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## Chapter 2

### Distribution of Lymphocyte Subsets in Healthy Human Immunodeficiency Virus-Negative Adult Ethiopians from Two Geographic Locales

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Immunological values for 562 factory workers from Wonji, Ethiopia, a sugar estate 114 km southeast of the capital city, Addis Ababa, Ethiopia, were compared to values for 218 subjects from Akaki, Ethiopia, a suburb of Addis Ababa, for whom partial data were previously published. The following markers were measured: lymphocytes, T cells, B cells, NK cells, CD4<sup>+</sup> T cells, and CD8<sup>+</sup> T cells. A more in depth comparison was also made between Akaki and Wonji subjects. For this purpose, various differentiation and activation marker (CD45RA, CD27, HLA-DR, and CD38) expressions on CD4<sup>+</sup> and CD8<sup>+</sup> T cells were studied in 60 male, human immunodeficiency virus-negative subjects (30 from each site). Data were also compared with Dutch blood donor control values. The results confirmed that Ethiopians have significantly decreased CD4<sup>+</sup> T-cell counts and highly activated immune status, independent of the geographic locale studied. They also showed that male subjects from Akaki have significantly higher CD8<sup>+</sup> T-cell counts, resulting in a proportional increase in each of the CD8<sup>+</sup> T-cell compartments studied: naïve (CD45RA<sup>+</sup>CD27<sup>+</sup>), memory (CD45RA<sup>-</sup>CD27<sup>+</sup>), cytotoxic effector (CD45RA<sup>+</sup>CD27<sup>-</sup>), memory/effector (CD45RA<sup>-</sup>CD27<sup>-</sup>), activated (HLA-DR<sup>+</sup>CD38<sup>+</sup>), and resting (HLA-DR<sup>-</sup>CD38<sup>-</sup>). No expansion of a specific functional subset was observed. Endemic infection or higher immune activation is thus not a likely cause of the higher CD8 counts in the Akaki subjects. The data confirm and extend earlier observations and suggest that, although most lymphocyte subsets are comparable between the two geographical locales, there are also differences. Thus, care should be taken in extrapolating immunological reference values from one population group to another.

#### Introduction

T-cell immunophenotyping by flow cytometry is an important tool in the evaluation of immunological status. It is especially of value in the management of diseases that involve alterations in lymphocyte subpopulations, such as human immunodeficiency virus (HIV) disease (12, 30, 31). For example, absolute CD4<sup>+</sup> and CD8<sup>+</sup> T-cell counts and the derived CD4/CD8 T-cell ratio are important for monitoring HIV infection progression (9, 36). CD4<sup>+</sup> T-cell counts are of additional value for the initiation of prophylactic treatment for opportunistic infections and for

the monitoring of responses to antiretroviral therapy in HIV-infected individuals, especially in industrialized countries (5). However, it should be kept in mind that these markers are still of limited use, especially in countries with little economical resources.

Since 1995, the Ethiopian Netherlands AIDS Research Project (ENARP) as part of its activities has initiated studies in the field of CD4<sup>+</sup> T-cell counting in Ethiopia with a view to eventually establish a nationwide network for reference purposes. A stepwise approach has been undertaken, including the establishment of reference values for CD4<sup>+</sup> and CD8<sup>+</sup> T cells and various subsets in healthy HIV-negative Ethiopians (37), the measurement of CD4<sup>+</sup> and CD8<sup>+</sup> T-cell counts in HIV-infected Ethiopians, and the establishment of their relations with World Health Organization (WHO)-defined clinical stages of the disease (19).

