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Late-emerging strains of HIV induce T-cell homeostasis failure by promoting bystander cell death and immune exhaustion in naïve CD4 and all CD8 T-cells

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ABSTRACT

The mechanisms involved in the decline of CD4 and CD8 T-cells that lead to HIV-induced immune dysregulation are not clearly understood. We hypothesize that late-emerging strains of HIV, such as CXCR4-tropic (X4) virions, induce T-cell homeostasis failure by promoting significantly more bystander cell death, and immune exhaustion in naïve CD4 and all CD8 T-cells, when compared to strain of HIV, such as CCR5-tropic (R5) virions, found early during the course of infection. In the reported study, inactivated X4 virions induced greater bystander cell death in sort-purified naïve CD4 T-cells compared to R5 virions, which was significant ($p = 0.013$), and in memory CD8 T-cells, though the latter was not significant. A clearer understanding of the mechanisms involved in HIV-induced depletion of T-cell numbers and function could lead to therapies that prevent T-cell death and restore immune function. These therapies could improve current anti-retroviral and cure-related treatments by boosting the immune system's own ability to combat the virus.

Introduction

Despite many years of intense research, the precise mechanisms that lead to the development of AIDS after infection with HIV are not well defined. Effective therapeutic strategies that enhance the immune system's ability to combat the disease over prolonged periods of infection can only be devised when a clearer understanding of these mechanisms is obtained.

One hallmark of HIV-1 infection is the gradual loss of CD4 T-cells over time. This loss begins with the establishment of HIV-1 infection and is initially counterbalanced by an increase in CD8 T-cells (except in individuals with very rapid disease progression). This allows for the maintenance of a constant level of total circulating CD3 T-cells, despite an inversion in the CD4/CD8 ratio, for many years, a phenomenon known as blind T-cell homeostasis (TCH) [1–8]. In the vast majority of cases, HIV-1 infection, if not treated, leads to AIDS, with TCH failure (i.e., the loss of both CD4 and CD8 T-cells) occurring an average of 1.5–2.5 years before clinically-defined AIDS [5,6,8]. The time between the establishment of HIV-1 infection and TCH failure is thus more variable than the time between TCH failure and the onset of clinically-defined AIDS. This suggests a common mechanism of disease progression between TCH failure and the development of AIDS. The emergence of variants of HIV that use CXCR4 as a co-receptor, which has long been associated with accelerated progression of HIV disease [9–11], most commonly occurs in the year immediately preceding TCH failure [6,12,13].

Naïve T-cells are distinguished by the expression of high levels of the CXCR4 receptor. They are considered critical for the replenishment of the immune system after an infection because they are long-lived and have the capacity to proliferate greatly and differentiate into memory and effector T-cells. The emergence of X4 virions coincides with accelerated CD4 T-cell decline and with the onset of overall CD8 T-cell decline. Naïve CD8 T-cell levels, in particular, have been shown to decline steadily throughout the course of HIV disease [14]. Despite controversy, there are an increasing number of reports in the literature that HIV can actually infect CD8 T-cells [15], including naïve CD8 T-cells [16].

Our data, along with other findings in the literature, suggest that late-emerging strains of HIV, such as X4 strains, may actively

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target naïve CD4 T-cells in particular and also directly affect CD8 T-cells overall. These events could be the key factors that tip the balance into the severe immune dysregulation that leads to AIDS.

Hypothesis

We hypothesize that, non-infectious virions, derived from late-emerging X4 and highly pathogenic R5 virions, contribute to T-cell homeostasis failure during HIV disease progression by depleting uninfected, naïve, CXCR4-positive, CD4 T-cells and affecting the viability and survival of CD8 T-cells overall. This hypothesis could help us understand the bystander immunological effects of HIV ligand binding and direct research towards new therapeutic strategies to inhibit these effects.

