Predicting the impact of CD8$^+$ T cell polyfunctionality on HIV disease progression

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Abstract

During the chronic phase of HIV-1 infection, polyfunctional CD8+ T cell responses, which are characterized by a high frequency of cells able to secrete multiple cytokines simultaneously, are associated with lower virus loads and slower disease progression. This relationship could arise for different reasons. Polyfunctional responses could simply be stronger. Alternatively, it could be the increased functional diversity in polyfunctional responses that leads to lower virus loads and slower disease progression. Lastly, polyfunctional responses could contain more CD8+ T cells that mediate a specific key function, which is primarily responsible for viral control. Disentangling the influence of overall strength, functional diversity, and specific function on viral control and disease progression is very relevant for the rational design of vaccines and immunotherapy using cellular immune responses. We developed a mathematical model to study how polyfunctional CD8+ T cell responses mediating lytic and non-lytic effector functions affect the CD4+ T cell count and plasma viral load. We based our model on in vitro data on the efficacy of IFN-γ and MIP-1β/RANTES against HIV. We find that the strength of the response is a good predictor of disease progression, while functional diversity has only a minor influence. In addition, our model predicts for realistic levels of cytotoxicity that immune responses dominated by non-lytic effector functions most positively influence disease outcome.

Importance

Why polyfunctional CD8+ T cell responses are associated with better viral control while individual functional correlates of protection have not been identified so far is still an open question in HIV-1 research. Identifying the role of CD8+ T cells in HIV-1 infection has important implications for the potential development of effective T cell based vaccines. Our analysis provides new ways to think about a causative role of CD8+ T cells by studying different hypotheses why polyfunctional CD8+ T cells might be more advantageous. We identify measurements that have to be obtained in order to evaluate the role of CD8+ T cells in HIV-1 infection. In addition, our method shows how individual cell functionality data can be used in population based virus dynamics models.
**Introduction**

CD8\(^+\) T lymphocytes are immune cells essential for the control or even eradication of viral infections (1, 2). After being activated, CD8\(^+\) T cells are able to recognize and kill infected cells. Besides their cytotoxicity, activated CD8\(^+\) T cells release a large number of cytokines, which either affect the dynamics of the immune response (e.g. IL-2, TNF-\(\alpha\)) or interfere with the viral pathogen itself (e.g. IFN-\(\gamma\), MIP-1\(\beta\)/RANTES) (3, 4). Absence of CD8\(^+\) T cells may lead to the inability of the organism to control an infection, as it is observed for lymphocytic choriomeningitis virus (LCMV) in mice and simian immunodeficiency virus (SIV) in monkeys (5, 6).

The role of CD8\(^+\) T cells in infection by human immunodeficiency virus type 1 (HIV-1) has not been determined so far (7). Although infected individuals are observed to exhibit high levels of HIV-specific CD8\(^+\) T cells (8–10), this response is not able to eradicate the virus. After a period of acute infection (~3-4 months after infection), high plasma viral load can persist for several years even in the presence of high levels of HIV-1 specific CD8\(^+\) T cells. In addition, the failure of HIV-1 vaccine trials based on the elicitation of strong cellular immune responses (11) questioned the importance of CD8\(^+\) T cells in HIV-1 infection despite previous observations on their influence on viral control in HIV-1 (1, 2) and SIV (5, 6).

The missing identification of a definitive mechanism by which CD8\(^+\) T cells might control HIV-1 infection hinders the evaluation of the role of this cell type. An immune correlate of protection of CD8\(^+\) T cells against HIV-1 has not been determined so far, as no single frequency of HIV-specific CD8\(^+\) T cells showing a certain functionality correlates with protection or viral control (7). However, it has been observed that the overall quality of HIV-1 specific CD8\(^+\) T cell responses measured by their polyfunctionality, i.e. the frequency of CD8\(^+\) T cells within the epitope specific response expressing several effector functions simultaneously, correlates with viral control: Betts et al. (12) showed that HIV non-progressors, who are HIV infected patients that are characterized by a stable viremia and CD4\(^+\) T cell count during the chronic phase of the infection, have significantly more polyfunctional CD8\(^+\) T cells than HIV-progressors who more rapidly progress to AIDS. The frequency of polyfunctional epitope-specific CD8\(^+\) T cells inversely correlated with the viral load (12). Several other studies addressed the
polyfunctionality of HIV-specific CD8+ T cell responses and analyzed their impact on
disease progression (for a review see (13, 14)).

Although the current prevailing assumption in the field is the more polyfunctional
CD8+ T cells the better viral control, it has not been determined so far if the increased
frequency of polyfunctional CD8+ T cells is the cause for or the consequence of
enhanced viral control. Better viral control in animal models of chronic infection after
restoring dysfunctional CD8+ T cell responses, as observed in LCMV (15) and SIV
(16), argues for a causative role of dysfunctional CD8+ T cells on disease progression.
In contrast, increased frequencies of polyfunctional CD8+ T cells were observed after
antiretroviral treatment (17) while their initial frequency had no impact on the clinical
outcome (18). Hence, the role of CD8+ T cells in HIV-1 infection still remains elusive
with the current experimental and clinical knowledge.

Even if we assume that a more polyfunctional CD8+ T cell response is the cause for
better viral control, there are still conceptual issues to be resolved. We can think of
three different reasons why a more polyfunctional response could lead to better viral
control than a less polyfunctional one with a comparable response size: [1.] A higher
frequency of polyfunctional CD8+ T cells within the HIV-1 specific response
indicates a stronger HIV-specific T cell response as more CD8+ T cells are capable of
performing several functionalities, increasing the total number of functions expressed
by the population. Thus, polyfunctionality would simply correlate with strength. [2.]
A higher frequency of polyfunctional CD8+ T cells could also indicate a more
functionally diverse CD8+ T cell response as many different functions are expressed,
which could synergize in their inhibitory effect on viral replication. [3.] A higher
frequency of polyfunctional CD8+ T cells also means that there is a higher frequency
of CD8+ T cells mediating a particular function, and this effector function could be
essential for controlling viral replication. In short, it is not yet clear if [1.] the
strength, [2.] the functional diversity, or [3.] a specific function mainly determines
disease progression. Separating the effect of each of these three different factors on
CD4+ T cell count and plasma viral load will help to reveal how HIV infection is
controlled by the CD8+ T cell response, and how a dysfunctional CD8+ T cell
response should best be modulated to enhance the outcome of the infection.

