negative serum controls were included for each plate.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA), unless specifically noted otherwise in the figure legends. All the statistical tests performed are described in the figure legends, and correlations were considered significant at a P value of <0.05.

RESULTS

Analytical Pipeline for Characterizing Influenza-specific Serological Repertoire

We previously developed a proteomics-based pipeline for the identification and semi-quantitative determination of the antigen-specific antibodies in human serum [94, 96, 98]. Utilizing this method, we delineated the composition and relative quantities of the antibody clonotypes comprising the serum IgG repertoire before (pre-) and after (post-) vaccination (days 0, 28 and 180) in four human donors who were immunized with the 2011–2012 IIV3 vaccine (Table 2.1 and Figure 2.1). For the analysis, serum IgG was digested with pepsin to generate F(ab')2 fragments, and F(ab')2 specific for each of the three vaccine strains was purified by three separate affinity chromatography columns, each using resins immobilized with one of the monovalent inactivated vaccine components (IIV1) that comprise the IIV3 (A/California/07/2009 X-179A, A/Victoria/210/2009 X-187 and B/Brisbane/60/2008; abbreviated as 'H1 A/CA09', 'H3 A/VI09' and 'Vic B/BR08', respectively). For peptide identification, custom databases were constructed for each donor by high-throughput sequencing of the V_H repertoires from day 7 peripheral B cells.

Table 2.1: Descriptions of the donors involved in the study. The vaccination and diagnosis history is based on self-reporting.

Donor	Age	Gender	Received 2010-2011 IIV3?	Received 2009 H1N1 IIV1?	Ever diagnosed with flu?
1	22	Female	No	No	No
2	22	Male	No	No	No
3	28	Male	No	No	No
4	32	Male	No	No	No

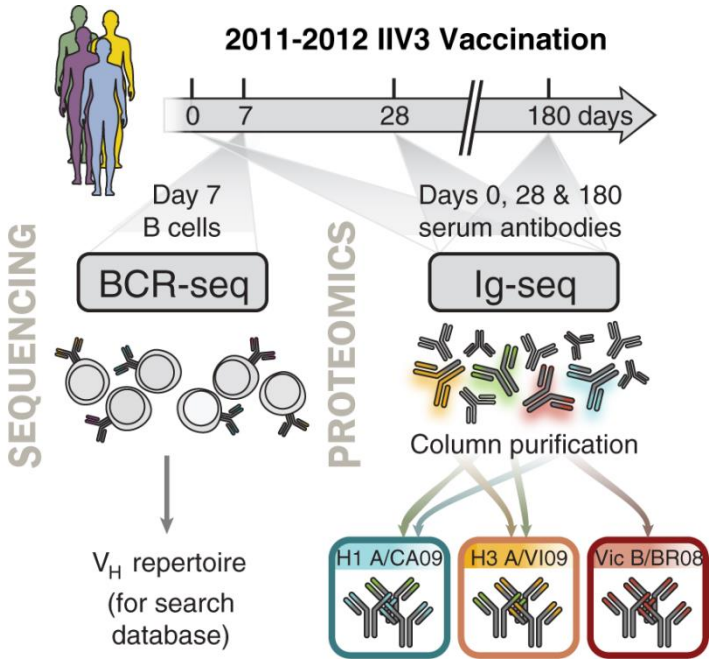

Figure 2.1: Experimental design. For the sequencing of B cell receptor (BCR)-encoding transcripts (BCR-seq), we used peripheral B cells isolated 7 d after vaccination to sequence the V_H repertoires for constructing custom databases of each donor for heavy-chain peptide identification. From sera of the donors, antibodies specific to each IIV1 were isolated through IIV1-immobilized column purification, and the purified immunoglobulins were subsequently analyzed proteomically (Ig-seq).

To ensure that the affinity chromatography conditions result in proper and complete isolation of serum antibodies that recognize IIV1, we performed several control experiments. First, because a pre-washing step with 2 M urea for the IIV1-immobilized resins was added to remove noncovalently-bound HAs shedding into the eluates which interfered with the subsequent LC-MS/MS analyses, we examined the effect of using 2 M urea. IIV1-immobilized resins were packed into two columns, and one column was washed with PBS and the other one with 2 M urea. After equilibrating the columns with PBS, F(ab')2 was applied to each column, and the flowthrough from each column was

collected. Based on the ELISA signal of IIV1-specific antibodies remaining in the flowthrough samples, both had a negligible amount, and the amount of antibody in the flowthrough from the 2 M urea-washed column was indistinguishable from the flowthrough of the column pre-washed with PBS (Figure 2.2). We also repeated the control experiment and analyzed the amount of antibody in each elution fraction with and without the 2 M urea-washing step, and our data showed that they were indistinguishable (Figure 2.3). From the two control experiments, we concluded that the additional pre-washing step with 2 M urea did not affect the integrity of the immobilized IIV1. Second, because of the nature of affinity chromatography, the eluate could be skewed for high affinity antibodies. To test, we checked for the depletion of IIV1-specific antibodies in flowthrough samples, and ensured that the flowthrough samples were depleted of antibodies capable of binding to IIV1 (Figure 2.4). These control experiments conclusively demonstrated that the conditions used for capturing the IIV1-specific antibodies resulted in thorough purification of serum antibodies with affinity toward IIV1. Each eluate samples enriched following the affinity chromatography conditions were subsequently trypsinized and analyzed by LC–MS/MS. In total, analysis of the serological repertoire for all of the time points and donors required 240 runs and >1,200 h of LC–MS/MS time, with collection of >7,000,000 mass spectra.

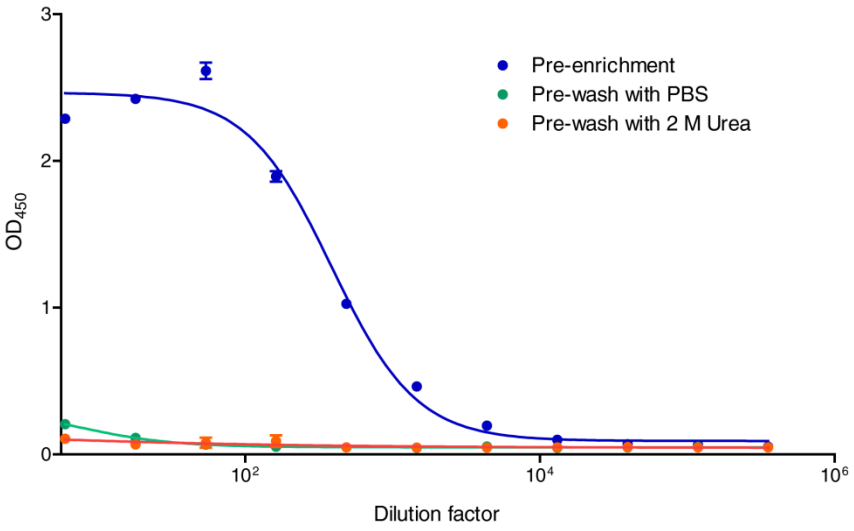

Figure 2.2: Analysis of IIV1-specific F(ab')2 in flowthrough samples after the affinity chromatography. The amounts of IIV1-specific antibodies in the pre-enrichment sample (pre-enrichment) as well as the flowthrough samples from PBS-washed column (pre-wash with PBS) and 2 M urea-washed column (pre-wash with 2 M urea) were analyzed by ELISA.

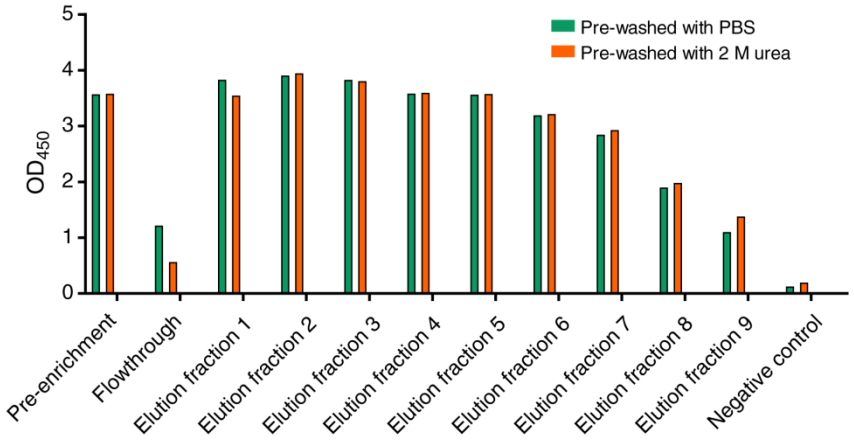

Figure 2.3: Analysis of IIV1-specific F(ab')2 in each elution fraction from the affinity chromatography. The amounts of IIV1-specific antibodies in each elution and flowthrough fractions from PBS-washed column (pre-wash with PBS) and 2 M urea-washed column (pre-wash with 2 M urea) were analyzed by ELISA.

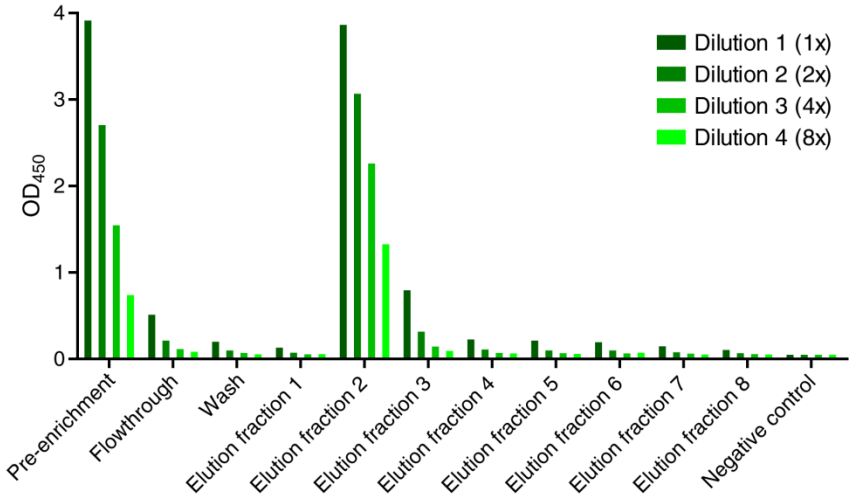

Figure 2.4: A representative experiment data showing the depletion of IIV1-specific antibodies in the flowthrough. ELISA signals for the samples 2-fold diluted are shown.

The Serological Repertoire to IIV3

Antibodies that share sequence similarity in the heavy-chain complementarity-determining region 3 (CDR-H3) and have the same binding specificity belong to a particular clonotype and are likely to recognize the same epitope. For our analyses, we identified high-confidence CDR-H3 peptides and grouped the peptides belonging to the same clonotype together. The corresponding LC peak intensities were used for relative quantification of the antibody clonotypes [97]. An estimated >80% of the CDR-H3 peptides within a sample are typically identified in this manner (detection limit around 0.4 ng/ml), and quantification calibrations using isobaric peptide spike-ins showed that peak intensities correlate well with absolute peptide concentrations [112]. Accordingly, our proteomic methodology enabled us to quantify changes in the abundance of different antibody clonotypes among the serum repertoire of antibodies that bind to each IIV1.

For each time point, the antibody repertoire composition and relative quantities at the clonotypic level were plotted as a histogram, with each bar representing an individual clonotype and the *y* axis showing its relative abundance (Figure 2.5). We defined antibody clonotypes present in both the pre-vaccination repertoire (day 0) and the post-vaccination repertoire (day 28 and/or day 180) as 'pre-existing', and clonotypes observed exclusively in the post-vaccination repertoire (not at day 0) as 'vaccine-elicited'. The term 'vaccine-elicited antibodies' refers only to the fact that these antibodies become detectable in the serum after vaccination and should not be interpreted in B cell ontogeny terms (i.e., whether they arise from stimulated naive or memory B cells). The temporal dynamics of the serum repertoire to each IIV1 at days 0, 28 and 180 for individual donors are represented as heat maps, with color intensity reflecting relative amount of the respective clonotype (Figure 2.6). The comparison of pre-existing and vaccine-elicited antibodies revealed that in the four donors we analyzed, on average pre-existing antibody clonotypes comprised 61.2% and 65.4% of the HA-binding repertoire in day 28 and day 180 sera, respectively (Figure 2.7). Thus, the post-vaccination response is dominated by pre-existing serum antibodies.

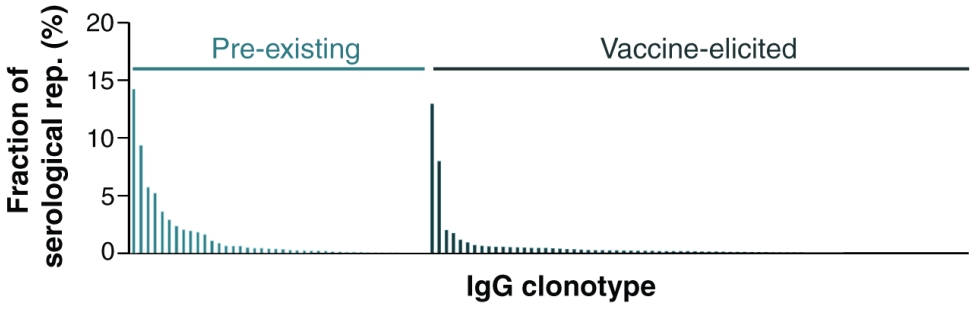

Figure 2.5: Representative serological repertoire (donor 1, day 28 anti-H1 A/CA09). Each bar represents a unique clonotype and the *y* axis indicates the relative abundance (fraction) determined by proteomic analysis.

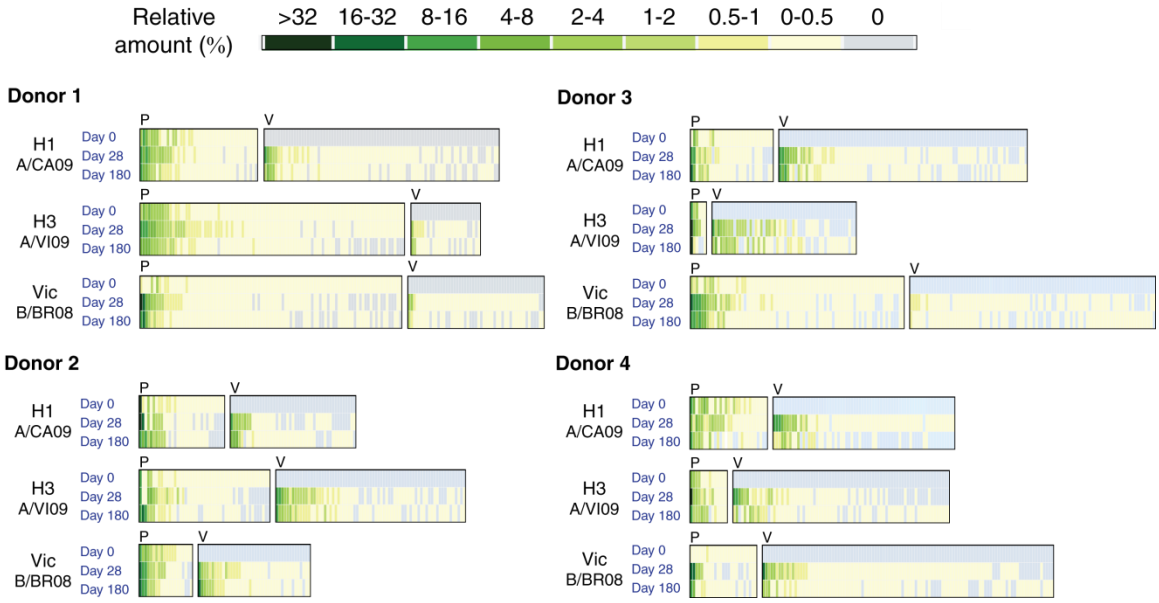

Figure 2.6 Heat maps of the relative amounts of antibody clonotypes (each column indicates distinct clonotype) comprising the serological repertoire to each IIV1 at different time points for each donor. The amount of each clonotype is determined by multiplying its relative abundance by the serum titer for that sample. Relative amounts are determined by normalizing the amounts to day 28 serum titers (refer to Materials and Methods).

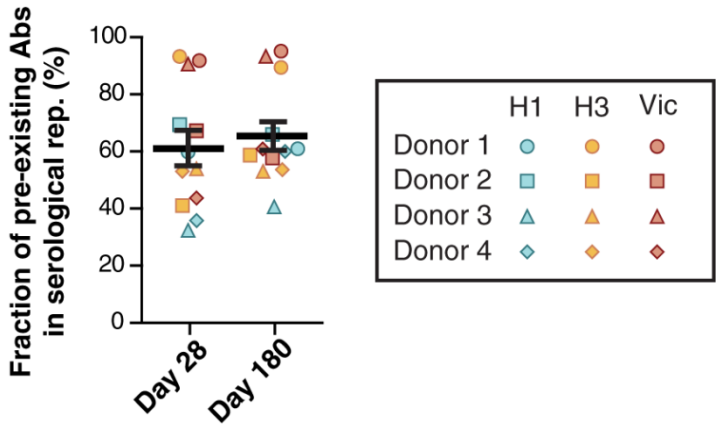

Figure 2.7: Predominance of pre-existing antibody clonotypes (Abs) in serum following vaccination. Each data point (see key, which is used throughout the chapter) corresponds to the fraction of pre-existing Abs in the serological repertoire to an IIV1 for a specific donor. Averages are calculated as mean, with error-bars indicating s.e.m. (n = 12 for each group).

Inspection of the serological repertoires for all of the donors also revealed several additional insights. First, the number of distinct antibody clonotypes specific for each IIV1 ranged from as low as 6 at day 0 to between 40 and 147 at day 28 (Table 2.2), revealing that the influenza-specific repertoire is highly restricted and oligoclonal. The restricted clonality of the serum repertoire observed here is in sharp contrast to the much more diverse repertoire encoded by influenza-specific peripheral B cells [75, 88]. Second, the serological repertoire is highly polarized, and on average, the top 6% of the most abundant clonotypes after vaccination account for more than 60% of the entire repertoire (Table 2.3). This polarization effect is also evident in the pre-vaccination serum IgG repertoires (top 12% antibody clonotypes constituting >60% of the repertoire). Third, the number of antibody clonotypes in the serum repertoire specific for a particular IIV1 correlates with the respective serum titer (Spearman correlation = 0.82, $P < 0.0001$; Figure 2.8 and Table 2.4). Fourth, the frequency of vaccine-elicited antibody clonotypes shows an inverse correlation to the serum titer before vaccination (Spearman correlation = –0.82, $P = 0.0010$; Figure 2.9). In other words, individuals with a lower serum titer before vaccination, and therefore a repertoire comprising fewer antibody clonotypes, produce a greater number of vaccine-elicited antibody clonotypes. These data are consistent with a model (Figure 2.10) in which the greater the number of pre-existing clonotypes (reflected as a higher pre-vaccination serum titer), the greater the number of antigenic epitopes that are bound by circulating antibodies resulting in a blockade of antigenic sites (or possibly, more effective clearance due to formation of HA immune complexes decorated with a larger number of antibodies), thereby reducing the magnitude and diversification of the B cell response [83, 113].

Table 2.2: Size of each IIV1 serological repertoire analyzed. Number of unique clonotypes in each repertoire is indicated. Total refers to the number of all the unique clonotypes that are identified for a particular donor and IIV1s.

Donor	Time point	H1 A/CA09	H3 A/VI09	Vic B/BR08
1	Day 0	44	99	98
	Day 28	118	116	127
	Day 180	110	99	116
	Total	132	125	149
2	Day 0	32	49	20
	Day 28	55	92	57
	Day 180	65	98	57
	Total	80	121	62
3	Day 0	31	6	80
	Day 28	103	40	147
	Day 180	101	47	138
	Total	124	60	172
4	Day 0	29	14	25
	Day 28	95	68	112
	Day 180	50	80	112
	Total	97	95	134

Table 2.3: D60 diversity indices. D60 diversity index refers to number of clonotypes that comprises the 60% (by abundance) of the serological repertoire. For each time point and IIV1 analyzed, D60 diversity index values were divided by the total number of clonotypes detected to determine the frequency of clonotypes that constitute D60 diversity indices.

Donor	Time point	H1 A/CA09	H3 A/VI09	Vic B/BR08
1	Day 0	6/44 (14%)	8/99 (8.1%)	8/98 (8.2%)
	Day 28	8/118 (6.8%)	9/116 (7.8%)	2/127 (1.6%)
	Day 180	8/110 (7.3%)	10/99 (10%)	2/116 (1.7%)
2	Day 0	1/32 (3.1%)	3/49 (6.1)	4/20 (20%)
	Day 28	3/55 (5.5%)	8/92 (8.7%)	3/57 (5.3%)
	Day 180	5/65 (7.7%)	6/98 (6.1%)	3/57 (5.3%)
3	Day 0	3/31 (9.7%)	1/6 (17%)	1/80 (1.3%)
	Day 28	6/103 (5.8%)	3/40 (7.5%)	6/147 (4.1%)
	Day 180	4/101 (4.0%)	3/47 (6.4%)	5/138 (3.6%)
4	Day 0	4/29 (14%)	2/14 (14%)	7/25 (2.8%)
	Day 28	8/95 (8.4%)	4/68 (5.9%)	3/112 (2.7%)
	Day 180	7/50 (14%)	8/80 (10%)	4/112 (3.6%)

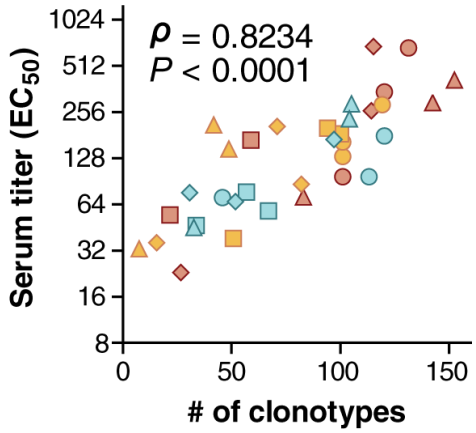

Figure 2.8: Correlation between ELISA serum titer to IIV1 with the number of clonotypes in the respective serum repertoires. Each data point corresponds to titers and the number of clonotypes in serological repertoire specific to an IIV1 for a donor at each time point (n = 36). Statistical analyses were performed using two-sided nonparametric Spearman rank correlation test.

Table 2.4: Serum binding titers to each IIV1 in each collected sera measured by ELISA, with EC_{50} values representing titers. Averages are calculated as mean with error-bars indicating s.d., repeated in triplicate.

Donor	Time point	H1 A/CA09	H3 A/VI09	Vic B/BR08
1	Day 0	70 ± 6	131 ± 7	96 ± 5
	Day 28	178 ± 3	285 ± 4	671 ± 19
	Day 180	96 ± 5	163 ± 8	346 ± 9
2	Day 0	46 ± 9	38 ± 2	54 ± 2
	Day 28	76 ± 3	201 ± 8	168 ± 14
	Day 180	57 ± 0.6	184 ± 8	167 ± 0.5
3	Day 0	45 ± 16	32 ± 9	71 ± 4
	Day 28	289 ± 33	210 ± 0.5	414 ± 22
	Day 180	231 ± 2	146 ± 4	294 ± 4
4	Day 0	76 ± 15	36 ± 12	23 ± 0.8
	Day 28	169 ± 3	205 ± 12	686 ± 25
	Day 180	66 ± 4	86 ± 3	261 ± 16

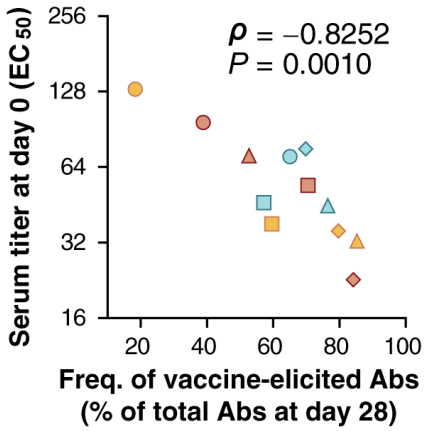

Figure 2.9: Inverse correlation between pre-vaccination ELISA serum titers and the frequency of vaccine-elicited clonotypes at day 28. Frequency is calculated as the number of vaccine-elicited clonotypes divided by the total number of unique clonotypes at day 28 (n = 12). Statistical analyses were performed using two-sided nonparametric Spearman rank correlation test.

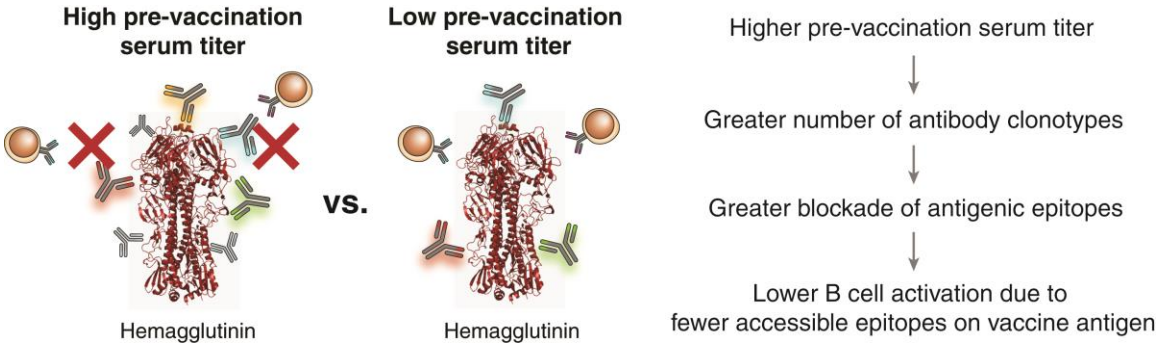

Figure 2.10: A model depicting the effect of high pre-vaccination serum titer on the number of antibody clonotypes elicited in the serological repertoire post-vaccination. As shown in Figure 2.8, serum titer correlates with size of serum repertoire and thus, a higher titer reflects a more diverse antibodies circulating in serum. This likely results in a greater epitope coverage of the antigen HA, leading to a lower B cell stimulations.

Prevalence of Cross-reactive Serum Antibodies

Comparing the repertoires specific to each IIV1, we noticed that a unexpectedly high number of the same antibody clonotypes were detected in both the H1 A/CA09 and H3 A/VI09 affinity chromatography eluates (Appendix A). We considered the possibility that these antibody clonotypes may have been isolated because they bound to constituents in the vaccine other than HA, or because of nonspecific binding. However, when we proteomically analyzed the affinity chromatography eluate from donor 1 day 28 serum using recombinant HA (rHA) of A/California/07/2009 X-181, >70% (by abundance) of all of the antibody clonotypes found in the eluate from affinity chromatography with immobilized H1 A/CA09 (IIV1) were also detected in the eluate from affinity chromatography with the rHA (Figure 2.11). In addition, the abundance of the H3 A/VI09–specific antibody that was also detected in the anti–H1 A/CA09 serological repertoire, on the basis of the proteomic analysis, was in excellent agreement with biochemical estimates of the abundance of H3 A/VI09–binding antibodies found in the eluate from H1 A/CA09 affinity purification (Figure 2.12). These data suggest that the antibodies found in the H1 A/CA09 and in the H3 A/VI09 affinity chromatography eluates overwhelmingly recognize both of the respective HAs and are not directed to other vaccine components, such as neuraminidase.

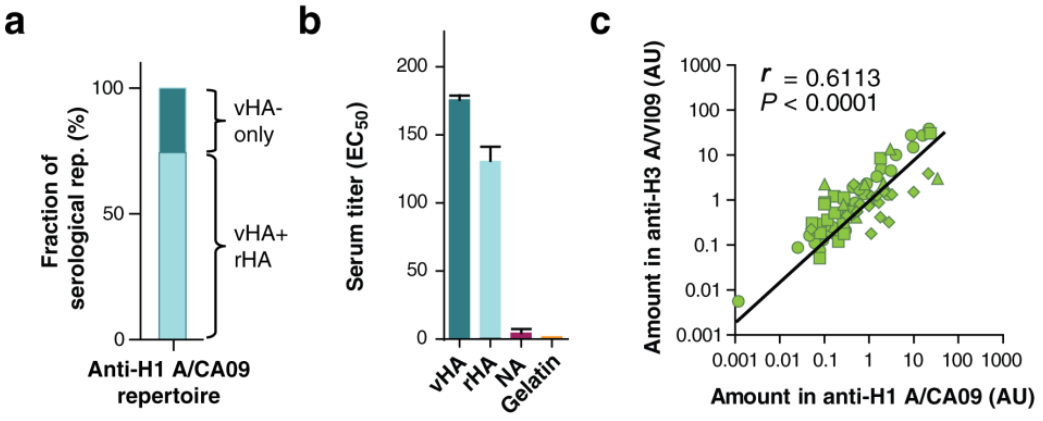

Figure 2.11: Proteomic and biochemical detection of H1 + H3 cross-reactive serum antibodies. (**a**) Comparison between anti-H1 A/CA09 (vaccine HA, vHA) and anti- A/California/7/2009 X-181 (recombinantly-expressed HA, rHA) serological antibody repertoires from the donor 1 day 28 serum. Antibodies clonotypes detected in LC-MS/MS following affinity chromatography with rHA constituted 74%, based on abundance, of the antibodies identified when affinity chromatography was carried out with IIV1 (H1 A/CA09). Antibody clonotypes comprising the remaining 26% were only detected in the eluate from the affinity chromatography with H1 A/CA09. Since the clonotypes detected only in the anti-H1 A/CA09 (26%) do not bind to rHA, such antibody clonotypes are specific to the IIV1 (vHA-specific). In the eluates from affinity chromatography with rHA, 94% of the antibody clonotypes, by abundance, were also found in the anti-H1 A/CA09. The antibodies that bind to both vHA and rHA (vHA + rHA) are directed to epitopes present in rHA that are also found in the IIV1, and these data suggest that the majority of the antibodies binding to IIV1 also binds to rHA. (**b**) Serum anti-influenza vaccine antibodies overwhelmingly react with rHA. In accordance with the data in a above, the ELISA signal against rHA was 75% of the signal against IIV1 (EC_{50} of 133 vs. 178). A very low titer to neuraminidase (NA) or to gelatin, present in the vaccine as a stabilizer, was detected. verages are calculated as mean with error-bars indicating s.d., repeated in triplicate. (**c**) Correlation between the amounts of H1 + H3 cross-reactive clonotypes detected by affinity chromatography with either H1 A/CA09 or H3 A/VI09. Each dot represents one H1 + H3 cross-reactive clonotype with *x* axis and *y* axis indicating amounts of each clonotype in the H1 A/CA09 and H3 A/VI09 affinity chromatography eluate, respectively. Amounts were calculated as described in Figure 2.6. Data points from all of the H1 + H3 cross-reactive clonotypes at day 28 in all four donors are shown. Statistical analyses were performed using two-sided Pearson correlation test.

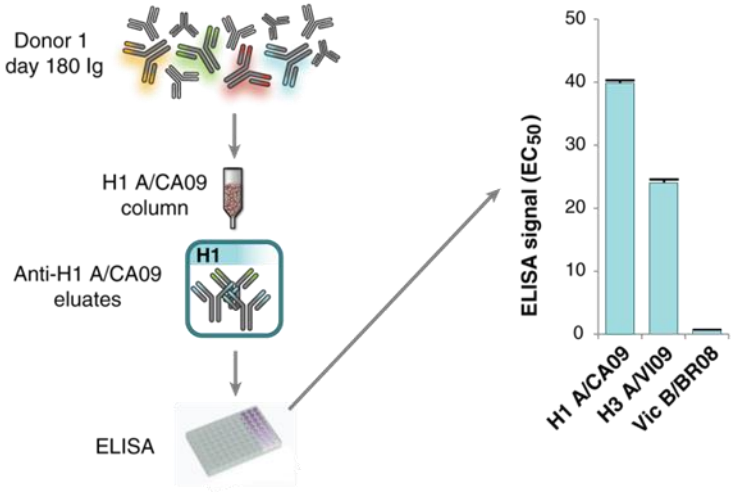

Figure 2.12: Biochemical detection of H1 + H3 cross-reactive antibodies in the eluate from the affinity chromatography with H1 A/CA09. Donor 1 day 180 serum. IgGs were enriched by affinity chromatography with H1 A/CA09, and the eluates were collected. Then, the binding signals of the IgGs in the eluate to H3 A/VI09 and Vic B/BR08 were determined by ELISA. The EC_{50} value of the antibodies in the eluate binding to H3 A/VI09 was ~60% of the EC_{50} value with H1 A/CA09 (EC_{50} of 40 vs. 24). In agreement with this biochemical analysis, the abundance, based on the LC-MS/MS analysis, of all anti-H1 A/CA09 antibody clonotypes that were also found in the anti-H3 A/VI09 repertoire comprised 56%. Averages are calculated as mean with error-bars indicating s.d., repeated in triplicate.

We designated the clonotypes detected in separate affinity chromatography eluates with H1 A/CA09 and H3 A/VI09 as 'H1 + H3 cross-reactive' (Figure 2.13). Notably, several highly abundant clonotypes were H1 + H3 cross-reactive, which was unexpected because previous reports on the HA specificity of antibodies isolated by single B cell cloning from IIV3 vaccines recipients had suggested that clones capable of binding to both group 1 and group 2 strains are rare [72, 79]. In the serum samples from the four donors we analyzed, the average fractions of H1 + H3 cross-reactive antibodies

among total IIV1-binding repertoire were 43.9%, 32.7% and 39.0% on days 0, 28 and 180, respectively (Figure 2.14). Also, across all donors, 63.1% of H1 + H3 cross-reactive clonotypes were pre-existing, and a higher amount of H1 + H3 cross-reactive antibodies in serum at day 0 correlated with a lower frequency of newly elicited H1 + H3 cross-reactive clonotypes at day 28 (Spearman correlation = –0.86, P = 0.0107; Figure 2.15), in agreement with the model in Figure 2.10. Comparison of the molecular features of H1 + H3 cross-reactive antibodies with those of antibodies detected only in the anti–H1 A/CA09 or anti–H3 A/VI09 repertoires (assigned as 'H1-specific' and 'H3-specific', respectively) showed no significant differences in immunoglobulin heavy chain variable (*IGHV*) gene usage, CDR-H3 length, hydrophobicity or charge, suggesting that there are no specific B cell evolutionary trajectories or germline features that predispose antibodies toward H1 + H3 cross-reactivity (Figure 2.16).

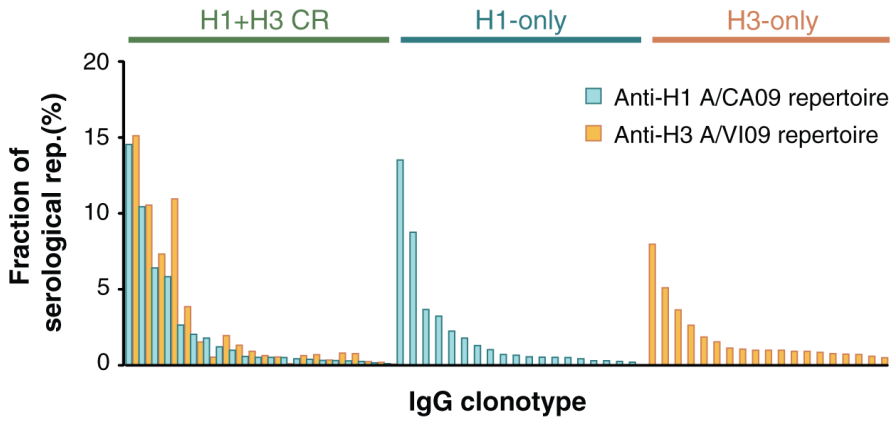

Figure 2.13: Representative histogram from donor 1, day 28 anti-influenza A serological repertoire. The fraction of each antibody clonotype in the anti–H1 A/CA09 and anti–H3 A/VI09 repertoires are indicated. CR, cross-reactive.

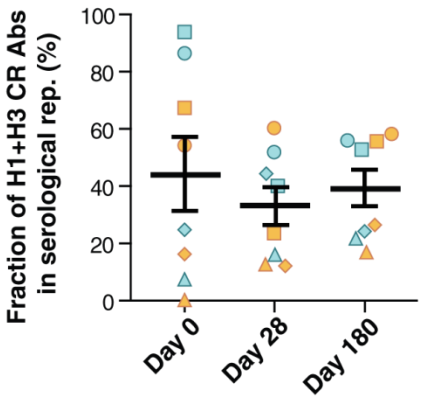

Figure 2.14: Relative abundance of H1 + H3 CR antibody clonotypes in the serum repertoires. Averages are calculated as the mean, with error-bars indicating s.e.m. (n = 8 for each group).

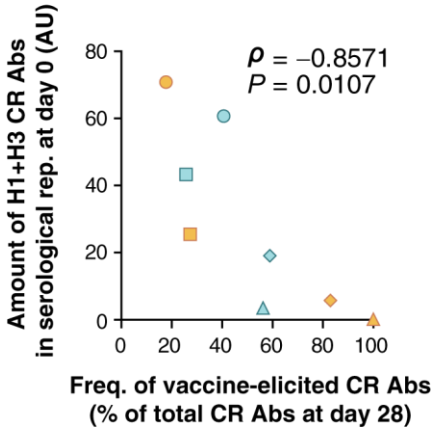

Figure 2.15: Inverse correlation between pre-vaccination amount of H1 + H3 CR antibodies and the frequency of vaccine-elicited CR antibodies at day 28. *y* axis indicates the amount of H1 + H3 CR antibodies in sera at day 0, and *x* axis indicates the frequency of vaccine-elicited CR antibodies, calculated as the number of vaccine-elicited cross-reactive clonotypes divided by the total number of cross-reactive clonotypes at day 28. Amounts were calculated as described in Figure 2.6, and statistical analyses were performed using a two-sided nonparametric Spearman rank correlation test. AU, arbitrary units.

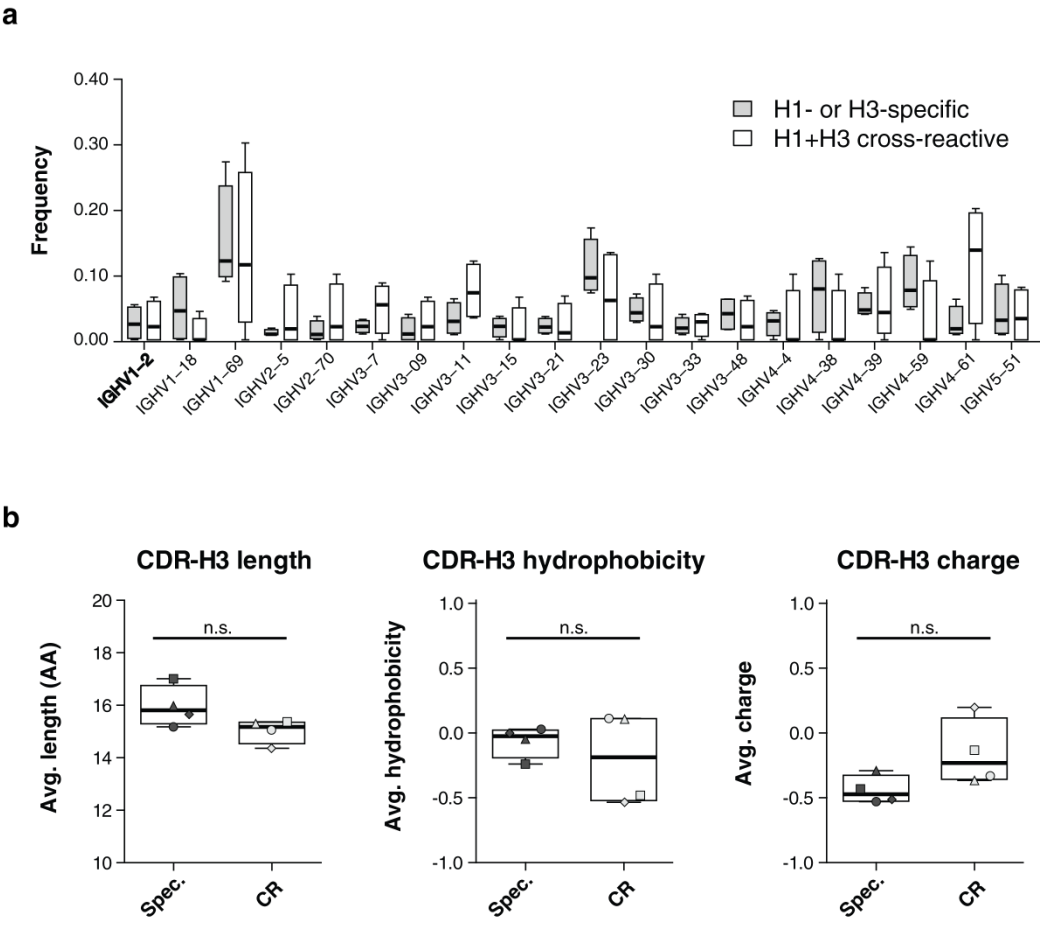

Figure 2.16: Analysis of antibody germline usage, CDR-H3 length, hydrophobicity and charge in H1+H3 cross-reactive vs. H1- or H3-specific antibodies. (**a**) Distribution of *IGHV* gene usage for H1- or H3-specific and H1 + H3 cross-reactive antibodies. The frequencies of *IGHV* gene usage are calculated for each individual. The center-lines represent the mean value of the frequencies across the donors, with the boxes extending from the 25th to 75th percentiles and the whiskers indicating min to max. Fisher exact tests were performed to identify differences in *IGHV* gene usage between H1- or H3-specific and H1 + H3 cross-reactive antibodies, and there was no statistically significant gene consistently found across the donors. (**b**) Box plot for the mean of CDR-H3 lengths, hydrophobicity indices and charge. For hydrophobicity index, Eisenberg scale was calculated using the "Peptides" package (version 1.0.4) in R. No significant difference was found between H1- or H3-specific (Spec.) and H1 + H3 cross-reactive (CR) antibodies as the length, hydrophobicity and charge *P*-values (Mann-Whitney U test) were 0.1143, 1 and 0.1143, respectively. The boxes extend from the 25th to 75th percentiles with the whiskers indicating min to max.

H1- or H3-specific Antibody Repertoire Analysis

Analysis of H1- or H3-specific antibodies revealed that vaccine-elicited antibody clonotypes (as opposed to pre-existing antibodies detected at day 0) in sera were overwhelmingly either H1 specific or H3 specific (Figure 2.17a). The total amount of these H1- or H3-specific antibody clonotypes correlated with the HAI titer (Spearman correlation = 0.85, $P < 0.0001$; Figure 2.17b and Table 2.5). Because a large fraction of the serum repertoire comprises pre-existing antibodies that bind to conserved epitopes in HA (Figure 2.14), the response to the vaccine appears to become focused on new epitopes in the head domain, the region of HA that experiences the highest degree of evolutionary drift.

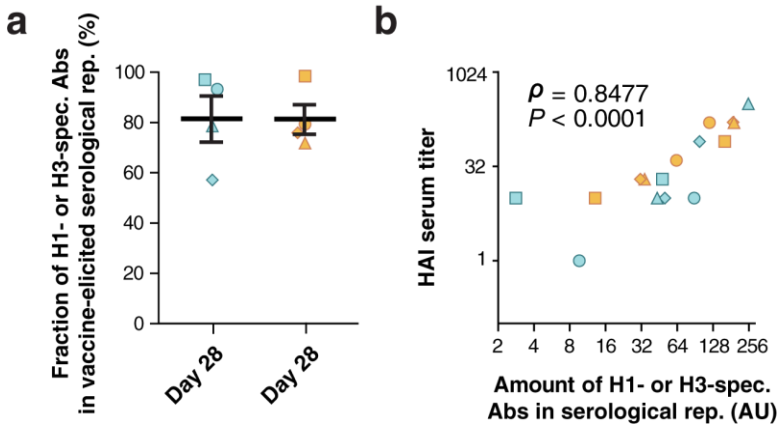

Figure 2.17: Analysis of H1- or H3-specific, and influenza B–specific, serological repertoire. (**a**) Quantification of H1- or H3-specific antibodies in the vaccine-elicited antibody repertoire at day 28. Averages were calculated as mean, with error-bars indicating s.e.m. ($n = 4$ for each group). (**b**) HAI serum titers before and after vaccination as a function of the amount of H1- or H3-specific antibodies in serum before and after vaccination. Amounts were calculated as described in Figure 2.6, and statistical analyses were performed using a two-sided nonparametric Spearman rank correlation test.

Table 2.5: Hemagglutination inhibition (HAI) titers against each viral strain included the 2011-2012 IIV3. The reciprocal dilutions of the sera containing non-agglutinated RBCs are used as HAI titers.

Donor	Time point	A/California/07/ 2009 (H1N1)	A/Perth/16/ 2009 (H3N2)	B/Brisbane/60/ 2008 (Vic)
1	Day 0	1	40	10
	Day 28	10	160	20
2	Day 0	10	10	10
	Day 28	20	80	80
3	Day 0	10	20	10
	Day 28	320	160	40
4	Day 0	10	20	10
	Day 28	80	160	40

Anti-influenza B Antibody Repertoire Analysis

Influenza B is antigenically distinct from influenza A strains. Globally, quadrivalent inactivated influenza vaccines (IIV4) that include both Yamagata and Victoria lineage HAs are gradually replacing the trivalent vaccine (IIV3), which contains one B lineage strain. Whether the IIV4 offers increased protection over IIV3 is actively debated [105, 106]. We evaluated the degree to which antibodies comprising the influenza B serum repertoires elicited by IIV3 containing a Victoria lineage strain ('Vic B/BR08' in Figure 2.1) also bound to a Yamagata lineage strain, specifically the influenza B component from the 2012–2013 IIV3 (B/Texas/6/2011, abbreviated as 'Yama B/TX11'). The fraction of antibody clonotypes detected in the eluate from Vic B/BR08 enrichment and also in the eluate with immobilized Yama B/TX11, designated as 'Vic + Yama cross-reactive', comprised 95.8%, 86.6% and 83.9% of the anti-influenza B serum repertoires at days 0, 28 and 180, respectively (Figure 2.18). Our data show that most of the serum antibodies elicited by vaccination with Vic B/BR08 also bind to Yama B/TX11. This finding raises the question of whether vaccination with the new IIV4 can offer significant additional protection benefit over that with IIV3.

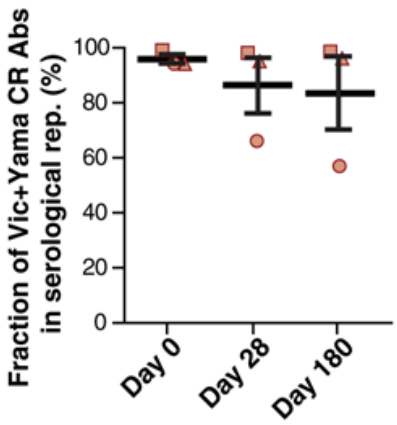

Figure 2.18: Prevalence of Vic + Yama CR antibodies in serum. Average was calculated as the mean, with bars indicating s.e.m. (n = 3 for each group).