Initial studies on the establishment of reference values for T-cell subsets (37) resulted in a striking observation of significantly lower CD4<sup>+</sup> T-cell counts, significantly higher CD8<sup>+</sup> T-cell counts, and a lower CD4/CD8 ratio in healthy HIV-negative Ethiopians than in healthy Dutch subjects. Some of these observations were confirmed by other studies comparing Ethiopians with populations like the Swedish (41) and Israeli (17, 26). In addition, healthy HIV-negative Ethiopians were also found to have a generally and persistently activated immune system, with increased memory and decreased naïve T cells compared to the Dutch (25). However, because of the importance of these observations and the potential consequences for clinical management of HIV-positive Ethiopians, we decided to extend our studies to other Ethiopian populations to get insight into the more general applicability of these data. The original observations were obtained from fiber factory workers living in Akaki, Ethiopia, a high-altitude (2,100 m) suburb of the capital city, Addis Ababa, Ethiopia. The present study presents data obtained from a second cohort of subjects living and working at a sugar estate in Wonji, Ethiopia, a medium-altitude (1,500 m) town 114 km southeast of Addis Ababa. It was demonstrated that most of the original observations done in Akaki could be confirmed in Wonji study subjects. However, there were also significant differences in certain T-cell subsets, like substantially higher CD8<sup>+</sup> T-cell counts in Akaki than in Wonji. These variations in CD8<sup>+</sup> T-cell counts were further investigated in an attempt to identify the particular T-cell subset(s) responsible for these differences.

## **Materials and Methods**

### **Subjects.**

The subjects involved in this cross-sectional study are factory workers participating in a long-term cohort study on the progression of HIV type 1 (HIV-1) infection in Ethiopia performed by ENARP at the Ethiopian Health and Nutrition Research Institute (EHNRI). A detailed description of the cohort studies has been reported elsewhere (33, 34). All study participants were examined by a medical doctor. Inclusion criteria for the present study were the absence of clinical conditions listed in the WHO staging system, looking apparently healthy (37, 40), and being negative for intestinal parasites and HIV-1 antibodies. Thus, 218 participants (131 males and 87 females) from Akaki (a suburb of the capital Addis Ababa at an altitude of 2,100 m) and 562 participants from Wonji (a sugar estate 114 km southeast of Addis Ababa at an altitude of 1,500 m) were enrolled. Only males participated in the Wonji cohort. For a more in depth immunological comparison between males from Akaki and Wonji, 60 age-matched, HIV-seronegative subjects were included: 30 from Akaki (median age 40, range 27 to 47) and

30 from Wonji (median age 40, range 29 to 47). In addition, data generated for T-cell subsets and activation markers from Dutch blood donors were used for comparison. Samples of the Dutch subjects were analyzed at the Department of Clinical Viro-Immunology, CLB and Laboratory for Experimental and Clinical Immunology of the University of Amsterdam, Amsterdam, The Netherlands, following the same laboratory protocol. The two laboratories are collaborating labs within ENARP.

#### **Stool microscopy.**

Stool examination for parasite infection was performed as part of the routine investigations on fresh stools at the study sites on the same date as blood sample collection. Direct microscopy in saline and iodine preparations and Formol-ether concentration methods were employed (2). The Baermann concentration method was also performed to detect *Strongyloides stercoralis* larvae (23). In addition, two kato thick smears from the same specimen on the same date as blood sample collection and another two kato smears plus one Baermann sediment on day 3 were analyzed in Wonji.

#### **Blood collection, plasma isolation, and HIV serology.**

Whole blood was collected into EDTA Vacutainer tubes between 8:30 and 11:30 a.m. and transported to the ENARP laboratory on the same day of collection. Upon arrival, the tubes were well mixed and 500 µl of the whole blood was transferred into Nunc tubes for FACScan and hematological analysis. The presence of HIV antibodies was detected on plasma using the HIV SPOT Rapid assay (Genelabs Diagnostics, Singapore) and the enzyme linked immunosorbent assay (Vironostika HIV Uni-Form II plus O; Organon Teknika, Boxtel, The Netherlands). Plasma samples which tested positive by one or both tests were confirmed by Western blot analysis (HIVBLOT 2.2; Genelabs Diagnostics).

#### **Hematological analysis.**

The absolute number of leukocytes per microliter of whole blood was obtained using a Coulter Counter T540 (Coulter Electronics, Hialeah, Fla.), which was standardized against a 4C plus blood control.