To evaluate the role of polyfunctionality, we extended a viral dynamics model
published previously (19) analyzing the effect of cytolytic and different non-cytolytic
effector functions of CD8+ T cells on viral control and immunopathology in HIV-1
infection. In our model, we explicitly consider three different effector functions of the CD8\(^+\) T cell response that directly affect the viral pathogen: (i) cytotoxicity as e.g. mediated by perforin and granzyme, (ii) inhibition of viral entry, e.g. mediated by MIP-1\(\beta\)/RANTES, which is observed to compete with HIV-1 for binding sites on the CCR5 chemokine receptor expressed by CD4\(^+\) T cells (20, 21), and (iii) inhibition of viral replication in infected cells, as it is e.g. suggested to be mediated by IFN-\(\gamma\) (22, 23) or the CD8\(^+\) T cell anti-HIV factor (CAF) (24–26) that has not been identified so far (27). Based on *in vitro* experimental data, we quantified the inhibition of HIV-1 infectivity and viral replication by MIP-1\(\beta\)/RANTES (28) and IFN-\(\gamma\) (23), respectively. We use these specific factors as surrogates for molecules mediating such functions, as uncertainties exist about their antiviral efficacy (see Discussion).

In our model, disease outcome is related to the level of polyfunctionality as has been observed experimentally (12). Manipulating the strength, polyfunctionality and individual functions separately we find that the strength of the CD8\(^+\) T cell response measured by the average number of different effector functions per CD8\(^+\) T cell is the best predictor for disease progression. In contrast, functional diversity of the response has only a minor influence. Investigating the influence of particular effector functions we found that CD8\(^+\) T cell responses with dominant non-lytic effector functions showed a better improvement in CD4\(^+\) T cell count and plasma viral load than more lytic-responses assuming reasonable levels of cytotoxicity. Especially effector functions inhibiting viral replication are predicted to have the most beneficial impact when trying to improve dysfunctional CD8\(^+\) T cell responses by immuno-modulatory interventions. Thus, individuals with an increased frequency of polyfunctional CD8\(^+\) T cells are most likely to show better viral control because of their stronger immune responses, rather than an increased functional diversity of the response.
Mathematical model

We extended a previously published model for viral dynamics (19, 29) incorporating the different functions of the CD8\(^+\) T cell response during chronic HIV-1 infection: The general model assumes that susceptible CD4\(^+\) T cells, \(T\), are constantly produced at a rate \(\lambda\), die with a natural death rate unrelated to the CD8\(^+\) T cell response of \(\delta_T\) and become productively infected, \(I\), by an infection rate \(\beta\) based on the density of free viral particles per \(\mu\)l of blood, \(V\). Virions are produced with rate \(\rho\) per productively infected cell and have an average lifetime of \(1/c\). The parameter \(\delta_I\) denotes the natural death rate of productively infected cells.

As an extension of the general model, we incorporated the different immune effector functions mediated by the CD8\(^+\) T cell response. As we are modeling viral dynamics during the chronic phase of HIV infection we assume the number of HIV-1 specific CD8\(^+\) T cells, \(E\), to be constant. The implication of this assumption is discussed in detail in the main text. The fractions of the CD8\(^+\) T cell population releasing cytokines that impair viral entry (MIP-1\(\beta\)/RANTES) and viral replication (e.g. IFN-\(\gamma\)/CAF), or the fraction that is capable of killing infected cells are denoted by \(f_{e}, f_{r}\) and \(f_{k}\), respectively. With this parameterization, infected cells get killed at a rate \(k f_k E\), where \(k\) denotes the killing rate (30). Based on a study by Cocchi et al. (28), we assume that the concentration of MIP-1\(\beta\)/RANTES reduces the efficacy of the infection rate \(\beta\) in a dose-dependent manner with efficacy \(\tau_e = f_{e} m E (f_{e} m E + M_{1/2})\). Here, the parameter \(m\) defines the cytokine production per CD8\(^+\) T cell, while \(M_{1/2}\) denotes the concentration of MIP-1\(\beta\)/RANTES where the impairment of infectivity is half of the maximum. Analogously, IFN-\(\gamma\) is assumed to impair viral replication (23), i.e. the production rate of viral particles, \(\rho\), by a factor \(1 - \tau_r\) with \(\tau_r = f_{r} \gamma E (f_{r} \gamma E + G_{1/2})\). The parameter \(\gamma\) defines the production of IFN-\(\gamma\) per CD8\(^+\) T cell, while \(G_{1/2}\) denotes the concentration of IFN-\(\gamma\) where the impairment of viral replications is half of the maximum. The full system is then formulated by:
\[
\frac{dT}{dt} = \lambda - \delta_T T - \beta \left(1 - \frac{mf_E}{mf_E + M \frac{1}{2}}\right) VT
\]

\[
\frac{dI}{dt} = \beta \left(1 - \frac{mf_E}{mf_E + M \frac{1}{2}}\right) VT - \delta_I I - k f E l
\]

\[
\frac{dV}{dt} = \rho \left(1 - \frac{\gamma_f E}{\gamma_f E + G\frac{1}{2}}\right) I - c V
\]

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The total density of CD4\(^+\) T cells per mm\(^3\) of blood at equilibrium, \(N^* = T^* + I^*\), is then defined by

\[
N^* = \frac{\lambda}{\delta_I + k f E} + \frac{c(\delta_I + k f E - \delta_I)}{\rho(1 - \tau_r)\beta(1 - \tau_e)}
\]

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The plasma viral load in virions per \(\mu l\) of blood at equilibrium, with one virion representing two HIV-1 RNA copies, is calculated by

\[
V^* = \frac{\lambda \rho(1 - \tau_r)}{c(\delta_I + k f E)} - \frac{\delta_I}{\beta(1 - \tau_e)}
\]

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**Parameterization**

The majority of model parameters in Eq. (1) is defined by values from the literature (see Tab. 1): We assume that the number of new CD4\(^+\) T cells per mm\(^3\) introduced into the blood per day is given by \(\lambda = 8\) day\(^{-1}\) (31), and that uninfected cells die by a natural death rate of \(\delta_I = 0.01\) day\(^{-1}\) (32). The average production rate of virions by infected cells is assumed to be \(\rho = 1000\) day\(^{-1}\), and virions have an average lifetime of \(1/c = 1/23\) days (33). The parameters defining the effector functions of the CD8\(^+\) T cell response are determined in the following.