DISCUSSION

We used LC–MS/MS proteomic spectrotyping of antigen-specific antibodies (Ig-seq) in combination with high-throughput sequencing of B cell transcripts (BCR-seq), to characterize, in a quantitative fashion, the serological repertoire to each of the monovalent components of the IIV3 influenza vaccine and how the serological repertoire changes before and after immunization. Although we analyzed a relatively small cohort because of limitations imposed by the labor involved and the LC–MS/MS instrument time requirements, our data reveal a number of findings that could not have been inferred from classical serology assays (i.e., ELISA and HAI titers) or from peripheral B cell cloning or sequencing. This is because bulk serological analysis cannot provide information on the properties or the persistence of the constituent individual antibodies. Similarly, the peripheral B cell repertoire cannot be used to infer the serological antibody repertoire because: (i) the former is much more diverse than the latter; (ii) at steady-state,

serological memory arises from antibodies produced by bone marrow plasma cells and not by peripheral B cells; (iii) blood samples capture a tiny fraction of the diversity of the peripheral B cell repertoire; and finally (iv) a clear negative correlation has been demonstrated between pre-existing serum antibody levels and the breadth of the B cell response, which is further correlated with age (or immune history) [114], an 'immunologic paradox' recognized a half-century ago [115].

It has been known for many years that influenza-specific antibody response after vaccination is shaped by pre-exposure history [83, 92, 113, 116]. However, what could not be evaluated from bulk serological studies is the relative contribution of pre-existing circulating antibodies that are boosted by vaccination relative to that of the new, vaccine-elicited antibodies. We now show that a substantial portion (on average ~60% across all IIV1 repertories in our cohorts as shown in Figure 2.7) of the serum response to influenza is due to the boosting effect of pre-existing antibody clonotypes. The fraction of the serum response that arises from the boosting of pre-existing antibodies and, conversely, the clonotypic diversity of 'new', or vaccine-elicited, antibodies are strongly correlated with the serum titer before vaccination. In other words, high serum titer before vaccination results in a more dominant boost effect of pre-existing antibodies and the emergence of fewer vaccine-elicited antibodies, and vice versa.

In the analysis of the serological repertoire to the group 1 and 2 monovalent vaccine strains in IIV3, we found an unexpectedly high prevalence of antibody clonotypes that were detected in both of the respective affinity chromatography eluates. The unexpectedly large fraction of H1 + H3 cross-reactive antibodies that was detected by LC–MS/MS could not have been inferred by classical serology metrics. Similarly, a high frequency of H1 + H3 antibodies had not been noted in previous analyses of peripheral B cells. There are several possible explanations for this. Single-cell cloning

typically involves the analysis of small samples of peripheral B cells, and therefore, plasmablasts or memory B cells that bind to H1 + H3 could have been missed. Additionally, the emphasis of B cell analysis studies has been on neutralization, which means that H1 + H3 cross-reactive antibodies that show little or no potency in neutralization assays could have been overlooked. An additional reason may be the kinetics of expansion of the memory B cells encoding these antibodies.

Finally, a very large fraction of the serum antibody repertoire elicited by immunization with a Victoria strain reacts with the following year's Yamagata strain. The data underscore the need for further serological and epidemiological studies to define the efficacy benefit that might be expected as the current IIV3 is being replaced with IIV4.

In summary, clonal-level analysis of the serological antibody repertoire revealed a wealth of unanticipated aspects of the response to the seasonal influenza vaccine. Because humans are repeatedly exposed to influenza, the serological repertoire becomes skewed towards antibodies that target epitopes conserved among vaccine strains, and these cross-reactive antibodies likely continue to be boosted every time the same conserved epitopes are encountered in the seasonal vaccine. A key to the universal influenza vaccine will be the design of immunogens that can elicit broadly protective antibodies that bind to a variety of different strains and subtypes and also provide long-term protection. Characterization of representative antibodies identified from sera would enhance our understanding of the serum response to the vaccination, which in turn could help engineer better immunogens.

Chapter 3: Recombinant Characterization of Abundant Antibodies Identified in Sera of Young Adults Following Influenza Vaccination

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INTRODUCTION

Influenza is a respiratory disease which causes over 5 million cases of severe illness and approximately half a million deaths globally [4]. While the most effective means currently available for controlling infection is vaccination, its efficacy remains limited even after almost eight decades of being in use [3, 27]. Vaccine-induced protection against influenza is conferred mainly through the adaptive immune system, which relies on the stimulation of B cells to produce a diverse repertoire of antibodies specific for hemagglutinin (HA) for an extended period of time. There are two groups of influenza A virus, groups 1 and 2, comprised of 18 different HA subtypes, and two lineages of influenza B virus, Victoria and Yamagata. In order to develop the next generation of influenza vaccines with higher and broader protective efficacy,

understanding the detailed features of the antibody response to the flu vaccine is of paramount significance.

Over the last couple of decades, studies involving cloning and characterization of antibodies from peripheral B cells has expanded our knowledge of key mechanisms for antibody-mediated neutralization of the virus [70, 72, 73, 81, 88, 90, 108]. Early studies focused on antibodies that can disrupt binding between the receptor-binding site (RBS) of HA and its target cell receptor. Hemagglutination inhibition (HAI) assay measures this ability of antibodies, and discovery of several RBS-targeting antibodies capable of neutralizing multiple strains of influenza A viruses belonging to group 1 or group 2, such as CH65 [73] and C05 [74], led to interest in developing vaccines that will elicit such antibodies that target the conserved receptor-binding regions. Later, several studies discovered that influenza can also be neutralized by other mechanisms by binding to the conserved stem region of HA [43, 81, 91], and notably one antibody, FI6 [70], neutralized virus by interfering with fusion between the virus and the host endosomal membranes after endocytosis of the virus. Because the stem regions are highly conserved across both groups 1 and 2, such stem-binding antibodies can target both group 1 and 2 influenza A viruses, making them broadly neutralizing antibodies (bNAbs). Recent studies examining peripheral B cells have shown that stem-binding bNAbs are rarely elicited following seasonal vaccination [72, 79, 117], although they have been detected at increased frequencies in individuals who were infected or vaccinated with divergent strains, such as H5N1 or the 2009 pandemic H1N1 strain [75, 91, 118-120]. For that reason, vaccination strategies aimed at increasing anti-stem, neutralizing responses are currently at the forefront of the quest for a universal influenza vaccine [84-86].

In the previous chapter, we directly analyzed the sera of four young adults following seasonal vaccination and determined the identities of influenza-specific

antibodies and their relative abundances. In order to better understand the serum response, it is essential to characterize the functions of constituent antibodies. Of the four donors analyzed in Chapter 2, donors 1 and 2 were selected for further analysis where we recombinantly expressed highly prevalent antibodies in sera and examined the functions of the antibodies.

MATERIALS AND METHODS

Reagents

Individual IIV1s comprising the 2011–2012 FluZone IIV3 (H1N1 A/California/07/2009 X-179A, H3N2 A/Victoria/210/2009 X-187 and B/Brisbane/60/2008), as well as the influenza B component of the 2012–2013 FluZone IIV3 (B/Texas/6/2011), were obtained from Sanofi-Pasteur. The following reagents was obtained through BEI Resources, NIAID, NIH: H1 Hemagglutinin (HA) Protein with C-Terminal Histidine Tag from Influenza Virus, A/Puerto Rico/8/1934 (H1N1), Recombinant from Baculovirus, NR-19240; H1 Hemagglutinin (HA) Protein from Influenza Virus, A/Brisbane/59/2007 (H1N1), Recombinant from Baculovirus, NR-15267; H1 Hemagglutinin (HA) Protein with C-Terminal Histidine Tag from Influenza Virus, A/New York/18/2009, Recombinant from Baculovirus, NR-19441; H2 Hemagglutinin (HA) Protein from Influenza Virus, A/Singapore/1/1957 (H2N2), Recombinant from Baculovirus, NR-2668; H5 Hemagglutinin (HA) Protein from Influenza Virus, A/Vietnam/1203/2004 (H5N1), Recombinant from Baculovirus, NR-10510; H9 Hemagglutinin (HA) Protein from Influenza Virus, A/Hong Kong/1073/1999 (H9N2), Recombinant from baculovirus, NR-654; H3 Hemagglutinin (HA) Protein with C-Terminal Histidine Tag from Influenza Virus, A/Perth/16/2009 (H3N2), Recombinant from Baculovirus, NR-42974; H3 Hemagglutinin (HA) Protein from Influenza Virus, A/New York/55/2004 (H3N2), Recombinant from Baculovirus, NR-19241; H3 Hemagglutinin (HA) Protein from Influenza Virus, A/Wisconsin/67/2005 (H3N2), Recombinant from Baculovirus, NR-15171; H3 Hemagglutinin (HA) Protein from Influenza Virus, A/Brisbane/10/2007 (H3N2), Recombinant from Baculovirus, NR-19238; H3 Hemagglutinin (HA) Protein from Influenza Virus, A/Uruguay/716/2007

(H3N2), Recombinant from Baculovirus, NR-15168; H7 Hemagglutinin (HA) Protein from Influenza Virus, A/Netherlands/219/2003 (H7N7), Recombinant from Baculovirus, NR-2633; H7 Hemagglutinin (HA) Protein from Influenza Virus, A/Anhui/1/2013 (H7N9), Recombinant from Baculovirus, NR-45118; Hemagglutinin (HA) Protein from Influenza Virus, B/Brisbane/60/2008, Recombinant from Baculovirus, NR-19239; Hemagglutinin (HA) Protein from Influenza Virus, B/Malaysia/2506/2004, Recombinant from Baculovirus, NR-15172; Hemagglutinin (HA) Protein from Influenza Virus, B/Ohio/1/2005, Recombinant from Baculovirus, NR-19243; Hemagglutinin (HA) Protein from Influenza Virus, B/Jilin/20/2003, Recombinant from Baculovirus, NR-19242; Hemagglutinin (HA) Protein from Influenza Virus, B/Florida/4/2006, Recombinant from Baculovirus, NR-15169; N1 Neuraminidase (NA) Protein with N-Terminal Histidine Tag from Influenza Virus, A/California/04/2009 (H1N1)pdm09, Recombinant from Baculovirus, NR-19234. A/California/7/2009-X181 and A/Solomon Islands/3/2006 rHAs were provided by Dr. Stephen Harrison (Harvard Medical School).

V_H:V_L Paired Sequencing

Paired heavy and light chain sequencing of single B cells from donor 1 day 7 and donor 2 day 7 samples was carried out as previously described [94, 121]. Briefly, B cells were isolated as single cells inside emulsion droplets using a custom flow-focusing apparatus. Droplets contained lysis buffer and poly(dT)-conjugated magnetic beads, as well as lysis buffer to capture mRNAs generated by genes encoding heavy and light chain molecules. Magnetic beads were collected and emulsified to serve as template for emulsion overlap extension RT–PCR. A follow-up nested PCR generated 850-bp linked V_H:V_L amplicons that were sequenced using the Illumina MiSeq platform. V_H and V_L regions were amplified separately for full-length V_H and V_L analysis for the recombinant

expression of monoclonal antibodies using the Illumina MiSeq platform as previously described [42, 102].

Recombinant Antibody Synthesis, Expression and Purification

Selection of antibody sequences for recombinant expression was based on the combination of V_H:V_L-paired databases and proteomics data. First, we identified antibody clonotypes identified in the proteomics analysis and searched for the same clonotype in the V_H:V_L-paired database. Full-length heavy- and light-chain-encoding sequences were then determined from the paired sequencing database.

For two of the recombinantly expressed antibodies (D1 H1-1/H3-1 and D1 H1-6/H3-2), their sequences were not found in the V_H:V_L paired database; thus their corresponding light chains were screened in phage as previously described [98]. Briefly, κ and λ light chain libraries were constructed for each heavy chain by amplifying V_L sequences from cDNA of day 7 PBMCs. Individual clones from the libraries were screened on Costar 96-well ELISA plates (Corning) coated with IIV1s, and wells with signals were sequenced to identify the light chain sequences. From each screening, multiple light chains were identified; they shared the same *IGKV* and *IGKJ* genes, and had homologous CDR-L3 sequences, indicating that they are likely similar to the endogenously paired V_L.

These genes were purchased as gBlocks (Integrated DNA Technologies) and cloned into the pcDNA3.4 vector (Invitrogen). Heavy- and light-chain-encoding plasmids for each monoclonal antibody were transfected into Expi293 cells (Invitrogen) at a 1:3 ratio. After incubating for 5 d at 37 °C with 8% CO_2, the supernatant containing the secreted antibodies was collected by centrifugation at 500*g* for 15 min at RT. The supernatant was passed over a column with 0.5 ml Protein A agarose resin (Thermo

Scientific) three times to ensure efficient capture. After washing the column with 20 cv of PBS, antibodies were eluted with 3 ml 100 mM glycine-HCl, pH 2.7, and immediately neutralized with 1 ml 1 M Tris-HCl, pH 8.0. Antibodies were buffer-exchanged into PBS using Amicon Ultra-30 centrifugal spin columns (Millipore).

ELISAs

EC_{50} values based on ELISA were used to determine the apparent affinities of the recombinant antibodies. First, costar 96-well ELISA plates (Corning) were coated overnight at 4 °C with 4 μg/ml IIV1 or rHAs and washed and blocked with 2% milk in PBS for 2 h at RT. After blocking, serially diluted recombinant antibodies were bound to the plates for 1 h, followed by 1:5000-diluted goat anti-human-IgG Fc horseradish peroxidase (HRP)-conjugated secondary antibodies (Jackson ImmunoResearch; 109-035-008) for 1 h. For detection, 50 μl TMB-ultra substrate (Thermo Scientific) was added before quenching with 50 μl 1 M H_2SO_4. Absorbance was measured at 450 nm using a Tecan M200 plate reader. Data were analyzed and fitted for EC_{50} using a 4-parameter logistic nonlinear regression model in the GraphPad Prism software. All ELISA assays were performed in triplicate.

Surface Plasmon Resonance

To determine the affinities of the influenza-specific recombinant antibodies toward the rHAs, Biacore 3000 instrument (GE Healthcare) was used. Bovine serum albumin (BSA) was immobilized in reference channels of the CM5 sensor chip to subtract buffer effect and non-specific binding signal, and the rHAs were immobilized in separate channels by amine-coupling at pH 5.5. All SPR measurements were performed

in HBS-EP running buffer (10 mM HEPES, pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% v/v surfactant P20; GE Healthcare). The serial diluted antibodies (400–12.5 nM) were injected in triplicates at 30 μl/min for 2 min, followed by a dissociation time of 10 min. The chip was regenerated after each binding event with 10 mM NaOH or 50 mM Tris, pH 11, with a contact time of 1 min. The resulting sensorgrams were fit with a bivalent model using Biaevaluation 3.0 software and the kinetic parameters were reported as the average of the three technical replicates.

In order to compare the binding epitopes of monoclonal antibodies, the antibodies in Na-acetate, pH 5.0 were immobilized on the CM5 sensor chips surface to a surface density of ~1,000 Response Units (RUs) by amine coupling. rHA at 100 μg/ml in HBS-EP were flowed over the immobilized-antibody surface at a flow rate of 30 μl/min for 2 min and, without the dissociation phase, each monoclonal antibody (at 100 μg/ml in HBS-EP) tested was immediately injected over the surface at 30 μl/min for 2 min. Between cycles, the flow cells were regenerated with 10 mM NaOH with a contact time of 1 min.

Binding Studies Using Biolayer Interferometry

A fortéBio Octet Red384 instrument was used to measure binding of H1+H3 CR antibodies (D1 H1-3/H3-3 and D1 H1-17/H3-18) to Influenza HA (full-length, RBD domain constructs, stem-only constructs). All the assays were performed with agitation set to 1,000 rpm in PBS buffer supplemented with 1% bovine serum albumin (BSA) in order to minimize nonspecific interactions. The final volume for all the solutions was 100 μl/well. Assays were performed at 30 °C in solid black 96-well plates (Geiger Bio-One). HA molecules (35 μg/ml) in PBS buffer was used to load anti-penta His probes for 300 s.

Typical capture levels were ~1 nm, and variability within a row of eight tips did not exceed 0.1 nm. Antibody binding was allowed to proceed for 300 s and normalized to a control antibody (5J8 for RBD, 39.29 for HA stem and full-length HA). Measurements were carried out in triplicate.

Antibody competition assays were carried out by first loading the HA molecule onto the probe for 300 s, followed by binding the competing antibodies (39.29, 5J8, or F005-126) for 300s. These loaded HA-IgG complexes were then allowed to bind to H1+H3 CR antibodies (D1 H1-3/H3-3 and D1 H1-17/H3-18) for 300 s. Competition was determined by comparison of H1+H3 CR antibody binding to uncomplexed HA molecules. Data analysis was carried out using Octet software, version 8.0.

Hemagglutination Inhibition Assay

Recombinantly expressed monoclonal antibodies were two-fold serially diluted in v-bottom microtiter plates. An equal volume of each influenza virus strains (A/California/07/2009, A/Perth/16/2009 and B/Brisbane/60/2008), adjusted to approximately 8 HAU/50 µl, was added to each well. The plates were covered and incubated at RT for 20 min followed by the addition of 1% turkey erythrocytes (RBC; Lampire Biologicals, Pipersville, PA, USA) in PBS. Red blood cells were stored at 4 °C and used within 72 h of preparation. The plates were mixed by agitation, covered, and the RBCs were allowed to settle for 1 h at RT. The HAI titer was determined by the reciprocal dilution of the last well that contained non-agglutinated RBC. Positive and negative serum controls were included for each plate.

Production of Pseudotyped Lentiviral Vectors and Measurement of Neutralizing Activity of Immune Sera and Monoclonal Antibodies

Influenza pseudotyped lentiviral vectors expressing a luciferase reporter gene were produced as described [122]. Briefly, the following plasmids: 17.5 μg of pCMVΔR8.2, 17.5 μg of pHR′CMV-Luc, 60 ng of pCMV Sport/h TMPRSS2, 1 μg CMV/R-HA and 0.125 μg of corresponding CMV/R-NA of a given strain of influenza virus, were transiently cotransfected into 293T cells by use of Fugene6 (Promega). Cells were transfected overnight and replenished with fresh medium. 48 h later, supernatants were harvested, filtered through a 0.45-μm PES membrane filter, aliquoted, and frozen at –80 °C.

For neutralization assays, recombinantly expressed monoclonal antibodies at various dilutions were mixed with 50 μl of pseudoviruses and then added to 293A cells in 96-well plates (10,000 cells per well). 48 h later, cells were lysed in cell culture lysis buffer (Promega, Madison, WI) before mixing with luciferase assay reagent (Promega). Light intensity was quantified with a Perkin Elmer microplate reader, and antibody neutralization resulted in lower light intensity.

Plaque Reduction Assay

Monolayers of MDCK cells in 6-well plates were infected with 100 pfu of influenza virus in 100 μl of DMEM in presence or without tested monoclonal antibodies and incubated for 1 h at 37 °C. The inoculum was removed from the cells and cells were washed with PBS buffer. DMEM supplemented with 1% agar and 1 μg/ml TPCK-trypsin was added to each well. After 48 h of incubation at 37 °C cells were fixed with 4% formaldehyde, the agar plugs removed and the cells stained with 0.1% crystal violet to estimate the titer of virus in presence and without neutralizing antibodies. Concentration

of tested antibodies with fifty percent of viral titer reduction is considered as IC_{50} (μg/ml) and performed in three independent experiments.

Testing of Binding of H1 + H3 Cross-reactive Antibodies to Infected Cells Using Flow Cytometry

MDCK cells plated in 6-well plate format (approx. 9×10^5 cells/well) were infected with pandemic H1N1 strain A/Netherlands/602/09 (NL09) at MOI of 1. They were incubated for 17 h at 37 °C before being trypsinized and fixed with 70% EtOH. Tested monoclonal antibodies were added at 5 μg/ml, incubated for 1 hr and detected with 1:1000-diluted goat anti-human IgG-Alexa-488 (Life Technologies; A11013) using flow cytometry. 50,000 events/sample were collected.

IgG Passive Transfer and Protection to Influenza Virus Challenge in Mouse Model

For passive transfer experiments, mice were randomized into groups of 5, and animal groups were not blinded to the investigators. Sample size was based on previous experience with similar experimental set-ups for antibody passive transfer influenza virus challenge experiments that used the same virus strains and challenge doses. Female 6- to 8-week-old BALB/c mice (n = 5 per group) were intraperitoneally injected with monoclonal antibodies diluted in PBS. Two hours post transfer mice were anesthetized with a ketamin/xylazine mixture (0.15 mg/kg and 0.03 mg/kg) and intranasally infected with ten murine lethal doses of the respective challenge virus in 50 μl of PBS. Weight loss and survival were monitored over a period of 14 d, and animals that crossed the humane end point of 75% of the initial body weight were euthanized. Initially, monoclonal antibodies D1 H1-1/H3-1, D1 H1-9/H3-7, D1 H1-3/H3-3, D1 H1-17/H3-18

and D2 H1-1/H3-1 were tested at 5 mg/kg for protection against the pandemic H1N1 strain A/Netherlands/602/09 (NL09). D1 H1-3/H3-3, D1 H1-17/H3-18 and D2 H1-1/H3-1 were also tested at lower doses (1 mg/kg and 0.5 mg/kg) against NL09. The protective breadth of these three mAbs was tested at 5 mg/kg against H3N2 strain X-31 (HA and NA from A/Hong Kong/1/68 and backbone of A/PR/8/34). The lung viral titers of mice (n = 3 per group) that were prophylactically treated with D1 H1-3/H3-3 and D1 H1-17/H3-18 2 h before an infection with NL09 were analyzed. Lungs were harvested on day 3 and day 6 after infection, homogenized and used for plaque assays.

In therapeutic-setting protection experiments, female 6- to 8-week-old BALB/c mice (n = 10 per group) were challenged first with NL09 and then received D1 H1-3/H3-3 and D1 H1-17/H3-18 monoclonal antibodies diluted in PBS, 24 and 48 h post infection (h.p.i.) at 5 mg/kg.

In all cases, irrelevant human IgG1 was used as negative control and bNAb CR9114 served as positive control. All animal experiments complied with the guidelines and protocols approved by the Icahn School of Medicine at Mount Sinai Institutional Animal Care and Use Committee.

Electron Microscopy and Single Particle Analysis

For the initial analysis and screening of HA/Fab mixtures, protein samples diluted to approximately 0.05 mg/ml were adsorbed to glow-discharged carbon-coated copper grids, washed with several drops of buffer containing 20 mM HEPES, pH 7.0, and 150 mM NaCl, and negatively stained with 0.75% uranyl formate, pH 5.0. Micrographs were recorded at a magnification of 100,000 on a FEI Tecnai T20 microscope operated at 200 kV and equipped with a 2k×2k Eagle CCD camera. The pixel size was 2.22 Å/px.

SerialEM [123] was used for data collection. Particles were picked using EMAN2.1 [124] automatically or manually. Particle alignment and reference-free classification were carried out in EMAN2 and SPIDER [125].

The initial analysis revealed that a subset of HA/Fab combinations contained highly heterogeneous mixtures of aggregates, free HA trimers and Fab fragments, partially unfolded HA trimers and dimers with varying numbers of Fab fragments bound, HA dimers and monomers, and, as a major component, the complex between a monomer of HA and a single Fab fragment (Figure 3.12a). Analysis of a dataset containing approximately 12,000 particles revealed that the latter complex was represented by about 50% of all particles and had a strong preferential orientation, with well-defined Fab fragments and head moieties of the HA and less well-defined membrane proximal regions ("tails"). The random conical tilt (RCT) approach [126] was utilized to obtain the three-dimensional map of the complex. Grids were prepared as described above, except that the sample was supplemented with tobacco mosaic virus to simplify alignment of untilted and tilted micrographs. To accelerate data collection, 5×5 to 7×7 montages of untilted and 45°-tilted images from same areas of the grid were obtained using SerialEM. All further analyses were performed with SPIDER. Montages were aligned automatically and pairs of untilted/tilted images were formed using a custom written script. 4,843 pairs of particles consistent in size with the HA monomer/single Fab complex were picked and centered manually with JWeb [125]. Reference-free classification of the particles from untilted micrographs windowed into 128×128 boxes into 20 classes revealed that at least 3,351 of them (9 classes) represented the complex (Figure 3.12b). The tilted particles corresponding to each 2D class were grouped, rotationally aligned, and used to compute three-dimensional reconstructions, which were then inspected in USCF Chimera [127], aligned, and merged. The resulting map was subjected to translational refinement,

yielding the RCT map of the complex (Figure 3.12c). This map was then used as the initial model for three-dimensional reconstruction and refinement using reference projections [128]. Particles from both the untilted and tilted micrographs were used for the refinement. No contrast transfer function (CTF) correction was performed. The "resolution" (level of self-consistency) of the final map was 22 Å when a Fourier shell correlation (FSC) threshold of 0.5 was used. Forward projections of the map corresponded well to two-dimensional classes (Figure 3.12d). The level of the map was chosen based on fitting a Fab domain into the corresponding density. The map was visualized in UCSF Chimera.

RESULTS

Recombinant Expression of Serum Antibodies

The proteomics-based identification of serum antibodies in Chapter 2 only determines the heavy chain (V_H) sequences. In order to acquire the endogenous light chain (V_L) information for the identified heavy chains, we used day 7 peripheral B cells from donors 1 and 2 to pair the transcripts of V_H and V_L from a single cell, and subsequently perform high-throughput sequencing. Using the proteomics data and the paired V_H:V_L sequence database, we recombinantly expressed 16 monoclonal antibodies for further biochemical and functional characterizations (Figure 3.1). Additionally, for two H1 + H3 cross-reactive antibodies for which the V_L sequence was not found in the paired V_H:V_L database, cognate light chains were identified by screening antigen-binding fragment (Fab)-encoding libraries constructed using the proteomically identified V_H combinatorially paired with V_L-encoding cDNA from day 7 B cells. For both V_H libraries, we isolated multiple V_L sequences with very similar CDR-L3 sequences with the same immunoglobulin kappa light chain variable and junctional genes (*IGKV* and *IGKJ*), suggesting that screening had converged onto a light chain that is likely to be clonally related to the respective natively paired sequences.

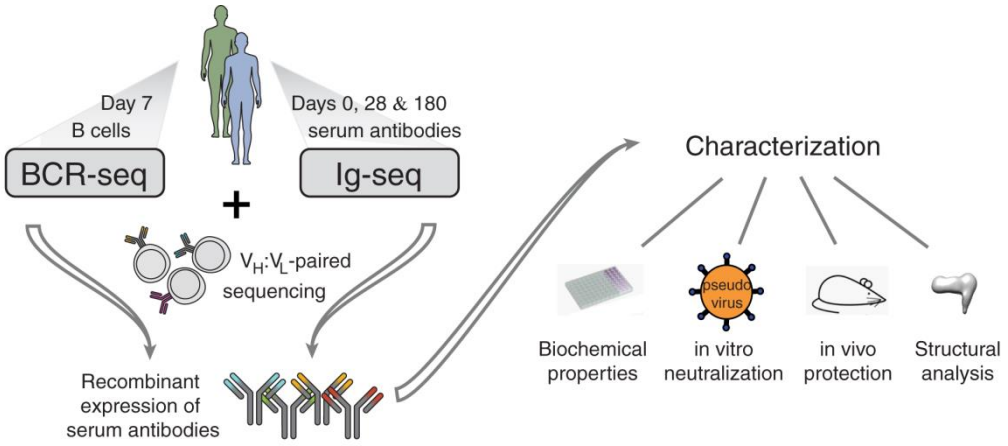

Figure 3.1: Experimental design. Day 7 peripheral B cells were used for sequencing the paired heavy and light chain (V_H:V_L) repertoire. Proteomics data and the V_H:V_L database were used to determine the full-length antibody sequences for recombinant expression, and these antibodies were characterized further.

Functional Characterization of H1 + H3 Cross-reactive Antibodies

We selected seven H1 + H3 cross-reactive antibodies (including some of the most abundant ones) from donors 1 and 2 for recombinant expression and subsequent characterization (Table 3.1). Seven of seven H1 + H3 cross-reactive antibodies we identified proteomically bound to both IIV1s. Furthermore, five of seven H1 + H3 cross-reactive antibodies had exceptional binding breadth toward a diverse panel of recombinantly expressed HAs (rHAs) from influenza groups 1 and 2, with 50% effective concentration (EC_{50}) values <100 nM, as assessed by ELISA (and apparent K_D values between 2.5 pM and 160 nM, on the basis of surface plasmon resonance analysis (Figures 3.2 and 3.3, and Table 3.2). One additional antibody isolated from donor 1 (D1 H3-11), which was originally identified only in the H3 A/VI09 eluate, also bound to rHAs from groups 1 and 2; however, because it bound to H1 A/CA09 (IIV1) with such low affinity, it was not detected in the H1 A/CA09 eluate.

Table 3.1: CDR-3 sequences, lengths, somatic hypermutation rates (SHM) and gene usages of the recombinantly expressed H1 + H3 cross-reactive serum antibodies. For each antibodies, heavy (V_H) and light (V_L) chain information is shown with SHM measured by mutation rates in *IGHV* gene.

Donor	rmAb ID	CDR-3 sequences		CDR-3 length	SHM (*IGHV*)	Gene usage
1	H3-11	V_H	AKEDSSGYPTRRPVDY	16	4.5%	*IGHV3-9, IGHJ4*
		V_L	QQYNNWPPEYT	11	1.8%	*IGKV3-15, IGKJ2*
1	H1-1/ H3-1	V_H	ARHFQERLGAARAGAFDV	18	5.4%	*IGHV4-34, IGHJ3*
		V_L	QQSYSDPPEG	10	3.9%	*IGKV1-39, IGKJ3*
1	H1-3/ H3-3	V_H	ARDFFEKLTGEDLNAFDV	18	1.7%	*IGHV4-61, IGHJ3*
		V_L	QETYSRG	7	2.1%	*IGKV1-39, IGKJ1*
1	H1-6/ H3-2	V_H	ARPSYTSGFAFDP	13	22.3%	*IGHV1-69, IGHJ5*
		V_L	QQSYSTPPT	9	0.4%	*IGKV1-39, IGKJ4*
1	H1-9/ H3-7	V_H	ARASIRSRPQFFDF	14	3.5%	*IGHV5-51, IGHJ4*
		V_L	QQYYDSTLT	9	5.0%	*IGKV4-1, IGKJ4*
1	H1-17/ H3-18	V_H	ARDFFEKLIADDLNAFDI	18	7.9%	*IGHV4-61, IGHJ3*
		V_L	QETYSRT	7	5.4%	*IGKV1-39, IGKJ1*
2	H1-1/ H3-1	V_H	ARRFVELLGGRSKPYDALDV	20	5.6%	*IGHV3-7, IGHJ3*
		V_L	QQSYSPVTT	9	2.5%	*IGKV1-39, IGKJ4*
2	H1-27/ H3-42	V_H	ARELPGNWFDT	11	6.2%	*IGHV4-30, IGHJ5*
		V_L	LQCKTYRT	8	7.9%	*IGKV1-5, IGKJ1*

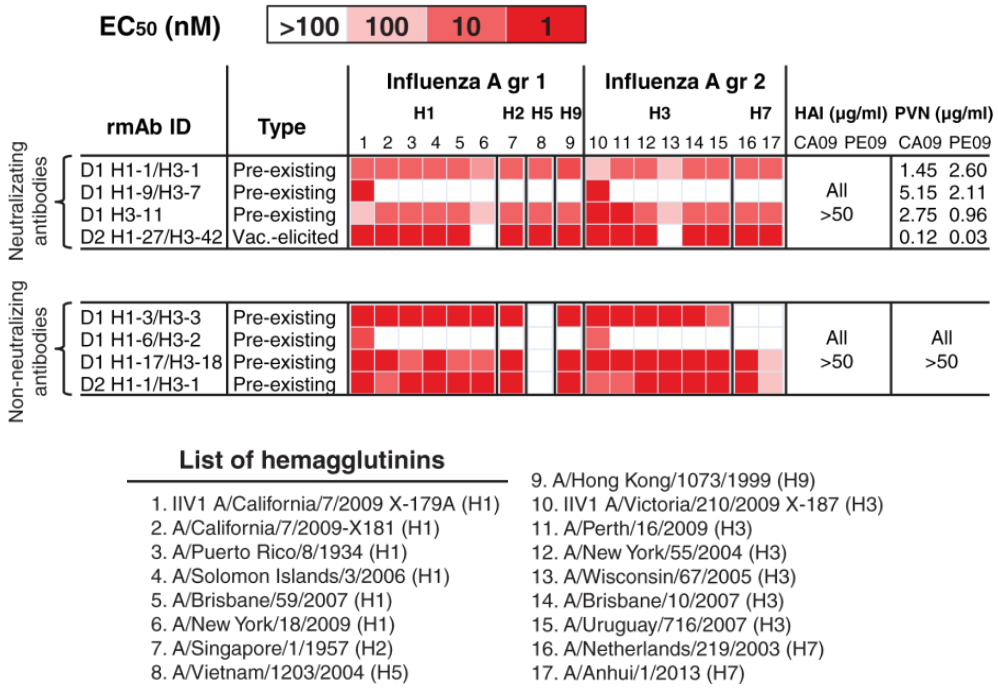

Figure 3.2: Biochemical and functional analysis of the recombinantly expressed H1 + H3 CR serum antibodies. Recombinant monoclonal antibody ID (rmAb ID) indicates the donor information, as well as the abundance rankings of the antibody at day 28 in respective repertoire (i.e., D1 H1-1/H3-1 indicates that this antibody is from donor 1 and ranked first in abundance in the anti–H1 A/CA09 repertoire and first in the anti–H3 A/VI09 repertoire). EC_{50} values, as determined by ELISA, for each recombinant antibody tested against IIV1s or rHAs as listed are shown according to the color scheme included. A/California/7/2009 (CA09) and A/Perth/16/2009 (PE09) strains were used for both of the HAI and PVN assays. All assays were repeated in triplicate.

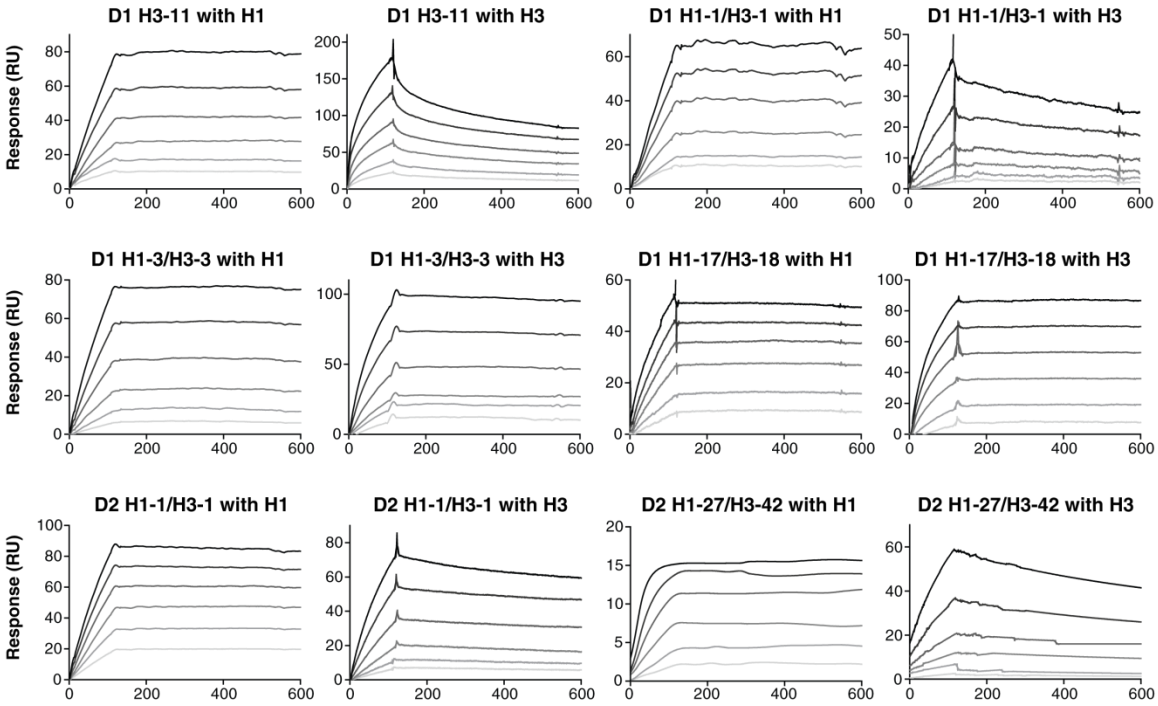

Figure 3.3: Surface plasmon resonance data for recombinant IgG binding to rHAs. Recombinant HAs were immobilized on CM5 sensor chips. Various concentrations of the recombinant monoclonal antibodies were tested (400, 200, 100, 50, 25, and 12.5 nM). H1, rHA from A/California/07/2009 X-181; H3, rHA from A/Perth/16/2009.

Table 3.2: K_d values (nM) of monoclonal antibodies to rHAs measured by surface plasmon resonance. Averages are calculated as mean with error-bars indicating s.d., replicated in triplicate. H1, rHA from A/California/07/2009 X-181; H3, rHA from A/Perth/16/2009.

Donor	rmAb ID	H1	H3	Vic
1	H3-11	0.62 ± 0.09	56 ± 2	>200
1	H1-1/H3-1	0.79 ± 0.08	79 ± 10	>200
1	H1-3/H3-3	0.028 ± 0.004	0.098 ± 0.01	>200
1	H1-17/H3-18	0.54 ± 0.07	0.75 ± 0.09	>200
2	H1-1/H3-1	3.8 ± 1	160 ± 3	>200
2	H1-27/H3-42	0.0025 ± 0.0009	24 ± 14	>200

Two antibodies, D1 H1-6/H3-2 and D1 H1-9/H3-7, bound only to the H1 A/CA09 and H3 A/VI09 monovalent vaccines, but not to the respective rHAs. Consistent with this finding, these two antibodies were detected in the proteomic analysis of the affinity chromatography eluate with H1 A/CA09 but not with rHA A/California/07/2009 X-181 (previously described in Chapter 2). These antibodies did not bind to neuraminidase, gelatin or other constituents of the monovalent vaccines. Notably, D1 H1-9/H3-7 neutralized group 1 and group 2 strains in pseudotyped particle-entry inhibition (PVN) assays with pandemic influenza A/California/07/2009 (H1N1) and A/Perth/16/2009 (H3N2) strains, and it bound to HA on the surface of infected Madin-Darby canine kidney (MDCK) cells (Figures 3.3, 3.4 and 3.5). We concluded that D1 H1-9/H3-7 recognizes an epitope that is present in the conformation HA assumes on the surface of infected cells but is inaccessible in purified rHA.

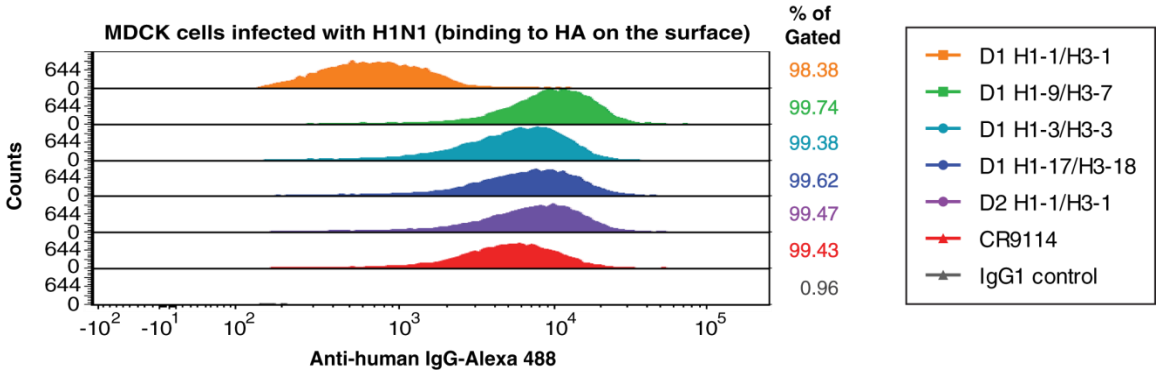

Figure 3.4: Binding of H1 + H3 CR antibodies to the HAs on the surface of the MDCKs infected with the 2009 pandemic H1N1 strain (A/Netherlands/602/09; NL09), as analyzed by FACS. 50,000 events/sample were collected.

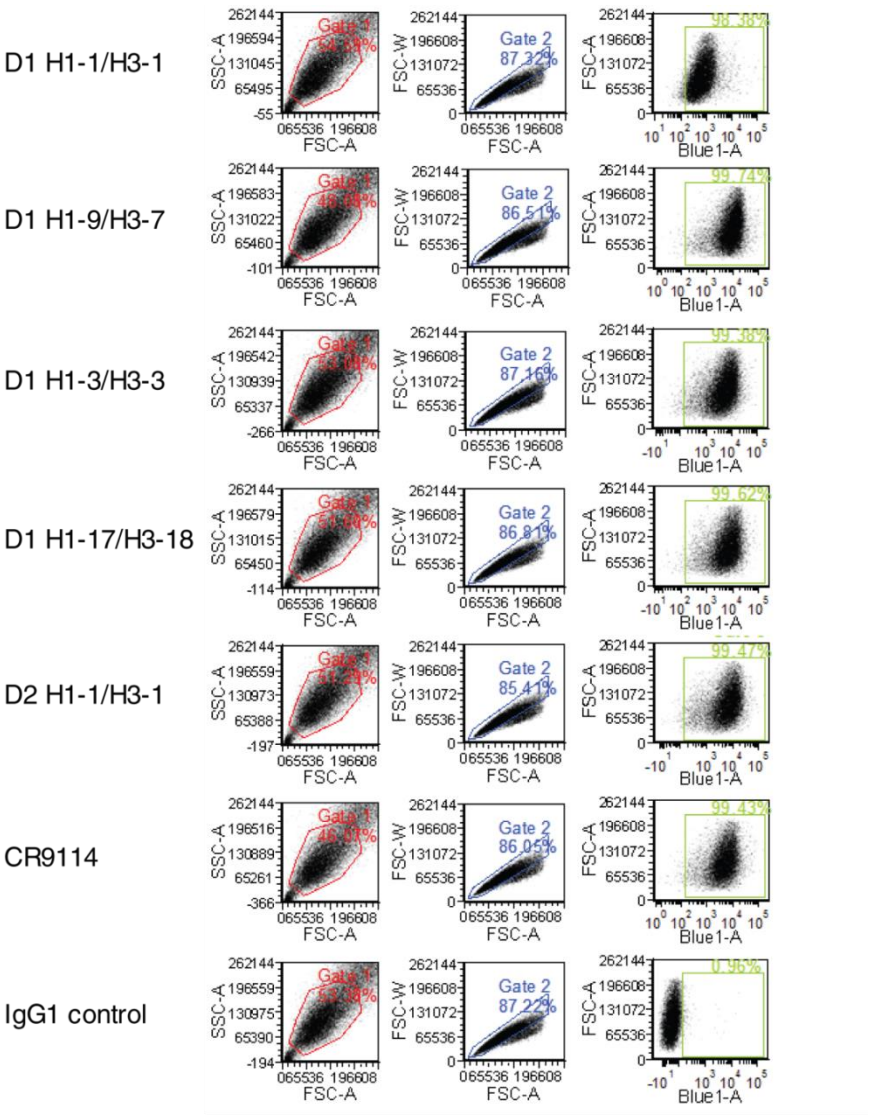

Figure 3.5: FACS analysis of monoclonal antibodies binding to influenza infected MDCKs. First, a relatively homogeneous cell population was selected in Gate 1. Gate 1 was applied to the second dot plot, where the Gate 2 was created to select for singlets and this was then applied to third dot blot, where the *x* axis represents the mean fluorescence intensity (MFI) after excitation by Blue-1 laser (for Alexa-488). 50,000 events are displayed per sample.

Although none of the eight H1 + H3 cross-reactive antibodies showed HAI activity, four of eight antibodies showed neutralization activity in PVN assays (Figure 3.2, top panel). Two of these (D1 H1-1/H3-1 and D1 H3-11) competed with the stem-binding bNAb FI6 [70] (Figure 3.6). One of these stem-binding H1 + H3 cross-reactive antibodies was prevalent in the serum of donor 1 as D1 H1-1/H3-1 accounted for 14.2% and 14.9% of the H1 A/CA09 and H3 A/VI09 repertoires at day 28, respectively. D1 H1-1/H3-1 in particular neutralized numerous influenza virus strains in PVN assays (Table 3.3). However, both antibodies had very weak or no activity in the more physiologically relevant plaque reduction assays (Table 3.4) and, notably, did not confer protection against infection with the H1N1 virus strain in lethal-challenge experiments in mice when administered at 5 mg per kg body weight (mg/kg) (Figure 3.7a).

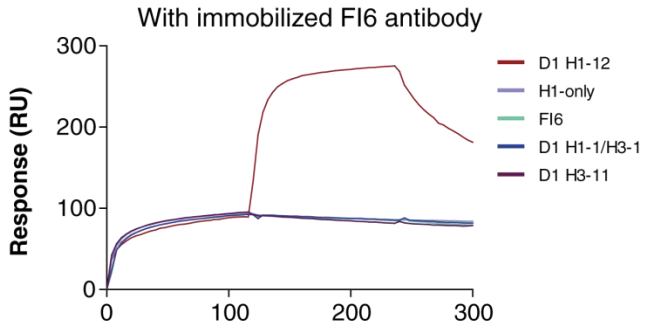

Figure 3.6: Epitope-blocking assay for the neutralizing H1 + H3 cross-reactive antibodies. FI6 was immobilized on a CM5 sensor chip, and recombinant H1 (A/California/7/2009 X-181) was bound to the immobilized FI6 before testing for binding of the monoclonal antibodies. Head-targeting antibody, D1 H1-12, and H1-only were used as controls. D1 H1-1/H3-1 and D1 H3-11 antibodies showed competition for binding epitope with FI6. The experiments were repeated in triplicate.

Table 3.3: IC_{50} (μg/ml) of the D1 H1-1/H3-1 on PVN assays against the listed influenza strains. 1: A/South Carolina/1/1918, 2: A/Puerto Rico/8/1934, 3: A/New Caledonia/20/1999, 4: A/Singapore/1/1957, 5: A/Canada/720/2005, 6: A/Vietnam/1203/2004, 7: A/Indonesia/05/2005, 8: A/Hong Kong/1073/1999, 9: A/Hong Kong/1/1968, 10: A/Netherlands/219/2003, 11: A/Anhui/1/2013).

Group 1								**Group 2**		
H1			**H2**		**H5**		**H9**	**H3**	**H7**	
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
3.00	4.25	1.88	>50	>50	0.435	3.59	0.465	5.90	5.08	0.917

Table 3.4: IC_{50} (μg/ml) of recombinant monoclonal antibodies with H1N1 A/California/07/09 and H3N2 A/Perth/16/09 viruses determined from plaque reduction assays performed in three independent experiments.