Hypothesis evaluation

HIV-induced bystander cell death by non-infectious mechanisms

Only a very small proportion of circulating T-cells are found to be infected with HIV at any one time. Most of the cell death that occurs is in uninfected, "bystander" T-cells [3,13,17–22]. Additionally, the vast majority of circulating HIV virions in vivo are defective and non-infectious [3,12,20,22–26]. They are, however, capable of triggering T-cell death and stimulating partial immune activation through interaction with surface receptors on T-cells [27,28] even without complete cell infection.

To verify this, we studied the survival of CD4 and CD8 T-cells after exposure to primary strains of HIV that had been inactivated with 2,2′-dithiodipyridine (aldrithiol). Aldrithiol covalently modifies essential zinc fingers in the HIV nucleocapsid protein and arrests HIV infectivity at the reverse transcription step [7,13]. Unlike other methods, such as exposure to heat or formalin, this method preserves the conformational and functional integrity of virion surface proteins so that virions can undergo cognate interactions with CD4 and perhaps CCR5 and CXCR4 [13,28,29]. These aldrithiol-inactivated virions have been shown to interact with T-cells without leading to active infection [28].

The emergence of highly pathogenic strains of HIV as a trigger for T-cell homeostasis failure

Variants of HIV that utilize the CXCR4 coreceptor have long been associated with accelerated disease progression [9–11]. The emergence of X4 variants most commonly occurs in the year immediately preceding TCH failure [6,12,13]. In longitudinal studies of Clade B HIV-1 infection, the average CD4 T-cell count at which X4 viruses are first detected is approximately 440 cells/μl [10,30], while that at the time of T-cell homeostasis failure is approximately 350 cells/μl [31], suggesting that the emergence of X4 viruses precedes TCH failure.

CXCR4 tropic strains of HIV have been shown to emerge in 50% or more of HIV infected people [32]. Discrepancies in the literature about the extent of X4 switching exist because assays and algorithms used to determine viral tropism differ considerably [33]. Despite these discrepancies, studying differences in the effect of CXCR4-tropic as compared to CCR5-tropic virions on the immune system should provide key insights into the pathogenic mechanisms of HIV.

The loss of naïve T-cells as a major determinant of AIDS development

A stable pool of naïve T-cells is ordinarily maintained by the thymus via the release of immature lymphocytes [34] and by the proliferation of peripheral naïve cells. Lymphocytes released by the thymus serve as peripheral precursor cells for regenerating mature naïve T-cells. Naïve T-cells have a major role in maintaining T-cell diversity, replenishing effector/memory T-cell populations and protecting the integrity of the total T-cell pool [34]. The disruption of CD4 and eventually CD8 T-cell homeostasis occurs most prominently in the naïve, CXCR4+ T-cell compartment. It is therefore conceivable that a key switch in the development of AIDS could occur when strains of HIV emerge that are more capable of targeting this particular subset of T-cells. The rhesus macaque animal model for HIV-1 pathogenesis corroborates this hypothesis in that some highly pathogenic strains of SHIV exclusively use CXCR4 for cell entry and target naïve CD4+ T-cells for infection. This is in contrast to natural SIV infection in macaques, which utilizes CCR5 and targets memory and effector CD4 T-cells.

Loss in CD8 T-cell numbers and function as a major determinant of AIDS development

CD4 T-cells are the main cells affected by HIV, because most viral replication takes place in them and their decline is the most clinically important feature of HIV infection. CD8 T-cells are also important in HIV infection, however, because they are essential for viral control and host survival through direct killing of infected cells and secretion of factors such as cytotoxic T-cell associated response factor (CNAR), which suppresses HIV replication non-cytolytically [32,35]. Their importance can be seen in that HIV-specific CD8 T-cells increase concurrently with the decline in viral load shortly after acute HIV infection. Experiments in animal models corroborate this in that depletion of CD8+ T-cells causes high viral loads and rapid disease progression in SIV infected monkeys [36,37]. Additionally, CD8 T-cells provide help to CD4 T-cells by secreting IL-2 and other cytokines required for the maintenance of their numbers and function [38].