**CD8\(^+\) T cells, \(E\):** The frequency (or density) of HIV-specific CD8\(^+\) T cells, \(E\), is parameterized based on the CD4:CD8 ratio observed in HIV infected individuals (34–36). The ratio of CD4\(^+\) to CD8\(^+\) T cells before the AIDS phase is of the order of 1.1-1.2 (34). Values smaller than 1 usually indicate the beginning of the AIDS phase. Around 0.5 to 22\% of CD8\(^+\) T cells are HIV-specific (35), meaning that the frequency of HIV-specific CD8\(^+\) T cells among all lymphocytes is between 0.17 - 7.5 \times 10^{-2}, assuming that 40\% of all lymphocytes are CD4\(^+\) T cells (36). Taking the maximum...
frequency of 0.075, the number of HIV-specific CD8$^+$ T cells per mm$^3$ of blood is roughly $E \approx 225$ cells.

For HIV infection, the observed numbers for CD4$^+$ and CD8$^+$ T cell populations are based on blood samples while the actual infection and immune processes preferentially occur in lymphatic organs, such as lymph nodes and the spleen. There, cell densities are much higher allowing close contacts between cells. In order to account for the difference in cell densities between blood and lymphoid organs, we have to introduce a scaling factor $\nu$ that scales the CD8$^+$ T cell response, $E$. The parameter $\nu$ is defined as follows. Based on Trepel (37), roughly 2 - 3% of all lymphocytes are in the blood, while the spleen comprises 12-20%. Assuming a total blood volume of 5 l and the average volume of a human spleen to be $\sim 200$ ml (38), the density of lymphocytes in the spleen is 100 - 250 times higher than in the blood, hence, $\nu \sim 100 - 250$. However, it is also observed that the CD4:CD8 ratio in lymphatic organs is roughly 2 - 3 times higher than in the blood (39). Because the frequency of HIV-specific CD8$^+$ T cells among lymphocytes is comparable between blood and lymphatic organs (40), we assume that the effective CD8$^+$ T cell density is given by $\nu E$ with $\nu \sim 30 - 125$. In the following, we use a scaling factor of $\nu = 75$, roughly the middle of the estimated interval for $\nu$. The sensitivity of the results with regard to the value of $\nu$ was analyzed.

**HIV suppressing factors (MIP-1β/RANTES):** Using *in vitro* cultures of HIV-infected cells Cocchi et al. (28) found a dose dependent inhibition of infection by HIV suppressing factors (SF), such as MIP-1α, MIP-1β and RANTES. Mathematically, we can describe this inhibiting effect on viral entry, $\tau_e$, as a function of the concentration of HIV-SF, $M$, by $\tau_e(M) = \frac{M}{M + M_{1/2}}$, with $M_{1/2}$ denoting the concentration at which the inhibiting effect is half of the maximum. Based on the data, $M_{1/2}$ is determined as $M_{1/2} = 5$ ng/ml. The concentration of HIV-SF given $10^6$ activated CD8$^+$ T cells per ml varies between 6 – 235 ng/ml (Table 1 in (28)), hence, the concentration per CD8$^+$ T cell is approximately $m = 6 \times 10^{-6} - 2.35 \times 10^{-4}$ ng.

**Effect of IFN-γ on viral production, $\rho$:** Already in 1986, Walker et al. (25) found a dose-response relationship between the number of CD8$^+$ T cells and the reverse transcriptase (RT) activity in HIV infected cells that was not attributable to CTL mediated killing of HIV infected cells. Studying the effect of IFN-γ on HIV infected
cells, Hammer et al. (23) found that roughly 100 units of IFN-γ (~ 6.25 × 10^3 pg) on 2 × 10^5 cells per ml lead to a 50% reduction in RT-activity. The production of IFN-γ per CD8^+ T cell is about 0.5 pg (41). With these values, we parameterized the effect of IFN-γ on the viral production rate ρ with $G_{1/2} = 6.25 \times 10^3$ pg/ml and $γ = 0.5$ pg.

**Killing rate constant, k:** Productively infected cells have an estimated lifespan of about 1 day (31, 42-44). This estimate includes the natural death rate of infected cells, $δ_i$, and the loss of infected cells due to CD8^+ T cell mediated killing. Therefore, the decay rate of productively infected cells is defined by $\tilde{δ}_i = δ_i + kE = 1$ day$^{-1}$. As $δ_i$ also comprises the natural death rate of target cells, $δ_T$, we always ensured $δ_i ≥ δ_T$.

Asquith et al. (45) estimated that HIV-specific CTL lysis is responsible for around 10%-20% of the observed decay in the number of infected cells. Using the upper limit with $kE = 0.2 \tilde{δ}_i$ and the density of CD8^+ T cells given by $E = 0.075$, we obtain a killing rate of $k = 2.7$ day$^{-1}$, comparable to previous estimates found for HIV (45) and HTLV (46).

**Production rate of new virions, ρ:** The number of viral particles produced by one infected cell in HIV or SIV infection is still debated. Experimentally obtained estimates vary quite substantially from 100 (47) to 700-1800 (48) and up to the order of 20,000-50,000 virions produced by one infected cell per day (49). Using different values for the viral production rate ρ in Eqs. (2) and (3), we found that reasonable CD4^+ T cell counts and plasma viral loads for HIV progressors and non-progressors are obtained when using viral production rates in the order of 1000 virions per cell per day (Figure S1). Here, the outcome of the model was compared to the observed CD4^+ T cell count and plasma viral load in patients belonging to the specific sub-groups (Table 2 and (12)). In the following, we used a viral production rate of $ρ = 1000$ virions per cell per day.

**Assessing the correlation of expression of specific functionalities**

To examine if effector functions are expressed independently, we analyzed the data by Betts et al. (12) who measured the expression of 5 different functions for each of 5 different HIV epitopes. If two functionalities, a and b, are independent of each other, then the measured frequency of CD8^+ T cells expressing both functions, $f_{ab}$, should be the product of the frequencies of CD8^+ T cells expressing the one or the other effector.
function, hence, \( f_{ab} = f_a \times f_b \). The difference \( \Delta = f_{ab} - (f_a \times f_b) \) indicates how far the expected and measured frequency of double positive cells are from each other. Because of the definition of \( f_a \) and \( f_b \) with \( f_{ab} \), \( f_a \) and \( f_b \), for large values of \( f_a \) and \( f_b \), \( f_{ab} \) would have less possible values than for small values of the two individual frequencies. Therefore, we have to scale the difference \( \Delta \) by the possible ranges for \( f_{ab} \) given \( f_a \) and \( f_b \) in order to analyze if \( \Delta \) is significantly different from 0, and, thus, would indicate a correlative expression of both effector functions. The maximal observable frequency of double positive cells, corresponding to the maximal overlap of \( f_a \) and \( f_b \), is given by \( f_{ab}^{\max} = \min\{f_a, f_b\} \). The minimal observable frequency is given by \( f_{ab}^{\min} = \max\{0, \max\{f_a, f_b\} - (1 - \min\{f_a, f_b\})\} \) (see also Figure S2). With this, we obtain a scaled difference of \( \Delta \), named \( \tilde{\Delta} \), by

\[
\tilde{\Delta} = \frac{\Delta}{f_{ab}^{\max} - f_{ab}^{\min}} = \frac{f_{ab} - (f_a \times f_b)}{f_{ab}^{\max} - f_{ab}^{\min}} \tag{4}
\]

