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Reduced naive and increased activated CD4 and CD8 cells in healthy adult Ethiopians compared with their Dutch counterparts

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SUMMARY

To assess possible differences in immune status, proportions and absolute numbers of subsets of $CD4^+$ and $CD8^+$ T cells were compared between HIV^- healthy Ethiopians ($n = 52$) and HIV^- Dutch ($n = 60$). Both proportions and absolute numbers of naive $CD4^+$ and $CD8^+$ T cells were found to be significantly reduced in HIV^- Ethiopians compared with HIV^-Dutch subjects. Also, both proportions and absolute numbers of the effector $CD8^+$ T cell population as well as the $CD4^+CD45RA^-CD27^-$ and $CD8^+CD45RA^-CD27^-$ T cell populations were increased in Ethiopians. Finally, both proportions and absolute numbers of $CD4^+$ and $CD8^+$ T cells expressing CD28 were significantly reduced in Ethiopians *versus* Dutch. In addition, the possible association between the described subsets and HIV status was studied by comparing the above 52 HIV^- individuals with 32 HIV^+ Ethiopians with CD4 counts $> 200/\mu$l and/or no AIDS-defining conditions and 39 HIV^+ Ethiopians with CD4 counts $< 200/\mu$l or with AIDS-defining conditions. There was a gradual increase of activated $CD4^+$ and $CD8^+$ T cells, a decrease of $CD8^+$ T cells expressing CD28 and a decrease of effector $CD8^+$ T cells when moving from HIV^- to AIDS. Furthermore, a decrease of naive $CD8^+$ T cells and an increase of memory $CD8^+$ T cells in AIDS patients were observed. These results suggest a generally and persistently activated immune system in HIV^-Ethiopians. The potential consequences of this are discussed, in relation to HIV infection.

Keywords Ethiopia HIV-1 T cells naive cells activation

INTRODUCTION

HIV infection is associated with profound changes in various T cell subsets of the immune system [1–5]. Loss of $CD4^+$ T cells is known to be the hallmark of HIV infection and it is a well accepted laboratory marker of HIV disease progression [6,7]. Also, changes in other T cell subsets have been implicated in HIV^+ persons from studies in Europe and North America: expansion of $CD8^+$ T cells [8], loss of naive $CD8^+$ T cells, increase of both $CD4^+$ and $CD8^+$ T cell memory subsets [9,10] and increased activated $CD4^+$ and $CD8^+$ T cell subsets [11–16].

In Africa, HIV infection is spreading fast and has become one of the major causes of mortality [17,18]. Studies have suggested that HIV disease progression is faster in Africa when compared with industrialized countries [19]. Pre-existent immune activation as a result of highly prevalent infectious diseases and also nutritional factors have been suggested as important contributors to this altered HIV disease progression in Africa [20–24]. There are not many reports detailing the immune status of HIV-infected and non-infected Africans. Moreover, studies on the immunological markers which are important for predicting HIV disease progression in Africans are scarce. We previously reported that CD4 counts are significantly lower and CD8 counts are higher in HIV^- Ethiopians compared with Dutch HIV^- controls (Tsegaye *et al*., submitted). In this study we compare several $CD4^+$ and $CD8^+$ T cell subsets in Ethiopian and Dutch HIV^-individuals. In addition, a cross-sectional study is performed to assess the association of several $CD4^+$ and $CD8^+$ T cell subsets with defined stages of HIV infection in Ethiopian individuals.

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SUBJECTS AND METHODS

Subjects

For comparison of Ethiopian and Dutch subjects, 52 HIV$^-$healthy Ethiopians and 60 HIV$^-$ healthy Dutch individuals were included. Two additional groups of Ethiopian subjects were included in a cross-sectional study: 32 HIV$^+$ with CD4 counts $>200/\mu$l and no AIDS-defining conditions (designated in this study as HIV$^+$) and 39 HIV$^+$ patients with CD4 counts $<200\,\mu$l or exhibiting AIDS-defining conditions based on the WHO staging system for HIV infection and disease [25] (designated in this study as AIDS). The HIV$^-$ and HIV$^+$ groups are residents of a suburb of Addis Ababa and factory workers participating in a cohort study presently performed in Akaki, a village 15 km to the south-east of Addis Ababa, the capital of Ethiopia. Subjects of the AIDS group were patients hospitalized in Addis Ababa. All the study subjects gave their informed consent.

