Treatment of primary HIV infection
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Early HIV treatment preserves cytotoxic T-cell functionality

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Submitted
Abstract

Background
In HIV infection, low viral setpoints correlate with slow disease progression. The Primo-SHM trial, a multicenter randomized trial comparing no treatment with 24- or 60-weeks of combination antiretroviral therapy (cART) during primary HIV infection (PHI), recently demonstrated that temporary early cART lowered the viral setpoint and deferred the need for reinitiation of cART during chronic HIV infection. We studied whether the beneficial effect of early treatment was caused by preservation of immunological responses.

Methods
Twenty-six treated and thirteen untreated PHI-individuals participating in the Primo-SHM study were compared at viral setpoint: 36 weeks after treatment interruption (TI) and randomization, respectively. We studied i) effector T-cell formation and function, by measuring terminal differentiation and ex vivo expression of the cytolytic molecules perforin and granzyme B in CD4 and CD8 T cells by flowcytometry, ii) poly-functionality of CD8 T cells, by measuring the cytokines TNFα, IFNγ, and IL-2, and the chemokine Mip1β after stimulation with an overlapping peptide-pool of the HIV-gag protein, and iii) regulation of the cellular immune response by measuring various inhibitory and regulatory markers on T, B and NK cells and dendritic cells. We also assessed, by measurement of the gut homing marker α4β7, whether early treatment prevented severe CD4 T cell depletion in the GALT and thereby prevented excessive immune activation.

Results
The polyfunctionality of the HIV specific T-cell response was more preserved in treated individuals; a median of 32% of Gag-specific CD8 T cells of treated individuals produced more than 1 cytokine whereas in untreated PHI individuals this was merely 12%. Also, approximately 14% of the total response was made up of 3 functions in treated individuals compared to 1% in the untreated individuals. In contrast, no differences due to treatment were found at the level of regulation, senescence, activation or effector function of the T cell response, nor on preservation of CD4 T cells in the gut.

Conclusions
Treatment during PHI led to the preservation of a more polyfunctional HIV-gag specific T cell response. These data suggest that early treatment may preserve important CTL functions, which are crucial in control of HIV viraemia.
Introduction

In HIV infection, a low viral setpoint correlates with slow disease progression [1]. The Primo-SHM trial, a multicenter randomized study comparing no treatment with 24- or 60-weeks of combination antiretroviral therapy (cART) during primary HIV infection (PHI), recently demonstrated that temporary early cART transiently lowered the viral setpoint and deferred the need for reinitiation of cART during chronic HIV infection [2]. Factors such as baseline CD4 T-cell count, stage of PHI, virological features, and HLA-background could not explain the differences observed in viral load [2, 3].

There are many immunological parameters that can play a role in HIV infection. Foremost, there is compelling evidence that gag-specific CTL responses correlate with HIV viral load and the rate of HIV disease progression [4-12]. Current consensus in the field is that a ‘protective’ T cell response in HIV infection is comprised of two elements, namely effective cytolytic killing and production of multiple cytokines. Recent work from Soghoian et al. highlights the importance of vigorous cytolytic activity by HIV specific CD4 T cells in controlling disease progression [9]. The presence of HIV specific CD4 T cells, which are able to express both perforin and high levels of granzyme A in PHI, was found to be highly predictive of slower disease progression and clinical outcome. Likewise, polyfunctionality (the capacity of cells to secrete multiple cytokines and chemokines upon antigenic stimulation) is a hallmark of a highly functional T cell response. For instance, HIV nonprogressors preferentially maintain more polyfunctional HIV specific T cell responses during infection [5]. In addition, a study by Almeida et al. demonstrated that superior control of HIV infection by CD8 T cells was reflected by a polyfunctional and high avidity phenotype [4].

The effect of these immune responses can be severely limited during disease progression, for the persistent viraemia has deleterious effects on HIV specific CD4 and CD8 T-cell immunity [7]. For instance, chronic antigen exposure leads to a terminally differentiated phenotype of the CD4 and CD8 T cells, which have upregulated CD57 expression and a diminished functional proliferative capacity [13]. HIV viraemia prevents the establishment of highly functional memory CD4 T cells that retain the capacity to proliferate upon antigen stimulation [14, 15]. And finally, chronic antigenic stimulation induces upregulation of inhibitory receptors, most notably PD-1 and CTLA-4, which may interfere with HIV specific T-cell responses and ultimately lead to T-cell anergy and loss of HIV specific T cells [16, 17].

