Flow Cytometric Analysis of Peripheral Blood Lymphocyte Immunophenotypes in Persons Infected with Treponema pallidum

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To characterize the human immune response to syphilis, we determined the effect of infection with Treponema pallidum on the percentage of the various lymphocyte subpopulations in the peripheral blood of infected and uninfected persons. Monoclonal antibodies labeled with either fluorescein isothiocyanate or phycoerythrin were used to perform dual color analysis on a FACScan with the following markers: CD3 for total T cells, CD4 for T helper cells, CD8 for T suppressor cells, CD19 for B cells, and CD16 plus CD56 for natural killer cells. Lymphocyte immunophenotype results were analyzed by the stage of untreated syphilis and by gender. Although they were within the ranges of the normal distribution of immunophenotypes, the percentages of CD4+ cells were significantly lower (P < 0.001) and those of CD8+ cells were higher (P = 0.03) in patients with syphilis than in the uninfected population. For infected versus uninfected subjects, both women and men, the differences in the mean percentages of CD3+ and CD4+ cells were significant (P ≤ 0.05). Significant differences were noted between the sexes in secondary syphilis only in the mean percentages of cells positive for CD3, CD4, CD8, and CD16 plus CD56. Gender had no effect on lymphocyte subpopulations in subjects with primary or latent syphilis. In the control population, significant differences due to gender were observed in the percentages of cells positive for CD3, CD4, and CD16 plus CD56.

As flow cytometric methods have improved, analysis of peripheral blood lymphocytes (PBL) in various disease states (11, 19) has become widely used. Flow cytometric methods are routinely used in evaluating the PBL of persons with acquired immunodeficiency syndrome, in which the effect of the human immunodeficiency virus on PBL is well established (11, 14). However, the effect of diseases such as syphilis on lymphocyte subsets has not been investigated.

Several investigators have shown that T cells are important in the immune response to Treponema pallidum (1, 14, 18, 20). Large numbers of T cells, both helper and suppressor, and some B cells and macrophages infiltrate syphilitic lesions. However, it is unclear how T. pallidum affects the response among various lymphocyte subsets in peripheral blood. In a preliminary study, Jensen and From (9) used rosetting techniques to determine total T cell, T helper (Th), cell, and T suppressor (Ts) cell subsets but did not evaluate B-cell or natural killer (NK) cell subsets.

To assess the effect of T. pallidum infection on populations of PBL from persons with primary, secondary, and latent syphilis, we evaluated PBL by dual color analysis with a FACScan (Becton Dickinson Immunocytometry Systems, San Jose, Calif.) fluorescence-activated cell sorter and monoclonal antibodies labeled with either fluorescein isothiocyanate (FITC) or phycoerythrin (PE). The Becton Dickinson FACScan with Simulset software (Becton Dickinson Immunocytometry Systems) was used for all analyses. Since the samples were sent by overnight mail, we were not able to do complete blood counts for the patients, and thus data for absolute counts were not available.

Heparinized blood was obtained from 235 healthy individuals participating as normal controls in the Centers for Disease Control and Prevention (CDC) serum bank donor program. These samples were used to establish normal ranges for PBL subpopulations. Heparinized blood was also collected, with informed consent, from 220 patients who had been diagnosed with and then treated for syphilis after the blood was collected and from 11 individuals without clinical signs or symptoms of any sexually transmitted diseases by the Richmond City Health Department Sexually Transmitted Diseases Control Program, Richmond, Va. The samples were sent by overnight express mail to CDC. Patients were classified as having primary, secondary, or latent syphilis of 2 years or less in duration. The 11 samples from patients without syphilis served as a control for the effect of shipping. Histories of infection with and treatment for syphilis were taken at the first clinic visit. Serum was collected for the rapid plasma reagin card test, the microhemagglutination assay for T. pallidum, and the fluorescent antibody-absorption test as well as for enzyme immunoassay and Western blot (immunoblot) tests for human immunodeficiency virus (HIV).

