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Concise Communication

HIV mediated immune aging in young adults infected perinatally or during childhood

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Running head: Immune aging in young HIV-1 infected adults

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Abbreviations

APC, allophycocyanin; FITC, fluorescein isothiocyanate; PE, phycoerythrin; IFN, Interferon; TNF, Tumor necrosis factor; CMV, cytomegalovirus

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Abstract

Background: HIV-infected patients progressing towards disease present a premature immune aging profile, characterized by the exhaustion of lymphopoiesis. The development of these anomalies may be prevented in young HIV-infected patients owing to their robust immune resources and lymphocyte regeneration capacities.

Methods: An immunomonitoring substudy was designed for young adults aged between 18 and 25 years, living with HIV since childhood included in the national ANRS Co19 COVERTE Cohort. We compared markers associated with immune aging, including the frequency of circulating hematopoietic progenitors and the phenotype of lymphocyte populations, with those of patients infected with HIV in adulthood.

Results: HIV-infected young adults displayed decreasing numbers of $CD34^+$ hematopoietic progenitors and mature lymphocytes, indicative of general lymphopenia and reminiscent of the alterations found in patients infected in adulthood or uninfected elderly people. This highlights the strong impact of HIV on the immune system despite patient young age at infection. Immune aging related alterations were particularly obvious in young patients who presented high viral loads.

Conclusions: HIV-infected young adults can present increased markers of immune activation and senescence, related to uncontrolled viral replication. This highlights the issue of non-compliance to antiretroviral therapy in patients at a young age, resulting in loss of viral control, premature immunosenescence, and potentially irreversible damage of their lymphopoietic system.

Keywords

Immunosenescence, lymphocytes, antiretroviral therapy, age

INTRODUCTION

Massive depletion of $CD4^+$ T-cells is the hallmark of HIV disease progression. However, this decline is not the only immune alteration occurring during the course of HIV-1 infection. HIV disease progression is also associated with a marked decrease in primary immune resources (encompassing $CD34^+$ hematopoietic progenitors[1, 2] and naïve T-cells[3, 4]), the failure to maintain adequate counts of all types of lymphocytes (including B cells[5, 6] and NK cells[7, 8]) and the accumulation of highly differentiated T-cells (i.e. $CD57^+$) often considered as cells approaching cellular senescence[9, 10]. These alterations highlight an exhaustion of lymphopoiesis, i.e. the capacity to produce or renew the lymphocyte compartment, and reflect a premature process of immune aging[11]. Systemic immune activation has emerged as a major driver of this altered lymphopoiesis, which can therefore be limited owing to antiretroviral therapy[1]. Nonetheless, HIV disease progression despite viral suppression, naturally (i.e. in HIV controllers) or due to antiretroviral treatment (i.e. also referred as to immunological failure), was associated with profound and persistent exhaustion of lymphopoiesis[1]. This highlights further the importance of preserving lymphopoietic capacity in HIV infected patients, which is a primary determinant for the maintenance of adequate $CD4^+$ T-cell levels during HIV infection.

In this context, young adults infected with HIV at birth or during childhood (YAHIC) represent a particularly interesting group to study. The development of immune aging-like anomalies related to HIV infection may possibly be limited in these patients because of the large immune resources and robust lymphocyte regeneration capacity associated with young age[12]. We therefore decided to assess the impact of HIV infection on the immune system of YAHIC, in comparison with patients infected later during adulthood, and age-matched uninfected donors. For this purpose, we analyzed HIV replication, HIV-DNA levels and blood frequencies of $CD34^+$ hematopoietic progenitor cells, T-, B-, NK lymphocytes, as well as naïve or highly differentiated $CD4^+$ or $CD8^+$ T-cells.

Immunological studies in these young HIV-infected patients provide important insights into the consequences of the infection at early stages of life onwards, which is important for the care of this vulnerable population.

