Effect of Maraviroc Intensification on HIV-1-Specific T Cell Immunity in Recently HIV-1-Infected Individuals

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Abstract

Background: The effect of maraviroc on the maintenance and the function of HIV-1-specific T cell responses remains unknown.

Methods: Subjects recently infected with HIV-1 were randomized to receive anti-retroviral treatment with or without maraviroc intensification for 48 weeks, and were monitored up to week 60. PBMC and in vitro-expanded T cells were tested for responses to the entire HIV proteome by ELISPOT analyses. Intracellular cytokine staining assays were conducted to monitor the (poly)-functionality of HIV-1-specific T cells. Analyses were performed at baseline and week 24 after treatment start, and at week 60 (3 months after maraviroc discontinuation).

Results: Maraviroc intensification was associated with a slower decay of virus-specific T cell responses over time compared to the non-intensified regimen in both direct ex-vivo as well as in in-vitro expanded cells. The effector function profiles of virus-specific CD8+ T cells were indistinguishable between the two arms and did not change over time between the groups.

Conclusions: Maraviroc did not negatively impact any of the measured parameters, but was rather associated with a prolonged maintenance of HIV-1-specific T cell responses. Maraviroc, in addition to its original effect as viral entry inhibitor, may provide an additional benefit on the maintenance of virus-specific T cells which may be especially important for future viral eradication strategies.

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Introduction

Maraviroc is an antiretroviral agent that blocks HIV-1 entry by binding the virus’ coreceptor CCR5. Given its molecular target, maraviroc treatment may modulate the natural expression and function of CCR5, and negatively affect chemotaxis and effector function of Th1-type CD4+ T cell and memory CD8+ T cells. Maraviroc may have additional immunomodulatory effects by blocking the binding of the natural ligands of CCR5 (MIP-1α, MIP-1β and, RANTES), yet little data exist on how maraviroc may interfere with the cellular host immunity, especially the one directed against HIV-1.

While CCR5 deficiency (in the form of a 32 base-pair homozygous deletion) can mediate resistance to HIV-1 infection [1–3], it also has the potential to impair control of other viral infections, such as West Nile virus (WNV), both in mouse and humans [4,5]. In particular, murine T cells lacking CCR5 expression have been shown to secrete lower amounts of IL-2 compared to CCR5+ T cells, and a similar phenotype has been observed in T cells from humans expressing the CCR5-32 mutation [6]. Furthermore, CD8+ T cell exhaustion during chronic Lymphocytic choriomeningitis virus (LCMV) infection is more severe in the absence of RANTES, one of the natural CCR5 ligands [7]. Thus, although CCR5-32 homozygosity does not
Flow cytometry for T cell phenotype analysis

Study design

In previous clinical trials, treatment with maraviroc has been shown to result in more extensive increases in CD4 counts in treatment-naive and -experienced subjects, though the mechanisms involved remain unknown [9–11]. In addition, some studies have indicated that adding maraviroc to suppressive combination antiretroviral treatment (cART) reduces markers of immune activation [12–15]. Also, in vitro exposure to maraviroc decreases some markers of immune activation on T lymphocytes [16]. While these findings suggest that maraviroc may have beneficial effects on global host immune status, maraviroc has also been found to increase T cell activation both in gut and peripheral blood [17]. Thus, it is still controversial whether maraviroc has net immunological benefits or disadvantages on host cellular immune responses. In addition, the impact of maraviroc on antigen-specific T cell responses, especially towards HIV-1-derived antigens, has not been assessed, despite its potential implications with regards to immune interventions, particularly therapeutic vaccination in maraviroc treated subjects. To address these issues, we analyzed in a longitudinal study the effects of cART versus maraviroc-intensified cART on the maintenance (breadth, magnitude and specificity) of HIV-1-specific T cell responses, their differentiation potential and their polyfunctionality.

