Old age and anti-cytomegalovirus immunity are associated with altered T-cell reconstitution in HIV-1-infected patients

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Objective and design: Increasing evidence supports a parallel between HIV-1 infection and immune aging, which is particularly apparent with common changes in naive versus memory T-cell proportions. Here, we aimed at refining the value of common T-cell-associated markers of immunosenescence during HIV disease progression or aging, and at exploring further the impact in this context of old age as well as cytomegalovirus (CMV) co-infection, which is predominant in HIV-1-infected individuals.

Methods: Frequencies of naive or $CD57^+$ memory T cells as well as the magnitude of CMV-pp65 T cells were measured in HIV-1-infected patients grouped according to disease progression status, treatment and age.

Results: Our results indicate that the decline in naive T-cell levels rather than the accumulation of $CD57^+$ senescent T cells identifies best the premature development of an immunosenescence phenotype with HIV disease progression. Moreover, advanced age or mounting of strong CMV-specific responses impact independently on $CD4^+$ T-cell counts and recovery with antiretroviral therapy.

Conclusions: The present findings indicate that HIV-1 infection amplifies the effect of age on naive T-cell levels, and highlight the constraint on the capacity of treated patients to reconstitute their $CD4^+$ T-cell compartment due to age and CMV co-infection.

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Introduction

The parallel between HIV infection and aging is raising increasing concerns. Collective evidence shows that HIV-1-infected patients suffer from a number of physiological alterations and comorbidities (i.e. atherosclerosis, malignancies, osteoporosis, cognitive impairment and frailty) that are usually observed during the human aging process

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[1]. The development of these clinical manifestations is thought to be the consequence of elevated immune activation and inflammation, hallmarks of HIV-1 pathogenesis [2]. HIV-1-infected individuals present also a number of immunological alterations that are reminiscent of old age [3]. These observations concern in particular decreased percentages of naive T cells, together with increased percentages of terminally differentiated ($CD28^-$) T cells with shortened telomeres [4–6]. Whereas elevated activation due to HIV and co-infections may drive the differentiation of memory T cells towards replicative senescence and their accumulation [7], reduced naive T-cell levels are likely to reflect the combining effects of reduced T-cell renewal capacity [8] together with the mobilization of naive T cells towards the memory compartment upon activation.

Overall, this supports the hypothesis that accelerated immune aging may occur during HIV-1 infection [9]. However, this is based on the comparison of independent studies performed in elderly donors or HIV-infected patients, and the association between the occurrence of markers related to immune aging and HIV disease progression (in contrast to HIV-1 infection *per se*) has not been addressed explicitly yet. Moreover, cytomegalovirus (CMV) co-infection, whose role in the development of immunosenescence has become more obvious in recent years [10], and which is highly prevalent in HIV^+ patients (between 75 and 90%) [11–13], may participate to shaping the parallel between HIV infection and aging. This remains to be determined. Our aim here is to provide a refined analysis of parameters commonly associated with immune aging in HIV-1-infected patients grouped according to progression state (i.e. the $CD4^+$ T-cell count), antiretroviral treatment and age. In addition to naive T cells, we focused our analysis on $CD57^+$ memory T cells. Although increased proportions of highly differentiated $CD28^-$ T cells have been considered as an established marker of immunosenenescence [5,14,15], the expression of CD57 on memory T cells has emerged as a refined mean to identify cells approaching senescence (i.e. with shortened telomeres and decreased proliferative capacity) [7,16]. The respective influence of biological age and CMV co-infection was studied in this context of $CD4^+$ T-cell reconstitution upon initiation of antiretroviral therapy (ART).

Methods

Study patients and samples

Blood samples were obtained from treatment-naive patients (aged 25–60 years) chronically infected with HIV-1 (positive for p24 ELISA and western blot), and HIV-1-infected patients (aged 25–81 years) treated with ART for more than 3 years, attending the Infectious Diseases Department of the Hôpital Pitié Salpêtrière (Paris, France). Patients were divided into distinct groups according to $CD4^+$ T-cell counts or age (Table 1). HIV elite controllers (i.e. with plasma HIV RNA levels below the level of detection by using conventional assays, in the absence of antiviral therapy) either nonprogressing (stable $CD4^+$ T-cell count) ($n = 12$) or progressing (decreasing $CD4^+$ T-cell count) ($n = 10$) were recruited from established cohorts in San Francisco (SCOPE) and Paris (ANRS CO18). For comparison, blood samples were obtained from young (18–24 years old), middle-aged (25–55 years old) or elderly (75–96 years old) healthy 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 was approved by the local institutional ethics committee (i.e. Comité de Protection des Personnes of the Pitié Salpétrière Hospital, Paris). Mononuclear cells were isolated over a Lymphoprep gradient and cryopreserved until use.

