

# The Effects of Cigarette Smoking on T Cell Subsets

## A Population-based Survey of Healthy Caucasians<sup>1-5</sup>

DAVID J. TOLLERUD, JEFFREY W. CLARK, LINDA MORRIS BROWN, CAROLYN Y. NEULAND, DEAN L. MANN, LUBA K. PANKIW-TROST, WILLIAM A. BLATTNER, and ROBERT N. HOOVER

### Introduction

Cigarette smoking is associated with a variety of alterations in the cellular immune system, including an elevated white blood cell (WBC) count and increased numbers of circulating lymphocytes (1-4). Advances in flow cytometry and monoclonal antibody technology have yielded new insights into the cellular immune system, allowing the identification of subpopulations of lymphoid cells with distinct functional and antigenic characteristics. Alterations in T cell subsets have been described in a variety of disease states (5, 6), but data on the influence of cigarette smoking are limited and conflicting. While early reports suggested a decrease in the ratio of T helper-inducer cells to T suppressor-cytotoxic cells (helper/suppressor ratio), other reports have shown an increase or no change in this ratio among smokers (7-11). These studies have been limited by their use of small, highly selected populations, with little information on clinical and demographic characteristics.

To investigate the effects of cigarette smoking on mononuclear cell subsets, we studied a population-based, random sample of healthy Caucasians from a large metropolitan area. Detailed medical and demographic information was collected, as well as socioeconomic data and information on the use of alcohol and tobacco products. Multivariate techniques were used to identify the effects of cigarette smoking habits on leukocytes and peripheral blood mononuclear cell subsets.

### Methods

#### Study Population

A stratified, random sample of subjects in the Washington, D.C. metropolitan area was selected by random digit dialing using the Waksburg method (12). The demographic characteristics of the resulting sample (i.e., race, sex, age group, and number of persons in the household) were consistent with 1980 census data for the Maryland-Washington, D.C. Standard Metropolitan Statistical Area (SMSA). For example, the percentages of

**SUMMARY** To investigate the influence of cigarette smoking on mononuclear cell subsets, we determined T cell, B cell, monocyte, and HLA-DR+ subsets in a population-based, stratified, random sample of healthy Caucasians using monoclonal antibodies and flow cytometry. The study population consisted of 282 subjects 20 to 69 yr of age, including 108 smokers and 174 nonsmokers. Multivariate analysis techniques were used to assess the influence of cigarette smoking status after controlling for the effects of age and gender. Cigarette smoking was associated with a nonspecific increase in the leukocyte count involving all major cell types (smokers:  $8.50 \pm 0.15$  versus nonsmokers:  $7.33 \pm 0.12$  cells/mm<sup>3</sup>;  $p \leq 0.0001$ ). In addition, cigarette smokers had a selective increase in CD4+ cells (helper-inducer T cells) compared with nonsmokers ( $55.3 \pm 0.8$  versus  $52.2 \pm 0.6\%$  of lymphoid cells;  $p = 0.002$ ), resulting in a statistically significant increase in the CD4+/CD8+ (helper/suppressor) ratio ( $2.42 \pm 0.1$  versus  $2.13 \pm 0.16$ ;  $p = 0.02$ ). There was no significant difference between smokers and nonsmokers in the level of CD3+ cells (total T cells:  $76.8 \pm 0.7$  versus  $76.1 \pm 0.5$ ;  $p = 0.5$ ), CD8+ cells (suppressor-cytotoxic T cells:  $25.7 \pm 0.8$  versus  $27.0 \pm 0.5\%$ ;  $p = 0.1$ ), CD19+ cells (B cells) ( $10.7 \pm 0.4$  versus  $10.0 \pm 0.3\%$ ;  $p = 0.2$ ), CD14+ cells (monocytes) ( $18.0 \pm 0.6$  versus  $17.0 \pm 0.4\%$ ;  $p = 0.2$ ), or HLA-DR+ cells ( $14.5 \pm 0.5$  versus  $14.0 \pm 0.4\%$ ;  $p = 0.4$ ). There were no significant differences in leukocyte count or mononuclear cell subset levels between ex-smokers and persons who had never smoked, suggesting that these effects resolved after smoking cessation. Further studies will be required to delineate the mechanisms that mediate such a reversible effect of cigarette smoke exposure on circulating immune cells.