Materials and Methods

Study design

The present study was performed as sub-study of the MaraviBoost study (ClinicalTrials.gov Identifier: NCT00808002). The MaraviBoost study was a multi-center, randomized, open-label, phase III clinical trial. The main goal of the parental clinical trial was to assess whether intensification with maraviroc in recently HIV-1 infected patients with standard triple therapy could accelerate the decay of the HIV-1 reservoir [18]. Thirty subjects recently infected with CCR5-tropic HIV-1 (subtype B) were recruited and randomized into 2 groups (n = 15 each), one being treated with triple therapy consisting of Raltegravir (RAL) plus Tenofovir (TDF)/Emtricitabine (FTC) (TDF/FTC) alone while the second group received additionally maraviroc (MVC) intensification for the first 48 weeks in the trial. The primary end point of the main study was week 48, but patients were followed until week 72 if possible. Frozen PBMC from pre-defined time points before starting cART (baseline, BL), 24 weeks after study initiation, and 12 weeks after maraviroc discontinuation (week 60), were analyzed in the present study. One patient without maraviroc intensification, who dropped out the study because of adherence problem, was excluded from the analysis. Three patients (01028, 01039, 23012) were lost at week 24 (n = 1) or 36 (n = 2), respectively. All patients received RAL plus TDF/FTC after week 48 except 4 patients (01021, 01031, 01034, 01043), who changed their anti-HIV drug regimen. Of the 29 individuals, peripheral blood mononuclear cells (PBMC) from at least one time point were available for 13 patients with maraviroc intensification (MVC arm) and 14 patients without MVC intensification (Control arm, Table 1). The study was approved by the ethics committee of Hospital Germans Trias i Pujol, Badalona, Spain. All patients gave their written informed consent before enrolling in the study.

Flow cytometry for T cell phenotype analysis

PBMC were thawed and rested overnight at 37°C in RPMI1640 supplemented with 10% heat-inactivated FCS, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM glutamine (R10). The following day, the cells were stained with LIVE/DEAD Fixable Dead Cell Stain Kits (Invitrogen), washed and stained with the following antibodies: anti-CD3-APC-Cy7, anti-CD4-V500, anti-CD8-PE-Cy7, anti-CD45RA-APC (BD Bioscience), and anti-CCR7-PE (e-BioScience). The cells were washed and fixed with 1% Formaldehyde in PBS. All data were collected on a BD LSR II flow cytometer (BD Bioscience) and analyzed using FlowJo 0.7.7 (TreeStar).

Peptides

A set of 410 overlapping-peptides (OLPs) was used to screen for HIV-specific T-cell responses [19]. The peptides spanned all HIV-1 proteins and were based on the HIV clade B consensus sequence of 2001, available at the Los Alamos National Laboratory HIV immunology database. For ELISpot analyses, peptides were used in a matrix layout of 6–12 peptides per pool for comprehensive screening as previously described [19]. Reconfirmations of all positive wells in the matrix screen were performed the following day on a single-peptide base. For multi-functional analysis by flow cytometry, peptide pools were used that contained peptides spanning either full-length Gag, Protease, RT, IN, gp120, gp41, or Nef. Peptides spanning Tat, Rev, Vif, Vpr, and Vpu were combined into one peptide pool (accessory proteins peptide pool. “Acp”).

IFN-γ ELISpot assay using ex-vivo PBMC and in-vitro expanded T cells

Thawed PBMC were rested for 3 hrs at 37°C in R10. If sufficient PBMC were recovered, thawed cells were used directly in IFN-γ ELISpot assays (11 and 7 samples at baseline, 6 and 7 samples at week 24, and 8 and 7 samples at week 60 in the CNT and MVC arm, respectively). In addition, 1 × 10⁶ thawed cells were stimulated with an anti-CD3 monoclonal antibody and cultured for 2–4 weeks in R10 supplemented with 50 U/ml of recombinant IL-2 [20]. Before use in ELISpot assays, the expanded cells were washed twice with R10 and incubated overnight at 37°C in the absence of IL-2. Per well, 75,000–100,000 cells were used and peptides were added as in the direct ex-vivo assay. Thresholds for positive responses were defined as 1) at least five spots (50–66 SFC/10⁶ PBMC) per well, 2) as responses exceeding the mean of negative wells plus 3 standard deviation and 3) responses exceeding three times the mean of negative (no peptide) wells; whichever was the highest. For reconfirmation ELISpot, the remaining cells and cells from negative wells from initial matrix screens were recycled as previously described [20].