Flow cytometry

Directly conjugated antibodies were obtained from the following vendors: BD Biosciences (San Jose, California, USA): CD4 (APC-cyanin7), CCR7 (PE-Cy7), CD45RA (V450), IFNγ (Alexa700), and TNFα (PE-Cy7); Beckman Coulter: CD57 (FITC); Caltag (Burlingame, California, USA): CD8 (Alexa405); Dako (Glostrup, Denmark): CD3 (Cascade Yellow); BioLegend (San Diego, California, USA): CD27 (AlexaFluor700). Overlapping peptides covering the entire pp65 protein were kindly provided by Dr Daniel Olive (Centre Paoli Calmettes, Marseille, France). Cell surface marker staining was performed by addition of the respective antibodies for 15 min at room temperature. After incubation, cells were washed in PBS and then fixed with 2% paraformaldehyde. For intracellular cytokine staining, peripheral blood mononuclear cells were incubated in the presence of pp65 overlapping peptides (5 μmol/l) for 1 h at 37°C in a 5% CO_2 incubator, followed by an additional 5 h in the presence of the secretion inhibitors monensin (2.5 μg/ml; Sigma–Aldrich) and brefeldin A (5 μg/ml; Sigma–Aldrich). Negative controls were obtained in absence of peptide. BD Cytofix/Cytoperm was used for permeabilization of the cells prior to staining for intracellular IFN-γ and TNF-α cytokines. Cells were analyzed on an LSR2 flow cytometer (Becton Dickinson) with appropriate isotype controls and colour compensation and the data using FlowJo v8.2 (Tree Star, Inc) and DIVA softwares.

Statistical analysis

Statistical analysis was performed using GraphPad prism software. Groups were compared using the nonparametric Kruskal–Wallis or Mann–Whitney tests. Spearman's rank test was used to determine correlations.

Table 1. Donor characteristics.

	Groups	Age	Sex (% of male)	CD4+ T-cell count (cells/μl)	Viral load (copies/ml)	Years since diagnosis	Years of treatment	CD4+ T-cell nadir (cells/μl)
Controls	Young ($n=21$)	**21.93** (18.62–25.75)	41%	937 (392–1494)	n.a.	n.a.	n.a.	n.a.
	Middle age ($n=33$)	**38.45** (28.45–54.99)	45%	831 (538–1509)	n.a.	n.a.	n.a.	n.a.
	Old ($n=34$)	**85.72** (75.4–96.92)	33%	500 (149–992)	n.a.	n.a.	n.a.	n.a.
HIV+ (no Tx)	H ($n=35$)	38.91 (21.8–56.4)	86%	**663** (514–1084)	18791 (40–2.e+6)	1.42 (0.01–20.9)	0	468 (182–973)
	I ($n=44$)	39.54 (24.38–54.98)	72%	**348.5** (211–495)	20745 (40–260258)	3.5 (0–25.68)	0	314.5 (166–552)
	L ($n=15$)	40.28 (23.68–47.48)	66%	**98** (26–198)	115639 (633–2.83e+6)	21.06[a] (9.87–24.07)	0	98 (23–198)
	Cp ($n=10$)	47.50 (43.00–54.00)	80%	**270.5** (124–337)	40 (40–75)	15.5 (11–21)	0	183[b] (6–300)
	Cnp ($n=12$)	47.50 (43.00–53.00)	83%	**980.5** (499–2604)	75 (40–318)	5.5 (1–23)	0	590[b] (391–1900)
HIV+ (Tx > 3y)	Mx ($n=38$)	**44.48** (33.16–55.72)	76%	440 (216–1224)	40 (40–216)	15.55 (3.24–21.9)	11.38 (3.08–18.96)	97.5 (3–218)
	Ox ($n=21$)	**68.95** (65.17–81.19)	85%	421 (179–1310)	40 (40–788)	15.06 (7.01–26.34)	12.03 (5.99–19.88)	97 (8–450)

The median and range for age (years), sex (% of male), $CD4^+$ T-cell count (cells/μl), viral load (copies/ml), time since diagnosis, duration of treatment and $CD4^+$ T-cell nadir (cells/μl) are indicated for three groups of individuals: healthy controls (divided according to age), untreated HIV-1-infected patients (age-matched but subdivided according to $CD4^+$ T-cell counts) and treated HIV-1-infected patients (grouped according to age).

[a]The median value for 'years since diagnosis' for the group L results from only four patients with documented date of infection.

[b]Values of '$CD4^+$ T-cell nadir' for grouped Cp and Cnp were self-reported.

Multivariate regression analyses were performed using JMP software. *P* values above 0.05 were considered not significant.