#### **Three-color immunophenotyping of lymphocyte subsets.**

Lymphocyte subsets and five-part differential counts were determined using Multitest kits and Multiset software (Becton Dickinson Immunocytometry Systems, San Jose, Calif.) as described in detail previously (37). Naïve, memory, and effector CD4<sup>+</sup> and CD8<sup>+</sup> T-cells were quantified by three-color flow cytometric analysis after staining with perdinin-chlorophyll *a* protein (PerCP)-conjugated CD4 or CD8 monoclonal antibodies (MAbs) in combination with fluorescein isothiocyanate-conjugated CD27 MAb and phycoerythrin-conjugated CD45RA (all from Becton Dickinson). In vivo activated and nonactivated CD4<sup>+</sup> and CD8<sup>+</sup> T cells were also quantified by three-color flow cytometric analysis after staining with PerCP-conjugated CD4 or CD8 MAbs in combination with fluorescein isothiocyanate-conjugated CD38 MAb and phycoerythrin-conjugated HLA-DR (Becton Dickinson). In brief, 50 µl of whole blood was mixed and incubated at room temperature with each combination of MAbs (5 µl of each) for 15 min in separate tubes in the dark. Erythrocytes were lysed by adding 1 ml of fluorescence-activated cell sorter lysing solution (50% diethylene glycol and 15% formaldehyde) (Becton Dickinson). After vortexing, the tubes were incubated in the dark at room temperature for 15 min and immediately centrifuged at 300 × g for 5 min. The supernatant was discarded, leaving approximately 50 µl of residual fluid

in the tube. Two milliliters of isoton (azide-free balanced electrolyte solution) was added to the cell pellet, mixed thoroughly, and centrifuged at the same speed and time interval. The supernatant was removed, and the residue was resuspended in 500  $\mu$ l of isoton. Events were acquired and analyzed using a FACScan flow cytometer with Cellquest software (Becton Dickinson). For acquisition and storage, a gate was set on side scatter and PerCP fluorescence to stop acquiring when 2,000 CD4<sup>+</sup> or CD8<sup>+</sup> T lymphocytes were collected. To analyze the events, a live gate was set first for all live events excluding debris, and then it was set for lymphocytes, monocytes, and granulocytes using the forward-versus-side light scattering property of the cells. CD4<sup>+</sup> and CD8<sup>+</sup> cells were gated on side scatter and PerCP fluorescence in order to get a minimum of 1,500 CD4<sup>+</sup> or CD8<sup>+</sup> T lymphocytes from the lymphocyte gate. Gated CD4<sup>+</sup> or CD8<sup>+</sup> bright events were used to quantify subsets and activation markers in dot plot-contour plot by setting quadrant markers. The percentage of events in each quadrant was used to calculate absolute values of the corresponding cell populations. The FACScan was calibrated with CaliBRITE fluorescent beads and FACScmp software (Becton Dickinson) weekly.

#### **Statistical analysis.**

Data were entered and analyzed using Dbase IV and STATA programs, respectively. The distribution of T-cell subsets and activation markers was compared between population groups using the Wilcoxon rank test. *P* values of <0.05 were considered significant.

#### **Ethics.**

This study is part of a long-term cohort study on the progression of HIV infection in Ethiopia which is ethically approved by both EHNRI and the National Ethical Clearance Committee. Informed consent was obtained from each subject.

### **Results**

#### **Lymphocyte subsets in HIV-negative Ethiopians from two study sites.**

Table 1 summarizes the median and 95th percentiles of lymphocyte subsets for adult Ethiopians from the Akaki and Wonji cohort sites. For comparison, values for healthy Dutch blood donors were included. First of all, it was confirmed that healthy Ethiopians have significantly lower ( $P < 0.0001$ ) CD4<sup>+</sup> T-cell counts than healthy Dutch subjects, independent of the geographical locale of blood sample collection. Second, CD8<sup>+</sup> T-cell counts from Akaki subjects were significantly higher ( $P < 0.0001$ ) than those from the other two groups. Since absolute CD4<sup>+</sup> T-cell counts did not differ between the two Ethiopian study groups, the higher CD8<sup>+</sup> T-cell counts resulted in significantly higher total T-cell and lymphocyte counts and a lower CD4/CD8 ratio in Akaki subjects. There were no statistically significant differences in the other lymphocyte subsets (B cells and NK cells) between the two Ethiopian groups. Akaki females showed increased CD4<sup>+</sup> T-cell percentages compared to Akaki males, resulting in a significantly higher CD4/CD8 ratio. There were no statistically significant gender differences in Akaki with respect to other white blood cell subsets.

