

Racial Differences in Serum Immunoglobulin Levels: Relationship to Cigarette Smoking, T-Cell Subsets, and Soluble Interleukin-2 Receptors

David J. Tollerud,¹ Linda Morris Brown,³ William A. Blattner,³ Scott T. Weiss,² Elizabeth M. Maloney,³ Carol C. Kurman,³ David L. Nelson,³ and Robert N. Hoover³

¹Department of Environmental and Occupational Health, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania; ²Channing Laboratory, Department of Medicine, Brigham & Women's and Beth Israel Hospitals and Harvard Medical School, Boston, Massachusetts; ³Epidemiology and Biostatistics Program and the Metabolism Branch, National Cancer Institute, Bethesda, Maryland

To investigate the influence of race, cigarette smoking, and immunologic parameters on serum immunoglobulins, we analyzed serum IgG, IgA, and IgM levels in 455 healthy adults. The study population ranged in age from 20 to 69 years, including 282 whites and 173 blacks, 181 never-smokers, 93 ex-smokers, and 181 current smokers. Race and smoking were independently associated with alterations in serum IgG levels. Blacks had significantly higher IgG levels than whites (1,587 vs. 1,209 mg/dl; $P < 0.001$), and never smokers had signifi-

cantly higher levels than current smokers (1,426 vs. 1,287 vs. mg/dl; $P < 0.001$). IgA and IgM levels were unrelated to race or smoking. Serum IgG was also found to be directly related to the proportion of HLA-DR⁺ cells and the level of soluble interleukin-2 receptors (sIL-2R) and inversely related to the proportion of CD4⁺ cells. Investigation of this racial heterogeneity may provide insights into the pathogenesis of immunologic diseases that exhibit unexplained racial variation. © 1995 Wiley-Liss, Inc.

Key words: blacks, whites, race, IgG, IgA, IgM, soluble interleukin-2 receptors

INTRODUCTION

We have previously reported an increase in circulating B cells and activated T cells among blacks compared to whites in a population-based study of healthy adults, suggesting that race may significantly influence baseline immunologic parameters (1). These observations, combined with reports of higher immunoglobulin levels, particularly IgG, among blacks compared to whites (2,3), suggest that blacks may indeed have increased B cell activity compared to whites. However, the genetic or environmental factors that may contribute to this variability have not been defined.

Cigarette smoking is also associated with immunologic alterations, including changes in immunoglobulin levels. For example, smoking appears to decrease the production of IgG after antigenic stimulation, an effect that may explain the significantly lower incidence of hypersensitivity pneumonitis in smokers compared to nonsmokers (4-6). To date, population studies on immunoglobulin levels have not included sufficient information on race and smoking to dissect the relative contribution of these important host characteristics. It is not possible, from the available data, to determine whether differences in smoking rates might account for some of the reported black/white differences in immunoglobulin levels.

Frozen sera from a previous population-based study of healthy black and white adults provided an opportunity to address the influence of race and smoking on serum immunoglobulin levels and to relate these levels to T-cell subsets and other immunologic data available on this cohort.

MATERIALS AND METHODS

Study Population

Sera for the present study were obtained from a population-based survey of healthy adults in the greater Washington, D.C., metropolitan area (7). Random digit dialing and a short household screening questionnaire were used to select a random sample of white and black adults stratified by age, race, gender, and smoking status. Selected subjects completed a 25-min telephone interview. Subjects were screened to exclude individuals with lifestyle characteristics (intravenous

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Address reprint requests to Dr. David J. Tollerud, Department of Environmental and Occupational Health, University of Pittsburgh, GSPH A-718, 130 DeSoto Street, Pittsburgh, PA 15261.

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. The final study population was composed of 282 white and 173 black adults, aged 20–69 years.

The study population was divided into three smoking categories: never-smokers had smoked less than 100 cigarettes in their lifetime; ex-smokers had previously smoked cigarettes but had quit smoking prior to phlebotomy; and current smokers were persons who smoked cigarettes at the time of the interview and phlebotomy. The usual number of cigarettes smoked per day (intensity) and the total number of years smoked (duration) were ascertained from the questionnaire.

Sample Preparation and Immunoglobulin Analysis

Blood for serum samples was collected in evacuated tubes and allowed to clot. After centrifugation, the serum was separated and divided into 1.0-ml aliquots, frozen, and maintained in a liquid nitrogen freezer. Samples were shipped, on dry ice, to a commercial laboratory (Metpath, Rockville, MD) for analysis of serum IgG, IgA, and IgM by nephelometry.