Results

Naive, rather than senescent memory, T-cell frequency characterizes best immune aging during HIV disease progression

We reasoned that an adequate approach to underline a potential parallel between aging and HIV disease progression would be to compare directly untreated HIV-1-infected patients at different disease progression stages (i.e. $CD4^+$ T-cell count) with age-matched, as well as young and elderly HIV-uninfected donors (see Table 1 for donor characteristics). On the basis of the expression of CD45RA, CCR7, CD27 and CD57, the percentages and absolute cell numbers, which may be seen as a more relevant information, of memory $CD4^+$ or $CD8^+$ T lymphocytes approaching senescence were measured in these different groups. As previously described [7,16], HIV-1-infected patients like elderly donors showed higher frequencies (both percentages and absolute counts) of $CD57^+$ memory lymphocytes, in particular within the $CD8^+$ T-cell compartment, compared to middle-aged healthy donors (Fig. 1a). Nonetheless, whereas increasing $CD57^+$ T-cell frequency (considering both percentages and absolute counts of $CD4^+$ or $CD8^+$ T cells) correlated relatively well with aging, it did only poorly with HIV disease progression: only the percentages of $CD57^+$ $CD8^+$ T cells augmented as HIV-1-infected patients progressed (Fig. 1a). The discrepancy between aging and HIV disease progression with regards to the evolution of $CD57^+$ T-cell frequency indicates that the latter may actually not be a relevant indicator of immune aging in HIV infection. In contrast, the naive T-cell frequency appeared to be a much more applicable marker of immune aging during HIV disease progression. HIV-infected patients showed indeed a gradual decline of the percentages or absolute counts of naive $CD4^+$ and $CD8^+$ T cells in relation to their progression stage, similar to healthy adults with increasing age (Fig. 1b). We also studied a very rare group of HIV-infected patients who presented evidence of disease progression (decreasing $CD4^+$ T-cell counts below 350 $CD4^+$ T cells/μl) despite persistent undetectable plasma HIV RNA levels in the absence of antiviral therapy. These elite controller progressors were characterized by lower proportions of naive T cells, but not increased levels of $CD57^+$ memory cells, compared to typical elite controller nonprogressors (Fig. 1c). Altogether, the comparison of elderly donors and untreated HIV-1-infected progressors stresses that the decline in naive T cells (rather than the enrichment in senescent memory T cells) represents a robust immunologic manifestation of the parallel between aging and HIV disease progression.