Effective control of HIV infection is associated with polyfunctional CD8+ T-cells that are more capable of degranulation and producing multiple functional molecules, such as interleukin 2 (IL-2), interferon-gamma (IFNγ) and tumor necrosis factor (TNF) [35]. HIV-specific CD8+ T-cells from HIV-infected progressors, for example, proliferate poorly and secrete fewer cytokines and chemokines [39]. The up-regulation of the programmed cell death molecule 1 (PD-1) and its ligand PD-L1, on both CD4 and CD8 T-cells is associated with decreased T-cell proliferation and cytokine production and poor T-cell survival [40]. This was corroborated in the present study in that up regulation of PD-1 was found in naive CD8 T-cells exposed to the aldrithiol-inactivated virions. For these reasons, it is important that we improve our understanding of the factors that affect CD8+ T-cell numbers and function during HIV disease progression.

HIV surface glycoproteins have a well-characterized specificity for the CD4 molecule and are not generally thought to interact directly with CD8 T-cells. How then might HIV virions affect CD8 T-cell numbers and function? There are several theories that could explain this. CD8 T-cell precursors within the thymus are CD4/CD8 double positive and are CXCR4-positive which presents an opportunity for interaction with HIV viral particles via the CD4 and CXCR4 receptors [16,41,42]. Indeed, HIV has been shown to infect and deplete CD4/CD8 double positive thymocytes [43]. Additionally, activated effector and memory CD8 T-cells have been shown to re-express the CD4 molecule [44,45], which could allow them to interact with HIV virions via this receptor. It has also been shown that HIV can infect CXCR4 positive cells in vitro in the absence of the CD4 molecule [46]. It is therefore conceivable that late-emerging strains of HIV, such as X4 virions, interact directly with CD8 T-cells via the CXCR4 receptor, depleting their numbers and causing a loss in their function in a more profound manner than the strains present earlier during infection.

Empirical data

See Table 1.

Consequences of the hypothesis and discussion

The most significant finding in the reported study was that sort-purified naïve CD4 T-cells exposed to inactivated X4 virions underwent significantly more cell death than those exposed to R5 virions (Table 1). It was also interesting that memory CD8 T-cells showed a greater decline, albeit statistically insignificant, when exposed to inactivated X4 virions, as compared to R5 virions. These results support previously published data showing that inactivated virions can in fact induce bystander T-cell death [28], and extend these findings by showing that this death can be induced differentially by different strains of HIV, among unstimulated T-cell subsets. These in vitro findings are also consistent with previous in vivo studies showing that naïve T-cells decline significantly in number around the T-cell inflection point [27] and with the hypothesis of this study that late-emerging strains of HIV contribute to T-cell homeostasis failure by inducing more cell death in naïve CD4 T-cells than the R5 strains present earlier during HIV infection. The findings also lend support to the hypothesis that late-emerging strains of HIV have a more deleterious effect on CD8 T-cells when compared to viral strains found earlier during the course of infection.

Data from the MACS and other studies show that the mean CD4 T-cell count at the time of TCH failure is about 350 cells/μl [6,10] and that after TCH failure CD4 T-cell counts fall to approximately 200 cells/μl over approximately 2 years, representing an accelerated cellular decline of 0.21 cells/μl/day [6,49]. In Table 1, the difference calculated for the decrease in viable naïve CD4 T-cells induced by X4 virions as compared to R5 virions was 0.09 ± 0.04 cells/μl/day. This is close to the value estimated in the above-mentioned studies, allowing for the influence of in vivo factors other than the virus itself that may augment the decline in CD4 T-cells.

It was originally believed that HIV required CD4 T-cells to be activated in order to infect or stimulate them [50]. In our study and in a study by Esser and colleagues, T-cells were exposed to aldrithiol-inactivated HIV virions without any exogenous stimulation. The cells became partially activated, CD95+CD95L+CD69−CD25−, as opposed to fully activated CD95+CD95L+CD69+CD25+, before undergoing programmed cell death [28] which suggests that complete activation of naïve T-cells is not required for the induction of bystander T-cell death. Additionally, the present study found that naïve CD8 T-cells exposed to the inactivated virions up-regulated PD-1, a marker for cellular activation and T-cell exhaustion, and down-regulated CD31 (data not shown). High expression of CD31 ($CD31^{bright}$) characterizes T-cells that have not undergone any activation or proliferation [51]. The down-regulation of CD31 is consistent with a "partially activated" phenotype.