Flow cytometric analysis of CD8+ T cell function

Thawed PBMC were rested overnight at 37°C in R10. The following day, costimulatory antibodies (anti-CD28 and anti-CD94d at 1 μg/ml; BD Biosciences) and monecin (GolgiStop; BD Bioscience) were added, and cells were stimulated with the different peptide pools (5 μg/ml per peptide) as indicated. A negative (no peptide) and a positive control (phorbol-12-myristate-13-acetate (PMA at 10 ng/ml and ionomycin, 1 μM) were included in each assay. Following incubation for 6 hrs, the cells were washed with PBS containing 1% FCS and the fluorescent reactive dye (Invitrogen) for dead cells was added. Cells were washed again, and stained with anti-CD3-V450, anti-CD8-PerCP, and anti-CD107a-PE (BD Bioscience). Following washing, the cells were fixed and permeabilized using Fix & Perm cell permeabilization reagents (Invitrogen). The cells were then stained with anti-MIP-1β-APC, anti-IL-2-PE-Cy7, anti-IFN-γ-APC (BD Bioscience). Data were collected on a BD LSR II flow cytometer (BD Bioscience).
## Table 1. Characteristics of participants.

| Patient ID | Estimated duration from infection (year-old) | Age (months) | VL (copies/ml) | CD4 (cells/µl) | CD8 (cells/µl) | VL (copies/ml) | CD4 (cells/µl) | CD8 (cells/µl) | VL (copies/ml) | CD4 (cells/µl) | CD8 (cells/µl) |
|------------|---------------------------------------------|--------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Control group (n = 14) |
| 01022 | 28 | 6.2 | 61,000 | 606 | 873 | 50 | 843 | 731 | 50 | 741 | 606 |
| 01025 | 32 | 5.8 | *36,000 | 287 | 691 | 50 | 446 | 809 | 50 | 432 | 527 |
| 01028 | 26 | 3.1 | 490,000 | 366 | 1,893 | 56 | 518 | 900 | 50 | 650 | 650 |
| 01030 | 21 | 4.0 | 19,000 | 297 | 1,124 | 50 | 435 | 464 | 50 | 650 | 650 |
| 01032 | 32 | 4.5 | *200,000 | 372 | 1,830 | 200 | 478 | 956 | 50 | 580 | 825 |
| 01036 | 42 | 5.1 | 40,000 | 273 | 1,034 | 50 | 352 | 641 | 50 | 429 | 617 |
| 01037 | 40 | 3.2 | *26,000 | 589 | 1,326 | 50 | 533 | 999 | 50 | 556 | 641 |
| 01039 | 26 | 8.6 | *63,000 | 450 | 1,125 | 50 | 654 | 1,162 | 50 | 620 | 589 |
| 01040 | 50 | 2.8 | 1,500,000 | 379 | 2,245 | *230 | 601 | 631 | 50 | 620 | 589 |
| 01044 | 35 | 3.8 | *54,000 | 454 | 1,127 | 50 | 962 | 1,683 | 50 | 396 | 834 |
| 23010 | 26 | 4.7 | *1,091 | 629 | 1,204 | *b50 | 624 | 559 | 50 | 649 | 593 |
| 23012 | 38 | 6.0 | 10,738 | 656 | 579 | 50 | 688 | 615 | 50 | 624 | 635 |
| 23013 | 39 | 4.1 | 30,210 | 492 | 1,624 | 94 | 644 | 984 | 50 | 624 | 635 |
| 23019 | 32 | 6.5 | 8,497 | 620 | 1,033 | *b50 | 688 | 899 | 50 | 543 | 688 |
| MVC intensified group (n = 13) |
| 01021 | 39 | 5.1 | 46,000 | 649 | 1,750 | 50 | 954 | 1,371 | 50 | 941 | 811 |
| 01027 | 32 | 6.6 | 120,000 | 558 | 888 | 50 | 767 | 684 | 50 | 832 | 407 |
| 01034 | 33 | 4.6 | 12,000 | 310 | 496 | 55 | 285 | 498 | 50 | 984 | 1,312 |
| 01035 | 35 | 4.2 | 320,000 | 384 | 1,024 | 50 | 706 | 1,169 | 50 | 984 | 1,312 |
| 01041 | 34 | 2.0 | 160,000 | 619 | 1,695 | 50 | 1,034 | 853 | 50 | 542 | 383 |
| 01042 | 33 | 2.3 | *140,000 | 280 | 1,595 | 50 | 602 | 1,228 | 50 | 654 | 782 |
| 01043 | 37 | 2.3 | 320,000 | 617 | 1,163 | 50 | 679 | 928 | 50 | 687 | 407 |
| 01045 | 49 | 1.1 | *470,000 | 639 | 408 | 60 | 1,077 | 661 | 50 | 832 | 407 |
| 23005 | 28 | 5.4 | 5,666 | 421 | 666 | 50 | 572 | 717 | 50 | 500 | 521 |
| 23007 | 26 | 2.2 | 149,556 | 641 | 1,124 | 50 | 770 | 1,183 | 50 | 839 | 896 |
| 23011 | 31 | 5.8 | 110,811 | 454 | 1,473 | 50 | 680 | 1,214 | 50 | 843 | 1,448 |
| 23015 | 42 | 8.2 | 54,216 | 283 | 2,384 | 61 | 492 | 1,638 | 50 | 743 | 1,974 |
| 23016 | 35 | 6.8 | 51,478 | 397 | 719 | 50 | 653 | 642 | 50 | 515 | 480 |
| median | 34 | 4.6 | 120,000 | 454 | 1,124 | 50 | 680 | 928 | 50 | 788 | 797 |
Biosience) and analyzed using FlowJo 8.7.7 (TreeStar). After gating for each effector function, a Boolean gate platform was used to create the full array of possible combinations and SPICE software (version 5.22) was used to analyze the polychromatic flow cytometry data. We applied a threshold for positive responses using negative values distribution after background subtraction (i.e. unstimulated cultures), as previously described [21].