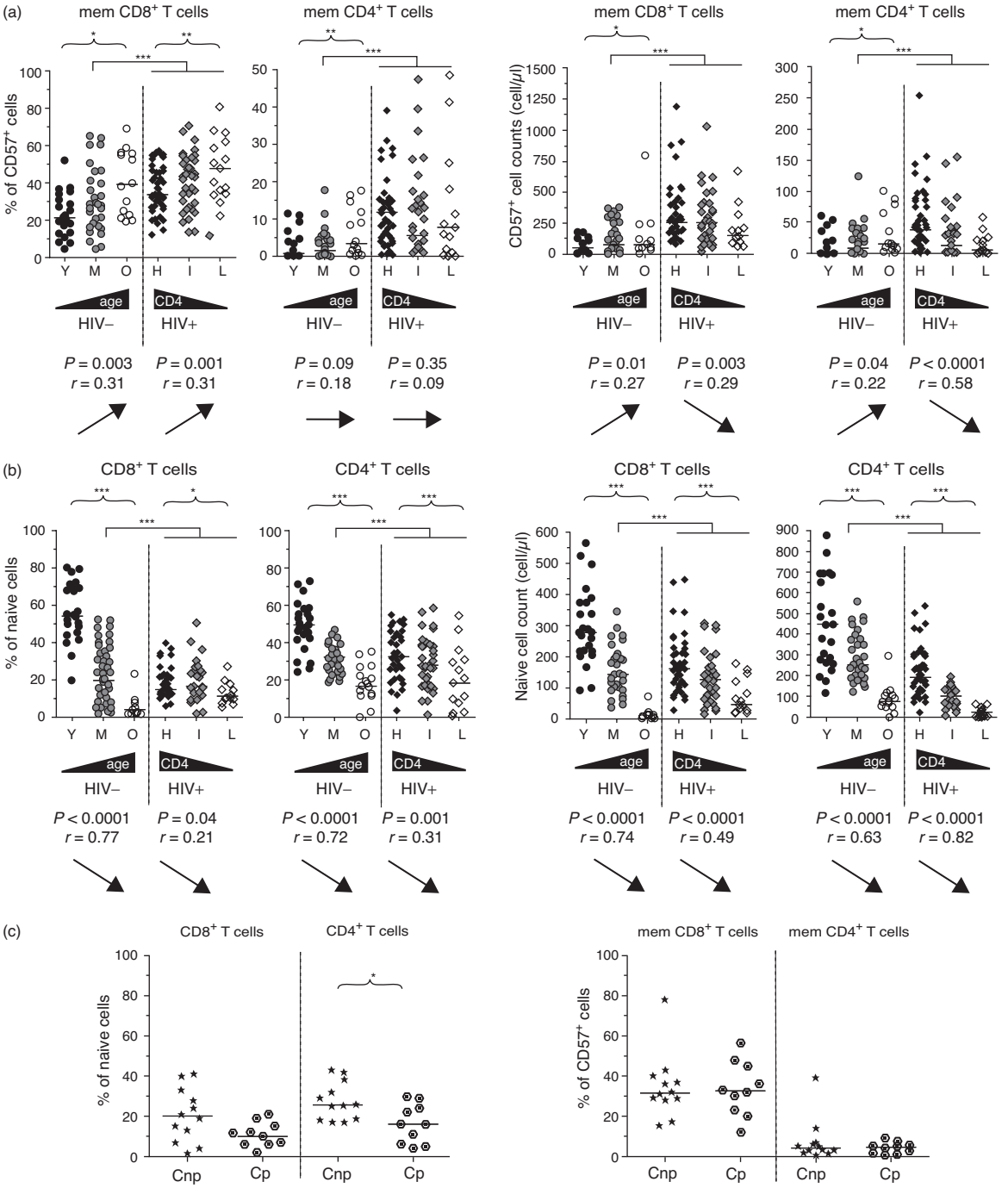

Fig. 1. Changes in CD57$^+$ or naive T-cell frequencies with aging or HIV disease progression. Percentages and absolute counts of (a) CD57$^+$ cells among CD4$^+$ and CD8$^+$ memory T lymphocytes or (b) naive cells (i.e. CD45RA$^+$ CCR7$^+$ CD27$^+$) among total CD4$^+$ and CD8$^+$ T lymphocytes in young (Y, $n=21$), middle-aged (M, $n=33$) or old (O, $n=34$) adults, and in treatment-naive HIV-1-infected patients grouped according to CD4$^+$ T-cell counts: above 500 (H, $n=45$), between 200 and 500 (I, $n=36$), or below 200 (L, $n=15$) CD4$^+$ T cells/μl. (c) Percentages of naive and CD57$^+$ memory CD4$^+$ and CD8$^+$ T cells in HIV elite controller nonprogressors (Cnp, CD4$^+$ T-cell count above 500 cells/μl, $n=12$) and progressors (Cp, CD4$^+$ T-cell count below 350 cells/μl, $n=10$). Bars indicate the median. The Mann–Whitney or Kruskall–Wallis tests were used for comparing two groups or 3$^+$ groups, respectively. *, ** and *** indicate *P* values below 0.05, 0.01 and 0.001, respectively. Below the graphs, *P* values and correlation coefficients (using Spearman's rank test) are also shown when the age or CD4$^+$ T-cell count are used as continuous parameters. Arrows indicate positive or negative correlations between T-cell subset frequency changes and aging or disease progression for healthy donors or HIV-1-infected patients, respectively.

Biased naive T-cell reconstitution in old HIV-1-infected patients receiving antiretroviral therapy

Studying the combined effect of advanced age and HIV-1 infection can be difficult due to the extreme rarity of treatment-naive HIV-1-infected patients with old age. We therefore decided to study the impact of advanced age on T-cell reconstitution upon ART. T-lymphocyte production capacity is central for the restoration of the T-cell compartment with the initiation of ART, which usually results in evident increases in naive $CD4^+$ and $CD8^+$ T-cell frequencies [17–19]. ART can thus result in a partial reversion of the immunosenescence like phenotype observed with HIV disease progression. However, earlier works showed an association between increasing age and slower reconstitution of the $CD4^+$ T-cell compartment in treated HIV-1-infected patients, thus indicating that advanced age could limit T-cell number recovery due to lower production of naive cells [20–22]. Here, we focused our study on old HIV-1-infected patients (more than 65 years old) who actually presented adequate $CD4^+$ T-cell recovery (relative to pre-ART low $CD4^+$ T-cell nadir) with treatment, equivalent to middle-aged patients (between 25 and 55 years old) (Fig. 2a and Table 1). Of note, middle-aged treated controls were selected for matching $CD4^+$ T-cell nadir and duration of treatment, which are known to affect naive $CD4^+$ T-cell level recovery [23]. Treated old HIV-1-infected patients presented reduced frequencies of both naive $CD4^+$ and $CD8^+$ T cells compared to middle-aged HIV-1-infected patients, despite equivalent total T-cell counts in the two groups (Fig. 2b). This certainly reflects the deficient production of new T cells [18], due to the involution of the thymus with age [8]; uninfected individuals beyond 60–65 years old usually have undetectable thymic activity. We found indeed strong inverse correlations between age and naive $CD4^+$ ($P < 0.0001$, $r = -0.6$) or $CD8^+$ ($P < 0.0001$, $r = -0.54$) T-cell counts in treated HIV-1-infected donors. Alike in untreated patients, the evolution of $CD57^+$ cell counts with age in treated HIV-infected patients was divergent in $CD4^+$ and $CD8^+$ T-cell compartments, supporting further that it may not be the most applicable marker of immune aging in HIV infection (Fig. 2c). The recovery of naive T cells and reversion of the HIV-associated immunosenescence phenotype with ART are thus limited by advanced age. Although this is expected, the present data show that, despite successful treatment and apparently adequate $CD4^+$ T-cell count recovery in old and middle-aged HIV-1-infected patients, T-cell reconstitution is actually not equivalent in both groups. Furthermore, we performed a multivariate regression analysis of naive $CD4^+$ and $CD8^+$ T-cell levels from treated HIV-1-infected patients or uninfected individuals to account for both age and HIV infection (Fig. 2d). It showed that both age and HIV-1 impact independently on the frequency of naive T cells. This was particularly evident with regards to the naive $CD4^+$ T-cell compartment and less with the naive $CD8^+$ T-cell compartment (for which the effect of HIV alone was observed only on percentages but not on absolute counts). Overall, this indicates that HIV amplifies the adverse effect of age on naive T-cell reduction, even in the context of ART.

