J Antimicrob Chemother 2012; **67**: 2474–2478
doi:10.1093/jac/dks216 Advance Access publication 7 June 2012

Journal of Antimicrobial Chemotherapy

Immune recovery and T cell subset analysis during effective treatment with maraviroc

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Received 23 January 2012; returned 22 March 2012; revised 8 May 2012; accepted 10 May 2012

Objectives: Patients treated with maraviroc frequently show high CD4+ T cell increases. The aim of this study was to detail the characteristics of maraviroc-induced immune recovery.

Patients and methods: We studied T cell subsets from frozen peripheral blood mononuclear cells of patients treated with raltegravir, etravirine and either maraviroc (REM, $n=24$) or darunavir/ritonavir (RED, $n=17$).

Results: The two groups showed a similar decrease in activated CD4+ and CD8+ T cells. A greater loss of naive CD4+ T cells and a reduction in cells expressing CXCR4 were observed in REM patients, while RED patients showed a greater loss of cells expressing CCR5.

Conclusions: Our findings do not support a role for reduction in activated T cell subsets to explain the greater maraviroc-induced immune recovery. Reduction in CXCR4+CD4+ and higher expression of CCR5+CD4+ T cells might represent a potential protection from non-R5 tropic viral strain overgrowth.

Keywords: HIV, immune activation, maraviroc, darunavir, CCR5, CXCR4, naive T cells

Introduction

Patients treated with maraviroc frequently show higher CD4+ T cell increases than patients in the comparator arm in randomized clinical trials. This has been observed in naive patients, in experienced patients achieving virological suppression and also in non-R5 patients starting a rescue treatment with maraviroc, suggesting a maraviroc-specific effect on immunological recovery.[1–3]

Greater reduction in immune activation has been associated with greater CD4+ T cell recovery during antiretroviral treatment.[4,5] The higher maraviroc-induced immune reconstitution could be associated with a greater reduction in activated T cell subsets.

Patients with a greater CD4+ T cell recovery also showed higher levels of naive T cells compared with patients with a standard CD4+ T cell recovery after treatment initiation.[6]

Furthermore, maraviroc acts by blocking the surface receptor CCR5, which is the most widely used coreceptor for HIV entrance in CD4+ cells. The implications of its use for the cellular expression of CCR5 are not clear.

Our aim was to detail the T cell subset profile of maraviroc-induced CD4+ T cell recovery. We compared T cell subsets in two groups of patients treated with either a maraviroc-including or a maraviroc-sparing, nucleoside reverse transcriptase inhibitor-sparing salvage highly active antiretroviral therapy (HAART).

Patients and methods

Triple-class-experienced HIV-infected patients showing virological failure to the current HAART were coscreened for darunavir/ritonavir (TMC114-C209 and TMC114-C226), raltegravir (MK0518-023), etravirine (TMC125-C214) and maraviroc (A4001050) Expanded Access Programmes (programmes designed for early access to investigational antiretroviral drugs for treatment-experienced patients who might benefit from new medications). These studies were approved by our institution's Ethics Committee and all patients signed informed consent to participate in the Expanded Access Programmes, to collect additional blood samples and to record clinical data in our clinic's database. Patients received a new regimen containing raltegravir, etravirine and either maraviroc (REM patients) or darunavir/ritonavir (RED patients), according to their genotypic drug resistance and viral tropism. Patients who harboured an R5-tropic virus received maraviroc and patients who harboured a non-R5 virus received darunavir/ritonavir.

Baseline and week 96 [or the closest available (week 72 or week 108)] samples were used for immunophenotyping. Median (IQR) follow-up at the time of second sampling (end of follow-up) was 98 (94–108) weeks.

Thawed peripheral blood mononuclear cells were directly labelled with fluorochrome-conjugated monoclonal antibodies and analysed by flow cytometry. The percentage of expression for each monoclonal antibody was based on morphological gated lymphocytes and on triple stainings. Activated (CD38+ and CD38+HLA-DR+), naive (CD45RA+) and memory (CD45RO+) CD4+ and CD8+ T cells were studied along with CD4+ and CD8+ T cells expressing CCR5 or CXCR4.

Comparisons between and within groups were performed with Mann–Whitney and Wilcoxon signed-rank tests, respectively. The Spearman correlation coefficient was used to assess linear relationships between continuous variables. Values are expressed as median (IQR) or frequency (%).