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white males from the telephone survey and from census data were 34.08% and 34.60%, respectively. A preliminary analysis of race-specific smoking rates predicted a marked relative shortage of black smokers in older age groups in the population to be studied. Therefore, budgetary and logistical constraints required exclusion of blacks and members of other racial groups from the pool of potential study subjects for analyses related to cigarette smoking. For similar reasons, the study population was restricted to adults between 20 and 70 yr of age.

A telephone questionnaire was administered to each potential study subject by a trained interviewer to collect demographic, lifestyle, and medical information. Initially, 3,888 households were screened, from which 741 potential white study subjects, stratified by age, sex, and smoking status, were ascertained. Telephone interviews were completed on 620 subjects (85%), of which approximately one-third were excluded from further consideration on the basis of lifestyle characteristics (intravenous drug use, homosexual activity) or medical conditions (blood product transfusion since 1975, recent hospitalization, severe allergies, use of steroid medications, history of connective tissue disease, or recent pregnancy) that might influence the immunologic parameters under investigation. At the completion of the interview, eligible subjects

were asked to undergo phlebotomy at a mobile field station near their home. The participation rate for phlebotomy was 74%. No significant differences were found between participants and nonparticipants with respect to demographic characteristics or socioeconomic status. However, response rates for phlebotomy were highest for subjects classified as nonsmokers (79%) versus smokers (71%), males (75%) versus females (72%), age group 40 to 49 yr (80%), education 1 to 4 years of college (78%), occupation skilled or semi-skilled (89%), and marital status divorced (86%); and lowest for subjects classified as

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<sup>4</sup> Requests for reprints should be addressed to William A. Blattner, M.D., Environmental Epidemiology Branch, NCI, NIH, Executive Plaza North—Room 434, Rockville, MD 20892.

<sup>5</sup> Correspondence should be addressed to David J. Tollerud, Channing Laboratory, 180 Longwood Avenue, Boston, Massachusetts 02115.

age group 20 to 29 yr (71%), education less than 12 yr (71%), occupation unskilled (69%), and marital status single (70%). Of the 304 persons who completed phlebotomy, 22 were excluded from further analyses for the following reasons: insufficient blood for analysis (4), homosexual activity (8), intravenous drug use (2), recent hospitalization (3), allergy treatment (1), use of steroids (2), and pregnancy (2). The final study population was composed of 282 white adults, 20 to 69 yr of age.

#### Specimen Processing

A venous blood sample was drawn by a nurse/phlebotomist in a specially equipped mobile van. Aliquots were submitted to a commercial laboratory for complete blood count (CBC) and differential WBC count. Peripheral blood mononuclear cells (PBMC) were separated from heparinized venous blood by Ficoll-Hypaque density gradient centrifugation (LSM; Litton Bionetics, Rockville, MD) and frozen in aliquots as previously described (13) using a CryoMed controlled-rate freezer and dimethyl sulfoxide. Immediately after freezing, the vials were transferred to the vapor phase of a liquid nitrogen storage freezer. After thawing the cells in preparation for flow cytometry analysis, viability by trypan blue exclusion was usually 90%, while total cell recovery ranged from 80 to 100% (13). Studies in our laboratory comparing fresh versus frozen cells showed no significant difference in the proportions of T cell subsets or B cells using this protocol (D. Tolperud, unpublished observations). The proportions of HLA-DR+ cells and ungated Leu M3+ cells showed minor but statistically significant differences between fresh and frozen cells, suggesting that caution should be exercised in extrapolating data for these subsets to analyses performed on fresh samples. Inclusion of length of time frozen as a covariate in multivariate analyses did not alter the results.