Strong CMV-specific cellular immunity is associated with altered T-cell reconstitution

The disequilibrium between memory and naive cell T-cell reconstitution in old patients is likely due to homeostatic perturbation associated with their defective T-cell renewal capacity, together with the expansions of antigen-specific memory cells. In line with this possibility, we recently showed that CMV infection and particularly the establishment of strong T-cell responses against this virus were associated with a significant reduction in naive T-cell levels and $CD4^+$ T-cell counts in HIV-uninfected young adults with reduced T-cell renewal capacity (i.e. due to thymectomy early after birth) [24]. It is therefore possible that CMV co-infection may also influence T-cell reconstitution during HIV-1 infection. Since the vast majority (97.6%) of HIV-1-infected donors studied in the present study are co-infected with CMV (serology data not shown), we were not able to directly compare CMV-seropositive and CMV-seronegative HIV-1-infected donors. Instead, we measured in all donors the T-cell response specific for the CMV immunodominant pp65 protein (i.e. the magnitude of IFN-γ or TNF-α producing T cells upon stimulation with pp65 overlapping peptides; Fig. 3a), shown to be representative of the total CMV-specific response [25]. As previously described [26–28], the magnitude of both CMV-specific $CD4^+$ and $CD8^+$ T-cell responses was generally amplified with age in uninfected individuals (Fig. 3b). HIV-1-infected donors presented increased levels of CMV-specific T cells ($P = 0.0014$ and $P = 0.0009$ for $CD4^+$ and $CD8^+$ responses, respectively), which was particularly obvious for patients on ART ($P < 0.0001$), in line with previous findings [29,30]. Old treated HIV-1-infected patients displayed generally the highest magnitude of CMV-specific T cells compared to all donors ($P < 0.0001$).

We hypothesized that strong expansions of CMV-specific T cells may impact on the recovery of the T-cell pool in treated HIV-1-infected patients, similarly to the situation described in HIV-uninfected but thymectomized young adults [24]. There was no obvious relationship between the magnitude of the CMV-specific T-cell response and naive T-cell frequencies or markers of disease progression (i.e. $CD4^+$ cell counts) in co-infected donors receiving no ART (i.e. with apparent ongoing HIV replication) (data not shown). However, when concentrating on treated HIV-1-infected patients, we observed that higher CMV responders presented significantly lower $CD4^+$ T-cell counts and recovery (relative to the $CD4^+$ T cell nadir) compared to lower CMV responders ($P = 0.019$) (Fig. 3c). To define higher versus lower CMV responder groups, treated HIV-1-infected patients were ranked