One of the earliest effects of HIV infection is a massive depletion of central memory CD4 T cells from the gut associated lymphoid tissue (GALT) [18]. The instant and massive early injury to the gut immune system, together with the subsequent damage exacted to the gut epithelial cells, is thought to induce gut permeability and translocation of microbial products such as LPS. This is thought to contribute to the systemic immune activation which characterizes HIV infection [19]. It is widely accepted that chronic immune activation drives progression to AIDS [20-23]. In fact immune activation is more strongly associated with the rate of CD4 T-cell loss in HIV infected individuals than viral load [20]. We and others hypothesize that viral suppression in PHI might prevent the excessive depletion of the GALT and the subsequent chronic immune activation, and would thereby contribute to the delay in CD4 T cell loss found in early treated individuals. Depletion of CD4 T cells in the GALT can indirectly be assessed in the blood, by measuring the...
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level of α4β7 expression on T cells. Indeed, in primary SIV infection the reduction of α4β7high CD4 T cells in the peripheral blood was shown to parallel the reduction of CD4 T cells in intestinal tract biopsies [24, 25]. Likewise, the finding that α4β7high CD4 T cells are preferentially depleted in the blood in PHI [26] likely reflects the depletion of CD4 T cells of the GALT.

To determine the cause of the lower viral setpoint in individuals treated during PHI, we evaluated whether i) effector T-cell formation, ii) inhibitory receptor expression on immune cells, or iii) effectiveness of the T cell response (in terms of cytolytic function, or polyfunctionality) were altered in individuals treated during PHI. Furthermore, to determine whether viral suppression in PHI caused preservation of CD4 T cells in the GALT we evaluated whether expression of the gut homing receptor α4β7 on CD4 T cells was altered in individuals treated during PHI. In concordance we assessed whether early treatment prevented excessive T cell activation and may have thereby contributed to a delay in disease progression.

Materials and Methods

Study Population

Blood samples were obtained from participants of the Primo-SHM trial. The Primo-SHM study was a multicentre, open-label randomized controlled trial comparing temporary early cART (24 or 60 wk) with no treatment. Detailed procedures have been described elsewhere [2]. Briefly, inclusion criteria were age over 18 years and a laboratory evidence of PHI, defined as a negative or indeterminate Western blot in combination with detectable plasma HIV-1 RNA (Fiebig stage I–IV) or, in case of a positive Western blot, a documented negative HIV screening test in the previous 180 days (Fiebig stage V–VI [21]).

Thirteen untreated and 26 treated (for either 24 or 60 weeks) PHI-individuals were selected based on sample availability and their immunological parameters were measured at randomization/treatment interruption (TI) and at ‘viral setpoint’, defined as 36 weeks after randomization/TI, respectively. In addition blood samples of 13 healthy donors were obtained via the ‘mini donor dienst’ of the UMC Utrecht. This was approved by the Medical Ethics Committee of the UMC Utrecht, and written informed consent was obtained from all donors.

T cell activation and phenotype

Expression of activation markers on CD4 and CD8 T cells was measured after staining of the cells with antiCD4-APC, antiCD8-PerCP, antiCD38-FITC and antiHLA-DR-PE monoclonal antibodies (BD). The phenotype of the cells was determined using antiCD27- APC-Cy7 (Biolegend) and antiCD45RO-PECy7 (BD). In the same sample, cells were also stained with antiPD-1-FITC. All incubations were performed at 4°C (20 minutes) after which cells were fixed in cellfix (BD) and analysed with the LSRII flow cytometer.

Ex vivo T cell function

Ex vivo surface staining was performed with antiCD3-eFluor450 (eBioscience), antiCD8-V500, antiα4β7-APC, antiCD57-FITC, antiCD45RO-PE-Cy7 (all BD) and antiCD27-APC-Cy7 (Biolegend) monoclonal antibodies for 20 min at 4°C. After fixation and permeabilisation (permeabilisation reagents, BD) for 10 min, cells were stained for cytotoxic molecules with antigranzyme.
B-Pe (Sanquin) and antiperforin-PerCP-CyS.5. Hereafter cells were fixed in cellfix (BD) and flowcytometry was performed.

