Increase in the Helper Inducer (CD4+CD29+) T Lymphocytes in Smokers

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Abstract: Previously, we reported an increase in the number of CD4+ T lymphocytes in the peripheral blood of smokers. In the present study, subpopulations of CD4+ T lymphocytes together with CD8+ T lymphocytes, B (CD19+) lymphocytes, natural killer (CD16+) cells and total lymphocytes were examined by two-color staining using anti-2H4 (CD45RA) and anti-4B4 (CD29) monoclonal antibodies in 8 male smokers and 22 age-matched male non-smokers. The number of CD4+CD29+ T lymphocytes in smokers was significantly higher than that in non-smokers. The total number of CD4+ T lymphocytes in smokers was also significantly higher. No significant differences in CD8+ T, CD19+ B lymphocytes and CD16+ NK cells were found between smokers and non-smokers. Thus, it is suggested that the increase in the number of CD4+CD29+ (helper inducer) T lymphocytes is responsible for the increase in total CD4+ T lymphocytes in smokers.

Key words: Smoking, CD4+CD29+ T lymphocytes, Helper inducer T lymphocytes, T lymphocyte subpopulation, Two color staining, Flow cytometry, Immune system

Recent advances in flow cytometry technology have enabled subpopulations of human lymphocytes in occupational and environmental health to be measured. Epidemiological data have been accumulated concerning the effects of lead, aluminum, asbestos, pentachlorophenol, mixture of organic solvents, 2,3,7,8-tetrachlorodibenzop-dioxin and toluene diisocyanate.

The increase in CD4+ T lymphocytes in smokers has also been reported. Miller et al. have reported that CD8+ T lymphocytes are increased in heavy smokers compared to light-to-moderate smokers and non-smokers. Torellud et al. reported an increase in CD4+ T lymphocytes in 282 smokers. The increase in CD4+ T lymphocytes in smokers has also been reported in Japanese.

In the present study, to identify increased CD4+ T lymphocyte subpopulations in smokers, we measured the minute subpopulations of CD4+ T lymphocyte subpopulations, CD4+CD45RA+ and CD4+CD29+ T lymphocytes, in peripheral blood. CD4+ T lymphocytes are classified into the CD4+CD45RA+ T lymphocytes (suppressor-inducer cells) and CD4+CD29+ T lymphocytes (helper-inducer cells) using anti-CD45RA (2H4) and anti-CD29 (4B4) monoclonal antibodies, respectively. In addition, CD8+ T lymphocytes (another component of T lymphocytes), CD3+ T (total mature T lymphocytes), B (CD19+) lymphocytes and natural killer (NK)(CD16+) cells were measured.

Thirty healthy male workers without past histories of exposure to hazardous substances were studied. They underwent a medical interview and health check-up by one...
of the authors and had neither histories nor symptoms that were related to immunological changes. The subjects were then divided into two groups. One group consisted of 8 current smokers aged 32 to 57 (mean 50 years) and the other 22 non-smokers aged 34 to 66 (mean 55 years). No significant difference in age between them was found (p>0.05, Wilcoxon's rank sum test). The study was conducted with their informed consent.

Two-color direct immunofluorescence surface maker analysis was performed using the following pairs of monoclonal antibodies: anti-Leu18 (equivalent to 2H4, CD45RA)/anti-Leu3 (CD4), anti-4B4 (CD29)/anti-Leu3 (CD4), anti-Leu3 (CD4)/anti-Leu2 (CD8), anti-Leu7 (CD57)/anti-Leu11 (CD16) and mouse IgG1/mouse IgG2a (negative control). In each pair of monoclonal antibodies, the former was conjugated with fluorescein isothiocyanate (FITC) and the latter with phycoerythrin (PE), respectively. Anti-4B4 antibody was purchased from Coulter Immunology (USA) and all the other monoclonal antibodies were purchased from the Becton Dickinson (USA).

Venous blood was taken from each subject between 8 and 10 a.m. A 100 microliter of 2K-EDTA added blood was incubated with each pair of monoclonal antibodies for 15 min in the dark at room temperature. All antibodies were used at optimal dilutions. After incubation, 2ml of 1 × FACS Lysing Solution (Becton Dickinson, USA) diluted 10 times with distilled water, was added to each sample and the samples were mixed gently, and incubated for a further 10 min in the dark to lyse the erythrocytes. Samples were centrifuged at 1,000 rpm for 5 min and the supernatant was aspirated. The pelleted cells were resuspended in 2 ml of phosphate buffered saline (PBS). After re-centrifugation, the pelleted cells were resuspended in 0.5ml of PBS and then analyzed for immunofluorescence on a flow cytometer (FACScan, Becton Dickinson, USA).

