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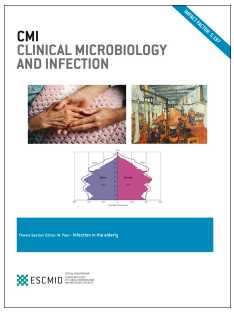

Dynamics of adaptive and innate immunity in patients treated during Primary HIV Infection: results from MAIN randomised clinical trial

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RESEARCH NOTE

FULL TITLE:

Dynamics of adaptive and innate immunity in patients treated during Primary HIV Infection: results from MAIN randomised clinical trial

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Adaptive and innate immunity in primary HIV infection

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AUTHORSHIP/CONTRIBUTION

MR and MP equally contributed to the work and wrote the manuscript. GT designed the clinical trial. SN, MP, SC, MR followed the patients. LG

supervised statistical analysis and helped to write the manuscript. SP, MC and GS performed B-cells and DC investigations. SDB and AC performed T-cells staining. DDB and MM performed NK-cells staining.

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ABSTRACT

We evaluated the dynamics of innate and adaptive immunity in patients treated with combined antiretroviral therapy (cART) during primary HIV infection (PHI), enrolled in a prospective randomised trial (MAIN, EUDRACT 2008-007004-29).

After 48 weeks of cART, we documented a reduction in activated B-cells and $CD8^+$ T-cells. Moreover, natural killer (NK) and dendritic cells (DC) frequencies were measured, finding a decrease in $CD16^+CD56^{dim}$ with a reciprocal rise in $CD56^{high}$ NK and an increase in myeloid and plasmacytoid DC.

In conclusion, 48 weeks of cART during PHI showed significant benefits on both innate and adaptive immunity.

KEYWORDS:

Primary HIV-1 infection; B cells; NK cells; dendritic cells; immune activation

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TEXT

During Primary HIV Infection (PHI) the huge amount of viral replication harms the immune system by a direct viral cythopatic effect, but mostly through massive T-cell activation [1,2], responsible for severe $CD4^+$ T-cell depletion; combined antiretroviral therapy (cART) can only partially restore this damage [3].

Few authors reported the dynamics of adaptive and innate immunity during PHI, the effect of early initiation of cART and their impact on $CD4^+$ T-cell recovery. It has been shown that not only T- [3,4] but even B- [5-8], natural killer (NK) [9,10] and dendritic cells (DC) [11] compartments are deranged since PHI and that cART can improve, although not completely, these impairments.

Aim of this study was to evaluate T, B, NK and DC subsets during PHI and to assess the effect of cART on the adaptive and innate immune system.

We analyzed 19 patients treated during PHI enrolled in a monocenter, randomised, open-label, proof-of-concept clinical trial (MAIN study, EUDRACT number: 2008-007004-29). Patients started a protease-inhibitor-based regimen and were randomized to maraviroc as associated drug. Blood was collected at baseline (BL), and at week (W) 48 and processed to preserve frozen Peripheral Blood Mononuclear Cells (PBMCs).

Staining and polychromatic flow-cytometry were performed on cryopreserved PBMCs at BL and W48. Gating strategies are described in Figure 1. $CD8^+$ and

$CD4^+$ T-cells were defined as naive (NV), central memory (CM), effector memory (EM), and terminally differentiated (TD) on the basis of CCR7 and CD45RA expression; activated cells were defined by HLA-DR expression. Treg were defined as $CD4^+CD8^-CD25^{bright}FoxP3^+CD127^-$. B-cells were divided in transitional (TR), naive (NV), resting memory (RM), activated memory (AM) and tissue-like memory (TLM) on the basis of CD27 and CD21 expression. Plasma cells (PC) were defined as $CD27^+CD38^+$. NK cells were divided in $CD56^{high}$ and $CD56^{dim}CD16^+$ subsets. Among DC, $CD11c^+$ myeloid (mDC) and $CD123^+$ plasmacytoid (pDC) DCs were identified.

An intention-to-treat analysis was performed. Results are described as median (interquartile range) or frequency (%). Wilcoxon signed-rank test and Spearman rank correlation coefficient were applied. All tests were two-sided and a P-value<0.05 was considered as significant. Statistical analysis was performed by SPSS 20 (IBM).