Results

Forty-one patients were studied: 24 REM patients and 17 RED patients. Baseline demographic, virological and immunological characteristics were similar between the two groups (Table 1).

Table 1. Patient demographic, virological and immunological characteristics; comparisons are between REM patients and RED patients

	Timepoint	Overall, $n = 41$	REM, $n = 24$	RED, $n = 17$	P value
Demographic characteristics					
males		35 (85)	22 (92)	13 (77)	0.212
age, years		47.7 (44.9–51.6)	46.3 (44.3–52.1)	48.7 (46.3–51.6)	0.606
Risk factor					
MSM		20 (48.8)	12 (50)	8 (47.1)	0.838
IVDU		11 (26.8)	7 (29.1)	4 (23.5)	
heterosexual contact		5 (12.2)	2 (8.3)	3 (17.6)	
other or unknown		5 (12.2)	3 (12.5)	2 (11.7)	
Duration of HIV infection, years		18.6 (16.4–22.6)	18.6 (16.8–23.1)	18.6 (16.1–21.8)	0.781
Duration of antiretroviral therapy, years		15.4 (13.8–17.9)	15.8 (13.9–18.4)	14.7 (13.6–16.3)	0.223
Patients with a previous AIDS diagnosis		23 (56)	14 (58)	12 (71)	0.519
Virological characteristics					
CD4+ T cell nadir, cells/mm^3		108 (30–175)	73 (28–195)	136 (46–165)	0.615
HIV-RNA, $\log_{10}$ copies/mL	BL	4.1 (3.8–5.1)	4.1 (3.8–5.1)	3.9 (3.6–4.9)	0.534
	EOS	1.69 (1.69–1.69)	1.69 (1.69–1.69)	1.69 (1.69–1.69)	0.379
CD4+ T cell counts, cells/mm^3	BL	243 (153–333)	269 (87–421)	215 (165–295)	0.308
	EOS	450 (353–562)	497 (441–608)	354 (291–454)	0.016
CD8+ T cell counts, cells/mm^3	BL	1029 (701–1637)	1311 (871–1709)	851 (488–1384)	0.152
	EOS	976 (739–1180)	980 (691–1215)	913 (757–1167)	0.968
Immunological characteristics					
CD38+ CD4+ T cells, % of CD4+ T cells	BL	33.9 (25.7–49.7)	33.4 (23.4–57.3)	38.0 (29.6–47.8)	0.534
	EOS	27.7 (21.9–35.2)	26.1 (20.9–34.9)	30.3 (23.4–37.0)	0.682
CD38+ CD8+ T cells, % of CD8+ T cells	BL	31.2 (23.9–52.4)	30.1 (20.5–54.5)	32.5 (26.3–50.6)	0.802
	EOS	14.9 (11.6–20.7)	13.9 (11.7–21.5)	17.7 (11.4–20.7)	0.624
CD38+HLA-DR+CD4+ T cells, % of CD4+ T cells	BL	7.1 (4.5–12.4)	6.3 (4.4–11.5)	8.6 (5.4–12.7)	0.368
	EOS	3.7 (2.9–5.8)	3.8 (2.9–6.0)	3.7 (2.6–4.8)	0.691
CD38+HLA-DR+CD8+ T cells, % of CD8+ T cells	BL	9.4 (5.1–13.1)	9.3 (5.3–15.1)	9.4 (5.1–12.7)	0.802
	EOS	2.9 (1.6–4.7)	2.8 (1.6–5.3)	2.9 (1.8–4.4)	0.999
naive CD4+ T cells, % of CD4+ T cells	BL	53.5 (43.9–66.1)	53.3 (44.0–65.9)	53.5 (43.8–68.2)	0.884
	EOS	53.6 (43.8–66.2)	49.1 (42.5–59.0)	60.5 (52.7–66.2)	0.029
memory CD4+ T cells, % of CD4+ T cells	BL	65.0 (51.9–76.9)	64.1 (45.7–78.4)	69.0 (55.1–79.6)	0.682
	EOS	68.6 (58.8–74.0)	68.6 (59.4–79.0)	65.6 (54.5–70.6)	0.420
CCR5+CD4+ T cells, % of CD4+ T cells	BL	16.9 (12.3–24.1)	16.7 (13.8–28.2)	16.9 (9.9–22.5)	0.420
	EOS	14.6 (10.0–19.5)	17.6 (13.8–20.9)	9.9 (6.5–13.9)	<0.001
CCR5+CD8+ T cells, % of CD8+ T cells	BL	40.3 (27.0–46.8)	40.8 (24.5–49.6)	39.9 (29.9–46.3)	0.968
	EOS	34.2 (23.7–42.7)	38.4 (30.0–47.4)	21.2 (17.6–34.1)	<0.001
CXCR4+ CD4+ T cells, % of CD4+ T cells	BL	38.1 (26.8–57.6)	42.9 (30.6–62.4)	34.8 (26.4–50.7)	0.296
	EOS	29.7 (22.9–38.1)	29.2 (22.4–37.1)	31.0 (23.0–38.2)	0.926
CXCR4+ CD8+ T cells, % of CD8+ T cells	BL	15.7 (9.5–26.6)	16.0 (9.7–30.3)	15.7 (9.5–19.0)	0.451
	EOS	18.2 (13.9–25.6)	18.6 (12.8–26.2)	18.2 (14.3–23.3)	0.905