Statistical analyses

Statistical analyses were performed using Graph Pad Prism 5.0. The results are given as medians and interquartile range (IQR) as indicated. Mann-Whitney test and Wilcoxon matched paired test were used for unpaired and paired comparisons, respectively. For multiple comparison analysis, we performed Bonferroni correction. Correlations between ex-vivo and in-vitro ELISpot data were analyzed by using Spearman’s rank correlation coefficient, and linear regression analysis.

Results

Changes in CD4⁺ and CD8⁺ T cell count and their differentiation status

HIV-1-specific T cell responses are known to decrease upon cART initiation, although not all responses and specificities may show similar decay kinetics [22,23]. To determine whether maraviroc-intensified cART would lead to an equally rapid or even faster decay of global T cell responses to HIV-1, longitudinal changes in the breadth and magnitude of total HIV-1-specific T cell responses were compared between the maraviroc and control study arms at week 24 and week 60, i.e. 12 weeks after stopping maraviroc intensification. As previously reported, plasma viral load decreased under the limits of detection within the first 4-week cART in most patients [18]. CD4⁺ T cell counts showed higher increases in the MVC subjects at week 12 and were significantly elevated in the MVC arm at week 60 when compared to the control subjects (p = 0.0378, Table 1 and ref [18]). At the same time, the decay in CD8⁺ T cells was significantly slower in MVC subjects than in the control subjects (Fig. 1A and [18]). To examine whether these effects on CD4⁺ and CD8⁺ T cell counts were associated with a modulation of T cell differentiation markers, the expression of CD45RA and CCR7 was assessed over time and compared between the two arms. The data show that the frequency of effector memory (EM, CD45RA⁻/CCR7⁻) CD8⁺ T cells was significantly decreased in both study arms at week 24 and week 60 compared to baseline, possibly reflecting the strong reduction in viral loads in both arms upon cART initiation (Fig. 1B). No significant changes for any other CD4⁺ or CD8⁺ T cell subset was observed, neither over time nor between study arms. These data indicate that maraviroc does not affect T cell differentiation during and after maraviroc intensification and that the different kinetics of CD4⁺ and CD8⁺ T cell counts between the arms are not reflected by gross alterations in differentiation markers.

Maraviroc intensification is associated with maintenance of HIV-1-specific T cell responses

To assess whether the effect of maraviroc intensification on cell homeostasis affected the magnitude, breadth and specificity of the HIV-1-specific T cell response, we performed IFN-γ ELISPOT assay on PBMC from individuals in both arms of the study using a 18-mer overlapping peptide (OLP) set covering the full HIV-1 proteome [19]. At baseline, the median magnitude of HIV-1-specific T cell responses in all patients was 2,708 SFC/10⁶ PBMC (range 395–13,860), with a median breadth of 6 (range 2-15).
Effect of Maraviroc on T Cell Response

A

Control

MVC

TDF/FTC/RAL

TDF/FTC/RAL

MVC

CD4+ T Cells/μL

CD8+ T cells/μL

weeks

weeks

0 4 12 24 48 60

0 4 12 24 48 60

B

Naive

CD4

CD8

(phenotype: CD45RA+CCR7+)