Analysis of Mononuclear Cell Subsets

Blood samples for cell subset analysis were drawn into preservative-free heparin and processed as previously described (1). The following directly fluorescein-conjugated monoclonal antibodies (mAbs), purchased from Ortho Diagnostics, Raritan, NJ (ORTHO) or Becton Dickinson Monoclonal Center, Mountain View, CA (BD), were used (2): OKT3 (CD3⁺ T cells; ORTHO); OKT4A (CD4⁺ helper-inducer T-cell subset; ORTHO); OKT8 (CD8⁺ suppressor-cytotoxic T-cell subset; ORTHO); anti-Leu 12 (CD19⁺ B cells; BD); anti-Leu M3 (CD14⁺ monocytes; BD); anti-Leu 11A (CD16⁺ natural killer [NK] cells; BD); anti-HLA-DR (nonpolymorphic HLA-DR antigen; BD); and mouse IgG1 (clones 11–63; BD) and IgG2(a+b) (clones 11–4.1 and MPC-11; BD) as negative control reagents. A direct immunofluorescence staining procedure was employed, after which samples were analyzed on a FACS II cell sorter (Becton Dickinson, Mountain View, CA) interfaced to a PDP 11/24 DEC computer (Digital Equipment, Landover, MD), as previously described (1).

sIL-2R Assay

Blood was collected by routine phlebotomy in evacuated tubes and allowed to clot. The serum was separated by centrifugation, divided into 1.0-ml aliquots, frozen, and stored in a liquid nitrogen freezer until withdrawal for soluble interleukin-2 receptor (sIL-2R) analysis. Serum soluble interleukin-2R (sIL-2R) concentrations were measured by a sandwich enzyme-linked immunosorbent assay (ELISA) as

previously described (8). Briefly, alternate columns of the inner 60 wells of microtiter plates were coated with anti-Tac antibody or buffer. After incubation at 4°C overnight, the plates were washed and 100 µl of sample was added to coated and control wells. After a 2-hr incubation at room temperature, the plates were washed, and FITC-labeled 7G7B6 monoclonal antibody (mAb) was added to each well. After a second 2-hr incubation at room temperature, the plates were again washed, and alkaline phosphatase-conjugated rabbit anti-FITC was added to all wells. After an additional 1-hr incubation at room temperature, *p*-nitrophenylphosphate (Sigma) was added, and the absorbance measured at 405 nM. Absorbance of the control wells was subtracted from the experimental wells, and the absorbance value was compared to absorbances determined for a standard curve generated for each plate from known concentrations of sIL-2R.

Statistical Analysis

Statistical analyses were performed using the SAS statistical analysis package (SAS Institute, Cary, NC). Student's *t*-tests were used to compare mean values for selected variables by race, sex, smoking category, and age group. Analyses were also performed using log-transformed values for serum immunoglobulin levels to achieve a more normal distribution of values. Linear regression analysis and analysis of variance (ANOVA) were used to test for significant differences in immunoglobulin levels by race or smoking status, independent of age, sex, or correlates of smoking (education and coffee and alcoholic beverage consumption). These approaches were also used to assess the relationship of serum IgG, IgM, and IgA to peripheral blood mononuclear cell subsets and to serum concentrations of sIL-2R concentrations independent of age, gender, race, and cigarette smoking status.

RESULTS

Characteristics of the Study Population

The study population consisted of 455 healthy adults, aged 20–69 years, including 282 whites and 173 blacks; 245 men and 210 women; and 181 never-smokers, 93 ex-smokers, and 181 current smokers (Table 1). Subjects were stratified by smoking status (current smokers, ex-smokers, and never-smokers), race, gender, and age in decades. Whites as a group were slightly older than blacks (43.9 vs. 39.7 years), due primarily to the younger age of black smokers. No significant

TABLE 1. Population Distribution by Cigarette Smoking Status

Subjects	Never-smokers	Ex-smokers	Current smokers
Whites	108	66	108
Blacks	73	27	73
All	181	93	181

age differences between blacks and whites were observed for never-smokers and ex-smokers.

Among current smokers, whites tended to smoke more heavily and had smoked for a longer duration than blacks (22.5 vs. 14.7 cigarettes per day). Similarly, white ex-smokers had smoked more heavily during the time they smoked than had black ex-smokers (23.5 vs. 14.8 cigarettes per day). Ex-smokers had stopped smoking an average of 10.2 years prior to the study, with no significant difference between blacks and whites.

Serum IgG, IgA, and IgM Levels

Blacks had significantly higher serum IgG levels than those of whites ($1,587 \pm 35$ vs. $1,209 \pm 16$ mg/dl; $P < 0.001$). This difference was present for all age groups (Fig. 1). No significant black/white differences were observed for serum IgA or IgM levels.