In general, for \( n \) different effector functions with individual frequencies \( f_i \), \( i = 1, \ldots, n \), the possible, observable frequency of polyfunctional T cells, \( P_n^{\pm} \), is in the range between the minimum of all individual frequencies, \( \min\{f_i\} \), and \( \max\{0, \sum f_i - n + 1\} \).
Results

Mathematical model

To study the influence of the functional composition of CD8+ T cell on HIV-1 disease progression, we extended a mathematical model that had been developed to investigate the impact of different level of CD8+ T cells on disease progression (19). The model describes the dynamics of uninfected cells, infected cells, and the plasma viral load (pVL) within a host. We specifically account for the effect of three different CD8+ T cell effector functions: (i) cytotoxicity, (ii) inhibition of infection, i.e., viral entry, and (iii) impairment of viral replication (see Figure 1). For each of the three different effector functions, we parameterized the effect of the chemokine/cytokine per CD8+ T cell based on previous estimates and experimental data of HIV-1 in vitro culture systems, using IFN-γ and MIP-1β as surrogates for the inhibition of viral replication and viral entry, respectively (23, 28, 45) (for a detailed description of the mathematical model and the parameterization see Materials & Methods). With the parameters used, between 0.4-4% of the CD4+ T cells are infected. These numbers are in agreement with the observed frequency of infected CD4+ T cells in HIV infected individuals (50).

The functionality of the CD8+ T cell response is defined by the frequencies of CD8+ T cells that are able to mediate one of the particular effector functions, with \( f_c \), \( f_k \), and \( f_r \) denoting the frequencies of functional CD8+ T cells for blocking viral entry, cytotoxicity, and impairment of viral replication, respectively. Using a fixed number of CD8+ T cells, we are able to create different responses with varying degrees of polyfunctionality by varying the values of \( f_c \), \( f_k \), and \( f_r \) (see below). Examining 79 HIV-1 progressors, Betts et al. (12) showed that their HIV-specific CD8+ T cell response is impaired with only fractions of the total response capable to mediate a specific effector function (see Figure 1B in (12)). Based on their observations across different HIV-specific epitopes, we set the average frequencies of the particular functionalities for CD8+ T cell responses in HIV progressors to \( f_c = 0.94 \), \( f_k = 0.59 \), and \( f_r = 0.62 \) (see Table 3).

Influence of strength and functional diversity of the CD8+ T cell response on disease outcome
To analyze the effect of the strength of the HIV-specific CD8+ T cell response on disease progression, we defined the parameter $\Psi$ as the number of effector functions that a single CD8+ T cell would have on average by

$$\Psi := \Psi(f_e, f_k, f_r) = \sum_{i \in \{e, k, r\}} f_i$$

A CD8+ T cell response with $\Psi = 3$ corresponds to a fully functional immune response, i.e. each CD8+ T cell is able to mediate each effector function. A completely dysfunctional or depleted CD8+ T cell response is characterized by $\Psi = 0$ (Figure 2).

We calculated the CD4+ T cell count and the pVL at equilibrium based on Eq. (2) and Eq. (3), using CD8+ T cell responses varying in their functional composition. Figure 2A shows the mean, maximal and minimal values for each of these outcome variables dependent on the strength of the CD8+ T cell response $\Psi$. The mean CD4+ T cell count increases with increasing strength, while the plasma viral load decreases. Variation in the mean CD4+ T cell count over $\Psi$ ranges from ~190 cells mm$^{-3}$ at $\Psi = 0$ to ~1000 cells mm$^{-3}$, while the mean plasma viral load varies between 0 and ~670 viral RNA copies $\mu$l$^{-1}$. For $\Psi < 0.5$, the mean CD4+ T cell count is close to 200 cells mm$^{-3}$, the threshold defining the AIDS phase of the infection. A value of $\Psi > 2$, as it is also observed for HIV-progressors ($\Psi_{HIV-p} = 2.15$), generally leads to CD4+ T cell counts that are above 400 cells mm$^{-3}$, and plasma viral loads with on average 23 RNA copies $\mu$l$^{-1}$ given our parameterization of the model (Figure 2A).

The parameter $\Psi$ is a measure for the strength, rather than the functional diversity of the CD8+ T cell response. A value of $\Psi = 1$ could mean $(f_e,f_k,f_r) = (1,0,0)$ or $(f_e,f_k,f_r) = (1/3,1/3,1/3)$ (Figure 2), two CD8+ T cell responses with probably different effects on CD4+ T cell count and plasma viral load. In order to investigate the influence of the functional diversity of the CD8+ T cell response on disease progression, we calculate the Simpson Index, $D$, as it is defined in ecology to calculate the biodiversity in a certain area (51) with

$$D = 1 - \sum_{i \in \{e,r,k\}} \left( \frac{f_i}{\sum_{i \in \{e,r,k\}} f_i} \right)^2$$

A value of $D = 0$ means no diversity, and only one particular function is mediated by the CD8+ T cell response. In our situation, the diversity can reach values between $D = 0$ and $D = 2/3$, with $D = 2/3$ indicating the maximal degree of diversity that is
reached if the frequencies of CD8+ T cells expressing the particular effector functions are the same, i.e. $f_e = f_i = f_k$. Figure 2B shows the effect of increasing functional diversity on the CD4+ T cell count and plasma viral load for three different subsets of CD8+ T cell responses with a predefined strength of $\Psi = 0.9$, $\Psi = 1.8$, and $\Psi = 2.4$, respectively. Stronger CD8+ T cell responses are also characterized by a higher level of diversity. However, among all CD8+ T cell responses with a certain strength $\Psi$, the influence of increasing diversity on the CD4+ T cell count and the plasma viral load is only minor. Within these groups, the mean CD4+ T cell count increases by approximately less than 1/3 of its starting value ($\sim$100 cells mm$^{-3}$) with increasing diversity, while the mean plasma viral load decreases to one third and down to one half of its starting value ($\sim$20 - 100 HIV-1 RNA copies $\mu l^{-1}$). Thus, the strength of the CD8+ T cell response has a stronger effect than the functional diversity of the response on viral control and disease outcome.