**CD8 T cell stimulation and intracellular cytokine staining**

Cryopreserved PBMC were thawed and aliquotted at 2x10^6 cells per ml in round bottom tubes (Becton Dickinson (BD), San Jose, California). CD8 T-lymphocytes were stimulated for 6 hours with a Gag-peptide pool (15mers with 11 overlap, final concentration of the individual peptides was 2 µg/ml, Consensus B 2007, NIH AIDS Research and Reagent program, Bethesda, Maryland, United States). As a positive control, PMA and ionomycin (Sigma-Aldrich, Zwijndrecht, The Netherlands; 5 ng/ml and 1 µg/ml respectively) were used. After 1.5 hours, Brefeldin A (3 µM, Sigma-Aldrich, Zwijndrecht, The Netherlands) was added. Surface staining was performed with antiCD3-PerCP, antiCD8-V500, antiα4β7-APC (all BD) and antiCD27-APC-Cy7 (Biolegend) monoclonal antibodies for 20 min at 4°C. After fixation and permeabilisation (permeabilisation reagents, BD) for 10 min, cells were stained with antiIFNγ-Pe-Cy7 (eBioscience), antiTNFα-FITC, antiMIP1β-PE and antiIL-2-PB (BD) for 20 min at 4°C. Cells were fixed in cellfix (BD) and flowcytometry was performed.

**Characterisation of inhibitory markers**

Expression of inhibitory markers was assessed on CD4 and CD8 T cells, B cells, NK cells and dendritic cells. A surface staining was performed for CD4 and CD8 T cells (antiCD3-eFluor450 (eBioscience), antiCD8-V500 (BD)), B cells (antiCD19-PerCP (BD)), NK cells (antiCD56-APC (BD)) and dendritic cells (antiHLA-DR-APC-Cy7 (BD), antiCD11c-PE-Cy7 (BD)). These sets were completed with either antiCD31–PE (BD)/3D3 antisirl-FITC or antiLAIR-PE/antiILT4-FITC or antiIREM-1-PE/antiKLRG-1-FITC or isotype controls. After staining for 20 minutes at 4 °C, cells were fixed in cellfix (BD) and flowcytometry was performed.

**Flow cytometry analysis**

At least 100.000 events were acquired after phenotypical staining and at least 300.000 events were acquired after intracellular cytokine stainings, using the LSRII flow cytometer (BD). Data were analyzed using the DIVA software (BD). The events were gated for either lymphocytes or monocytes in a FSC-A versus SSC plot. Following this, events were gated using the markers described above. T-cell polyfunctionality was analysed by Flowjo software (version 9.2). After determining the lymphocyte gate in a FSC-A versus SSC plot, cells were sequentially gated for CD3 and CD8. Subsequently, within the CD8 T cell population a gate was created for the 4 respective functions; IFNγ, TNFα, MIP1β, and IL-2. Hereon a Boolean gating was performed resulting in 20 different combinations. All data were background-subtracted using the unstimulated samples.

**Statistical analysis**

Differences between the 2 groups were analyzed using the Mann-Whitney test. Groups of >2 were analysed with a Kruskal –Wallis test. All statistical analyses were performed using the software program SPSS 19.0 (SPSS Inc, Chicago, Illinois).
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Results

Patient characteristics
The baseline characteristics and the viral setpoint measurements of the patients selected for this study (Table 1) resembled those of the Primo-SHM trial. The median viral loads, CD4 and CD8 T cell counts were similar at baseline in the treated and untreated patients. The viral load at setpoint was significantly lowered by treatment during PHI: a median 4.4 versus 5.2 log_{10} copies/ml for treated and untreated individuals, respectively. At that moment, median CD4 T cell counts were significantly higher in treated patients than in untreated patients: 645 (range 290-950) versus 325 (180-560) CD4 T cells/mm^3 respectively. CD8 T cell counts were also significantly higher in early treated individuals.

