Immunological profile of an infant treated with integrase inhibitor from the neonatal period

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Young children on antiretroviral therapy (ART) exhibit superior immune reconstitution to adults due partly to high frequencies of naïve T cells and preserved thymic function [1]. We report on immunological investigations in a child commenced on ART during the neonatal period by selecting markers previously associated with viraemic control in adults [2–5].

In 2013, a male infant was diagnosed with presumably intrauterine-acquired HIV [6] (confirmed integrated HIV DNA 1750 copies/10^6 peripheral blood mononuclear cells [PBMCs] at age 8 days). The infant was initiated on zidovudine/lamivudine/ritonavir-boosted lopinavir at age 2 days, and raltegravir (RAL) was added at age 5 days due to the high HIV RNA load (>10,000,000 copies/mL). His HIV DNA at that time was 1750 copies/10^6 PBMCs [6]. The infant's viral load dropped to <200 copies/mL after 6 months and to <50 copies/mL after 12 months of ART. At 18 months, while on RAL, lamivudine and abacavir, he lost maternal HIV antibodies (non-reactive fourth generation HIV-1/2 assay, Vironostika HIVAg/Ab, BioMérieux, and negative Western blot), indicating low HIV RNA viral burden [7,8]. At that time, he had protective antibody titres to hepatitis B virus, tetanus, poliomyelitis, diphtheria and pertussis vaccines and normal total immunoglobulin levels. His CD4 T lymphocyte count was 2030 cells/mm^3 (26%), CD4:CD8 ratio was 1.5, HIV RNA was <50 copies/mL and HIV DNA was 123 copies/10^6 PBMCs.

We investigated innate and adaptive immune markers reported to be associated with viraemic controllers including elite controllers (ECs) and post-treatment controllers (PTCs) in the Visconti cohort of adults treated during primary HIV infection and subsequently able to control viraemia after treatment interruption [9].

HLA class I and II typing did not show the HLA-B35 and HLA-B07 alleles seen in PTCs or the HLA-B27 and B57 alleles associated with ECs.

There were high frequencies (35%) of activated (HLA-DR+) natural killer (NK) cells. Inducibility was low for NKp30 but high for NKp44 (1.09 and 103.8, respectively and reported as fold change in the proportion of activated NK cells expressing NKp30/NKp44 after 4 days of *in vitro* activation with rh-IL2). NK cells are activated in adults with untreated HIV [10], and can be persistent following successful ART and CD4 immune reconstitution [11,12]. Persistent NK cell activation is also reported in EC adults [5]. The reasons are unclear but could be due to virus replication or production in secondary lymphoid tissues with insufficient antiretroviral drug concentrations [13].

We analysed the proliferative activity and cytokine profile (percentage of antigen-specific IL-2 and IFN-γ producing cells) by flow cytometry of CD4 and CD8 T lymphocytes after *in vitro* stimulation with recall antigens: tetanus toxoid (TT), *Candida albicans* (Ca), influenza virus (FLU) and deca-pools of 20mer overlapping peptides of HIV retrotranscriptase (RT). There was a positive response to recall stimuli in both T cell subsets, and a weak response to some peptide deca-pools of RT by CD8 and CD4 T lymphocytes. A high frequency of CD4+ IL-2+ cells specific for RT peptides was observed in comparison with those for recall antigens. There were also low frequencies of CD4+ IFN-γ+ cells except for Ca antigens. There were higher frequencies of CD8+ IL-2+ cells against pool 1 of RT peptides compared to those observed for recall antigens and other RT peptide pools, and low levels of CD8+ IFN-γ+ cells, except for Ca antigens. These data indicate maintenance of circulating memory CD4+ and CD8+ T cells specific for HIV antigens [5,8,14,15].

The frequencies of CD45RA+CCR7+ naïve cells were high on both CD4+ and CD8+ T lymphocytes (58% and 43%). The distribution of antigen-experienced T cells showed: (a) higher percentage of CD45RA-CCR7- effector memory (EM) (40%) cells compared to CD45RA-CCR7+ central memory (CM) (5%) and CD45RA+CCR7- terminally effector memory (TEM) (11%) cells on CD8 T cells; (b) similar percentages of CM, EM, TEM populations on CD4+ (16%, 14% and 11%, respectively) and CD3+CD8- T cell subsets (Figure 1a).

Eomes transcription factor expression was evaluated by flow cytometry on total CD4+ and CD8+ T lymphocytes: 25% of Eomes+ CD8+ T cells (32% T-bet^{low}Eomes$^+$, 68% T-bet^{high}Eomes$^+$ respectively); 64% of Eomes+ cells were CD28+ and 44% were CD127+. The data on CD4+ T cells showed: 8% Eomes+ (34% T-bet^{low}Eomes$^+$ and 66% T-bet^{high}Eomes$^+$); 67% of Eomes+ cells were CD28+ and 39% were CD127+ (Figure 1b). We observed PD-1 expression on 4.4% CD4+ and 8.1% CD8+ T cells. Adults with HIV were reported to have a subpopulation of HIV-restricted CD8$^+$T-bet^{lo}Eomes^{hi} exhausted T lymphocytes [2]. Our case had a profile similar to healthy individuals with a high proportion of Eomes^{hi} cells including Eomes+ cells expressing CD127+ and CD28+ [4].