**TABLE 1. Median values and 95th percentile reference ranges of leukocyte subsets of HIV-negative Ethiopian factory workers from Akaki and Wonji compared to those of Dutch blood donors**

Parameter	Median value <sup>a</sup> (95th percentile reference range) for:			
	Akaki females ( <i>n</i> = 87)	Akaki males ( <i>n</i> = 131)	Wonji males ( <i>n</i> = 562)	Dutch subjects ( <i>n</i> = 678) <sup>e</sup>
Lymphocytes	1,825 (937-2,993)	1,840 (1,046-3,280) <sup>b</sup>	1,653 (855-3,239)	2,000 (1,120-3,370)
CD4 cells	749 (386-1,355)	671 (391-1,145)	675 (337-1,257)	930 (490-1,750)
% of CD4 cells	42 (29-55) <sup>a</sup>	39 (23-54) <sup>b</sup>	41 (27-54)	48 (34-63)
CD8 cells	617 (258-1,301)	619 (249-1,933) <sup>b</sup>	475 (180-1,253)	460 (200-1,100)
% of CD8 cells	34 (16-54)	35 (17-61) <sup>b</sup>	29 (16-51)	24 (13-41)
CD4/CD8 ratio	1.20 (0.52-2.60) <sup>a</sup>	1.10 (0.41-2.57) <sup>b</sup>	1.40 (0.60-2.80)	1.96 (0.89-4.67)
T (CD3) cells	1,407 (741-2,329)	1,344 (759-2,742) <sup>b</sup>	1,224 (587-2,377)	1,470 (800-2,590)
B (CD19) cells	181 (63-331)	167 (47-437)	165 (64-451)	290 (110-670)
NK (CD16 <sup>+</sup> 56 <sup>+</sup> ) cells	183 (55-496)	211 (69-646)	195 (56-614)	NA <sup>d</sup>

<sup>a</sup> Significant difference compared with Akaki males, *P* value of <0.05, using the Wilcoxon rank test.

<sup>b</sup> Significant difference compared with Wonji males, *P* value of <0.001, using the Wilcoxon rank test.

<sup>c</sup> Significant difference compared with Ethiopian subjects from both sites, *P* value of <0.001, using the Wilcoxon rank test.

<sup>d</sup> NA, not available.

<sup>e</sup> Absolute counts are per microliter of whole blood.

### **Naïve, memory, and memory/effector subsets in HIV-negative Ethiopians from two study sites.**

To further study the significantly higher numbers of CD8<sup>+</sup> T cells in Akaki males than in Wonji males, CD4<sup>+</sup> and CD8<sup>+</sup> T cells were analyzed for numbers of naïve, memory, and memory/effector subsets as defined by differential expression of CD45RA and CD27 (1, 14) in 30 subjects from each site. Also in these smaller groups, the observations of low CD4<sup>+</sup> T-cell counts in Ethiopians from both sites compared to the Dutch and the increased CD8<sup>+</sup> T-cell counts in Akaki males compared to Wonji males were confirmed (data not shown).

As shown in Table 2, the significantly higher number of CD8<sup>+</sup> T cells in Akaki males compared to Wonji males resulted in a proportional increase in almost all CD8<sup>+</sup> T-cell subsets: naïve (CD45RA<sup>+</sup>CD27<sup>+</sup>, *P* = 0.007), memory (CD45RA<sup>+</sup>CD27<sup>+</sup>, *P* = 0.03), and cytotoxic effector (CD45RA<sup>+</sup>CD27<sup>-</sup>, *P* = 0.008). Only the memory/effector (CD45RA<sup>-</sup>CD27<sup>-</sup>) CD8<sup>+</sup> T-cell subsets showed no statistically significant difference in the two population groups. However, when percentages of CD8<sup>+</sup> T-cell subsets were compared, no statistically significant differences

were detected. All four compartments of the CD4<sup>+</sup> T cells, naïve (CD45RA<sup>+</sup>CD27<sup>+</sup>), memory (CD45RA<sup>-</sup>CD27<sup>+</sup>), memory/effector (CD45RA<sup>-</sup>CD27<sup>-</sup>), and CD45RA<sup>+</sup>CD27<sup>-</sup>, appeared comparable, both in percentages and absolute numbers (Table 2).