Testing of samples

The samples from the Ethiopian subjects were analysed at the ENARP laboratory in Addis Ababa, Ethiopia, and samples from the Dutch subjects were analysed at the clinical viro-immunology department, CLB, Amsterdam. The two laboratories are collaborating labs within ENARP. Similar protocols of sample processing and testing are used in both laboratories. Furthermore, samples are shared between both laboratories for quality control purposes.

Three-colour immunophenotyping of lymphocyte subsets

In vivo activated, non-activated, naive and memory CD4$^+$ and CD8$^+$ T cells were quantified by three-colour flow cytometric staining using perdinyl chlorophyll-A protein (PerCP)-conjugated CD4 or CD8 MoAbs in combination with PE-conjugated HLA-DR and FITC-conjugated CD38 MoAbs and PerCP-conjugated CD4 or CD8 MoAbs in combination with PE-conjugated CD27 and FITC-conjugated CD45RA MoAbs, respectively. All the MoAbs were purchased from Becton Dickinson (San Jose, CA), except the CD38 MoAb, which was purchased from Immunotech (Marseille, France). The immunophenotyping was performed on whole blood. EDTA blood (100 μl) was incubated with each combination of MoAbs for 15–20 min at room temperature in the dark. Erythrocyte lysing was done by adding 2 ml lysing solution per tube (FACSlyse; Becton Dickinson) and incubating for 10 min at room temperature in the dark. The cells were centrifuged at 300 ***g*** for 5 min and then washed twice with Isoton (Becton Dickinson). The stained samples were analysed the same day using a FACScan flow cytometer with Cellquest software (Becton Dickinson). A live gate was set around the CD4$^+$ and CD8$^+$ cells in order to acquire a minimum of 1500 CD4 or CD8 cells for analysis.

Table 1. Proportions and absolute numbers of T cell subsets in HIV$^-$ Ethiopian and Dutch individuals

T cell subset	Ethiopians, $n=52$	Dutch, $n=60$	P (Mann–Whitney U-test)
CD4$^+$	33 (8–48)*	50 (33–60)	<0·0001
	667 (219–1185)*	1067 (533–2059)	<0·0001
CD8$^+$	33 (10–72)	23 (8–41)	<0·0001
	635 (152–1476)	510 (196–1140)	NS
CD4/8 ratio	0·97 (0·12–3·87)	2·09 (0·94–5·69)	<0·0001
CD4$^+$CD45RA$^+$CD27$^-$	1 (0–16)	0 (0–4)	<0·0001
	8 (0–90)	0 (0–66)	<0·0010
CD4$^+$CD45RA$^+$ CD27$^+$	15 (2–49)	37 (14–82)	<0·0001
(naive)	96 (19–290)	389 (108–1181)	<0·0001
CD4$^+$ CD45RA$^-$ CD27$^-$	23 (6–69)	5 (2–13)	<0·0001
(contains Th2)	140 (30–279)	60 (14–174)	<0·0001
CD4$^+$CD45RA$^-$CD27$^+$	58 (24–75)	58 (13–77)	NS
(memory)	396 (104–719)	618 (127–1407)	<0·0001
CD4$^+$CD28$^+$	94 (80–100)	99 (91–100)	<0·0001
	627 (180–1158)	1060 (530–2049)	<0·0001
CD8$^+$CD45RA$^+$CD27$^-$	29 (8–65)	4 (0–23)	<0·0001
(cytotoxic effector)	153 (13–898)	20 (0–160)	<0·0001
CD8$^+$CD45RA$^+$CD27$^+$	17 (3–41)	40 (16–85)	<0·0001
(naive)	97 (26–243)	209 (41–760)	<0·0001
CD8$^+$CD45RA$^-$CD27$^-$	24 (2–49)	7 (1–28)	<0·0001
(memory)	132 (8–809)	34 (2–203)	<0·0001
CD8$^+$CD45RA$^-$CD27$^+$	24 (4–46)	44 (6–72)	<0·0001
(memory)	127 (52–383)	203 (22–608)	<0·0010
CD8$^+$CD28$^+$	43 (15–95)	82 (54–96)	<0·0001
	211 (87–533)	404 (178–975)	<0·0001

Values are medians, 95 percentiles are in parentheses. Absolute values are per μl of whole blood. Absolute counts were performed on 44 Ethiopians, except absolute CD28$^+$ subsets, which were done on 42 Ethiopians.