MATERIALS AND METHODS

Study subjects and samples

Blood samples of young adults living with HIV since childhood (n=134) were obtained from the ANRS COVERTE-CO19, a national prospective cohort of adults diagnosed with HIV infection before 13 years of age, enrolled in 88 sites since 2010, annually followed, with multiple clinical and physiopathological objectives[13, 14]. Patients were infected perinatally or during childhood, and were diagnosed with HIV on average three years after birth (with a 4 year SD) and were aged 18 to 25 years at study inclusion. Frozen PBMC samples (n=84) were collected for in depth immunological evaluation at inclusion. For comparison, blood samples of older adults aged 25 to 55 years (n=61) or aged 65 to 81 years (n=28) infected with HIV-1 during adulthood were obtained from patients attending HIV clinics in France and treated with antiretroviral therapy for more than 3 years. Blood samples were also obtained from age-matched uninfected individuals, including young (n=24), middle aged (n=34), and elderly (n=13) adults. Elderly individuals with malignancies, acute diseases, or advanced stages of severe chronic diseases, such as chronic inflammatory disease, atherosclerotic disease, congestive heart failure, poorly controlled diabetes mellitus, renal or hepatic disease, or chronic obstructive pulmonary disease, as well as individuals under immunosuppressive therapy were excluded from the study. All participants gave their written informed consent. The study received the approval of the local institutional ethics committees (at Cochin and Pitié Salpêtrière Hospitals, Paris). Peripheral blood mononuclear cells (PBMCs) were isolated over a Lymphoprep gradient and cryopreserved until use. High-sensitivity C-reactive protein (hsCRP) was

measured from frozen serum samples by immunonephelometry on an IMMAGE analyzer (Beckman-Coulter, Brea, California, USA).

Quantification of plasma HIV-RNA and cell-associated HIV-DNA

HIV-RNA was quantified in blood plasma with the Cobas Ampliprep Cobas Taqman assay v2 (Roche, France). Total DNA was extracted from thawed PBMCs by using the QIAamp DNA microkit (Qiagen, Courtaboeuf, France) and HIV-DNA was quantified with an ultrasensitive real-time PCR method (Generic HIV-DNA assay, Biocentric, Bandol France) with a detection limit of five copies per PCR[15]. To standardize results, the total DNA in extracts was quantified using fluorescence readings at 260 nm (Nanodrop, Labtech,Ringmer, UK). Each entire DNA extract was tested in two replicates. Results were reported as the number of HIV-DNA copies per 10^6 PBMCs. DNA extracts were stored at -20°C.

Flow cytometry

Directly conjugated antibodies were obtained from the following vendors: BD Biosciences (San Jose, CA): CD4 (APC-cyanin7), CCR7 (PE-Cy7), CD38 (APC), CD21 (PE-Cy7), CD34 (FITC and PE), CD56 (FITC), CD45RA (V450), Ki67 (FITC), and lineage cocktail (CD3, CD14, CD16, CD19, CD20, CD56 / FITC); Beckman Coulter: CD45 (ECD), CD57 (FITC); Caltag (Burlingame, CA): CD8 (Alexa405); Dako (Glostrup, Denmark): CD3 (Cascade Yellow); BioLegend (San Diego, CA): CD27 (AlexaFluor700). Stainings were performed on thawed PBMCs by addition of the live/dead Aqua marker (Thermofisher, Illkirch, France) and the respective antibodies for 15 min at room temperature. After incubation, stained PBMCs were washed in PBS and then fixed with 2% paraformaldehyde. CD4/CD8 ratios were calculated based on the absolute counts of $CD4^+$ and $CD8^+$ T-cells obtained from the routine clinical assessment of patients. Cells were analyzed on an

LSR Fortessa flow cytometer (Becton Dickinson) and data analyzed using FlowJo v10.6 (Tree Star, Inc).

Statistical analyses

Statistical analysis was performed using GraphPad prism software. Groups were compared using the non-parametric Kruskal Wallis or Mann-Whitney tests. Spearman's rank test was used to determine correlations. *P* values above 0.05 were considered not significant.

RESULTS

Altered lymphopoiesis and premature immune aging in YAHIC

Young adults HIV infected during childhood (YAHIC) were aged between 18 and 25 years and all received antiretroviral therapy. For the analyses, patients were divided according to disease progression stage (i.e. $CD4^+$ T-cell count >500, <500-200> and <200 cells/μl) and compared with equivalent groups of older HIV-infected patients, including middle aged (between 25 and 55 years - mean of 44 y) and old (above 65 years - mean of 70 y) individuals, as well as age-matched HIV-uninfected adult controls. All HIV-infected patient age groups presented with equivalent time since diagnosis (mean of 17 y) and time since treatment initiation (mean of 11 y).