	Virus strains	
Antibodies tested	**H1N1 A/California/07/09**	**H3N2 A/Perth/16/09**
D1 H1-1/H3-1	>100	>100
D1 H1-9/H3-7	100	>100
D1 H3-11	>100	100
D1 H1-3/H3-3	>100	>100
D1 H1-6/H3-2	>100	N/A
D1 H1-17/H3-18	>100	>100
D2 H1-1/H3-1	>100	>100

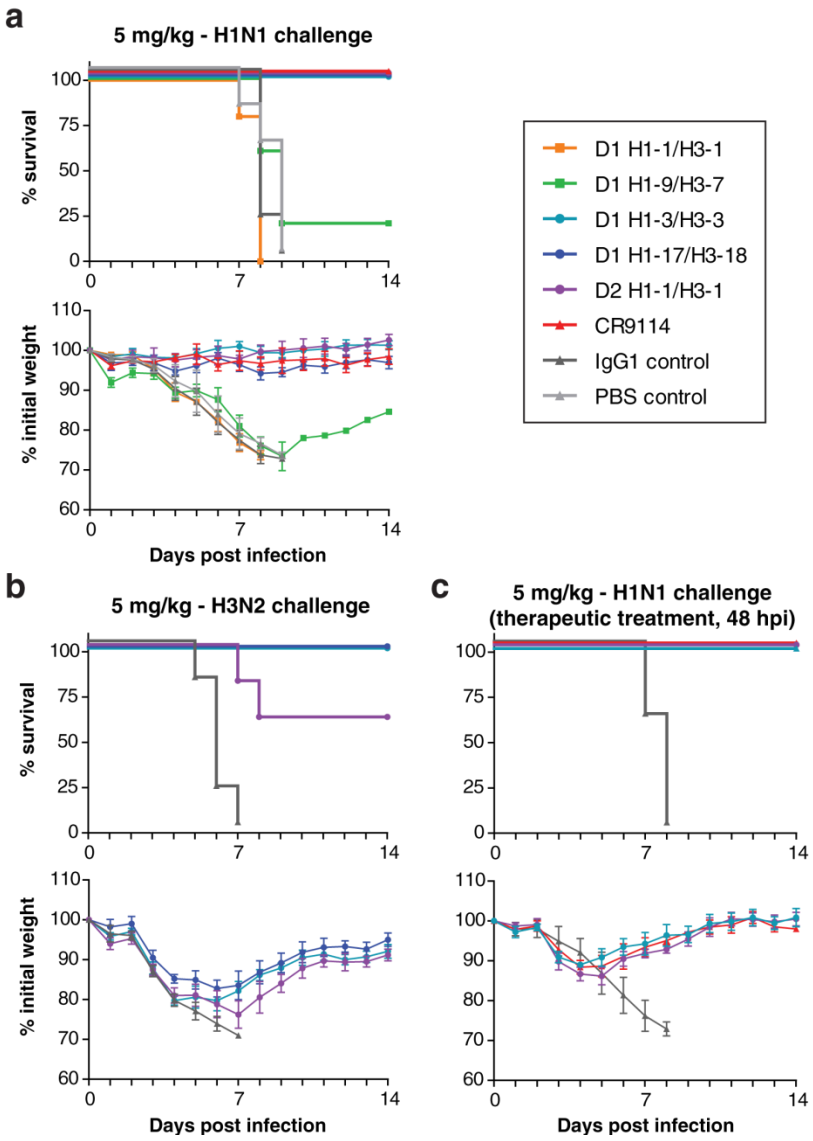

Figure 3.7: Protective efficacy of the H1 + H3 cross-reactive antibodies in mice. (**a**) Survival data (Kaplan–Meier plots) (top) and weight loss data (bottom) for BALB/c mice (n = 5 per group) after passive immunization (5 mg/kg) with H1 + H3 CR antibodies followed by challenge with NL09. (**b**) Survival (top) and weight loss (bottom) data for BALB/c mice (n = 5 per group) after passive immunization (5 mg/kg) with the protective antibodies from **a** followed by challenge with H3N2 strain X-31 (A/Hong Kong/1/68 in the backbone of A/PR/8/34). (**c**) Survival (top) and weight loss (bottom) data for BALB/c mice (n = 10 per group) challenged with NL09, followed by therapeutic administration of with antibodies (5 mg/kg) at 48 h.p.i. For **a**–**c**, averages were calculated as mean, with error-bars indicating s.d.

The remaining four of eight H1 + H3 cross-reactive antibodies showed no neutralization activity in HAI, PVN or plaque reduction assays (Figure 3.2, bottom and Table 3.4). Three of these (D1 H1-3/H3-3, D1 H1-17/H3-18 and D2 H1-1/H3-1), which were highly abundant in both donors, competed with each other for binding to HA (Figure 3.8), indicating that they target either the same or overlapping epitopes. Of note, administration of these three antibodies at 5 mg/kg, and even at lower doses (1.0 and 0.5 mg/kg), conferred excellent protection in mice that were challenged with the H1N1 strain (Figures 3.7a and 3.9) Furthermore, D1 H1-3/H3-3 and D1 H1-17/H3-18 also conferred 100% protection (although transient weight loss was observed) in challenge experiments with the H3N2 strain (Figure 3.7b). Finally, treatment with D1 H1-3/H3-3 and D2 H1-1/H3-1 both led to a reduction in viral titers in the lower lung, following H1N1 challenge, and also conferred protection when administered therapeutically 24 and 48 hours after infection with the H1N1 strain (Figures 3.7c and 3.10).

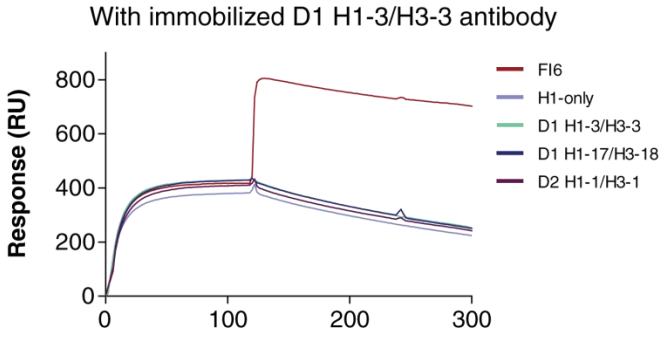

Figure 3.8: Epitope-blocking assay for the non-neutralizing H1 + H3 cross-reactive antibodies. D1 H1-3/H3-3 was immobilized on a CM5 sensor chip, and H1 was bound to the immobilized D1 H1-3/H3-3. Stem-biding antibody, FI6, and H1-only were used as controls. D1 H1-17/H3-18 and D2 H1-1/H3-1 antibodies showed competition for binding epitope with D1 H1-3/H3-3. The experiments were repeated in triplicate.

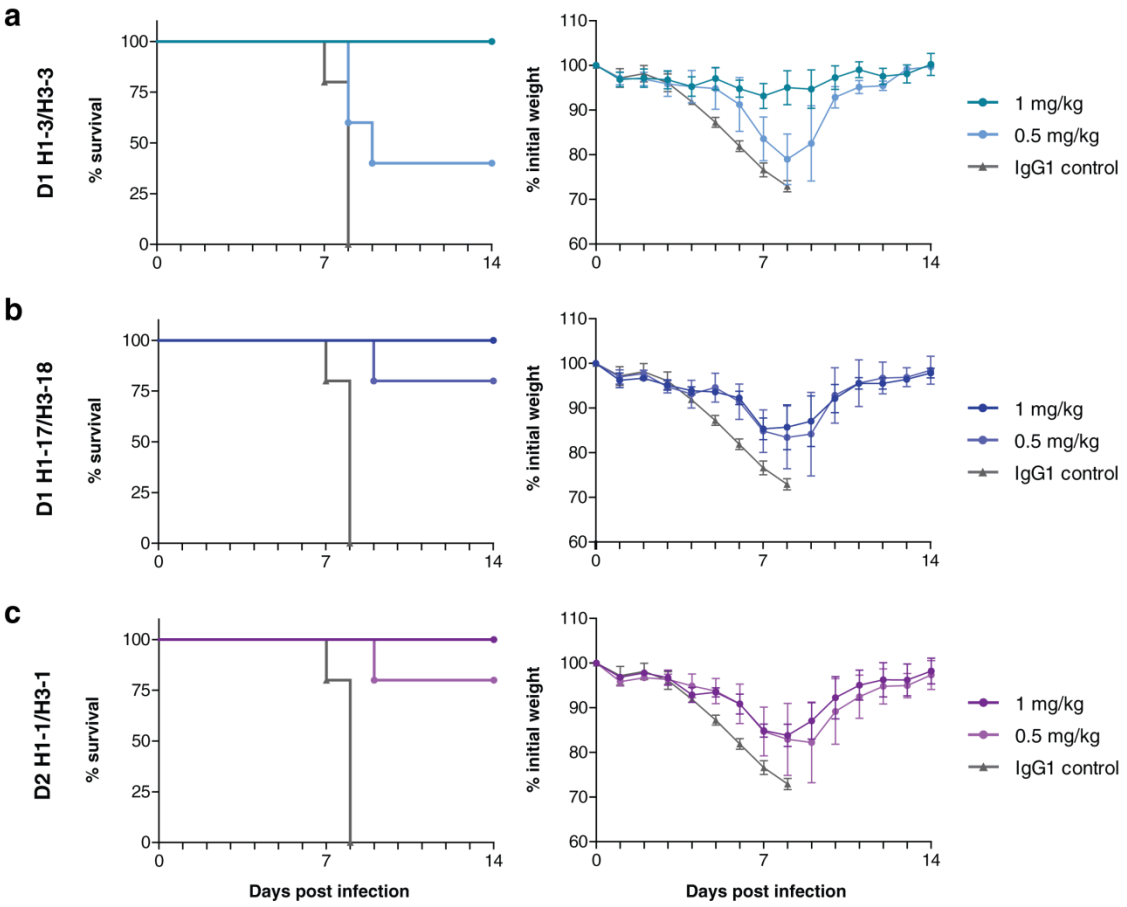

Figure 3.9: Protection in mice challenged with H1N1 live influenza following passive immunization with low dose of select H1 + H3 cross-reactive, non-neutralizing antibodies. Survival data for BALB/c mice (*n* = 5 per group) after passive immunization (intraperitoneally) followed by challenge with 2009 pandemic H1N1 (A/Netherlands/602/09) 2 hours later and corresponding weight loss for (**a**) D1 H1-3/H3-3, (**b**) D1 H1-17/H3-18 and (**c**) D2 H1-1/H3-1. Each antibody was tested at 1 mg/kg and 0.5 mg/kg, and survival (left) and body weight loss (right) were monitored for 14 days. Averages are calculated as mean with error-bars indicating s.d.

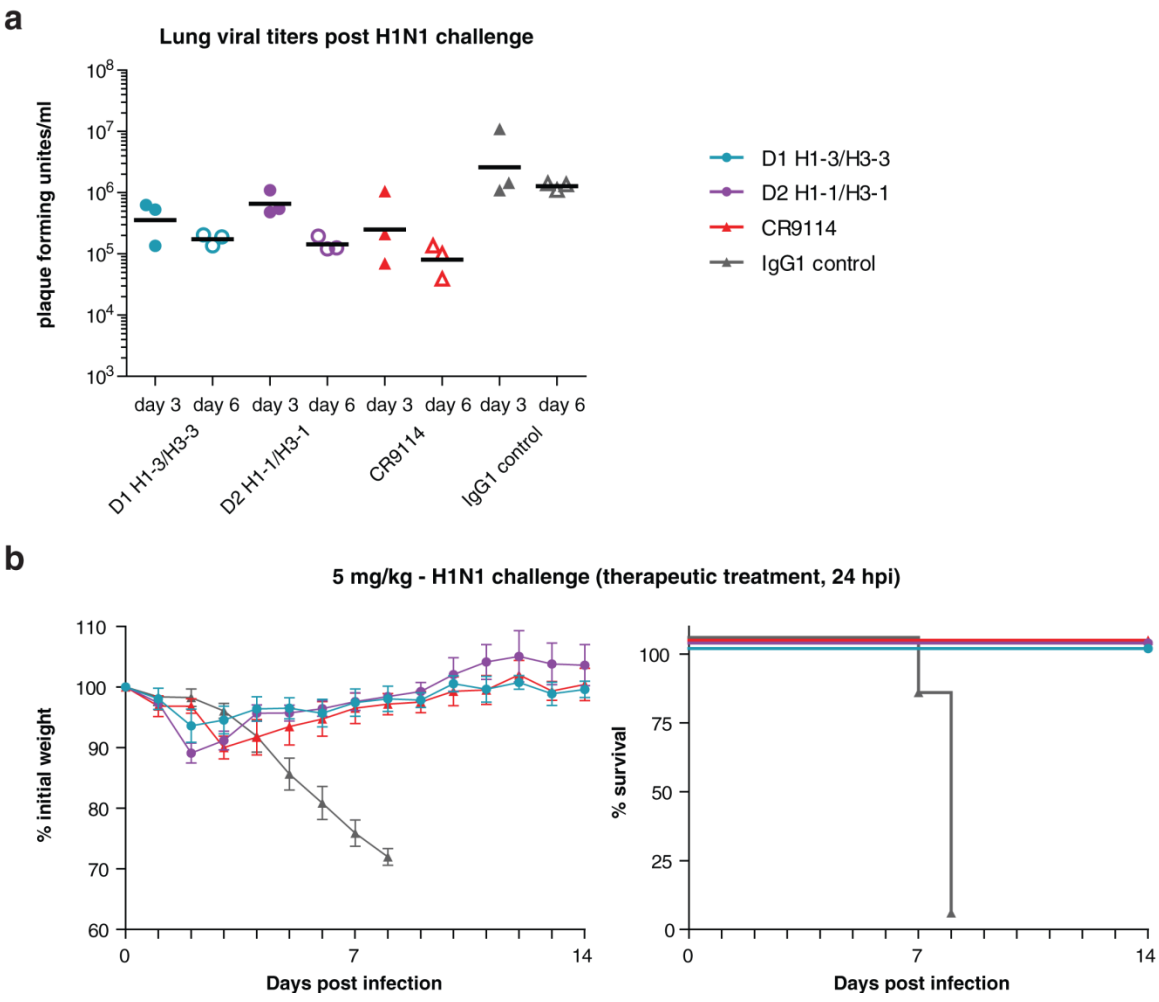

Figure 3.10: (**a**) Lung viral titers in mice (*n* = 3 per group) that had been passively immunized with monoclonal antibodies (5 mg/kg) followed by challenge with NL09. Lungs were harvested at days 3 and 6 after passive immunization. Lung viral titers were measured by plaque assay. (**b**) Post-infection (therapeutic treatment) survival data. Survival and weight loss data for BALB/c mice (*n* = 10 per group) challenged with NL09 with antibody (5 mg/kg) administered 24 h.p.i. Average calculated as mean, bars indicate s.d.

Structural Characterization of H1 + H3 Cross-protective Antibodies

To identify the epitope targeted by the three non-neutralizing yet *in-vivo*-protective antibodies (D1 H1-3/H3-3, D1 H1-17/H3-18 and D2 H1-1/H3-1), we used

negative-staining electron microscopy (EM). We first tested the binding of these antibodies to multiple rHA proteins, as well as to smaller rHA domains, and although no binding was observed to a trimeric rHA stem molecule[84], strong binding was observed to the rHA RBS [129]. The antibodies bound to the full-length rHA molecule (A/Hong Kong/1-4-MA21-1/1968) but not to a disulfide-bonded covalent trimer version of the rHA (A/Hong Kong/1-4-MA21-1/1968 212Cys-216Cys) [130] (Figure 3.11a). When we mixed Fab fragments with the trimeric, full-length rHA molecules (A/Hong Kong/1-4-MA21-1/1968), we observed dissociation of the rHA trimer molecule with ~50% of the mixture, forming a 1:1 complex of one HA protomer with one Fab molecule as shown by negative-stain EM (Figures 3.11b and 3.12). To visualize the epitope on the HA protomer, a three-dimensional negative-stain EM map at ~22-Å resolution was determined by using random conical tilt. A single HA protomer and an Fab molecule could be fit to the EM map (Figure 3.11c,d), indicating that the antibody's mode of binding was incompatible with an intact HA trimer molecule due to steric clashes. Competition assays with the F005-126 antibody [80] (Figure 3.13) and analysis of the Fab contact site on the HA molecule indicated that the antibodies Asn171, Arg208, Pro239, Gly240 and Asp241, which is occluded in the intact HA trimer (Figure 3.11e). These residues are highly conserved in the HA sequence database of over 4,468 sequences from human-infecting strains, especially for H1 and H3 strains.

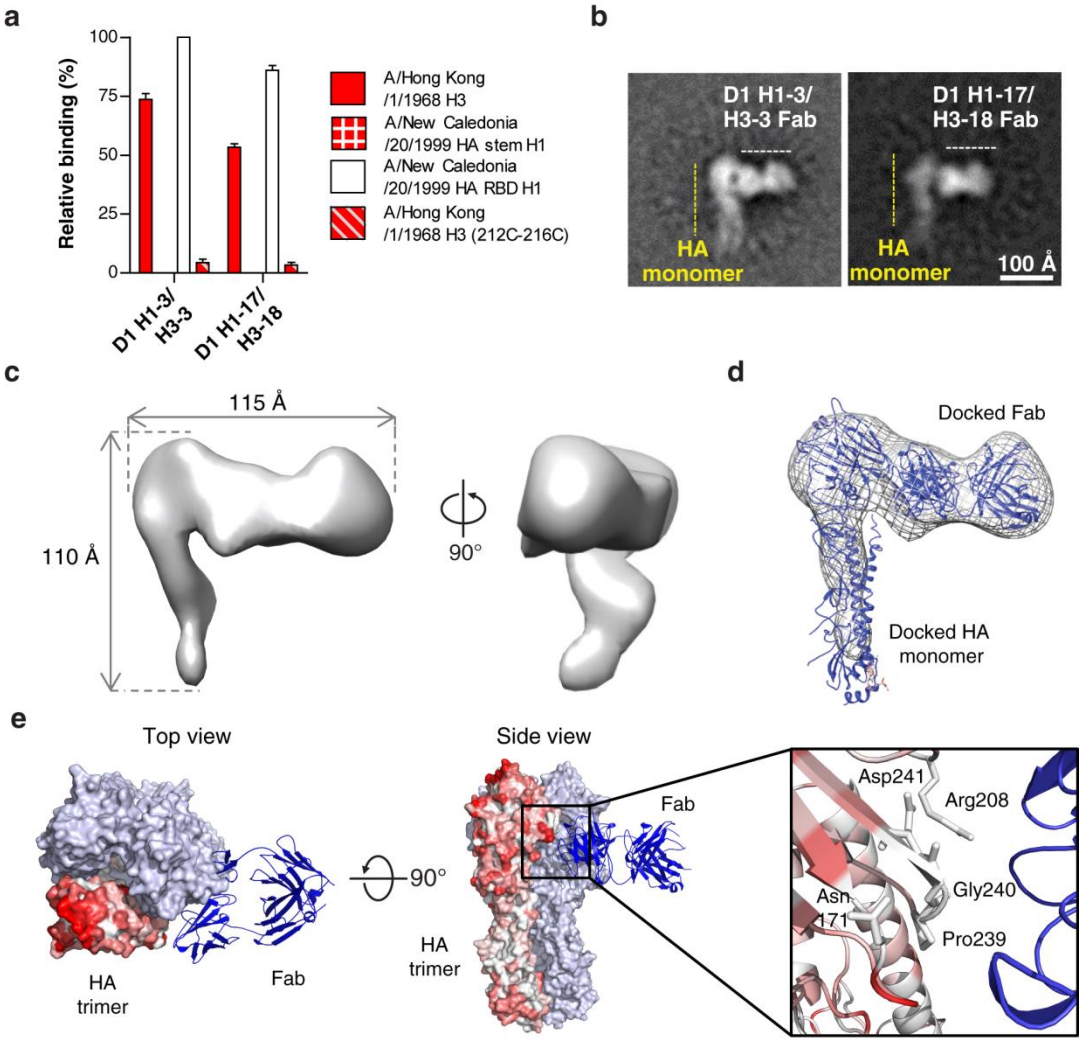

Figure 3.11: Structural analysis of H1 + H3 CR antibodies. (**a**) Binding characteristics to head versus stem HA, as determined by biolayer interferometry, repeated in triplicate. (**b**) Analysis of HA–Fab complexes by negative-stain EM shows that the antibody binds to a HA monomer. Scale bar, 100 Å. (**c**) EM structure of D1 H1-3/H3-3 in complex with HA, determined to 22 Å using the random conical-tilt method. (**d**) Combine electron-density map of a single HA protomer and antibody Fab that were docked separately. (**e**) The fitted HA protomer–Fab complex was overlaid with an intact HA trimer (colored red-to-white indicating influenza HA conservation for one protomer; light blue for the remaining two protomers) showing that major overlap occurs between the Fab and an adjacent protomer molecule, which precludes binding of the antibodies to intact trimers. A set of highly conserved residues are located in this region (inset), which likely form part of this antibody epitope.

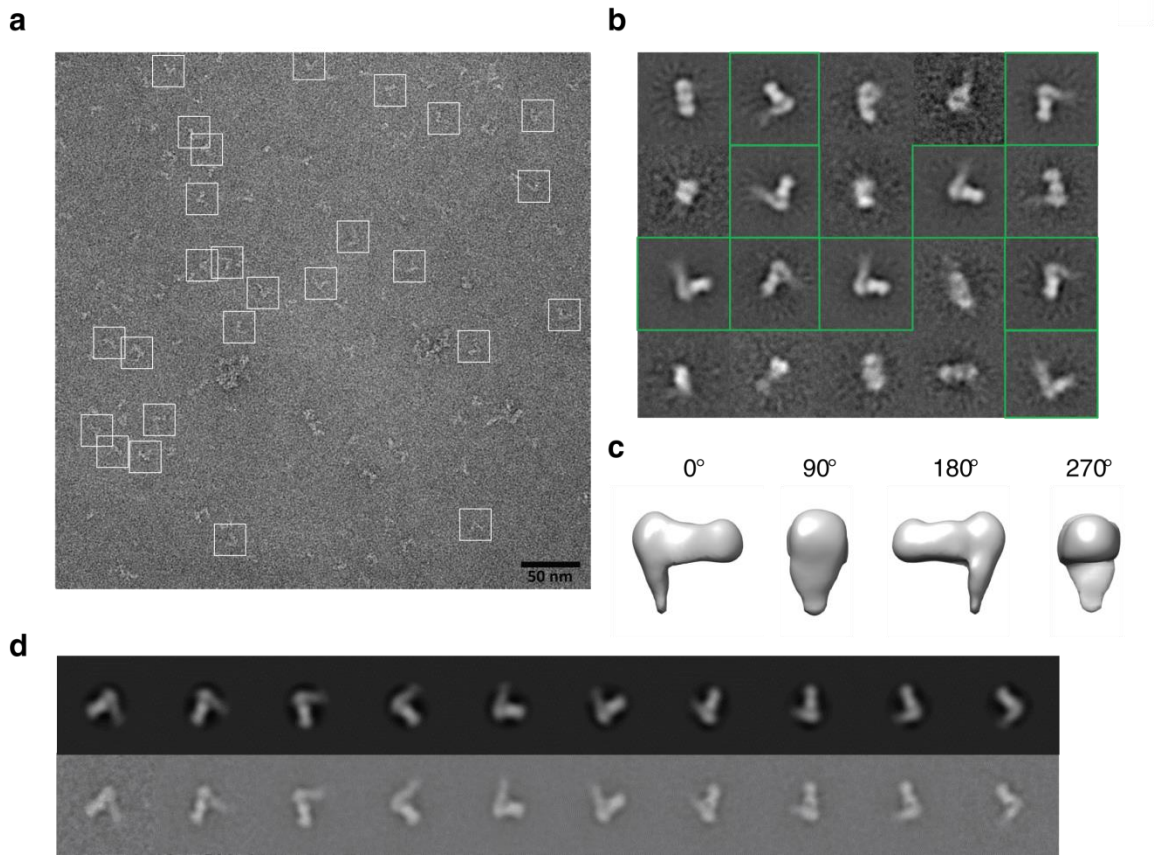

Figure 3.12: Negative-stain electron microscopy and single particle analysis for D1 H1-3/H3-3 in complex with HA. (**a**) A representative micrograph illustrating high compositional and structural heterogeneity of the antibody/HA mixture. Examples of L-shaped 1:1 complexes between the Fab fragment and the HA monomer are enclosed in white squares. (**b**) Results of reference-free classification of untilted particles. The classes used for random conical tilt (RCT) structural analysis are enclosed in green squares. (**c**) Angular views of the final RCT map, which was used as the initial model for three-dimensional reconstruction and refinement using reference projections (Figure 3.11c). (**d**) Examples of forward projections of the final map (Figure 3.11c) (top) and corresponding 2D average views (bottom).

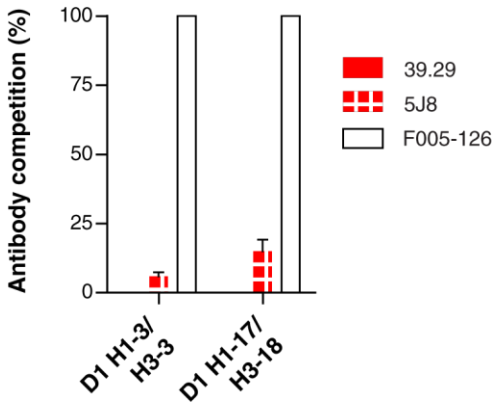

Figure 3.13: Binding-competition assay using stem or head directed antibodies. Biolayer Interferometry showed that the binding of D1 H1-3/H3-3 and D1 H1-17/H3-18 to HA can be fully blocked by F005-126 antibody, indicating that they compete for overlapping binding epitope (but not with 39.29 [117] or 5J8 [78]). Averages are calculated as mean with error-bars indicating s.d., repeated in duplicate.

Functional Characterization of H1- or H3-specific Antibodies

We selected four H1-specific and one H3-specific antibodies from donor 1 for recombinant expression and subsequent characterization (Table 3.5). As expected, recombinant antibodies identified exclusively in the eluate from H1 affinity chromatography bound only to H1 A/CA09 and not to the H3 strains, and vice versa (Figures 3.14 and 3.15, and Table 3.6). All of the H1-specific antibodies competed for the same epitope as the RBS-targeting antibody C05 [74] (Figure 3.16), and most of them showed activity in HAI assays, indicating that they target the RBS. One antibody, D1 H3-9, neutralized the A/Perth/16/2009 (H3N2) strain in PVN assays and also bound with high affinity to a number of recent historical group 2 rHAs (Figure 3.14).

Table 3.5: CDR-3 sequences, lengths, SHM and gene usages of the recombinantly expressed H1- and H3-specific serum antibodies. For each antibodies, heavy (V_H) and light (V_L) chain information is shown with SHM measured by mutation rates in *IGHV* gene.

Donor	rmAb ID	CDR-3 sequences		CDR-3 length	SHM (*IGHV*)	Gene usage
1	H1-2	V_H	AREYRILDERSNYFDA	16	5.9%	*IGHV4-38, IGHJ5*
		V_L	QTWDISSGVV	10	4.7%	*IGLV3-1, IGLJ2*
1	H1-12	V_H	ARLYMVRGELWGFYFDH	17	3.9%	*IGHV4-59, IGHJ4*
		V_L	CSYAGSSTSWV	11	3.8%	*IGLV2-23, IGLJ3*
1	H1-34	V_H	ARDDTSGELPRGLDY	15	4.5%	*IGHV4-38, IGHJ4*
		V_L	QAWDSSTVV	9	1.1%	*IGLV3-1, IGLJ2*
1	H1-53	V_L	ARGERFGESPYYSYHMDV	18	4.6%	*IGHV4-59, IGHJ6*
		V_L	QAWDSSTVV	9	1.1%	*IGLV3-1, IGLJ2*
1	H3-9	V_H	TTGDHYNDPTTPAHRNW	17	6.9%	*IGHV1-69, IGHJ5*
		V_L	QQSYSTPPVT	10	0.4%	*IGKV1-39, IGKJ2*

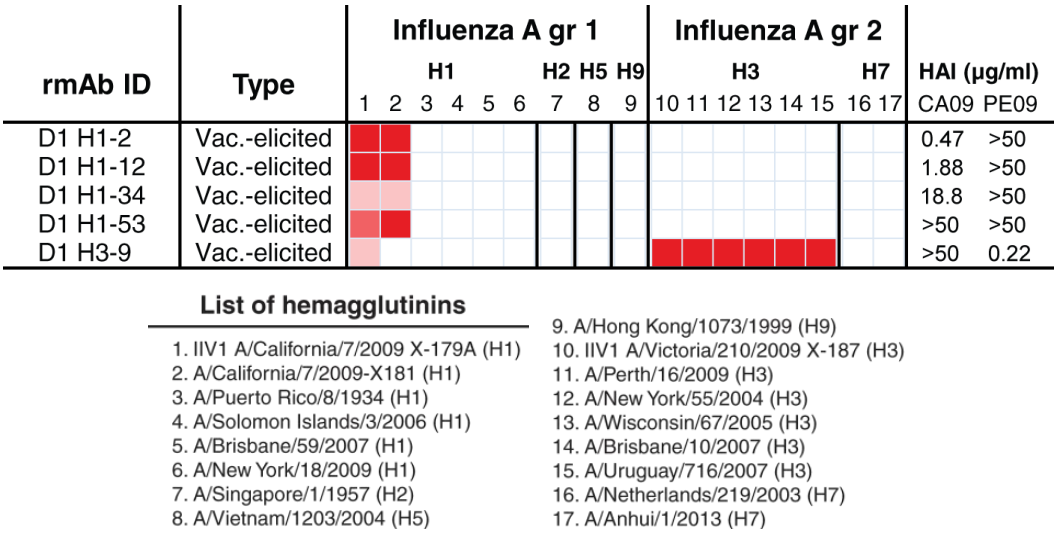

Figure 3.14: Biochemical and functional analysis of the recombinantly expressed H1- or H3-specific serum antibodies. EC_{50} values, as determined by ELISA, for each recombinant antibody tested against IIV1s or rHAs are shown according to the list of hemagglutinins and color scheme included in Figure 3.2. A/California/7/2009 (CA09) and A/Perth/16/2009 (PE09) strains were used for both of the HAI and PVN assays. All assays were repeated in triplicate.

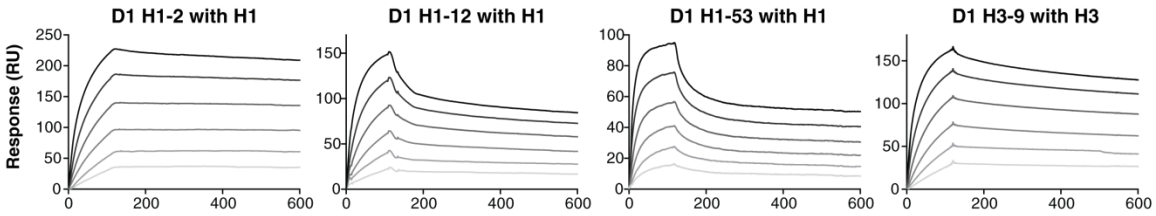

Figure 3.15: Surface plasmon resonance data for recombinant IgG binding to rHAs. Recombinant HAs were immobilized on CM5 sensor chips. Various concentrations of the recombinant monoclonal antibodies were tested (400, 200, 100, 50, 25, and 12.5 nM). H1, rHA from A/California/07/2009 X-181; H3, rHA from A/Perth/16/2009.

Table 3.6: K_d values (nM) of monoclonal antibodies to rHAs measured by surface plasmon resonance. Averages are calculated as mean with error-bars indicating s.d., replicated in triplicate. H1, rHA from A/California/07/2009 X-181; H3, rHA from A/Perth/16/2009.

Donor	rmAb ID	H1	H3	Vic
1	H1-2	0.013 ± 0.0003	>200	>200
1	H1-12	4.3 ± 0.5	>200	>200
1	H1-53	5.0 ± 0.3	>200	>200
1	H3-9	>200	4.9 ± 0.4	>200

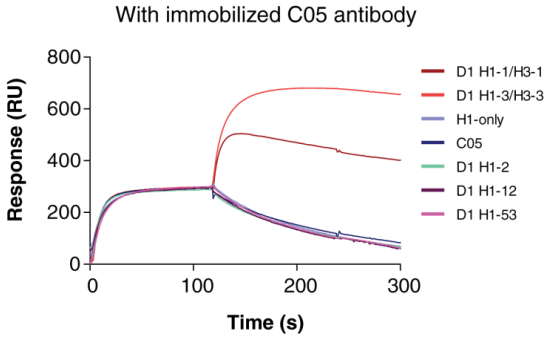

Figure 3.16: Epitope-blocking assay for the H1-specific antibodies. C05 was immobilized on a CM5 sensor chip, and H1 was bound. Stem-biding antibody, D1 H1-1/H3-1, and H1-only were used as controls. D1 H1-2, D1 H1-12 and D1 H1-53 antibodies showed competition for binding with C05 while not competing for binding with the head-binding cross-reactive antibody, D1 H1-3/H3-3. The experiments were repeated in triplicate.

Functional Characterization of Influenza B-specific Antibodies

Five antibodies that were identified as Vic + Yama cross-reactive from donor 1, including one antibody that constituted ~40% of the anti-Vic B/BR08 repertoire, were recombinantly expressed (Table 3.7), and their binding affinities to a panel of Victoria and Yamagata rHA proteins and their HAI titers to the vaccine strain were determined (Figures 3.17 and 3.18, and Table 3.8). Four of five antibodies bound with high affinity to all influenza B rHAs tested, whereas one antibody bound only to both Vic B/BR08 and Yama B/TX11 IIV1s but not to the respective rHAs. Our data show that most of the serum antibodies elicited by vaccination with Vic B/BR08 also bind to Yama B/TX11. This finding raises the question of whether vaccination with the new IIV4 can offer significant additional protection benefit over that with IIV3.

Table 3.7: CDR-3 sequences, lengths, SHM and gene usages of the recombinantly expressed influenza B-specific serum antibodies. For each antibodies, heavy (V_H) and light (V_L) chain information is shown with SHM measured by mutation rates in *IGHV* gene.

Donor	rmAb ID	CDR-3 sequences		CDR-3 length	SHM (*IGHV*)	Gene usage
1	Vic-1/ Yama-1	V_H	KCLLTTFPEKWFDP	14	7.1%	*IGHV3-15, IGHJ5*
		V_L	QSYDSNTQEI	10	4.1%	*IGLV6-57, IGLJ2*
1	Vic-4/ Yama-3	V_H	ATNLDYYDRSGYYVRDAFDI	20	3.1%	*IGHV5-51, IGHJ3*
		V_L	QKYDSAPWT	9	5.7%	*IGKV1-27, IGKJ1*
1	Vic-8/ Yama-20	V_H	AKDNYGGNLSGWFDP	15	2.1%	*IGHV3-23, IGHJ5*
		V_L	QSADSSGIYVV	11	0%	*IGLV3-25, IGLJ2*
1	Vic-9/ Yama-5	V_H	ARDLKYYGSVRMGWFGP	17	10.9%	*IGHV4-59, IGHJ5*
		V_L	QQYYSSPPT	9	1.7%	*IGKV4-1, IGKJ1*
1	Vic-20/ Yama-18	V_H	ARVSPGGIGTGYGDY	15	5.9%	*IGHV4-38, IGHJ4*
		V_L	QQYNSNPWT	9	2.9%	*IGKV1-5, IGKJ1*

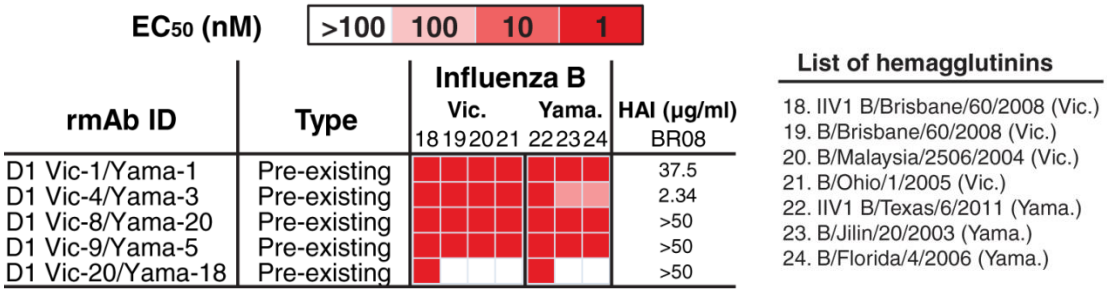

Figure 3.17: Biochemical and functional analysis of the recombinantly expressed influenza B-specific serum antibodies. EC_{50} values, as determined by ELISA, for each recombinant antibody tested against IIV1s or rHAs is listed. HAI assays were performed with B/Brisbane/60/2008 (BR08). All assays were repeated in triplicate.

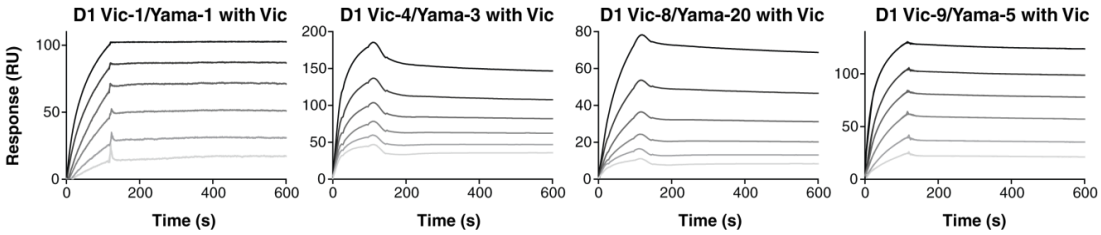

Figure 3.18: Surface plasmon resonance data for recombinant IgG binding to rHAs. Recombinant HAs were immobilized on CM5 sensor chips. Various concentrations of the recombinant monoclonal antibodies were tested (400, 200, 100, 50, 25, and 12.5 nM). Vic, B/Brisbane/60/2008).

Table 3.8: K_d values (nM) of monoclonal antibodies to rHAs measured by surface plasmon resonance. Averages are calculated as mean with error-bars indicating s.d., replicated in triplicate. H1, rHA from A/California/07/2009 X-181; H3, rHA from A/Perth/16/2009.

Donor	rmAb ID	H1	H3	Vic
1	Vic-1/Yama-1	>200	>200	0.0086 ± 0.001
1	Vic-4/Yama-3	>200	>200	0.14 ± 0.01
1	Vic-8/Yama-20	>200	>200	1.4 ± 0.2
1	Vic-9/Yama-5	>200	>200	0.21 ± 0.01

DISCUSSION

We used the proteomics data with the V_H:V_L-paired databases to recombinantly express and characterize the functions of the representative antibodies identified from sera of two donors following IIV3 vaccination. Recombinant expression of a set of H1 + H3 cross-reactive antibodies show a remarkable breadth of binding, recognizing not only historical H1 and H3 strains but also binding to H2, H5, H7 and H9. The unexpectedly large fraction of H1 + H3 cross-reactive antibodies that was detected by LC–MS/MS could not have been inferred by classical serology metrics. Similarly, a high frequency of H1 + H3 antibodies had not been noted in previous analyses of peripheral B cells. There are several possible explanations for this. Single-cell cloning typically involves the analysis of small samples of peripheral B cells, and therefore, plasmablasts or memory B cells that bind to H1 + H3 could have been missed. Additionally, the emphasis of B cell analysis studies has been on neutralization, which means that H1 + H3 cross-reactive antibodies that show little or no potency in neutralization assays could have been overlooked. An additional reason may be the kinetics of expansion of the memory B cells encoding these antibodies.

We distinguished two classes of H1 + H3 cross-reactive antibodies on the basis of their epitope specificity. One class recognized H2, H5, H7 and H9 with good to moderate affinity (in addition to H1 and H3), and these antibodies were found to compete with the stem-binding bNAb FI6. Because these antibodies are present at high levels at day 0 and bind to epitope(s) that overlap those recognized by antibodies such as FI6, their existence could be interfering with the recognition of the critical protective epitopes in the stem and the elicitation of high-potency broadly protective antibodies. The second class of H1 + H3 cross-reactive antibodies, identified in multiple donors, were all shown to bind to a highly conserved epitope on monomeric HA and to confer prophylactic and therapeutic

protection against challenge by both group 1 and group 2 strains despite their complete lack of neutralization activity *in vitro*. Such antibodies have not been previously described. Given that they bind to the HA molecules on the surface of infected cells (Figure 3.4) but lack any detectable *in vitro* neutralization activity, the ability of these antibodies to confer protection *in vivo* is probably dependent on effector functions mediated by crystallizable fragment (Fc), namely complement activation and/or antibody-dependent cellular cytotoxicity and phagocytosis [131, 132]. Studies to determine the precise mechanism for protection are under investigation.

Our data on the existence of broadly protective, non-neutralizing antibodies, which are present in relatively high amounts in serum, raise interesting questions in terms of their role in the prevention of influenza infections and whether it might be desirable to design universal influenza immunogens that stimulate the production or boosting of this class of antibodies, perhaps in addition to the elicitation of neutralizing antibodies.

Chapter 4: Longitudinal Analysis of Serum Antibody repertoires after Repeated Exposures to Influenza

INTRODUCTION

Influenza is a rapidly evolving, enveloped virus globally infecting over a billion people every year, approximately 500,000 of which resulting in death [4, 5]. Among the three types, influenza A virus especially poses a global threat as it accounts for pandemics and the majority of seasonal outbreaks [9, 14]. There are two groups of influenza A virus, group 1 and group 2, and they are differentiated based on the antigenic properties of two surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA) [9]. HA, in particular, is the major target of humoral response due to its high antigenicity and abundant presence on the surface [133, 134].

HA consists of two domains, head and stem, and they mediate attachment to host cells via the receptor-binding site (RBS) and fusion with the host cell upon infection [10]. HA-targeting antibodies are known to confer protective immunity, but the influenza virus is equipped with an ability to rapidly evolve and evade the host's immune system through a process called 'antigenic drift' [85, 104, 135]. Because of the necessity for the virus to maintain its cell-binding capability and assemble into the infective viral structure, however, not every amino acid residue is equally inclined to mutate. For instance, the stem region and RBS tend to be conserved while the residues around the RBS diverge substantially across different strains of virus [20].

There are two main classes of HA-targeting antibodies that confer protection against influenza. First class of antibodies neutralize virus by binding to the RBS and preventing the viral attachment to host cells [73, 108, 129, 136]. Due to the

aforementioned high variability in the areas around the RBS, these antibodies tend to neutralize a narrow range of homologous strains. On the other hand, another group of antibodies neutralize infectivity of the virus by targeting conserved sites on the stem of HA [70, 75, 76, 81, 91, 118, 137] and interfering with the membrane fusion required for infection. Because those antibodies bind to highly conserved epitopes, they neutralize a wide range of influenza strains, including viruses within group 1 [75, 77, 91], group 2 [74, 138, 139], and across group 1 and group 2 [70, 81, 117, 140]. Currently, the stem-binding broadly-neutralizing antibodies (bNAbs) have garnered the most interest as therapeutics [26], and development of vaccination strategies aimed at increasing the elicitation of such anti-stem bNAbs are at the forefront of the quest for a universal influenza vaccine [84-86].

Because influenza is a widespread virus, most individuals develop antibodies against influenza at an early age, and the pre-existing repertoire of influenza-specific antibodies affects the succeeding exposure to influenza. Over five decades ago, Thomas Francis first recognized this phenomenon and reported that sera from patients collected after being infected with an H1N1 strain showed higher neutralizing potency against a drifted variant that had circulated during their childhood than the strain responsible for the existing infection [92, 141]. The observation, which was termed ‘original antigenic sin’ (OAS), suggested that a sequential exposure to closely-related viral strains diminished antibody response to the unique antigenic determinants in the subsequent virus. In other words, the antibody response elicited from the first influenza infection or vaccination in childhood governed the future immunity against influenza. Subsequent studies examined the extent of the OAS effect in humans and other animals [142-144], using bulk serologic metrics, such as ELISA and neutralization titers. These assays measure the amounts of antibodies in serum that can bind to or neutralize the virus, respectively. The data available so far suggest that recurring exposures to influenza

preferentially induce antibodies targeting conserved regions [114, 116, 138, 145, 146], including the stem [146]. However, a molecular-level analysis of the serum antibodies is required to identify the constituent individual antibodies and determine their persistence in order to deconvolute the polyclonal nature of such aggregate metrics.

To address the question above, the HA-specific serum antibody repertoire was determined across a period of 6 years in a donor from whom analysis of peripheral B cells had yielded seminal insights on the emergence of broadly neutralizing antibodies active against both group 1 and group 2 viral strains [70, 137, 140, 147, 148].

Specifically, we determine the extent of variation in the clonotypic identity, diversity, and abundance of the antibodies in the sera, identify antibodies that persist for many years as well as others that are produced transiently, and compare the persistence of head- and stem-binding antibodies in serum. Insights gained from this study could help understand the determining factors for persistence in serum, which is critical for developing improved vaccination strategies.

MATERIALS AND METHODS

Purification of Total IgG from Serum and Subsequent Digestion into F(ab')2

Each serum samples analyzed in this study was passed through a 1 ml Protein G Plus agarose (Pierce) affinity column in gravity mode. Serum flow-through was collected and passed through the column three times. The column was washed with 15 cv of PBS prior to elution with 5 cv of 100 mM glycine-HCl, pH 2.7. The eluate, containing total IgG from serum, was immediately neutralized with 2 ml of 1 M Tris-HCl, pH 8.0. Purified IgG was digested into F(ab')2 with 10 μg of IdeS protease (Genovis) per 1 mg of IgG in PBS for 4 h on inverter at 37 °C.

Antigen-enrichment of F(ab')2 and Mass Spectrometry Sample Preparation

CA09pdm (A/California/07/2009pdm) rHA provided by Dr. Stephen Harrison (Harvard Medical School) was immobilized on N-hydroxysuccinimide (NHS)–activated agarose resins (Pierce) by overnight rotation at 4 °C. The coupled agarose resins were washed with PBS, and unreacted NHS groups were blocked with 1 M ethanolamine, pH 8.3 for 30 min at RT. The beads were further washed with PBS, and packed into a 0.5 ml chromatography column (Clontech). The column was equilibrated with 10 cv of PBS.

For each sample, F(ab')2 was applied to the individual antigen affinity columns in gravity mode. Flow-through was collected and reapplied to the column three times, and the column was washed with 10 cv of PBS and 10 cv of diluted PBS (1:2 in ddH_20). Antigen-enriched F(ab')2 was eluted with 2% (v/v) formic acid in 0.5 ml fractions. 20 μl

from each elution fraction, neutralized with NaOH/Tris, and flow-through were assayed by indirect ELISA with the rHA. Depletion of ELISA signal in each flow-through sample was checked. Elution fractions showing an ELISA signal were pooled and concentrated under vacuum to a volume of ~3 µl before neutralizing with NaOH/Tris. The neutralized elution samples were concentrated to 50 µl under vacuum.

For each enrichment, elution and flow-through samples were denatured in 50% (v/v) 2,2,2-trifluoroethanol (TFE), 50 mM ammonium bicarbonate, and 10 mM dithiothreitol (DTT) at 60 °C for 1 hr, then alkylated by incubation with 32 mM iodoacetamide (Sigma) for 1 hr at RT in the dark. Alkylation was quenched by the addition of 20 mM DTT. Samples were diluted 10-fold with 50 mM ammonium bicarbonate and digested with trypsin (1:30 trypsin/protein) for 16 hr at 37 °C. Formic acid was added to 1% (v/v) to quench the digestion, and the sample volume was reduced to ~100 µl under vacuum. Peptides were then bound to a C18 Hypersep SpinTip (Thermo Scientific), washed three times with 0.1% formic acid, and eluted with 60% acetonitrile, 0.1% formic acid. C18 eluate was dried under vacuum centrifugation and resuspended in 50 µl in 5% acetonitrile, 0.1% formic acid.

LC-MS/MS Analysis

Samples were analyzed by liquid chromatography-tandem mass spectrometry on a Dionex Ultimate 3000 RSLCnano uHPLC system (Thermo Scientific) coupled to an LTQ Orbitrap Velos Pro mass spectrometer (Thermo Scientific). Peptides were first loaded

onto an Acclaim PepMap RSLC NanoTrap column (Dionex; Thermo Scientific) prior to separation on a 75 μm×15 cm Acclaim PepMap RSLC C18 column (Dionex; Thermo Scientific) using a 5–40% (v/v) acetonitrile gradient over 250 min at 300 nl/min. Eluting peptides were injected directly into the mass spectrometer using a nano-electrospray source. The LTQ Orbitrap Velos Pro was operated in data-dependent mode with parent ion scans (MS1) collected at 60,000 resolutions. Monoisotopic precursor selection and charge state screening were enabled. Ions with charge ≥+2 were selected for collision-induced dissociation fragmentation spectrum acquisition (MS2) in the ion trap, with a maximum of 20 MS2 scans per MS1. Dynamic exclusion was active with a 45-s exclusion time for ions selected more than twice in a 30-s window. Each sample was run three times to generate technical replicate datasets.