* Top value = proportions, bottom value = absolute numbers.

Statistical analysis

Statistical analyses were performed using the STATA program (Stata Corp., TX) The distribution of T cell subset proportions was compared between two groups using non-parametric methods (Mann–Whitney *U*-test). When comparisons involved three groups (Ethiopian HIV^-, HIV^+ and AIDS) the level of significance (α) was adjusted using the Bonferroni correction ($\alpha = 0{\cdot}033$).

RESULTS

Naive, memory and effector T cell subsets compared between HIV^- Ethiopian and Dutch subjects

Naive, memory and effector $CD4^+$ and $CD8^+$ T cells were measured using a combination of CD45RA and CD27 MoAbs, according to Hamann *et al*. [26]. Table 1 shows both the proportions and absolute values of $CD4^+$ and $CD8^+$ T cell subsets in 52 HIV^- Ethiopian *versus* 60 HIV^- Dutch subjects.

Both proportions and absolute values of $CD4^+$ T cells were significantly decreased in Ethiopians compared with the Dutch subjects. Proportions of $CD8^+$ T cells were increased in Ethiopians, with borderline significance for absolute values. As a consequence, the CD4/8 ratios of HIV^-healthy Ethiopians were low.

The proportions and absolute values of both $CD4^+$ and $CD8^+$ naive ($CD45RA^+CD27^+$) T cells were significantly reduced in Ethiopians compared with Dutch subjects. In contrast, the proportions and absolute values of both $CD4^+$ and $CD8^+$ memory ($CD45RA^-CD27^-$) T cells were increased in Ethiopians. For the second memory subset ($CD45RA^-CD27^+$), the absolute values were decreased, both in $CD4^+$ and $CD8^+$ T cells of Ethiopians. This decrease was also reflected in the proportions of the pertinent $CD8^+$ memory subset, but not of the $CD4^+$ subset. Both proportions and absolute values of $CD8^+$ cytotoxic effector T cells were increased in Ethiopians compared with the Dutch subjects. Finally, both proportions and absolute values of $CD4^+$ and $CD8^+$ T cells expressing CD28 were decreased in Ethiopians *versus* Dutch.

The combination of the above observations points towards a highly activated immune status of HIV^- Ethiopian *versus* HIV^- Dutch individuals.

Naive, memory and effector T cell subsets in Ethiopian HIV^-, HIV^+ and AIDS subjects

In order to assess the effect of HIV infection on the above-defined immune status of Ethiopian individuals, a cross-sectional study was performed, comparing three groups of subjects. A provisional classification is used in this study, naming HIV non-infected individuals 'HIV^-', HIV-infected individuals with $CD4^+$ T cell counts $>200/\mu$l and no AIDS-defining conditions 'HIV^+', and HIV-infected individuals with $CD4^+$ T cell counts $<200/\mu$l or AIDS-defining conditions 'AIDS'.

Table 2 presents the median age, the male-to-female ratio, the proportions and absolute values of lymphocytes, $CD4^+$, $CD8^+$ T cell subsets and the CD4/CD8 ratios of 123 Ethiopian individuals, grouped according to the above HIV status designation. Figure 1a,b depict representative dot plot analyses of changes in CD45RA/CD27 proportions of the various T cell subsets, according to HIV status, as defined above.