0 20 40 60 80

0 10 20 30 40 50 60

0 10 20 30 40 50 60

weeks

CM

(phenotype: CD45RA+CCR7+)

0 20 40 60 80

0 10 20 30 40 50 60

0 10 20 30 40 50 60

weeks

EM

(phenotype: CD45RA+CCR7+)

0 20 40 60 80

0 10 20 30 40 50 60

0 10 20 30 40 50 60

weeks

Effector

(phenotype: CD45RA+CCR7+)

0 20 40 60 80

0 10 20 30 40 50 60

0 10 20 30 40 50 60

weeks
responses per individual (Fig 2, left hand panels). The magnitude and the breadth in this cohort were considerably lower than that of chronically infected patients reported previously but in line with described breadth of responses in early, untreated HIV-1 infection [19,24]. No significant difference was observed in magnitude and breadth of HIV-1-specific response between the arms at any time point (Fig 2, right panels). When we assessed changes in the virus-specific response in each arm, the magnitude of the HIV-1-specific response in the control arm was significantly reduced by week 24 (median 454 SFC/10⁶ PBMC (range 27–7584), p = 0.0042) and even more so by week 60 (median 115 SFC/10⁶ PBMC (range 0–1,475), p = 0.0043, Fig 2A). In contrast, subjects in the MVC arm did not show a significant reduction until week 60 when their median magnitude was still more than 5-fold higher than responses in the control arm (median 691 SFC/10⁶ PBMC (range 0–3,535), Fig 2A). Similarly, the breadth of response was reduced over time as well, with significant reductions seen by week 60 in the control arm but not in the maraviroc intensified group (Fig 2B).

There was no difference between the arms in regards to protein specificity of the HIV-1-specific CD8⁺ T cells that remained at 24 and 60 weeks after starting cART (data not shown).

To extend the longitudinal analyses of responses between the intensified and non-intensified arms of the study to additional individuals for whom sample availability was limiting, we performed the same analysis using in vitro expanded cells. Aside from including additional individuals into the analyses, this also offered the opportunity to test for potential differences in the proliferative capacities of HIV specific T cells in the two arms. Thawed PBMC were expanded using an anti-CD3 mAb and kept in culture until sufficient cell numbers were reached. The culture time needed between the two study arms was comparable (both arms a median of 19 days), indicating intact proliferative capacities of T cells in maraviroc intensified cART treated individuals. For samples for which direct ex-vivo PBMC and in vitro expanded cells were tested, the ELISpot results were compared to validate the approach of using in vitro, unspecifically expanded cells. Overall, the breadth of responses in expanded cells correlated well with the direct ex-vivo results (Fig 3A, r = 0.78, p<0.0001). The magnitude of responses was generally increased in expanded cells, with later time points (week 24 and 60) showing stronger recovery of responses when compared to unexpanded cells (Fig 3B). Of note, the correlation between results from direct ex-vivo analyses and in vitro stimulated cells became stronger over time (r = 0.5235, 0.8455, 0.8720, and p = 0.0374, 0.0018, 0.0004 for comparisons at BL, w24, and w60 respectively). No differences were observed in proliferative capacity between the arms. These data indicate that in both arms, HIV-1-specific T cell responses showed intact in vitro proliferative capacities after prolonged cART and that in settings with limited sample availability, the in vitro expansion approach produces reliable data [23].

HIV-1-specific T cell responses measured in expanded cells showed a significant decline in their magnitude during first 24 weeks in all subjects together (Fig 3C, left panel). However, the reduction was generally less than three-fold (median 8,110 SFC/10⁶ PBMC in BL and 2,656 SFC/10⁶ PBMC in week 24) and thus not as dramatic as in unexpanded cells (median 6.3 fold, 2,708 SFC/10⁶ PBMC in BL, and 424 SFC/10⁶ PBMC in w24) (Fig 2A and 3C, left panel). When the longitudinal changes in magnitude and breadth of responses were analyzed for each treatment arm separately, no significant reductions at week 24 and week 60 were noted (Fig 3C, D). However, when in vitro stimulated responses were compared between the two arms, there was a trend that MVC-intensified subjects maintained stronger HIV-1-specific response at week 24 than control individuals (median 1,450 (IQR 277–2,965) in the control arm, 3,957 (1,714–13,018) in MVC, p = 0.0625, Fig 3C, right panel). In addition, the median HIV-specific response was three-fold higher in MVC (median 3,957 (275–4,691)) compared to the control arm (1,114 (2,394–6,882)) until week 60. These data further support the notion that HIV-1-specific T cell responses are maintained for longer at higher levels in subjects with maraviroc intensification compared to individuals receiving non-intensified cART.