Patients BL characteristics are shown in table 1. Median HIV-RNA load was $5.8\log_{10}$cp/mL (5.2/6.4), and CD4:CD8 ratio was very low [0.2 (0.15/0.42)], mainly because of massive $CD8^+$ T-cell activation.

After 48W of cART, $CD4^+$ T-cell significantly increased [349cell/μL (241/398), p<0.001], while HIV-RNA decreased [$-5.8\log_{10}$cp/ml (-6.4/-5.2), p<0.001], reaching <50cp/ml in 18/19 patients.

At W48, among $CD4^+$ T-cells we observed a decrease of NV and CM [NV: -18.1% (-29.9/-8.0), CM: -28.9% (-41.5/-21.6); p<0.001] and an increase in TD [62.5% (40.3/70.1), p<0.001]; among $CD8^+$ T-cells, we documented an increase in EM [20.8% (-10.6/32.7), p=0.030] and TD [9.7% (3.5/20.4), p=0.005].

Cell activation was measured in both $CD4^+$ and $CD8^+$ T-cells. While $CD4^+HLA\text{-}DR^+$ T-cells did not significantly change (p=0.053), we found a significant reduction in activated $CD8^+$ T-cell [-45.2% (-55.4/-30.1), p<0.001].

At baseline, both $CD4^+HLA\text{-}DR^+$ and $CD8^+HLA\text{-}DR^+$ T-cells were inversely correlated with CD4:CD8 ratio (r=-0.505, p=0.027 and r=-0.728, p<0.001, respectively). At W48 a greater reduction in $CD4^+HLA\text{-}DR^+$ T-cells was associated with a higher gain of $CD4^+$ T-cells (r=-0.460, p=0.048).

The T-reg population frequency did not significantly change after 48W of treatment (p=0.091).

The analysis of the B-cell populations revealed a 48-week decrease in activated subsets, TLM, AM and PC [TLM: -7.1% (-10.5/-3.0), p<0.001; AM: -5.2% (-15.8/-3.3), p<0.001; PC: -8.7% (-13.0/-2.2), p<0.001], and an increase in RM [9.9% (7.5/17.4), p<0.001].

At baseline, CD4:CD8 ratio inversely correlated with AM and TLM [r=-0.571, p=0.011 and r=-0.715, p=0.001, respectively], while higher HIV-RNA was associated with higher PC frequencies (r=0.519, p=0.023). Notably, after 48W a greater reduction of HIV-RNA was associated to a more significant decrease of PC (r=0.647, p=0.003).

Among NK cells, at 48W we observed a decrease in $CD16^{+}CD56^{dim}$ [-9.3% (-18.4/2.9), p=0.011] and an increase in $CD56^{high}$ cells [0.4% (-0.2-1.0), p=0.033].

Finally, both pDC and mDC percentages increased at W48 [pDC: 0.1% (0.1-0.2), p=0.001; mDC: 0.2% (0.1-0.3), p=0.003].

Aims of this study were to assess alterations of innate and adaptive immunity during PHI and to evaluate cART impact on different cellular subsets. Antiretroviral therapy was able to decrease $CD8^{+}$ T-cell activation in patients with PHI, while no effect was documented on $CD4^{+}HLA\text{-}DR^{+}$ T-cells. A greater decrease of $CD4^{+}HLA\text{-}DR^{+}$ was correlated to a more significant increase in $CD4^{+}$ T-cells, while no relationships were found between decrease in $CD8^{+}HLA\text{-}DR^{+}$ T-cells and $CD4^{+}$ T-cell gain. Interestingly, we observed a decrease in NV and CM CD4+ T-cells, coupled with an increase in TD CD4+ and CD8+ T-cells, in contrast to what is described in chronic infected patients [12].

Few reports described B-cells kinetics in PHI [13-15]. Notably, at the onset of infection we observed increased frequencies of activated B-cells. Dysregulated B-cell subsets, such as TLM, AM and PC, decreased significantly at W48, suggesting a possible role of cART in preserving humoral immunity. Moreover, we documented a significant increase in RM B-cells, responsible for an efficient secondary immune response.