All samples from Richmond were processed on the day that they were received, while control samples from CDC were usually held overnight at room temperature before being processed. We used several monoclonal antibody combinations: anti-CD45-FITC and anti-CD14-PE for determination of lymphocyte gate; anti-mouse gamma 1-FITC and antimouse gamma 2-PE as nonfluorescent and nonspecific fluorescence controls; anti-CD3-FITC and anti-CD19-PE for T and B cells; anti-CD3-FITC and anti-CD16-PE plus anti-CD56-PE for NK cells; and anti-CD4-FITC and anti-CD8-PE for T+ and...
TS cells. Standard procedures for the lysed whole blood method were used, with FACSLyse (Becton Dickinson Immunocytometry Systems) as the lysing reagent. The final cell pellet was resuspended in 0.5 ml of 1% formaldehyde, and the cells were stored at 5°C until they were analyzed (within 18 h). Blood from a presumed normal subject was processed as described above and included in each run as a control.

The data for diagnosis and treatment, serologic tests for syphilis, and percentage of each immunophenotype were entered into EpiInfo software (CDC, Atlanta, Ga., and World Health Organization, Geneva, Switzerland) and analyzed with the EpiInfo and Proc Univariate (PC-SAS) software. Because the data were not evenly distributed, Kruskal-Wallis P values were used.

All serologic tests for syphilis and HIV infection were done at CDC. The rapid plasma reagin card test, microhemagglutination assay for antibodies to T. pallidum, and fluorescent treponemal antibody-absorption test were all performed according to the standardized procedures in the 1990 Manual of Tests for Syphilis (3, 8, 10). HIV testing was done with an enzyme-linked immunosorbent assay (Abbott Laboratories, Abbott Park, Ill.) for screening and Western blotting (Cambridge-Biotech, Rockville, Md.) for confirmation of reactive specimens.

Normal ranges for the subsets of lymphocytes were determined. The ranges, covering the 5 to 95% portion of the frequency distribution, and the means are shown in Table 1. The results were also analyzed by gender (Table 1). The ranges obtained were similar to those in other studies (2, 4, 6, 7, 19).

Our healthy female subjects tended to have a higher percentage of total T cells (P = 0.004) and TH cells (P = 0.01) but lower percentages of NK cells (P = 0.02) than the healthy male subjects.

Patients from the Richmond clinic were excluded from the analysis if they had received an antibiotic within 1 month prior to the initial visit, if they had any other sexually transmitted disease, or if they tested positive for HIV antibodies. This left 19 samples from patients with primary syphilis, 78 samples from patients with secondary syphilis, and 69 samples from patients with latent syphilis. Because only six patients had a previous history of syphilis, they were not singled out for separate analysis but were included with the others.

The immunophenotypes expressed in the peripheral blood were also analyzed by gender for the clinic patients. The ranges and mean percentages of lymphocyte subsets of syphilis patients, for all the individuals and by gender, are listed in Table 1. For individuals for whom gender was indicated, we had 12 patients with primary, 59 with secondary, and 48 with latent syphilis. Infection with T. pallidum appeared to have a minor effect on the mean percentages of lymphocyte immunophenotypes compared with those of the healthy subjects (Fig. 1). When the results were analyzed by gender for each stage of syphilis, there were significant differences only in secondary syphilis for CD3+ (P = 0.03), CD4+ (P < 0.001), CD8+ (P = 0.007), and CD16+ plus CD56+ (CD16,56+) (P = 0.015) cells (Table 2). When we considered only gender and not the stage of syphilis, there were only significant differences between men and women in the mean percentages of CD4+ (P = 0.02) and CD8+ (P = 0.01) cells compared with healthy individuals.

We also compared the data generated for the various stages of syphilis with the results obtained for the normal population by gender. Females and males with syphilis both had significant differences in the mean percentages of CD3+ (P = 0.04 and 0.008 for women and men, respectively) and CD4+ (P = 0.02 for both) cells compared with the normal group.

When Jensen and From (9) looked at changes in T-cell subtypes using rosetting methods on Ficoll-Hypaque-purified