#### Monoclonal Antibodies and Flow Cytometry

The following directly fluorescein-conjugated monoclonal antibodies, purchased from Ortho Diagnostics (Raritan, NJ) (Ortho) or Becton Dickinson Monoclonal Center (Mountain View, CA) (BD) were used: OKT3 (CD3+ T cells; Ortho) (14, 15); OKT4 and OKT4A (CD4+ helper-inducer T cell subset; Ortho) (14-18); OKT8 (CD8+ suppressor-cytotoxic T cell subset; Ortho) (15-17); anti-Leu 12 (CD19+ B cells; BD) (19); anti-Leu M3 (CD14+ monocytes; BD) (20, 21); anti-HLA-DR (nonpolymorphic HLA-DR antigen; BD) (22, 23); and mouse IgG1 (clone 11-63; BD) and IgG(a+b) (clones 11-4.1 and MPC-11; BD) as negative control reagents.

Prepared samples were analyzed on a fluorescence-activated cell sorter, FACS II (Becton Dickinson, Mountain View, CA) interfaced to a PDP 11/24 DEC computer (Digital Equipment Corporation, Landover, MD). Viable lymphocytes were selected for fluores-

cence analysis using a combination of forward- and right-angle scatter. The forward-angle light scatter window was set to exclude electronic noise, debris, and damaged or dying cells, while the right-angle light scatter window excluded monocytes. Standard window settings were determined for each monoclonal antibody. After gating, only  $1.9 \pm 0.1\%$  of cells were Leu M3+, with no significant differences between men and women, smokers and nonsmokers, or older and younger subjects. For analysis of CD14+ cells (monocytes), the right-angle light scatter window was opened to allow for viewing of all mononuclear cells (24). A total of 10,000 gated events were collected for each monoclonal antibody tested. The percentage of immunofluorescence positive cells was determined by subtracting the negative control fluorescence from each monoclonal reagent, determined by analysis of cells stained with mouse IgG of the appropriate isotype for the monoclonal reagent tested. A series of quality-control experiments was carried out to evaluate intra-analysis variation. Reproducibility was excellent, with a coefficient of variation ranging from 2 to 5% for the major T cell subsets to a maximum of 15% for B cells. Samples were analyzed in random order, without knowledge of the demographic characteristics or smoking history of the study subject.

#### Statistical Analysis

The study population was grouped into two smoking categories, nonsmokers and current smokers, to assess the influence of cigarette smoking on leukocyte count and mononuclear cell subset proportions. Persons who smoked cigarettes at the time of the interview were considered "current" smokers; all others were considered nonsmokers. The usual number of cigarettes smoked per day (intensity) and the total number of years smoked (duration) were ascertained from the questionnaires, and a lifetime estimate of the number of packs of cigarettes smoked (pack-years) was calculated as the product of these two variables.

Student's *t* tests were used to compare mean values for selected variables by age in 10-yr categories (20 to 29 . . . 60 to 69), sex, and smoking category. Linear regression analysis and analysis of variance were used to test the independence of associations found in the univariate analysis. Analyses were also per-

formed using log-transformed values for leukocyte count, and arcsin-transformed values for mononuclear cell subset proportions, to achieve a more normal distribution of values. The resulting significance estimates were similar to the analyses presented for the nontransformed values. Analysis of variance was used to test whether there were significant differences for mean WBC count and mononuclear cell subset proportions by smoking status, usual number of cigarettes smoked per day (1 to 10, 11 to 20, 21 to 50), and total number of years smoked (1 to 10, 11 to 20, 21 to 60) independent of age, sex, or correlates of smoking (education and coffee and alcoholic beverage consumption) (25).