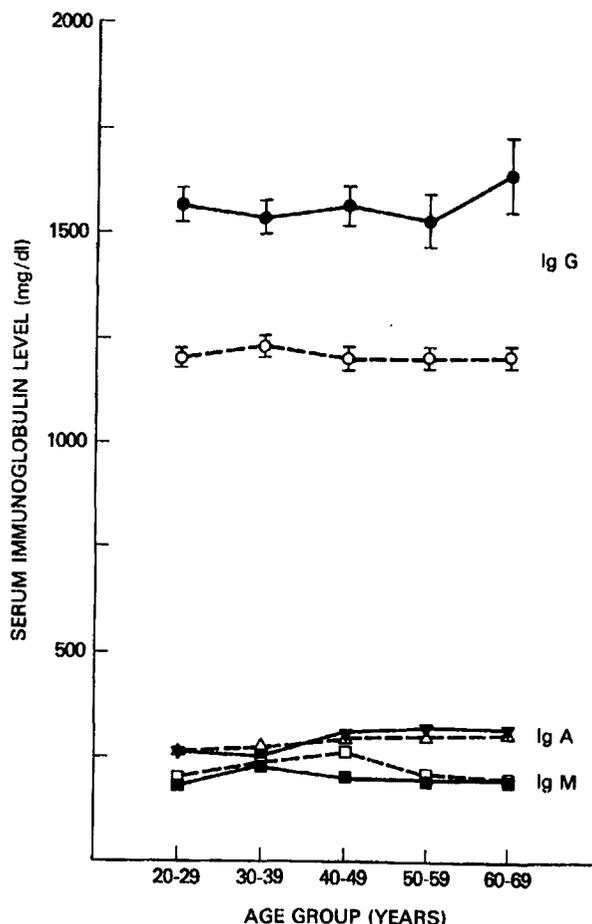


Fig. 1. Serum immunoglobulin levels by age group and race. Note that the lines are not meant to imply a continuum. Each point represents the mean value (\pm SEM) for that 10-year age group. Whites, open symbols; blacks, filled symbols.

Never-smokers had significantly higher serum IgG levels than those of current cigarette smokers ($1,426 \pm 26$ vs. $1,287 \pm 24$ mg/dl; $P < 0.001$). The independent relationship of serum IgG level to both race and cigarette smoking was confirmed in ANOVA and linear regression analyses, adjusting for age and gender. Black never-smokers had the highest levels, and white current smokers had the lowest levels (Fig. 2). Among current smokers, no consistent relationship between serum IgG levels and the intensity (cigarettes smoked per day) or duration (years) of cigarette smoking could be detected.

To investigate whether differences in smoking pattern might account for the observed black/white differences, we repeated the analyses, excluding all subjects who smoked more than 20 cigarettes per day. In this subset of light smokers, smoking intensity was similar for the 73 white and 68 black smokers (15.5 vs. 16.0 cigarettes per day, respectively). The relationship of serum IgG level to race, smoking, and age category for this subgroup was similar to that observed in the total population.

Similar results were obtained when these analyses were repeated using log-transformed immunoglobulin values to correct for small deviations from normality in the data. Analyses were also repeated after adjustment for correlates of smoking (level of education, coffee consumption, and alcohol consumption), with no significant change in the results.

Multiple linear regression analysis was used to assess the relationship between log serum IgG level and peripheral blood mononuclear cell (PBMC) subset levels and log serum sIL-2R adjusted for race, gender, and smoking (Table 2). Log serum IgG level was directly related to the proportion of circulating HLA-DR⁺ cells, CD8⁺ cells, and log serum sIL-2R and inversely related to the proportion of circulating CD3⁺ cells, CD4⁺ cells, and to the CD4:CD8 ratio. The relationships of IgG level to sIL-2R level and T-cell subset proportions were statistically independent, and sIL-2R level itself was unrelated to T-cell subset proportions.

DISCUSSION

This study, which used a well-defined population-based sample of healthy adults from a single U.S. metropolitan area, was able to demonstrate that race and cigarette smoking are independently associated with alterations in serum IgG levels and that IgG levels are correlated with T-cell subset and sIL-2R levels. The approximately 30% higher serum IgG level in blacks compared to whites was observed in all age categories and was not significantly altered by adjustment for gender, smoking, alcohol, caffeine, or socioeconomic status.

These findings, as well as those from previous analyses (1,9), suggest that race (or ethnicity) may have an important influence on numerous immunologic parameters, independent of

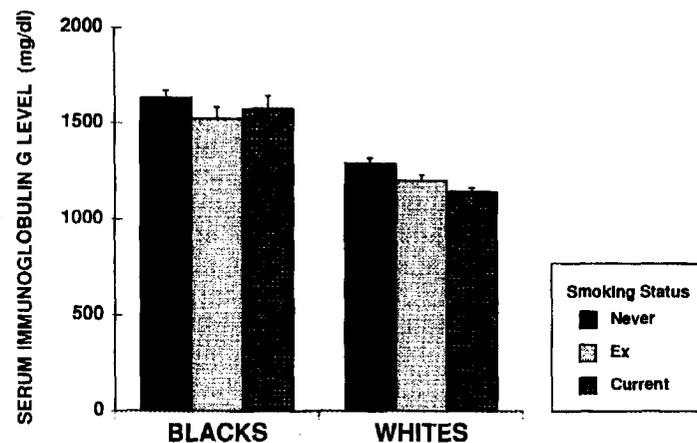


Fig. 2. Serum IgG levels by smoking status and race. Each bar represents the mean (\pm SEM) value for that stratum of race and cigarette smoking status.

environmental exposures such as cigarette smoke. Exploration of this naturally occurring racial heterogeneity may provide clues to explain race-related differences in diseases, such as multiple myeloma, which arise in the immune system (10).