Table 3a summarizes the observations for subsets defined by CD45RA and CD27 MoAbs. Absolute values of naive ($CD45RA^+CD27^+$) $CD4^+$ and $CD8^+$ T cells were significantly reduced only in the AIDS group (for $CD8^+$ T cells this also applied to proportions). The Th2 cell containing memory ($CD45RA^-CD27^-$) $CD4^+$ T cells [27,28] were reduced in absolute numbers in HIV^+ and AIDS patients, but remained stable in proportions, comparing the three groups of subjects. The equivalent $CD8^+$ memory T cells were increased in proportions in the AIDS group compared with the HIV^- group, whereas the absolute values remain unchanged in the three groups. The second subset of memory ($CD45RA^-CD27^+$) $CD4^+$ T cells showed identical patterns to the specialized memory $CD4^+$ T cells. However, the memory ($CD45RA^-CD27^+$) $CD8^+$ T cells showed a significant increase in absolute numbers in the HIV^+ group compared with the two other groups, while this was not reflected in their proportions. The cytotoxic effector ($CD45RA^+CD27^-$) $CD8^+$ T cell proportions were significantly reduced in both HIV^+ and AIDS groups compared with the HIV^- group. The absolute values of this subset were only significantly decreased in the AIDS group. Both absolute values and proportions of $CD4^+$ T cells expressing these markers were very low in all groups.

Table 2. Characteristics of study populations, designated HIV^-, HIV^+ and AIDS

	HIV^- $n=52$	HIV^+ $n=32$	AIDS $n=39$
Age (years)	37 (15–50)	36 (20–73)	32 (18–52)[a]
Male/female	36/16	18/14	21/16[a]
Lymphocyte percentage	37 (18–62)[b]	42 (18–67)[c]	29 (7–47)[d]
Abs. lymphocyte	1841 (819–2945)[b]	2268 (1260–4891)[c]	1070 (371–3388)[de]
CD4 %	33 (8–48)[e]	18 (7–39)[e]	7 (1–44)[e]
Abs. CD4	667 (219–1185)[be]	382 (227–1468)[ce]	73 (4–586)[de]
CD8 %	33 (10–72)[e]	48 (24–84)	40 (7–81)
Abs. CD8	635 (152–1476)[b]	1000 (416–3130)[ce]	422 (78–2744)[d]
CD4/CD8 ratio	0·97 (0·12–3·87)	0·34 (0·1–1·6)	0·15 (0·02–1·46)

Values are medians (except for male/female ratio), 95 percentiles are in parentheses. Absolute values are per μl of whole blood and based on: [a]$n=37$, [b]$n=44$, [c]$n=31$, [d]$n=38$. [e]$P<0{\cdot}03$ compared with the other two groups.

The CD28 costimulatory molecule in Ethiopian HIV$^-$, HIV$^+$ and AIDS subjects

The percentages of CD4$^+$ T cells expressing CD28 were equally high in the HIV$^-$, HIV$^+$ and AIDS groups, whereas the absolute values showed a decrease from HIV$^-$ to HIV$^+$ to AIDS. As shown in Table 3a, the proportions of CD8$^+$ T cells expressing CD28 were significantly reduced in the AIDS group. When absolute values were assessed, this particular CD8$^+$ T cell subset showed a significant increase in HIV$^+$ *versus* HIV$^-$subjects and a decrease in the AIDS group.

Activated and resting T cell subsets in Ethiopian HIV$^-$, HIV$^+$, AIDS subjects

Activated and resting T cell subsets were measured using a combination of HLA-DR and CD38 MoAbs, as described [12,14]. Figure 1c,d depict representative dot plot analyses of the changes in CD38/HLA-DR proportions according to HIV status. Table 3b summarizes the observations.

Proportions of activated (HLA-DR$^+$ CD38$^+$) T cells of both CD4$^+$ and CD8$^+$ T cells were increased from HIV$^-$ to HIV$^+$ to AIDS groups. This increment was more pronounced for CD8$^+$ T