No change in T cell subsets after early treatment
To investigate the immunological cause of the lower viral setpoint in individuals treated during PHI, we determined whether T cells of treated individuals had a less mature and/or less exhausted phenotype, and could consequently form a more effective immune response. Therefore we analyzed the level of terminal differentiation (based on the markers CD45RO and CD27) and replicative senescence (based on the marker CD57) of the CD4 and CD8 T cells of treated and untreated individuals (Figure 1). We found that irrespective of treatment, CD4 T cells of HIV infected individuals had a more terminally differentiated phenotype (CD45RO-CD27-) and increased replicative senescence (CD57+) than healthy volunteers (p=0.0002 and p<0.0001, respectively). The level of senescence of CD8 T cells was also higher in HIV infected individuals compared to healthy volunteers (p=0.008). However, no differences could be observed in treated versus untreated individuals. To reveal whether the HIV specific cells also

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>A. Patient characteristics at baseline</th>
<th>Treatment^a (N=26)</th>
<th>No Treatment (N=13)</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>Age (years), median (range)</td>
<td>43 (30-59)</td>
<td>47 (25-55)</td>
<td>0.314</td>
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<tr>
<td>Male</td>
<td>24 (92%)</td>
<td>13 (100%)</td>
<td>0.544</td>
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<td>MSM</td>
<td>20 (76%)</td>
<td>12 (92%)</td>
<td>0.388</td>
</tr>
<tr>
<td>Caucasian</td>
<td>22 (84%)</td>
<td>12 (92%)</td>
<td>0.648</td>
</tr>
<tr>
<td>Stage of PHI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Fiebig I-IV</td>
<td>20 (77%)</td>
<td>11 (85%)</td>
<td>0.694</td>
</tr>
<tr>
<td>- Fiebig V-VI</td>
<td>6 (23%)</td>
<td>2 (15%)</td>
<td></td>
</tr>
<tr>
<td>CD4 cell count (cells/mm^3), median (range)</td>
<td>510 (280-1050)</td>
<td>455 (200-680)</td>
<td>0.163</td>
</tr>
<tr>
<td>CD8 cell count (cells/mm^3), median (range)</td>
<td>1285 (300-1050)</td>
<td>1350 (380-1940)</td>
<td>0.639</td>
</tr>
<tr>
<td>Plasma HIV-1 RNA (log_{10} copies/ml), median (range)</td>
<td>5.28 (2.91-6.70)</td>
<td>5.45 (2.49-6.14)</td>
<td>0.438</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Patient characteristics at viral setpoint</th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>CD4 cell count (cells/mm^3), median (range)</td>
<td>645 (290-950)</td>
<td>325 (180-560)</td>
<td>0.026</td>
</tr>
<tr>
<td>CD8 cell count (cells/mm^3), median (range)</td>
<td>1270 (380-1800)</td>
<td>885 (560-1080)</td>
<td>0.043</td>
</tr>
<tr>
<td>Plasma HIV-1 RNA (log_{10} copies/ml), median (range)</td>
<td>4.37 (2.36 -5.35)</td>
<td>5.16 (4.30-5.44)</td>
<td>0.036</td>
</tr>
</tbody>
</table>

^a Treatment consists of either 24 or 60 weeks of cART. MSM, men who have sex with men; PHI, primary HIV infection.
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displayed differences in replicative capacity, an in vitro proliferation assay was performed. Cells were stimulated with an overlapping gag-peptide pool and after 6 days the stimulation index was determined. Early treatment had no effect on the gag-specific proliferative capacity of either CD4 or CD8 T cells (data not shown).

Ex vivo cytolytic T cell activity is not enhanced after treatment

Next, direct ex vivo cytolytic T cell functionality was assessed by measurement of the levels of granzyme B and perforin expression in the total CD4 and CD8 T-cell pool. The percentage of cells expressing perforin, granzyme B and a combination of both was elevated in CD4 T cells of HIV infected individuals compared to healthy individuals (Figure 2). Granzyme B expression in CD8 T cells of HIV infected individuals was also increased. However, no differences were found between untreated and treated individuals, suggesting that treatment did not preserve the cytolytic activity of T cells.

Preservation of a more polyfunctional T cell response due to treatment

Another measure for an effective T cell response is its polyfunctionality. We performed a stimulation assay with an overlapping gag-peptide pool (Figure 3A) and measured which proportion of cells produced one or more of the cytokines TNFa, MIP1b, IFNy and IL-2. No
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Differences were observed in the total amount of CD8 T cells that produced cytokines/chemokines to HIV gag in treated and untreated PHI individuals (median 1.0 % vs 0.5% of CD8 T cells, respectively; NS, data not shown). When we compared the functional profiles of the CD8 T-cell responses by expressing each functional component as a proportion of the total response [5], we found that treated individuals exhibited a more polyfunctional response than untreated PHI-individuals (Figure 3A). In treated individuals, a median of 32% of the gag-specific CD8 T cells displayed more than 1 function compared to 12 % of CD8 T cells in the untreated individuals. Moreover, a median of 14% of the responding CD8 T cells in treated individuals produced 3 functions simultaneously compared to 1% in the untreated individuals. The differences in the T cell polyfunctionality profiles were HIV-gag specific, for when the cells were stimulated with the ‘superstimuli’ PMA and ionomycin (Figure 3B), both treated and untreated PHI individuals showed a similar functional profile to that of healthy controls.