Similar to patients infected with HIV during adulthood, YAHIC displayed decreasing CD4/CD8 ratio, numbers of progenitor cells ($CD34^+CD45^+Lin^-$), as well as B ($CD21^+$) and NK ($CD56^+CD3^-$) lymphocytes with lowering $CD4^+$ T-cell counts (Figure 1A). This suggests a disruption of lymphopoiesis with disease progression in YAHIC. Decreasing naive ($CD45RA^+CD27^+CCR7^+CD57^-$) $CD4^+$ or $CD8^+$ T-cell numbers characterized also YAHIC as they progressed towards disease, similar to observations in older HIV-infected patients (Figure 1B). In

parallel with reduced naive T-cell counts, frequencies of highly differentiated $CD57^+$ effector/memory $CD4^+$ or $CD8^+$ T-cells, often considered as a marker of immune senescence, were significantly increased, compared to age-matched healthy donors. This parameter reflects the impact on the T-cell compartment of persistent viruses, like HIV, but also CMV, which YAHIC were frequently (i.e. 80%) co-infected with, and which is a major driver of the accumulation of these cells. Altogether, these data support the development of a premature immune aging profile associated with HIV disease progression in YAHIC despite their young age and *a priori* robust immune resources.

Persistence of strong viral replication in YAHIC

The establishment of a premature immune aging profile in HIV-infected patients has been linked to elevated chronic systemic activation, which results in particular from HIV replication. Unexpectedly, a high proportion (33%) of YAHIC presented with viral replication. These individuals included in particular patients progressing towards disease (i.e. with $CD4^+$ T-cell counts below 200 cells/µl), in whom plasma viral load was always detectable (Figure 2A). This was in clear contrast with older HIV-infected patients, who had no detectable viral replication (data not shown). In YAHIC, viral replication was inversely correlated with the CD4/CD8 ratio (Figure 2B), low values of which are classically associated with immune activation and declining immunity[16]. We found associations between viral replication and the frequency of $Ki67^+CD38^+$ memory $CD8^+$ T-cells (Figure 2C), and serum levels of hsCRP (Figure 2D), which are established markers of immune activation and inflammation in HIV-infected patients. High plasma viral load was also correlated with the frequency of $CD57^+$ $CD8^+$ T-cells (Figure 2E). Last, viral replication correlated with cell-associated HIV-DNA levels in YAHIC (Figure 2F). Altogether, these data suggest that uncontrolled viral replication in YAHIC is a main cause of disease progression, elevated immune activation, resulting in a premature immune aging profile, as well as seeding of the HIV reservoir.

DISCUSSION

Young adults infected with HIV at birth or during early childhood represent a small yet very particular group of the HIV infected population, due to their young age but long experience of HIV. We report here that YAHIC display a general exhaustion of lymphopoiesis with decreasing counts of $CD34^+$ hematopoietic progenitors, naïve $CD4^+$ and $CD8^+$ T-cells, B-lymphocytes and NK cells, and increased frequency of memory senescent T-cells as they progress towards disease. These alterations are similar to those observed in older HIV-infected patients and reminiscent of a premature immune aging process despite their young age. This highlights the potential strong impact of HIV infection on the immune system despite *a priori* robust immune resources. Immune aging-like alterations were most obvious in YAHIC with lowest $CD4^+$ T-cell counts, who also had high viral loads and chronic activation markers, in contrast to older treated HIV-infected patients. While the establishment of a premature immune aging profile in HIV-infected adults with good viral suppression is likely due to long term exhaustion of primary immune resources[1], our present data suggest that its primary cause in YAHIC is uncontrolled viral load and related immune activation.

This situation in YAHIC underlines the problem of non-compliance to antiretroviral therapy in this age group. In young HIV-infected patients prescribed anti-HIV drugs, this represents a rather frequent and particularly sensitive issue[13]. Lack of adherence to antiretroviral treatment not only results in loss of viral control and increased risk of HIV transmission, but our data indicate that it can also promote HIV reservoir seeding and the establishment of premature immunosenescence in YAHIC, therefore potentially leading to irreversible damage to the lymphopoietic system. It is thus very important to enforce treatment compliance in teenagers and young adults, in order to prevent disease progression and preserve their immune system, which may otherwise prematurely resemble

the one of elderly people. This is essential not to jeopardize hopes for remission in these young patients. These should serve as strong arguments to convince HIV-infected teenagers and young adults to adhere to antiretroviral therapy recommendations.

AUTHOR CONTRIBUTION

Solène FASTENACKELS performed the research and analyzed data.
Delphine SAUCE performed the research and analyzed data.
Corinne VIGOUROUX designed the research and analyzed data.
Véronique AVETTAND-FÈNOËL performed the research and analyzed data.
Jean-Philippe BASTARD performed the research and analyzed data.
Soraya FELLAHI performed the research and analyzed data.
Laura NAILLER analyzed clinical data and recruited patients.
Elisa AREZES analyzed clinical data and recruited patients.
Christine ROUZIOUX designed the research and wrote the paper.
Josiane WARSZAWSKI designed the research and wrote the paper.
Jean Paul VIARD designed the research and wrote the paper.
Victor APPAY designed the research, analyzed data and wrote the paper.