Non-lymphocytic cells contaminating the preparations were excluded from analysis using scatter gates set on the 90° and the straight light scatter profile. Two-color fluorescence data were displayed as log green fluorescence on the X axis and log red fluorescence on the Y axis. The two color dot plot was divided into four quadrants by setting the X and Y cursors. The right shift was found in all samples of cell populations stained with FITC-conjugated anti-4B4 antibody. As a result, the Y axis cursor was set at the narrowest position of the dot pattern between 4B4+ and 4B4− cell populations. A representative pattern of two-color staining of lymphocytes by monoclonal antibodies anti-4B4 (CD29) and anti-Leu3 (CD4) is shown in Figure 1. Thirty thousand cells were analyzed for each sample and the number of fluorescinated cells were expressed as a percentage of the total lymphocytes. Total lymphocyte counts in peripheral blood were obtained by an automated cell count analyzer (Coulter Counter SP-VI, Coulter Electronics, USA). Non-specific staining, assessed using the negative control was always less than 1%.

The number of each lymphocyte subpopulation was calculated by multiplying lymphocyte counts by the percentage of positive cells in each category, as determined by the flow cytometer. Differences in the number of all lymphocyte subpopulations in this study between smokers and non-smokers were analyzed by the Wilcoxon's rank sum test.

The number of all measured lymphocyte subpopulations in 8 smokers and 22 non-smokers are shown in Table 1 and Figure 2. The number of CD4+CD29+ T lymphocytes in the smokers was significantly higher than that in the non-smokers. The total number of CD4+ T lymphocytes in the smokers was also significantly higher. No significant differences in CD8+ T, CD19+ B lymphocytes and CD16+ NK cells were found between the smokers and the non-smokers. No significant difference in total white blood cell counts was found between the two groups.

In the present study, an increase in CD4+CD29+ T lymphocytes was found in smokers. It is suggested that the increase in total CD4+ T lymphocytes in smokers reported previously8,10 may be due to a selective increase in the helper
inducer CD4+ T lymphocytes.

Sanders et al.\textsuperscript{13} designated CD4+CD45RA+ T lymphocytes as naïve T cells, and CD4+CD29+ T lymphocytes as well as CD4+CD45R0+ T lymphocytes as memory T cells. Chavance et al.\textsuperscript{14} examined the relationships between smoking and CD4+ T lymphocyte subpopulations, namely CD4+CD45RO+ T and CD4+CD45RA+ T lymphocytes by trend analysis. They concluded that the effect of smoking on memory (CD4+CD45RO+) T lymphocytes and naïve (CD4+CD45RA+) T lymphocytes seems to be equivalent. However, the trend between daily cigarette consumption and the number of T lymphocytes was stronger for memory T lymphocytes (p<0.001) than for naïve T lymphocytes (p<0.05). Their finding together with our present result suggests that the effect of smoking on memory T lymphocytes is stronger than that on naïve T lymphocytes.

The mechanism of why there is an increase in CD4+CD29+ T lymphocytes in smokers remains unclear. It may be due to a continuous local inflammation in the respiratory system induced by chronic smoking. Another possible explanation is that antigenic substances such as cigarettes containing glycoprotein might act as an antigen leading to an increase in CD4+CD29+ T lymphocytes as the tobacco glycoprotein induces the production of interleukin (IL) 1 alpha and IL-1 beta by peripheral blood and adherent cells\textsuperscript{15}. To examine the effects of smoking on the function of CD4+CD29+ T lymphocytes, function assay such as pokeweed mitogen induced immunoglobulin synthesis by B lymphocytes should be further conducted in smokers.

Despite the accumulation of studies on the evaluation of T lymphocyte subpopulations in workers exposed to work related substances\textsuperscript{1-7}, smoking status on subjects has not been described nor taken into consideration. To draw an exact conclusion on the effects of occupational and environmental factors on T lymphocyte subpopulations, it is necessary to control the smoking habit as an essential confounding factor.

<table>
<thead>
<tr>
<th>Lymphocytes</th>
<th>Smokers</th>
<th>Non-smokers</th>
</tr>
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<tbody>
<tr>
<td>CD4+ cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4+CD45RA+ cells</td>
<td>460 (210–1,225)</td>
<td>464 (121–778)</td>
</tr>
<tr>
<td>CD4+CD29+ cells</td>
<td>711 (368–936)*</td>
<td>409 (270–745)*</td>
</tr>
<tr>
<td>Total CD4+ cells</td>
<td>1,142 (603–1,588)*</td>
<td>783 (439–1,231)*</td>
</tr>
<tr>
<td>CD8+ cells</td>
<td>654 (305–1,192)</td>
<td>678 (319–2,116)</td>
</tr>
<tr>
<td>CD3+ (total) T cells</td>
<td>1,682 (911–2,135)</td>
<td>1,230 (621–2,808)</td>
</tr>
<tr>
<td>B (CD19+) cells</td>
<td>322 (141–677)</td>
<td>191 (84–484)</td>
</tr>
<tr>
<td>Natural killer (CD16+) cells</td>
<td>497 (216–874)</td>
<td>526 (156–1,188)</td>
</tr>
<tr>
<td>Total Lymphocytes</td>
<td>2,656 (1,490–3,451)</td>
<td>1,964 (1,570–4,192)</td>
</tr>
</tbody>
</table>

*p<0.01 (Wilcoxon’s rank sum test).

Fig. 2. CD4+CD29+ T lymphocytes per mm\(^3\) whole blood in 8 smokers and 22 non-smokers (p<0.01, Wilcoxon’s rank sum test) Transverse lines indicate median values.
References