The separate responses showed distinct cytokine profiles when stimulated with specific antigen. For instance, in treated individuals IL-2 production by HIV specific CD8 T cells made up approximately 20% of the total response while its median contribution did not exceed 4% in untreated individuals.

No changes in expression of regulatory and inhibitory molecules after early treatment

In addition we analysed markers of regulation of the immune response. We selected a panel of ‘well recognized’ but also recently characterized and largely unexplored inhibitory receptors...
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Figure 3. Preservation of a more polyfunctional T cell response due to treatment. PHI- individuals with treatment (n=10), no treatment (n=8) and uninfected healthy donors (n=6) were compared. The piecharts depict the relative contribution of the number of cytokines/chemokines that are produced by CD8+ T cells in response to either HIV-gag (figure 3A) or PMA/ionomycin (figure 3B). The graphs zoom in on the relative contribution of the cytokine (Mip1β, INFγ, TNFα and IL-2) combinations to the total CD8 T cell response. In all graphs medians (with ranges) are shown.
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with different expression aspects [27] and analysed their level of expression on CD4 and CD8 T cells, B cells, NK cells and dendritic cells.

Pecam-1 (CD31), known to inhibit apoptosis [28], was expressed less in HIV infected individuals compared to healthy controls in all measured cell types (Figure 4 & S1 first panel). Similarly, a distinct down regulation of LAIR-1 (known to inhibit cytolytic function) in HIV infected individuals was seen on T-, B- and dendritic cells (Figure 4 & S1 third panel). KLRG-1, an NK cell inhibitor, was increased on CD4 T cells in HIV infected individuals, reflecting an exhausted phenotype. On dendritic cells no difference was seen for ILT4 expression, a molecule that inhibits CTL function (Figure 4 & S1 forth panel). IREM-1, which inhibits TLR signalling in dendritic cells (Figure 4 & S1 fifth panel), was the only receptor that showed a significant effect of early treatment. However, instead of mirroring the healthy controls, the expression of this receptor was even more decreased in the treated group compared to the untreated group (median 78 vs 88% of dendritic cells positive for IREM-1, respectively). Finally, an upregulation of SIRL-1 was found on dendritic cells and B cells in HIV infected individuals (Figure 4 & S1 second panel). Remarkably, so far, this receptor was described as an inhibitory signaling molecule found solely on monocytes and neutrophils. No differences were found in the well characterized PD-1 expression in treated compared to untreated HIV infected individuals (data not shown).

No effect on GALT T cell depletion and T cell activation after early treatment

To study whether treatment in PHI could overcome the rapid depletion of T cells from the GALT that is typically observed in untreated HIV infection, we evaluated the level of expression of the gut homing receptor α4β7 on CD4 and CD8 T cells of treated and untreated individuals (Figure 5A & B). Both treated and untreated HIV infected individuals exhibited a marked decrease in the percentage of α4β7high cells in the naive CD4 T cell compartment compared to healthy controls. Also, in the effector CD4 T cell compartment the percentage of α4β7 expression was lower in treated individuals than healthy controls. In contrast, no significant differences in α4β7 expression were found on CD8 T cells. Despite the lower viral setpoint in patients treated during PHI, there were no significant differences between treated and untreated individuals in the percentages of α4β7high cells; thus treatment did not lead to the preservation of the α4β7 high CD4 T-cells.

Because immune activation levels are known to be even more predictive of the rate of disease progression than viral loads, we analyzed whether treatment affected the overall level of immune activation, by measuring CD38 and HLA-DR expression on T cells. Again, no differences could be found between treated and untreated individuals in the expression of CD38 and HLA-DR on CD4 or CD8 T cells (data not shown).