BL, baseline; EOS, end of study; MSM, men who have sex with men; IVDU, intravenous drug users.
Values are expressed as median (IQR) or frequency (%), as appropriate.

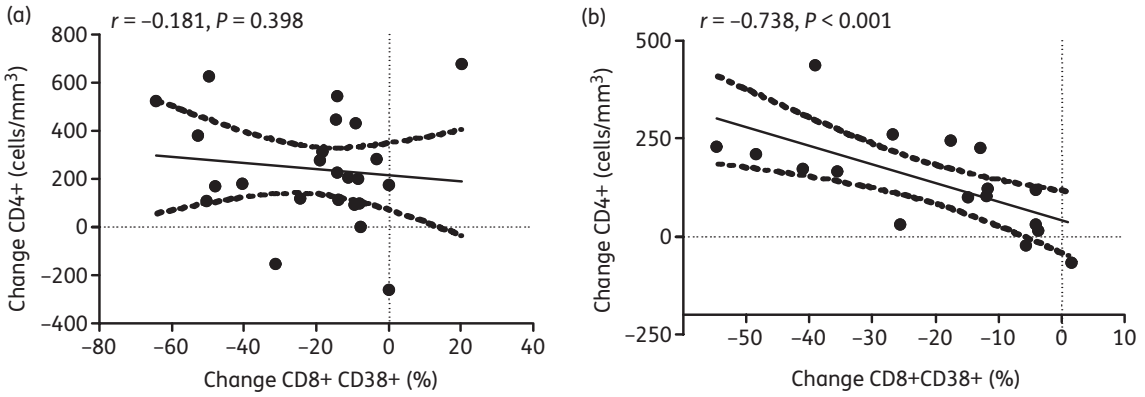

Figure 1. Correlation between CD4+ T cell increase and decrease in T cell activation: (a) REM patients and (b) RED patients.

After 96 weeks of treatment, virological success (HIV-RNA < 50 copies/mL) was achieved by 96% of REM patients (23/24) and 100% of RED patients (17/17).

REM patients had a greater immunological recovery during follow-up: CD4+ T cells at week 96 were 497 (441–608) cells/mm^3 in REM patients versus 354 (291–454) cells/mm^3 in RED patients (P=0.016); CD4+ T cell gain was almost double in REM patients compared with in RED patients [221 (141–316) versus 132 (57–215) cells/mm^3, P=0.062]. CD8+ T cells were not different between groups either at baseline or at the end of follow-up (Table 1).

We analysed the T cell subset profile in the two groups of patients. REM and RED patients had similar levels of each subset at baseline (Table 1).

CD38+CD4+ T cells decreased in both groups during follow-up: −6.9% (−14.3%/+6.11%) in REM patients (P=0.081) and −3.7% (−20.17%/−1.1%) in RED patients (P=0.013). Similarly, CD38+CD8+ T cells decreased significantly in both groups compared with baseline: −14.3% (−35.9%/−8.2%) in REM patients and −14.8% (−35.7%/−5.7%) in RED patients (P<0.0001 for both groups). At the end of follow-up, levels of both subsets were similar between the two groups (Table 1).

We also observed a significant decrease in levels of CD38+HLA-DR+CD4+ and CD38+HLA-DR+CD8+ T cells during follow-up: −3.3% (−7.1%/+0.2%) and −5.7% (−6.7%/−1.7%) for CD38+HLA-DR+CD4+ T cells in REM patients (P=0.010) and RED patients (P<0.0001), respectively, and −4.8% (−9.6%/−2.3%) and −5.9% (−8.6%/−2.2%) for CD38+HLA-DR+CD8+ T cells in REM patients and RED patients, respectively (P<0.0001 for both groups). No differences in the levels of these activated subsets were observed between groups at the end of follow-up (Table 1).