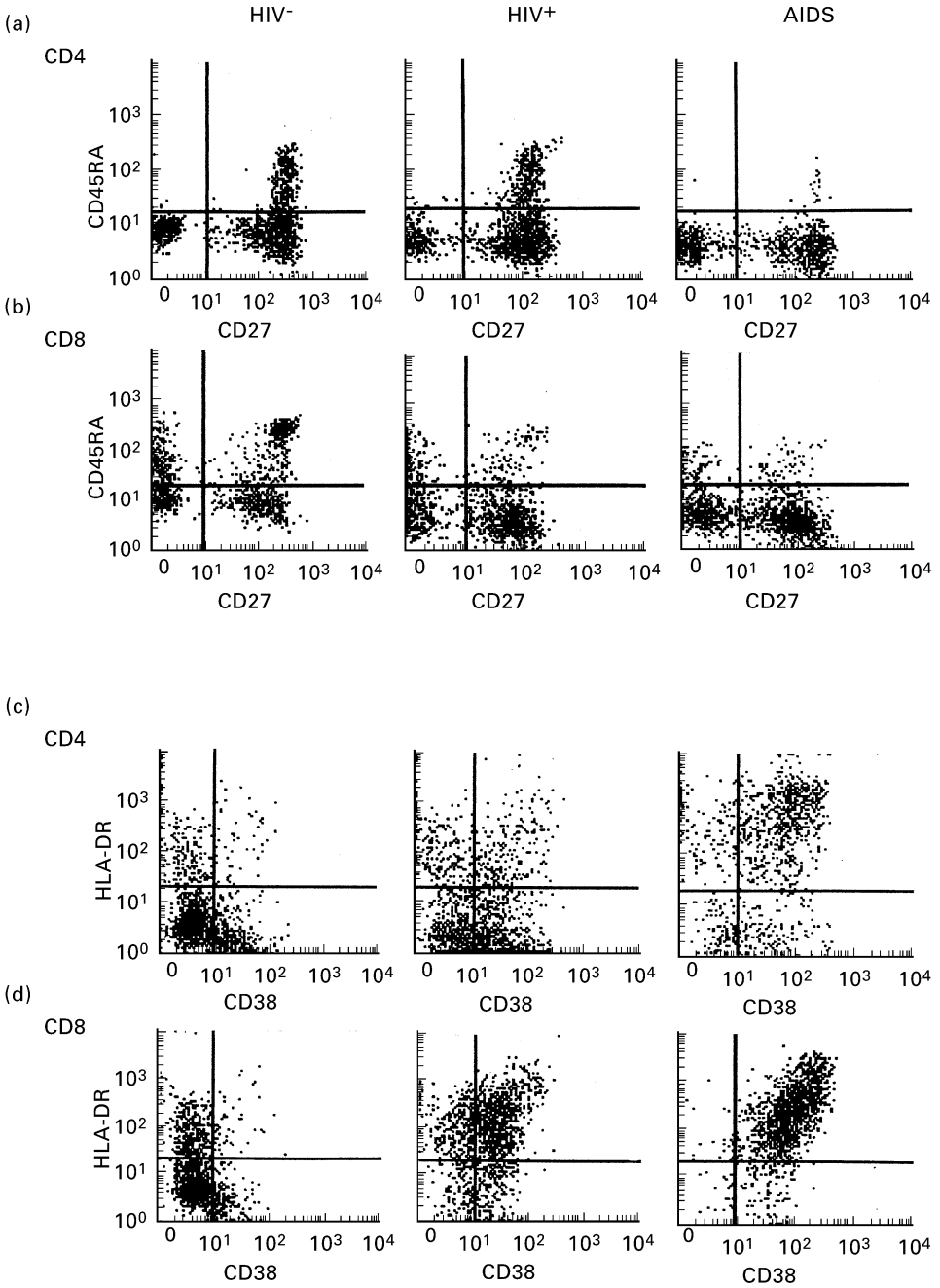

Fig. 1. Examples of dot plots of CD4$^+$ and CD8$^+$ T cell subsets in the three groups of Ethiopians (HIV$^-$, HIV$^+$ and AIDS). CD4$^+$ and CD8$^+$ T cell subsets are defined as follows: activated (HLA-DR$^+$CD38$^+$), resting (HLA-DR$^-$CD38$^-$), 'non-progression associated' (HLA-DR$^+$CD38$^-$), naive (CD45RA$^+$CD27$^+$), memory (CD45RA$^-$CD27$^+$), effector (CD45RA$^+$CD27$^-$) and memory + effector (CD45RA$^-$CD27$^-$).

