Due to the importance of determining the proportions of lymphocyte subpopulations in Mexicans as reference values for flow cytometry, the aim of this study was to establish CD4⁺ and CD8⁺ T cell reference values for healthy Mexicans according to gender and age. Our results may serve as reference standards for the Mexican city population.

The quantification of CD4⁺ and CD8⁺ T lymphocytes is the most important laboratory test used to evaluate and monitor the success of antiretroviral treatment for HIV infection (1, 2). In Mexico, this test has been performed for more than 12 years. However, the reference values for healthy people are adopted from textbooks or the flow cytometry supplier and are usually based on Caucasian populations. Studies in different countries have reported variations in T lymphocyte subset reference values, emphasizing the importance of considering gender, age, environment, and ethnicity when evaluating cell counts (3, 4). The objective of this study was to determine reference values for absolute CD4⁺ and CD8⁺ T cell counts for the adult Mexican population according to sex and age.

**Subjects.** A total of 400 healthy Mexicans (200 women and 200 men) participated in this study. A demographic and epidemiological questionnaire was administered to all participants to gather information on age, address, education level, and family history. All individuals who were born in Mexico City and had ancestors born in Mexico City for at least three generations were included. Individuals from well-delimited ethnicities were not included in this analysis. The epidemiology of HIV in Mexico has been reported to be 4 HIV-infected adults for every 1,000 Mexicans. With regard to age group, people between 25 and 34 years of age represent the highest percentage of AIDS cases. Thus, the participants in the present study were grouped into the following age ranges: 20 to 25, 26 to 30, 31 to 35, and 36 to 40 years old. We analyzed 50 individuals in each age and gender group. All study participants were examined by a medical doctor. The health statuses of the participants were assessed by reviewing their medical records and conducting a physical examination and clinical laboratory tests, including a negative HIV probe. The participants were recruited from private medical care facilities and medical societies. The samples were analyzed at the National Reference Institute Instituto de Diagnostico y Referencias Epidemiologicos in Mexico City. Patient information and informed consent letters were signed by all participants.

**Specimen characteristics.** Whole blood was collected with a Vacutainer system in 5-ml tubes containing EDTA. The samples were processed within 10 h of collection.

**Flow cytometric analysis.** Lymphocyte subsets were analyzed with a FACSCount (Becton, Dickinson) with monoclonal antibodies (MAbs) against the following proteins: immunoglobulin G1 and immunoglobulin G2 as controls, CD3, CD3-CD4, and CD3-CD8. In addition to the monoclonal antibodies, the reagent tubes also contained fluorochrome-labeled reference beads, which act as a fluorescence standard to identify lymphocytes and a quantification standard (CD3 PE-Cy5 and CD4 phycoerythrin [PE], CD3 PE-Cy5 and CD8 PE). To evaluate accuracy and linearity, the instrument was calibrated with control beads at four different concentrations (0, 50, 250, and 1,000 particles/50 µl). In brief, 50 µl of whole blood was mixed with 10 µl of each MAb in separate tubes and incubated at room temperature for 60 min. Subsequently, 50 µl of a fixative solution was added, and after being vortexed, the tube was incubated for 30 min. The samples were then vortexed and analyzed with the FACSCount.

The data were analyzed using SPSS statistical software, version 15. The mean, median, and standard deviation (SD) were calculated for each parameter.

Blood samples from a total of 400 healthy Mexican adults (200 women and 200 men) were analyzed. The median weights were 59.95 kg for women and 70.62 kg for men, and the median heights were 159.35 cm for women and 169.45 cm for men. The total median lymphocyte percentages ranged from 17.8 to 46.6% for

**TABLE 1** Means and standard deviations of CD4⁺ and CD8⁺ cells by age group and gender

| Gender and age group (no. of subjects) | Mean no. of cells/µl (SD) | CD4⁺ | CD8⁺ | CD4/CD8 ratio (SD) |
|---------------------------------------|---------------------------|------|------|-------------------|
| **Women**                             |                           |      |      |                   |
| 20–25 (50)                            | 783.75 (198.06)           | 555.86 (180.09) | 1.40 (0.5) |
| 26–30 (50)                            | 766.94 (244.48)           | 532.02 (191.67) | 1.43 (0.7) |
| 31–35 (50)                            | 851.00 (286.06)           | 559.80 (192.11) | 1.52 (0.6) |
| 36–40 (50)                            | 871.28 (222.50)           | 464.89 (168.47) | 1.87 (0.5) |
| Total (200)                           | 818.24 (220.50)           | 528.14 (188.37) | 1.5 (0.4) |
| **Men**                               |                           |      |      |                   |
| 20–25* (50)                           | 739.42 (211.48)           | 513.25 (210.86) | 1.44 (0.5) |
| 26–30 (50)                            | 810.43 (280.88)           | 607.08 (307.09) | 1.33 (0.6) |
| 31–35 (50)                            | 853.9 (249.05)            | 497.56 (181.64) | 1.71 (0.4) |
| 36–40* (50)                           | 944.84 (323.8)            | 621.07 (275.76) | 1.52 (0.5) |
| Total (200)                           | 837.14 (260.80)           | 559.74 (230.75) | 1.5 (0.4) |

* Significant difference between these groups (P < 0.001).
women and from 17.8 to 46.4% for men. The mean leukocyte counts ranged from 3.5 to 9.03 cells/mm³ for women and from 3.6 to 7.4 cells/mm³ for men.