**TABLE 2. Medians and 95th percentile ranges of CD4<sup>+</sup> and CD8<sup>+</sup> T-cell subsets in HIV-negative Ethiopian factory workers in comparison with those of Dutch blood donors**

T-cell subset	Absolute count (cells/ $\mu$ l), % of total for <sup>a</sup> :		
	Akaki subjects (n = 30)	Wonji subjects (n = 30)	Dutch subjects (n = 108) <sup>b</sup>
CD4 <sup>+</sup> CD45RA <sup>+</sup> CD27 <sup>+</sup> (naïve)	134 (40-361), 22 (3-43)	128 (27-574), 20 (5-47)	380 (60-1,087), 38 (9-74)
CD4 <sup>+</sup> CD45RA <sup>-</sup> CD27 <sup>+</sup> (memory)	330 (191-889), 55 (17-67)	316 (110-610), 53 (40-66)	500 (200-1,004), 53 (20-76)
CD4 <sup>+</sup> CD45RA <sup>+</sup> CD27 <sup>-</sup>	10 (0-95), 1 (0-6)	7 (0-36), 1 (0-5)	0 (0-73), 0 (0-10)
CD4 <sup>+</sup> CD45RA <sup>-</sup> CD27 <sup>-</sup> (memory/effector)	130 (61-1,132), 21 (12-79)	137 (47-289), 21 (9-45)	60 (10-203), 6 (1-19)
CD8 <sup>+</sup> CD45RA <sup>+</sup> CD27 <sup>+</sup> (naïve)	166 (55-366), 27 (8-53)	117 (26-282) <sup>c</sup> , 29 (5-60)	225 (45-491), 44 (13-80)
CD8 <sup>+</sup> CD45RA <sup>-</sup> CD27 <sup>+</sup> (memory)	98 (29-419), 19 (7-44)	74 (22-254) <sup>c</sup> , 21 (6-48)	180 (40-383), 35 (9-71)
CD8 <sup>+</sup> CD45RA <sup>+</sup> CD27 <sup>-</sup> (cytotoxic effector)	217 (52-734), 35 (16-74)	103 (19-692) <sup>c</sup> , 34 (8-78)	30 (0-328), 6 (1-46)
CD8 <sup>+</sup> CD45RA <sup>-</sup> CD27 <sup>-</sup> (memory/effector)	51 (9-1,057), 9 (2-47)	37 (4-765), 9 (2-53)	20 (0-183), 4 (0-21)

<sup>a</sup> Values are medians. Values in parentheses are 95th percentile reference ranges. Asterisks denote *P* values of <0.05, using the Wilcoxon rank test.

<sup>b</sup> Significant difference compared with Ethiopian subjects from both sites.

<sup>c</sup> Significant difference compared with Akaki subjects.

Compared to Dutch subjects, Ethiopians had significantly reduced naïve and increased memory/effector subsets in both CD4<sup>+</sup> and CD8<sup>+</sup> T-cell compartments and increased cytotoxic effector cells in the CD8<sup>+</sup> T-cell compartment (*P* < 0.001).

#### Activated and resting T-cell subsets in HIV-negative Ethiopians from two study sites.

Finally, a combination of HLA-DR and CD38 MAbs was used to measure activated and resting T-cell subsets (13) in 20 subjects (10 from each site). Again, for CD4<sup>+</sup> T cells, no statistically significant difference was seen in absolute numbers or percentages of activated, resting, HLA-DR<sup>+</sup>CD38<sup>-</sup>, and HLA-DR<sup>-</sup>CD38<sup>+</sup> subsets between Akaki and Wonji participants (Table 3). Within the CD8<sup>+</sup> T-cell compartment, the higher absolute counts in Akaki subjects were reflected

by higher absolute counts of resting ( $P = 0.02$ ), HLA-DR<sup>+</sup>CD38<sup>-</sup> ( $P = 0.02$ ), and HLA-DR<sup>-</sup>CD38<sup>+</sup> T cells ( $P = 0.03$ ). No statistically significant differences were seen when percentages of these cells were compared.