**Strength, diversity and polyfunctionality**

In the clinical studies, the polyfunctionality of a CD8+ T cell response is defined as the fraction of pathogen-specific cells that express multiple functional markers simultaneously (7). How is this definition related to the frequencies $f_e$, $f_k$, and $f_i$ that characterize the functionality of responses in our virus dynamics model?

Assuming that all three effector functions are expressed independently, we can calculate the expected frequency of polyfunctional CD8+ T cells, $P^{3+}$, for a particular CD8+ T cell response based on our population dynamics model by $P^{3+} = f_ef_kf_i$. Similarly, the fraction of CD8+ T cells that can perform two functions is given by $P^{2+} = (1 - f_e)f_kf_i + f_e(1 - f_k)f_i + f_e f_k(1 - f_i)$. The expected functionality profile of the CD8+ T cell response in HIV-progressors based on the average functional frequencies (Table 3) can be seen in Figure 3A.

How is the conventional measure of polyfunctionality, i.e., $P^{3+}$, related to our concepts of strength and diversity? For a given strength of the response, $\Psi$, the expected frequency of polyfunctional T cells, $P^{3+}$, is highest when all the functional frequencies are equal: $f_e = f_k = f_i$. In this situation, the response is also maximally diverse. In general, the polyfunctionality of a CD8+ T cell response increases with increasing strength of the response as expected by the definition of the strength, i.e., denoting the average number of effector functions per CD8+ T cell (Figure 3B).

However, there is no linear relationship between these two quantities defined by $P^{3+} =$
and $\Psi = f_e + f_k + f_r$, respectively. Comparing the performance of the conventional measure of polyfunctionality, the strength and the diversity in predicting disease progression, we find that the strength of the response is a slightly better predictor of disease progression (CD4: $R^2 = 0.9$, pVL: $R^2 = -0.84$, Pearson-Correlation) than the conventional measure of polyfunctionality (CD4: $R^2 = 0.83$, pVL: $R^2 = -0.69$) while the diversity has the least predictive power (CD4: $R^2 = 0.52$, pVL: $R^2 = -0.53$).

Based on the data of Betts et al. (12) characterizing the functional profile of CD8$^+$ T cell responses in HIV progressors and non-progressors, we examined if the particular effector functions are expressed independently. Betts et al. measured the expression of 5 different functions: IL-2, TNF-$\alpha$, IFN-$\gamma$, MIP-1$\beta$, and the degranulation marker CD107a for 5 different HIV epitopes: env, pol, gag, nef and trvrvv. If two functionalities, $a$ and $b$, are independent of each other, then the measured frequency of CD8$^+$ T cells expressing both functions, $f_{ab}$, should be the product of the frequencies of CD8$^+$ T cells expressing the one or the other effector function, hence, $f_{ab} = f_a \times f_b$. Figure 3C shows the measured frequencies of CD8$^+$ T cells being positive for one or two markers for each different combination of CD107a, IFN-$\gamma$ and MIP-1$\beta$ exemplarily for the gag-epitope for each patient from (12). Analyzing $\Delta = f_{ab} - (f_a \times f_b)$ scaled for the range of possible frequencies for $f_{ab}$ (Materials & Methods), our data indicate that we cannot generally reject the null-hypothesis that the different functionalities are expressed independently ($p$-value $> 0.05$, Mann-Whitney U-test, and Table S1, Figure S2, S4). However, for cells expressing three or more functions simultaneously, our analysis shows that the individual functions positively affect each other ($p<0.05$) throughout all epitopes. This does not hold for combinations including IL-2. In general, we find that on average the expression of triple functional CD8$^+$ T cells is higher than expected based on the individual frequencies of CD8$^+$ T cells specific for one functionality ($\bar{\Delta} > 0$). This would indicate a correlative expression of specific effector functions of CD8$^+$ T cells as observed before (12, 52, 53).

**Influence of particular effector functions on disease outcome**

In Figure 2A we can see that the CD4$^+$ T cell count and plasma viral load still varies substantially for intermediate strengths of the CD8$^+$ T cell response. The variation is not explained by varying diversities of the responses (Figure 2B) leading to the
question if some effector functions might be more effective than others in controlling disease progression.

To disentangle the impact of particular functionalities from the strength and the functional diversity of the response, we analyzed all CD8$^+$ T cell responses with a predefined functional diversity $D$ and strength $\Psi$.

In Figure 4 we show exemplarily for a CD8$^+$ T cell response with a frequency ratio of 2:1:1 between the three different effector functions and changing dominant effector functions how CD4$^+$ T cell count and plasma viral load are impacted. CD8$^+$ T cell responses with a dominant non-lytic response (Figure 4A,B) show better maintenance of CD4$^+$ T cell count and better viral control than responses with dominant cytotoxicity (Figure 4C), irrespective of the strength $\Psi$. Inhibition of viral replication suppresses the viral load more efficiently than inhibition of viral entry. These observations are in agreement with previous findings in HIV and SIV infection showing that non-lytic effector functions are more important for viral control than lytic responses (45, 54–57). The observed hierarchy in effector functions can also be seen when looking at the impact of different compositions of a CD8$^+$ T cell response (with strength $\Psi = 1$) on CD4$^+$ T cell count and plasma viral load (Figure 4D,E). A response skewed towards non-lytic effector functions, especially where the inhibition of viral replication is the dominant effector function, would maintain a higher CD4$^+$ T cell count than CD8$^+$ T cell responses with dominant cytoxicity. Responses dominated by lytic effector functions are advantageous if more than 80% of the death in infected cells is assumed to be due to cytotoxicity, assuming higher killing rates ($k \sim 10.6$ day$^{-1}$) than estimated before (45).

**Optimizing immunomodulatory interventions**

Therapeutic approaches involving the restoration of CD8$^+$ T cell function during chronic infections are being explored (15, 16). Drugs that increase specific effector functions in chronic HIV infection could be available in the future. This prospect leads to the question which effector function constitutes the most promising target.