## Results

### Study Population

The study population consisted of 108 smokers and 174 nonsmokers, with approximately equal numbers of men and women in each 10-yr age group (table 1). The mean age was  $42.9 \pm 1.5$  yr for smokers and  $44.5 \pm 1.0$  yr for nonsmokers, with no significant difference between men and women. The smoking characteristics of current smokers are shown in table 2. Smoking intensity, duration, and pack-years were equivalent in men and women.

Smokers and nonsmokers differed with respect to educational level and occupational category. The proportion of current smokers decreased markedly as the level of education increased. Among persons without a high school education, 68% were smokers, compared to 22% of those with 5 or more yr of college. A higher proportion of nonsmokers held professional or managerial positions or jobs in the technical category, whereas current smokers were more likely to have jobs that were classified as sales work/clerical or skilled, semiskilled, or unskilled. No major differences were apparent according to marital status. The findings were similar when smoking group comparisons were made for men and women separately.

### Leukocyte Count

Cigarette smoking was associated with an increase in WBC count relative to nonsmokers, including all major cell types (figure 1). This increase in WBC count was apparent across all age groups and for men and women separately. Neither age nor gender was significantly associated with WBC count after controlling for smoking status using multivariate techniques. The differential cell count was similar in smokers and nonsmokers, although smokers had a slightly higher proportion of eosinophils (2.3%) than

TABLE 1  
AGE DISTRIBUTION OF THE STUDY  
POPULATION

Age Group (yr)	Smokers		Nonsmokers		Total
	Men	Women	Men	Women	
20-29	11	8	12	16	47
30-39	9	19	23	16	67
40-49	12	10	25	15	62
50-59	12	10	23	16	61
60-69	9	8	14	14	45
All ages	53	55	97	77	282

TABLE 2  
CIGARETTE CONSUMPTION BY CURRENT SMOKERS\*

Age Group (yr)	Cigarettes per Day		Pack-years	
	Men	Women	Men	Women
20-29	19.5 ± 2.4	15.4 ± 3.3	4.8 ± 1.2	7.0 ± 1.6
30-39	35.0 ± 7.2	22.8 ± 4.4	27.6 ± 3.7	17.4 ± 3.4
40-49	24.3 ± 3.4	24.5 ± 4.9	33.0 ± 7.0	22.9 ± 4.7
50-59	20.0 ± 3.0	26.2 ± 3.2	40.1 ± 7.0	41.2 ± 7.5
60-69	23.0 ± 3.1	25.0 ± 5.3	49.2 ± 6.3	41.2 ± 14.6
All ages	23.9 ± 1.9	23.0 ± 2.1	30.6 ± 3.2	27.8 ± 3.7

\* Values are expressed as mean ± SE.

did nonsmokers (1.9%) ( $p = 0.003$ ). Because of this nonspecific smoking-related leukocytosis, current smokers had a significantly higher absolute lymphocyte count than did nonsmokers ( $p \leq 0.0001$ ). There was no detectable significant effect ( $p > 0.05$ ) of smoking intensity, smoking duration, or total pack-years of smoking on the WBC count or differential. Adjustment for correlates of smoking (i.e., age, educational level, and alcohol and coffee consumption) did not alter the observed associations between cigarette smoking and leukocyte levels.

#### Mononuclear Cell Subsets

The range and distribution of mononuclear cell subsets in the combined study population are shown in table 3. As a group, cigarette smokers had a small but significant increase in the proportion of