**TABLE 3. Medians and 95th percentile ranges of activated and resting T-cell subsets in HIV-negative Ethiopian factory workers in comparison with those of Dutch blood donors**

T-cell subset	Absolute count (cells/ $\mu$ l), % of total for <sup>a</sup> :		
	Akaki subjects ( $n = 10$ )	Wonji subjects ( $n = 10$ )	Dutch subjects ( $n = 40$ )
CD4 <sup>+</sup> HLA-DR <sup>+</sup> CD38 <sup>-</sup>	92 (41-309), 13 (8-37)	86 (37-124), 13 (7-18)	50 (0-160), <sup>b,c</sup> 6 (1-20) <sup>b,c</sup>
CD4 <sup>+</sup> HLA-DR <sup>+</sup> CD38 <sup>+</sup> (activated)	55 (32-123), 7 (5-12)	52 (34-94), 8 (5-11)	20 (0-60), <sup>b,c</sup> 2 (0-6) <sup>b,c</sup>
CD4 <sup>+</sup> HLA-DR <sup>-</sup> CD38 <sup>-</sup> (resting)	326 (240-831), 48 (33-62)	290 (181-583), 43 (28-62)	430 (50-1,340), <sup>c</sup> 49 (4-75)
CD4 <sup>+</sup> HLA-DR <sup>-</sup> CD38 <sup>+</sup>	188 (109-558), 28 (17-38)	230 (66-558), 39 (13-46)	390 (50-730), <sup>b,c</sup> 42 (6-69) <sup>b</sup>
CD8 <sup>+</sup> HLA-DR <sup>+</sup> CD38 <sup>-</sup>	224 (82-827), 32 (20-51)	101 (29-311), <sup>b</sup> 30 (18-53)	60 (0-630), <sup>b</sup> 14 (1-65) <sup>b,c</sup>
CD8 <sup>+</sup> HLA-DR <sup>+</sup> CD38 <sup>+</sup> (activated)	93 (20-459), 12 (4-28)	57 (24-84), 15 (8-29)	10 (0-80), <sup>b,c</sup> 3 (0-23) <sup>b,c</sup>
CD8 <sup>+</sup> HLA-DR <sup>-</sup> CD38 <sup>-</sup> (resting)	297 (96-666), 38 (20-67)	120 (37-281), <sup>b</sup> 30 (15-60)	320 (40-620), <sup>c</sup> 75 (7-91) <sup>b,c</sup>
CD8 <sup>+</sup> HLA-DR <sup>-</sup> CD38 <sup>+</sup>	79 (17-285), 12 (3-29)	49 (20-183), <sup>b</sup> 16 (7-37)	20 (0-140), <sup>b,c</sup> 6 (0-24) <sup>b,c</sup>

<sup>a</sup> Values are medians. Values in parentheses are 95th percentile reference ranges. Asterisks denote  $P$  values of  $<0.05$ , using the Wilcoxon rank test.

<sup>b</sup> Significant difference compared with Akaki subjects.

<sup>c</sup> Significant difference compared with Wonji subjects.

Compared to Dutch subjects, Ethiopians had significantly higher numbers of activated and HLA-DR<sup>+</sup>CD38<sup>-</sup> CD4<sup>+</sup> and CD8<sup>+</sup> T cells, lower resting CD8<sup>+</sup> T cells, and increased HLA-DR<sup>-</sup>CD38<sup>+</sup> CD8<sup>+</sup> T cells ( $P < 0.05$ ).

## Discussion

The present study was performed to assess the applicability of previously determined (37) reference values for immunohematological markers in the Ethiopian setting.

In agreement with earlier studies (17, 19, 25, 26, 37, 41), the remarkably lower CD4<sup>+</sup> T-cell counts of Ethiopians was confirmed in subjects from a second cohort site (Wonji). This could have consequences for HIV-1 disease progression in Ethiopians. Previous studies have confirmed the applicability of the WHO staging system for HIV infection progression in Ethiopia (19), and provisional values were established for CD4<sup>+</sup> T-cell counts, representing the various stages. However, it should be kept in mind that the numbers of observations for these analyses

were low, and it remains to be proven whether, for example, a lower preinfection CD4 count would be associated with a faster progression to AIDS. Thus, a more detailed analysis of clinical data and T-cell subset counts in a large number of individuals representing the various stages of HIV disease progression is recommended.