Poly-functionality of HIV-1-specific CD8⁺ T cells is maintained under MVC intensified cART

The ability of HIV-1-specific T-cells to respond to antigenic stimulus with multiple different effector functions has been associated with the relative control of HIV-1 infection [26,27]. Since therapeutic strategies that aim at prolonged treatment interruptions or even viral eradication, will possibly depend on such polyfunctional T cell responses, we assessed the effector functions of HIV-1-specific CD8⁺ T cells in cART treated subjects with and without maraviroc intensification. To this end, direct ex vivo isolated PBMC were stimulated using peptide pools covering each of the viral proteins and analyzed for the expression of the degranulation marker (CD107a) or the production of intracellular cytokines, including IFN-γ, MIP-1β, and IL-2. The frequency of IFN-γ producing T cell responses correlated well with the data from the ex-vivo ELISpot analyses (Fig 4A, r = 0.8265, p<0.0001). The magnitude of the total HIV-1-specific CD8⁺ T cell responses with at least one effector function by flow analysis varied widely in baseline samples (0.43% to 16.44% of total CD8⁺ T cells across arms) and, as expected, was reduced at week 24 and week 60 (Fig 4B). Although the magnitude of total HIV-specific CD8⁺ T cells between the arms was comparable at the different time points, a significant reduction in the strength of the ex-vivo response was seen in the control arm but not in MVC treated, as observed in direct ex vivo ELISpot analysis (Fig 1A and 4B). Also, as the reduction in frequency of HIV-specific CD8⁺ T cell fractions with different cytokine secretion pattern was similar between the two arms, the data indicate that maraviroc intensification does not skew HIV-specific CD8⁺ T cell function (Fig 4C). The same was observed when the relative contribution of T cell populations with different numbers of effector functions to the total HIV-specific CD8⁺ T cell responses was compared between arms and over time (Fig 4D) in line with previous reports [26,28].

Discussion

Since its development as a HIV entry inhibitor, CCR5 has been used as a target in several clinical studies of HIV infection as well as in other applications, including auto-immune diseases, cancer and transplantation [15,29–34]. Although some results remain still controversial [11–15,17,32] blocking the CCR5 co-receptor is
thought to suppress adverse immune activation and inflammation by blocking the chemotactic activity via its inhibition of CCR5-mediated signals. Due to its potential immune-modulatory properties, maraviroc may thus also affect the HIV-specific immune response, not necessarily only in a beneficial manner. While a number of studies have described effects on total T cell counts, CD4⁺ and CD8⁺ T cell kinetics and outcome of vaccination to other pathogens [35–39], no study has, to our knowledge, investigated the effect of MVC on the total HIV-1-specific CD4⁺ and CD8⁺ T cell response. In the present study, we investigated the effect of maraviroc intensification on HIV-specific T cell responses in primary HIV-1 infected subjects treated with standard cART or maraviroc intensified regimen. Although there was no gross difference in specific T cell subsets, maraviroc intensification showed extended maintenance of stronger HIV-1-specific T cell responses when compared to non-intensified treatment in PBMCs. Our data in recently infected and early treated individuals showed that maraviroc intensification accelerated recovery of CD4⁺ T cell counts and maintained higher CD4⁺ T cell count after its discontinuation (Table 1 and [18]). As CD4⁺ T cell help is critical for maintenance of memory CD8⁺ T cells [40], this early increase of CD4⁺ T cells may also provide the basis for the extended maintenance of virus-specific T cell responses. Alternatively, the maintenance of higher HIV-1-specific T cell responses in maraviroc intensified subjects may be a reflection of a slower reduction in the total CD8⁺ T cells in the peripheral blood. This would be in line with clinical data showing that maraviroc intensification increase CD4⁺ T cells faster and reduce CD8⁺ T cell slower than non-intensified regimen (Fig. 1A and [18]). In addition, others have recently reported that maraviroc intensification increased CD8⁺ T cell counts in peripheral blood and decreased CD8⁺ T cells in rectal tissue in chronically HIV-infected subjects on stable cART [17], suggesting a possible in vivo redistribution of T cells by maraviroc. However, the relative changes of total CD8⁺ T cell counts between control arm and intensified group were less pronounced than the extensive changes in HIV-specific CD8⁺ T cell frequencies, making it unlikely that a MVC-driven redistribution of virus-specific CD8⁺ T cells would be the sole driving force behind the prolonged maintenance of these cells in the peripheral blood. Maintenance of virus-specific T cells has also been linked to the availability of cognate antigen[22]. As the reduction in virmeia in both arms was comparable, additional mechanisms may be at work in maraviroc-intensified individuals that lead to extended presence of cells. As shown in previous analyses, not all HIV-specific T cell response contract with the same kinetics and some even expand after cART initiation[23]. As there were no differences in the specificity of HIV-specific T cell response between the two arms in the present study, the mechanism for the maintenance of responses in the MVC intensified group remain unclear. One possibility is that the slower CD4⁺ T cell decline in the intensified arm [18], together with a