MS/MS Data Analysis

Protein sequence databases were constructed using the V_H sequences obtained from the donor. Specifically, HA-specific antibody sequences obtained across 7 years [70, 137] were combined with high-throughput sequencing of V_H transcripts from 2014 days 7, 14 and 21 post-vaccination B-cells with ≥2 reads. These sets of V_H sequences were concatenated to a database of background proteins comprising non-donor derived V_L sequences (HD1 [98]), a consensus human protein database (Ensembl 73, longest sequence/gene), and a list of common protein contaminants (MaxQuant). Spectra were searched against the database using SEQUEST (Proteome Discoverer 1.4; Thermo Scientific). Searches considered fully-tryptic peptides only, allowing up to two missed

cleavages. A precursor mass tolerance of 5 ppm and fragment mass tolerance of 0.5 Da were used. Modifications of carbamidomethyl cysteine (static) and oxidized methionine (dynamic) were selected. High-confidence peptide-spectrum matches (PSMs) were filtered at a false discovery rate of <1% as calculated by Percolator (q-value <0.01, Proteome Discoverer 1.4; Thermo Scientific).

Iso/Leu sequence variants were collapsed into single peptide groups. For each scan, PSMs were ranked first by posterior error probability (PEP), then q-value, and finally XCorr. Only unambiguous top-ranked PSMs were kept; scans with multiple top-ranked PSMs (equivalent PEP, q-value, and XCorr) were designated ambiguous identifications and removed. Observed precursor masses were recalibrated according to the previous method [109], and the average mass deviation (AMD) for each peptide was calculated as described [97] using data from elutions only. Peptides with an AMD >1.5 ppm were removed. Additionally, only peptides identified in ≥2 replicate injections for at least one elution sample were kept as high-confidence identifications.

Peptide abundance was calculated from the extracted-ion chromatogram (XIC) peak area, as described [98], using peak area values generated by the Precursor Ions Area Detector node in Proteome Discoverer. For each peptide, a total XIC area was calculated as the sum of all unique XIC areas of associated precursor ions, and the average XIC area across replicate injections was calculated for each sample. For each antigen dataset, the eluate and flow-through abundances were compared and peptides with ≥10-fold higher signal in the elution sample were considered to be antigen-specific.

Clonotype Indexing and Peptide-to-clonotype Mapping

V_H sequences were grouped into clonotypes based on single-linkage hierarchical clustering as described [98]. Cluster membership required ≥90% identity across the CDR-H3 amino sequence as measured by edit distance. High-confidence peptides identified by MS/MS analysis were mapped to clonotype clusters, and peptides uniquely mapping to a single clonotype were considered "informative". Abundance of each antibody clonotype was calculated by summing the XIC areas of the informative peptides mapping to ≥4 amino acids of the CDR-H3 region.

Quantitating Abundances of Individual Antibody Clonotypes

For each serological repertoire, relative abundance of a clonotype was calculated by dividing the XIC area for a particular clonotype by the sum of the XIC areas for all clonotypes. Fraction of serological repertoire refers to this relative abundance based on XIC.

ELISAs

EC_{50} values based on ELISA were used to determine the binding titers of CA09pdm-specific antibodies in the serum samples. First, costar 96-well ELISA plates (Corning) were coated overnight at 4 °C with 4 μg/ml CA09pdm HA and washed and blocked with 2% milk in PBS for 2 hr at RT. After blocking, serially diluted recombinant antibodies or serum samples bound to the plates for 1 h, followed by 1:5000 diluted goat anti-human IgG Fc HRP-conjugated secondary antibodies (Jackson ImmunoResearch; 109-035-008) for 1 h. For detection, 50 μl TMB-ultra substrate (Thermo Scientific) was

added before quenching with 50 µl 1 M H_2SO_4. Absorbance was measured at 450 nm using a Tecan M200 plate reader. Data were analyzed and fitted for EC_{50} using a 4-parameter logistic nonlinear regression model in the GraphPad Prism software. All ELISA assays were performed in triplicate.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA), unless specifically noted otherwise in the figure legends. All the statistical tests performed are described in the figure legends, and correlations were considered significant at a *P* value of <0.05.

RESULTS

Blood samples were collected from the donor during each encounter with influenza through infection or vaccination (Table 4.1). Peripheral B cells were isolated from the collected blood, immortalized with Epstein Barr Virus [69] and individually cultured, secreting antibodies to media which were subsequently screened for binding to HA. B cells encoding HA-binding antibodies were sequenced and recombinantly expressed to characterize their biochemical and functional properties. It should be noted that the donor was repeatedly exposed to pandemic A/California/04/2009 (CA09pdm) viral strain as summarized in Table 4.1. ~500 CA09pdm HA-binding monoclonal antibodies were discovered of which ~200 of the antibodies targeted the stem region, and several of them showed remarkably broad neutralization activities. One of the antibodies, named FI6, is a pan-influenza A neutralizing antibody currently under clinical trials as therapeutics for influenza [70]. Additionally, ~300 antibodies that bound to the HA-head were also identified. The exhaustive analysis of HA-specific antibodies from the donor across a decade also led to details regarding the developmental pathways of CA09pdm HA-specific antibody-encoding B cells.

Table 4.1: List of the collected samples and the encountered viral strains. IIV1, monovalent inactivated influenza vaccine; IIV3, trivalent inactivated influenza vaccine; CA09pdm, A/California/04/2009pdm; VI09, A/Victoria/210/2009; BR08, B/Brisbane/60/2008; VI11, A/Victoria/361/2011; WI10, B/Wisconsin/1/2010; TX12, A/Texas/50/2012; MA12, B/Massachusetts/02/2012.

Exposure history	Viral strains encountered	Sera analyzed (collection dates)
2009 infection	**CA09pdm**	21 d post-exposure (Dec. 1, 2009)
2010 vaccination 1	IIV1 (**CA09pdm**)	14 d post-exposure (May 6, 2010)
2010 vaccination 2	IIV3 (**CA09pdm**, VI09, BR08)	Pre-exposure (Dec. 6, 2010) 14 d post-exposure (Dec. 20, 2010)
2011 vaccination	IIV3 (**CA09pdm**, VI09, BR08)	Pre-exposure (Nov. 11, 2011) 17 d post-exposure (Nov. 28, 2011)
2012 vaccination	IIV3 (**CA09pdm**, VI11, WI10)	Pre-exposure (Nov. 14, 2012)
2013 vaccination	IIV3 (**CA09pdm**, VI11, MA12)	Pre-exposure (Dec. 3, 2013) 16 d post-exposure (Dec. 19, 2013)
2014 vaccination	IIV3 (**CA09pdm**, TX12, MA12)	14 d post-exposure (Dec. 10, 2014)

It is important to note, however, that the investigation of the peripheral B cells does not provide information regarding the individual antibodies' relative abundances in serum. In previous studies directly analyzing serum antibodies, we have observed that the presence of B cells in the periphery is not always an indication for the corresponding immunoglobulin molecules circulating in serum [98]. Furthermore, the steady-state serum antibody response, referring to antibodies that persist for many months or years after they were elicited as a result of infection or vaccination, is maintained by long-lived plasma cells (LL-PCs) in the bone marrow [65, 66]. Given the difficulties and limitations associated with analyzing LL-PCs isolated from bone marrow, a direct analysis of the antibodies in serum at steady-state can provide valuable information regarding the antibody repertoire formed by the subset of B cells in the bone marrow. Such information is imperative for a longitudinal study to investigate the OAS effects at a molecular level and understand how the serological antibody repertoire is shaped by repeated exposures to influenza.

Using our previously developed proteomics-based method [98, 99], we delineated the composition and relative abundances of the antibody clonotypes comprising the serum IgG repertoire specific to CA09pdm in each of the collected serum samples. To briefly summarize, we sequenced B cells isolated from the donor 7, 14 and 21 days after the vaccination in 2014 and combined the high throughput sequencing data with the aforementioned ~500 CA09pdm-HA-specific antibody sequences to compile a list of antibodies encoded by the B cells from the donor. Then, the immunoglobulin molecules specific to CA09pdm HA were isolated from each serum sample through two separate affinity chromatography steps, first using protein G resins to capture the total IgG and second using agarose resins with immobilized CA09pdm HA. The eluates from the final purification were analyzed by high-resolution liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) (Figure 4.1). We identified complementarity-determining region 3 (CDR-H3) peptides and used the corresponding LC peak intensities to determine the relative abundance of each CDR-H3 peptide. Antibodies that have high similarity in the CDR-H3 were grouped together into a clonotype. Accordingly, we delineated the longitudinal landscape of the anti-CA09pdm serum antibody repertoire in an individual across 6 years (Appendix B).

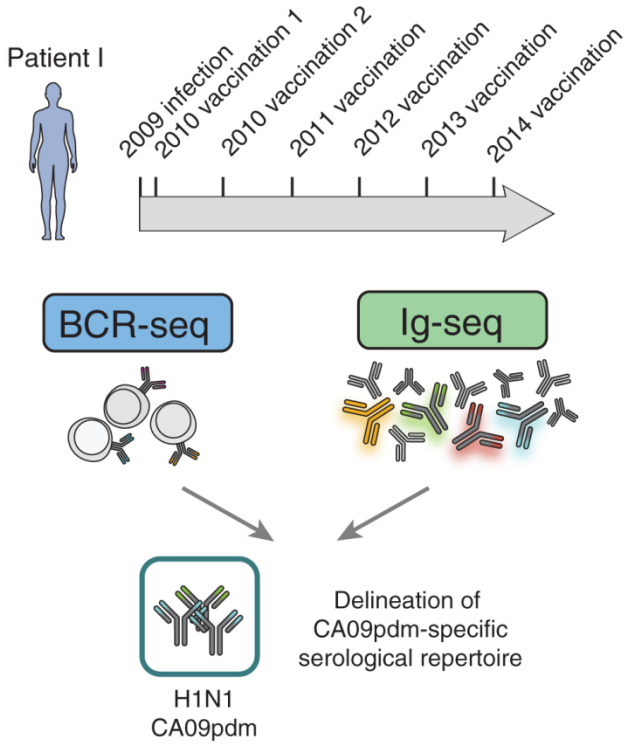

Figure 4.1: Experiment design. For sequencing of B cell receptor (BCR) transcripts (BCR-seq), we used peripheral B cells isolated 7, 14 and 21 days after the vaccination in 2014 to sequence the heavy chain variable (V_H) regions. These sequences were added the V_H sequences of the ~500 antibodies obtained from the longitudinal B cell analysis for constructing custom database for the heavy-chain peptide identification. From each serum sample, antibodies with binding affinities to CA09pdm were isolated through column purification and the eluates were subsequently analyzed proteomically (Ig-seq).

Longitudinal Analysis of the Serological Repertoire to CA09pdm

For each sample analyzed, the clonotypic antibody composition and relative abundances were plotted as a histogram, with each bar indicating an individual clonotype and the *y* axis displaying its relative abundance (Figure 4.2, left). To compare the serological repertoires from different time points, each histogram was represented as a

subdivided bar graph with individual divisions representing the relative quantities of antibody clonotypes (Figure 4.2, right). Individual subdivided bar graphs were combined together, and the same colors were used for the same clonotypes across the time points to illustrate the longitudinal changes of the individual antibody clonotypes (Figure 4.3). We determined the total amounts of CA09pdm-binding IgG in each serum sample by measuring ELISA serum titers (Table 4.2), and dashed lines were used to indicate the total quantities of the CA09pdm-specific antibody in the sera. Lastly, the subdivided bar graphs were scaled accordingly to the corresponding serum titer values. Therefore, the height of each bar indicates the total amounts of antibodies specific to CA09pdm with the divisions representing the relative abundance of each antibody clonotype comprising the serological repertoire.

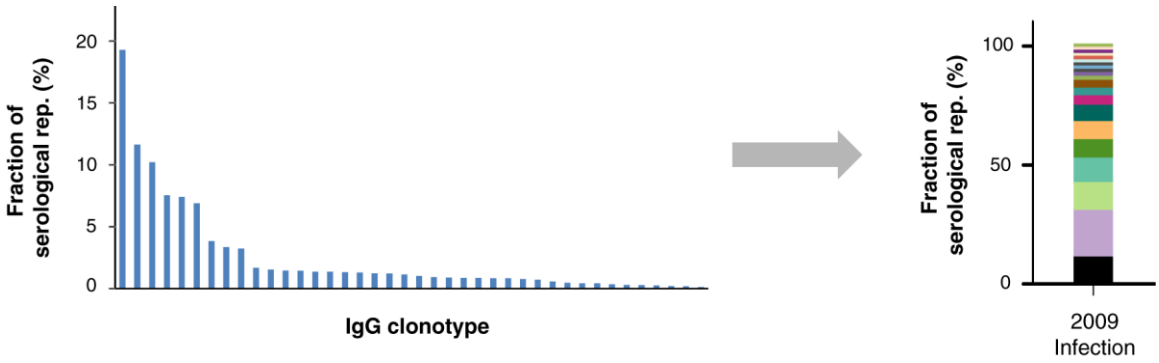

Figure 4.2: Representative serological repertoire (2009 infection, 21 d post-exposure). Each bar represents a unique clonotype and the *y* axis indicates the relative abundance (fraction) determined by the proteomic analysis. Based on the histogram, a subdivided bar graph was generated where individual divisions represent the top-20 most abundant unique clonotypes and their relative abundances. Different colors were used for different antibody clonotypes, and the black division at the bottom indicates the fraction of the rest of the antibody clonotypes.

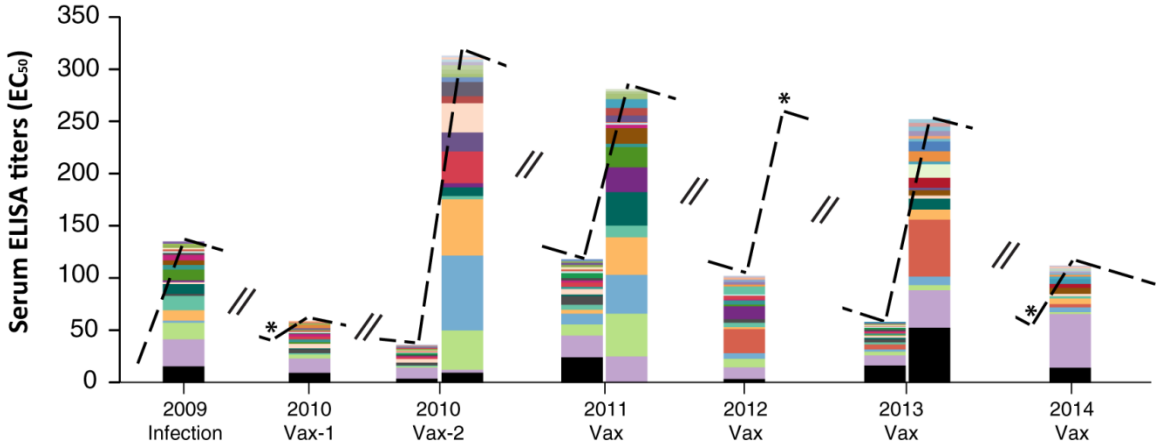

Figure 4.3: Longitudinal landscape of the serological repertoires across 6 years. For each time point, a subdivided bar graph was used to represent the relative abundance of individual antibody clonotypes as described in Figure 4.2. The top-20 most abundant antibody clonotypes are individually colored with the black shade indicating the abundances of the rest of the antibodies. The same colors are used for the same antibody clonotypes across the time points. Dashed lines represent serum titers to CA09pdm, with '*' indicating predicted titers based on the exposure history of the donor.

Table 4.2: ELISA titer to CA09pdm for each serum sample.

Exposure history	Serum samples	Serum titer (EC_{50})
2009 infection	21 d post-exposure	135
2010 vaccination 1	14 d post-exposure	59
2010 vaccination 2	Pre-exposure 14 d post-exposure	36 313
2011 vaccination	Pre-exposure 17 d post-exposure	118 281
2012 vaccination	Pre-exposure	102
2013 vaccination	Pre-exposure 16 d post-exposure	58 252
2014 vaccination	14 d post-exposure	112

Examination of the longitudinal serum samples revealed some general features of the serum antibody repertoire to influenza. First, the number of unique antibody

clonotypes in each serum analyzed ranged from 19 to 115 (Table 4.3), agreeing with our earlier observation that the influenza-specific serological repertoire is restricted and oligoclonal. In addition, we observed a high degree of polarization as the top-10 most abundant clonotypes accounted for on average ~73% of the antibody response by abundance. These results indicate that the protection mediated by serum antibodies is conferred by a limited number of serum antibody clonotypes. Interestingly, ~50% of the antibody repertoires was comprised of the antibodies that had been identified through the B cell immortalization.

Table 4.3: Size and fraction of the top-10 most abundant clonotypes of each serological repertoire analyzed. Number of unique clonotypes in each repertoire is indicated, and the abundances of the top 10 antibody clonotypes were added to determine the % fraction of the top-10 clonotypes.

Exposure history	Serum samples	Number of antibody clonotypes	Relative abundance of top-10 clonotypes (%)
2009 infection	21 d post-exposure	40	75.1
2010 vaccination 1	14 d post-exposure	44	66.8
2010 vaccination 2	Pre-exposure 14 d post-exposure	37 30	71.6 87.2
2011 vaccination	Pre-exposure 17 d post-exposure	60 19	63.6 88.5
2012 vaccination	Pre-exposure	27	81.4
2013 vaccination	Pre-exposure 16 d post-exposure	85 115	54.9 65.6
2014 vaccination	14 d post-exposure	73	76.1

Persistent and Intermittent Antibody Clonotypes

To analyze the antibodies persisting in circulation, we identified the antibody clonotypes that are detected in serum samples collected before encountering influenza (pre-exposure serum samples). These antibodies circulating in serum prior to exposure events represent the steady-state antibodies since the peak response generated by the

previous encounter with influenza has waned, and they are likely being secreted by the LL-PCs in the bone marrow [65]. Based on the proteomic analysis of the four pre-vaccination serum samples (2010 vaccination 2, 2011 vaccination, 2012 vaccination and 2013 vaccination), we defined the antibody clonotypes detected in all four samples as 'persistent', and the rest as 'intermittent' antibody clonotypes. It should be noted that these definitions are strictly based on the detection in our LC-MS/MS data, and it is possible that some of these clonotypes were present at low quantities in serum but not detected.

Overall, we identified 15 persistent antibody clonotypes across all the time points (Table 4.4). Two persistent antibody clonotypes, clonotype-633 and clonotype-324, were of particular interest. As shown in Table 4.5, clonotype-633 antibodies comprised substantial fraction of the serological repertoire in each serum sample, indicating how the serological repertoire can be constantly dominated by one antibody clonotype during the 6 years of repeated exposure to CA09pdm. In contrast, while clonotype-324 is a persistent clonotype, it comprised one of the major clonotypes only in 2 of the 10 serum samples analyzed (i.e. present as one of the top 10 antibody clonotypes). However, it is important to note that one of the antibodies belonging to clonotype-324 is the aforementioned pan-influenza A neutralizing antibody, FI6. Our data suggest that while FI6 persisted in circulation, it did not constitute one of the dominant components of the antibody response to CA09pdm, further highlighting the importance of analyzing the relative abundances of the antibodies to better understand humoral immunity.

Table 4.4: Size and fraction of the persistent antibody clonotypes in each serological repertoire analyzed.

Exposure history	Serum samples	Number of persistent clonotypes detected	Fraction of the persistent clonotypes (%)
2009 infection	21 d post-exposure	13	66.4
2010 vaccination 1	14 d post-exposure	15	62.0
2010 vaccination 2	Pre-exposure 14 d post-exposure	15 7	60.7 35.3
2011 vaccination	Pre-exposure 17 d post-exposure	15 7	55.8 70.3
2012 vaccination	Pre-exposure	15	50.2
2013 vaccination	Pre-exposure 16 d post-exposure	15 10	47.6 26.2
2014 vaccination	14 d post-exposure	10	56.8

Table 4.5: Fraction of clonotype-633 and clonotype-324 in each serological repertoire analyzed.

Exposure history	Serum samples	Fraction of Clonotype-633 (%)	Fraction of Clonotype-324 (%)
2009 infection	21 d post-exposure	19.3	Not detected
2010 vaccination 1	14 d post-exposure	23.1	1.0
2010 vaccination 2	Pre-exposure 14 d post-exposure	29.0 0.9	0.2 1.3
2011 vaccination	Pre-exposure 17 d post-exposure	17.6 8.9	0.4 8.4
2012 vaccination	Pre-exposure	10.8	12.1
2013 vaccination	Pre-exposure 16 d post-exposure	16.6 14.2	0.1 Not detected
2014 vaccination	14 d post-exposure	45.8	Not detected

On average, these 15 persistent antibody clonotypes comprised 53.1% of the CA09pdm HA-binding antibodies in serum, and this surprisingly high abundance indicates that during the repeated exposure to CA09pdm, approximately half of the serum antibodies did not change, and the variation in different serum samples resulted from the elicitation of the intermittent antibody clonotypes. The high fraction of antibody response comprised by the persistent antibody clonotypes reveal the high degree of the OAS effect observed in serum at a molecular-level.

Next, we quantitatively determined the extent of overlap between the antibody repertoires. For the analysis, we separated the serum samples into two groups. The pre-exposure group contained the four serum samples collected before each exposure (2010 vaccination-2 pre-exposure, 2011 pre-exposure, 2012 pre-exposure, and 2013 pre-exposure), and the post-exposure group consisted of the six serum samples collected after (2009 post-exposure, 2010 vaccination-1 post-exposure, 2010 vaccination-2 post-exposure, 2011 post-exposure, 2013 post-exposure, and 2014 post-exposure). We determined the fraction of antibody clonotypes that is overlapping between all the samples in the same group. As illustrated in Figure 4.4, the overlap between the pre-exposure serum samples was on average 78.2% of the antibody repertoires. The degree of overlap between the post-exposure samples was significantly lower than the pre-exposure samples, at 61.5% on average. Considering that the 15 persistent antibody clonotypes comprised about half of the serological repertoire, this data suggest that the intermittent antibody clonotypes changed greatly between the post-exposure samples as those antibody clonotypes were elicited and quickly decayed. However, the steady-state repertoires existing prior to each exposure to CA09pdm are much similar to one another. In other words, the immediate antibody response varied for each instance of being exposed to influenza, but the serum antibody repertoire returns back to its pre-exposure state. This finding strongly support the notion that the antibody repertoires generated from previous exposures govern the future antibody response to influenza, again demonstrating the degree of the OAS phenomenon in human.

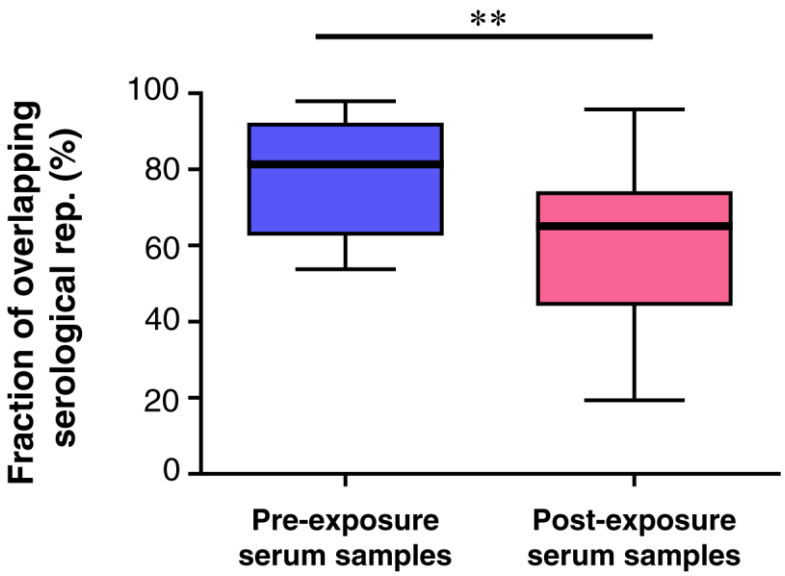

Figure 4.4: Fraction of overlapping antibody clonotypes between the pre- and post-exposure serum samples. For each serum sample analyzed, the abundances of the antibodies overlapping between each sample in the group were determined. The center-lines represent the mean value of the overlapping fractions across the time points, with the boxes extending from the 25th to 75th percentiles and the whiskers indicating min to max. Unpaired t test with Welch's correction was performed to identify the differences in the degree of overlap between the two groups.

The Persistent Antibody Clonotypes Are Likely to be Stem-binding

As mentioned earlier, the longitudinal investigation of the donor's B cell receptors and subsequent characterization of the recombinant antibodies enabled us to determine the binding specificity of some of the antibody clonotypes detected from the serum samples. We focused on these antibody clonotypes with the knowledge about whether they are stem-binding or head-binding to investigate the differential persistence of the antibodies in serum based on their binding specificities. It is important to note that due to the variation in the head resulting from antigenic drift, antibodies that target the HA-head are likely to react with narrow range of viral strains while stem-binding antibodies tend to

have broad recognition due to the high degree of conservation in the HA-stem, including across group 1 and group 2. From our proteomics data, we identified 20 stem-binding antibody clonotypes and 10 head-binding antibody clonotypes. The fraction of head- and stem-specific antibodies in each serological repertoire is summarized in Table 4.6.

Table 4.6: Fraction of the head- and stem-binding antibody clonotypes in each serological repertoire analyzed.

Exposure history	Serum samples	% of head-binding antibody clonotypes	% of stem-binding antibody clonotypes
2009 infection	21 d post-exposure	0.4	48.0
2010 vaccination 1	14 d post-exposure	2.0	52.6
2010 vaccination 2	Pre-exposure 14 d post-exposure	1.4 47.4	50.9 2.2
2011 vaccination	Pre-exposure 17 d post-exposure	15.4 38.3	36.5 20.4
2012 vaccination	Pre-exposure	7.7	31.3
2013 vaccination	Pre-exposure 16 d post-exposure	8.3 13.1	33.2 17.8
2014 vaccination	14 d post-exposure	11.2	54.0

It is important to note, the donor was infected with the CA09pdm viral strain in 2009. The HA-head of this strain diverged considerably from the viral strains that circulated previously, and this was the reason for the pandemic that followed because most of the population had not been exposed to a similar strain in the past, lacking pre-existing immunity. It is established that because of its divergent head domain, exposure to CA09pdm boosted the antibodies with binding specificity toward the stalk domain [149]. In agreement with these observations, the abundance of head-binding antibody clonotypes in serum 21 days post-infection was very low. The fraction of the repertoire resulting from head-specific antibody clonotypes did not substantially increase until the

second vaccination in 2010. In contrast, the stem-binding clonotypes dominated the post-infection repertoire to CA09pdm in 2009.

The relative abundances of the head-binding and stem-binding antibody clonotypes in the pre-exposure samples and the post-exposure samples are presented in Figure 4.5. Our data showed that the stem-binding antibodies consistently dominated the pre-vaccination over the head-specific antibody clonotypes (Figure 4.5a). This indicates that the persistent antibody clonotypes are likely to be stem-binding antibodies. When we analyzed the post-exposure sera, the head-specific antibodies significantly increased following the vaccination in 2010 and 2011, but their relative abundances waned and the stem-binding antibody clonotypes' abundances increased. Addition to the fact that 6 clonotypes are stem-binding while 2 clonotypes are head-binding, our data illustrate that the stem-binding antibody clonotypes are likely to be persistent in serum while the head-binding antibodies are intermittent.

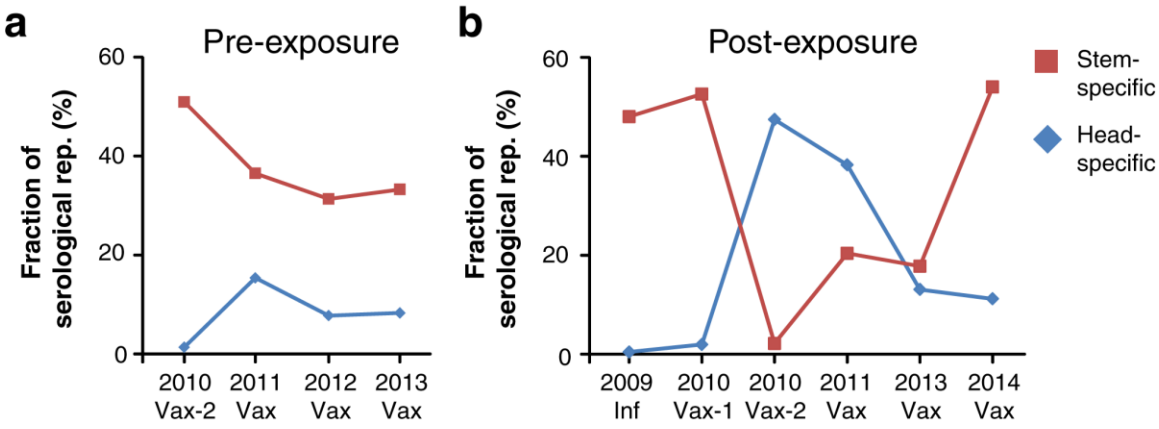

Figure 4.5: Fractions of head- and stem-binding antibody clonotypes in the pre-exposure and post-exposure serum samples. Antibody clonotypes with known binding specificities are indicated and the rest is of antibody clonotypes with unknown binding specificities.

DISCUSSION

We have performed a longitudinal analysis of the serum antibodies at the molecular-level to analyze the persistence and decay of CA09pdm-specific serum antibodies across 6 years. Our analysis identified 15 persistent antibody clonotypes that dominated the pre-exposure serological repertoires and on average comprised approximately half of the identified antibody repertoire to CA09pdm. Our analysis illustrate that the serum repertoire changes after each exposure to CA09pdm resulting from the elicitation of the intermittent antibody clonotypes. But after a period of time, the serum antibody repertoire returns back to the steady-state that existed prior to the exposure. Given that half of the antibody repertoire stays the same and that the serological repertoire returns back to its pre-existing state, our study clearly illustrates the extent of the OAS effects in serum. It also highlights the importance of vaccination in childhood as the antibodies elicited during the early exposure to influenza will likely dominate the future antibody responses.

The general understanding of the OAS is that it is a 'sin' because it is unfavorable; however if repeated exposure could lead to the persistence of neutralizing antibodies targeting conserved regions, such as the stem, this phenomenon could be beneficial and should be exploited by vaccines. Our finding showed that through the multiple exposures to CA09pdm, the immune system selectively promoted the persistence of the stem-binding antibody clonotypes. It is worth noting that the majority of studies illustrating the detrimental effect of the OAS relied on the aggregate serologic metrics, like the HAI titer. However, the HAI assay does not measure the neutralizing activity of the stem-binding antibodies since the assay quantifies only the antibodies neutralizing the infection through binding to the head region. If the repeated exposure promoted the antibodies

targeting the conserved stem domains, it could have decreased the HAI activity without compromising the level of protection conferred by the antibodies in serum.

A key to the universal influenza vaccine will be the design of immunogens that can elicit broadly-protective antibodies to handle a variety of different strains and also to provide long-term protection. It is important to note that just elicitation of broadly neutralizing antibodies is not the end point. Given how polarized serological repertoire is, the objective of a universal vaccine might need to focus on promoting the generation of antibodies with desired functions so they constitute a major fraction of the serum antibodies. For instance, we observed FI6 in the sera of the donor across many years, but it was not one of the abundant antibody clonotypes. The next step in the field should be focused on understanding what are the determining factors for certain antibody clonotypes becoming one of the most abundant antibody clonotypes. With the stem-binding antibodies showing a tendency to persist in serum at a detectable level across multiple years, the repeated exposure to influenza promotes longevity and increase of such antibodies. The differential longevity of stem-binding and head-specific antibodies presented here could be highly informative for developing effective future vaccination strategies.

Chapter 5: Future Directions and Perspectives

Vaccine-induced protection against influenza is conferred mainly through the stimulation of B cells to produce a diverse repertoire of antigen-specific immunoglobulin molecules. These antibodies circulate in serum and afford immunity by binding and neutralizing the virus, and characterizing them is the essence of understanding serological immunity. Until recently, serological studies relied on the aggregate metrics, but the inability to deconvolute the polyclonal nature of serum antibody has restricted the depth of information obtainable from such studies. The recent rapid advancement of novel technologies for analyzing functional antibody repertoires has enabled high-through and deeper characterizations of humoral immune responses. In particular, with the combination of the high-throughput sequencing of B cell receptor-encoding transcripts (BCR-seq) and the high-resolution proteomics analysis of immunoglobulin molecules (Ig-seq), we are now entering a new era of serologic studies as the technical advancements have enabled us to directly interrogate the serum antibodies at an unprecedented high resolution.

In this dissertation, we used the proteomics-based serum antibody repertoire analysis pipeline to identify and delineate, in a quantitative fashion, the monoclonal antibodies comprising the human serum response to influenza. We first interrogated the influenza HA-specific IgG molecules circulating in sera of four young adults before and after seasonal vaccination. Our study led to several new findings including the dominance of pre-existing antibodies in the vaccine response and the higher than expected abundance of cross-reactive antibodies in serum. Subsequent recombinant analysis led to the discovery of non-neutralizing but protective cross-reactive antibodies targeting a newly identified conserved epitope in the HA-head. We also examined the serum

antibodies from one individual across 6 years to show the degree of change, or the lack thereof, in the antibody repertoire through the repeated exposure to influenza. Our analysis also illustrated the likelihood of the stem-binding antibodies persisting over the head-binding antibodies. These findings provide important data to help describe the immunological mechanisms underlying cross-reactivity and persistence, which have direct implication for the evaluation and design of future vaccine for influenza.

As we utilize these new technologies to better understand serological immunity to influenza at the molecular-level, there are numerous questions waiting to be answered. We so far have investigated a small number of young adults. There is a need to explore a cohort in different age groups, in particular the elderly since the majority of the influenza-related death occurs in people over the age of 65. A comparison of the serological repertoire specific to influenza between different age groups could provide useful information.

In addition to IgG, it is known that IgA also plays a role in protection against influenza. The relationship between the IgG repertoire and IgA repertoire remains to be explored. For example, to what degree are the influenza specific IgG and IgA repertoires clonally relate to each other? Characterization of the antibodies that are found in serum as IgA and IgG isotypes could elucidate about the relationship between class switching and serum immunity.

Furthermore, these approaches are not only applicable for studying the vaccine-elicited antibody repertoires. The analytic tools can be extended to other infectious disease as well as autoimmunity and cancer. In infectious diseases, pathogen-specific antibodies could be directly isolated from serum and further developed as therapeutics. Also, new epitopes being targeted by abundant serum antibodies could be identified, which would be valuable for developing vaccines. In autoimmune diseases and cancer,

characterization of the serological repertoire would enable the identification of novel biomarkers, as well as therapeutic or diagnostic antibodies. Such studies could provide insights into the underlying mechanisms of disease. There are many directions we could take as the new technologies for the direct analysis of serum antibodies has resulted in an unprecedented capacity to investigate serological immunity.

Appendix A

Donor 1

Anti-H1 A/CA09 serological repertoire clonotypic antibodies

Representative CDR-H3 Sequence	Total PSM	Day 0 PSM	Day 28 PSM	Day 180 PSM	Day 0 XIC	Day 28 XIC	Day 180 XIC
ARHFQERLVAARAGAFDV	557	57	248	252	1.63E+07	3.55E+08	6.91E+08
ARDFFEKVTGDDVNAFDI	368	48	135	185	1.77E+07	2.33E+08	4.97E+08
ARVPPYSSGWLRPMDV	263	75	119	69	4.10E+07	1.43E+08	3.63E+08
ARLYMVRGELWGFYFDH	246		68	178		4.35E+07	1.77E+08
ARGGETSEIPNGMDV	177		78	99		1.99E+08	5.58E+08
ARDGIAVPGVDWFDS	172	2	76	94	2.48E+05	7.23E+07	7.77E+07
AREYRILDERFNYFDA	142		96	46		3.23E+08	1.53E+08
KCLLTTFAESWFDP	129	7	75	47	8.47E+05	4.89E+07	6.66E+07
ARDLAIPIDSLTYYLRAFDS	105	33	34	38	1.04E+07	4.08E+07	3.23E+07
ARDRRDLLTGYDQNH	92	24	44	24	9.83E+06	8.99E+07	1.39E+08
ARDFFEKLIADNLNAFDI	86	11	37	38	1.42E+06	2.20E+07	4.45E+07
AKDLAQWPPGAGALDY	76	16	25	35	1.40E+05	4.43E+05	8.38E+05
ARDVAMGGFDS	71	19	23	29	1.26E+07	5.10E+07	9.89E+07
ASGPGGLLRLGELLFEY	66	18	27	21	2.17E+06	1.56E+07	1.59E+07
ARPSYTSGFAFDP	65	14	20	31	6.86E+06	1.30E+08	1.97E+08
ARASIASRPQFFDF	64	13	24	27	3.95E+06	5.91E+07	1.01E+08
ARASNLYPRVGATDF	56		30	26		1.30E+07	2.70E+07
ATNLEYYDRSGYYIRDAFGI	51	18	11	22	4.42E+06	1.62E+07	3.41E+07
ARAACSLCPLDY	48	8	22	18	1.53E+06	4.57E+07	4.45E+07
VRKGLCDY	44	21	13	10	3.45E+06	8.89E+06	2.32E+07
ARDFFEKLGGEDHNAFDI	41	8	13	20	1.22E+06	9.47E+06	3.49E+07
VKDPLGSLEGDH	38		19	19		1.38E+07	1.17E+07
ARDLGGGELLDS	35	8	14	13	1.07E+06	7.22E+06	1.14E+07
ARDDTSGELPRGLDY	33		23	10		1.22E+07	3.09E+07
TREFQSTFDP	30	8	11	11	4.90E+06	2.73E+07	4.23E+07
ITSLGTYYRARVDY	29	7	8	14	4.24E+06	1.16E+07	4.85E+07
ARVLGRPYSYAMDV	29	1	23	5	6.62E+05	1.59E+07	7.65E+06
ARDSDSYNFYYMDV	29		20	9		1.74E+07	5.72E+06
TTRIYNNYMDI	28		8	20		2.26E+06	1.78E+07
ARGRGSYPIRGAFDI	27	12	6	9	2.88E+06	5.57E+06	8.95E+06
ARINQWEDAYNSYPARYFDS	27	11	6	10	8.05E+05	3.53E+06	9.57E+06
ARQLGYSYSIGDY	26		24	2		1.05E+07	6.46E+05
ARDRGDILTGGQSDY	25	9	10	6	8.79E+05	1.15E+07	7.43E+06
ARGTFSSSNNWFDS	24		11	13		2.31E+07	3.95E+07
ARDKAALRFLEGYGMDV	24	9	10	5	1.05E+06	5.62E+06	5.87E+06
AKDRGSGWYENWFHP	23		10	13		1.49E+07	6.03E+07
AKDNARVVPNAGWVDY	23	7	7	9	7.65E+05	1.23E+07	8.96E+06
ARGGHGDYSNYFDP	23		13	10		1.17E+07	1.01E+07

ARDTDRYSYGYVLEH	22		12	10		1.19E+07	1.54E+07
ARTERGYYYYHDGMDV	21	6	7	8	5.66E+05	2.63E+06	9.83E+06
ARHFLERLGAARVGAFDI	21	3	12	6	8.73E+05	4.18E+06	5.54E+06
ARVRSSLHIDGFDM	21	1	17	3	3.41E+05	5.71E+06	1.88E+06
AKLAGAVSEYTYQDF	20		11	9		9.42E+06	7.75E+06
ARSSRLYYHGLDV	20	3	8	9	1.99E+05	1.22E+06	2.40E+06
TSGVGKTDVDY	20	7	6	7	3.90E+05	2.67E+05	2.79E+06
ARPEGEWWLRPFDY	19	7	5	7	8.61E+05	2.48E+06	7.35E+06
TRALYGDYVGGYDH	18		8	10		2.90E+07	7.11E+07
ARSGGFGESDFYNWFDP	17		8	9		8.25E+06	7.14E+06
ARDFFEKLTNEDHNAFDV	16		8	8		6.77E+06	2.15E+07
AKSQGSGSYQDT	16		5	11		6.53E+06	1.12E+07
ARDFFEKLIGEDLNAFDI	16	2	7	7	1.77E+05	6.44E+06	1.07E+07
VKDGKLPYSSGWYPA	16		14	2		5.74E+06	3.66E+06
AREWAAADLVHAFDI	16		5	11		4.89E+05	2.22E+05
TRAWLLYGDPDYYVDV	15		10	5		7.71E+06	6.52E+06
ARGERFGESPYYSYHMDV	15		8	7		4.58E+06	8.96E+06
ARAEGYTTYHNALDV	15		8	7		4.79E+06	3.81E+06
AKTFGQQAYPWDSFDL	15			15			7.04E+06
VKFIADTGMVH	14		7	7		1.39E+07	1.78E+07
ARDKRDILTGYSTDH	14	6	6	2	1.38E+06	1.03E+07	6.61E+06
ARDCFEKLTGHGDDLNAFDI	14		6	8		5.13E+06	4.78E+06
ARGERFGESPYYAYSVDV	13		2	11		1.53E+06	6.16E+07
ARGLRYSPTYGMDV	13			13			4.27E+07
ARFGGFGDSDFYNWIDP	13		5	8		1.15E+07	2.26E+07
ARRGGYTFDYAY	13	4	3	6	1.73E+06	4.92E+06	2.03E+07
ARGGGLPSRSNNWIDP	13		12	1		8.75E+06	1.25E+06
ARGGEAAEIPNSMDV	12		5	7		2.97E+06	2.26E+07
ARTTEHGDYLF	12	5		7	7.30E+05		1.77E+07
ARDRRDILTGPLLDY	12		6	6		5.71E+06	9.54E+06
ARDYMAGESPYNIYNMDV	12		6	6		2.69E+06	5.89E+06
ARELLRGEGRLYHGMDV	12		9	3		5.20E+06	5.99E+05
ARDRPNILSPPGFDV	12		6	6		3.49E+06	2.24E+06
AKDRLRYFDWSFAS	12	4	6	2	6.80E+05	2.82E+06	1.58E+06
AREFSQGALDV	11		5	6		1.15E+06	6.39E+06
AKGLMIFGGSHENWFDP	11		6	5		1.43E+06	2.51E+06
AKDLPASGISVYSDMLTADYTRH	11		2	9		4.43E+05	2.31E+06
ARDSVAAPRVFDS	10		7	3		5.32E+06	3.00E+07
AKDRGIWGGMDV	10		7	3		4.58E+06	1.04E+06
AREVWDNQDGVDV	9		4	5		5.02E+07	1.75E+08
ARMGGFSYGGWFDP	9		7	2		3.44E+06	6.59E+06
ARDLHGDYSNYFDY	9		9			4.52E+06	
ARDRGNILIGGHSDY	9		8	1		2.39E+06	1.80E+06
ARVPPYGSEVNWFDP	9		9			2.52E+06	
ATDRSIGGDVPVVP	9		9			8.60E+05	

ARDFFERLSGEDHNAFDI	8		4	4		1.16E+07	4.14E+07
ASGPGNLLRLGELSFEH	8		5	3		2.41E+06	1.34E+06
AGAPSRGDSYSLAV	8			8			1.51E+06
ASHDGYNLKGYMDV	8		8			9.19E+05	
ARDGAGDGYLATNAFDI	8		6	2		3.75E+05	5.20E+05
VRGAGFLHDH	7		5	2		4.60E+06	7.07E+06
ARERWVQFLFDY	7		4	3		1.88E+06	7.29E+06
MRDAGY	7		1	6		1.08E+06	5.10E+06
ARASGYHLLGNWFDP	7		6	1		4.40E+06	1.11E+06
ASDLRLGGYDTPSYYFDY	7		7			5.26E+06	
AREIESGEHVGGFGLDV	7			7			4.01E+06
ARDCSRTNCHGFDV	7		7			1.95E+06	
ASPGRDYGDRGGFYFYGMDV	7	3	4		3.84E+05	1.17E+06	
ARQQGPLDY	7	1	6		7.13E+04	1.04E+06	
AHSITKYHYGLDV	6		3	3		3.49E+06	1.41E+07
AREFNDSSGWRAGFDI	6	1	4	1	3.73E+06	1.48E+06	3.43E+06
AKSQGSGSFEED	6			6			3.01E+06
ASPSGLVRHNYYYAMDV	6		5	1		1.34E+06	7.14E+05
ARTRNVGGGSYWLGLDN	5		5			1.57E+07	
ARVLKDYYGMDV	5		2	3		9.21E+05	3.53E+06
ARGRPAYYFNWFDP	5	1	2	2	1.47E+03	8.04E+05	1.75E+06
ARQPQRNWINNWFDP	5			5			2.31E+06
TSSRITIFGVVRMYFDL	5		4	1		9.03E+05	8.82E+05
AKDGTGYIPLTGSFDQ	5		3	2		6.72E+05	7.87E+05
ARDRRDVLTGYDLNN	5		5			8.79E+05	
ARGENYHDSGGWVY	5		5			6.36E+05	
ARESTATTTRHFDY	4		1	3		6.61E+06	1.04E+07
ASLRMGFDP	4		3	1		1.60E+06	1.06E+07
EGYYVWGSYRQYYYGMDV	4		1	3		1.16E+06	8.83E+06
ARGTGPNYQYYYYMDV	4		2	2		1.42E+06	3.08E+06
ARDSGHSSGPRERAGFDY	4	1	2	1	3.68E+05	1.54E+06	2.57E+06
ARDTDRYRNGYVLEH	4			4			3.80E+06
AKMGSSPMAGQLDY	4		3	1		1.77E+06	2.00E+06
ARERGNWGLDL	4			4			3.36E+06
ARSEHVHYSSGWPFDS	4		4			2.53E+06	
ARDTSAWSVDF	4			4			2.13E+06
VKRVGDY	4			4			1.98E+06
ARDFFERLTGDDHNAFDV	4		4			1.38E+06	
ARDGGYNFVGRFDP	4		4			3.60E+05	
ARDFFERLTDDDLNAFDI	4		4			2.76E+05	
ARDPGIYEEEWFFDV	3		3			4.10E+06	
TSGVGKTDVDY	3	1	2		1.27E+05	1.55E+04	
ATSTLMVRGVRHAFDP	2		2			1.39E+06	
ARDRGNLLTGFSPDF	2		2			6.81E+05	
ASSYRSGGYYFHY	2	1		1	2.61E+05		

ARAEGGSYLRTRAFDV	1		1			1.06E+06	
TSTTVTKGPPGAFDI	1			1			2.01E+06
GRIKPRKLGMDV	1		1			1.01E+06	
ARNPHYFAMDV	1			1			2.68E+06