Table 3. Proportions and absolute numbers of T cell subsets in Ethiopian HIV$^-$, HIV$^+$ and AIDS groups

a. CD45RA, CD27 subpopulations

T cell subset	HIV$^-$ $n=52$	HIV$^+$ $n=32$	AIDS $n=39$
CD4$^+$CD45RA$^+$CD27$^-$	1 (0–16)†	1 (0–22)	1 (0–19)
	8 (0–90)†	4 (0–67)	1 (0–35)*
CD4$^+$CD45RA$^+$CD27$^+$	15 (2–49)	21 (2–46)	17 (1–67)
(naïve)	96 (19–290)	81 (11–329)	12 (0–122)*,**
CD4$^+$CD45RA$^-$CD27$^-$	23 (6–69)	24 (10–60)	24 (4–47)
(contains Th2)	140 (30–279)	76 (32–470)*	15 (1–227)*,**
CD4$^+$CD45RA$^-$CD27$^+$	58 (24–75)	51 (33–68)	52 (19–84)
(memory)	396 (104–719)	193 (107–837)*	34 (2–287)*,**
CD4$^+$CD28$^+$	94 (80–100)	96 (69–99)	95 (56–100)
	627 (180–1158)	344 (200–1248)*	52 (4–227)*,**
CD8$^+$CD45RA$^+$CD27$^-$	29 (8–65)	22 (12–62)*	18 (5–55)*
(cytotoxic effector)	153 (13–898)	225 (55–1781)	76 (5–502)**
CD8$^+$CD45RA$^+$CD27$^+$	17 (3–41)	18 (3–39)	9 (1–29)*,**
(naïve)	97 (26–243)	174 (52–349)	38 (3–200)*,**
CD8$^+$CD45RA$^-$CD27$^-$	24 (2–49)	20 (4–63)	31 (10–71)**
(memory)	132 (8–809)	165 (25–845)	121 (16–892)
CD8$^+$CD45RA$^-$CD27$^+$	24 (4–46)	37 (10–59)	39 (1–69)*
(memory)	127 (52–383)	360 (91–1047)*	163 (5–1147)**
CD8$^+$CD28$^+$	43 (15–95)	39 (22–58)	28 (2–52)*
	211 (87–533)	375 (113–1068)*	98 (17–666)*,**

Values are medians, 95 percentiles are in parentheses. Absolute values are per μl of whole blood and based on data from HIV$^-$ ($n=44$), HIV$^+$ ($n=31$), AIDS ($n=36$), except the CD28$^+$ subsets which are based on HIV$^-$ ($n=42$), HIV$^+$ ($n=25$), AIDS ($n=29$).

* $P<0{\cdot}03$ compared with HIV$^-$ group; ** $P<0{\cdot}03$ compared with the HIV$^+$ group.

† Top value = proportions, bottom value = absolute numbers.

b. HLA-DR, CD38 subpopulations

T cell subset	HIV$^-$ $n=52$	HIV$^+$ $n=32$	AIDS $n=39$
CD4$^+$HLA-DR$^+$CD38$^-$	16 (2–39)†	19 (5–41)	14 (3–35)
	108 (13–294)†	64 (11–602)	10 (0–89)*,**
CD4$^+$HLA-DR$^+$CD38$^+$	2 (0–21)	10 (4–31)*	28 (11–69)*,**
(activated)	9 (1–83)	40 (16–235)*	17 (1–251)
CD4$^+$HLA-DR$^-$CD38$^-$	74 (45–94)	42 (16–69)*	30 (2–54)*,**
(resting)	486 (107–829)	163 (49–573)*	17 (0–151)*,**
CD4$^+$HLA-DR$^-$CD38$^+$	5 (1–31)	27 (4–65)*	27 (6–63)*
	32 (4–333)	104 (22–314)*	14 (1–181)
CD8$^+$HLA-DR$^+$CD38$^-$	37 (4–71)	34 (12–70)	9 (1–38)*,**
(non-progression)	234 (16–971)	375 (50–944)	32 (2–568)*,**
CD8$^+$HLA-DR$^+$CD38$^+$	2 (0–54)	32 (6–62)*	65 (18–91)*,**
(activated)	13 (0–415)	284 (63–1941)*	285 (58–1707)*
CD8$^+$HLA-DR$^-$CD38$^-$	57 (12–88)	19 (4–53)*	7 (0–43)*,**
(resting)	297 (82–1086)	160 (54–840)‡	36 (0–400)*,**
CD8$^+$HLA-DR$^-$CD38$^+$	2 (0–31)	12 (1–39)*	16 (2–56)*
	8 (0–172)	125 (9–1120)*	46 (5–834)*