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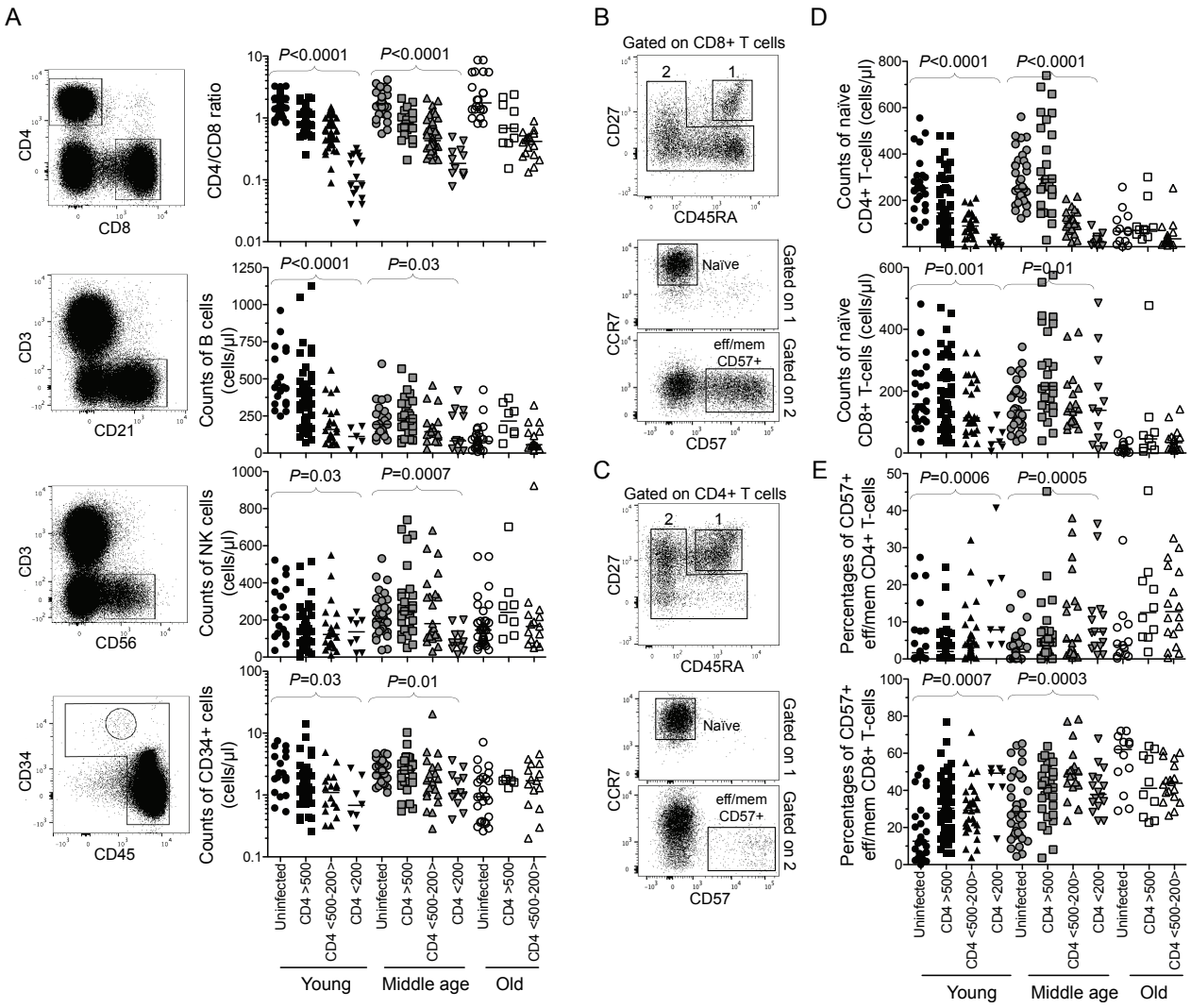
FIGURES AND LEGENDS