Anti-H3 A/VI09 serological repertoire clonotypic antibodies

Representative CDR-H3 Sequence	Total PSM	Day 0 PSM	Day 28 PSM	Day 180 PSM	Day 0 XIC	Day 28 XIC	Day 180 XIC
ARHFQERLGAAPVGAFDV	400	45	208	147	3.48E+07	3.60E+08	5.10E+08
ARDFFETLTGDDLNAFDV	365	70	142	153	9.44E+07	2.32E+08	4.30E+08
ARVPPYSSGWLRPMDV	167	65	53	49	1.15E+08	1.56E+08	3.21E+08
ARACSSSCHYYYAMDV	150	43	34	73	6.01E+07	7.82E+07	1.31E+08
ARLVATARDYFGLDV	130	28	33	69	3.55E+07	3.31E+07	6.80E+07
KCQLTTFAERWFDP	119	25	47	47	2.54E+07	4.03E+07	8.81E+07
ARASIRSRPQFFDF	88	32	30	26	5.40E+07	8.55E+07	1.59E+08
ARKGLGDF	83	35	23	25	1.78E+07	1.48E+07	4.45E+07
ARDLAIPIDSLTYYLRAFDS	76	24	30	22	2.15E+07	3.24E+07	2.39E+07
ARDFFEKLIADDLNAFDI	73	17	31	25	8.44E+06	2.83E+07	3.65E+07
ATGDYYNDPTTPAHRNWFDP	72		32	40		5.65E+07	2.05E+08
ARDPSRGDYFPYYSCYMDV	69	23	15	31	8.77E+07	1.09E+08	1.60E+08
ARPSYTSGFALDP	68	17	22	29	1.31E+08	2.35E+08	3.54E+08
ARDVAMGGFDA	64	24	14	26	5.13E+07	6.27E+07	1.36E+08
ARAVALSGYYGSGTPYNWFDP	52	13	20	19	8.67E+07	1.71E+08	2.24E+08
TRLRGYNILTGDLTAAEFDP	51	23	23	5	4.43E+06	6.45E+06	5.96E+05
ARLNMVRGEFWGYYVDD	49	23	15	11	1.71E+07	2.48E+07	6.69E+07
AGGRGWSYYMDV	45	20	20	5	1.01E+07	2.42E+07	1.69E+06
ARGGGRKPFDP	43	12	12	19	1.13E+07	2.13E+07	1.51E+08
ARINQWEDGYYSYPARYFDN	41	13	10	18	6.01E+06	6.58E+06	3.50E+07
ARASNLYPRVGATDF	39		26	13		1.96E+07	1.56E+07
ARAACSLCPLDY	39	14	10	15	1.98E+07	2.03E+07	2.43E+07
ARDFFEKLGGEDHNAFDI	39	7	13	19	6.81E+06	1.35E+07	3.16E+07
TLLRWGAVAGRDWFDP	34	10	8	16	1.55E+06	2.46E+06	8.48E+06
TRQRQNQPGATDY	33	10	8	15	6.04E+06	6.18E+06	2.63E+07
ARVCSSSCHYFYAMDV	33	12	12	9	7.80E+06	1.06E+07	4.68E+06
ARDLGGGELLDS	31	7	14	10	2.48E+06	7.38E+06	6.44E+06
AKEDRSGYPTRRPIEY	30	4	15	11	1.19E+06	3.99E+07	4.69E+07
ARDYLGSGREFDP	30	1	19	10	4.78E+05	1.52E+07	2.69E+07
ARGRFDIVVVPGAMPGPYMDV	29	2	13	14	1.04E+06	2.14E+07	5.44E+07
TREFQSTFDP	28	7	7	14	1.89E+07	4.18E+07	8.19E+07
STRGIVRRTNYDY	28	10	9	9	6.66E+06	7.60E+06	3.09E+07
ARNRPGGDYYQYGMDV	28	7	11	10	5.60E+06	1.82E+07	1.73E+07
ARDEKRVRGVGRMALDP	25	8	9	8	3.21E+06	4.16E+06	1.10E+07
ARDFFEKLTNEDHNAFDV	24	8	9	7	3.61E+06	1.72E+07	2.54E+07
VKDAVWFGDSLTHNYFDP	24	7	13	4	2.62E+06	1.96E+07	1.22E+07
ARDFFEKLIGEDLNAFDI	23	8	7	8	3.12E+06	1.63E+07	1.30E+07

ARVSLRYCSGGSCANWFDP	23	8	8	7	8.40E+06	9.20E+06	1.01E+07
VRLPGAAMDYYYGLDV	23	8	15		3.95E+06	1.85E+06	
AKGLVRFGESHENWFGP	22	2	3	17	6.40E+05	1.65E+06	2.18E+07
ARVRSSLHIDGFDM	22	6	6	10	3.59E+06	4.08E+06	9.18E+06
ARERNYFDPVPYQYSFDF	22	7	11	4	9.16E+06	2.13E+07	1.18E+07
ARGRGVMVWFGELAY	21		7	14		1.40E+07	3.41E+07
ARDLPQAPVDGEGVTTV	21		21			8.49E+05	
ARRGGYTFEYAY	20	10	3	7	1.06E+07	1.01E+07	2.08E+07
VRSGGHTILTDYCECPDY	20		7	13		1.57E+07	1.15E+08
AREPGDYLSVYRGYEGYSGFDV	20	6	12	2	5.23E+05	3.56E+06	1.33E+06
ATNLEYYDRSGYYIRDAFGI	19	8	6	5	2.67E+06	4.12E+06	8.71E+06
ARDGAGDGYLATNAFDI	19	7	9	3	5.43E+05	7.42E+05	7.94E+05
ARDSGHSSGPRERAGFDY	19	7	6	6	2.19E+06	2.29E+06	4.16E+06
ARGPEYVRGYYGMDV	19	7	8	4	3.82E+06	3.48E+06	4.09E+06
AIEVKLSLRDMDTRSWFDP	18	9	7	2	7.00E+06	1.04E+07	4.51E+06
VNSRDYYDNSGYRD	18	3	6	9	2.10E+06	2.13E+07	4.47E+07
ARDGKFSGFDWNFDY	18	6	6	6	3.56E+06	7.41E+06	2.81E+07
TSDAEGDVVVPASMRDDLDYYYYMDV	18	7	5	6	1.63E+06	2.88E+06	1.68E+07
AKDWSYYYFKGIFDS	18	7	6	5	3.01E+06	3.11E+06	1.30E+07
ARDNTAWSVDF	18	9	5	4	2.45E+06	3.71E+06	2.09E+06
ITSLGTYYRARVDY	17	2	5	10	8.52E+06	1.16E+07	3.54E+07
ARERENVQFLFDY	17	5	4	8	1.51E+06	2.29E+06	5.73E+06
LGVGAIGISDI	16	8	6	2	4.53E+06	5.37E+06	1.45E+07
ARDIPNYDCLSGRNMDV	16	2		14	8.58E+05		1.70E+07
ARDRSSGYYPHFDY	16	6	5	5	2.19E+06	2.93E+06	5.12E+06
ARSSRLYYHGLDV	15		9	6		2.48E+06	2.31E+06
ARGGDTAEIPNGMDV	15	5	6	4	3.95E+06	8.52E+06	1.30E+07
ARLSGTNGWYPDY	15	7	4	4	1.61E+06	1.07E+06	4.95E+04
ARVLGRPYSYGMDV	14	2	10	2	1.65E+06	1.11E+07	5.14E+06
ARAIEYYYGMDV	14	4	3	7	5.32E+06	6.52E+06	1.25E+07
ARDVPHVPGTAGNS	14		6	8		3.33E+06	3.65E+06
ARTERGYYYYHDGMDV	13	5	5	3	1.57E+06	1.81E+06	6.55E+06
ARAEGYIDYHYGMDV	13	2	7	4	9.43E+06	9.65E+06	9.80E+06
ARDCSRTNCHGFDV	12	6	6		1.08E+06	1.73E+06	
ATSTLMVRGVRHAFDP	12		5	7		1.47E+06	6.37E+06
ARADYDILTAHIDPPYYYYMDV	12		6	6		1.98E+07	2.54E+07
ARAIGGYDSH	11		11			2.47E+06	
ARVQGRGYTYGLDY	11	8	3		1.31E+07	1.28E+07	
ASPIPKTPHFEASDT	11		7	4		3.41E+06	2.24E+06
ARDSVAAPRVFDS	10	2	1	7	5.83E+03	4.84E+06	3.86E+07
ARVYPPSAIRGYFDL	10	7		3	1.76E+06		1.12E+07
STFDGVSGWH	10	1	4	5	1.26E+06	2.32E+06	7.80E+06
ARPMVRGVFYMDV	10	4	4	2	1.70E+06	1.85E+06	2.83E+06
ARPEGEWWLRPFDY	9	6	2	1	2.09E+06	1.53E+06	5.63E+06
VRGAGFLHDH	9	3	3	3	3.45E+06	5.31E+06	1.70E+07

AKEYSSGFPTRRPIDF	9		7	2		5.66E+06	6.79E+06
AKDRLRYFDWSFAS	9	5	4		1.03E+06	1.67E+06	
ARDFFERLSGEDHNAFDI	8	3	4	1	1.99E+06	1.36E+07	1.51E+07
TSSRITIFGVVRMYFDL	8	3	5		4.51E+05	9.36E+05	
ARAMDV	8			8			3.13E+06
ARAIGAAGAF	8	3	1	4	1.19E+06	1.66E+07	1.89E+07
ARDRHSTSRDD	8	3	1	4	4.97E+04	3.81E+04	3.52E+06
AKPDRSGWFFDF	8		8			1.94E+06	
ARLGRGYSYGPLGY	8	6	2		5.96E+05	9.04E+05	
ARESTATTTRHFDY	7	1		6	2.52E+06		1.46E+07
TKGQLLYDMLTGSFDF	7	1	6		7.33E+05	9.57E+05	
AREPGSYLSVYRGYPGTSGFDV	7	2	5		2.01E+06	2.80E+06	
ARVALRYCISDDCANWFDP	7	6	1		9.59E+05	1.20E+06	
ARQIYHCDY	7	5	2		1.04E+06	1.10E+06	
ARDCFEKLTGHGDDLNAFDI	6	1	5		4.99E+05	5.80E+06	
ARDDTSGELPRGLDY	6	1	3	2	7.03E+03	4.63E+06	3.50E+07
ARGLRYSPTYGMDV	6			6			1.52E+07
ARDPGIYEEEWFFDV	6	3	2	1	2.95E+06	3.50E+06	9.33E+06
AILQKNVIRYFDWGMDV	6		1	5		7.10E+03	6.64E+06
TRARPGSYVDY	6	1	2	3	4.26E+06	5.32E+06	6.97E+06
ARPIASSGYYGSGTPYNWLDP	6	2	3	1	1.26E+06	1.73E+06	3.24E+06
VREYYDHYHFYMDV	6	1	2	3	9.36E+05	2.07E+04	2.80E+06
ARGLLHYDSSGSGNGFDI	6		5	1		1.40E+06	1.21E+04
ARDRGNLLTGFSPDF	5	3	1	1	6.14E+05	1.38E+06	1.92E+06
ASSIAVAGTGHG	5		2	3		1.96E+06	5.75E+06
ARDRRDSLTGYDQNQ	5		4	1		3.73E+06	1.08E+06
ARLTTVPTDQYYGMDV	5		5			3.47E+06	
AKGGPVYLDIFDY	5	2	3		2.97E+05	8.75E+05	
ARGRGSYPIRGAFDI	4		4			4.98E+06	
TSGVGKTDVDY	4		3	1		4.74E+04	6.98E+04
ASSYRSGGYYFHY	4	2	2		1.80E+06	6.82E+04	
ARNPHYFAMDV	4	3	1		1.49E+06	3.26E+06	
TSTTVTKGPPGAFDI	4	1		3	2.76E+06		1.63E+06
GRIKPRKLGMDV	4	1	3		1.23E+06	1.68E+06	
ARAEGGSYLRTRAFDV	4	1	3		1.06E+06	1.59E+06	
AKDDSESYLVAGYFDT	4			4			1.67E+07
VRPLSASGYYGSGTPYNWLDP	4	3	1		1.08E+06	2.56E+06	
ARDLEVAATADY	4	2	2		2.92E+05	1.06E+04	
ARAGDFGDYAGARDAFHV	4	3	1		6.60E+03	1.63E+04	
ASPGRDYGDRGGFYFYGMDV	4	2	2		9.15E+05	6.34E+05	
VKRVGDY	2			2			2.08E+06
ARQPQRNWINNWFDP	2		1	1			2.78E+06
ARDFFERLTGDDHNAFDV	1		1			1.10E+06	

Anti-Vic B/BR08 serological repertoire clonotypic antibodies

Representative CDR-H3 Sequence	Total PSM	Day 0 PSM	Day 28 PSM	Day 180 PSM	Day 0 XIC	Day 28 XIC	Day 180 XIC
KCLMTTIPEHWCDP	2148	60	1209	879	1.46E+08	1.63E+10	1.04E+10
AKGLVIFGESHENGFGP	842		514	328		1.08E+08	4.97E+07
TTDGSVGGDVPVVP	556	16	242	298	1.78E+07	1.04E+10	1.44E+10
ARIHGYDLDYYFGMDV	440		365	75		2.55E+08	1.43E+08
ARINQWEDGYYSDPAKYFDY	426	4	263	159	1.09E+06	2.26E+09	1.22E+09
ATNLDYYDSSGYYVRDAFDV	339	11	120	208	8.80E+06	1.63E+09	3.13E+09
ARDPSRGDYFPYYSYYMDV	257		137	120		1.30E+09	8.87E+08
VRASMGSYPDS	156	39	51	66	3.22E+08	4.16E+08	6.30E+08
AKDNYGGNLSGWFDP	115	54	25	36	3.09E+08	8.56E+08	5.01E+08
ARDLKYYGSTKMGWFDP	114	29	40	45	1.37E+07	5.05E+08	4.67E+08
ARDHGATFYYYYGMDV	113		113			1.76E+07	
ARDLKYYGSLRMGWFDP	106	24	43	39	6.19E+07	9.42E+08	9.36E+08
ARVGDDSGAYDKGYYYYPMDV	104	29	37	38	4.59E+07	2.24E+08	2.33E+08
ARDNFGGNLSGWFDP	104	20	41	43	4.42E+07	2.20E+08	1.68E+08
ARGDHVGDY	101	15	47	39	2.20E+06	1.08E+08	8.25E+07
TTDGVHAYDFRSGYASAYRYFGLAV	97		59	38		9.02E+08	5.59E+08
AKDLAKWLVTPYFDS	86	40	22	24	1.21E+08	2.27E+08	3.14E+08
ARGLVTTGPTSQF	82	29	25	28	1.33E+08	2.42E+08	2.80E+08
ARDERLYYYGLGSTQPRT	77	27	22	28	5.39E+07	1.17E+08	1.71E+08
ARASSTSWDFDH	69	19	24	26	1.47E+08	4.10E+08	4.19E+08
ARAAAEGLILTNWLDP	61	1	36	24	2.40E+03	1.58E+07	7.24E+06
ARLKRVRGELWGYHFDY	60	26	19	15	5.43E+07	1.05E+08	1.73E+08
ARDVAMGGFDA	57	50	1	6	1.29E+08	1.14E+05	7.14E+07
ARMRFGECLDY	56	25	14	17	2.00E+07	2.25E+07	1.61E+07
AQDRGGGNFWGWFDP	49	7	22	20	3.70E+06	2.98E+08	2.02E+08
ARDMGYYGSVRMGWFGP	47	13	17	17	1.28E+07	3.11E+08	3.80E+08
ARGVVTTGPTSYD	47	18	9	20	4.75E+07	3.59E+07	6.73E+07
ARSPGGMNWFDP	46	20	12	14	6.86E+07	1.22E+08	1.79E+08
TRQMQNQPGATDY	45	7	20	18	6.50E+06	4.32E+07	5.60E+07
ARHGVWTLRYFDW	45	26	7	12	2.92E+07	1.55E+07	1.94E+07
ARAEGGSYLLTRAFEI	43	28	8	7	2.75E+07	2.31E+07	2.43E+07
KFLVTTMMEKWIDP	42	2	29	11	1.79E+06	7.18E+07	2.42E+06
ARVSPGGIGTGYGDY	41	7	17	17	1.72E+07	1.33E+08	1.01E+08
STFYGGRGGH	37	15	9	13	9.87E+06	1.68E+07	2.94E+07
ARRGNYYRALDY	36	22	8	6	1.59E+07	4.22E+07	1.64E+07
ARHSGYHFRVNWFDP	34	12	7	15	1.39E+07	3.16E+07	5.23E+07
ARRGGYTFDYAY	29	6	13	10	1.69E+07	7.76E+07	3.89E+07
ARGHVTGMAPHNGMDV	28	10	6	12	1.33E+07	3.30E+07	2.50E+07
ARGLISTGPTSEY	28	8	6	14	9.86E+06	1.61E+07	2.56E+07
ARENWADYGDHCLGY	25	6	14	5	9.24E+06	2.46E+07	1.80E+07
ARDLMQWQLWSGLDD	25		16	9		2.73E+07	1.20E+07
ARREWVQFLFDR	24		16	8		7.28E+07	1.51E+07
VKSLDYYDSSGYRD	23		16	7		8.93E+07	9.03E+07

ARDSVTAPRVFDA	23	12	5	6	6.18E+06	6.59E+06	3.37E+06
ARPFDSAQRGGFHV	22	5	6	11	4.00E+06	1.05E+07	1.82E+07
VRVELDGDSPLVSRGYYYYPMDV	21	3	9	9	3.38E+06	2.56E+07	3.03E+07
ARDGSGGSTGWGYDH	20	7	5	8	6.54E+06	4.18E+07	7.28E+07
ARDLVGRTMVRGVPNDY	20	2	3	15	1.71E+06	9.84E+06	1.16E+07
VKDHHRPYRYAKNGLDV	20	10	4	6	3.26E+06	1.88E+06	1.11E+06
AKDLSEDNLRGFDFWSGYVF	19	7	6	6	1.60E+07	4.33E+07	6.29E+07
ARQDGLVAAPIDY	19	2	2	15	1.23E+06	5.44E+06	4.94E+07
ARAACTLGPLEY	19	8	2	9	7.91E+06	2.15E+07	2.61E+07
ARDERMYYYGLGSTQPRN	19	15	4		3.94E+06	1.16E+07	
GSPGRDYGARGGYYYFGMDV	19	6	4	9	1.06E+06	2.57E+06	9.26E+06
ARFRMTSVGVLFGVLDAFDI	18		11	7		6.41E+05	2.79E+06
ARVGPGAIGLGYMDV	17	2	8	7	6.89E+06	1.10E+08	1.33E+08
ARDPTYDILAGYSVGAFPYFDL	17		17			1.43E+07	
ARDLRIAVDSLTYYLRAFDS	17	6	6	5	1.37E+06	5.87E+06	5.28E+06
AREWAAADLFHAFDI	16		8	8		6.07E+06	6.29E+06
AKEPLTYRVLDV	16	5	5	6	1.69E+06	4.43E+06	5.96E+06
VRDRPYGDYAKTVDA	15	12	3		2.88E+06	3.75E+06	
ARVRTSNYYYGMDV	14	2	8	4	4.72E+07	7.87E+07	9.65E+07
ARLRGYGYYYFDS	14		8	6		5.03E+07	1.47E+07
ARVKNYDIFTGINPVDY	14		5	9		3.93E+07	2.03E+07
GRVPTGYYRNDY	14	8	3	3	4.41E+06	6.39E+06	9.33E+06
ARDSSSGYYSAFDH	14	9		5	7.94E+06		7.53E+06
ARDPFPTSISFGYFDP	14	6	2	6	3.96E+05	4.29E+06	8.21E+06
ARSQKYYGSGTHNWFDP	14	7	3	4	5.87E+04	4.58E+06	4.59E+05
ARGFGLGEIFYYMDV	13		6	7		5.48E+06	7.10E+06
ARRSYDY	13	6	1	6	7.24E+05	3.28E+06	4.25E+06
ARAMDV	13	11		2	8.13E+05		5.18E+06
TRKIGEY	12	4	4	4	3.99E+06	2.20E+07	1.43E+07
ARSVVAAIYDF	12	4		8	7.13E+05		1.19E+07
ARHGAWELRSFDY	12	4	6	2	4.56E+06	1.65E+06	8.60E+03
AKFLRGDELDH	11	6	4	1	6.53E+07	2.19E+08	2.78E+08
TTDGSIGGDEIVVP	11		5	6		7.36E+07	2.76E+07
ARQSVLLQLRYFDWPFDS	11		6	5		1.21E+07	2.45E+07
ARDSGYSYGFMVGDY	11	6	3	2	5.76E+06	8.64E+05	1.36E+07
ARVGMDVESVMVGRGYYYYAMDV	11		5	6		3.10E+06	9.64E+06
ARLYGDALDV	11	11			5.64E+06		
ARGVASGWFDYFDY	11	4	2	5	3.79E+05		4.34E+04
TLLRWGAVAGRDWFDP	10		10			5.22E+07	
VRGAGFLHDH	10	5	1	4	1.65E+06	1.27E+07	2.93E+07
ARLGQWLVPAYFDL	10		5	5		1.61E+07	1.01E+07
ARDHLLDERSCDL	10	2	2	6	1.10E+06	6.02E+06	8.64E+06
ARSNFRQLNAFDI	10	10			4.76E+06		
ARHFITDYYWGGFDY	10		8	2		6.37E+05	1.89E+04
ARDYCGETYPVPFEK	9		7	2		1.03E+07	1.45E+07

ARAMYSSSLGGLTTSHPDY	9	3	1	5	1.09E+06	6.77E+06	1.16E+07
ARDAVGAIKALDI	9	5	1	3	1.32E+07	3.99E+04	5.72E+06
ARTRPYDILTGFSMDV	9		5	4		8.05E+06	6.18E+06
ARDFGLPTITPFDY	9	1	3	5	3.24E+03	5.60E+06	8.07E+06
TRATLPSADRQTDD	9	7		2	1.77E+06		3.65E+06
ARGSLVGESSSYYMDV	9	1	4	4	2.32E+03	6.31E+05	7.37E+04
ARDNGIAVPRSPQGLFDY	9		4	5		1.43E+05	7.42E+03
ARRTFDS	8	2	2	4	1.03E+06	1.89E+07	7.99E+06
ARDGSRYDYYPYYSYYMDV	8		4	4		1.29E+07	1.36E+07
ARDELLWFDYYAMDV	8		7	1		2.21E+07	1.86E+06
AREMGSRWYSEGMDV	8	1	7		8.49E+03	2.17E+07	
ARDGSGGSSGWGYDY	8	8			5.06E+06		
ARGGEYNPLTGSPGRNGLDI	8	7		1	2.12E+06		1.32E+06
ARDHYGGNRDYYYYYMDV	8		7	1		1.78E+06	8.46E+03
KFLVTTIMEDWFDP	8		2	6			3.27E+04
ARSYSIGNSFDI	7	4		3	5.36E+06		4.51E+07
ATSRGAGSYEED	7		2	5		1.24E+07	2.99E+07
IRLKGDAFEI	7	6		1	1.46E+07		1.48E+07
ARMGGFSYGGWFDP	7		7			2.49E+07	
ARDDTSGELPRGLDF	7	4	1	2	5.02E+06	1.82E+07	6.08E+04
ARAITGNVHFHH	7	5	2		2.53E+06	1.62E+07	
VRLHGAAMDYYYGLDV	7		7			1.61E+07	
ARTERGYYYYHDGMDV	7	6	1		5.89E+06	7.92E+06	
ARGRGVMVWFGELAY	7		7			1.35E+07	
ARVGMEEESVMLRRGYYYYAMDV	7	4	2	1	5.34E+05	3.41E+06	3.97E+06
VKDTCRSGTYCGGLWFDP	7		7			6.01E+06	
ARDFGIAVAASDY	7	6		1	2.54E+06		3.33E+06
ARHFQERLGAARAGAFDL	7	5		2	2.87E+06		3.37E+04
AREFPPLSGDYYHGMDV	7		7			3.96E+04	
ARRGYDL	6	3	2	1	1.05E+07	2.89E+07	4.35E+07
AREKNYLTDY	6		2	4		9.57E+06	1.23E+07
ARGAYDYYYFRMDA	6		2	4		9.17E+06	8.37E+06
ARVGIDMEKTMLSRGYYYYPLDV	6		2	4		8.77E+06	7.07E+06
ATYRAMIYFGFDY	6		2	4		1.50E+06	3.37E+06
ARDRSDILTGGHSDY	6	2	4		1.10E+06	3.15E+06	
ARTYSSSSPFDY	6		4	2		6.91E+05	4.49E+04
ARDRLGLLTGYNPGDY	6	3	3		1.08E+04	1.38E+05	
AKPMIVLVSSQLFQH	6	1	4	1		7.11E+04	
ASARESNTHHFIEY	5	2	1	2	1.47E+06	6.90E+06	3.85E+07
ARDHGASPYYYHGMDV	5		5			3.05E+07	
ARVVGATMDYHHGMDV	5		3	2		2.34E+07	5.76E+04
ARGSGWYDVYYMDV	5	1	1	3	1.16E+07	1.46E+06	6.07E+06
ARSVWNGYHFFDY	5		5			1.38E+07	
ARVKEWLATNCFDL	5		5			8.98E+06	
ARPWSRALDV	5	2	1	2	8.86E+05	2.36E+04	3.36E+06

VKEDMSKGYEY	5	3		2	1.91E+06		1.62E+06
ARGGGTAGIPNGMDV	5		4	1		2.78E+06	5.44E+05
AREYRILDERANYFDP	5	5			2.95E+06		
ARAADDYSDYDRTRTCFWFDP	5	4		1	2.22E+06		
ARDKVGARAFDI	5	5			1.94E+06		
ARNSGFGEVANN	5	5			1.15E+06		
ARVHMVPGEGWYFGMDL	5		5			2.57E+05	
ARRIDAWLGGDYFDY	4		4			2.50E+07	
ARDPPAIRSGTYG	4	3	1		3.22E+06	4.48E+06	
ARTARTRVMYYFDL	4		1	3		7.46E+06	5.02E+04
ARGRSGSGWFKM	4	4			4.32E+06		
ARVYPTLEAPAASPRNPNHQYYYYNWDV	4		4			2.31E+06	
ARQIYHGDY	4		1	3		5.97E+04	2.25E+06
SRESEAVPNDY	4	4			1.50E+06		
VSATVG	4		4			5.54E+04	
ARQTKIAVVLKPYNWFDP	4			4			2.57E+04

Anti-Yama B/TX11 serological repertoire clonotypic antibodies

Representative CDR-H3 Sequence	Total PSM	Day 0 PSM	Day 28 PSM	Day 180 PSM	Day 0 XIC	Day 28 XIC	Day 180 XIC
KSLLTTFPEKGFDP	1635	161	714	760	4.26E+08	1.95E+10	2.74E+09
ARIKTRGYEGSGSVVDAFDL	739	238	44	457	1.38E+08	4.14E+07	9.61E+07
ATNLDYYDSSGYYVRDAFDV	465	3	253	209	1.95E+07	1.50E+09	1.40E+09
ARDPSRGDYFPYYSYYMDV	163	32	99	32	9.41E+07	1.34E+09	4.40E+07
VRASMGSYPDS	154	66	68	20	6.36E+08	5.91E+08	8.74E+07
ARDLKYYGSLRMGWFDP	135	25	58	52	6.49E+07	1.28E+09	2.94E+08
AKDNYGGNLSGWFDP	119	38	32	49	3.63E+08	7.24E+07	3.21E+08
ARDNFGGNLSGWFDP	110	37	38	35	5.56E+07	6.65E+07	9.52E+07
ARHLRSFASYFDD	89	37	24	28	9.75E+08	7.27E+08	1.21E+08
TTDGVHAYDFRGGYSSAYRYFGLGV	79		63	16		2.06E+09	2.32E+07
ARVGDDSSAYDKGYYYYPMDV	71	29	30	12	1.17E+08	1.58E+08	3.10E+07
AQDRGGGNFWGWFDP	59	22	18	19	9.89E+07	2.19E+08	3.10E+07
AKDLAKWLVTPYFDS	59	24	20	15	2.62E+08	2.03E+08	3.07E+07
ARASSTSWDFDH	59	27	17	15	4.74E+08	4.48E+08	4.42E+07
ARLYRVRGELWGFNFDY	53	23	9	21	1.60E+08	7.43E+07	3.89E+07
LGVGAIGISDI	52	1	2	49	6.05E+06	1.94E+07	3.27E+06
AKGLVIFGESHEDWFDP	50	1	38	11	2.43E+07	4.12E+06	4.64E+07
ARDLAIPVDSLTYYLRAFDS	46	12	19	15	1.04E+07	7.93E+07	5.99E+06
TRAWFQFFDS	45	13	18	14	2.64E+07	7.04E+07	1.93E+07
ARSPGGMNWFDP	42	17	13	12	1.39E+08	1.79E+08	2.05E+07
ARQDGLVAAPIDY	40	10	11	19	5.45E+07	4.89E+07	1.71E+07
ARAAAEGLVLTNWLDP	39		13	26		6.81E+05	4.75E+06
ARDLDYDILTGYKPNNWFDP	34	12	10	12	4.25E+07	2.02E+07	5.98E+06
TTDGSVGGDVPVVP	34		18	16		2.63E+08	4.84E+07
ARPFDSAQRGGFYV	34	18	11	5	3.24E+07	2.12E+07	7.37E+05
TRQMQNQPGATDY	34	12	7	15	2.35E+07	7.20E+06	1.09E+07

VRVELDGDSPLVSRGYYYYPMDV	28	10	10	8	4.46E+07	6.02E+07	9.34E+06
VRGAGFLHDH	28	9	19		2.33E+06	1.01E+07	
ARVSPGGIGTGFGDY	27	4	12	11	1.16E+07	1.16E+08	1.58E+07
ARGHVTGMAPHNGMDV	26	13	4	9	1.52E+07	6.36E+06	7.72E+06
ARDGSGGSTGWGYDH	25	10	9	6	1.54E+07	3.10E+07	1.06E+07
ARAACTLGPLEY	25	9	7	9	5.18E+07	5.87E+07	1.50E+07
AREWAAADLFHAFDI	23	2	11	10	9.12E+04	1.14E+07	5.98E+06
ARRGDSGWSLQY	23	22	1		3.23E+07	6.81E+06	
ARDERLYYYELGSTQPRT	21	12	6	3	3.87E+07	2.30E+07	7.75E+06
AREYRILDERSNYVDA	21	4	9	8	2.88E+06	4.31E+07	7.67E+06
ARDSVAAPRVFDS	20	4	9	7	3.44E+06	7.04E+07	8.10E+06
ASPGRDYGDRGGFYFYGMDV	19	2	8	9	4.01E+06	1.28E+07	5.54E+06
ARRGGYTFDYAY	19	6	4	9	3.49E+07	6.81E+07	1.42E+07
ARDMGYYGSVRMGWFGP	18		10	8		2.72E+08	2.24E+07
ARDLVGRTMVRGVPNDY	18	8	1	9	1.05E+07	6.06E+06	3.93E+06
ARVKNYDIFTGINPVDY	17		8	9		4.65E+07	5.63E+06
AKAEGGSYLLTRAFDV	16	10		6	2.28E+07		7.06E+06
GRPSVGAKEDY	16			16			1.88E+07
TLLRWGAVAGRDWFDP	15	5	3	7	1.90E+07	5.20E+06	4.94E+06
AKERHVEGIAALSVDC	15	7	4	4	8.88E+06	7.53E+06	1.33E+06
VKRGGDY	15		15			2.96E+07	
ARGPRGPWELPQPPWSYFPMDV	14	3	1	10	1.07E+07	4.18E+07	1.33E+06
ARGRFDIVVVPGAMPGPYMDV	14		8	6		9.66E+06	2.46E+06
ARFRMTSVGVLFGVLDAFDI	14		14			1.27E+06	
ARDNGIAVPRSPQGLFDY	14	1	12	1		4.40E+05	1.67E+03
ARSQKYYGSGTHNWFDP	14	6	4	4	4.16E+04	9.12E+05	1.93E+05
ARHSGYHFRVNWFDP	13	4	3	6	3.22E+07	4.48E+07	9.99E+06
ARVLGRPYSYGMDV	13			13			8.70E+06
ARHGIWVLRYLDW	13	12		1	2.25E+07		2.35E+06
ARLTGYSGYHERIGDYYYYYMDV	12	5	1	6	1.71E+07	1.20E+07	2.03E+07
AREKNYLTDY	12	5	7		7.80E+06	2.33E+07	
ARSGIVGPMELPNDAFDI	11	4	4	3	3.00E+06		8.26E+05
ARQSVLLQLRYFDWPFDS	11			11			1.02E+07
ARDHYGGNRDYYYYYMDV	11		8	3		2.72E+06	1.08E+03
ARDERMYYYGLGSTQPRN	11	3	2	6	1.74E+06	1.97E+05	2.80E+06
ARGFGLGEIFYYMDV	11	7	2	2	5.74E+06	1.70E+07	1.66E+05
ARSYSIGNSFDI	11	4	1	6	3.84E+07	2.34E+07	3.33E+07
AREGGGLGYVDY	11	11			3.48E+07		
ARRPFSSSWTRPARYFDY	10			10			5.47E+06
ARVGPGAIGLGYMDV	10		6	4		1.19E+08	1.12E+07
TTDGSIGGDEIVVP	10		4	6		6.50E+06	3.16E+07
ARGVASGWFDYFDY	10	2	1	7	7.19E+04	2.55E+05	1.22E+06
ARDFGLPTITPFDY	10	4		6	1.62E+06		2.04E+06
ARGLVTTGPTSYD	10	7	3		2.35E+07	1.21E+07	
ARGLVTTGPTSQY	10	2	2	6	9.32E+07	1.26E+08	2.59E+07

ARGDRHPYYCDY	10	6		4	5.77E+06		6.86E+06
ASRRYGGLDP	10	8	2		2.81E+07	1.21E+05	
ARSQGYYYESSGLTRNPLDY	9	8	1		2.81E+07	1.59E+05	
ARTERGYYYYHDGMDV	9			9			6.16E+06
VNSLDYYDKSGYRD	9		9			4.23E+05	
AKDRLRYFDWALNY	9	1	3	5	3.93E+06	8.44E+05	3.82E+06
ARPQTVPYNAMDV	9	3		6	1.71E+07		6.81E+06
ARTRPYDILTGFSMDV	8		8			1.69E+07	
AKPMIVLVSSQLFQH	8	5		3	3.90E+04		5.41E+04
ATLFGGNSRYYLDV	8	1	7		1.26E+06	1.35E+07	
AREGDKLGNFDY	8	8			1.94E+07		
VKDHHRPYRYAKYVLDV	7	2	5		4.07E+06	7.95E+06	
ARENWADYGDHCLGY	7	3	4		9.69E+06	2.08E+07	
ARDDTSGELPRGLDY	7	1	2	4	5.48E+04	9.07E+07	1.07E+07
ARVRTSNYYYGMDV	7	3	4		2.49E+07	2.31E+07	
AKFLRGDELDH	7	3	4		6.15E+06	1.78E+08	
ARRTFDS	7		7			9.20E+06	
ARWGATFGGVIVSGSYFDS	6	1	3	2	5.09E+05	1.52E+05	6.00E+03
ATYRAMIYFGFDY	6			6			1.57E+06
ARGLISTGPTSEY	6	6			6.55E+06		
VKSRMGLAIDY	6		1	5		2.92E+06	4.31E+06
ARDLHSTSLDY	6	5		1	4.43E+06		1.38E+03
ARVGIDMEKTMLSRGYYYYPLDV	5	5			7.55E+06		
ARINQWEDAYNSYPARYFDS	5		5			2.66E+07	
ARAEYYYDNDYYYYIDV	5	4	1		2.13E+07	1.59E+07	
ARPLTYSGYDGPFHY	5	5			2.88E+08		
ARGGDTSELPNGMDG	5	5			2.19E+07		
ARTYSSSSPFDY	5	3	1	1	5.45E+05		8.24E+05
AKEPLTYRVLDV	5	5			7.07E+06		
ARSYGSGEIDY	5			5			2.38E+06
ARQVGGGDSH	5	4	1		3.54E+07	4.24E+04	
ARGTYYDSSGSSVQYSVRYFQH	4	3	1		1.01E+07	9.23E+05	
ARDQTTSDWGAAKGRFDY	4	4			9.63E+06		
AKGPASMGVGGVFEY	4	3		1	2.36E+07		
KFLVTTIMEDWFDP	4		4			3.10E+04	
AKEAIAVAGDWFDP	4	4			1.34E+07		
AKDRLRYFDWSLDY	4		4			2.44E+05	
GRVPTGYYRNDY	4	2		2	7.11E+06		1.58E+06
ARRGNYYRALDY	4	2	2		4.86E+06	2.07E+07	
ARDVAMGGFDS	4	4			3.36E+07		

Donor 2

Anti-H1 A/CA09 serological repertoire clonotypic antibodies

Representative CDR-H3 Sequence	Total PSM	Day 0 PSM	Day 28 PSM	Day 180 PSM	Day 0 XIC	Day 28 XIC	Day 180 XIC
ARRFVELLGGRSKPYDALDV	208	63	75	70	8.39E+08	1.18E+09	4.02E+08

ARDKGYSVSNNCLDP	198	15	116	67	1.56E+07	8.29E+08	2.13E+08
AKHFPEWLLRANDY	94	9	13	72	3.33E+06	1.33E+07	2.37E+08
ARTLSGGESPYYYYHMDV	82		25	57		1.31E+08	1.96E+08
ANHLPEWLLRANDH	74	7	22	45	1.75E+06	1.73E+07	3.72E+08
AGRGNVAYDQGSYHHMDV	70		42	28		4.57E+08	2.59E+08
ALPGAYRTVLYYYYFMDV	62	8	20	34	8.44E+06	5.07E+07	8.19E+07
AGRGNLASGQGYYAHMDV	61		24	37		8.44E+07	2.19E+08
AMPGSFREVKYYYFHMDV	50	12	19	19	1.93E+07	1.28E+08	3.43E+07
ARAFKGGEGPYYYYHEDV	49		27	22		3.79E+07	7.36E+06
ARHVLERLVATRVDGFDT	48		29	19		1.61E+07	1.44E+07
ARTFGGGESPYYYYYMDV	46		24	22		6.98E+06	7.09E+06
AKDRVGHQPPYEGSFDY	37		24	13		6.86E+07	1.71E+07
ARVDYGGEGPYYYYSMDV	37		31	6		4.52E+07	5.54E+06
ARVPGTEYPPFGWFDP	36	10	14	12	1.55E+06	4.86E+06	1.01E+06
ARQNGELEGYNWFDP	35		29	6		1.50E+07	2.40E+06
ARRFLETLWGQSRPYDAFDL	32		11	21		1.39E+07	1.86E+07
ARELGELLGYRLFDA	31	11	9	11	1.56E+07	4.85E+07	3.79E+07
VRDSGEGLDP	30	11	13	6	2.44E+06	5.51E+06	1.72E+06
TRLYYLRTSATMDY	28	10	11	7	1.32E+07	1.00E+07	7.74E+06
ARETWTDVDLRTDSYYMDV	24		12	12		1.04E+08	3.83E+07
ARAFRVRDEGNYYMDV	24		24			7.36E+07	
AGQGEGRLVDELSNWFDP	24	6	14	4	1.52E+06	1.37E+07	1.38E+06
ARHVREVLVATPLDSFDT	24	7	11	6	2.05E+06	1.03E+07	1.81E+06
ARAEYYYGRYYYMGV	23	5	11	7	2.16E+06	5.19E+06	4.55E+06
VRLFNTGVGGRGYFDP	21	2	8	11	6.29E+05	3.93E+06	5.47E+07
ATAGLTKYCVGDGHV	20	6	6	8	2.81E+06	5.87E+06	3.68E+06
AKFEGELRAFEV	18	6	7	5	1.33E+07	5.94E+07	3.44E+07
ARSYTSGWSGPFFDP	17	6	4	7	1.57E+06	9.93E+05	2.05E+06
AKKLSSKVPYSGYLDS	15	1	8	6	2.78E+05	4.13E+06	3.89E+06
AKDRGPGGSYSFDD	14	12	2		1.29E+07	2.63E+06	
AWTKLRYFSGGGCSTRWFDP	14		6	8		3.03E+06	5.55E+06
GVASRGPLGGYMDV	14		14			2.66E+06	
AREGKKGVWFDP	14			14			2.50E+06
ARRGVYMGDAFDL	13	5	3	5	4.49E+06	2.40E+06	4.78E+06
AARRRGGTLVRADYYLDG	12	2	6	4	5.93E+07	9.03E+07	6.34E+07
ARSSGANTRPFDY	12		6	6		8.49E+06	7.80E+06
ARKRYSRGPNDY	12		6	6		4.28E+06	3.05E+06
ARGTTDRYYMDV	12	1		11	9.37E+05		5.98E+06
ARDEVAAYHY	12		6	6		3.48E+06	2.93E+06
AREPGDWAPAGQDWFDP	12		7	5		2.72E+06	8.14E+05
ARDGRSAGHGPFAY	11			11			6.07E+06
ALPGPFRELLYYYHFTDV	11		6	5		3.08E+06	9.08E+05
GLAVRGPLGGYMDV	11		4	7		9.42E+05	2.41E+06
SQQLGGRRDDVWSCDSRYFDA	10	3	7		5.17E+03	4.07E+06	
ARFYRSDYMDV	9	3	4	2	4.69E+06	1.16E+07	7.35E+06

ARVEMGGERYYYYSMDV	9		7	2		2.72E+06	1.96E+06
ASGVRGGEAPYYYYHMDV	9		9			2.54E+06	
ATTGTTPWKGSRDY	9		9			1.82E+06	
ARCSNSWYDFDS	9			9			9.28E+05
ARVVAGGESPYYYYSMDV	8		7	1		4.71E+06	1.25E+06
ATGARRSLPVAGSFDF	8		3	5		5.41E+05	1.11E+06
AREGRWEQQGKSKRGALDY	8			8			8.67E+05
ATVLTGGERPYYYCHMDA	7		5	2		8.31E+06	1.37E+06
AKETSYYDSRGYRHMRFPDY	7	6		1	5.12E+06		1.22E+06
ARLPMRSPYHMDV	7			7			5.89E+06
ATRDTASGYDYYMDV	7			7			5.58E+06
ASDPDDTYSYDVRGYSDY	7			7			3.81E+06
ATVLTGGERPYYYCHMDA	7		5	2		8.31E+06	1.37E+06
ARETMVTPGRFDN	6			6			5.29E+06
ARDVWGGTLAFDV	6			6			3.92E+06
ARELSGNWFDL	6			6			3.90E+06
ARGNRFGESPYYSYYVDV	6		6			1.52E+06	
ARDLGAWNDPRDAFDL	6	6			1.16E+06		
ARVHKFWEGEGSDAFDL	6		6			3.11E+04	
ARAAGSVAADFDY	5	5			8.83E+06		
ARARSGSEPYFFDH	5			5			3.97E+06
AKEGLTSTTREGSGLYYMDV	5	2	3		8.83E+05	2.10E+06	
ASFTTGRTGDY	4	3		1	2.06E+06		1.07E+06
ARVVVAAPESWFDP	4			4			1.09E+06
ARGAYSTASGGLFDY	4			4			7.43E+05
ARVGRLGVWNGNADV	4	4			6.99E+05		
ARRPLYSGRQQAPYFDS	4	4			6.55E+05		
ARANGGLEAFDL	4		1	3		3.11E+03	5.92E+04
ASRLAVTAKAQFFDD	3			3			1.52E+06
ARHLGSRVFRGMDV	2	2			4.94E+05		
ARSPPGLRLSSSNSPYYFDY	1			1			1.09E+06
TRRTPSASGTRAADV	1			1			1.33E+06
TRRTPSASGTRAADV	1			1			1.33E+06
ARVQRRGEGAADLDP	1		1			1.52E+06	

Anti-H3 A/VI09 serological repertoire clonotypic antibodies

Representative CDR-H3 Sequence	Total PSM	Day 0 PSM	Day 28 PSM	Day 180 PSM	Day 0 XIC	Day 28 XIC	Day 180 XIC
ARRFVELLGGRSKPYDALDV	199	79	51	69	2.70E+08	1.46E+08	1.73E+08
AKDSAFNWDPSGPYNYYYMDV	178		94	84		2.44E+07	3.67E+07
ARQQEVADSTAVEPYYFYYMDV	153		72	81		4.03E+07	6.48E+07
AREALLWFEELTPNYMDV	127	42	39	46	4.46E+06	2.41E+06	7.19E+06
AKHFPEWLLRANDY	125	3	7	115	6.97E+05	1.42E+06	2.00E+08
ARAQRKYDDGAYRAEYPFYYSTDV	107	38	41	28	6.27E+07	5.84E+07	2.33E+07
AATRRPLRGDLVTNAFDL	78	33	27	18	1.05E+07	1.46E+07	6.89E+06

ANHLPEWLLRANDH	65	6	10	49	6.39E+05	2.66E+06	4.37E+08
ARSVVTGGRHFDY	64		34	30		1.45E+07	1.70E+07
ARGQRRGMGAADLDP	54	38	7	9	2.81E+07	1.68E+06	2.52E+06
ARLPLYSGSQQAPYFDS	49		23	26		1.43E+08	1.96E+08
SRHAPLLQGSPPAFDL	42		28	14		2.45E+07	1.73E+07
AKDSKWRFSDSGPYYSYYMDV	39		19	20		1.28E+07	1.83E+07
ARLKYDYDTPEGYMDV	38		25	13		3.79E+07	3.98E+07
ARRGVYMGDAFDL	36	16	15	5	1.29E+06	2.19E+06	2.78E+06
AREYVDLLTGPAMLDH	34		14	20		1.10E+07	1.61E+07
ARELGELLGYRLFDA	33	9	11	13	9.83E+06	2.43E+07	3.84E+07
GRTNVAVSGGPYFDH	28		12	16		9.56E+06	5.70E+07
AGQGEGRLVDELSNWFDP	28	5	11	12	3.43E+06	5.46E+06	1.18E+07
ATAGLTKYCVGDGHV	28	10	8	10	2.36E+06	1.74E+06	2.45E+06
ATDSRVWGWGNQDDSYFYYMDV	27		12	15		1.21E+07	1.04E+07
ATDGRFRDEFDTRAPYYFYHMDA	27		15	12		2.34E+06	1.51E+06
ARLYYDSSDSSFGLRAFDL	26		20	6		1.52E+07	1.97E+06
ARVGDYYDSGSYSARGEYPPYYYYYMDV	26	2	24		1.04E+06	3.95E+06	
ARRMNYGSGSYDY	25	2	13	10	9.53E+05	7.81E+06	7.97E+06
AKLRPDGSPYYYYMDV	22		11	11		4.05E+06	1.04E+07
VKDRVVTWELPHPNFDY	22		14	8		1.26E+06	5.08E+06
ARHVREVLVATPLDSFDT	22	6	4	12	1.70E+06	5.76E+05	3.07E+06
ARRGAGGPPRSMDV	21		10	11		9.62E+07	7.59E+07
ALWRHCSTPSCLDPVPEMDV	21		12	9		8.36E+06	7.69E+06
VREFYDVLTGDSEPGP	19	5	9	5	1.23E+07	1.12E+07	4.89E+06
ATDTKVAGSLDGPYYYYYTDV	18		7	11		8.62E+06	1.69E+07
VREYYDVMTGDSEPGP	18	6	5	7	6.40E+06	4.34E+06	4.22E+06
LSGTLKGFDY	18	6	6	6	2.56E+06	1.13E+06	1.91E+06
ARGTTDRYYMDV	18	3		15	5.62E+05		4.34E+06
APSEPMVACTNGVCSRNSDVFDM	18		18			3.53E+06	
AARRRGGTLVRADYYLDG	18	5	7	6	4.41E+07	4.09E+07	3.64E+07
AREPGDWAPAGQDWFDP	17	6	5	6	7.73E+05	6.57E+06	2.88E+06
AKFEWELRAFDV	17		11	6		5.29E+06	3.85E+06
VKAARFFSGSGSYFDY	17	5	6	6	2.88E+06	3.28E+06	2.41E+06
VTDSHFRLGESTEPHHFYYMDV	17		11	6		4.43E+06	3.19E+06
VRLFNTGVGGRGYFDP	16		2	14		2.45E+05	2.35E+07
ARVSGNYLERNYYYYYMDV	16	6	6	4	1.28E+06	2.34E+06	2.17E+06
ARLNYDVLTGADGGFAP	16	7	4	5	1.77E+06	1.39E+06	2.11E+06
ARELGDDELGYYYDHMDV	15		10	5		6.52E+06	1.20E+07
AKDRGPGGSYSFDD	15	12	3		8.37E+06	1.49E+06	
ARAEYYYGRDYDMGV	15	2	8	5	1.36E+06	4.29E+06	2.56E+06
SQQLGGRRDDVWSCDSRYFDA	15	3	3	9	9.37E+05	8.93E+05	5.08E+06
ARDGCNHVAFDY	14	6	4	4	9.91E+06	2.21E+06	1.18E+06
ARHVGQLVPDALDF	14		11	3		7.91E+06	1.24E+06
ATDRRLGVEAYYFYYMAV	14	7	6	1	8.22E+05	5.57E+05	6.22E+05
ARFYRSDYMDV	13	4	4	5	1.39E+07	8.31E+06	1.45E+07