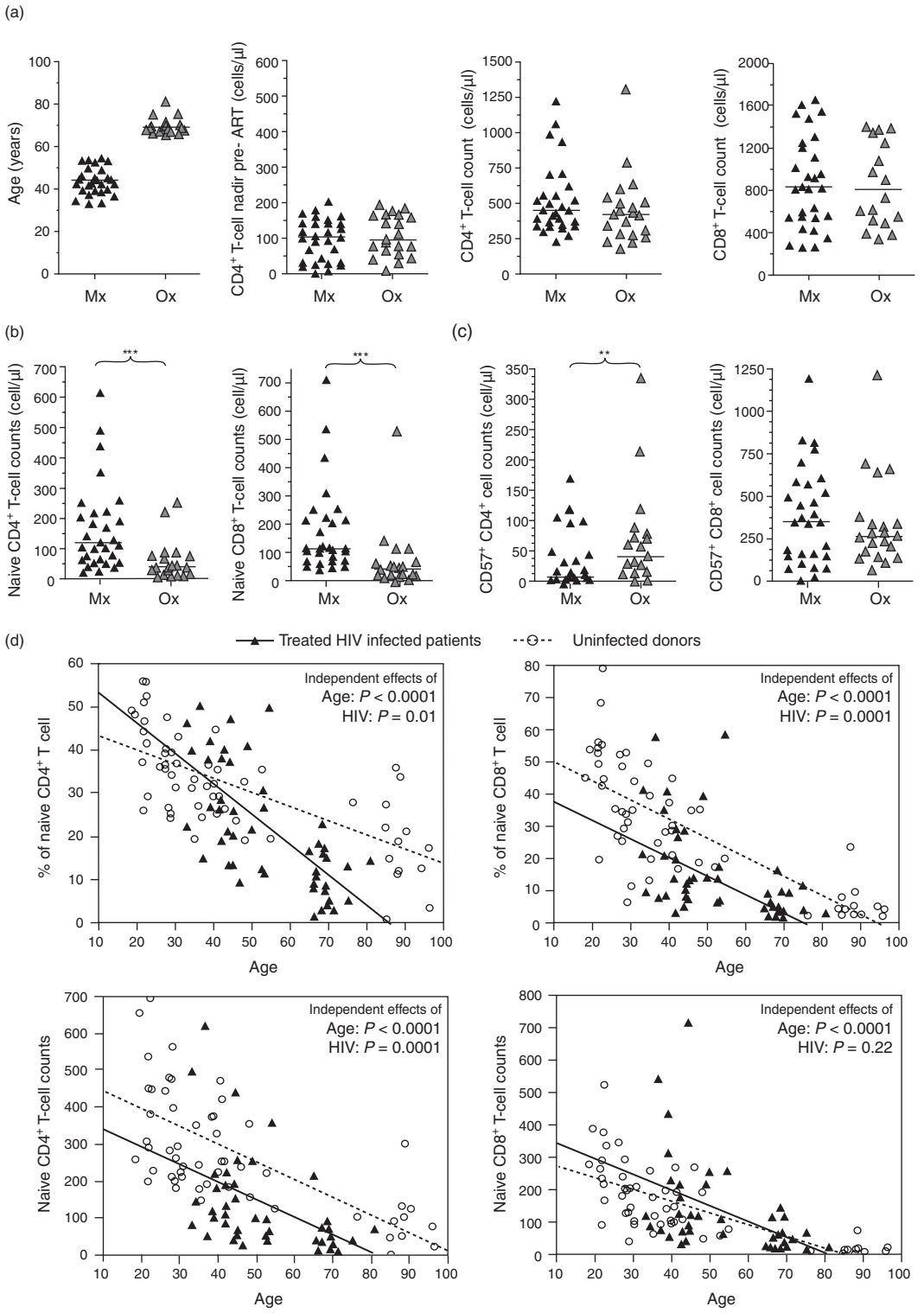

Fig. 2. Naive T-cell frequency in old HIV-1-infected patients with T-cell recovery after ART. (a) Age, $CD4^+$ T-cell nadir (pre-ART), and total $CD4^+$ or $CD8^+$ T-cell counts in old treated HIV-1-infected patients (>65 years old – Ox, $n = 21$) and matching middle-aged, HIV-1-infected patients (Mx, $n = 38$). (b) Absolute counts of naive (i.e. $CD45RA^+$ $CCR7^+$ $CD27^+$) $CD4^+$ or $CD8^+$ T cells in old versus middle-aged treated HIV-1-infected patients. (c) Absolute counts of $CD57^+$ cells among $CD4^+$ and $CD8^+$ memory T lymphocytes in old versus middle-aged treated HIV-1-infected patients. Bars indicate the median. The Mann–Whitney test was used for group comparison. ** and *** indicate P values below 0.01 and 0.001, respectively. (d) Inverse correlations between naive $CD4^+$ and $CD8^+$ T-cell levels (counts or percentages) and age in treated HIV-1-infected patients (full triangle and line) or uninfected donors (open circles and dashed line). P values were calculated using multivariate regression analyses and refer to the probability of age or HIV-1 infection to influence independently naive T-cell levels.