Values are medians, 95 percentiles are in parentheses. Absolute values are per μl of whole blood and based on data from HIV$^-$ ($n=44$), HIV$^+$ ($n=31$), AIDS ($n=36$), except the CD4 subsets for HIV$^+$ ($n=29$).

* $P<0{\cdot}03$ compared with the HIV$^-$ group; ** $P<0{\cdot}03$ compared with the HIV$^+$ group.

† Top value = proportions, bottom value = absolute numbers.

‡ Borderline significant compared with HIV$^-$ ($P=0{\cdot}04$).

cells than for $CD4^+$ T cells. For absolute values, there was an increase of activated $CD4^+$ and $CD8^+$ T cells in HIV^+ *versus* HIV^- subjects. However, in the AIDS group there was no further increase of these T cell populations. Conversely, the proportions and absolute values of resting ($HLA\text{-}DR^-CD38^-$) T cells of both $CD4^+$ and $CD8^+$ subsets were decreased from HIV^- to HIV^+ to AIDS. The proportions of $CD4^+$ and $CD8^+$ T cells expressing CD38 but not HLA-DR were increased in both HIV^+ and AIDS groups compared with the HIV^- group. This was also true for absolute values, with the exception of $HLA\text{-}DR^-CD38^+CD4^+$ T cells of AIDS patients compared with HIV^-individuals.

Both proportions and absolute values of $HLA\text{-}DR^+CD38^-CD8^+$ T cells were significantly reduced in the AIDS group compared with the HIV^- and HIV^+ groups. There was no significant difference on proportions of $CD4^+$ T cells expressing this marker combination between the three groups studied.

DISCUSSION

In this study we demonstrate that the representation of several T cell subsets is different, comparing HIV^- Ethiopian and Dutch subjects. Most significantly, naive T cells were found to be considerably reduced in healthy HIV^-Ethiopians. The naive T cell subsets are responsible for mounting immune responses to newly encountered antigens and *in vitro* studies have shown that these cells have a better capacity for proliferation in response to mitogenic stimuli [29]. T cells which were previously shown to be of memory type, in terms of cytokine production and antigen expression [26], were demonstrated to be reduced in Ethiopian $CD8^+$ T cells, but not in $CD4^+$ T cells, compared with the Dutch. The $CD8^+$ effector T cell subset, which was shown to exhibit cytolytic properties and poor responses to most *in vitro* stimuli [26], was found to be increased in Ethiopians. The latter observation confirms previous reports of increased effector ($CD8^+CD57^+$) T cells in Ethiopians [30]. Finally, both $CD4^+$ and $CD8^+$ T cells with $CD45RA^-CD27^-$ memory phenotype were found to be significantly increased in Ethiopians compared with the Dutch group. In previous studies the $CD45RA^-CD27^-$ T cell subsets have been shown to constitute a very small proportion of T cells and are suggested to arise as a result of repeated antigenic stimulation *in vivo* [26,31,32]. $CD4^+CD27^-$ T cells have been shown to be increased in parasitic infections and to contain Th2 cells, producing IL-4 and IL-5 [27,28]. In the present study, this population of T cells was found to be significantly increased in Ethiopians *versus* Dutch, probably pointing towards a high prevalence of parasitic infections in the studied Ethiopian subjects.

CD28 is a transmembrane glycoprotein that provides an essential costimulatory signal to T cells [33]. $CD4^+$ and $CD8^+$ T cells expressing CD28 were reduced in Ethiopians compared with the Dutch. Loss of CD28 on T cells has been associated with increased cytolytic T cell activity, impaired response to costimulation via B7, anergy to anti-CD3-induced proliferation and also shorter telomere length, which is indicated to be a result of chronic immune system activation [26,34–39].