CD4+ cells (helper-inducer T cells) compared to nonsmokers (table 4). However, increasing age and female gender were also associated with a significant increase in percent CD4+ cells, as well as a decrease in percent CD8+ cells (suppressor-cytotoxic T cells) and an increased CD4+/CD8+ (helper/suppressor) ratio. The association between cigarette smoking and increased CD4+ cells remained highly significant ( $p = 0.002$ ) after controlling for age and gender through multivariate analysis (table 5). Among current smokers, the percentage of CD4+ cells tended to increase with the number of cigarettes smoked per day ( $p = 0.06$  by linear regression analysis). There was no evidence of a decrease in CD4+ cells or the CD4+/CD8+ ratio, even among heavy smokers who consumed 2 to 3 packs of cigarettes per day. Smoking was

also independently associated with a small increase in the CD4+/CD8+ ratio without affecting the proportion of CD8+ cells. There were no significant associations between cigarette smoking and the proportion of CD19+ cells (B cells), CD14+ cells (monocytes), or HLA-DR+ cells, although significant age and gender effects were noted (tables 4 and 5). As in the analysis of WBC count, no significant dose effects of cigarette smoking duration or pack-years were detected, and adjustment for correlates of smoking did not alter the results.

#### Analysis of Ex-smokers

Among the 174 nonsmokers in the study, 66 subjects (43 men and 23 women) were ex-smokers who had smoked cigarettes at some time in the past but were not currently smokers at the time of the interview. Ex-smokers had a mean age of  $48.5 \pm 1.5$  yr and had quit smoking an average of 10.7 yr prior to the study. For both sexes combined, the usual number of cigarettes smoked per day was similar for ex-smokers (23.5 cigarettes per day) and current smokers (22.5 cigarettes per day;  $p = 0.7$ ), although female ex-smokers had smoked less than male ex-smokers (15.7 versus 27.8 cigarettes per day,  $p \leq 0.01$ ). There were no significant differences between ex-smokers and never smokers for WBC count, differential cell count, or any of the mononuclear cell subsets in this analysis. To evaluate the effect of smoking cessation on leukocytes and T cell subsets, the data from ex-smokers were analyzed by duration of time since cessation of smoking. The WBC count among ex-smokers was similar to never smokers, even for ex-smokers who had stopped smoking less than 1 yr before the study. In contrast, the elevated proportion of CD4+ cells observed among smokers was still present in ex-smokers who had quit smoking within the previous year. By 2 to 4 yr after smoking cessation, however, CD4+ cell levels and the helper/suppressor ratio among ex-smokers were no longer elevated relative to persons who had never smoked.

#### Discussion

These data indicate that the major effect of cigarette smoking on T cell subsets is a relative increase in the number and proportion of CD4+ (T helper-inducer) lymphocytes. This smoking-related increase in CD4+ cells, with no significant change in CD8+ (T suppressor-cytotoxic) cells, resulted in a small but significant increase in the CD4+/CD8+ (help-