The development of HIV immune-restorative therapies

In the light of these hypotheses, future studies into HIV immune-restorative, therapeutic vaccine or cure-related therapies should focus more on preventing cell death in conjunction with preventing T-cell exhaustion and restoring immune function. Treatment evaluation should be done in both CD4 and CD8 T-cells. Future studies should focus more on bystander mechanisms of immune dysregulation and how ligand binding by viral particles, even in a non-infectious manner, affects immune status. Studies should be designed to evaluate whether possible immune therapies prevent highly pathogenic strains of HIV from exerting their damaging effects when compared to less pathogenic HIV variants. Studies should determine whether immune therapies specifically arrest or reverse T-cell homeostasis failure based on more extensively defined biomarkers.

IL-15, IL-7, IFNα, IL-21, anti-TNFRII, PD-1/PD-L1 blockers, gp41 fusion inhibitors, HIV Env surface glycoprotein binding agents and IL-2, for example, have all been suggested as possible immune-therapies against AIDS development. Specifically, IL-15 and IFNα have been shown to restore CNAR activity in a number of studies [32]. IL-15 has also been found to have anti-apoptotic effects in CD8 T-cells [52]. The in vivo secretion of IL-21 by HIV-specific CD8+ T-cells from elite controllers has been linked with the maintenance of CD8 T-cell levels and secretion of IL-2 [39]. Culture and human studies show that CD8+ T-cell apoptosis can be inhibited by antibodies against TNFRII [53]. PD-1 and PD-L1 blockers have been shown to be effective at reviving CD8 effector function during HIV infection [54]. HIV gp41 fusion inhibitors such as T20 and C34 have been shown to inhibit bystander cell death in vitro [55]. HIV envelope glycoprotein (Env) binding agents such as plant lectins and glycopeptide antibodies could potentially prevent the bystander effects cause by HIV particles and soluble gp120 [23].

IL-2 therapy, as a final example, has been shown to cause an early increase in CD45RO+ effector memory CD4 T-cells, followed by a rise in CD45RA+ naïve and central memory CD4 T-cells [38]. It has also been shown to restore CNAR activity in CD8 T-cells in a number of studies [32]. When combined with HAART, IL-2 immunotherapy has been shown to increase HIV-1 specific CD8+ T-cell responses in infected subjects. In 2010, Margolick et al. entered a 29 year old, HIV+ male patient into an Ultra Low Dose (UDL) IL-2 therapy study with 2 ART (zidovudine, lamivudine and efavirenz) interruptions, with relative success. Viral load had been undetect-

Table 1
Multi-variate analysis of the effect of aldrithiol-inactivated HIV virions on survival of purified T-cell populations. PBMCs were obtained from 3 HIV-negative laboratory staff and 4 HIV-seropositive men enrolled in the Baltimore/Washington site of the Multicenter AIDS Cohort Study (MACS) (http://www.statepi.jhsph.edu/macs/macs.html) [47]. Cells were purified by cell sorting into naïve (CD27+CD45RA+) and memory or effector (CD27−CD45RA−&+; CD27+CD45RA−) CD4 and CD8 T-cell fractions. Ex-vivo cultures were treated with mock virions (untreated); 10 ng/ml aldrithiol-inactivated CCR5-tropic HIV virions (CCR5); and 10 ng/ml aldrithiol-inactivated CXCR4-tropic HIV virions (CXCR4). Absolute cell numbers per microwell were measured every day, for 9 days and the mean of 3 separate culture replicates was used for the above computation at each time point. Changes in T-cell numbers were assessed by random effects generalized estimating equations models (GEE) [48]. Statistical analyses of the data were conducted using SAS/STAT statistical software version 9.1 (SAS Institute Inc., Cary, NC) and Stata 8.2 (StataCorp, College Station, TX). (For more detailed methods and additional results see [41]).