To investigate this question, we compared the gain in CD4$^+$ T cells that our model predicts when we improve one, two or three effector functions. In all three cases, the improvement was chosen such that the overall strength increased by the same amount. We observe that concentrating on the improvement of non-lytic effector functions for our parameterization would lead to a more substantial improvement in the CD4$^+$ T
cell count and plasma viral load than increasing each effector function equally. Hereby, increasing the inhibition of viral replication leads to a stronger improvement than inhibiting viral entry (Figure 5). This is also due to the fact that HIV progressors maintain a very high frequency of functional CD8+ T cells mediating MIP-1β/RANTES (Table 3 and (12)) which can be observed throughout all different HIV epitopes (Fig. S2). Hence, an increase in this specific effector function has only a minor additional effect. Focusing on lytic effector functions would lead to less substantial improvements in CD4+ T cell count and plasma viral load than compared to increasing the strength of the response in general (Figure 5). However, an increase in the frequency of CD8+ T cells mediating cytotoxicity does not increase immunopathology as indicated by the development of the CD4+ T cell count (Figure 4). This finding is in agreement with previous observations made for acute infection (58). In general, each improvement of the functionality of CD8+ T cell responses improved disease outcome.
Discussion

The more functions CD8+ T cells can perform in chronically HIV infected individuals, the lower the virus load and the better the prognosis (7, 12). This observed association has lead to “the prevailing paradigm [...] that the more functions a CD8+ T-cell performs, the more antiviral it must be” (7). Adopting this paradigm, we developed a virus dynamics model describing the relationship between the polyfunctionality of CD8+ T-cell responses, virus load, and disease.

Our virus dynamics model allowed us to dissect and quantitatively investigate the relationship between polyfunctionality and viral control. In particular we posed the following questions: Is it that in polyfunctional responses one simply has a higher fraction of functional CD8+ T cells? Or is it important to mount a functionally broad response to optimally control virus replication? Or is there a key function that is more prevalent in more functional responses?

To answer these questions empirically would require a large cohort of HIV-1 infected individuals. In the individuals of such a cohort, one would have to determine the functional composition of the CD8+ T cell response (corresponding to the frequencies $f_i$ in our model) in addition to the virus load and disease progression, as has been done for the 88 individuals enrolled in the study by Betts et al. (12). The cohort would have to be large enough such that it contains sizable subgroups of individuals with each function present at different levels. The virus load and disease progression in these subgroups would then have to be correlated with their functional composition.

Because such a large cohort is not yet available, we took a computational approach that incorporates empirical information on the effect of CD8+ T cells on virus replication. To disentangle the effect of the strength and functional diversity of CD8+ T cells on HIV-1 disease progression, we extended a theoretical model on viral dynamics used previously (19). Our model explicitly considers three specific functionalities of the CD8+ T cell response that directly interact with the virus and the infected cells, and is parameterized according to previous estimates (31, 33, 42–44) and in vitro experimental observations (23, 28). We assume a constant number of CD8+ T cells as this allows us to determine the effect of different levels of polyfunctionality on CD4+ T cell count and pVL at steady-state. Furthermore, the
actual size of the CD8+ T cell response is not critical in our qualitative analysis on the importance of strength, functional diversity and specific functions on the disease outcome.

In agreement with Betts et al. (12), our virus dynamics model showed that increased polyfunctionality correlates with the CD4+ T cell count, and correlates inversely with the plasma viral load. Hence, we did not find evidence for immunopathology caused by the CD8+ T cell response as measured by the CD4+ T cell count. Restoring the functionality of a dysfunctional CD8+ T cell response is predicted to be always beneficial, as already observed in acute infections (58).

Varying the functional profile of a CD8+ T cell response, we found that the strength of the response, i.e. the average number of effector functions a CD8+ T cell expresses simultaneously, has the largest explanatory power on the inverse correlation between the polyfunctionality of the immune response and the plasma viral load (Figure 2). The functional diversity, i.e. the ability of the CD8+ T cell response to mediate many different effector functions, is of less importance. This finding would support the assumption that indeed the increased overall functionality in polyfunctional CD8+ T cell responses is essential for viral control rather than the functional breadth of the response. In general, an increase in both lytic and non-lytic responses improves the outcome (59, 60).

However, when looking at the abundance of specific functions in more detail, we found that CD8+ T cell responses with a dominant non-lytic response show better maintenance of CD4+ T cell count and better viral control than responses with dominant cytotoxicity (Figure 4D,E). This hierarchy of effector functions was observed independently of the strength of the response.

Our observation is in line with several other studies suggesting that CD8+ T cells mainly control HIV infection by non-lytic mechanisms (45, 54-57). The depletion (54, 55) or the level (61, 62) of virus-specific CD8+ T cell responses is observed to have a strong effect on total viral loads but does not change the decay rate of infected cells significantly. Recent studies showed evidence that viral replication in rhesus macaques persistently infected with SIV is unlikely to be controlled by cytotoxicity (54, 55). In addition, CD8+ T cells in persistent infections while loosing their effector functions and getting exhausted, maintain lytic capabilities (63-65) that nevertheless are assumed to be insufficient to allow a systematic control of the infection. In our model responses dominant for lytic effector functions would only lead to better viral...
control and CD4$^+$ T cell maintenance if we assume that more than 80% of the loss in infected cells is due to cytotoxicity ($k \sim 10.6 \text{ day}^{-1}$). However, also in this case the general qualitative conclusions on the importance of strength, and the neglecting influence of functional diversity on disease outcome stay valid.

Using realistic levels of cytotoxicity (45), among the non-lytic responses studied in our model, the inhibition of viral replication leads to lower viral loads and the maintenance of higher CD4$^+$ T cell counts than the inhibition of viral entry. Besides depending on the relative parameterization of both effector functions, this observation also depends on the assumed viral production rate and turnover of uninfected cells. If the viral production rate is high and uninfected cells have a long half-life, inhibiting viral replication is more effective in reducing viral load as a larger number of cells would need to be protected from getting infected (see Eq. 3).

In our model, we used IFN-γ as a surrogate for the effect of a cytokine inhibiting viral replication in infected cells and hindering the production of new diffusing HIV virions, i.e., representing the CD8$^+$ T cell anti-HIV factor (CAF) which has not been identified so far (24, 27). The role of IFN-γ against HIV is still controversial (66-68). While in some studies IFN-α and IFN-β, but not IFN-γ have been observed to inhibit viral replication in vitro (69), other studies showed a suppressive effect of IFN-γ on HIV replication in certain cell lines (23, 70). Koyanagi et al. (66) observed that the timing of IFN-γ treatment might be important: While treatment with IFN-γ before infection enhanced viral replication, IFN-γ treatment after infection reduced HIV expression. As we are focusing on the steady state of the CD4$^+$ T cell count in chronic HIV infection, the assumption of an inhibiting effect of IFN-γ on viral replication is reasonable.