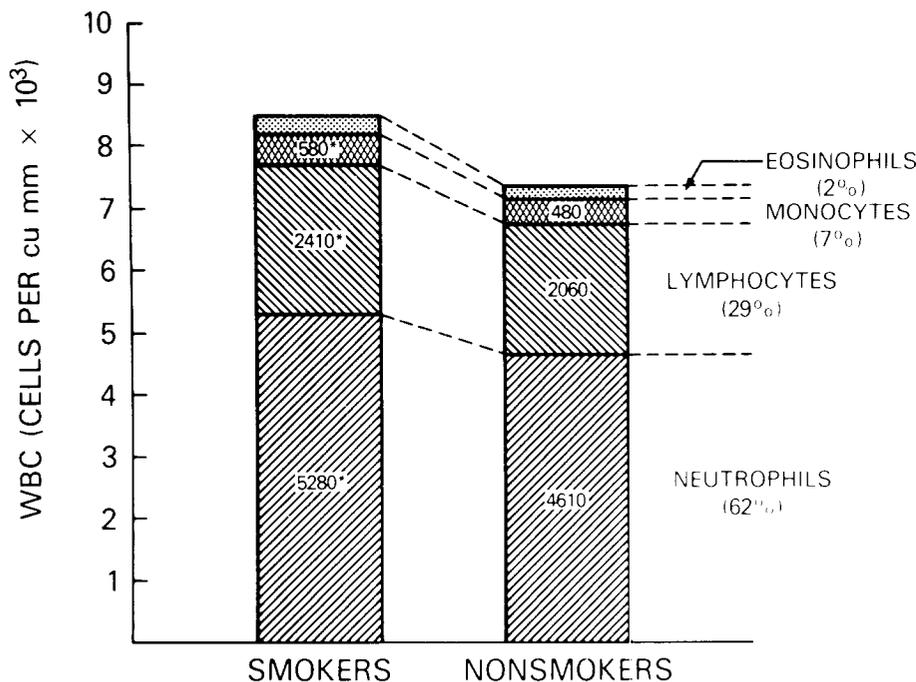


Fig. 1. White blood cell (WBC) count and differential in smokers and nonsmokers. Total WBC count was  $8,244 \pm 199$  cells/mm<sup>3</sup> for smokers versus  $7,360 \pm 193$  cells/mm<sup>3</sup> for nonsmokers ( $p < 0.0001$ ). Values within bars indicate cell count (cells/mm<sup>3</sup>) for each cell type (asterisks indicate  $p < 0.0001$  compared to nonsmokers). Corresponding values for eosinophils: 190 (smokers) versus 140 (nonsmokers) ( $p < 0.0001$ ). Values in parentheses indicate relative proportion of each cell type.

TABLE 3  
MONONUCLEAR CELL SUBSETS IN THE COMBINED STUDY POPULATION

Cell Surface Antigen	Monoclonal Antibody	Cell Proportion*			Cell Number†		
		Mean	SD	Range	Mean	SD	Range
CD3	OKT 3	76.4	7.2	46.9–89.4	1,682	476	600–3,234
CD4	OKT4A	53.4	8.1	32.0–77.0	1,177	374	409–2,723
CD8	OKT8	26.5	7.5	7.1–54.5	584	238	127–1,722
CD4:CD8 ratio		2.26	1.04	0.65–10.07	2.26	1.04	0.65–10.07
CD19	Anti-LEU 12	10.3	4.0	3.0–22.8	226	111	40–732
CD14	Anti-LEU M3‡	17.4	5.8	4.9–35.4	376	158	110–1,127
HLA-DR	Anti-HLA-DR	14.2	4.7	5.7–35.9	310	136	63–973

\* Percentage of lymphoid cells.

† Cells per mm<sup>3</sup>.

‡ Right-angle light scatter gate removed for determination of Leu M3+ cells.

er/suppressor) ratio. Cigarette smoking was not associated with alterations in the proportion of total T cells, B cells, monocytes, and HLA-DR+ cells. In contrast to the specificity of effects at the level of cellular subsets, the smoking-related increase in leukocyte count observed was nonspecific, involving all major cell types to a similar degree. These smoking-related effects all appeared to be readily reversible. The leukocyte count among subjects who had stopped smoking less than 1 yr prior to the study was similar to never smokers, while the smoking-related increase in CD4+ cells appeared to require somewhat longer to resolve. The number of ex-smokers who had recently quit smoking was insufficient to further evaluate the first year after smoking cessation.

Previous reports of cigarette smoking and T cell subsets have expressed variable and conflicting results. Ginns and associates (7) reported a decreased helper/suppressor ratio among a small group of heavy smokers. Other investigators have reported no change or a slight increase among smokers compared to non-smokers (8–11). These analyses have all been based on small groups of subjects, without sufficient descriptive data to al-

low careful interstudy comparisons or extrapolation to other populations. As demonstrated in this study, age, gender, and smoking-related effects may be significant, particularly for CD4+ subset determinations, and small differences in the makeup of comparison populations may significantly influence the interpretation of study results. To avoid these limitations, the current study incorporated rigorous epidemiologic methods to select a random, population-based sample of healthy study subjects, stratified to assure a similar age and sex distribution among smokers and nonsmokers. Because so little was known at the time of initial study design about the influence of age, gender, and smoking on T cell subset levels and the variability introduced by testing samples over a prolonged time period, the choice of population size was largely empiric. The present population size would be expected to reliably detect a 5 to 10% difference in T cell subset proportions between smokers and nonsmokers using the described equipment and protocols.