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We also studied some parameters to assess the innate immunity in patients treated during PHI: a reciprocal variation of $CD16^{+}CD56^{dim}$ and $CD56^{high}$ NK was observed, while among DC we documented an increase in both mDCs and pDCs, usually reduced during CHI [16].

We showed that during PHI a lower CD4:CD8 ratio was significantly associated not only to a more significant $CD4^{+}$ and $CD8^{+}$ T-cell activation, but also to higher levels of B-cell dysregulated subsets, as measured by AM and TLM. This finding underlines the importance of CD4:CD8 ratio as a surrogate marker of immune function during HIV infection.

Despite the small sample size, due to the well-known underestimated incidence of patients diagnosed during PHI, our study showed that during PHI both the adaptive and the innate immune system cell subsets were skewed toward an activated phenotype. Immune activation was significantly dampened by cART, in particular for $CD8^{+}$ T-cells, B-cells and NK cells. Moreover, our findings suggested that CD4:CD8 ratio could be a useful parameter to indirectly assess baseline immune activation during primary HIV infection.

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REFERENCES

1. Cohen MS, Shaw GM, McMichael AJ, Haynes BF. Acute HIV-1 Infection. N Engl J Med **2011**; 364(20):1943–54.

2. Mattapallil JJ, Douek DC, Hill B, Nishimura Y, Martin M, Roederer M. Massive infection and loss of memory CD4+ T cells in multiple tissues during acute SIV infection. Nature **2005**; 434(7037):1093–7.

3. Streeck H, Jessen H, Alter G, et al.. Immunological and virological impact of highly active antiretroviral therapy initiated during acute HIV-1 infection. J Infect Dis **2006**; 194(6):734–9.

4. Tilling R, Kinloch S, Goh LE, et al. Parallel decline of CD8+/CD38++ T cells and viraemia in response to quadruple highly active antiretroviral therapy in primary HIV infection. AIDS **2002**; 16(4):589–96.

5. Moir S, Fauci AS. B cells in HIV infection and disease. Nat Rev Immunol **2009**; 9(4):235–45.

6. Moir S, Fauci AS. Insights into B cells and HIV-specific B-cell responses in HIV-infected individuals. Immunol Rev **2013**; 254(1):207–24.

7. Amu S, Ruffin N, Rethi B, Chiodi F. Impairment of B-cell functions during HIV-1 infection. AIDS **2013**; 27(15):2323–34.

8. Moir S, Ho J, Malaspina A, Wang W, et al. Evidence for HIV-associated B cell exhaustion in a dysfunctional memory B cell compartment in HIV-infected viremic individuals. J Exp Med **2008**; 205(8):1797–805.

9. Alter G, Teigen N, Davis BT, et al. Sequential deregulation of NK cell subset distribution and function starting in acute HIV-1 infection. Blood **2005**;

106(10):3366–9

10. Mavilio D, Lombardo G, Benjamin J, et al. Characterization of CD56-/CD16+ natural killer (NK) cells: a highly dysfunctional NK subset expanded in HIV-infected viremic individuals. Proc Natl Acad Sci U S A **2005**; 102(8):2886–91.

11. Huang J, Yang Y, Al-Mozaini M, et al. Dendritic cell dysfunction during primary HIV-1 infection. J Infect Dis **2011**; 204(10):1557–62.

12. Robbins GK, Spritzler JG, Chan ES, et al. Incomplete reconstitution of T cell subsets on combination antiretroviral therapy in the AIDS Clinical Trials Group protocol 384. Clin Infect Dis. **2009;** 1;48(3):350-61.

13. Titanji K, Chiodi F, Bellocco R, et al. Primary HIV-1 infection sets the stage for important B lymphocyte dysfunctions. AIDS **2005**; 19(17):1947–55.

14. Moir S, Buckner CM, Ho J, et al. B cells in early and chronic HIV infection: evidence for preservation of immune function associated with early initiation of antiretroviral therapy. Blood **2010**; 116(25):5571–9.

15. Pensieroso S, Galli L, Nozza S, et al. B-cell subset alterations and correlated factors in HIV-1 infection. AIDS **2013** ; 27(8):1209–17.