Based on our parameterization, our results would support the use of non-cytolytic cytokines as therapeutic agents against HIV, as it is done in other chronic diseases (71). However, while MIP-1β mainly blocks the CCR5 chemokine receptor on CD4$^+$ T cells, this effector function might be ineffective in individuals who harbor HIV strains that use a co-receptor different from CCR5 (72). MIP-1β/RANTES might even mediate the selection pressure forcing this co-receptor switch (73).

Immunomodulatory interventions have been found to be successful in persistent infections. Barber et al. (15) showed that blocking the immunoregulatory PD-1 receptor (programmed death 1) on exhausted CD8$^+$ T cells restores functionality of these cells and reduces plasma viral load in mice persistently infected with the
LCMV-Clone 13 strain. They suggest the blocking of this PD-1 inhibitory pathway as a potential therapeutic strategy in chronic infections, as for example done recently in patients chronically infected with the hepatitis C virus (74).

Our model neglects dynamical changes in the CD8+ T cell response as e.g. the occurrence of CTL escape. As lytic, as well as non-lytic responses, if acting locally, can drive CTL escape (73, 75), more polyfunctional responses might also mediate increased selective pressure. To determine which combination of functions would minimize the emergence of escape would be an interesting topic of future study, but is beyond the scope of the present analysis.

In our model, the strength of the response defined as the average number of functionalities expressed by an HIV-1 specific CD8+ T cell showed a slightly better predictive power than the expected frequency of polyfunctional CD8+ T cells, as it is used in experimental and clinical studies today. However, we do not make any assumptions about the correlative expression of effector functions as we follow frequencies of cell populations rather than individual cells. When analyzing the data of Betts et al. (12), we find that within CD8+ T cells of HIV infected patients, the independent expression of both functionalities by bi-functional CD8+ T cells cannot be ruled out. For cells expressing three or more functions simultaneously, a correlative expression of the different effector functions was determined, excluding combinations involving IL-2. This observation could be made for each HIV-specific epitope examined. A correlative expression of certain effector functions among HIV-1 specific CD8+ T cells has also been observed elsewhere (12, 52, 53). In addition, multifunctional T cells seem to be optimized for effector function as the expression level of certain cytokines per cell measured in mean fluorescent intensity is up to 10-fold higher in multifunctional T cells compared to monofunctional T cells only expressing one particular effector function (76–78). If this difference in expression level also translates into a higher efficacy in performance, the frequency of polyfunctional CD8+ T cells might already represent the fraction of the response having by far the biggest impact on viral control, i.e. explaining the observed correlation.

To evaluate the role of CD8+ T cells in HIV-1 infection and to determine possible immune correlates of protection in more detail, current measurements of the CD8+ T cell response have to be refined. In order to account for the actual quality of the CD8+ T cell response, the antiviral impact of each effector function and for each specific
epitope has to be quantified in more detail. In addition, the relationship between
cytokine expression level and antiviral effect needs to be determined, also in terms of
the correlative expression of certain effector functions. These missing links would
allow us to combine cell frequencies and expression levels of certain chemokines in
individual cells to calculate a functional correlative of the CD8\(^+\) T cell response in
HIV-1 infected individuals, and to determine if this strength of the response can be
used to predict disease progression.

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Figure Legends

Figure 1: Model of viral and immune dynamics considering target cells, $T$, infected cells, $I$, virions, $V$, and CD8$^+$ T cells, $E$. The functional profile of the CD8$^+$ T cell response is denoted by the frequencies of CD8$^+$ T cells capable (i) to inhibit viral entry, $f_e$, (ii) to impair viral replication, $f_r$, and to kill infected cells, $f_k$. In a fully functional CD8$^+$ T cell response, all CD8$^+$ T cells would express all different effector functions considered. With a dysfunctional profile, not all effector functions are expressed by all CD8$^+$ T cells. The functional profile of the CD8$^+$ T cell response influences the infection rate, $\beta$, the viral production rate, $\rho$, or the death rate of infected cells by the additional killing term $k_fE$, respectively as schematically indicated. A detailed description of the model and the individual parameters is given in Materials & Methods.

Figure 2: Strength and diversity of the CD8$^+$ T cell response: (A) CD4$^+$ T cell count and plasma viral load dependent on the strength of the CD8$^+$ T cell response. The grey shaded areas indicate the minimum and maximum of all calculated values with the corresponding strength, $\Psi$, the solid line denotes the mean. The horizontally shaded areas indicate the CD4$^+$ T cell count and plasma viral load as measured for HIV-progressors (light-gray) and non-progressors (dark-gray) (see Table 2). (B) Relationship between the functional diversity of the CD8$^+$ T cell response measured by the Simpson Index, $D$, for the CD4$^+$ T cell count and plasma viral load. All possible combinations for three different values for the strength of the response, $\Psi = 0.9, \Psi = 1.8$ and $\Psi = 2.4$, are analyzed separately. Lines denote the mean CD4$^+$ T cell count and plasma viral load, while shaded areas indicate the minimum and maximum of all calculated values with the corresponding diversity index $D$.

Figure 3: Strength and polyfunctionality of the CD8$^+$ T cell response: (A) Estimated functionality profile of the CD8$^+$ T cell response for HIV-progressors in terms of multifunctionality of single cells based on the measured frequency of individual effector functions, $f_e = 0.94, f_k = 0.59, f_r = 0.62$. (B) Relationship between the strength of the CD8$^+$ T cell response and the multifunctional profile assuming maximal diversity of the response, i.e., $f_e = f_k = f_r$. (C) Relationship of the strength and the polyfunctionality of the CD8$^+$ T cell response for each patient of (12) exemplarily for...
the gag-epitope considering three (CD107a, IFN-γ, MIP-1β) of the 5 measured effector functions. (D) For each patient out of (12), the frequency of CD8⁺ T cells expressing two of the three functions indicated by CD107a, IFN-γ and MIP-1β, fab, is plotted against the total frequencies of CD8⁺ T cells expressing the corresponding specific function, i.e., fa and fb. The grey lines indicate the profile of the expected frequency of combined expression under the assumption that the particular effector functions are expressed independently, E[fab] = fa × fb. Observations are shown exemplarily for the gag-epitope.