Discussion

We investigated whether the reported beneficial effect of treatment during PHI [2] was caused by preservation of immunological responses, and whether early treatment could overcome the rapid depletion of CD4 T cells from the GALT, and its immune activating effect. Treatment in PHI-individuals seemed to preserve the quality of HIV specific CD8 T cell responses in terms of their cytokine polyfunctionality, which could contribute to a lower viral setpoint. In contrast, our results
Figure 4. No changes in regulatory and inhibitory molecules after early treatment. To assess the level of inhibitory receptor expression, the % (y-axis) of inhibitory receptors on CD4+ T cells, CD8+ T cells, B cells, NK cells and DC’s was determined. The parameters were compared between; healthy individuals (n=13, black), treated individuals (n=8, dark grey) and untreated individuals (n=10, white). Bars represent the median with range. *P-value = 0.01-0.05, **P-value =0.01-0.001, ***P-value<0.001
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We were surprised that treatment did not decrease the level of senescence of the effector T cells. As previously, chronic antigen exposure was shown to lead to a terminally differentiated phenotype of CD4 and CD8 T cells, with a diminished functional proliferative capacity [13]. In addition, neither levels of inhibitory receptor expression (after correction for multiple testing) or cytolytic function of T cells were significantly altered in treated compared to untreated individuals.

It is possible that the immune parameters measured were preserved by treatment, but regained the features of a disrupted immune response rapidly after treatment interruption. If so, we have missed the effect of treatment on the immune response by measuring too late. Though, for the cytolytic T-cell functions, the discrepancy between our results and existing literature is likely to be found in the T cell subsets measured. In the literature, proliferated HIV

Figure 5. No differences in T cell activation between treated and untreated patients. The expression of gut homing marker α4β7 within the T cell compartments (naïve, central memory, effector memory and effector) on CD4 T cells (figure 5A) and CD8 T cells (figure 5B), is depicted. The mean fluorescent intensity of α4β7 was compared for treated (n=8, light gray), untreated (n=10, dark grey) and healthy (n=12, black) individuals. Bars represent the median with range. *P-value = 0.01-0.05, ** P-value = 0.01-0.001.
specific CD8 T cells of long term non-progressors were more prone to produce perforin in comparison with the T cells of progressors [7]. Also, increased granzyme production after HIV specific stimulation was predictive of outcome [9]. We, on the other hand, looked into perforin and granzyme expression of the total CD4 and CD8 T cell pool. Additionally, changes might have been missed due to our limited sample size.

Equally, treatment had no effect on preservation of CD4 T cells in the GALT and subsequent overall immune activation. As there is an acute and severe depletion of CD4 T cells in the GALT due to HIV infection, we expected treatment in PHI to prevent this. However, the initiation of treatment might have been too late to preserve gut immunity, even though all individuals in the Primo-SHM study were recruited within 100 days of HIV infection and 73% was even recruited within 30 days. In SIV infection severe depletion of CD4 T cells in the GALT (up to 65%) was seen within days [29] and not weeks.

Reflecting the unaffected CD4 T cell depletion of the GALT, systemic immune activation was also not diminished after treatment. However, immune activation is not only induced by gut permeability and translocation of microbial products such as LPS, but also HIV itself is a strong driver of immune activation [22]. Therefore, one would expect a lower viral load to have an impact on the level of immune activation. Immune activation is an even stronger predictor of disease progression than viral load, and a diminished immune activation would therefore strongly contribute to delay of cART, as seen in the Primo-SHM study [2].

Finally, treatment in PHI-individuals seemed to preserve the quality of HIV specific CD8 T cell responses, in terms of their cytokine polyfunctionality. So far, not one single function of HIV specific CD8 T cells has been proven to correlate with control of HIV infection. Therefore, the current consensus is that the more functions a CD8 T cell performs (HIV specific), the more protective it must be. Indeed, HIV specific CD8 T cells of long term non-progressors are more prone to perform 5 functions simultaneously (IL-2, IFN-γ, TNF-α, MIP-1β, and CD107a) compared to HIV progressors [5]. Moreover, the cytolytic response is thought to be more antiviral when producing a distinct profile of perforin and various granzymes against HIV-gag [9]. In addition, while the antigen-specific polyfunctional CD8 T cells do not produce more of a single cytokine per cell basis, overall they produce more cytokines on a per cell basis than monofunctional CD8 T cells [30]. Therefore, it seems that the preservation of a polyfunctional HIV specific CD8 T cell response could be involved in lowering the viral setpoint as a result of treatment.

In conclusion, our results showed that early treatment during PHI might preserve a more polyfunctional HIV-gag T-cell response. These data thereby suggest that early treatment may preserve important CTL functions, which are crucial in control of HIV viraemia.
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