### TABLE 1. Percentage ranges and means of lymphocyte immunophenotypes

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Total T (%CD3+)</th>
<th>TH (%CD4+)</th>
<th>TS (%CD8+)</th>
<th>B (%CD19+)</th>
<th>NK (%CD16,56+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All (246)</td>
<td>54–85 (72.1)</td>
<td>32–58 (44.1)</td>
<td>19–44 (31.2)</td>
<td>6–23 (13.3)</td>
<td>5–23 (12.2)</td>
</tr>
<tr>
<td>Females (52)</td>
<td>66–87 (76.6)</td>
<td>31–59 (45.0)</td>
<td>19–44 (33.2)</td>
<td>5–21 (12.2)</td>
<td>4–19 (10.4)</td>
</tr>
<tr>
<td>Males (51)</td>
<td>54–86 (71.1)</td>
<td>29–52 (41.1)</td>
<td>20–44 (34.3)</td>
<td>7–26 (13.8)</td>
<td>4–26 (13.2)</td>
</tr>
<tr>
<td>P</td>
<td>0.004</td>
<td>0.01</td>
<td>0.64</td>
<td>0.23</td>
<td>0.02</td>
</tr>
<tr>
<td>Syphilis cases</td>
<td>59–83 (71.2)</td>
<td>27–55 (40.5)</td>
<td>22–54 (34.0)</td>
<td>5–25 (14.3)</td>
<td>3–24 (10.9)</td>
</tr>
<tr>
<td>All (166)</td>
<td>59–82 (72.4)</td>
<td>30–55 (42.1)</td>
<td>22–48 (32.0)</td>
<td>7–25 (14.9)</td>
<td>3–22 (10.0)</td>
</tr>
<tr>
<td>Females (87)</td>
<td>54–84 (71.2)</td>
<td>23–55 (38.7)</td>
<td>21–58 (36.2)</td>
<td>4–26 (13.6)</td>
<td>4–26 (12.0)</td>
</tr>
<tr>
<td>Males (79)</td>
<td>0.54</td>
<td>0.02</td>
<td>0.01</td>
<td>0.12</td>
<td>0.08</td>
</tr>
</tbody>
</table>

* Because gender was not indicated for every subject, only selected subjects were included in the analysis by gender.

* For females versus males.
mononuclear cells, they found that patients with primary syphilis had decreased T\textsubscript{H} cell and increased T\textsubscript{S} cell levels, whereas T\textsubscript{H} cell levels were normal and T\textsubscript{S} cell levels decreased in persons with secondary syphilis. We examined the effects of syphilis on T, B, and NK cells circulating in the peripheral blood system. Lymphocyte subsets varied by clinical stage of syphilis. We found that in all stages of syphilis, there were decreases in the percentages of T\textsubscript{H} cells. The numbers of T\textsubscript{S} cells were increased in persons with secondary and latent syphilis but unchanged in persons with primary syphilis. Whether the differences in our results from those of Jensen and From were due to technique or patient population are undetermined at this time. Ficoll-Hypaque separation has been reported to artificially reduce the number of T\textsubscript{S} cells, but whether CD8\textsuperscript{+} cells from someone infected with T. pallidum are depleted to a greater degree is unknown.

Several groups have demonstrated that T cells were the predominant infiltrating cell in syphilitic lesions (5, 15, 16). Engelkens et al. (5) reported that primary syphilitic lesions had predominantly CD4\textsuperscript{+} cells, while secondary syphilitic lesions had either an equal number of CD4\textsuperscript{+} and CD8\textsuperscript{+} or more CD8\textsuperscript{+} cells. We found that patients with secondary and early latent syphilis had a significantly increased percentage of CD8\textsuperscript{+} cells in the peripheral blood, while the CD8\textsuperscript{+} cell levels of those with primary syphilis were unchanged from those of healthy individuals. The percentages of circulating CD4\textsuperscript{+} cells were decreased in all stages, but those of total T cells were unaffected. This finding suggests that the presence of CD8\textsuperscript{+} cells in the lesions, especially in secondary syphilis, was not due solely to CD8\textsuperscript{+} cells migrating from the peripheral blood supply but rather to an increased proliferation of these cells. An increased number of CD8-positive cells is not unexpected in latent syphilis, since there is a decrease in antibody levels and CD8\textsuperscript{+} cells act to suppress or modulate the antibody production.

In our study, we found that the mean percentages were within the normal ranges for each immunophenotype, even when the differences were significant. However, gender differences were significant for both healthy subjects and those with syphilis. In secondary syphilis, there were significant differences between men and women in the mean percentages of T\textsubscript{H} and T\textsubscript{S} cells. In primary and latent cases of syphilis, gender had no effect on the mean percentages of the various lymphocyte subsets. Because the number of persons with primary syphilis in our sample was very small, the significance of these results may change as PBL from more people with primary syphilis are examined in future studies. However, in summary, we found that infection with T. pallidum had very little significant effect on the lymphocyte subpopulations in the peripheral blood.

REFERENCES