ARVQRRGEGAADLDP	13	6		7	1.98E+06		1.63E+06
ASPKVRPGAYAFDL	13		13			3.56E+06	
ARLDGGNSRGPFFDY	13		13			1.24E+06	
AWTKLRYCSGGGCSTRWFDP	13		8	5		1.92E+05	2.48E+05
ARVPLVGGVTGPYFDD	12		6	6		9.92E+06	8.03E+06
ARGTVVPQNVPWFDP	12		6	6		7.36E+06	9.29E+06
ARQPRLGDQKGTLDY	12	6	3	3	1.50E+06	6.68E+05	6.99E+05
AREGKKGVWFDP	12			12			4.24E+06
ARLGFDYDTPEGYMDV	11		5	6		9.58E+06	1.63E+06
ARHMDLVVVPPPPPLDV	11		2	9		1.91E+06	5.50E+06
ARDEVAAYHY	11	3	6	2	9.18E+05	2.77E+06	2.09E+06
ARRFLETLWGQSRPYDAFDL	11		3	8		8.49E+05	2.01E+06
ARESAGWKNYLDN	10	4	2	4	2.19E+07	8.21E+06	1.11E+07
ARELYRYFDS	10	6	1	3	3.01E+06	9.83E+05	1.38E+06
ARDGRSAGHGPFAY	10			10			5.23E+06
ARTPLTAGDAGWFDP	10		8	2		2.16E+06	4.71E+05
ARDKGYRVSKNCLDP	9	4	2	3	2.42E+06	7.65E+05	1.63E+06
AKGLTLGSFDS	9	6	3		1.90E+06	2.16E+06	
AAERHYYESTGGPGYMDV	9		6	3		1.42E+06	1.43E+06
ARDAFCGGDCSPDY	9		1	8		5.75E+03	2.81E+06
TTDRGYDLYDR	9		3	6		1.05E+06	1.59E+06
ATEEGYGANSRVFDM	9		8	1		1.09E+06	8.20E+05
ATDGRFSTGDVTGPYYYYYMDV	9		8	1		1.07E+06	1.90E+05
ARETSRTVSGLVLLPPTFDP	8		5	3		1.43E+07	2.45E+07
ARERESSGGKRYFDH	8		6	2		3.09E+06	3.73E+06
ATRDTASGYDYYMDV	8			8			4.22E+06
ARVSDFWSGKTQYYFDY	8		2	6		1.24E+06	1.65E+06
ARGAVGAKHPGLDF	7		1	6		2.78E+03	1.64E+07
ARLPMRSPYHMDV	7			7			4.91E+06
ARLPVVPGAPYFDQ	7		1	6		1.72E+06	1.13E+06
ASRLAVTAKAQFFDD	7			7			1.60E+06
ARRANYKSDAFDL	7	7			1.50E+06		
ARTPLATADAGWFDP	7		5	2		6.97E+05	5.30E+05
ARLQEVADSSATEPFYFYYLDV	7		5	2		4.47E+05	3.90E+05
ARALLRPPPGPFDL	7		4	3		6.79E+05	9.13E+03
ARLDWNDRGAVDY	6	4		2	1.20E+07		7.41E+06
ARSSGANTRPFDY	6		5	1		2.48E+06	5.26E+06
ARETMVTPGRFDN	6			6			6.60E+06
ARCSNSWYDFDS	6			6			5.36E+06
AKETSYYDSRGYRHMRFPDY	6	6			4.38E+06		
ARVVVAAPESWFDP	6			6			2.58E+06
ARGAMVAGPYYFDY	6		6			2.45E+06	
ARTGHGDYSNFVDY	6	6			1.65E+06		
ARPRSPSSNPSNKKMKMKPRGYYFDS	6		6			1.64E+06	
ARLQKFWEGEGSDAFDL	6	5		1	1.45E+06		1.74E+05

ARASWFGDSERLLDQYYYYYYMDV	6		6			1.57E+06	
ATSRTKYYKSSGYVRVFDP	6		6			1.39E+06	
ATDPTVLRCLEWSAPTEWYFDV	6			6			1.22E+06
AREEGWFGELSTFRYFNL	6		6			1.14E+06	
ARLHYDSPDYSFGLRAFDL	6		6			8.91E+05	
ARDSSGSYLEAFDM	6	2		4	2.95E+05		4.38E+05
ANYGGRGYVYRYPLHGSHMNV	6		6			6.37E+05	
ARPVYYMDV	5	3	1	1	2.07E+07	8.64E+06	1.02E+07
TRRTPSASGTRAADV	5			5			6.62E+06
ARSPPGLRLSSSNSPYYFDY	5			5			2.51E+06
AREPMVRKVPFDS	5			5			2.14E+06
ASDPDDTYSYDVRGYSDY	5			5			2.12E+06
ARQGLPPPRGGFDP	5	3	2		1.17E+06	8.42E+05	
ARGTAGGYDFWNGYRFFFDY	5			5			1.47E+06
ARSKPLSACTNGVCSRNSDVFDM	5		5			3.78E+05	
AKSGEDGKSKGGLDY	4	2	2		1.23E+06	1.04E+07	
ASFTTGRTGDY	4	4			2.23E+06		
ANFGGRGYLYGYPLHGGDYSMDV	4	4			8.71E+05		
ARAPDEWNRDVYNYCASDL	4	4			8.51E+05		
GVASRGPRGGYMDV	4			4			6.93E+05
ARELSGNWFDL	2			2			4.47E+06
ARARSGSEPYFFDH	2			2			1.54E+06
ATVLTGGERPYYYCHMDA	2		1	1		5.84E+06	1.91E+06
ARVGRLGVWNGNADV	1	1			1.22E+06		

Anti-Vic B/BR08 serological repertoire clonotypic antibodies

Representative CDR-H3 Sequence	Total PSM	Day 0 PSM	Day 28 PSM	Day 180 PSM	Day 0 XIC	Day 28 XIC	Day 180 XIC
ARDWGEDSSRPPFDY	493	25	265	203	2.88E+06	1.74E+08	2.38E+08
ARDGEPNTSMDYYYYYHLDV	217		93	124		7.81E+07	1.82E+08
ARGNVDSVMDYYSFYSLDV	216		90	126		3.35E+06	1.69E+07
ARDWGESTDEPPFLY	175		84	91		1.72E+07	5.31E+07
ATFKGDYGDSSTNY	163	15	85	63	8.42E+05	8.69E+07	1.11E+08
ARLEYGDYLRDVDL	61	16	27	18	1.26E+06	1.29E+07	1.20E+07
AKDPFGGNLGAYFDF	54		40	14		2.50E+06	1.74E+06
ARHNLRSGELLWFDP	53	8	26	19	1.94E+05	7.95E+06	7.36E+06
ARDWGEDTSRPPFAF	52		28	24		3.06E+06	3.91E+06
ARGGGDGYTFGFAPCFDA	50		25	25		4.62E+06	5.82E+06
ARMGQTE	42	13	14	15	6.02E+05	5.02E+06	6.30E+06
AREDLEMGTGTAGAPFDY	41	7	17	17	1.71E+06	4.92E+07	7.03E+07
VRDWGEDTFPPPFDY	39		19	20		8.07E+05	2.54E+06
VRDWGESTDEPPFLF	29		13	16		4.53E+05	1.26E+06
ARDDGFFDD	29		16	13		5.70E+06	2.35E+06
AREPGDWAPAGQDWFDP	24	6	10	8	3.62E+05	5.59E+06	3.09E+06
ARELGELLGYRLFDA	23	6	9	8	3.04E+05	6.78E+06	1.27E+07

LSGTLKGFDY	23	11	6	6	2.66E+05	2.68E+06	3.57E+06
ARDWGENLYEPPFDS	22		11	11		3.08E+06	2.60E+06
ARHTSPYYFFLDV	22		11	11		5.40E+05	2.25E+06
ARDWGESLYESPFDS	20		9	11		1.38E+06	2.80E+06
SRRQYGDYVRDLDL	20		10	10		6.29E+06	9.93E+06
AKFEGELRAFEV	19	6	5	8	6.16E+05	6.31E+06	1.27E+07
ARDEVAAYHY	18	6	6	6	3.25E+05	6.40E+06	1.50E+05
ARGNLDSLMDYYYYDYMDV	16		5	11		2.35E+05	1.32E+06
AKDRARNVLCSLYSCPTDAFDM	16		9	7		2.24E+06	2.14E+06
ARPASLGYCSSTTCPFDY	15	2	6	7	3.09E+05	2.78E+06	3.88E+06
ARDWGEDTSRPPFDL	15		7	8		8.80E+06	9.74E+06
ASPKLRPGADAFDL	14	1	8	5	4.60E+04	1.12E+06	2.15E+06
ALRSPYTLD	14		7	7		3.81E+06	1.93E+06
ARDLMGLDCLGGSCNWFDP	13		9	4		5.03E+05	2.39E+05
ALRGYSGLGNWNY	13	3	6	4	3.01E+04	1.55E+06	2.04E+06
ARDAEPHYADYRSVYWYSYMDV	12		6	6		9.27E+05	1.06E+06
ARDPRVYGEGYDWFDP	12		5	7		2.18E+06	1.92E+06
ARELPENWFDN	12	3	4	5	2.96E+05	2.23E+06	1.65E+06
ARAVLTGGRHFDY	10		2	8		1.05E+06	1.20E+06
ARENGEREGYKWFDP	9		7	2		5.17E+06	9.67E+04
AKETSYYDSRGYRHMRFPDY	9		1	8		6.96E+02	1.19E+06
ARDLLLRRGSSYLHYYMDV	9		3	6		3.54E+06	6.64E+06
ARDWGESLSAPPFDS	9		6	3		7.86E+05	7.84E+05
ARDPSGAQNWYFDL	9		5	4		4.04E+05	6.70E+05
ARRGVYMGDAFDL	8	2		6	6.31E+04		2.50E+06
ARFFRSYYMDV	8		7	1		8.44E+05	1.76E+06
ARVSGNYLERNDYYYYMDV	7			7			8.09E+05
VRWAGEAKYDFWRGPYDN	7		4	3		3.20E+06	1.07E+06
ARENYDCLTGSSDGFDL	7		4	3		1.98E+06	2.58E+06
AKDPEGAYYYDSGLDY	7		6	1		5.25E+05	3.08E+03
AKANGDQRNHFFDS	7		2	5		3.74E+06	5.44E+06
ALPGSVREVVYYYHYMDV	6			6			2.90E+06
ARGPGRDYLGYWYQRNFDY	6	1	5		5.49E+04	1.39E+06	
ARGDHDLLTGYYRGHFDY	6		1	5		1.96E+06	8.15E+06
AKDPDGAYYYDGGFDF	6		5	1		2.59E+03	8.05E+02
ARGPLLSGSAPLDY	6		6			1.26E+06	
ARHGAGGPPRSLDV	6		5	1		8.96E+05	3.77E+06
ARDNSQSPRFFQH	6	2		4	1.09E+03		6.60E+05
VTRAVPDS	6		6			7.47E+05	
AREEGWVGELSTFRYVDL	5	5			7.54E+04		
AKVGGNYYGAFES	5	2	2	1	2.90E+05	2.38E+06	2.82E+06
ARCSWFGDSERLLDCDLYYYDRDV	4		2	2		4.96E+05	4.76E+06
ARRKGELLGYNWFDP	4		2	2		2.15E+03	1.02E+04
ARGGFLQWFFRWFDP	4		4			1.39E+05	
ARGGGRYYYMDV	4		2	2		9.74E+06	1.73E+07

Anti-Yama B/TX11 serological repertoire clonotypic antibodies

Representative CDR-H3 Sequence	Total PSM	Day 0 PSM	Day 28 PSM	Day 180 PSM	Day 0 XIC	Day 28 XIC	Day 180 XIC
AREDLEMGTGTAGAPFDY	973	196	439	338	1.55E+08	5.51E+08	5.43E+08
ARDGEPNTSMDYFYHYYLDV	344	60	114	170	1.63E+07	2.65E+08	2.45E+08
ARWEYNDRSGYFQLYDY	156	54	52	50	2.48E+07	9.54E+07	1.25E+08
AREWGEDNYAPPFDY	149	42	57	50	1.26E+07	6.83E+07	4.84E+07
ARAFPGTSGGWYYFDY	135	50	43	42	5.72E+07	1.11E+08	1.01E+08
ARSYTSGGSGPLFDP	130	76	25	29	4.43E+06	6.08E+06	1.49E+07
ALGGLGELPSGFGY	121	51	34	36	2.37E+07	5.07E+07	9.52E+07
ARGGGDGYTFGFAPCFDA	119	55	18	46	2.40E+07	2.93E+07	1.48E+08
ARNWARLDD	115	34	49	32	3.45E+06	1.25E+07	1.26E+07
ARQDGELEGYNWFDP	97	7	44	46	2.34E+06	4.39E+07	4.31E+07
ARAYRSFSSVHFDF	97	27	37	33	3.70E+07	2.57E+08	2.70E+08
ARGNVDAVMDYYSFYSLDV	95	23	20	52	4.33E+06	1.10E+07	3.31E+07
AAFKGDYGDASTNF	91	34	27	30	1.34E+08	1.88E+08	2.49E+08
AVPGSFREVLYYYHYMDA	88	19	28	41	3.82E+06	8.03E+06	2.30E+07
AREFPDFSEFYFDS	84	38	27	19	6.34E+07	1.91E+08	1.30E+08
ARKYWGTEPSSFYMDA	76	27	24	25	8.31E+06	3.18E+07	3.27E+07
ARDQTVAGPRTVDY	73	12	31	30	3.18E+06	6.10E+07	8.97E+07
ARRSHDTLD	70	21	23	26	2.72E+06	7.67E+06	1.15E+07
ARYRASGYVPCDF	65	30	10	25	2.95E+06	1.59E+06	7.32E+06
ARSTWGELLLRSGGYFDL	64	20	25	19	7.26E+06	2.01E+07	2.05E+07
ARGNLDSLMDYYYYDYMDV	63	18	23	22	2.68E+06	1.15E+07	9.64E+06
ARGTRERFFDC	58	18	18	22	7.46E+06	3.21E+07	7.23E+07
ARHLGSSGFRGMDG	55	16	18	21	2.22E+07	2.12E+07	2.00E+07
TAQWELRFPKRVMDY	54	23	14	17	9.39E+07	1.92E+08	1.48E+08
ARRGVYMGDAFDL	53	11	25	17	2.92E+06	8.65E+06	1.53E+07
ARDRLGEYQMMYQWAY	52	12	20	20	1.98E+06	2.12E+07	2.24E+07
SRHHGDYSSTPPPLDY	48	11	18	19	1.22E+06	3.13E+07	2.94E+07
AGRGNVADDQGSDHHMDV	47	7	14	26	1.07E+06	1.36E+08	2.19E+08
AREEMYYLDSSGSFDY	45	16	14	15	7.65E+06	2.21E+07	1.64E+07
ARFYRSDYMDV	43	1	9	33	3.13E+07	6.37E+06	5.51E+07
ARELPENWFDN	43	13	15	15	3.79E+06	6.24E+06	5.96E+06
ARDKGYSSSNNWRDP	40	6	14	20	6.32E+05	1.10E+08	2.20E+08
ARAGRGGELPNYSYYMDV	40	23	7	10	3.29E+06	1.58E+06	2.16E+06
AKTHQRHSGTYGVLDM	39		15	24		2.47E+06	5.59E+06
ATDSRWRFASLGPNYSYYMDV	39	14	14	11	8.22E+06	1.27E+07	2.45E+07
ARDCGESSDEPPFLY	38	4	16	18	1.45E+06	1.19E+07	4.02E+07
AHRRTPGPDGTFDY	36	12	12	12	5.05E+06	1.25E+07	2.02E+07
AREDYGSGVNDPPFDY	34	15	11	8	5.73E+05	1.00E+06	1.19E+06
ARRTWHNAFDS	34		16	18		1.60E+06	4.71E+06
ARDVWGGTLAFDV	33	9	15	9	7.03E+06	1.33E+07	1.56E+07
ARDWTKLVEVPPLGPFDP	32	4	16	12	2.60E+05	1.07E+07	1.09E+07

ARHHEEVGLPEGGFAY	32	4	18	10	3.08E+06	1.18E+08	7.11E+07
ARDLGKGLAVLPASMKARGTGDFDF	31	5	9	17	7.49E+05	1.70E+07	1.47E+07
ARAPGQWLTGRESYFDY	30	18	6	6	3.20E+07	8.23E+07	1.18E+08
AKDPEGAYYYDSGLDY	30	7	15	8	2.14E+06	7.88E+06	6.29E+06
ARAVGTGGRHFDS	29	4	6	19	5.95E+05	7.18E+05	1.55E+07
ARVSEWLVVMPAARDRPSPQFDF	28		16	12		1.20E+07	7.62E+06
VSGDYFGH	28	14	6	8	2.84E+07	4.78E+06	1.45E+07
ARCSWFGDSERLLDCDLYYYDRDV	27	5	8	14	1.06E+06	7.68E+06	3.54E+06
ARDWGEYLYEPPFDS	27	2	18	7	7.54E+05	2.97E+07	3.82E+06
TRRPNTFYGDWFDP	27	8	10	9	2.95E+06	9.89E+06	1.10E+07
TRLRLRDCTLGVCDLWYFDY	26		12	14		9.22E+06	1.87E+07
ARDSVTTLTHDAFDV	25	9	12	4	7.94E+05	5.05E+06	3.48E+06
ARDRGTTGGAFDL	25	3	11	11	2.28E+05	6.84E+06	5.10E+06
ARDNSQSPRFFQH	25	10	8	7	2.31E+05	1.27E+06	8.96E+05
AKEGGSSGTDRYHLDD	23	6	4	13	1.64E+07	3.49E+07	2.93E+07
ARGRSFYYDSSGFSWWFDP	23	11	6	6	1.26E+06	1.95E+06	2.74E+06
ARRRDYYDSSGSDTAVHL	22	2	13	7	5.99E+02	7.72E+06	1.38E+06
AKDDFSNTFVTYDYDYMDV	21	6	3	12	6.96E+05	8.93E+05	8.25E+06
ARGVTVAGLYYYKYMDV	21		12	9		9.54E+06	6.92E+06
TRDRLFYDFWNAQFAL	21	11	8	2	7.44E+05	9.12E+04	1.40E+03
GRRDYDLLTACEGAFDV	20	6	4	10	1.21E+06	9.46E+06	9.87E+06
AKDKGMQVWLQCYFEH	20	10	2	8	1.73E+05	3.62E+05	8.28E+05
AKDRARNVLCSLYSCPTDAFDM	20	1	11	8	2.25E+05	1.21E+07	1.60E+07
AKFEWELRAFDV	20	7	6	7	5.08E+04	5.29E+06	4.56E+06
ARDRPLRLLDS	20	5	8	7	3.22E+05	3.42E+06	1.37E+06
AREPGDWAPAGQDWFDP	19		9	10		3.12E+06	5.28E+06
ARLLRGGERPYDYYHMDV	19		11	8		7.17E+06	5.37E+06
ARLSPGLKFHYFDYMDV	19		1	18		4.74E+05	7.97E+06
ARHGHSSGGFHGGAFDL	19		9	10		3.57E+07	3.29E+07
ARVKGAVAGDD	19	10	1	8	5.36E+05	3.04E+05	1.00E+06
ARHFMCRGGLAWFDS	18		8	10		5.75E+06	7.74E+06
ARDLLLRRGSSYLHYYMDV	18	1	11	6	1.01E+05	2.85E+06	1.71E+06
AKVHGRRGQAYFYYYMDV	18	15		3	4.82E+05		5.82E+05
ARVVFALGRYYYYMDV	18	6	6	6	5.63E+05	6.20E+05	7.07E+05
TRGNPSTMKSDHFDS	18		11	7		8.67E+06	7.50E+06
ARAAGVLECLSYYSDS	17	7	2	8	1.74E+05	6.84E+05	2.80E+06
ARVSGNYLERNDYYYYMDV	17	7	4	6	6.65E+05	1.94E+06	6.15E+06
ARRRPHCTPGRCTPYFDY	17		5	12		2.02E+06	5.30E+06
ARVEGVGVTRGAFDD	17	5	5	7	3.94E+05	1.35E+06	1.61E+06
AKANGDQRNHFFDS	16		4	12		5.30E+06	5.11E+06
ASFTTGRTGDY	16		10	6		5.79E+05	1.37E+06
ARDPRGYGEGYDWFDP	15	1	7	7	2.46E+05	7.21E+06	8.86E+06
AGRGNLASGQGYDSHMDV	15	9	1	5	5.55E+05	7.95E+05	8.44E+05
ASSPRLGTYYYLDV	15		10	5		6.93E+05	4.36E+05
AKETSYYDSRGYRHMRFPYY	14	6	2	6	1.96E+06	1.29E+06	2.42E+07

ARNTSWGLRAVAGTRAPDSFDL	14			14			1.81E+08
EREDGATVDGPSHYFDY	14	6	5	3	3.04E+05	1.42E+06	6.26E+05
VKAARFCSGSGSYFDY	14	7	2	5	1.90E+06	9.33E+05	7.84E+05
AGGFSYAVPYYYMDV	14	6	2	6	4.78E+06	1.67E+07	8.05E+06
ARVMQWLDSHYMDV	14	3	7	4	5.53E+06	6.09E+07	6.46E+07
GRYMYGFFDYYMAV	14		6	8		4.33E+05	3.66E+05
ARESAGWKNYLDN	14		7	7		4.26E+06	1.17E+07
AKRGNYTGGALDY	14	1	6	7	3.35E+03	1.98E+06	5.45E+06
ARMGQTE	14	2	6	6	2.72E+05	4.02E+06	5.15E+06
ARHVTPGWEKHTFDY	13		4	9		3.26E+05	2.61E+06
AKDRAAMGAFDL	13		7	6		1.50E+05	9.77E+05
AREDREKTTLTAGGPFDN	12		6	6		7.92E+06	8.93E+06
AREERLLEWLLSSFDY	12	8	3	1	1.18E+06	3.12E+06	2.39E+06
ARTPLTAGDAAWFDP	12			12			1.03E+06
ASPKLRPGADAFDL	12		5	7		1.04E+06	4.71E+06
ATAPLGTRNTVVVPAARSGYYMYV	11	2	8	1	2.38E+05	7.58E+05	2.72E+05
VHRPSGMNSATVLGRFDG	11	1	1	9	1.86E+05	1.20E+06	3.91E+06
ASFAVTSDRPTYLDYMDG	11	5	4	2	5.84E+06	1.29E+07	4.16E+06
ARGLGDDELGYDYDNMDV	11	7	2	2	7.21E+05	3.81E+06	2.57E+06
ATMYYNFWSDGPSADF	11			11			1.08E+07
AREYRMFGVDGAFDL	11	5		6	3.65E+06		6.34E+06
ARDPGHTAALLMGD	11		7	4		1.98E+06	1.27E+06
VRRRLDMDGAFDL	11	6	4	1	5.45E+05	6.96E+06	1.10E+06
ASKGTYALGWRY	11	5	3	3	4.19E+06	4.16E+05	5.95E+06
LSGTLKGFDY	11	6	1	4	2.37E+06	4.56E+06	1.72E+07
ARDMWSGYSGYNVFY	10	2	6	2	5.32E+06	3.64E+06	1.23E+07
ARQGAGGPPRSMDV	10	3	5	2	7.32E+05	1.84E+06	3.78E+06
ARAVREKATSTTYGKTLLGWYFEY	10		6	4		1.76E+06	1.26E+06
ARGLVGDRSGYYFDGYFDY	10		1	9		3.72E+05	2.09E+06
VTQGEERLVDELSTWFDP	10		5	5		8.54E+05	2.65E+06
AKDQNGQLLPDAFDV	10	3	3	4	9.90E+05	3.98E+06	3.22E+06
ARGSVAGRYFDY	10		4	6		8.38E+06	6.71E+06
ARDLGEDSLTGPGAFDL	9		6	3		1.15E+06	1.05E+06
ARERGDFWSGYTALGV	9	2	5	2	2.91E+05	4.32E+06	4.75E+06
ALRGYSGLGNWNY	9	2	1	6	1.53E+07	8.20E+07	3.37E+07
AKGTLYTTSGLDY	9		7	2		1.69E+06	2.16E+06
ARRQPAANFDY	9		1	8			1.49E+06
ARARQYSYGHPYFDF	8	7		1	1.98E+05		6.33E+05
ARGLGHGQNWFDP	8		6	2		3.12E+06	1.45E+06
ARRHLVLMAVDY	8			8			8.43E+05
ARGVTGYSYGS	8	1	4	3	1.14E+06	2.08E+06	2.54E+06
AWTKLRDCSGGGCSTRWFDP	7			7			5.33E+06
AKEGLTSTTREGSGLYYMDV	7	3	3	1	1.96E+06	2.67E+06	4.60E+06
ARHVSERLESTPLDGFDF	7			7			5.02E+05
ARLGCRGDCYHLGFDF	7		3	4		8.24E+05	1.39E+06

VKDRRVGLGFYFDS	7			7			9.11E+05
ALRGLSGYTDGYGY	7		1	6		1.74E+06	3.74E+06
ARGPHFDWPLASY	7		6	1		4.10E+06	1.53E+06
ARLEYGDYLRDVDL	6		2	4		1.10E+06	1.72E+06
ARDDGRYCTSTTCYRGPCDL	6			6			3.33E+06
ARAPDECNRDVYNYCAFDL	6	6			2.32E+06		
ARLQKFWEGEGSDAFDL	6			6			3.25E+06
ARENYDCLTGSSDGFDL	6			6			2.53E+06
VRGLLGTDYAADYFDY	6		2	4		4.52E+07	1.64E+07
ARGAYSTASGGLFDY	6			6			4.71E+06
ARDWGEDTSRPPFAF	6		2	4		1.60E+06	2.23E+06
ARLGHYGGNDAFDL	6		5	1		7.60E+06	1.31E+07
ARGPPRYSYMDV	6			6			1.26E+06
ARDGCNHVAFDY	6		1	5		8.99E+05	1.18E+06
ARLRGDAFDF	6	1	3	2	8.25E+02	2.41E+05	3.22E+06
VRGVQGMDV	6		4	2		5.23E+06	1.89E+06
AREHNWPAPPTWFDS	5			5			1.92E+06
ARVGKQPGASYFDG	5	2		3	3.95E+05		6.59E+05
ARRANYKSDAFDL	5			5			1.57E+06
VKDRVVMWESPHPNVDH	4		1	3		3.37E+06	4.44E+06
VKEGGKGLVNRALDL	4	1	1	2	2.34E+06	1.86E+07	1.39E+07
ARLSVVGATNC	4		3	1		1.07E+07	1.99E+07

Donor 3

Anti-H1 A/CA09 serological repertoire clonotypic antibodies

Representative CDR-H3 Sequence	Total PSM	Day 0 PSM	Day 28 PSM	Day 180 PSM	Day 0 XIC	Day 28 XIC	Day 180 XIC
ARSKWNVPETGLGAFDM	941		500	441		5.65E+08	4.17E+08
ARHFSEYLSPLWGTPMTCFDY	686		261	425		3.21E+08	6.64E+08
VYSGEETLFGSGTSHKWFDP	293		123	170		5.07E+08	1.13E+09
AHARLANRGYDFFDY	288	87	99	102	1.48E+08	4.14E+08	5.88E+08
ARPGIGPADMSNYYYMDV	218	7	88	123	2.72E+06	5.29E+08	1.33E+09
AKGDTTFAVGEVEMGDWYFDH	149		22	127		2.90E+07	8.17E+07
ARGPYDFWSGYPAYDY	112		102	10		1.55E+08	1.50E+07
AKDQSDVGPLGGIIAS	88		53	35		6.01E+07	3.51E+07
ARAYHDILTGYENPLGYYGMDV	82	1	24	57	3.44E+03	4.52E+07	1.03E+08
AKDARRISIPATGDFDY	71		49	22		1.05E+08	5.35E+07
ARVRQSYYIDY	68	23	20	25	9.86E+06	4.78E+07	3.98E+07
AGGPTRDFNIFAPATY	54	24	13	17	3.49E+06	3.15E+07	7.62E+07
ARGSNYYDSSGPDN	51		20	31		1.45E+07	6.14E+07
ATGEMATISARFCMTN	49		19	30		1.67E+07	2.78E+07
ARRLGTNYADPLGRFDP	43		23	20		2.02E+08	2.83E+08
ARDHSPADPLVDGGSWFDP	42		22	20		4.43E+07	2.69E+07
ARGSSSWPLDY	38	16	10	12	3.40E+06	1.19E+07	1.50E+07
AKDWTHMIPLSGFFDY	38		19	19		8.41E+06	8.36E+06
ARLGGKIVVGEGWNYCYMDV	36		17	19		9.71E+06	9.72E+06

AREGGELRGYNWFDP	33	2	13	18	4.18E+03	4.04E+07	9.15E+07
AKDLYSLPPDSGSLDY	33		33			2.89E+06	
AIFSPRGYQLVSYYFYMDV	31		10	21		1.20E+07	4.03E+07
VKESGDDQGYYIGAFHI	31		15	16		4.68E+06	6.48E+06
ASRFDDILAGYRLGPFDI	28		13	15		1.78E+07	4.24E+07
ARLKDYVWGS	28	9	10	9	8.69E+06	2.06E+07	1.16E+07
ARDMGELVGYRWCDP	28	4	12	12	3.53E+06	1.74E+07	1.69E+07
AHLISPRKLDLLGTYFGN	26		12	14		5.15E+07	5.13E+07
ARGGYSRSNNWFDS	26		10	16		1.85E+07	8.38E+06
TTVVVGGTSYRHY	26	4	2	20	1.52E+06	2.68E+06	1.52E+06
AKDLSRPSLRYFDS	24	6	7	11	3.91E+06	1.45E+07	1.54E+07
AKDHVDTIMVPFSGYFDY	24		24			4.03E+06	
VKAGPGQYDSSGYYHY	23		10	13		1.00E+07	6.12E+06
ARGVIYGGYVPAPLNKRHSMDV	22		5	17		1.66E+07	1.48E+08
AKAQFRRSDLYDY	22		9	13		1.23E+07	2.21E+07
ARGERRSSDFDF	21	11	5	5	1.22E+07	2.36E+07	2.63E+07
AKDLARYIPTSGSFDY	21		2	19		8.54E+06	2.14E+07
ARGGTIFGLVRWYCDL	21		10	11		9.96E+06	6.68E+06
AGGRGYHLQGARADFDS	20		13	7		3.91E+07	2.36E+07
ATDPRVAAARLLLSD	20		8	12		7.37E+06	3.16E+07
AKEKGGVDY	19	17	1	1	3.76E+07	2.91E+07	1.03E+07
AKDPFGGNIGSDFDS	19	7	5	7	5.02E+06	1.12E+07	1.57E+07
ARDLTYRGDASHI	18		11	7		2.87E+07	5.54E+06
ALLISPRKLDLLGTYFDY	18		7	11		1.23E+07	8.81E+06
AKDLGRRVWENGYFDL	17		6	11		1.18E+07	2.29E+07
AKDLVTAAHIPLAGTADY	17		13	4		2.80E+07	3.85E+06
ARHSAYSSGWIDY	17	5	6	6	1.95E+06	7.31E+06	1.48E+07
AGNRNYDNSAYYST	17		8	9		5.20E+06	2.92E+06
AKDQILPPATGLFDY	17		14	3		6.83E+06	4.65E+05
ARHFSEYLSPPWDTPISTFDH	16		8	8		2.18E+07	1.90E+07
ARVWPAANPGDY	16	5	5	6	1.54E+06	1.57E+07	1.93E+07
ARPLWSGYNPNYVMDV	16		10	6		3.03E+07	4.57E+06
AKGGRLIPINGILDS	16		8	8		7.90E+06	4.61E+06
AGGSGYDLRTSLDY	15		3	12		7.99E+06	2.38E+07
ARGPHYYSSYMDV	15		6	9		4.87E+06	1.36E+07
ARRTGDY	15	7	6	2	2.05E+06	5.49E+06	1.57E+06
ATLASGRLLGDYYYYYMDV	15			15			8.50E+05
AHSRMYYDDSSGWVYYFDY	14		7	7		1.60E+07	1.12E+07
ARAYHDVLTGNENSLGYYGLDV	13		7	6		1.22E+07	2.47E+07
ARGRDTTYVDTLHVDH	13	4	2	7	2.87E+06	2.97E+04	1.59E+07
TRGRNIVGAAHY	13			13			1.17E+07
TRGSVS	12		9	3		4.64E+06	2.98E+07
ARGRRGSYFSNSIFDD	12		7	5		1.15E+07	1.70E+07
AKLMVLRVSEWSHDY	11		2	9		3.57E+04	1.45E+07
ATEKRAVNYYDSTGYFDY	11	4	1	6	2.64E+06	2.77E+06	6.29E+06

ARGITIFGLIRNRLDP	11		5	6		3.90E+06	7.19E+06
ARDRGRRGALDDWYFDL	11		9	2		4.17E+06	1.32E+06
ASSTAKIANYYYYMDV	11			11			5.88E+05
AREEENTSLNDY	10	3	6	1	2.97E+06	2.48E+07	1.42E+07
AKDLSRFIPAAGNFDC	10		7	3		1.72E+07	5.39E+06
ARKMGGGSAFDA	10			10			1.51E+07
ARGSGYHTVGNYFDD	10			10			1.29E+07
AKDGSRVVPTTGYVDY	10		10			5.53E+06	
AKDRSDYIPATGTLAS	9		6	3		1.57E+07	8.59E+06
GRASGYHLRHNWFDP	9		2	7		2.31E+06	4.39E+06
ARSGGYNHGRGFDL	9	1	1	7	7.74E+05	2.52E+06	2.07E+06
ARGTAGIAVAGTLGVPYYRYYMDV	9	3		6	1.02E+06		2.33E+06
AGNAFRSGWELPPWGARLGWFDP	9	7		2	9.60E+05		1.86E+06
AKDRMGYTARGAFDI	8	2	3	3	4.18E+06	1.27E+07	1.26E+07
ATNVRNDQYNYMDV	8		2	6		3.11E+06	4.50E+06
ARHPRNLQQSYFDL	8		2	6		2.49E+06	4.23E+06
ARVNTGWLEY	8		8			5.64E+06	
ASPDSLRFLYYYAMDV	8		8			4.31E+06	
ARGQTNSLYY	8		8			3.40E+06	
ARAGDSATWKAPLDH	8		7	1		1.51E+06	4.79E+03
VKDRLEWTLRGAFET	7	5		2	3.04E+07		1.72E+04
ARVDGTDHLDY	7		7			1.83E+07	
ATRYEFPLMRF	7		7			1.27E+07	
ARDKGVPTYFYHGMDV	7	5	2		8.22E+06	4.24E+06	
ARHFSENLFPAWQTPMSCFDS	7		1	6		5.31E+06	6.96E+06
ARSRTYYPSYCDY	7			7			8.61E+06
ASQGGVDGTYFRNA	7		7			4.45E+06	
AGLSKVPNYYYFMDV	7			7			3.24E+06
ARAMGATKYYFDY	7		3	4		1.32E+06	8.44E+05
ARVATFVQRGAISSLAGGGPYDY	6		2	4		5.13E+03	1.55E+07
ARVFQDYLMDV	6		2	4		3.10E+06	5.84E+06
AKTRTYYDILTGYLGRNSYYYMDV	6			6			8.32E+06
ARERSFFSQGFDS	6		5	1		3.42E+06	2.88E+06
AAPARGRGLINYHYLLDV	6			6			6.00E+06
ARDPLPGRPAATTVNWFDP	6			6			4.48E+06
AREGGYNFGLPFDY	6		6			3.78E+06	
ASPHCSGGSCYSDAVVSRGFDY	6		6			3.35E+06	
ARDRTGIGDLNAFDY	6	6			2.53E+06		
ARRSQGGYHDVWSAYYASGWAYDY	6		2	4		1.02E+05	1.48E+04
ARDLRGPGDY	5		3	2		1.42E+07	2.30E+06
ARREPLFDY	5	4	1		3.99E+06	3.15E+06	
ARVDTVIKYYYYYMDV	5			5			6.41E+06
TKDITSVYSSGSYYPS	5			5			3.86E+06
AKDTPFGGY	5		4	1		3.38E+06	3.04E+04
ATRRCTISDCYASSYKSFDY	5	5			1.77E+06		

ARVTFPDGDNPNNWFGP	5		2	3		1.40E+04	6.42E+03
AKDLSLRRPTTGFFDF	4		4			9.72E+07	
ARDPLWLVVDF	4		4			1.75E+07	
GRSGSDPHRDYPTYNWFAP	4		3	1		5.26E+06	3.59E+06
ARRLDYSGSGGFYSNSRLYFHH	4		4			7.01E+06	
ARAVEWTVSLRFDY	4	2	1	1	1.25E+06	2.63E+06	2.36E+06
VKDIQPWSPGRGYCDS	4		3	1		5.00E+06	
ARSLASYGDFDS	4		3	1		3.16E+06	1.42E+06
ARHPCISSTPEGFIDS	4		4			4.41E+06	
ASPPTLHSRGYLDVRAFDL	4		4			1.99E+06	
ARHPTYTYTGSYFDN	4			4			1.79E+06
ASPSYDRGRPLKF	4			4			9.16E+05
AREHIDTITAARGAFDT	4			4			8.65E+03
ARMSKYRSSVYYFDF	2	2			4.66E+06		
ARDYGDALLDY	2		2			3.97E+06	

Anti-H3 A/VI09 serological repertoire clonotypic antibodies

Representative CDR-H3 Sequence	Total PSM	Day 0 PSM	Day 28 PSM	Day 180 PSM	Day 0 XIC	Day 28 XIC	Day 180 XIC
ARVIVEVYGRAGTGDY	298	52	121	125	8.36E+06	4.73E+07	5.20E+08
VYSGEETLFGSENSYKGFDP	90		14	76		1.51E+06	8.56E+07
AGGPTRDFNIFAPATY	81		19	62		1.19E+06	3.34E+07
ARMSGLYYMDV	71		57	14		1.07E+07	1.80E+07
ARDHSPADPLVDGGSWFDP	50		33	17		6.82E+06	5.61E+06
ARSAPMVQGASGWFDP	47		9	38		1.13E+06	2.31E+07
TTVVVGGTSYRHY	36			36			2.10E+07
ARAPLGVTGSPPAFDY	32		12	20		2.88E+06	1.98E+07
ARNQKYSGYDLPSEDYYYYYMDV	32			32			1.76E+07
AKEKGGVDY	31	20	2	9	1.57E+06	2.38E+06	6.84E+06
ASPPTLHSRGYLNVRAFDV	31	11	20		1.50E+05	1.24E+06	
ARHPITQSTPEGFIDY	29		9	20		2.16E+06	4.47E+07
ARTRTGYHYGSGSFGDYYYTDV	24		9	15		9.58E+05	7.36E+06
AGVRTARGDDAFDI	23		14	9		9.78E+05	2.92E+06
ARDWLGTSSAYGQSRGTEY	23		10	13		2.52E+05	1.74E+06
ARGEPYSDSGGYYDGPNRIPDYWYFDL	22	3	12	7	9.32E+05	5.20E+06	1.17E+07
ARVGSVPGRPPFDY	22		12	10		4.64E+05	9.30E+06
ARGERRSSDFDF	18			18			2.35E+07
ARGSSSWPLDY	17		7	10		1.12E+06	3.31E+06
ATRESGATEYYYYTYMDV	15			15			6.06E+06
ARGQLVVQDAGWFDP	14			14			3.54E+06
ARGYASYNFDY	13		10	3		1.83E+06	2.13E+07
ARLITMIQGTRGEFDS	12		6	6		2.90E+05	5.86E+06
ARMTMMVQGLKGDFDL	12		2	10		5.61E+05	4.04E+06
AGDRDSYARLDY	12		9	3		1.14E+06	1.29E+06
AGLNARYYFDS	12		7	5		2.04E+05	4.85E+05

AKTPPPTISAQLVFDR	11			11			1.04E+07
GRGGVAATPPGMDV	11		8	3		5.60E+05	2.40E+06
ARIYDNSWYRYFDL	11	1	6	4	9.16E+04	2.09E+05	2.13E+06
AKAPHSVVGYDLDY	11		11			1.55E+06	
ARAGDSATWKAPLDH	11		11			1.13E+06	
AREDIDTITAARGAFDA	11		11			2.53E+05	
AGERDSSSSFQFDH	10		4	6		3.09E+05	1.59E+07
ARSPKFRGVVIPPTYFFDL	10			10			1.34E+06
ARGSEWKIRAETPEYYFDF	10			10			1.20E+06
AKDRGLSLHSSYAMDR	9			9			7.92E+06
ARAYHDVLTGNENSLGYYGLDV	9			9			3.86E+06
TRDAWEYTKSSYGDS	9			9			1.83E+06
ARDNIIFGVISGLDS	9			9			8.23E+05
ARDQLHCSGGSCDTGLDV	9		9			7.62E+05	
ARAGVMVFGVIMKGGLDV	9	4	5		1.35E+05	5.24E+05	
ARDYGDALLDY	9		9			4.00E+05	
ARGSNFGVTPIPSFDY	8		3	5		1.36E+06	1.09E+07
ARDRGGTTTPDFDY	7			7			3.09E+06
ARARGVYNYYFDP	7		7			1.91E+06	
TKDELSSPRGYFVD	7		7			3.39E+05	
ARVDSGSLDS	7		7			2.89E+05	
ARDPKAGYIYGYPGAIRGVIGHEGGIDY	6			6			3.17E+07
AFSSSSSRRPFDS	6			6			1.04E+07
ARMSKYRSSVYYFDF	6		1	5		4.79E+05	4.41E+06
ARVNTGWLEY	6			6			9.91E+05
AKTPPPSIAAQLVFDY	6			6			4.30E+05
ATHMLRERAIDY	5			5			8.97E+06
ATQGRTIWGSYRVFDS	5		3	2		1.40E+06	5.75E+06
ARDPTSFGFDF	5		2	3		1.10E+06	1.29E+06
ATEKRAVNYYDSTGYFDY	5			5			2.03E+06
ARGPYDVWSGYPAFDY	5			5			4.47E+05
ARVEPMIQGTRGRIDP	5		5			5.73E+04	
AKDPHSIAVYPPDH	4		4			2.02E+06	
ARGDPVGATTTGFDY	4		4			3.94E+05	

Anti-Vic B/BR08 serological repertoire clonotypic antibodies

Representative CDR-H3 Sequence	Total PSM	Day 0 PSM	Day 28 PSM	Day 180 PSM	Day 0 XIC	Day 28 XIC	Day 180 XIC
AKSLQQLADPEFDY	1626	65	784	777	9.53E+07	1.72E+09	1.65E+09
AKLIVLRFSEWSYDL	881	8	349	524	3.97E+07	6.66E+08	1.89E+09
ARGYASYNFDY	697	72	225	400	8.48E+07	4.91E+08	7.00E+08
AGDPSRGYGQEPPHAPFDF	364	1	202	161	4.24E+04	6.04E+08	7.67E+08
VKEVGDDHGYYIGAFDI	313	3	118	192	1.92E+06	9.31E+07	6.76E+07
ARGLMGHYYDSSGSYD	249	32	106	111	8.84E+07	7.80E+08	1.09E+09
VRQFSEYLSPPWNTPMACFDY	113	7	41	65	5.85E+06	1.81E+07	3.46E+07

ARVTFPYGDNPNTWFDP	90	21	30	39	1.07E+07	8.14E+08	1.17E+09
AKDSGSNIYYDFLGYYMNL	76	1	54	21	2.10E+04	2.28E+07	1.03E+07
ARDVAARPGALQH	72	14	26	32	1.41E+07	4.75E+07	5.83E+07
ARGPSRSGYYYGYFQQ	71		29	42		3.90E+05	7.35E+05
ARDLGAGHYTEGVPDY	69	6	38	25	1.07E+07	1.99E+08	5.01E+07
ARDVKYHYDTSDNYYTGGLDS	58	14	25	19	6.92E+07	1.34E+08	9.45E+07
ARGGGYQLRGGHFDY	58		20	38		1.66E+06	7.46E+05
ARSPSDLWNGYRLYFDY	56	9	27	20	8.03E+06	3.67E+07	2.85E+07
ARDRTPDCYDCTPTFDY	54	7	18	29	2.02E+07	9.92E+07	1.14E+08
ARSFASYGDVDS	50	6	10	34	1.19E+06	4.57E+06	1.74E+06
AGRRAGTVD	44	4	23	17	1.10E+07	5.31E+07	3.16E+07
AREDIDTIMAARGAFDI	43	4	18	21	9.07E+06	7.72E+06	3.13E+04
ARDVAYQAFDY	42		6	36		7.72E+04	3.82E+06
GRDKGTGWSPIDH	38		28	10		5.68E+07	1.39E+07
ARDLGGGFYGMDV	38			38			4.72E+05
AKSSSSGWDWSGAADS	37	3	14	20	7.47E+06	3.28E+07	6.25E+07
ARAAWAYEADSGYSLGPLDH	37		20	17		2.30E+07	2.79E+07
ARGRRGNNYYFDY	37		13	24		1.39E+04	2.49E+05
ARDWGSGSYNLDH	36	11	12	13	1.44E+07	4.28E+07	4.97E+07
ARDKDGYTPGDY	35	8	16	11	4.99E+07	2.36E+08	2.23E+08
ARDKGVPTYSYSGMDV	34	5	19	10	2.01E+06	4.45E+07	2.44E+07
AKQANCYEDNGYFKGNWFGP	33		17	16		6.57E+07	7.60E+07
ARGLNYYDYSDQGVRCDY	32	6	14	12	4.97E+06	9.65E+07	2.60E+07
ARSTKDHLVRGIGSFDP	32	6	14	12	6.12E+06	2.04E+07	1.64E+07
ARDFESYFMDV	31	7	10	14	1.06E+07	1.58E+07	1.86E+07
AKGRSTVVTTAGNWFDP	30	9	12	9	7.43E+06	1.22E+07	2.31E+07
ARDDYYHSSGYHVH	30	5	14	11	6.35E+06	1.44E+07	1.27E+07
ATGGRRLRANYYFAMDV	30		21	9		1.22E+07	4.06E+06
VPLLARPLVEERMRD	28	1	6	21	1.90E+06	5.86E+07	1.61E+08
ARGERRSSDFDF	28	11	11	6	3.41E+07	2.84E+07	2.53E+07
AREYQSYAFSV	28	4	6	18	1.60E+06	6.67E+04	5.08E+05
AKDREIAVPRTGCFDY	27		6	21		4.69E+05	2.17E+04
ARGGVATTPPGMDV	26	8	2	16	4.25E+06	1.14E+07	1.46E+07
VKDRLEWTLRGAFET	25	11	4	10	6.22E+07	2.78E+07	1.68E+08
ARTDLSHSNYFDP	23	12	5	6	1.33E+07	3.11E+07	3.79E+07
ARDRRGSGDY	23	2	13	8	2.39E+06	2.66E+07	1.45E+07
ARDRDGYHTLDV	23		13	10		7.31E+06	1.56E+07
AGQRLSRVARCDS	22	2	12	8	1.19E+07	3.68E+07	2.34E+07
ARVWPAANPGDY	21	8	6	7	8.53E+06	2.02E+07	3.72E+07
ARDSGYCGGDCYLPGGY	21	7	7	7	1.13E+07	1.50E+07	2.24E+07
AQGYGEHPHTTSTFS	20		13	7		3.24E+07	2.46E+07
GLSRYSRPSWFDP	20	5	14	1	3.11E+06	4.62E+06	1.09E+06
ARIYGDSSTVDP	20		6	14		3.14E+04	5.35E+04
ASLSAVQGGRGHNWLDP	18	6	6	6	1.18E+07	3.44E+07	4.37E+07
ARGGDSGPGDKLDN	18	5	4	9	4.48E+06	7.11E+06	3.86E+07