Variable	CD4 T-cell subset		CD8 T-cell subset	
	Naïve 27+RA+	Memory 27-RA+/− & 27+RA−	Naïve 27+RA+	Memory 27-RA+/− & 27+RA−
HIV serostatus (reference HIV−)	[a]0.21 (.30)	0.10 (.32)	[a]0.12 (.44)	0.53 (.52)
Time (per day)	−0.21 (.03)**	−0.37 (.06)**	−0.33 (.03)**	−0.21 (.03)**
$Time^2$(per day^2)	–	0.02 (.01)**	_	_
Virion treatment (X4 vs. reference R5)	−0.09 (.04)*	0.04 (.04)	0.02 (.05)	−0.04 (.04)

[a] The unit of coefficients are cells/μl. (SE – standard error).
* $p < 0.01$.
** $p < 0.001$.

able for 4 years on ART prior to the study. After the first ART interruption, viral load became detectable within 2 weeks to a set point of 39,000 copies/ml of plasma RNA. The patient was then put back on the ART therapy for approximately 5 months with the addition of UDL IL-2. IL-2 therapy was continued for 1.5 months after a second ART interruption. Five months after the second interruption (3.5 months after IL-2 therapy ceased), the patient's viral load was only 4 copies/ml of plasma RNA and the patient remained clinically undetectable for HIV for a total of 14 months off all therapy [56].

Unfortunately, none of these studies have resulted in the universal acceptance or application of these treatments because results vary considerably among different patients. It is still not clear how best to administer the therapies. It is not clear what immune profile distinguishes patients who are good candidates for and responders to each particular treatment [57]. It is also unclear when to intervene with these treatments and how to monitor and evaluate their success. These factors will become easier to determine once biomarkers for HIV disease progression and immune decline, in addition to CD4 T-cell levels and viral load, have been characterized more precisely and become widely acceptable.

Based on the hypothesis described in this article, additional biomarkers that could be useful for defining immune status in HIV infected individuals and that could help in developing and evaluating immune-restorative and cure-related treatments include markers for T-cell activation and death; markers for "partial" T-cell activation and immune exhaustion; peripheral IL-2 levels; naïve CD4 and CD8 T-cell levels; overall CD8 T-cell levels and T-cell function, including the proliferative capacity of naïve T-cell subsets, the cytotoxic capacity of CD8 T-cells and the ability of CD4 and central memory CD8 T-cells to secrete IL-2 and other cytokines and chemokines that are important for their function.

Despite the great strides in anti-retroviral therapy, it is important that we continue to study and develop immune-restorative therapies. This can be exemplified by the first case of a sterilization cure from HIV. A German HIV positive patient with acute myeloid leukaemia received a bone marrow transplant from an HIV-negative donor homozygous for a deletion in the CCR5 gene in 2009. The patient ceased HIV treatment soon after the procedure. There was a reconstitution and great improvement in the patient's immune profile. He currently remains undetectable for HIV nucleic acids and asymptomatic for HIV-related disease. This is especially encouraging considering that the patient did harbor X4 virus that his immune system was able to contain and eradicate after the immune-reconstructive therapy [58–60]. Elite controllers of HIV infection, who manage to keep viral loads below the limit of detection despite not being on any treatment, also point towards the possibility of a "functional cure" from HIV if researchers can determine how to boost the patient's own immune system [61]. Therapies that augment current anti-retroviral treatments are needed because generally, patients experience a viral rebound if they are taken off ART, even when their viral loads have become undetectable using ultra-sensitive molecular assays [62,63]. Moreover, long-term ART therapy is not readily available worldwide and is often unsustainable and prohibitively expensive to the local people in areas of the world that are the most affected by the epidemic.

Conflict of interest

There are no conflicts of interest to declare.

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