The analysis of regulatory T cells (Treg) revealed a normal frequency of CD25^{high}FoxP3$^+$ cells on CD4+ Treg population (5%), and no CD8+Treg circulating cells, consistently with an undetectable HIV RNA load [16]. The frequency of resting or naïve Treg (3% CD45RA$^+$FoxP3^{low}) was higher than those of secreting T cells (1.2% CD45RA$^-$FoxP3^{low}) and activated Treg (0.7% CD45RA$^-$FoxP3^{high}, Figure 1c).

We therefore concluded that early and suppressive ART initiated in infancy in this child led to immune preservation of CD4+ and

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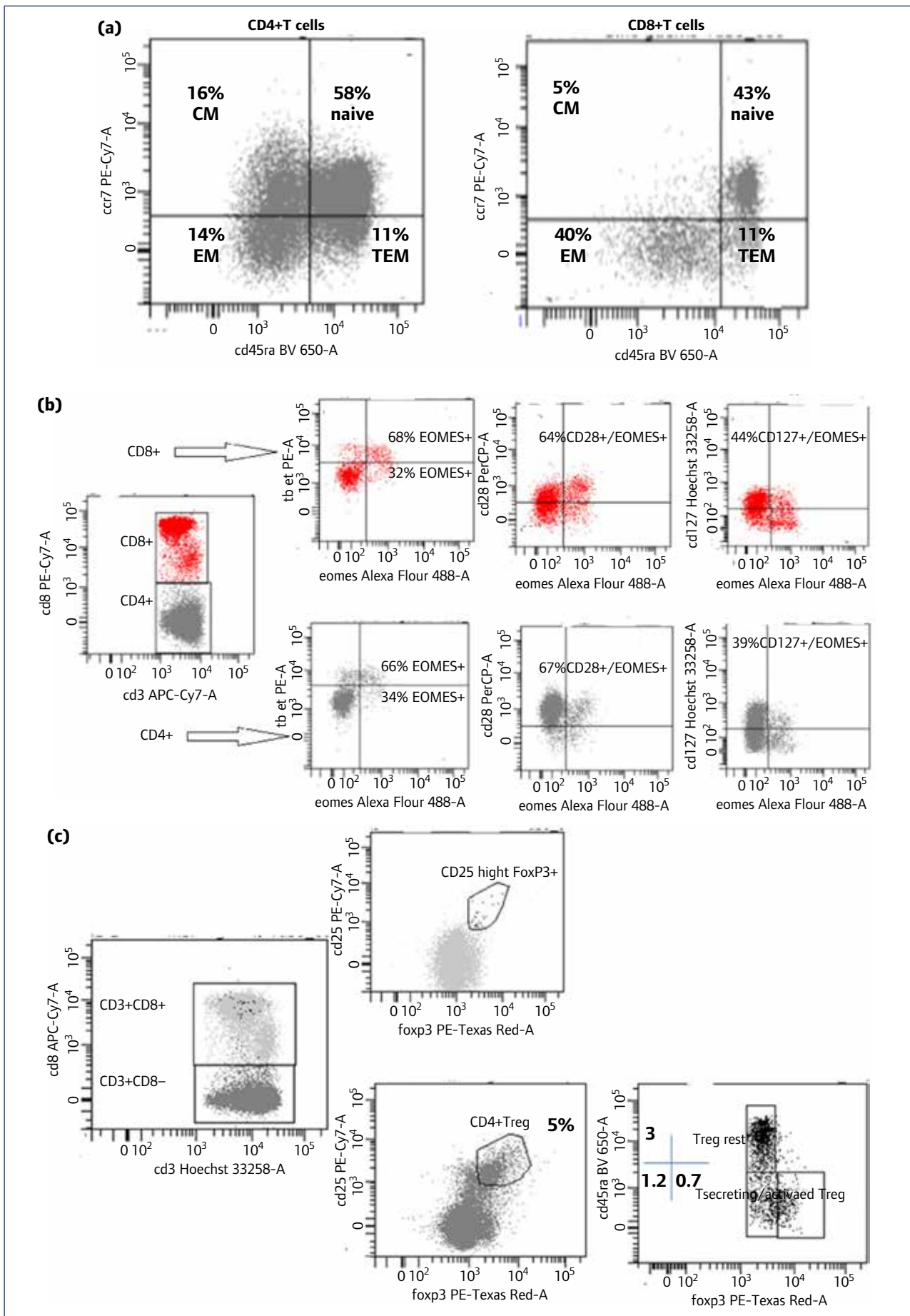Email: paolatatarelli@gmail.com

Figure 1. Immunological status at age 18 months in a child who initiated antiretroviral therapy from age 2 days. (a) Evaluation of naïve and central memory (CM), effector memory (EM) and terminal effector memory (TEM) populations on CD4+ and CD8+ T cell subsets. (b) Expression of Eomes, T-bet transcription factors and CD28, CD127 markers on circulating CD8+ and CD4+ T lymphocytes. (c) Analysis of circulating CD4+ Treg population. Treg: regulatory T cells.

CD8+ T cells with high frequencies of naïve cells and low expression of activation and exhaustion markers. HIV DNA continued to be detected. The RAL used in this case may have contributed to the rapid plasma viral load decline and immunological recovery. This case is an example of an early-treated child who might benefit from future immunotherapies to boost HIV-specific immunity.

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Declaration of interests

The authors declare no conflict of interest.

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