ARDLMPDHYLYDSSADNGMDV	18		11	7		3.37E+07	1.62E+07
VVSLSVGGRLEVP	18		12	6		1.42E+07	2.44E+07
ARVGREVAGQYFFDF	18		4	14		3.85E+06	2.82E+06
ARLKGGNSGGYFDY	18	3	8	7	8.28E+05	1.50E+06	2.75E+06
ARDLTALAYGSSPDFYEYGMDV	17		7	10		7.44E+06	1.74E+07
AQDSGSNIYYDFLGKYMVG	17	6	7	4	5.51E+06	7.53E+04	1.34E+07
ARVFQDYFMDV	17		6	11		1.82E+07	1.98E+05
ARDFYGGNSLSLDS	17	8	8	1	2.72E+06	3.05E+06	2.09E+06
AREEENTSLNDY	16	3	7	6	2.24E+06	2.48E+07	3.25E+07
ASEKFAVTTISGFEM	16			16			1.37E+05
ARRGGYSNHGDGRNGFDS	15	5	7	3	9.64E+07	9.94E+07	1.22E+08
AKEKGGVDY	15		9	6		3.87E+07	3.65E+07
VRFSGGNPKAETDY	15		8	7		1.01E+07	1.30E+07
CRVYNSYYMDV	15		10	5		1.19E+07	7.07E+06
ARRTGDY	15	2	8	5	4.75E+06	8.18E+06	3.74E+06
ATHSGDHYRSMYMDV	14	3	8	3	7.87E+06	3.38E+07	7.94E+06
ARWGVVSSDSYYGRSKDAFDI	14	4		10	1.25E+06		3.34E+07
ATGPFVF	14			14			3.46E+07
ARTMESYFLDH	14		6	8		9.33E+06	1.24E+07
ARSNWNLPETGFGAFDM	14		4	10		4.94E+05	4.41E+05
TRGPLMRGGVWFGELRDPWDT	13		7	6		1.78E+06	6.83E+06
ANDRGVVIGPRNDPFET	12	1	7	4	8.67E+05	4.05E+07	3.57E+07
AKDLSRPSLRYFDS	12	3	6	3	5.55E+06	2.08E+07	2.02E+07
ARGVGEYDYLNTYFPAQNFDY	12		7	5		1.73E+07	1.40E+07
ATESHGEVERSGFFDN	12		9	3		7.02E+06	3.75E+06
TTDYGDYVTGGR	11		11			4.41E+07	
ARLKDYVWGS	11	4	7		1.89E+07	2.45E+07	
ARSRTYYPTYCDY	11	7	4		9.92E+05	3.89E+07	
ARLNNGGYNLGLLNY	11		3	8		3.74E+06	2.97E+07
AGDYK	11		6	5		1.91E+07	1.33E+07
ARGTKWALGRGVGWFDP	11	3	6	2	1.38E+06	1.68E+07	1.22E+07
TTDRKWDDSSMS	11	11			2.03E+07		
AKDPFGGNIESYFDS	11		4	7		9.03E+06	9.64E+06
ARTGMASVTPGALDV	11		4	7		5.92E+06	5.48E+06
VKDHTNWNYGY	11		4	7		8.17E+06	1.92E+06
ARESDGDYSPGPFDY	11		5	6		5.33E+06	4.40E+06
ARGGWLRDGVGGNFFDY	11		1	10		3.14E+03	1.32E+06
ARDGDRSSYYGTNYFYMDV	10	4	6		3.61E+06	2.74E+07	
ARQVEDPYYYDSSGFLDY	10	2	6	2	2.25E+05	1.62E+07	1.35E+07
ARDEAQGRNYDYIWGRSNGMDL	10		8	2		1.11E+07	8.26E+06
VQDTGRSGLY	10	2	3	5	2.66E+06	6.07E+06	6.96E+06
TKDITSVYGSGSYYPS	10		1	9		3.90E+03	1.52E+07
ARGSYSTIWGSYRAFDY	10	8		2	1.45E+07		3.56E+04
AGSLRGNWFGP	10	5	4	1	2.48E+06	3.92E+06	6.95E+06
ARGDSSGYLPFDY	10			10			2.32E+05

TTQGKQWLLFDY	9	5	3	1	9.88E+06	1.31E+07	4.41E+07
AIRVSTGGIH	9		9			6.03E+06	
ARLQQGLIGDFSMDV	9		5	4		3.30E+04	1.78E+05
ARDLNHYHSSGFFYKGAHYFDD	8	5		3	3.29E+07		6.50E+07
ARGGVAAPPHFDY	8	1	4	3	4.09E+06	6.72E+06	2.48E+06
AKGGVLRFSEFHMDV	8		8			8.78E+06	
ARQVVDGDDNWFDP	8		2	6		1.60E+06	7.17E+06
ARCTKWAMVRGVGWFGP	8		7	1		4.37E+06	3.63E+06
ARDYGYDSSGYLPGY	8	2	6		1.83E+06	4.88E+06	
ARGHPDTAMAPDY	8			8			6.34E+06
CREGDLSSFYLGSGSPNRHLDS	8		1	7		1.49E+05	3.76E+06
ARDRHYYVSVFDH	7	1	2	4		6.25E+07	2.26E+07
AFSSSSSRRPFDS	7	1	3	3	3.83E+04	4.38E+07	1.10E+07
ARVSNVVVEAAGGQIDY	7		3	4		1.08E+07	7.89E+06
ATKGQSSRWKSFDV	7	1	3	3	2.41E+06	8.89E+06	3.84E+06
ARDTGELLGYRWLDP	7	5	1	1	4.47E+06	4.28E+06	3.39E+06
AQGYGERPYTRSTFS	7		7			1.17E+07	
ARSHSYYHNCFEF	7		3	4		1.74E+06	9.46E+06
ARDAGYSKDWEPGY	7	3	2	2	2.74E+06	2.90E+04	5.79E+06
AREKDGDYSPGPFDS	7			7			8.12E+06
ARETHDYDSRGPPYPFDI	7		7			8.05E+06	
ARTWGGNPDY	7	4	3		1.57E+06	4.26E+06	
ARAIMDYNFLPFFDS	7		7			2.09E+06	
ARHNRDGYDYDWRLDY	7	5	2		1.76E+06	3.38E+04	
VREYDSSGYYQTGAFDI	7		4	3		1.60E+05	1.28E+04
ARGTYSTMWGSYRAFDF	6	1	4	1	5.36E+07	1.58E+07	9.34E+03
ACPGDTPMARRVFDY	6	6			8.35E+06		
ASPPTLHSRGYLNVRAFDV	6	5	1		4.45E+06	2.64E+06	
ARSRPGARITIFGVSVSRNPFDI	6	4		2	3.34E+06		1.98E+06
ARHGKLDHPWAYDTSGRGWFDP	6	6			4.46E+06		
ARSVGGASSNAFYYIDV	6		6			3.68E+06	
ARSTKHHLIRGVGYFDP	6		6			2.59E+06	
TLRAYGGYGPRAH	6	6			7.78E+05		
ARVDGNYHLDY	6		2	4		1.77E+05	1.15E+04
RVYNSYYFDY	6		1	5			1.86E+05
ARDRTYRGDAAHI	5		2	3		6.49E+07	6.12E+07
ARVGGSYGKEN	5		5			5.92E+06	
AKLMILRFAEWSYDS	5		4	1		3.37E+06	2.41E+06
VRDGASGWNFDY	5		2	3		2.68E+04	5.13E+06
AKDLDDDRGIFDS	5		5			4.84E+06	
ASRYSSRWSPFYF	5			5			4.19E+06
TRGRNIVGAAHY	5		5			4.04E+06	
AKDPDGQYYYDSSGYN	5		5			3.89E+06	
ARDPKQGLGSYNWFDS	5		5			2.25E+06	
ARDGGWIAGMDV	5		4	1		1.14E+06	9.04E+04

AKTSRQFLEWLSPFDY	5		4	1		1.66E+05	3.17E+04
ARDLMADHYRYESSADHGMDG	4		3	1		3.61E+06	4.40E+07
AREFGEFLGGEFSCVIDL	4		3	1		1.23E+07	1.82E+07
VKAFGSAWSFDY	4	1	3		2.55E+06	1.40E+07	
ASGRVLYGDYTSRYYLDY	4		2	2		1.49E+06	1.27E+07
ARDAGYSINWEPGY	4			4			7.90E+06
ARAGNTGYSYGSNFDY	4			4			7.11E+06
FTSGHDPRADDYSYMDG	4		4			6.33E+06	
ARTGFIFRDFDL	4			4			5.95E+06
ARSSRYDSSGYYVF	4		4			4.48E+06	
ARGDIVVGPVDKGGWFDP	4		4			4.01E+06	
ARSGGYNHGRGFDL	4	4			3.57E+06		
ARLEVFGSSWHAAYFDY	4		2	2		8.72E+03	3.17E+06
ARAMGATKYYFDY	4	2	2		1.97E+06	1.15E+06	
ARIVYRWGGDCYYYLDL	4		4			2.67E+06	
ARGTVGRMATVPGFDH	4		1	3			1.66E+06
VRMEGSYYMDV	4		2	2		1.59E+06	
ARDRVSGSGSSDY	4	4			9.63E+05		
ARGQTNSLYY	4		3	1		3.12E+05	4.27E+05
LKGPFYNMLTGDYGPWNNWFDP	4		1	3		2.15E+05	3.28E+05
ARSAPMVQGASGWLDP	4		3	1		9.35E+03	4.54E+05
VRDPAPYSGSPFDA	4		2	2		4.04E+05	2.33E+04
ARTKLLYDTYFDY	4	1	2	1	1.15E+04	3.11E+05	1.03E+04
ASGSGSRMLFDY	4		1	3		8.30E+03	2.84E+05
ARDSDSYSRLDY	4			4			2.70E+05
AKFSGSLHHDYYMDV	4			4			1.74E+05
ARPLWSGYKPNYVMDV	4		1	3			7.95E+04
ARGGTDSSGYYLSL	4	1	1	2	2.12E+03	4.08E+03	2.59E+04
AHTSIIVGDEAQLLGYGLDV	4		2	2		1.10E+04	7.03E+03
ARGASGSYVNVLAFDI	4		3	1		1.05E+04	

Anti-Yama B/TX11 serological repertoire clonotypic antibodies

Representative CDR-H3 Sequence	Total PSM	Day 0 PSM	Day 28 PSM	Day 180 PSM	Day 0 XIC	Day 28 XIC	Day 180 XIC
AGDPSRGYGQEPPHAPFDF	774		492	282		1.31E+09	1.03E+09
AKLMVLRVSEWSHDY	751	17	471	263	1.24E+08	5.00E+08	8.22E+08
ARVFLSYSFDD	519	73	229	217	6.92E+07	3.93E+08	4.11E+08
ARGLMGHYYDSSGSYD	267	47	116	104	1.75E+08	6.23E+08	7.81E+08
ARDRTPDCYDCTPTFDY	106	21	45	40	8.17E+07	2.93E+08	3.55E+08
ARVTFPYGDTPNNWFDP	95	14	40	41	2.82E+07	6.32E+08	6.93E+08
ARAAWAYEADSGYSLGPLDH	93	10	48	35	7.54E+04	1.78E+08	8.29E+07
ARDVAARPGALQH	73	30	10	33	4.90E+07	5.65E+06	6.70E+07
ARDLGAGHYTEGVPDY	64	12	36	16	1.46E+07	1.73E+08	3.82E+07
AGRRAGTVD	51		32	19		1.07E+08	3.24E+07
VRQFSEYLSPPWNTPMACFDY	51		31	20		3.47E+06	5.37E+06

AGDPSRGYGQLPPHGPFDY	50		33	17		7.00E+07	4.84E+07
ARARRGFYERGGGFFDPFDF	50	10	21	19	1.70E+06	1.28E+07	8.97E+06
TRDPFYYDTSGNPGGY	47	23	14	10	1.16E+08	9.73E+07	6.32E+07
ARSPSDLWNGYRLYFDY	46	5	29	12	1.02E+07	3.41E+07	2.64E+07
ARDVKYQYDTSDNFYRGGLDS	43	12	12	19	4.78E+07	3.15E+07	1.10E+08
ARDKGVPTYAYSGMDV	41		25	16		8.20E+07	1.50E+08
ARGLNYYDYSDQGVRCDY	41	5	14	22	9.20E+06	8.84E+07	1.98E+07
ARGGGDTYDYRVYFDY	39	12	14	13	2.23E+07	2.23E+07	2.55E+07
ARDKDGYTPGDY	36	8	17	11	7.13E+07	2.03E+08	1.19E+08
VRAIVEMYSRAGTGDY	35	11	10	14	2.41E+07	4.35E+07	2.80E+07
ARGRPTAVAGQMDV	35	18	12	5	1.55E+07	1.23E+07	1.61E+07
ARSTKDHLVRGIGSFDP	35	13	15	7	6.12E+06	1.43E+07	8.12E+06
ARDWGSGSYNLDH	34	13	10	11	4.24E+07	3.13E+07	2.18E+07
ARGGVATTPPGMDV	34	10	11	13	1.82E+07	1.09E+07	2.08E+07
AREDGTSWYYFDY	33	3	15	15	4.46E+06	2.69E+07	3.50E+07
AKQANCYEDNGYFKGNWFGP	31		18	13		5.28E+07	7.18E+07
ARVWPAANPGDY	31	8	17	6	1.94E+07	3.33E+07	2.27E+07
ARSLQQLADPEFEY	30	8	15	7	1.48E+07	1.19E+08	5.09E+07
AKGRSTVVTTAGNWFDP	30	10	8	12	8.19E+06	6.95E+06	2.88E+07
ARVTSDYDSRGSDPFDI	29		18	11		3.69E+07	2.62E+07
ARDFESYFMDV	27	7	12	8	2.75E+07	3.65E+07	2.33E+07
AKDSGSNIYYDFLGYYMNL	27		26	1		7.18E+06	6.21E+05
ARSLASYGDFDS	27		18	9		1.59E+06	4.02E+04
ARDLNHYHSSGFFYKGAHYFDD	25	12	6	7	8.19E+07	1.06E+08	1.14E+08
AGQRLSRVARCDS	24	6	8	10	7.46E+06	2.42E+07	2.13E+07
ARDDYYHSSGYHVH	24	13	2	9	1.58E+07	1.96E+06	1.94E+07
ARAWGGSPAAKDNWFDP	24	10	7	7	8.22E+06	4.71E+06	5.34E+06
VKDRLEWTLRGAFDT	23	4	7	12	2.59E+07	3.15E+07	1.31E+08
ASLSAVQGGRGHNWLDP	23	6	10	7	2.90E+07	4.13E+07	5.00E+07
ARDLMPDHYLYDSSADNGMDV	23		10	13		2.44E+07	1.93E+07
ARGPGYNVGWSPVRWIY	23	2	9	12	9.45E+04	1.17E+07	1.07E+07
VPLLARPLGEERMRD	21	1	10	10	4.91E+06	9.95E+07	6.61E+07
GRDKGTGWSPIDH	21	1	12	8	2.18E+06	2.53E+07	2.05E+07
ARETHDYDSRGPPYPFDI	21		15	6		2.53E+07	1.16E+07
AHSRMYYDDSSGWVYYFDY	21	17	4		1.73E+07	1.19E+07	
ARDSGYCGGDCYLPGGY	20	8	6	6	1.99E+07	1.83E+07	2.08E+07
VVSLSVGGRLEVP	20		14	6		2.58E+07	2.33E+07
AGSLRGNWFGP	19	8	2	9	9.20E+06	5.82E+06	1.07E+07
ARARGYTYVQERGGFDP	19	6	6	7	6.83E+06	4.75E+06	7.88E+06
GLSRYSRPSWFDP	19	8	10	1	5.59E+06	3.65E+06	8.00E+03
ARGASGSYVNVLAFDI	19		15	4		2.29E+04	3.12E+04
ARERVPYGGNLAEDACDI	18		15	3		3.57E+07	8.06E+06
ATESHGEVERSGFFDN	18		11	7		9.57E+06	5.04E+06
AQGYGEHPHTTSTFS	17		7	10		1.68E+07	1.70E+07
VRFSGGNPKAETDY	17		9	8		1.09E+07	8.22E+06

AKTSRQFLEWLSPFDY	17		13	4		3.38E+04	7.70E+05
AKDLSRPSLRYFDS	16	9	5	2	3.44E+07	2.75E+07	1.08E+07
ARVNLGTTRGLEY	16	6	5	5	9.35E+06	7.28E+06	2.77E+06
ARDRDGYHTLDV	16	1	11	4	5.66E+05	6.91E+06	1.11E+07
AREKSTCDGDCNDV	16			16			4.22E+06
VRERHSADSGGYYDAFDI	15		9	6		3.58E+07	2.48E+07
ARDPSRGYNQQPPPAPFDF	15		13	2		7.80E+06	4.37E+06
ARDYGYDSSGYLPGY	15	8	7		5.81E+06	5.68E+06	
ARGTKWALGRGVGWFDP	14	5	7	2	4.38E+06	3.37E+07	9.59E+06
AIRVSTGGIH	14		14			2.94E+07	
ARSVGGASSNAFYYIDV	14		8	6		1.53E+07	1.80E+06
ARLKGGNSGGYFDY	14	7		7	6.83E+05		2.74E+06
ARRGGYSNHGDGRNGFDS	13	3	7	3	4.24E+07	2.39E+07	1.44E+08
AREEENTSLNDY	13		8	5		3.45E+07	2.71E+07
ARQVEDPYYYDSSGFLDY	13		8	5		3.06E+07	1.69E+07
ARDFYGGNSLSLDS	13	9	2	2	3.44E+06	2.03E+06	2.53E+06
AIRGPIEDV	13		13			5.92E+06	
GRAMEA	12		2	10		5.27E+07	7.28E+07
ARDEAQGRNYDYIWGRSNGMDL	12		6	6		1.00E+07	3.05E+06
VKDHTNWNYGY	12	3	2	7	2.49E+06	2.76E+06	7.19E+06
ARRTGDY	12	4	8		5.91E+06	5.31E+06	
AQGYGERPYTRSTFS	12		12			6.30E+06	
TTQGKQWLLFDY	11	2	2	7	1.75E+07	1.75E+07	1.01E+08
TTDYGDYVTGGR	11		11			2.36E+07	
TKSVAGFHYYMDG	11	5	6		7.56E+06	5.72E+06	
AFSSSSSRRPFDS	10	1	4	5	3.54E+07	2.38E+07	1.49E+07
ARTDLSHSNYFDP	10	5		5	1.75E+07		3.76E+07
ARGGDSGPGDKLDN	10	5		5	2.35E+06		1.42E+07
FTSGHDPRADDYSYMDG	10		10			1.18E+07	
ARNRCTASYYFDY	10		8	2		7.42E+06	3.32E+06
ARVHSGSYYL	10	2	4	4	1.20E+04	4.59E+04	6.41E+05
AKEKGGVDY	9		9			4.46E+07	
ARGVGEYDYLNTYFPAQNFDY	9		7	2		1.83E+07	5.29E+06
VRGFLATRQAGWFDP	9	5	2	2	3.64E+06	3.60E+06	6.00E+06
AGDYK	9		5	4		8.46E+06	1.94E+06
VRTLSYENALDS	9	4	1	4	3.56E+06	7.20E+05	4.75E+06
ARGDIVVGPVDKGGWFDP	9		9			6.23E+06	
AGGHPDTAMAPDY	9		4	5		1.14E+04	4.31E+05
ARVDKSYHFDS	9		4	5		4.15E+04	1.95E+04
ARIYGDSSTVDP	9		5	4		1.22E+04	3.04E+04
ARRGGGALSVASYHGMDV	8		4	4		2.44E+06	1.95E+07
ARTGMASVTPGALDV	8		4	4		5.35E+06	8.23E+06
VYSGEETLFGSEKAYKWFDP	8	1	6	1	1.56E+06	8.94E+06	1.38E+04
ATDKYHSYYDLAKYFQH	8		2	6		1.44E+06	8.55E+06
ARSTKHHLIRGVGYFDP	8		8			4.04E+06	

ARQPFRGTTVEGWFDP	8	2	5	1	1.10E+05	2.35E+06	3.43E+05
AGVATFVQRVAISSLAGGGPYDY	7		4	3		1.11E+07	5.95E+06
AKSSSSGWDWSGAADS	7			7			1.39E+07
ATQGRTIWGSYRVFDS	7	5		2	8.16E+06		4.68E+06
ARGPYEFGSGYPAFDY	7	5	2		5.24E+06	1.38E+06	
ARASQEYTRTSFAFDI	7	7			6.43E+06		
ARDLTAMSNQDYNHYYAMDV	7		7			4.53E+06	
TRGRNIVGAAHY	7		7			4.22E+06	
AKGGVLRFSEFHMDV	7		7			3.30E+06	
AKVAGYHYENYCDY	7		5	2		9.92E+03	2.31E+05
ARGDSSGYLPFDY	7		1	6			9.72E+04
ATHSGDHYRSMYMDV	6	2	3	1	1.10E+07	2.21E+07	9.17E+06
ARVGNSSAWLAAFDY	6	1	3	2	1.61E+06	2.34E+07	3.51E+06
AGEDIDTITAARGAFDF	6		6			8.05E+06	
AHVFRGGVTVAEYVKGFTSFDQ	6		6			5.30E+06	
ARQVEDPYYAHSSGFLDC	6		6			4.83E+06	
ARCTKWAMVRGVGWFGP	6		6			4.46E+06	
ASAPPIFCSRPKCYKYYGLDL	6			6			3.94E+06
VKGPCYNSVTGYYGPWNNWFDP	6	1	5		1.60E+06	1.61E+06	
ARGGFCSGGSCYYVS	6			6			1.24E+05
ARDRTYRGDASHI	5		3	2		4.50E+07	5.98E+07
ARDLMADHYRYESSADHGMDG	5	3	1	1	4.15E+06	1.05E+07	3.89E+07
ARRKNGSGGYWYYLDY	5	2	3		2.60E+07	1.90E+07	
ARRAGLMVYAMNAFDI	5		4	1		6.41E+06	1.18E+07
ARGRDTTYVYTLHFDS	5		5			1.04E+07	
ARDGDRSSYYGTNYFYMDV	5		5			9.84E+06	
AKDLARYIPTSGSFDY	5	3	2		6.28E+06	2.36E+06	
CRVYNSYYMDV	5		5			6.56E+06	
ARDDVRGVNKAMDC	5		5			5.84E+06	
ARGPHYYNRYLDY	5	5			5.74E+06		
AREDVTGYNPDY	5		4	1		2.79E+06	2.03E+06
ARERDSSSAFQFDH	5	4		1	9.53E+05		3.83E+06
ARSHSYYHNFFEC	5		5			4.31E+06	
RVYNSYYFDY	5		2	3		6.65E+05	4.84E+05
VRDRDSYGHFDY	5	2		3	6.94E+04		7.77E+05
ARHRGFDYLLSFDY	4	3		1	6.87E+06		8.13E+06
ARGRYLFGTDV	4		1	3		3.34E+06	1.14E+07
ARTMESYFLDH	4		3	1		8.64E+06	4.58E+06
AKDTSIAVRTVDY	4	1		3	3.85E+06		9.22E+06
ARDQIQARGYFDL	4			4			7.19E+06
TKDITSVYGSGSYYPS	4			4			5.95E+06
ARDAGYSKDWEPGY	4	3	1		2.60E+06	2.24E+04	
ARDPSSFPQQNYFDY	4	3		1	1.46E+06		4.84E+04
ARDDSGPENLFDY	4	4			1.29E+06		
VKEIGDDHGYNIGGFDS	4		4			2.20E+04	

Donor 4

Anti-H1 A/CA09 serological repertoire clonotypic antibodies

Representative CDR-H3 Sequence	Total PSM	Day 0 PSM	Day 28 PSM	Day 180 PSM	Day 0 XIC	Day 28 XIC	Day 180 XIC
ARDWQEKLVSDLVNSFDF	519		487	32		4.20E+08	1.54E+07
ANSKGKYSTSAYSYALDS	165		130	35		3.60E+08	1.28E+07
ARDDYVDTDLKNWYPDI	96	25	33	38	2.56E+07	2.36E+08	1.38E+08
AALIGVGVTSIRNRWRDWFDP	69	10	24	35	3.13E+06	9.47E+07	4.98E+07
VRAIGDHESY	66	5	37	24	4.81E+05	2.21E+08	1.76E+07
ARQSPIALGAFDI	65		46	19		1.98E+08	4.49E+07
VRAIAAADST	64		44	20		2.91E+08	1.08E+07
SRDRGDYRYSPGSH	61	22	11	28	7.93E+06	3.01E+07	5.47E+07
ARISTRDYDTVTAYRVYGMDV	44		40	4		6.56E+07	1.32E+06
ARRGGSSGDFLD	40	13	15	12	5.55E+06	2.29E+07	6.49E+06
ARLTMSPPWLLDS	39	1	27	11	1.55E+05	6.41E+07	5.62E+06
ARDRGNVLTGCSIDN	39		17	22		3.65E+07	3.23E+07
ARTDGGYCSTTRCPRVFDQ	35	10	25		1.70E+06	3.49E+07	
VRAIGGYNSY	34		17	17		1.50E+07	8.63E+06
ARDFLQYASGSGAYYPDV	33		11	22		1.07E+07	1.02E+07
VKDAAGWERVFDN	32	6	26		1.67E+06	3.83E+07	
AKDRITFTLRGAFDV	31	6	18	7	6.93E+05	2.26E+07	3.22E+06
ARRMGATDLGYYFDY	29	1	20	8	2.92E+05	4.81E+07	8.31E+06
ARDRGDYRYSPGHY	25	6	9	10	4.80E+05	1.05E+07	6.10E+06
AREYYYDVDLHQFYMDV	24		13	11		7.77E+06	3.85E+06
AKDVNRGIVDRPWVSGFDS	22		15	7		6.84E+07	3.69E+07
ARTYSSERVGWFDP	22	1	12	9	1.33E+05	4.28E+07	3.96E+06
AKDLGVTPPTAGFFDH	21		21			4.99E+07	
AKTHSWLPTGA	20		13	7		6.81E+07	3.43E+06
ARVYRSYYFDS	20		7	13		4.50E+06	2.69E+07
VRDDYADTDLKNFYFDV	18		13	5		8.27E+06	1.23E+06
ARGTTGSYGID	18	2	7	9	6.86E+05	4.29E+06	1.90E+06
ARNMDY	18	4	8	6	6.43E+05	2.56E+06	2.40E+06
ARNLVGAVESRAFDI	16	3	10	3	3.97E+06	1.26E+08	1.97E+07
TRLYDFWNAYSNMDV	16		11	5		8.33E+07	8.89E+06
AKDLGVTPAAAGFFDY	15		12	3		4.96E+06	7.97E+05
AGWNPENYYDRSGWGWFDP	14		10	4		1.51E+07	1.18E+06
ATSHGSGSYPIR	14	2	8	4	8.50E+05	7.79E+06	4.00E+06
AKDADTRVWLSGNFDS	14		14			6.05E+06	
ARRSRDGYNLDY	14		14			1.24E+06	
ARDQHSYCDGGRCRHDAFDI	13	5	7	1	9.85E+05	1.97E+07	3.73E+06
ATTNRRKYDSSGYFYDY	13		9	4		4.98E+06	1.52E+07
ARDRPHLLTGSIFDY	13		13			1.57E+07	
ARHRGRYHPFDV	13		13			8.79E+06	
AKDLVTVLPLSGAFDI	13		13			4.84E+06	
ARDRITFTLRGAFNV	12		12			3.54E+07	

AKDLDGNVPRGVDS	12	8	2	2	8.68E+06	1.89E+07	4.73E+06
ARDTGTFGRNNYYMDV	12		10	2		1.41E+07	1.89E+06
ARAARYQNVDF	12		12			1.15E+07	
ARAEGYHDSSALPL	12		12			1.05E+07	
ARISPTARSYWGFDY	12		12			2.15E+06	
ARDHCTSPNCYMDP	11	1	2	8	2.96E+05	6.57E+06	1.11E+07
ARGKILTAYTRNVNFDY	11		11			9.03E+06	
ARGDSSGWDVDY	11	6	4	1	4.31E+05	3.23E+06	1.01E+06
ARDPPEDGTEFDY	11	6	5		7.50E+05	2.86E+06	
ANFRLGP	11		10	1		2.41E+06	4.76E+05
TRDPADTERGYFGLDV	10		9	1		9.06E+06	1.26E+06
ARDNYYDVDLKQFYMDV	10	1	4	5	1.07E+06	4.70E+06	4.51E+06
ARTGAYGDSLPTYPMDV	10		8	2		8.43E+06	1.31E+06
AKRHSSGWYIDY	10		10			8.61E+06	
ARDRAFGHSVYRGAFDI	10		6	4		7.16E+06	1.22E+06
ATRDYYDRTGTINS	10		10			3.00E+06	
ARLGYCYRSTCYFREAAKGLDP	10	6	4		4.37E+05	2.34E+06	
ARMLYGDSLYNFDS	10		9	1		1.93E+06	2.09E+04
ARPYASDDY	9	4	4	1	8.38E+05	1.25E+07	9.43E+03
ARPRGVTTVVTPDDAFDV	9		9			1.32E+07	
ARVVGETADGYYSDY	9			9			7.38E+06
TRHMSVAAARNPYYMDV	9	4	5		2.27E+06	5.10E+06	
GRVRYSRASSGMAA	8		4	4		9.00E+06	1.27E+06
ARSIVDQLEAFDI	8		8			9.54E+06	
AKDWAKVPSWQGYLDY	8		8			2.13E+06	
GGYWQERLGADLVNAFDM	8		8			1.83E+06	
ARGAGYYPLHFDS	7		7			2.28E+07	
ASPPGGREQLEAFDI	7		7			8.19E+06	
ASHSSFYYDRSGHFQQ	7		7			5.88E+06	
AKEDYADVDLKNWYCDL	7		1	6		1.49E+06	2.50E+06
TRDRPNPIAVAAPGWFDP	7		7			3.08E+06	
AREDVKPMAGRQCFDI	7		6	1		1.76E+06	5.56E+05
ARDRAADYGDEPANWYFDL	7		7			1.17E+06	
ARGLGTRPPFDY	6	1	3	2	3.57E+03	3.47E+07	3.98E+07
AKRHQIMWWFDN	6		5	1		2.20E+07	8.30E+06
VRPHKYPPNWNFDN	6		6			1.09E+07	
AGGWGARWFRFDP	6		6			1.08E+07	
ARGSSLKLDY	6		6			5.54E+06	
ARGVTYHYGSGLLY	6		6			3.79E+06	
AKDQVHTSMVPVYGYFAH	6		6			3.25E+06	
AREIGGIGSS	6		6			1.72E+06	
ARPERASMFDIFDI	6		6			5.79E+05	
AISKGKYSTSWYSYVYDI	5		5			5.52E+06	
VREQQGYSYGNIVVAQFRNALDI	5		5			4.77E+06	
ARGYSDSSGYFYRYYFES	5		5			2.70E+06	

ARDRGNILTGPSFDY	5		5			2.24E+06	
ARRLGRAFDV	5		5			1.89E+06	
AHEAYSYGSRQVDY	5	1	4		1.30E+05	1.25E+06	
AREDYADTDLKNWYIDL	5		5			1.05E+06	
ARGSRGLADFDY	5		5			6.79E+05	
AKALDTSMVFCFDY	4	1	3		1.39E+06	1.06E+07	
ANFLNRLIVLPTAGDTIDH	4		4			3.44E+06	
AKDIFSGKYYDRSGYFDS	4		4			1.62E+04	
ARHGACRFYFDY	2			2			1.11E+06
ARGIGGSSWVDY	2	1	1		1.75E+06	6.33E+06	
AKKGPGSQYKTFDA	1		1			1.66E+06	

Anti-H3 A/VI09 serological repertoire clonotypic antibodies

Representative CDR-H3 Sequence	Total PSM	Day 0 PSM	Day 28 PSM	Day 180 PSM	Day 0 XIC	Day 28 XIC	Day 180 XIC
ARDGRQGVVMVDARRGLDS	152	32	54	66			
AGDWQEKLVSDLVNAFDF	142	16	22	104		3.82E+08	1.54E+07
ARQSPIALGAFDI	95	3	10	82		1.98E+08	4.49E+07
GRAIGNHESY	75		32	43	4.81E+05	2.21E+08	2.19E+07
SRDRGDYRYSPGSH	75	18	17	40	7.93E+06	3.01E+07	5.47E+07
ARAHYEYGSSGYRGESPPGASFDY	52	3	6	43			
ARLTMSPPWLLDS	48		17	31	1.55E+05	6.41E+07	5.62E+06
ARQTNSGDQVDY	47	8	11	28			
AALIGVGVTSIRNRWRDWFDP	45		25	20	3.13E+06	8.45E+07	3.96E+07
VRAIAATDST	45		22	23		2.83E+08	1.08E+07
ARIQTGGFDY	45	12	15	18			
ARDPPAGEFSPNWYFDL	43		15	28			
ASIRRIDGGHYVGALKI	41	10	7	24	2.42E+06	3.53E+06	1.92E+07
ASLGRTETEIPAAGIIGSGIDP	36		20	16			
ARGGDSGDIPVVDFDH	34		17	17			
ARVRGDTETGKYFDP	33		33				
AKDTQIWFGELDRRAFDY	32		6	26			
AKIAYCGPGCYRSSNYFDY	31		16	15			
ARTDGGYCSTTRCPRVFDQ	30	2	15	13	1.70E+06	3.49E+07	
AKDRITFTLRGAFDV	29		9	20	6.93E+05	2.26E+07	3.22E+06
ARRVGATDLGYYSDY	28	3	8	17	2.92E+05	3.27E+07	8.30E+06
VRAIGGYNSY	27		6	21		1.50E+07	8.63E+06
ARDFLQYASGSGAYYPDV	27		8	19		1.07E+07	5.21E+06
ARCQLRGSETGHNGMDV	26		13	13			
ARRGGSSGDFLD	26	3	13	10	5.55E+06	2.29E+07	6.49E+06
ATERGWEFHHGGILDY	25		6	19			
ARDRGDSSGYNEFPVPDVLDF	25		5	20			
ARDGRQGVVMVDANRGCDY	25		7	18		2.94E+06	
ARTYSSERVGWFDP	24		11	13	1.33E+05	4.28E+07	3.96E+06
ARGQYYDGTGYWRGGQYYDGMDV	24		14	10			

ATERGWEFHHGGIIDH	23		6	17			
ARDGDGIDY	21	8	11	2			
ASPPGGREQLEAFDI	20	6	7	7		3.59E+06	1.49E+07
ARQKQNSSGSLDS	19		4	15			
GRCSRRVVNYYYYMDV	19		2	17			
AKKGPGSQYKTFDA	19			19		1.66E+06	
TRDPADTERGYFGLDV	17		12	5		9.06E+06	1.26E+06
VRVKTGALDY	14		5	9			
ARDRITFTLRGAFNV	13		7	6		3.54E+07	
VKDAAGWERLFDN	12		5	7	1.21E+06	2.95E+07	
ARDRGDYRYSPGHY	11		2	9	4.80E+05		6.10E+06
ARGERSSGWYFDL	11		6	5			
ARLVVAPTALPYGMDV	11		6	5			
ARGGESGDIPVADFDY	11		9	2			8.41E+05
ARDQHSYCDGGRCRHDAFDI	10		1	9	9.85E+05	1.97E+07	3.73E+06
ARLTTQPTDDYYGMDV	9			9		1.69E+06	
AKDLDGNVPRGVDS	9	2	7		8.68E+06	1.89E+07	4.73E+06
AGGYCGSTRCHNWYSDL	9		3	6			
ARHLVDTAMANAFDI	9			9			
ATYYGDYARSAYDT	9			9			
ARDQLEEVQYCSGGSCPRNDY	9			9			
VRDQLEDLQYCSGGRCPHNDY	8			8			
ARGMDDSSGHNYHYFYMDV	8			8			
AREARRVEAGTLGGFDP	8		8				
ARQVDPGVANAQEGMDV	8			8			
VRRTGDY	7		3	4			
ARPYASDDY	7		5	2	8.38E+05	1.25E+07	9.43E+03
ARVSGPSGPGGMDV	7		6	1			
ARAGIAPGTEYGMGV	7		7				
AHEAYSYGSRQVDY	6			6			
AKRHQIMWWFDN	6		1	5		2.20E+07	8.30E+06
ATRDYYDRTGTINS	6			6			
ARWPVTHLEYSRSGFDY	6		6				
ARGAEGQWFADY	6		5	1			
ARHGACRFYFDY	6			6			1.11E+06
AKDADTRRWFSGNFDY	6			6			
ARIRPAISGTGFDP	6		6				
AKARGSGIAPPLDY	6		6				
ARDAIPKDVEDGMDV	6		6				
ARAGDSSGDWYFDL	6		6				
AREGYYDVMTGEEGNWFDP	5			5			
ARHVGSGWGFDH	5		1	4			
AKEDWEPATGGGNDAFDI	5			5			
TTRISVAGPDAFDI	5			5			
ARDRAFGHSVYRGAFDI	5			5		7.16E+06	1.22E+06

ARRVWSD	5		5				
ARDRWARYESPSFDS	5			5			
VRVKIPEMTTLDH	5		3	2			
ARGSEGGWGVDY	5		5				
ARGIGGSSWVDY	5		3	2	1.75E+06	6.33E+06	
TRLYDFWNAYSNMDV	5			5		2.73E+06	5.43E+05
ARAATISVSGLVRRKPYYMDV	5			5			
ARESAIVYFDH	5			5			
AKDLEW	5		4	1		2.06E+04	
TTERGWEVHNGGILDH	4			4			
AREFQSYNFDH	4			4			
ARHEAVVATYYMAV	4			4			
ARHPLYYSDNRAISDGYFDN	4		4				
ARGRGTRPPFDY	4		4			1.01E+06	
VRISSGSYLGFFDS	4		4			2.21E+05	
AREDVKPMAGRQCFDI	3			3		1.76E+06	5.56E+05
TRHMSVAAARNPYYMDV	2			2	2.27E+06	5.10E+06	
ARRSRDGYNLDY	2		1	1		1.24E+06	
ARGKILTAYTRNVNFDY	1			1		9.03E+06	
AKALDTSMVFCFDY	1		1		1.39E+06	1.06E+07	

Anti-Vic B/BR08 serological repertoire clonotypic antibodies

Representative CDR-H3 Sequence	Total PSM	Day 0 PSM	Day 28 PSM	Day 180 PSM	Day 0 XIC	Day 28 XIC	Day 180 XIC
ASFATSGSYYDIFSK	1195	15	414	766	6.01E+05	1.90E+09	5.05E+09
ARVKWDRLTGAPTDLDS	506	5	369	132	1.67E+06	1.92E+09	1.34E+09
AGHLLRGVPDALDI	342		92	250		2.04E+08	7.02E+08
ARDVEGQSYGSSWFDP	321	2	255	64	3.01E+05	3.51E+08	2.47E+07
ARVYCVADCYRISETYGYHYGMDV	298		280	18		8.91E+07	5.88E+07
ARDIEAGDYDSVGYPDH	210		155	55		5.07E+08	3.79E+08
ARGQYYDSSGYWPAGYHYFGMDV	165		68	97		9.77E+07	3.53E+08
ARHGVATRGGLLYYGMDV	148	10	77	61	1.41E+06	1.50E+08	1.94E+08
AREGGSQVLPYSWFDP	138		109	29		3.03E+08	5.15E+07
AKDFFQYASGSGAYYPDF	134		80	54		2.70E+09	7.83E+08
ARVKWDASTGAPSDLDF	116		90	26		1.31E+08	2.67E+07
ARESSDWDLLSPDD	111		70	41		1.49E+08	1.27E+08
VKDAAGWERVFDN	105		42	63		5.69E+07	1.71E+08
ARHGAWRFYLDY	95		79	16		2.75E+07	1.35E+07
AKDIARVSMDVVDIIAGFDS	91		61	30		1.64E+07	6.27E+06
AKDISPVNMDSVVFGVFDI	90		60	30		2.34E+07	1.10E+07
AKDGRTEYCSTISCSYFDP	73		50	23		8.68E+07	7.94E+07
ARTRGRYLPLDY	64		51	13		1.44E+08	2.77E+07
ARKMVTTWRWVNAMDV	60	2	26	32	9.17E+04	4.21E+07	2.05E+08
VRAIAATDST	56		33	23		7.50E+07	4.32E+07
ARDNPHGYSTFGWLDP	53		40	13		2.27E+08	7.41E+07

SRDRGDYRYSPGSH	51	12	11	28	6.58E+05	6.36E+06	7.12E+07
VKDIWEDTYGGELPVGAFDL	50		20	30		2.68E+07	4.09E+07
AKKLVDY	48	17	10	21	6.67E+05	1.25E+07	5.54E+07
ARGHDGLDV	46		19	27		1.02E+07	3.42E+07
ARDRGDYRYSPGHY	44	3	13	28	1.75E+05	2.51E+06	3.25E+07
GRAIGNHESY	43		27	16		4.28E+07	6.93E+07
ARLRAWNLDAFDI	43		39	4		2.49E+07	4.12E+06
VRSIPGAGYFYYMDV	39		21	18		2.03E+07	1.18E+08
AREMGYDDGVYYLSDALDV	38		16	22		5.33E+06	1.59E+07
ARGDGVATVYWFDP	36		10	26		7.10E+06	3.19E+07
ARHRGRYHPFDV	36		28	8		1.56E+07	7.21E+06
ALAPFGDKRLDS	34		22	12		3.00E+07	2.18E+07
AALIGVGVTSIRNRWRDWFDP	32	13	2	17	1.45E+06	2.53E+08	1.29E+08
ARDNSGYGHNAY	32		14	18		5.63E+07	2.80E+08
ARHLVDAAMANGFDV	32		8	24		2.80E+07	6.48E+07
ARAWVLEMATPLDY	28		15	13		7.05E+07	6.99E+07
ARERDDYFFDY	28		20	8		2.13E+07	1.44E+07
ARALSDYGDLQYDF	28		14	14		1.19E+07	2.26E+07
ARDEDTPFLRVFDH	27	8	7	12	4.66E+05	1.84E+07	4.04E+07
ARTRGKLFGFDY	26		15	11		1.69E+07	2.31E+07
ARATSYYYDSLPDRAFDI	25		10	15		2.30E+07	1.40E+07
ARLGFDSSTGSNQEFDL	25		21	4		1.74E+07	8.76E+06
ARDDFALTPRVLDV	23	12	8	3	2.61E+06	5.19E+06	2.07E+07
VRAIGGYNSY	23	1	9	13	2.85E+05	5.55E+06	1.11E+07
TKDISAVAVGTETPTFDY	23		23			3.71E+06	
ARHEQWLFPSNFDY	22		16	6		3.06E+07	2.85E+07
ARDRAYYDSSGYYPDFKIFDY	20		8	12		7.03E+06	2.11E+07
AKGPGYSNSWKAFDI	20	11	5	4	2.02E+05	1.47E+06	6.98E+06
ATVSGGRGFV	20			20			5.76E+05
ARESEDYGDNFFDP	19		13	6		2.70E+07	1.21E+07
ARGLFDSATLTDH	18		8	10		5.03E+06	1.01E+08
ARVSSGGHLGYFDY	18		10	8		3.27E+07	1.15E+07
GRHLIWRLGSFDY	18		6	12		1.99E+06	1.09E+07
AREVPSDTPAQRRLNGFDP	17	4		13	1.28E+05		5.25E+07
ANSKGKYRTSSYSYALDS	17		7	10		1.26E+07	2.50E+07
AKDAWTTVPRLYFED	17		13	4		1.90E+07	8.66E+06
ARMLLDYYSTGSYPLEDV	16		9	7		1.03E+07	2.03E+07
AREFQSYNFDH	16		9	7		2.73E+06	1.91E+07
ARDENGWSGGFDS	16		15	1		1.71E+07	4.37E+06
ARELGYDDGDYYLSDALDV	16		8	8		4.20E+06	9.44E+06
ARRGGSSGDFLD	16	6	8	2	1.34E+06	2.47E+06	5.59E+06
AREPGELGYNWFREGMDV	15		7	8		1.02E+07	2.23E+07
ARGNPGLDPTWFDL	15		11	4		2.25E+07	5.39E+06
ARATSDYGDLYFDY	15		14	1		6.54E+06	2.04E+06
ARNLVGAVESRAFDI	14		4	10		5.66E+07	2.48E+08

ARGGESGDMPVADFDY	14		4	10		3.46E+07	6.95E+07
ARGLGTRPPFDY	14	3		11	6.58E+05		8.73E+07
ARGCSGGSCHRPGFDI	14	7		7	1.07E+06		1.84E+07
ARVFEYSSGWPFDH	14		13	1		6.46E+06	2.19E+06
VRRTGDY	13		5	8		7.01E+06	5.33E+07
ASPPGGREQLEAFDI	13	5		8	5.93E+05		3.77E+07
ARTDGGYCSTTRCPRVFDQ	13	9	4		4.95E+05	3.53E+06	
ARIPFYESSGLRGNCFDP	12		12			1.59E+07	
AKVGVTDAYDHLSGAVDV	12		5	7		9.84E+06	2.96E+06
ARDPLEDYNDYAGGAFDI	12		6	6		5.72E+06	5.37E+06
ARDGRRWLQLMPVY	12		4	8		7.12E+05	9.88E+06
ARDEDTPFVRAFDR	12	10		2	3.79E+05		5.03E+06
AKTVVADPGVYYYMHV	12		12			4.23E+06	
ARGWGHHYDWFDP	11		7	4		1.82E+07	7.44E+06
AREKADYGDNWFDP	11		11			1.29E+07	
AKDIGYTSGWSEAFHV	11		10	1		1.07E+07	1.49E+06
ARDLRLASGWSLGNDY	11		11			9.78E+06	
ARMLYGDSLYNFDS	11		2	9		2.08E+06	4.58E+05
TRDDLMTTVTTCVH	11		1	10			1.16E+05
ASRPPNLRADSY	10		4	6		3.67E+06	5.44E+07
SRGDFWSDRHYYKGMDV	10	1		9	1.97E+05		2.61E+07
ARHGPWSWYSDN	10		5	5		1.06E+07	6.04E+06
ATDGEFRLGELAKGIPDH	10		4	6		6.02E+06	8.25E+06
VRDWGDTVLFPAMDV	10		3	7		4.32E+06	9.44E+06
AAADDARTDAFDI	10		10			1.01E+07	
ARNMDY	10	4		6	5.13E+05		8.71E+06
ARGFSNSFTDFLVRKKRGTFDF	10			10			8.47E+06
ATVLPRDGEQDYYYFYMDV	10	1	1	8	1.68E+05	8.10E+05	6.70E+06
ARVVGETADGYYFDS	10		3	7		1.23E+06	2.88E+06
ARHEAVVATYYMAV	10			10			2.52E+06
AKVSTKGDYVPPWFDP	9		2	7		2.15E+05	1.85E+07
ARHPAWLYPGFFDY	9		9			9.60E+06	
ARSDSSGWSLEDYFDY	9		9			3.24E+06	
VRDRETDFGDYFDY	8		2	6		2.49E+07	5.52E+07
ARHQSWGLHSFDY	8		5	3		1.28E+07	1.58E+07
ARENYDSSGFYPPDAFDI	8		8			9.21E+06	
ARESAIVYFDH	8		5	3		4.11E+06	2.85E+06
SRTYGSGSSENFDY	8		8			4.22E+06	
ARGTTGSYGID	8	2	6		2.98E+05	2.56E+06	
ARELGYDGGDYYLSDALDF	8			8			2.83E+06
ARIYSGGYLGYFDP	8		8			1.76E+06	
ARDPSDTAIVNQLDAFDI	7		2	5		1.31E+07	2.68E+06
ARIFSGGHLGYFDS	7		5	2		1.23E+07	3.37E+06
ARENSDGSGYYPPDAFDI	7		7			1.07E+07	
ARISPTAGSYWGFDY	7		7			4.19E+06	

ARDSPPRNGVEI	6	2	4		3.09E+05	3.91E+06	
ANFRLGP	6			6			4.16E+06
ARRWEEQYCRSTSCLWYFDF	6		6			1.48E+06	
ARCPGYYLEYFPH	5		3	2		1.31E+08	3.83E+06
ARERDYDSSGYWFEY	5		4	1		2.74E+06	3.08E+06
ARRSWQQEGIYYYYGMDV	5		5			4.01E+06	
ARTYSSGWRGFDH	5		2	3		3.24E+05	3.39E+06
ARELGYDGGVYYLSDAFDI	5			5			2.62E+06
ARYTLTPDHVSGMDV	5		2	3		1.84E+06	5.65E+05
ANGRFESFDY	5		5			2.05E+06	
ARQGGSGSYYLSFDY	4		3	1		4.37E+07	1.25E+08
ARWAGDTETGYDVDV	4		4			2.30E+07	
ARWGSVSSGYYYYYGMDV	4		3	1		1.26E+07	7.79E+06
ARDPGQHLLSINDLGLFDS	4			4			8.58E+06
ARRLGRAFDV	4			4			8.15E+06
ATYYGDYARSAYDT	4			4			4.21E+06
ARDKDSYGTLDN	4			4			4.04E+06
ARPTGLGASWDWFDP	4			4			3.82E+06
ARESSDWELLSADF	4		4			3.73E+06	
AKIFLGHRGGPGDDS	4			4			2.07E+06
ARVYCGGDCFRVSEAYGYYYGMDV	4		4			5.34E+05	
ARGMVRAPTYYYYYMDV	4			4			3.25E+04
VRMVAYTIGHGEFDC	4			4			2.13E+04

Appendix B

Anti-CA09pdm serological repertoire clonotypic antibodies. Values indicate the fraction (%) of the serological repertoire based on XIC.