The above observations reflect a generally and persistently activated immune system in Ethiopians. This activation has been attributed to an increased load of environmental pathogens, especially intestinal parasites in Ethiopia, compared with industrialized countries [20]. The activated state of the immune system in Ethiopians is reflected by increased effector $CD8^+$ T cells, increased T cells associated with repeated antigenic stimulation (containing Th2 cells). The increase of these subsets is at the expense of memory $CD8^+$ T cells, as well as naive T cells and finally T cells expressing CD28 costimulatory molecules in general. This would predict that, as a consequence, the immune system of Ethiopians most probably has a reduced ability to build effective immune responses to newly encountered infections. Also, the immune response against recurrent infections could be impaired. It has been suggested that the HIV-1 co-receptor CCR-5 is more abundantly expressed on memory T cells than on naive T cells [40]. In this light, it can be speculated that the observed reduction in the ratio of naive/memory $CD4^+$ T cells in Ethiopians *versus* Dutch will potentially increase the proportion of CD4 T cells expressing CCR-5 and thus might facilitate infection of CD4 T cells of Ethiopians with HIV-1. In addition, the increase of effector $CD8^+$ T cells in HIV^- Ethiopians *versus* HIV^- Dutch could result in increased production of cytokines such as tumour necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ), potentially stimulating HIV-1 replication after infection. The above assumptions could contribute to the reported faster progression of HIV-1 infection in an African context [19,20].

In light of this, the observations on the three groups designated HIV^-, HIV^+ and AIDS in this study could be interpreted as follows. Although in general Ethiopians already harbour less naive $CD4^+$ and $CD8^+$ T cells than the Dutch, there was no further proportional decrease of these cells after HIV infection. Only at AIDS was a significant further decrease of naive $CD8^+$ T cells observed. In contrast to previous reports [9], we did not observe significant changes in the proportion of naive CD4 subsets in the three groups of Ethiopians that were compared. The memory $CD8^+$ T cell subset, being smaller in HIV^- Ethiopians than in the Dutch, was found to be increased in HIV^+ and AIDS Ethiopians. Conversely, the effector $CD8^+$ T cell subset, being higher in HIV^- Ethiopians than in HIV^- Dutch, decreased in Ethiopian HIV^+ and AIDS subjects. This could result in less production of CCR-5 ligands (MIP-1a, MIP-1b, RANTES) and thus the potential inhibitory effect on HIV-1 cell entry would be decreased [41].

The reduced CD28-expressing subsets of HIV^-Ethiopians *versus* HIV^- Dutch were only further lost at AIDS for $CD8^+$ T cells. No differences in proportions of $CD4^+$ T cells expressing this marker were observed. This is in agreement with previous reports which showed that hyporesponsiveness of T cells during HIV infection to costimulatory signals is limited to the $CD8^+$ T cell subset and the response of $CD4^+$ T cells is intact [42].

The activated T cell subsets co-expressing CD38 and HLA-DR were found to increase, on both $CD4^+$ and $CD8^+$ T cell subsets, when comparing the HIV^-, HIV^+ and AIDS groups. The opposite was true for resting T cell subsets. The observation that $CD8^+$ T cells expressing only HLA-DR but not CD38 are lost in Ethiopian AIDS patients is in agreement with a previous prospective study which demonstrated that the presence of this subset is associated with long-term survival and stable $CD4^+$ T cell counts [14]. Apart from being an activation marker, CD38 is also detected in immature cells [43].The representation of $CD4^+$ and $CD8^+$ T cells expressing only CD38 but not HLA-DR is increased in both HIV^+ and AIDS groups compared with the HIV^-group. This observation may reflect that in HIV infection, apart from immune activation, there is also an increased flow of immature cells into the periphery due to accelerated destruction and replacement of mature T cells (see also [44]).

In conclusion, several of the presently used T cell subset

markers could be of predictive value for HIV-1 progression in Ethiopians. However, the value of these markers remains to be evaluated in future prospective studies.

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