The finding of low CD4<sup>+</sup> T-cell counts in Ethiopians is comparable to those reported for Chinese adults (18) but lower than the values reported for other Africans (22, 38, 42). Interestingly, the CD4 values reported by Urassa et al. (39) for healthy adults from Dar es Salaam, Tanzania, are lower than those reported for rural Tanzania (19), indicating the heterogeneity of the African population, though methodological and other sources of variability in CD4 counting could also account for the observed differences.

In the present study, females were found to have significantly higher percentages of CD4<sup>+</sup> T cells and CD4/CD8 ratios ( $P < 0.05$ ) and relatively higher absolute CD4<sup>+</sup> T-cell counts than males. Several studies have reported similar observations of higher CD4<sup>+</sup> T-cell counts in females compared to males in both Africans and Caucasian populations (Clinical monograph no. 1, Becton Dickinson Immunocytometry Systems) (8, 18, 21, 24, 27, 28, 38). It has been suggested that a sex hormone effect could be one possible explanation for the reported gender difference in CD4 counts (24). A statistically significant difference in CD4 counts has also been observed between males from Akaki and Wonji. However, the clinical relevance of these differences in terms of patient management remains to be elucidated. The most interesting finding of the present study is the significant differences in CD8<sup>+</sup> T cells between Akaki and Wonji males. The higher CD8<sup>+</sup> T-cell counts in Akaki agree with previous reports for Ethiopians (17, 25, 37, 41), but Wonji subjects had significantly lower CD8<sup>+</sup> T-cell counts than those detailed in these reports.

Further analysis of CD8<sup>+</sup> T-cell subsets using different combinations of MAbs, well known to separate T cells into functional subsets, like naïve, memory, effector, activated, and resting cells, however, did not detect qualitative differences between Akaki and Wonji males. The quantitative differences detected for CD8<sup>+</sup> T cells as a whole were reflected in the absolute numbers of most CD8<sup>+</sup> T-cell subsets, which made these subsets of limited informative value for explaining the observed differences in the two population groups. A dramatic change in the CD8 subset composition, mainly expansion of the memory cell types, has been observed in acute viral infections (32). In addition, chronic antigenic stimulation has been shown to result in the loss of CD27 antigen expression (15, 16). Since we observed no specific expansion of certain T-cell subsets, our data suggest that the difference in CD8<sup>+</sup> T-cell counts may not be attributed to a specific response of these cells to an endemic infectious disease. Such imbalances in the CD8 subset composition would have been expected in the Akaki subjects if acute or chronic viral infections were the underlying causes for the observed differences between the two populations. However, other environmental factors, like nutrition or altitude, or genetic differences could cause the higher total CD8 count in the Akaki subjects and should be the subject of further studies. In this regard, it would be of interest to study children and females from both sites. In summary, it can be concluded that caution should be taken in presenting immunological reference data on particular groups of Ethiopians as valid for the entire population. In this respect it could be mentioned that the Ethiopian population is extremely heterogeneous, living at high altitudes of up to 4,000 m and in lowlands at sea level, with more than 120 ethnic groups speaking over 80 different languages and being from Semitic, Cushitic, and Nilotic origins.

Remarkably, although the total CD8 count was different between the two geographical areas, the present study confirmed that the immune system of the Ethiopians is in a highly activated state, independent of the geographic locale of sample collection. This is in agreement with earlier reports (17, 25) and makes this observation likely to be more generally applicable in Ethiopia. Similar observations have been reported for Ugandans (29). It has been hypothesized that the higher activation state of the immune system of Ethiopians reflects an increased load of intestinal parasitic infections, particularly helminthes (3, 4, 17). Although the subjects of this study were found to be negative for intestinal parasites at the time of the investigation, it is highly likely that they might be infected and dewormed several times in their lifetime, resulting in chronic and persistent antigenic stimulation. This would be possible since intestinal parasitism is common in Ethiopia, and prevalence rates as high as 70% have been reported (11, 20). Nutritional factors (10, 35) or ethnic composition (6) could also be involved. Although genetic factors were reported to play a role (6), recently Clerici et al. (7) demonstrated that immune activation in Africans is environmentally driven and not genetically predetermined.

In conclusion, this study confirms and extends earlier observations on fundamental differences between the immune systems of Ethiopians and others (17, 19, 25, 26, 37, 41). It also indicates that caution should be taken in extrapolating immunological reference values from one population group to another.

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