A potential source of the conflicting results in previous reports of T cell subsets in smokers may be a failure to control for nonspecific effects of smoking

on the leukocyte count. Comparisons of absolute cell counts will tend to show an increase among smokers due to the well-described, nonspecific leukocytosis associated with cigarette use. This potential source of error is largely eliminated by the use of cellular proportions or ratios rather than cell counts.

It is unlikely that sampling bias or technical factors could be responsible for the observed smoking-related differences. The study population was drawn from a population-based sample with a reasonable cooperation rate, and subjects were carefully screened for conditions or exposures that might alter mononuclear cell subsets. Only subjects meeting a series of predetermined health criteria were included in the study. Detailed intergroup comparisons demonstrated previously observed differences between smoking groups with respect to demographic characteristics and socioeconomic status. However, controlling for these variables did not affect the results. All specimens were handled according to the same protocol, and all samples were maintained under the same conditions of liquid nitrogen storage for similar lengths of time prior to flow cytometry analysis.

The smoking-related increase in CD4+ cells was extremely consistent across all groups and for males and females separately. We were unable to document a clear dose effect, although the level of CD4+ cells among current smokers tended to increase as the number of cigarettes smoked per day increased ( $p = 0.06$ ). It is noteworthy that the expected dose effect of smoking on WBC count was also not detected. Our study population may have been too small to detect a true dose-response effect, particularly at low levels or cigarette exposure. Alternatively, the full impact of cigarette smoking on T cell subsets may occur at a relatively low exposure

TABLE 4  
MONONUCLEAR CELL SUBSETS IN SMOKERS AND NONSMOKERS, STRATIFIED BY GENDER\*

Cell Surface Antigen	Monoclonal Antibody	Smokers		Nonsmokers		p† Smokers (versus nonsmokers)
		Men	Women	Men	Women	
CD3	OKT3	75.6 ± 1.0	77.9 ± 1.0	75.9 ± 0.7	76.5 ± 0.8	0.47
CD4	OKT4A	53.2 ± 1.1	57.2 ± 1.0‡	50.8 ± 0.8	54.1 ± 0.9‡	0.002
CD8	OKT8	25.9 ± 1.2	25.4 ± 1.0	28.1 ± 0.8	25.7 ± 0.8	0.14
CD4:CD8 ratio		2.36 ± 0.16	2.48 ± 0.12	2.02 ± 0.09	2.34 ± 0.13‡	0.04
CD19	Anti-LEU 12	11.2 ± 0.6	10.2 ± 0.6	10.2 ± 0.4	9.8 ± 0.4	0.19
CD14	Anti-LEU M3§	18.9 ± 0.8	17.1 ± 0.8	17.6 ± 0.6	16.2 ± 0.7	0.17
HLA-DR	Anti-HLA-DR	15.7 ± 0.7	13.6 ± 0.6‡	14.5 ± 0.5	13.4 ± 0.5	0.42

\* Values are expressed as mean ± SE percentage of lymphoid cells.

† p value derived from two-tailed Student's *t* test comparing all smokers to all nonsmokers.

‡  $p < 0.05$  comparing men versus women within the same smoking category.

§ Right-angle light scatter gate removal for determination of LEU M3+ cells.