We found an inverse correlation between CD4+ T cell recovery and CD38+CD8+ decrease in RED patients, but not in REM patients, as shown in Figure 1.

Naive CD4+ T cells tended to decrease in REM patients during follow-up [median change −1.7% (−16.5%/+2.3%), P=0.086] while they remained stable in RED patients [median change 1.7% (−3.5%/+9.9%), P=0.548], thus leading to higher levels of naive CD4+ T cells in RED patients at the end of follow-up (Table 1). Conversely, we did not observe significant changes in the levels of memory CD4+ T cells during follow-up in either REM patients [median change +2.8% (−4.5%/+11.9%), P=0.166] or RED patients [median change −3.0% (−9.5%/+5.3%), P=0.404]. Levels of memory CD4+ T cells were also similar at the end of follow-up (Table 1).

We then analysed the expression of CCR5: during follow-up, CCR5+CD4+ T cells remained stable in REM patients [median change 0.4% (−10.9%/+4.1%), P=0.523] while they decreased significantly in RED patients [median change −7.6% (−10.5%/−1.4%), P<0.001]. At the end of follow-up, CCR5+CD4+ T cells were significantly higher in REM compared with RED patients (Table 1). Since blocking of the CCR5 molecule by maraviroc may lead to a decreased migratory response to natural chemotactic peptides, there exists the possibility that the increase in CD4+ T cells might be attributed to a diminished trafficking of lymphocytes in tissues. However, when we took into consideration the changes in CD4+CCR5+ T cells, CD4+ increase again tended to be higher in REM patients compared with RED patients, both considering the crude mean change and the mean change adjusted for the increase in CCR5+CD4+ T cells (crude mean CD4+ change ± SEM was 243 ± 46 cells/mm^3 in REM patients versus 141 ± 30 cells/mm^3 in RED patients, P=0.112; adjusted mean CD4+ change ± SEM was 247 ± 39 cells/mm^3 in REM patients versus 135 ± 46 cells/mm^3 in RED patients, P=0.074). In addition, no correlation was found between the change in CD4+CCR5+ and either the overall increase in CD4+ T cells (r=−0.169, P=0.431) or memory CD4+ T cells (r=0.200, P=0.349) in REM patients.

We also looked at changes in CCR5+CD8+ T cells, which remained stable in REM patients during follow-up [median change +2.9% (−10.4%/+12.3%), P=0.523] while decreasing significantly in RED patients [median change −15.3% (−18.9%/−8.3%), P=0.001]. At the end of follow-up, CCR5+CD8+ T cells were significantly higher in REM patients compared with in RED patients (Table 1).

The expression of CXCR4 showed opposite modifications within groups in the CD4+ T cell compartment: CXCR4+CD4+ T cells decreased significantly in REM patients [median change −11.3% (−30.9%/+3.9%), P=0.005] while remaining substantially stable in RED patients [median change −8.3% (−15.7%/+3.2%), P=0.109]. At the end of follow-up, however, CXCR4+CD4+ T cells were similar in the two groups (Table 1).

Changes in CXCR4+CD8+ T cells during follow-up were similar in REM and RED patients (Table 1).

Discussion

As previously reported, we observed a greater CD4+ T cell increase in patients treated with maraviroc compared with patients who did not receive maraviroc (i.e. those treated with darunavir/ritonavir in our study).

Viral tropism itself might have influenced immune recovery, since patients harbouring an R5 virus have a greater CD4+ T cell increase on therapy[7] and in our study only REM patients harboured an R5-tropic virus.

The greater CD4+ T cell recovery we observed during maraviroc treatment did not appear to be related to a greater decrease in levels of activated T cell subsets: in line with what was shown by others,[8] we observed a strong decrease in activated T cell subsets in both treatment groups during follow-up; however, such a decrease was not different between the groups. A significant reduction in activated T cell subsets is a hallmark of effective antiretroviral treatment and suppression of viral replication,[9] and CD4+ recovery has been shown to correlate with the extent of T cell activation reduction[4] in patients starting an effective antiretroviral treatment. We observed a significant correlation between immune recovery and the reduction in activated subsets only in RED patients, which is in line with what was previously reported for immunological non-responders.[5] RED patients might be considered more similar to immunological non-responders while REM patients do not meet the criteria for such a definition, and thus the same correlation might not apply.