**Figure 4:** Influence of particular effector functions on CD4⁺ T cell count and plasma viral load: For the example of a CD8⁺ T cell response with a frequency ratio of 2:1:1 between the three different effector functions, and corrected for different strengths of the CD8⁺ T cell response, we show the influence of particular effector functions on CD4⁺ T cell count and plasma viral load. CD8⁺ T cell responses with a dominant non-lytic response (A, B) show better maintenance of CD4⁺ T cell count and better viral control than those responses with a dominant cytotoxic response (C) irrespective of the strength Ψ. Inhibition of viral replication suppresses the viral load more efficiently than inhibition of viral entry. This hierarchy of effector functions can also be seen when looking at the simplex-plots showing the CD4⁺ T cell count (D) and the plasma viral load (E) dependent on the composition of a CD8⁺ T cell response with strength Ψ = 1. Lighter colors indicate better disease control. The black dot indicates an immune response where each effector function is equally present, hence, fe = fk = fr = 1/3.

**Figure 5:** (A) Additional gain (orange) or loss (grey scaling) in the CD4⁺ T cell count when increasing the frequencies of particular effector functions compared to a situation where the same increase in the strength of the response is equally distributed among all three different effector functions. Positive values indicate that concentrating on this particular effector function would be more beneficial than increasing the polyfunctionality for each effector function in the same way. White boxes indicate no difference to an equal increase in polyfunctionality. The red framed box indicates the starting functionality of the CD8⁺ T cell response with fe = 0.94, fk = 0.62 and fr = 0.59. (B) Corresponding plots for the effect on the plasma viral load with blue colors indicating a more substantial reduction of the viral load by focusing on the
frequency of this particular effector function when increasing the strength of the CD8+ T cell response.
Diversity of CD8+ T cell responses:

- $D = 0$
- $D = 23$
- $D = 3$

Strength of CD8+ T cell response, $\psi$:

- Fully functional
- Dysfunctional
- Exhausted/depleted

Viral load (RNA copies $\mu l^{-1}$)

CD4+ T cell count (cells $mm^{-3}$)

A

B

- Simpson diversity of CD8+ T cell responses, $\phi$

- $\phi = 0$
- $\phi = 0.1$
- $\phi = 0.2$
- $\phi = 0.3$
- $\phi = 0.4$
- $\phi = 0.5$
- $\phi = 0.6$

(Charts and graphs showing trends and data distribution)
Ratio of frequencies in the CD8$^+$ T cell response: $2 : 1 : 1 \rightarrow$ Diversity, $D = 0.625$

**Dominant response:**

**A** inhibition of viral replication

**B** inhibition of viral entry

**C** cytotoxicity

**Impact of composition of CD8$^+$ T cell response for $\Psi = 1$:**

**D**

**E**
Table 1: Parameter used for the simulation of the model in Eq. (1). The different parameters to simulate HIV-1 dynamics during the chronic phase of the infection are obtained from the indicated literature.

<table>
<thead>
<tr>
<th>parameter</th>
<th>definition</th>
<th>unit</th>
<th>value</th>
<th>ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda$</td>
<td>number of newly produced CD4$^+$ T cells (per $\mu l$)</td>
<td>day$^{-1}$</td>
<td>8</td>
<td>(31)</td>
</tr>
<tr>
<td>$\delta_T$</td>
<td>death rate of CD4$^+$ T cells</td>
<td>day$^{-1}$</td>
<td>0.01</td>
<td>(32)</td>
</tr>
<tr>
<td>$\delta_i$</td>
<td>death rate of productively infected cells</td>
<td>day$^{-1}$</td>
<td>1</td>
<td>(31, 42-44)</td>
</tr>
<tr>
<td>$c$</td>
<td>viral clearance rate</td>
<td>day$^{-1}$</td>
<td>23</td>
<td>(33)</td>
</tr>
<tr>
<td>$\beta$</td>
<td>infection rate per virion</td>
<td>day$^{-1}$</td>
<td>10-4</td>
<td></td>
</tr>
<tr>
<td>$E$</td>
<td>density of HIV specific CTL</td>
<td>cells ml$^{-1}$</td>
<td>225 (0.075)</td>
<td>(34-36)</td>
</tr>
</tbody>
</table>
Table 2: Median of the CD4+ T cell count and plasma viral load in RNA copies per μl for HIV-progressors and non-progressors based on the data published in (12) (Table 1). The numbers in brackets represent the 10% and 90% percentiles.

<table>
<thead>
<tr>
<th>Individuals</th>
<th>CD4+ T cell count (cells mm⁻³)</th>
<th>plasma viral load (RNA copies μl⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>progressors</td>
<td>79</td>
<td>522.5 (209.7, 863.0)</td>
</tr>
<tr>
<td>non-progressors</td>
<td>9</td>
<td>972.0 (690.2, 1682.0)</td>
</tr>
</tbody>
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**Table 3:** Frequencies of the functional HIV-specific CD8+ T cell response among 79 HIV-1 progressors for different HIV-epitopes. The median, as well as the 25% and 75% quantiles were taken out of Figure 1B in (12).

<table>
<thead>
<tr>
<th>Epitope</th>
<th>CD107a, $f_k$ (in %)</th>
<th>MIP-1β, $f_r$ (in %)</th>
<th>IFN-γ, $f_r$ (in %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAG</td>
<td>71.8 (50.0, 86.5)</td>
<td>94.7 (87.6, 98.3)</td>
<td>67.6 (50.5, 88.9)</td>
</tr>
<tr>
<td>POL</td>
<td>62.4 (40.0, 85.3)</td>
<td>95.3 (88.2, 98.9)</td>
<td>69.4 (46.4, 88.3)</td>
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<tr>
<td>ENV</td>
<td>46.5 (15.2, 84.7)</td>
<td>93.5 (67.0, 99.5)</td>
<td>50.6 (20.0, 92.4)</td>
</tr>
<tr>
<td>NEF</td>
<td>60.0 (31.7, 83.6)</td>
<td>94.1 (82.3, 99.5)</td>
<td>71.2 (42.9, 92.4)</td>
</tr>
<tr>
<td>TRVV</td>
<td>55.9 (20.0, 84.7)</td>
<td>90.0 (76.4, 99.5)</td>
<td>50.6 (28.2, 90.0)</td>
</tr>
<tr>
<td>mean %</td>
<td>59</td>
<td>94</td>
<td>62</td>
</tr>
</tbody>
</table>