Figure 2. Longitudinal analyses of HIV-1-specific T cell responses in PBMC. The total magnitude (A) and breadth (B) of ELISpot responses at baseline (BL), week 24 (w24) and week 60 (w60) are shown for all subjects together (left panels) and for each study arm separately (right panels, crossed lines for control arm, circles for MVC arm). Horizontal lines represent median values of Spot-forming cells (SFC)/10⁶ PBMC and the IQR, respectively. Mann-Whitney test was used in all statistical analysis. Only p values with significance after Bonferroni correction was shown. The numbers in parenthesis below the x-axis represent the median value.

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Figure 3. Longitudinal assessment of HIV-specific T cell responses with in vitro expanded T cells. A. Relationship of the breadth between responses detected by direct ELISpot and ELISpot using in vitro expanded cells. Responses on the x-axes represent the total HIV-1-specific responses in direct ELISpot, and the y-axes indicate total HIV-1-specific responses in expanded ELISpot for samples taken at baseline (circle), week 24 (square), and week 60 (triangle). B. Relationship of the magnitude between direct ELISpot and expanded ELISpot at each time point. Cross: control arm, circle: MVC arm. The lines in A and B show linear regression lines. C, D. Changes in magnitude and breadth of total HIV-specific T cell responses in expanded ELISpot are shown as in Figure 2.

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reported increased in T cell activation upon maraviroc intensification [17] maintains activated CD8 T cell for longer. Although a number of studies show conflicting data in terms of immune activation [11–15], it is important to note that the present data were generated in early treated subjects, which may yield different results than the analyses in maraviroc intensification during chronic HIV infection.

Blocking CCR5 signaling \textit{in-vivo} might inhibit migration of memory T cells expressing CCR5 to the site of the cognate antigen, thus preventing these memory T cells to be stimulated properly to acquire effector functions and exert effective anti-viral immunity. In fact, maraviroc has been shown to inhibit chemotactic activity of lymphocytes and monocytes \textit{in vitro} and to reduce the risk of Graft-versus-host disease (GVHD) in patients.

Figure 4. Longitudinal assessment in functional profile of HIV-specific CD8$^+$ T cells during cART with MVC intensification. A. Correlation between the total HIV-specific responses determined by direct \textit{ex-vivo} ELISpot analysis (as spot-forming cells (SFC)/10$^6$ PBMC) and by ICS analysis (% of IFN-$\gamma^+$ CD8$^+$ T cells). Linear regression line, and correlation coefficient and p-values (Spearman’s rank correlation test) are shown. B. The change in total HIV-specific CD8$^+$ T cell frequency over time by ICS analysis. Horizontal lines indicate median values of all positive responses. P values were determined by Mann-Whitney tests and shown if the significance remains after Bonferroni correction. C, D. Effector function profiles of HIV-specific CD8$^+$ T cells over time in controls and MVC treated subjects. (left, baseline; middle, week 24; right, week 60). The median and IQR are indicated by horizontal lines and boxes, respectively. Differences relative to baseline values in each arm were tested for statistic significance by Mann-Whitney tests, and shown as # for $p<0.05$, ## for $p<0.01$.

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isolated PBMC and replication. However, we didn't observe any difference in T cell mount HIV epitope-specific DTH reactions [43] may offer profiles in maraviroc intensified therapy, including their ability to cation on response to vaccination and immune profile in HIV-1 infection.

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