TABLE 5  
MULTIPLE LINEAR REGRESSION ANALYSIS OF THE EFFECTS OF SMOKING,  
GENDER, AND AGE ON MONONUCLEAR CELL SUBSETS\*

Cell Surface Antigen	Intercept	Current Smoking		Gender		Age		Model r-Square
		Coefficient	(p)	Coefficient	(p)	Coefficient	(p)	
CD3	76.85	0.72	(0.43)	1.01	(0.25)	-0.048	(0.14)	0.02
CD4	41.61	3.08	(0.0016)	3.61	(0.0001)	0.121	(0.0006)	0.12
CD8	33.08	-1.24	(0.18)	-1.84	(0.0420)	-0.076	(0.0246)	0.04
CD4:CD8	1.032	0.269	(0.0348)	0.258	(0.0368)	0.0170	(0.0003)	0.08
CD19	12.40	0.58	(0.25)	-0.65	(0.18)	-0.032	(0.0811)	0.02
CD14	20.61	0.95	(0.19)	-1.52	(0.0303)	-0.033	(0.20)	0.03
HLA-DR	11.43	0.72	(0.20)	-1.51	(0.0053)	0.107	(0.0001)	0.12

\* Linear regression model in the form:  $Y = A + B_1X_1 + B_2X_2 + B_3X_3$ , where Y represents the cell subset proportion (%), A is the linear intercept,  $X_1$  represents smoking status (0 = nonsmoker, 1 = smoker),  $X_2$  represents gender (1 = male, 2 = female),  $X_3$  represents age in years, and  $B_1$ ,  $B_2$ , and  $B_3$  are the respective coefficients. A positive coefficient indicates an increase in subset proportion for increasing values of X (e.g., % CD4+ cells is higher in females than in males), while a negative coefficient indicates a decrease in subset proportion for increasing values of X (e.g., % CD8+ cells decreases with increasing subject age).

level or may be related to smoking characteristics (i.e., depth of inhalation) which we did not determine. Finally, it is possible that smoking is simply a marker for another as yet unknown factor or exposure associated with an increase in CD4+ cells. Although a quantitative dose effect could not be conclusively demonstrated, the trend toward an increase in CD4+ cells with increasing smoking intensity and the apparent reversibility of these changes with smoking cessation is strong evidence for a specific effect of cigarette smoking on CD4+ cells or their progenitors.

A recent report of T cell subsets in pregnant women illustrates the necessity of including data on cigarette smoking in analyses of lymphocyte subsets (26). The investigators noted significant T cell subset alterations with pregnancy, but the pattern was significantly different in women who smoked during pregnancy compared with nonsmoking pregnant women. Failure to ascertain and analyze the smoking status of study subjects might have substantially altered the interpretation of this investigation. Even greater problems may arise in comparisons of men and women of different ages. For example, if middle-aged female smokers from the current study population (60% CD4+ cells; CD4+/CD8+ = 2.5) were compared to a "reference" population of 20- to 29-yr-old nonsmokers from this study (50% CD4+ cells; CD4+/CD8+ = 1.8), the resulting "statistically significant" difference might erroneously be ascribed to some unrelated characteristic of the study population.

Extrapolation of these results to the general population is limited by the exclusion of blacks and other racial groups. Cancer incidence and mortality rates differ, for example, between blacks and whites in the United States, leading to

speculation that race-related host factors, as well as differences in socioeconomic factors and access to health care, may influence disease development. Investigations are currently under way to address these important issues.

In summary, cigarette smoking is associated with a selective increase in the number and proportion of circulating CD4+ cells, with relatively little effect on other mononuclear cell subsets. The mechanisms responsible for this effect, like those responsible for the nonspecific leukocytosis observed among smokers, are obscure. These results emphasize, however, that information on cigarette smoking, as well as age and other potential confounders, should be included in analyses of T cell subsets, particularly in large populations. Careful stratification or multivariate analysis techniques are mandatory to allow proper interpretation of results in heterogeneous study groups. The "normal range" of values for these subsets may vary according to the specific techniques and equipment used by each laboratory, but the relationships reported here should be independent of such methodological differences. These analyses provide vital baseline data for the design and analysis of future clinical and epidemiologic studies of mononuclear cell subsets in health and disease.

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