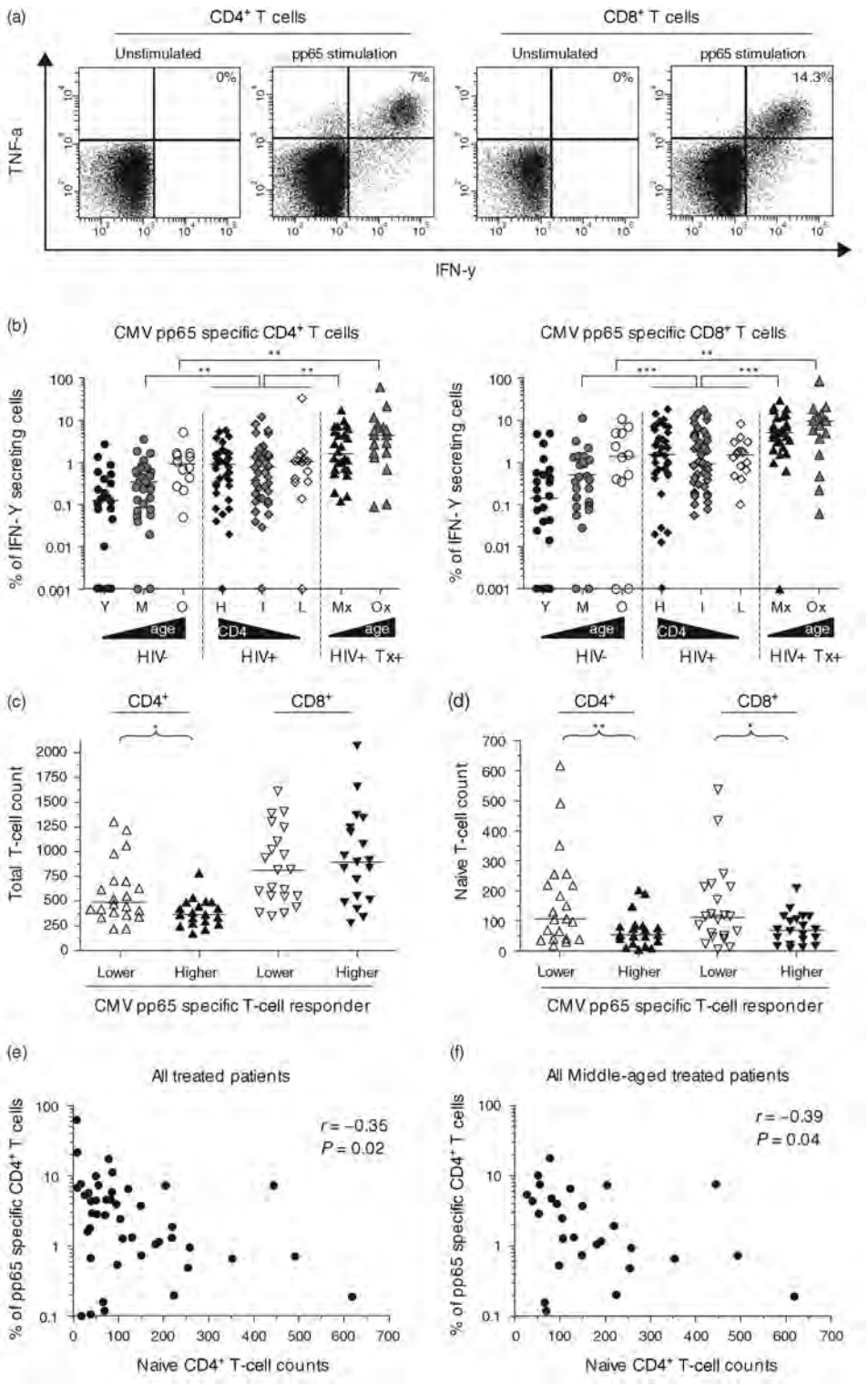

Fig. 3. Magnitude of the CMV pp65-specific T-cell response and T-cell reconstitution. (a) Representative staining for IFN-γ and TNF-α in $CD4^+$ or $CD8^+$ T cells from HIV-seropositive elderly upon stimulation with pp65 overlapping peptides. (b) Percentages of IFN-γ secreting $CD4^+$ or $CD8^+$ T cells upon pp65 stimulation from each group of patients included in the study, that is healthy controls and HIV-1-infected patients (treated or not). Absolute counts of (c) total or (d) naive $CD4^+$ or $CD8^+$ T cells from treated HIV-1-infected patients, grouped into higher or lower CMV responders. Bars indicate the median. The Mann–Whitney test was used for comparison of each group. *, ** and *** indicate *P* values below 0.05, 0.01 and 0.001, respectively. Inverse correlations between percentages of pp65-specific $CD4^+$ T cells and counts of naive $CD4^+$ T cells for (e) all treated HIV-1-infected patients, or for only (f) middle-aged treated HIV-1-infected patients. Spearman's rank test was used to determine correlations.

according to the magnitude of their total pp65-specific T-cell response, and separated into two groups of equal size. The difference between higher and lower CMV responders was particularly obvious with regards to naive T-cell counts, as both naive $CD4^+$ and $CD8^+$ T-cell numbers were reduced in patients with higher pp65-specific T-cell responses ($P = 0.009$ and $P = 0.04$ for $CD4^+$ and $CD8^+$ T cells, respectively) (Fig. 3d). We even found a modest but significant inverse correlation between the naive $CD4^+$ T-cell counts and magnitude of the pp65-specific $CD4^+$ T-cell response (Fig. 3e). This correlation was not conditional to the old HIV-1 patients, as it remained valid when considering solely middle-aged treated donors (Fig. 3f). On the whole, altered $CD4^+$ T-cell recovery and homeostasis with ART appears to be associated with age or CMV independently. This was supported by a multivariate analysis of our data set in HIV-1-infected treated donors ($P = 0.03$ for the age and $P = 0.04$ for pp65-specific responses as independent predictive factors of naive $CD4^+$ T-cell counts).