Lymphocyte subset reference range parameters may be influenced by factors such as age and gender. Indeed, when we analyzed lymphocytes by age group, we found a significantly higher CD4⁺ T cell count in men 36 to 40 years old than in men between 20 and 25 years old (Table 1). Similar results were found in a study of 208 Chinese individuals, in whom an increase of CD4 of 1.6% per decade was reported (5). Additionally, a comparison between newborn infants, infants aged 2 days to 11 months, children aged 1 to 6 years, children aged 7 to 17 years, and adults aged 18 to 70 years showed an increase in both CD4⁺ and CD8⁻ subsets with age (6). However, other studies have shown different results. In a German study, a significant decline in CD8⁻ T cells was observed beyond the age of 50 years old (7). In a study conducted in Burkina Faso, there was no statistically significant influence of age on the distribution of lymphocyte subpopulations (8). These discrepancies may be due to the age cohorts analyzed in the studies. The impact of age on CD4⁺ and CD8⁻ T cells and the senescence of the immune response must be studied. Regarding gender, a higher number of CD4⁺ cells have been reported in women than in men in Indian, Spanish, and Chinese populations (4, 5, 9); however, in the present study, we did not find a significant difference between women and men (Table 1).

To identify marked differences in world populations, we compared our results with those of unrelated populations, including Ethiopian, Saudi (males), Dutch, and Indian populations (Table 2). Our results were similar to those obtained from the male Saudi population (10). Compared to the Ethiopian population, we observed that Mexicans had a lower median CD8⁻ count, resulting in a slightly higher proportion of CD4⁺/CD8⁻ cells in Mexicans than in Ethiopians (1.5 ± 0.6 versus 1.2 ± 0.5). In comparison to the Dutch population, Mexicans had a lower median CD4⁺ count (11). Finally, Indian and Ugandan (19- to 24-year-old) females had a higher median CD4⁺ count than Mexicans (4, 12).

It is important to note two characteristics. (i) Mexicans have lower median CD4⁺ and CD8⁻ counts than at least 3 populations. These results can be reflected in people with HIV. In this context, it has been reported that on their first clinical visit, Hispanic HIV patients had lower median cell counts (median, 220 cells/mm³) than black (median, 318 cells/mm³) or white (median, 372 cells/mm³) patients (13). (ii) The differences in CD4⁺ cell counts are found principally in women. The number of CD4⁺ T cells in circulation is influenced by race and environmental factors. Therefore, when we compared our results with those of other populations, we expected to find differences. It would be interesting to analyze the relationship between CD4⁺ cell counts and female hormone variations.

The differences between our results and those from other populations may be due to well-defined confounding variables (14), such as genetic background, lifestyle (exercise and nutrition), environmental exposure (pollution and infectious pathogens), or even vaccination history. In this context, a Spanish study demonstrated the influence of lifestyle factors on lymphocyte counts and reported correlations between physical activity and an increased percentage of lymphocytes and a higher percentage of CD4⁺ cells in those who consume alcohol (9).

The need to estimate Mexican lymphocyte subpopulation reference values has increased due to the importance of monitoring CD4 T cells during the progression of human immunodeficiency virus infection. For this reason, the aim of this study was to establish CD4⁺ and CD8⁻ T cell reference values according to gender and age in healthy Mexicans living in Mexico City. Our results may serve as reference standards for the Mexican city population.

TABLE 2 CD4⁺ and CD8⁻ counts in healthy individuals from different populations

| Gender and cell subset | Present study (n = 400) | Saudi | Ethiopian | Dutch | Indian |
|------------------------|------------------------|-------|-----------|-------|-------|
| Women                  |                        |       |           |       |       |
| CD4⁺                   | 818.24 ± 220.50        | 14    | 13        |
| CD8⁻                   | 528.14 ± 188.37        | 14    |           |
| CD4/CD8 ratio          | 1.5 ± 0.5              | 14    |           |
| Men                    |                        |       |           |       |       |
| CD4⁺                   | 837.14 ± 260.80        | 11    | 14        | 13    |
| CD8⁻                   | 559.74 ± 230.75        | 11    | 14        |       |
| CD4/CD8 ratio          | 1.50                   | 11    | 14        |
| Overall                |                        |       |           |       |       |
| CD4⁺                   | 800 ± 230.10           | 14    | 14        | 13    |
| CD8⁻                   | 526 ± 210.70           | 14    | 14        |
| CD4/CD8 ratio          | 1.5 ± 0.6              | 14    | 14        |

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