Representative CDR-H3 Sequence	2009 Inf D21	2010 Vax1 D14	2010 Vax2 D0	2010 Vax2 D14	2011 Vax D0	2011 Vax D17	2012 Vax D0	2013 Vax D0	2013 Vax D16	2014 Vax D14
ARGQGYCLSASCPNYFDP	19.28	23.13	29.02	0.90	17.62	8.90	10.80	16.60	14.17	45.80
ARDRFGQRLRLFGPDY	7.42	6.46	2.92	12.01	9.01	14.59	8.08	6.26	1.96	1.65
ARGDRGGYEDY	0.92	2.40	2.60	1.04	3.98	3.97	4.09	3.23	0.88	1.84
AKASQYCRGGSCYANWFDP	1.15	2.55	0.72	0.29	0.80	6.85	1.55	1.35	0.34	0.38
ARNNYFDSGYGMDV		0.65	0.54	17.16	3.25	12.85	1.70	1.01	3.79	4.86
ARTLLDQGEAFHL	0.44	1.32	0.82	2.66	1.44	11.43	0.61	2.54	4.20	
ARGAPTHDFWSAYYPYGMDV	10.22	3.69	2.26		1.47	1.22	0.78	1.61	0.39	0.29
AKERPLRLLRYFDWLSGGANDY	1.44	4.59	0.51		1.80	1.18	4.03	1.84	0.14	0.38
VKDLGDGGGFSCLES	1.37	1.00	0.95		0.48	5.27		0.72	2.01	5.06
ARNPSHGSGSYYNARRADY	1.53	1.14	1.55		1.21		0.43	1.30	0.20	0.64
ARLVNQMAAAGSLPGKRRFDS	0.14	0.22	1.75		1.10		0.60	1.31	0.17	0.39
ARLPYGDFGRMFDD				22.94	8.72	13.20	5.43	3.68	3.24	4.01
ARHPTYHPSGSPFDY	11.64	6.32	8.40		6.93		3.38	5.21		0.51
ATSPTYYLSHLDS	6.91	7.49	9.35		4.45			3.65	1.17	2.09
AKDSQLRSLLYFEWLSQGYFDP		0.98	0.19	1.28	0.39	8.42	12.14	0.12		
AKGRRVGRLQTASDD	1.22	1.67	3.67		2.05			3.20	0.09	0.20
VKDSEYSSSSLEVTR	3.36	2.01	2.84		1.61	0.24	0.11	1.43		
VREREHLTVSGMLSETVKLHRHFDP	1.34	2.02	1.87		1.54	0.66	1.31	1.66		
ARGGNYYHTSLDY	3.23	2.25	0.74		1.17			0.27	0.14	0.64
AGSRLTDLGYCSGGSCYMYYFDY	0.57	0.43	2.47		1.67			1.86	0.21	0.70
ARDAGYYSGSYYEQ	1.29	0.65	1.07		0.44			0.78	0.41	2.18
ARQHSSGWYPWFDP	3.84				1.04		22.62	8.19	21.65	2.87
AKDLGTVLDYYGMDV	7.55	4.50	4.67		3.63		0.60	2.17		
AREDGTVTSGGGHYFDL		1.55	2.10		1.85			1.64	0.26	0.71
AKEGGARLLRYFDWLAHDALDV	1.37	2.80			1.75			1.59	0.14	0.02
ARQARGDYVGGLFDY	0.21			2.13	0.67	2.55		0.23	0.49	
AKEMVQGVLMNLFAY				5.80	0.76	2.23		0.18	0.93	
ARGRGYHFGDLVS	1.69	1.60	1.28		0.74			0.59		
ARGFDTATRPYDH	1.47	1.21	1.48		0.69			0.91		
ARDRTGINSLNALDV					0.62		1.67	1.05	0.77	1.49

ARNGYGDFVDWFDP				1.03		1.79		0.07	0.57	0.13
TTDLVRYYGMDV				0.16	1.02			0.49	0.61	1.03
AGVGLRPSVAGTETNY	0.89	0.88	0.70		0.38			0.28		
ARWKAMDV	0.35			0.36			0.84	0.18		0.09
ARSSGYHYSKYFDY	0.19	0.11	0.52		0.55			0.39		
ARDRGYGEVY	1.02	0.58	0.58				2.57			
ARVLTPGDSLYYFDY	0.88	1.26	1.01					0.20		
ATNKDSLGGYEAYDI					0.72			0.36	0.89	1.00
ARRGRQPEDAFDL		0.79	0.38		0.42		1.01			
AIARGHNLNAPHCDY					1.05			0.79	0.03	0.32
ARDWIV					0.37			0.18	0.27	0.29
AKVRRGSHECLDH			4.69		3.93			2.35		
ARAEGVQRGYYDILTAYYPTPDAFDI					0.40		7.41	0.70		
ATEGRGRPHGANPLDF								1.70	0.45	4.08
ARGLLVTARPPLDFDC				0.34					3.87	0.74
ARAPRYCTNGVCYREACDY		0.63			0.40				3.68	
ARTLYGDYAGGNFDS				4.36					0.04	0.07
ARGFLETARPPLDFDS						3.04			1.05	0.16
ARRLHGDTLYHFDY				1.44	1.62			0.98		
TRKDLTTAGGFDY	0.83	1.30							1.00	
ARVEDGSYYFDS		0.42	2.13					0.24		
ARARCSDCISTMDV								1.02	1.35	0.40
AGAGRTPLP								0.94	0.44	1.38
ARDRGRPPYFDLLTGYHKVAEGWFDP							1.55	0.83	0.22	
TTGYFPDTETDTAPQREQIFIVTPYDAFDI								0.01	1.07	1.22
ARGGSVREYDRNFDY				1.28		0.82		0.19		
ARDRGGAGYLDY					1.01			0.81	0.26	
ARYDCTNGVCRKNFDS					0.33			1.34	0.25	
ARETLRIPYSSGWHQATDY			0.93		0.34			0.22		
ARDPPYRTGHLDY				0.15		0.78		0.07		
ARAENYHGSGSYYRH	0.30	0.59							0.02	
AKSSGYSYIKEIFDS			0.50		0.22			0.13		
ARGRGKDLTSN		0.38			0.19			0.16		
ARDGRPDWTSRGYFDY								0.11	0.44	0.18
AKDARTPIRISWFDY					0.29			0.18	0.04	
TTGRGVLRFLEWLDYFDY			0.21		0.12			0.14		
AREEVDLLTGYYRLPGFDY				9.69			3.45			
AHRRSSVGGSPFDY									3.80	3.35

ARGGKRGSYYGSGSLFDH			3.48					0.52		
ARASGLNFYY		0.50					2.23			
ARDGGTGGSGFDH									1.82	0.75
TRDVKGWPPTVDY	1.25	1.13								
ARPTLEMATIPVH									1.77	0.09
ARAPDRRFSMTTFGGGSFAPTGLDV	0.78	1.00								
ARVAVAGTWFDAFDL				1.40					0.10	
SRHRDYSSGWGFDQ			0.77		0.59					
AKDRTRWSGPPGHYYGMDV									0.51	0.84
ARGLPTVTTPQAFDI								0.75	0.54	
ARDKLAAAGTSYYYYGMDV	0.84							0.35		
ARGIGYYDSSYFDY									0.34	0.78
ASGVAATRRTKYYYYGMDV									0.09	0.89
ARYQTKWLRWDLYYFDY		0.91								0.07
ARSPLGGDPSLPGGTAFDS	0.28	0.64								
ARVRFYKNNRQAVFDY					0.23			0.63		
ARGVDDYGDLPYFDY									0.79	0.05
ASPRLRRRGKVYDAFDL	0.73								0.05	
VKGYSNGWSEGYLDN				0.65					0.08	
ASELERPPMYYFDY				0.43					0.23	
ATDASLMARQSGMDV	0.27	0.37								
ATDSGSYRGCDY		0.47						0.12		
ARGPNHDFWSSYYPGGMDV					0.18			0.40		
VHSFYGSGRSPNEFDY				0.42					0.14	
ARDMAAAGTGGPDY									0.31	0.16
IAAGRGGYYYGMDV									0.09	0.36
AHRTTPPGGNNKDYFDY									0.10	0.34
ARALVLSTPLVYFDP									0.21	0.20
AKDSGTYPYFFDS									0.14	0.27
AREKYTSDWYYFDY								0.16		0.22
TTSRKSYYYLMDV					0.22			0.14		
ARTKSTIYPPYYFDY					0.20			0.15		
VRPRMIVSGGLAPDAFDI									0.10	0.18
ANDRGDCHYCLHY								0.17	0.04	
AKDGWGRGRWLQLGFLDY									0.04	0.17
AKDRRGAARRNFDY									0.02	0.10
ARDVTSMVREVPFAFDI									0.04	0.05
ARAPYSSSSDYFDY									0.08	0.01

ARLGGLQRIYYFDY								0.00		0.01
ARAALGGSSSWLN				8.96						
ARDTDTAMVTLPFDY		5.30								
ARGPPIYSNTWNRGYYFYGLDV									5.17	
AKERGRKGLRSRTFDY									1.41	
ARARGGGNSVFGSFVDY				0.95						
ARWRGGGHNDY	0.87									
VKGTSYDDYGTWLDP										0.82
ARVVDFWSGYSSFDY				0.77						
ASSREYYDFWSGYHR									0.72	
ARERRDSSTFDP								0.72		
AKAQYYDSPRDAADFYFYAMDV					0.71					
AKSRNKYNSYPYFFDY								0.66		
GREREPLFDY					0.58					
ARPAYSSGWAPFDY							0.57			
ARDRPLVGEVTDDAFDL				0.51						
AKVIDKHSSPRRVALDY								0.51		
ATLPVAATRYYGMDV									0.50	
AREAIQKWELRLIGDY								0.50		
ARGSLGSRRRPMAFDY									0.47	
ARDLYPWGTMDV	0.47									
AKDRGGSGWYGY							0.44			
AREGGSSRAYDY	0.44									
ARDDEGMRV									0.40	
ARVETTHSSSYSFDY									0.40	
ARVDRWGGTHFDH								0.39		
AKVAGYSLGGCDY									0.39	
ARDMRDCVTTRSCYRGPFDY								0.38		
AHSHYASGRSPKEFDY				0.36						
ARHRKQWMVSDAFDI					0.36					
AKELGHAKTFDF				0.34						
ARALHGDYAHF									0.33	
ARHSITFMDS			0.33							
ARGGQLGFLEWFSSREYHGMDV								0.33		
TTDRSYGYFAVH									0.30	
AREAKDSSGWNGETFFDY									0.29	
ARPRMIMSRGLAPDAFEV								0.29		
AKDRGYGLGSYRWIDY					0.29					

ASEPGRFREGGSWFDP										0.28
AKRTGEYSYGSDYFDD								0.28		
ARDLVLTANAEYFQH									0.28	
ASLRGYSYARDFDY									0.27	
ARSMVVTLRGKYYGLDV									0.27	
ARHVEKKTDNDAFDI										0.27
ARDKSGFGWFDP								0.26		
ARDRYDSSGLGGAFDI										0.26
VRDRSVNAFDI									0.26	
ARGVGARVMDY									0.25	
ARDSDPFYYDSSGYVRQGNWFDP									0.25	
ARQVPRDVGWFDP										0.24
ARAPYYYDSSGDAEYFQH								0.24		
GRDGGTITPRGWFDP									0.24	
AKDGRSSSWYYGSYFDY										0.24
ARVHNYCYYSMDV									0.23	
VKRQNNWFDL								0.22		
ARDREIVPKVIGDYDGMDV								0.21		
GKDNWNDCSKGFDI										0.21
ATDKDYLFAS					0.20					
ARDRDSYGYRLFDP				0.19						
ARGPGYYYYMDV										0.18
VKVNGYAFGEFDH									0.17	
ARGDYTGLEYYFDY									0.16	
ARFSGISSGGAFDI										0.16
ARDRGVVVAASFDY								0.16		
AHMTVDRSDTVFSTDAFEV									0.15	
ARQTLGV					0.15					
AHRGDGDREGLFDY								0.14		
ASQKGVLVDY										0.14
ARRFRFSGTHLDYFDY										0.13
AREYFIHQVFDY									0.11	
AREPVSNRNWFDP									0.11	
ARIYGTHYYYGMDV									0.11	
AKGALPHDSTASRFYFDY								0.11		
ATVYCSGGRGYFTGSH									0.11	
ARDGGTGGAPSDY										0.11
TTDPAYYRVDY									0.11	

AKVAPILTGYFDY									0.10	
ARDSGGWSYFDY									0.10	
ASGRVGSSL		0.10								
AKDRHESRHFDTPRAEGFES										0.09
ARDGGQLANWVDP									0.09	
ARVGRTTVTTLTGFGP									0.09	
ARLSYLTIHRAHGWFDP									0.08	
ARDSSAFDRSLAY										0.07
ARVGRNNWNARHAFDV					0.06					
AREQHNGNSFFDS									0.06	
ARHARDGYNLLEAFDI									0.06	
ARSRYRILTGYYPKYFYYFDY										0.06
AKGACSNTIYSRPFDP									0.05	
AREARESNWNAMTYFDS										0.03
ARVIVGATTLRPRYYFDC									0.03	
ARRKRENWNDGRVGAFDI									0.03	
ARSRNIAALYFQH									0.03	
ASSRVTQVWLQGYFDF									0.03	
AREPITTVRGAIINYYGMDV									0.02	
AKDMNSSPPYSFEN									0.01	
ARDRIYYYDPSGYYNFDY										0.01
AGGYSYGYIDY									0.01	
ARDSSSWSSDY									0.01	
ARHRGACGDYLDY									0.00	
ARVRASHPYAFDI									0.00	
ARHGVSTGTGGFDA										0.00
ARDQNPPEYSSGWYP									0.00	
ARGHPDGAGVDY									0.00	
AKGYDFWSGYYDA									0.00	

References

1. Roush, S.W., T.V. Murphy, and a Vaccine-Preventable Disease Table Working Group, *HIstorical comparisons of morbidity and mortality for vaccine-preventable diseases in the united states.* JAMA, 2007. **298**(18): p. 2155-2163.
2. Ehreth, J., *The global value of vaccination.* Vaccine, 2003. **21**(7–8): p. 596-600.
3. Osterholm, M.T., et al., *Efficacy and effectiveness of influenza vaccines: a systematic review and meta-analysis.* The Lancet Infectious Diseases, 2012. **12**(1): p. 36-44.
4. Lambert, L.C. and A.S. Fauci, *Influenza Vaccines for the Future.* New England Journal of Medicine, 2010. **363**(21): p. 2036-2044.
5. Molinari, N.-A.M., et al., *The annual impact of seasonal influenza in the US: Measuring disease burden and costs.* Vaccine, 2007. **25**(27): p. 5086-5096.
6. Smith, W., C.H. Andrewes, and P.P. Laidlaw, *A Virus Obtained from Influenza Patients.* The Lancet, 1933. **222**(5732): p. 66-68.
7. Zambon, M.C., *The pathogenesis of influenza in humans.* Reviews in Medical Virology, 2001. **11**(4): p. 227-241.
8. Webster, R.G., Monto, Arnold S., Braciale, Thomas J., Lamb, Robert A., *Textbook of Influenza*. 2nd ed. 2013, Hoboken, NJ: Wiley-Blackwell.
9. Wright, P.F., Webster, R.G., *in Fields Virology 4th edn*, ed. D.M.H. Knipe, P.M. 2001: Lippincott, Williams & Wilkins, Philadelphia, PA.
10. Skehel, J.J. and D.C. Wiley, *Receptor Binding And Membrane Fusion In Virus Entry: The Influenza Hemagglutinin.* Annual Review of Biochemistry, 2000. **69**(1): p. 531-569.
11. Suzuki, Y., *Sialobiology of Influenza: Molecular Mechanism of Host Range Variation of Influenza Viruses.* Biological and Pharmaceutical Bulletin, 2005. **28**(3): p. 399-408.
12. Wu, Y., et al., *Bat-derived influenza-like viruses H17N10 and H18N11.* Trends in Microbiology, 2014. **22**(4): p. 183-191.
13. Lynch, J.P. and E.E. Walsh, *Influenza: Evolving Strategies in Treatment and Prevention.* Semin Respir Crit Care Med, 2007. **28**(02): p. 144-158.
14. Hay, A., Gregory, V., Douglas, AR, and Lin, YP, *The evolution of human influenza viruses.* Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 2001. **356**(1416): p. 1861.
15. Bouvier, N.M. and P. Palese, *The Biology of Influenza Viruses.* Vaccine, 2008. **26**(Suppl 4): p. D49-D53.
16. Stech, J., et al., *A new approach to an influenza live vaccine: modification of the cleavage site of hemagglutinin.* Nat Med, 2005. **11**(6): p. 683-689.
17. Karlsson Hedestam, G.B., et al., *The challenges of eliciting neutralizing antibodies to HIV-1 and to influenza virus.* Nat Rev Micro, 2008. **6**(2): p. 143-155.
18. Harrison, S.C., *Viral membrane fusion.* Nat Struct Mol Biol, 2008. **15**(7): p. 690-698.

19. Wagner, R., M. Matrosovich, and H.-D. Klenk, *Functional balance between haemagglutinin and neuraminidase in influenza virus infections.* Reviews in Medical Virology, 2002. **12**(3): p. 159-166.
20. Corti, D. and A. Lanzavecchia, *Broadly Neutralizing Antiviral Antibodies.* Annual Review of Immunology, 2013. **31**(1): p. 705-742.
21. Connor, R.J., et al., *Receptor Specificity in Human, Avian, and Equine H2 and H3 Influenza Virus Isolates.* Virology, 1994. **205**(1): p. 17-23.
22. Trifonov, V., H. Khiabanian, and R. Rabadan *Geographic Dependence, Surveillance, and Origins of the 2009 Influenza A (H1N1) Virus.* New England Journal of Medicine, 2009. **361**(2): p. 115-119.
23. von Itzstein, M., et al., *Rational design of potent sialidase-based inhibitors of influenza virus replication.* Nature, 1993. **363**(6428): p. 418-423.
24. Kim, C., Lew, W, Williams, MA, Liu, H, Zhang, L, Swaminathan, S, Bischofberger, N, Chen, MS, Mendel, DB, Tai, CY, Laver, WG, Stevens, RC., *Influenza neuraminidase inhibitors possessing a novel hydrophobic interaction in the enzyme active site: design, synthesis, and structural analysis of carbocyclic sialic acid analogues with potent anti-influenza activity.* J Am Chem Soc, 1997. **119**(4): p. 681-690.
25. de Jong, M.D., et al., *Oseltamivir Resistance during Treatment of Influenza A (H5N1) Infection.* New England Journal of Medicine, 2005. **353**(25): p. 2667-2672.
26. Sparrow, E., et al., *Passive immunization for influenza through antibody therapies, a review of the pipeline, challenges and potential applications.* Vaccine, 2016. **34**(45): p. 5442-5448.
27. Hannoun, C., *The evolving history of influenza viruses and influenza vaccines.* Expert Review of Vaccines, 2013. **12**(9): p. 1085-1094.
28. Release, S.P.P. *Sanofi Pasteur Presents Pediatric Data On Investigational Quadrivalent Influenza Vaccine*. May 1, 2012 [cited 2016 September 16]; Available from: http://sanofipasteurus.mediaroom.com/index.php?s=11069&item=127633.
29. CDC. *ACIP votes down use of LAIV for 2016-2017 flu season*. June 22, 2016 [cited 2016 September 28]; Available from: http://www.cdc.gov/media/releases/2016/s0622-laiv-flu.html.
30. CDC. *Cell-Based Flu Vaccines*. August 1, 2016 [cited 2016 September 28]; Available from: http://www.cdc.gov/flu/protect/vaccine/cell-based.htm.
31. Cox, N.J. and K. Subbarao, *Influenza.* The Lancet, 1999. **354**(9186): p. 1277-1282.
32. Sedova, E.S., et al., *Recombinant Influenza Vaccines.* Acta Naturae, 2012. **4**(4): p. 17-27.
33. Broberg, E., Snacken, R., Adlhoch, C., Beaute, J., Galinska, M., Pereyaslov, D., Brown, C., Penttinen, P., WHO European Region and the European Influenza Surveillance Network, *Start of the 2014/15 influenza season in Europe: drifted influenza A(H3N2) viruses circulate as dominant subtype.* Euro Surveill., 2015. **20**: p. 21023.

34. D'Mello, T., Brammer, L., Blanton, L., Kniss, K., Smith, S., Mustaquim, D., Steffens, C., Dhara, R., Cohen, J., Chaves, S.S., Centers for Disease Control and Prevention (CDC), et al., *Update: influenza activity - United States, september 28, 2014-february 21, 2015.* MMWR Morb. Mortal. Wkly. Rep., 2015. **64**: p. 206–212.
35. Murphy, K.P., *Janeway's Immunobiology*. 8th ed. 2012, New York: Garland Science.
36. Roux, K.H., *Immunoglobulin Structure and Function as Revealed by Electron Microscopy.* International Archives of Allergy and Immunology, 1999. **120**(2): p. 85-99.
37. Wu, T.T. and E.A. Kabat, *An analysis of the sequences of the variable regions of Bence Jones proteins and myeloma light chains and their implications for antibody complementarity.* The Journal of Experimental Medicine, 1970. **132**(2): p. 211-250.
38. Kabat, E.A., and T. T. Wu, *Construction of a three-dimensional model of the polypeptide backbone of the variable region of κ-immunoglobulin light chains.* Proc. Natl. Acad. Sci. , 1972(69): p. 960–964.
39. Chothia, C. and A.M. Lesk, *Canonical structures for the hypervariable regions of immunoglobulins.* Journal of Molecular Biology, 1987. **196**(4): p. 901-917.
40. Zemlin, M., et al., *Expressed Murine and Human CDR-H3 Intervals of Equal Length Exhibit Distinct Repertoires that Differ in their Amino Acid Composition and Predicted Range of Structures.* Journal of Molecular Biology, 2003. **334**(4): p. 733-749.
41. Xu, J.L. and M.M. Davis, *Diversity in the CDR3 Region of VH Is Sufficient for Most Antibody Specificities.* Immunity, 2000. **13**(1): p. 37-45.
42. Wu, T.T., Johnson G., Kabat E. A., *Length distribution of CDRH3 in antibodies.* Proteins, 1993. **16**(1): p. 1-7.
43. Okuno, Y., et al., *A common neutralizing epitope conserved between the hemagglutinins of influenza A virus H1 and H2 strains.* Journal of Virology, 1993. **67**(5): p. 2552-2558.
44. Ravetch, J.a.B., S, *IGG FC RECEPTORS.* Annual Review of Immunology, 2001. **19**(1): p. 275-290.
45. Rus, H., C. Cudrici, and F. Niculescu, *The role of the complement system in innate immunity.* Immunologic Research, 2005. **33**(2): p. 103-112.
46. Tonegawa, S., *Somatic generation of antibody diversity.* Nature, 1983. **302**(5909): p. 575-81.
47. Matsuda, F., et al., *The Complete Nucleotide Sequence of the Human Immunoglobulin Heavy Chain Variable Region Locus.* The Journal of Experimental Medicine, 1998. **188**(11): p. 2151-2162.
48. Zachau, H.G., *The immunoglobulin kappa locus-or-what has been learned from looking closely at one-tenth of a percent of the human genome.* Gene, 1993. **135**(1-2): p. 167-173.

49. Fippiat, J.-P., et al., *Organization of the human immunoglobulin lambda light-chain locus on chromosome 22q11.2.* Human Molecular Genetics, 1995. **4**(6): p. 983-991.
50. Alt, F.W. and D. Baltimore, *Joining of immunoglobulin heavy chain gene segments: implications from a chromosome with evidence of three D-JH fusions.* Proceedings of the National Academy of Sciences, 1982. **79**(13): p. 4118-4122.
51. Sanz, I., *Multiple mechanisms participate in the generation of diversity of human heavy chain CDR3 regions.* Journal of Immunology, 1991(147): p. 1720.
52. Schroeder Jr, H.W. and L. Cavacini, *Structure and function of immunoglobulins.* Journal of Allergy and Clinical Immunology, 2010. **125**(2, Supplement 2): p. S41-S52.
53. LeBien, T.W. and T.F. Tedder, *B lymphocytes: how they develop and function.* Blood, 2008. **112**(5): p. 1570-1580.
54. McHeyzer-Williams, M., et al., *Molecular programming of B cell memory.* Nat Rev Immunol, 2012. **12**(1): p. 24-34.
55. Legge, K.L. and T.J. Braciale, *Accelerated Migration of Respiratory Dendritic Cells to the Regional Lymph Nodes Is Limited to the Early Phase of Pulmonary Infection.* Immunity, 2003. **18**(2): p. 265-277.
56. Belz, G.T., et al., *Distinct migrating and nonmigrating dendritic cell populations are involved in MHC class I-restricted antigen presentation after lung infection with virus.* Proceedings of the National Academy of Sciences of the United States of America, 2004. **101**(23): p. 8670-8675.
57. Victora, G.D. and M.C. Nussenzweig, *Germinal Centers.* Annual Review of Immunology, 2012. **30**(1): p. 429-457.
58. Sablitzky, F., Wildner, G., Rajewsky, K. , *Somatic mutation and clonal expansion of B cells in an antigen-driven immune response.* EMBO J., 1985. **4**(2): p. 345-350.
59. Rajewsky, K., I. Forster, and A. Cumano, *Evolutionary and somatic selection of the antibody repertoire in the mouse.* Science, 1987. **238**(4830): p. 1088-1094.
60. Berek, C., A. Berger, and M. Apel, *Maturation of the immune response in germinal centers.* Cell, 1991. **67**(6): p. 1121-1129.
61. Jacob, J., et al., *Intraclonal generation of antibody mutants in germinal centres.* Nature, 1991. **354**(6352): p. 389-392.
62. Frölich, D., et al., *Secondary Immunization Generates Clonally Related Antigen-Specific Plasma Cells and Memory B Cells.* The Journal of Immunology, 2010. **185**(5): p. 3103-3110.
63. Yoshida, T., et al., *Memory B and memory plasma cells.* Immunological Reviews, 2010. **237**(1): p. 117-139.
64. Radbruch, A., et al., *Competence and competition: the challenge of becoming a long-lived plasma cell.* Nat Rev Immunol, 2006. **6**(10): p. 741-750.
65. Amanna, I.J. and M.K. Slifka, *Mechanisms that determine plasma cell lifespan and the duration of humoral immunity.* Immunological Reviews, 2010. **236**(1): p. 125-138.

66. Amanna, I.J., Slifka, M.K., *Contributions of humoral and cellular immunity to vaccine-induced protection in humans.* Virology, 2011. **411**(2): p. 206-215.
67. Dowdle, W.R., Coleman, M.T., Mostow, S.R., Kaye, H.S., Schoenbaum, S.C., *Inactivated vaccines. 2. Laboratory indices of protection.* Postgrad Med J. , 1973. **49**(569): p. 159-163.
68. Plotkin, S.A., *Correlates of Protection Induced by Vaccination.* Clinical and Vaccine Immunology, 2010. **17**(7): p. 1055-1065.
69. Traggiai, E., et al., *An efficient method to make human monoclonal antibodies from memory B cells: potent neutralization of SARS coronavirus.* Nat Med, 2004. **10**(8): p. 871-875.
70. Corti, D., et al., *A Neutralizing Antibody Selected from Plasma Cells That Binds to Group 1 and Group 2 Influenza A Hemagglutinins.* Science, 2011. **333**(6044): p. 850-856.
71. Scheid, J.F., et al., *Broad diversity of neutralizing antibodies isolated from memory B cells in HIV-infected individuals.* Nature, 2009. **458**(7238): p. 636-640.
72. Wrammert, J., et al., *Rapid cloning of high-affinity human monoclonal antibodies against influenza virus.* Nature, 2008. **453**(7195): p. 667-671.
73. Whittle, J.R.R., et al., *Broadly neutralizing human antibody that recognizes the receptor-binding pocket of influenza virus hemagglutinin.* Proceedings of the National Academy of Sciences, 2011. **108**(34): p. 14216-14221.
74. Ekiert, D.C., et al., *Cross-neutralization of influenza A viruses mediated by a single antibody loop.* Nature, 2012. **489**(7417): p. 526-532.
75. Wrammert, J., et al., *Broadly cross-reactive antibodies dominate the human B cell response against 2009 pandemic H1N1 influenza virus infection.* The Journal of Experimental Medicine, 2011. **208**(1): p. 181-193.
76. Wei, C.-J., et al., *Induction of Broadly Neutralizing H1N1 Influenza Antibodies by Vaccination.* Science, 2010. **329**(5995): p. 1060-1064.
77. Throsby, M., et al., *Heterosubtypic Neutralizing Monoclonal Antibodies Cross-Protective against H5N1 and H1N1 Recovered from Human IgM⁺ Memory B Cells.* PLoS ONE, 2008. **3**(12): p. e3942.
78. Krause, J.C., et al., *A Broadly Neutralizing Human Monoclonal Antibody That Recognizes a Conserved, Novel Epitope on the Globular Head of the Influenza H1N1 Virus Hemagglutinin.* Journal of Virology, 2011. **85**(20): p. 10905-10908.
79. Moody, M.A., et al., *H3N2 Influenza Infection Elicits More Cross-Reactive and Less Clonally Expanded Anti-Hemagglutinin Antibodies Than Influenza Vaccination.* PLoS ONE, 2011. **6**(10): p. e25797.
80. Iba, Y., et al., *Conserved Neutralizing Epitope at Globular Head of Hemagglutinin in H3N2 Influenza Viruses.* Journal of Virology, 2014. **88**(13): p. 7130-7144.
81. Dreyfus, C., et al., *Highly Conserved Protective Epitopes on Influenza B Viruses.* Science, 2012. **337**(6100): p. 1343-1348.

82. Henry Dunand, Carole J., et al., *Both Neutralizing and Non-Neutralizing Human H7N9 Influenza Vaccine-Induced Monoclonal Antibodies Confer Protection.* Cell Host & Microbe, 2016. **19**(6): p. 800-813.
83. Andrews, S.F., et al., *High Preexisting Serological Antibody Levels Correlate with Diversification of the Influenza Vaccine Response.* Journal of Virology, 2015. **89**(6): p. 3308-3317.
84. Yassine, H.M., et al., *Hemagglutinin-stem nanoparticles generate heterosubtypic influenza protection.* Nat Med, 2015. **21**(9): p. 1065-1070.
85. Krammer, F. and P. Palese, *Advances in the development of influenza virus vaccines.* Nat Rev Drug Discov, 2015. **14**(3): p. 167-182.
86. Impagliazzo, A., et al., *A stable trimeric influenza hemagglutinin stem as a broadly protective immunogen.* Science, 2015. **349**(6254): p. 1301-1306.
87. Jackson, Katherine J.L., et al., *Human Responses to Influenza Vaccination Show Seroconversion Signatures and Convergent Antibody Rearrangements.* Cell Host & Microbe, 2014. **16**(1): p. 105-114.
88. Vollmers, C., et al., *Genetic measurement of memory B-cell recall using antibody repertoire sequencing.* Proceedings of the National Academy of Sciences, 2013. **110**(33): p. 13463-13468.
89. Jiang, N., et al., *Lineage Structure of the Human Antibody Repertoire in Response to Influenza Vaccination.* Science Translational Medicine, 2013. **5**(171): p. 171ra19.
90. Laserson, U., et al., *High-resolution antibody dynamics of vaccine-induced immune responses.* Proceedings of the National Academy of Sciences, 2014. **111**(13): p. 4928-4933.
91. Li, G.-M., et al., *Pandemic H1N1 influenza vaccine induces a recall response in humans that favors broadly cross-reactive memory B cells.* Proceedings of the National Academy of Sciences, 2012. **109**(23): p. 9047-9052.
92. Francis, T.J., *On the Doctrine of Original Antigenic Sin.* Proc Am Philos Soc, 1960. **104**(6): p. 572–578.
93. DeKosky, B.J., et al., *High-throughput sequencing of the paired human immunoglobulin heavy and light chain repertoire.* Nat Biotech, 2013. **31**(2): p. 166-169.
94. DeKosky, B.J., et al., *In-depth determination and analysis of the human paired heavy- and light-chain antibody repertoire.* Nat Med, 2015. **21**(1): p. 86-91.
95. Sanchez-Freire, V., et al., *Microfluidic single-cell real-time PCR for comparative analysis of gene expression patterns.* Nat. Protocols, 2012. **7**(5): p. 829-838.
96. Georgiou, G., et al., *The promise and challenge of high-throughput sequencing of the antibody repertoire.* Nat Biotech, 2014. **32**(2): p. 158-168.
97. Boutz, D.R., et al., *Proteomic Identification of Monoclonal Antibodies from Serum.* Analytical Chemistry, 2014. **86**(10): p. 4758-4766.
98. Lavinder, J.J., et al., *Identification and characterization of the constituent human serum antibodies elicited by vaccination.* Proceedings of the National Academy of Sciences, 2014. **111**(6): p. 2259-2264.

99. Wine, Y., et al., *Molecular deconvolution of the monoclonal antibodies that comprise the polyclonal serum response.* Proceedings of the National Academy of Sciences, 2013. **110**(8): p. 2993-2998.
100. Cheung, W.C., et al., *A proteomics approach for the identification and cloning of monoclonal antibodies from serum.* Nat Biotech, 2012. **30**(5): p. 447-452.
101. Sato, S., et al., *Proteomics-directed cloning of circulating antiviral human monoclonal antibodies.* Nat Biotech, 2012. **30**(11): p. 1039-1043.
102. Stephenson, I., et al., *Cross-Reactivity to Highly Pathogenic Avian Influenza H5N1 Viruses after Vaccination with Nonadjuvanted and MF59-Adjuvanted Influenza A/Duck/Singapore/97 (H5N3) Vaccine: A Potential Priming Strategy.* Journal of Infectious Diseases, 2005. **191**(8): p. 1210-1215.
103. Thomas, P.G., et al., *An unexpected antibody response to an engineered influenza virus modifies CD8+ T cell responses.* Proceedings of the National Academy of Sciences of the United States of America, 2006. **103**(8): p. 2764-2769.
104. Rimmelzwaan, G.F. and J.E. McElhaney, *Correlates of protection: Novel generations of influenza vaccines.* Vaccine, 2008. **26, Supplement 4**: p. D41-D44.
105. Jain, V.K., et al., *Vaccine for Prevention of Mild and Moderate-to-Severe Influenza in Children.* New England Journal of Medicine, 2013. **369**(26): p. 2481-2491.
106. Tinoco, J.C., et al., *Immunogenicity, reactogenicity, and safety of inactivated quadrivalent influenza vaccine candidate versus inactivated trivalent influenza vaccine in healthy adults aged ≥18 years: A phase III, randomized trial.* Vaccine, 2014. **32**(13): p. 1480-1487.
107. Monto, A.S., et al., *Comparative Efficacy of Inactivated and Live Attenuated Influenza Vaccines.* New England Journal of Medicine, 2009. **361**(13): p. 1260-1267.
108. Lee, P.S., et al., *Receptor mimicry by antibody F045–092 facilitates universal binding to the H3 subtype of influenza virus.* Nat Commun, 2014. **5**.
109. Cox, J., A. Michalski, and M. Mann, *Software Lock Mass by Two-Dimensional Minimization of Peptide Mass Errors.* Journal of the American Society for Mass Spectrometry, 2011. **22**(8): p. 1373-1380.
110. Gillim-Ross, L. and K. Subbarao, *Emerging Respiratory Viruses: Challenges and Vaccine Strategies.* Clinical Microbiology Reviews, 2006. **19**(4): p. 614-636.
111. Ross, T.M., et al., *C3d enhancement of antibodies to hemagglutinin accelerates protection against influenza virus challenge.* Nat Immunol, 2000. **1**(2): p. 127-131.
112. Wine, Y., et al., *Serology in the 21st century: the molecular-level analysis of the serum antibody repertoire.* Current Opinion in Immunology, 2015. **35**: p. 89-97.
113. Sasaki, S., et al., *Influence of Prior Influenza Vaccination on Antibody and B-Cell Responses.* PLoS ONE, 2008. **3**(8): p. e2975.
114. Andrews, S.F., et al., *Immune history profoundly affects broadly protective B cell responses to influenza.* Science Translational Medicine, 2015. **7**(316): p. 316ra192-316ra192.

115. Morris, J.A., et al., *Immunity to Influenza as Related to Antibody Levels.* New England Journal of Medicine, 1966. **274**(10): p. 527-535.
116. Fonville, J.M., et al., *Antibody landscapes after influenza virus infection or vaccination.* Science, 2014. **346**(6212): p. 996-1000.
117. Nakamura, G., et al., *An In Vivo Human-Plasmablast Enrichment Technique Allows Rapid Identification of Therapeutic Influenza A Antibodies.* Cell Host & Microbe, 2013. **14**(1): p. 93-103.
118. Ellebedy, A.H., et al., *Induction of broadly cross-reactive antibody responses to the influenza HA stem region following H5N1 vaccination in humans.* Proceedings of the National Academy of Sciences, 2014. **111**(36): p. 13133-13138.
119. Whittle, J.R.R., et al., *Flow Cytometry Reveals that H5N1 Vaccination Elicits Cross-Reactive Stem-Directed Antibodies from Multiple Ig Heavy-Chain Lineages.* Journal of Virology, 2014. **88**(8): p. 4047-4057.
120. Mariana, B., et al., *Seroconversion to Seasonal Influenza Viruses after A(H1N1)pdm09 Virus Infection, Quebec, Canada.* Emerging Infectious Disease journal, 2012. **18**(7): p. 1132.
121. McDaniel, J.R., et al., *Ultra-high-throughput sequencing of the immune receptor repertoire from millions of lymphocytes.* Nat. Protocols, 2016. **11**(3): p. 429-442.
122. Yang, Z.-y., et al., *Selective Modification of Variable Loops Alters Tropism and Enhances Immunogenicity of Human Immunodeficiency Virus Type 1 Envelope.* Journal of Virology, 2004. **78**(8): p. 4029-4036.
123. Mastronarde, D.N., *Automated electron microscope tomography using robust prediction of specimen movements.* Journal of Structural Biology, 2005. **152**(1): p. 36-51.
124. Tang, G., et al., *EMAN2: An extensible image processing suite for electron microscopy.* Journal of Structural Biology, 2007. **157**(1): p. 38-46.
125. Frank, J., et al., *SPIDER and WEB: Processing and Visualization of Images in 3D Electron Microscopy and Related Fields.* Journal of Structural Biology, 1996. **116**(1): p. 190-199.
126. Radermacher, M., et al., *Three-dimensional reconstruction from a single-exposure, random conical tilt series applied to the 50S ribosomal subunit of Escherichia coli.* Journal of Microscopy, 1987. **146**(2): p. 113-136.
127. Pettersen, E.F., et al., *UCSF Chimera—A visualization system for exploratory research and analysis.* Journal of Computational Chemistry, 2004. **25**(13): p. 1605-1612.
128. Shaikh, T.R., et al., *SPIDER image processing for single-particle reconstruction of biological macromolecules from electron micrographs.* Nat. Protocols, 2008. **3**(12): p. 1941-1974.
129. Schmidt, Aaron G., et al., *Viral Receptor-Binding Site Antibodies with Diverse Germline Origins.* Cell, 2015. **161**(5): p. 1026-1034.

130. Godley, L., et al., *Introduction of intersubunit disulfide bonds in the membrane-distal region of the influenza hemagglutinin abolishes membrane fusion activity.* Cell, 1992. **68**(4): p. 635-645.
131. DiLillo, D.J., et al., *Broadly neutralizing hemagglutinin stalk-specific antibodies require Fc[gamma]R interactions for protection against influenza virus in vivo.* Nat Med, 2014. **20**(2): p. 143-151.
132. DiLillo, D.J., et al., *Broadly neutralizing anti-influenza antibodies require Fc receptor engagement for in vivo protection.* The Journal of Clinical Investigation, 2016. **126**(2): p. 605-610.
133. Taylor, H.P., S.J. Armstrong, and N.J. Dimmock, *Quantitative relationships between an influenza virus and neutralizing antibody.* Virology, 1987. **159**(2): p. 288-298.
134. Wrigley, N.G., *Electron Microscopy of Influenza Virus.* British Medical Bulletin, 1979. **35**(1): p. 35-38.
135. Li, C.K.-f., R. Rappuoli, and X.-N. Xu, *Correlates of protection against influenza infection in humans — on the path to a universal vaccine?* Current Opinion in Immunology, 2013. **25**(4): p. 470-476.
136. Knossow, M. and J.J. Skehel, *Variation and infectivity neutralization in influenza.* Immunology, 2006. **119**(1): p. 1-7.
137. Pappas, L., et al., *Rapid development of broadly influenza neutralizing antibodies through redundant mutations.* Nature, 2014. **516**(7531): p. 418-422.
138. Henry Dunand, C.J., et al., *Preexisting human antibodies neutralize recently emerged H7N9 influenza strains.* The Journal of Clinical Investigation, 2015. **125**(3): p. 1255-1268.
139. Tan, Y.-C., et al., *High-throughput sequencing of natively paired antibody chains provides evidence for original antigenic sin shaping the antibody response to influenza vaccination.* Clinical Immunology, 2014. **151**(1): p. 55-65.
140. Kallewaard, Nicole L., et al., *Structure and Function Analysis of an Antibody Recognizing All Influenza A Subtypes.* Cell, 2016. **166**(3): p. 596-608.
141. Davenport, F.M., A.V. Hennessy, and T. Francis, *Epidemiologic and Immunologic Significance of Age Distribution of Antibody to Antigenic Variants of Influenza Virus.* The Journal of Experimental Medicine, 1953. **98**(6): p. 641-656.
142. Webster, R.G., *Original Antigenic Sin in Ferrets: the Response to Sequential Infections with Influenza Viruses.* The Journal of Immunology, 1966. **97**(2): p. 177-183.
143. de St. Groth, S.F. and Webster, R.G., *Disquisitions on Original Antigenic Sin.* The Journal of Experimental Medicine, 1966. **124**(3): p. 347-361.
144. Virelizier, J.-L., A.C. Allison, and G.C. Schild, *Antibody Responses to Antigenic Determinants of Influenza Virus Hemagglutinins*
. II. Original Antigenic Sin: A Bone Marrow-Derived Lymphocyte Memory Phenomenon Modulated by Thymus-Derived Lymphocytes, 1974. **140**(6): p. 1571-1578.

145. Li, Y., et al., *Immune history shapes specificity of pandemic H1N1 influenza antibody responses.* The Journal of Experimental Medicine, 2013. **210**(8): p. 1493-1500.
146. Miller, M.S., et al., *Neutralizing Antibodies Against Previously Encountered Influenza Virus Strains Increase over Time: A Longitudinal Analysis.* Science Translational Medicine, 2013. **5**(198): p. 198ra107-198ra107.
147. Pinna, D., et al., *Clonal dissection of the human memory B-cell repertoire following infection and vaccination.* European Journal of Immunology, 2009. **39**(5): p. 1260-1270.
148. Corti, D., et al., *Heterosubtypic neutralizing antibodies are produced by individuals immunized with a seasonal influenza vaccine.* The Journal of Clinical Investigation, 2010. **120**(5): p. 1663-1673.
149. Krammer, F. and P. Palese, *Influenza virus hemagglutinin stalk-based antibodies and vaccines.* Current opinion in virology, 2013. **3**(5): p. 521-530.