16. Manches O, Frleta D, Bhardwaj N. Dendritic cells in progression and pathology of HIV infection. Trends Immunol **2013**; pii: S1471-4906(13)00156-7

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FIGURE CAPTIONS:

Figure 1 – Gating strategies

FSC: forward scatter; SSC: side scatter.

A) T-cells. NV: naïve; CM: central memory; EM: effector memory. TD: terminally differentiated.

B) Treg.

C) B-cells. TR: transitional; NV: naïve; RM: resting memory; AM: activated memory; TLM: tissue-like memory; PC: plasma cells.

D) NK cells.

E) Dendritic cells. pDC: plasmacytoid dendritic cells; mDC: myeloid dendritic cells.

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Table 1 - Baseline characteristics

Characteristics (N=19)		
Age, years	39 (33/41)	
Sex		
M	18 (95%)	
F	1 (5%)	
Risk factor		
MSM	12 (63%)	
Heterosexuals	7 (37%)	
Fiebig		
III	7 (37%)	
IV	1 (5%)	
V	11 (58%)	
	W0	**W48**
$CD4^+$ T-cells, cells/µL	382 (269/470)	705 (614/854)
$CD4^+$ T-cells %	12.7 (10.1/22.1)	35.2 (32.2/39.3)
$CD8^+$ T-cells, cells/µL	1539 (884/2697)	733 (587/1263)
$CD8^+$ T-cells %	70.7 (53.0/72.9)	36.7 (32.7/45.1)
CD4:CD8 ratio	0.20 (0.15/0.42)	1.01 (0.72/1.13)
HIV-RNA, log_{10} cp/ml	5.8 (5.2/6.4)	0.0 (0.0/1.6)

M: males; F: females; MSM: men who have sex with men; W: week

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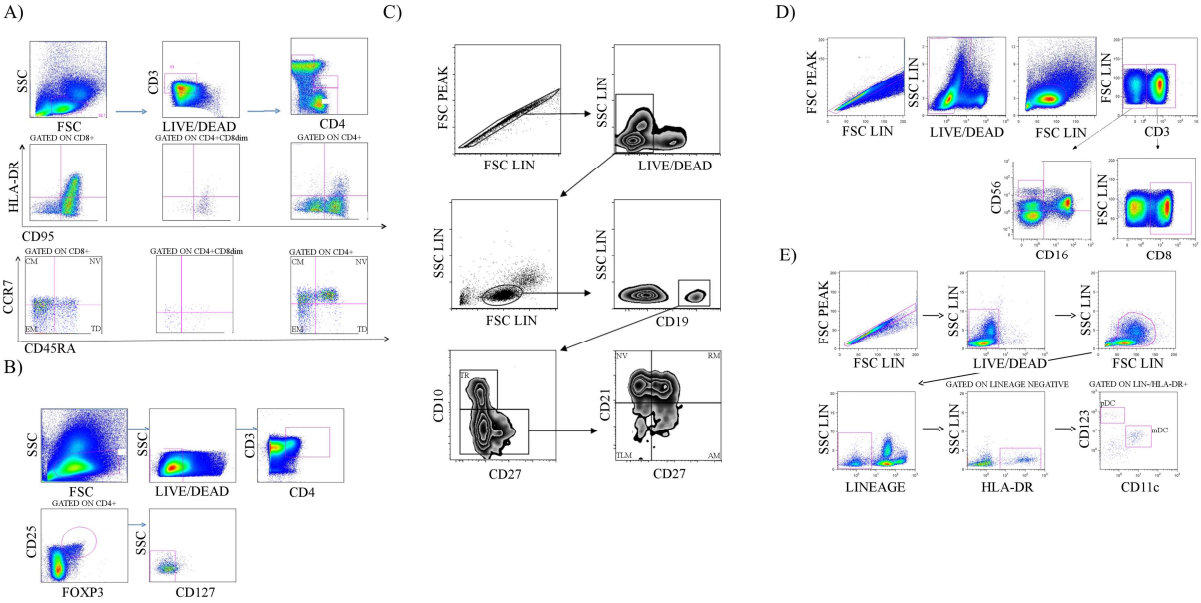

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