We observed a decrease in naive cells in patients treated with maraviroc compared with darunavir, which is in contrast to what was previously observed in the context of improved immune recovery.[6] A decrease in naive T cell proliferation after starting antiretroviral treatment and an inverse correlation with CD4 recovery, consistent with an homeostatic mechanism, was previously reported.[10] Also, a significant reduction in naive CD4+ T cell apoptosis was observed in patients switching to a protease inhibitor-based HAART due to limited immune reconstitution, which might explain the stability of naive CD4+ T cells in our patients who received darunavir/ritonavir.[11]

We also found a stability in the levels of CCR5 expression both in CD4+ and CD8+ cells in REM patients, while it decreased significantly in RED. We did not observe similar changes or differences between groups in the expression of CXCR4.

The maintenance of higher levels of T cells expressing CCR5 might be related to a decreased migratory response to natural chemotactic peptides that bind to CCR5, which is now blocked by maraviroc.[12] However, the increase in CD4+ T cells was still higher in REM patients compared with RED patients when adjusting for CD4+CCR5+ T cell changes, and no significant correlation between CD4+CCR5+ T cells and the overall CD4+ T cell increase was observed. Although we do not have experimental data on chemotaxis, our results suggest that the increase in CD4+ T cells in REM patients cannot be only attributed to a diminished trafficking of such cells from plasma to peripheral tissues.

One of the major concerns during antiretroviral treatment with maraviroc is the potential selection of CXCR4-tropic viruses. The significant decrease in the expression of CXCR4 on CD4+ T cells we observed in REM patients could prevent the overgrowth of CXCR4-tropic viral strains by allowing for a less favourable substrate in which to replicate. Recent findings showing a greater and faster replication of CXCR4-tropic viruses in cell lines expressing high levels of CXCR4 on CD4+ T cells[13] would support the need for a CXCR4-tropic virus to have a favourable substrate for an efficient replication that might lead to virological failure.

Our findings do not support a role for a greater reduction in activated T cell subsets to explain the greater increase in CD4+ T cells, not even when considering the expression of CCR5 as a marker of immune activation, since REM patients did not show any decrease in T cells expressing CCR5. However, maintaining a higher expression of CCR5+CD4+ T cells and reduction in CXCR4+CD4+ T cells might represent a potential protection from non-R5 tropic viral strain overgrowth during treatment with maraviroc.

Acknowledgements

This study was partially presented at the Eighteenth Conference on Retroviruses and Opportunistic Infections, Boston, MA, USA, 2011 (Abstract n. 573).

We are thankful to all patients who donated supplementary blood samples, and to Elisabetta Carini and Vega Rusconi who coordinated the Expanded Access Programme coscreenings.

Funding

The present study was carried out as part of the routine work at our institution. Additional internal funding was only used for the purchase of monoclonal antibodies and laboratory reagents.

Transparency declarations

F. C. has received research grants, travel grants or payment for educational grants from Abbott, Boehringer Ingelheim, Bristol-Myers Squibb, Tibotec (Johnson & Johnson), GlaxoSmithKline, Merck Sharp & Dohme, ViiV Healthcare and Gilead Sciences. L. G. has received payment for educational grants from Bristol-Myers Squibb. N. G. has acted as a consultant to and received research grants from Bristol-Myers Squibb, Abbott, Roche, Boehringer Ingelheim, Pfizer, Virco (Johnson & Johnson), Gilead Sciences and GlaxoSmithKline. V. S. has received payment for educational grants from Bristol-Myers Squibb and Merck Sharp & Dohme. A. L. and A. C. have received grants, travel grants, consultancy fees and payment for educational grants from Abbott, Boehringer Ingelheim, Bristol-Myers Squibb, Gilead Sciences, GlaxoSmithKline, Merck Sharp & Dohme, Pfizer, Roche, Tibotec (Johnson & Johnson) and ViiV Healthcare. S. N. has received research grants, consultancy fees, travel grants and payment for educational grants from Bristol-Myers Squibb, Pfizer, GlaxoSmithKline, ViiV Healthcare, Merck Sharp & Dohme, Pfizer, Tibotec (Johnson & Johnson) and Boehringer Ingelheim. All other authors: none to declare.

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