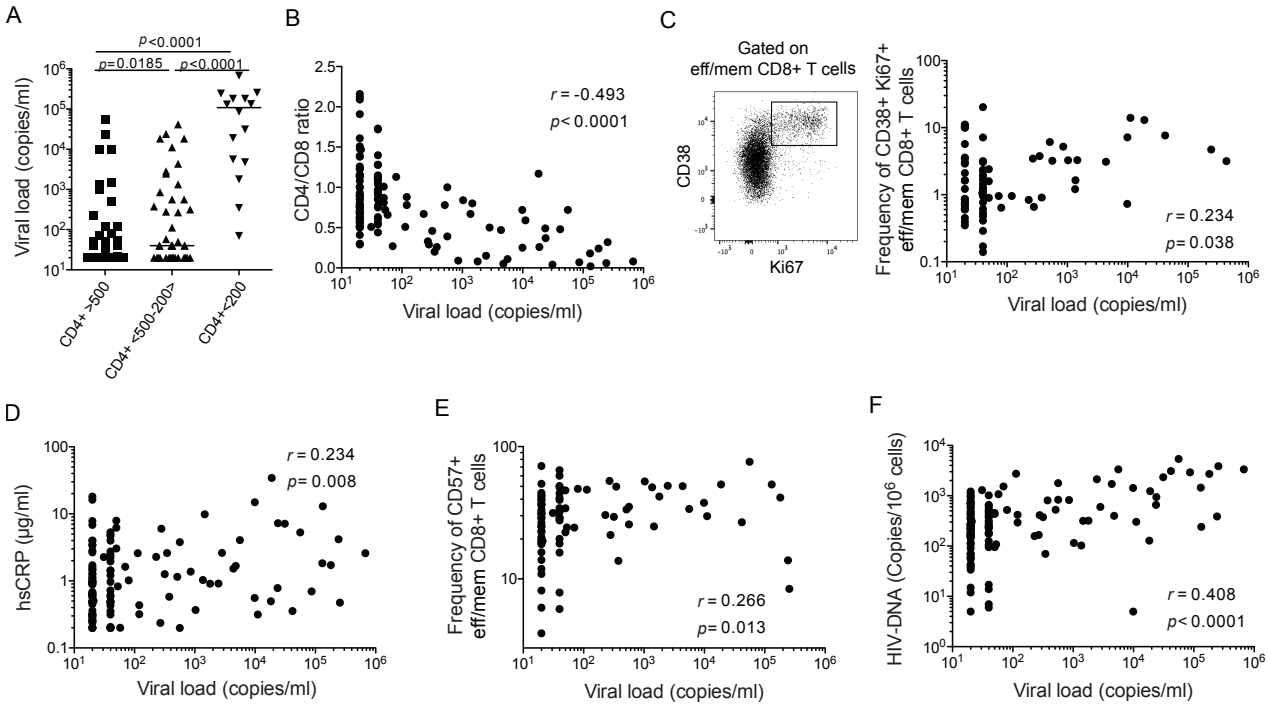
Figure 1. Immune parameters of YAHIC and older HIV infected patients grouped according to CD4 counts



Immunophenotyping of HIV-1-infected patients grouped according to age (young or YAHIC, 18 to 25 years old (black symbols); middle aged, 25 to 55 years old (grey symbols), and old, 65 to 81 years old (white symbols)) and $CD4^+$ T-cell counts: above 500 (square symbols), between 200 and 500 (upward triangle symbols), or below 200 $CD4^+$ T-cells/µl (downward triangle symbols), and their respective age matched HIV uninfected controls (circle symbols). **(A)** CD4/CD8 ratio, absolute counts of B cells, NK cells, $CD34^+$ cells are shown in all groups. Left panels show

representative flow cytometry stainings. **(B, C)** Representative flow cytometry stainings and gatings to identify naïve (i.e. $CD45RA^+$ $CCR7^+$ $CD27^+$ $CD57^-$) or $CD57^+$ effector/memory among total $CD8^+$ (B) or $CD4^+$ (C) T lymphocytes. **(D)** Absolute counts of naïve $CD8^+$ and $CD4^+$ T-cells. **(E)** Percentages of $CD57^+$ effector/memory $CD4^+$ and $CD8^+$ T-cells. Bars indicate the median. Statistical differences were calculated using the non-parametric Kruskal Wallis test, which compares together and ranks the four groups of young or middle aged subjects.

Figure 2. Relationship between viral replication, progression, activation and reservoir size in YAHIC



(A) Viral load in YAHIC grouped according to $CD4^+$ T-cell counts. The non-parametric Mann-Whitney test was used to compare groups with one another. Correlations between viral load and **(B)** CD4/CD8 ratio, **(C)** frequency of $Ki67^+CD38^+$ memory $CD8^+$ T-cells (the left panel shows a representative flow cytometry staining), **(D)** serum hsCRP levels, **(E)** frequency of $CD57^+$ memory $CD8^+$ T-cells, **(F)** cell-associated HIV-DNA in YAHIC. The Spearman's rank test was used to determine correlations.