Cigarette smoking has numerous effects on immune function (11–13). Decreased levels of circulating immunoglobulins and NK cells support the hypothesis that the predominant effect is immunosuppression (14,15). However, other data suggest that tobacco smoke may exert a stimulatory role on the immune system. For example, smokers have higher serum IgE levels, higher leukocyte counts, and a higher proportion of circulating CD4⁺ “helper” T cells than those found in nonsmokers (3,16). In vitro studies confirm that chemical constituents purified from tobacco leaf may exert a direct stimulatory effect on cultured mononuclear cells (17). These observations emphasize the complex relationships between various components of the immune system and the important

contribution of rigorously defined population-based analyses to an understanding of these relationships.

A number of previous studies have related immunoglobulin levels to race, ethnicity, immunologic factors, and other parameters such as ABO blood group. Higher levels of IgG among blacks compared to whites have consistently been found among adults (2,3) and infants (18). These observations have been extended through analysis of IgG subclasses and molecular biologic analysis of immunoglobulin allotypes (19,20), with particular attention to the influence on immunologic responses to infection (21–24). The relationship of various immunoglobulins to ABO blood groups and other genetic factors has historically been controversial (25), and the complex relationships among immunoglobulins, genetics, and disease susceptibility have been extensively reviewed (26).

The rich database of immunologic information available for this population-based study provided an opportunity to extend our understanding of the interrelationships between immunoglobulin levels and other immunologic parameters. Serum IgG level was positively related to the proportion of circulating HLA-DR⁺ cells. Among circulating mononuclear cells, this surface marker is primarily expressed on B cells, monocytes, and activated T cells. Since serum IgG was not significantly related to the number or proportion of B cells, and monocytes were electronically excluded by light-scatter gating, this finding suggests that serum IgG level may be related to the proportion of activated T cells. The relationship of IgG to immune activation is further supported by the positive association between IgG and the level of circulating sIL-2R, an in vivo marker of immune activation (27).

The biracial nature of this well-characterized population has allowed us to investigate the independent influence of age, gender, race, and cigarette smoking on immunologic parameters. However, several potentially important differences between blacks and whites in this population need to be addressed. White smokers tended to smoke more heavily

TABLE 2. Multiple Linear Regression Analysis of Association Between Log Serum IgG Level and Peripheral Blood Mononuclear Cell Subsets or Log Serum IL-2R Level Adjusted for Age, Race, Gender, and Smoking Status^a

Cell surface antigen	Coefficient (SE)	P-value
CD3	-0.0025 (0.0010)	0.01
CD4	-0.0027 (0.0011)	0.01
CD8	0.0026 (0.0013)	0.04
CD4/CD8 ratio	-0.0109 (0.0061)	0.08
CD14	0.0009 (0.001)	0.61
CD16	0.0017 (0.0027)	0.53
CD19	0.0027 (0.0018)	0.15
HLA-DR	0.0061 (0.0018)	0.001
Log-sIL-2R	0.0453 (0.0189)	0.02

^aThe coefficient represents the amount of change in percentage of each cell subset for each one unit change in log serum IL-2R, after adjusting for the influence of age, race, gender, and cigarette smoking. A negative coefficient indicates a decrease in cell subset proportion associated with an increase in sIL-2R level.

than black smokers, an observation also reported in a study of data from the 1985 National Health Interview Survey (28). The relationship of race and smoking to serum IgG levels, however, remained when the analysis was restricted to subjects who smoked 20 or fewer cigarettes per day. Although the participation rate for blacks in this study was lower than for whites, the potential bias resulting from this disparity is unknown. Nevertheless, adjustment for potential confounders, including measures of socioeconomic status, did not significantly alter the results.

In summary, we were able to demonstrate the independent relationship of serum IgG levels to race and cigarette smoking and to correlate immunoglobulin levels with sIL-2R levels and the proportion of circulating HLA-DR⁺ cells. The challenge for the future will be to determine which of these immunologic relationships, alone or in concert with other factors, are related to the risk of developing clinical disease. Application of the rapidly developing laboratory techniques to analyze immunologic differences between population groups may provide insight into the mechanisms involved.

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