Discussion

The present study, which focuses on general attributes of the peripheral T-lymphocyte compartment, furthers our understanding of the aspects of HIV pathogenesis related to immune aging. Due to activation and high antigen load conditions, memory T cells expand and eventually differentiate into CD57 expressing cells. The high apoptosis resistance of these cells [31] may confer them survival advantages, resulting in their persistence and accumulation within the T-cell compartment. The frequency of $CD57^+$ memory T cell is generally increased with age or HIV-1 infection, and these cells are likely to play a role in the different inflammatory disorders occurring during aging or HIV-1 infection (i.e. in relation to their strong effector capacity like cytotoxicity and release of pro-inflammatory cytokines) [32]. However, our observations indicate that their frequency does not necessarily characterize well the parallel between immune aging and HIV disease progression. In contrast, declining $CD4^+$ and $CD8^+$ naive T-cell levels, which reflect mobilization and lack of renewal capacity of this compartment, emerged as a more relevant factor to highlight the similarity between aging and HIV disease progression. Preservation of the naive T-cell compartment is particularly important to mount adequate response to new antigens (including vaccination) as recently shown in old vaccinated primates [33]. Of note, despite their younger age (i.e. mean of 40 years), most HIV-1-infected individuals with a $CD4^+$ T-cell count below 200 cell/μl were comparable to elderly donors (i.e. mean of 85 years) when considering naive $CD4^+$ and $CD8^+$ T-cell blood frequencies. It will be important to address in future studies the influence on the development of immune aging in HIV infection of factors like sex and ethnic background, which are known to alter T-cell count and progression towards HIV disease [34].

T-cell renewal capacity is central for T-cell reconstitution upon the initiation of ART, and age is thought to have a significant impact in this context. Here, we studied for the first time HIV-1-infected patients with satisfactory $CD4^+$ T-cell recovery upon ART despite advanced age. Compared to middle-aged treated controls, these patients displayed nonetheless signs of altered T-cell reconstitution, characterized by significantly lower naive T-cell numbers. Considering the extent of thymic involution at this age, it is not surprising that the recovery of naive T-cell levels is considerably limited in old HIV-1-infected patients with ART. However, the present data show that altered homeostasis is taking place in these patients, with a preferential recovery of the memory T-cell pool. A similar phenomenon can be observed with the T-cell compartment reconstitution in donors after bone marrow transplantation [35,36]. Despite comparable recovery in $CD4^+$ T-cell numbers with ART, T-cell reconstitution in old HIV-1-infected patients is therefore not equivalent to one of the younger patients. This is important since distinct drug regimens may thus be envisaged according to the age, in order to achieve optimal T-cell reconstitution. Of note, the use of IL-7, a potent homeostatic regulator and thymopoiesis inducer, may be particularly relevant in this context, since studies performed in humans have shown that its administration resulted in a replenishment of the naive T-cell pool [37–39]. Future studies will need to assess the effects of regimens combining antiretroviral and immunomodulatory drugs in old HIV-1-infected patients specifically in order to design the most adapted treatments for this population.

CMV infection and in particular the mounting of a robust CMV-specific T-cell response are recognized factors associated with the development of the immunosenescence phenotype and homeostatic changes with the establishment of memory T-cell inflation [40,41]. Since HIV–CMV co-infection is highly prevalent in patients, we thought of investigating the potential role of CMV infection in the parallel between HIV-1 infection and aging. We did not find any clear relationship between the magnitude of the CMV-specific T-cell response and total or naive T-cell counts in untreated HIV-1-infected patients. This lack of association may be due to the impact of HIV-mediated immune activation on the development of the immunosenescence phenotype, which may conceal the potential weight of CMV infection in patients receiving no therapy. In line with this possibility, an effect of CMV infection was more evident in treated patients, that is once HIV-mediated immune activation is abrogated with ART. Strong anti-CMV T-cell responses were associated with lower $CD4^+$ T-cell count (in particular naive T cell) under treatment. Although the present data are cross-sectional and does not demonstrate

cause and effect, it is reminiscent of the observations made in young adults thymectomized during early childhood, when strong anti-CMV T-cell immunity was associated with the exhaustion of T-cell resources (i.e. the naive T-cell pool) [24]. In HIV-1-infected patients, the mounting of strong anti-CMV T-cell responses may mobilize a significant part of the resources, which may result in lower naive T-cell numbers in the absence of adequate T-cell renewal capacity, thus altering T-cell reconstitution upon ART. Although this is independent of age, it may be particularly evident in old treated HIV-1-infected patients, due to the synergic effect of thymic involution and the inflation of the CMV-specific response in these donors. Loss of naive T cells with CMV-mediated memory inflation may provide potential mechanistic insights underlying the association between CMV infection and more rapid HIV disease progression as described previously [42,43]. Comparative studies of T-cell reconstitution in CMV-seropositive versus CMV-seronegative HIV-1-infected donors starting ART will be necessary to confirm the impact of CMV in this context.

In summary, the premature development of an immunosenescence like phenotype during HIV-1 infection is better reflected by the decrease in naive T cells than the accumulation of senescent memory T cells. HIV appears to amplify immune aging, adding to the effect of age, as evidenced with further reductions in naive T-cell frequencies in HIV-1-infected patients. Under ART age as well as the mounting of robust anti-CMV T-cell responses independently alter T-cell reconstitution in treated patients, likely due to thymic involution or mobilization of the resources, respectively. This knowledge is important for the long-term clinical management of the aging HIV-1-infected population.

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Conflicts of interest

There are no conflicts of interest.

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