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Cigarette Smoking: A Modifier of Human Immunodeficiency Virus Type 1 Infection?

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Summary: Two hundred and two homosexual men enrolled in a prospective cohort study of AIDS risk were assessed for differences in the occurrence and progression of human immunodeficiency virus type 1 (HIV-1) infection with respect to cigarette smoking. Among subjects who were initially seronegative, smokers were more likely than nonsmokers to become HIV-1 seropositive ($p = 0.03$). After seroconversion, serum β_2-microglobulin and $CD4^+$ lymphocyte levels were elevated in cigarette smokers relative to nonsmokers ($p = 0.02$ for both comparisons), but both of these differences disappeared within 2 years. There was no detectable difference in the risk of AIDS or *Pneumocystis carinii* pneumonia with respect to smoking. Our data suggest that cigarette smoking may alter the immune response to HIV-1 infection, but it appears to have no marked effect on clinical outcome. They also suggest that cigarette smoking may be a surrogate marker for continued high-risk sexual behavior in homosexual men. **Key Words:** Human immunodeficiency virus—Smoking—Homosexual men.

AIDS has been documented to occur <18 months to >9 years after human immunodeficiency virus type 1 (HIV-1) seroconversion (1,2). Little is known about the factors responsible for this wide range in the time to AIDS, although both viral and host factors are being investigated.

The objective of this study was to determine whether there was any identifiable association between one behavioral factor, cigarette smoking, and the progression of HIV-1 infection. We were intrigued by the accumulating evidence of alterations in the immune system associated with smoking (3–17) and hypothesized that cigarette smoking might modify the effect of HIV-1 infection by one or more mechanisms.

METHODS

A cohort of homosexual men, previously described in detail (18–21), was enrolled during May and June 1982 in a study designed to identify factors associated with the development of AIDS. The men were consecutive patients of three primary care

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physicians in Washington, D.C. and New York, NY. Patients with known AIDS were excluded; >90% of the remaining eligible patients agreed to participate. Identical data collection instruments and procedures were used at all sites. Subjects were reevaluated approximately annually by self-administered questionnaires, physical examinations, and blood samples. The questionnaires assessed current sexual and other behavioral activities, including cigarette smoking ("ever/never," defined as more than/less than 5 packs in entire life, and current average number of cigarettes per day), as well as current health status. The diagnosis of AIDS was made according to the 1987 revision of the Centers for Disease Control surveillance case definition (22).

The present analysis included all subjects who were found to be HIV-1 antibody positive at the time of enrollment (prevalent positives) as well as all those who seroconverted during the observation period, 1982–1988. Fifty-eight percent of prevalent positives and 77% of seroconverters were still alive and in follow-up at the end of 3 years. At the end of 6 years, 33% of prevalent positives and 53% of seroconverters remained in active follow-up. The control group consisted of all subjects who were still in follow-up and who remained HIV antibody negative through 1988. This group represented 65% of all subjects who were HIV antibody negative at the time of their last evaluation.

Subjects who seroconverted were estimated to have done so at the midpoint between their last negative test and first positive test (i.e., in most cases, 6 months before their first positive test). Using back-calculation probabilities, Washington, D.C., and New York City prevalent positives were estimated to have seroconverted 1 and 2 years prior to the onset of the study, respectively (23–24). These estimates have been found to predict the decline of $CD4^+$ lymphocyte levels and the onset of AIDS rather accurately when compared with subjects with known seroconversion dates (25).

HIV antibodies were determined by a commercially available enzyme-linked immunosorbent assay (ELISA) (Electronucleonics Inc., Columbia, MD, U.S.A.). If positive, a Western blot assay (Biotech, Inc., Rockville, MD, U.S.A.) was performed for confirmation. T-lymphocyte subset enumeration was performed on frozen-thawed lymphocytes with a fluorescence-activated cell sorter using OKT4 and OKT8 monoclonal antibodies (Ortho Diagnostics Co., Raritan, NJ, U.S.A.) as measures of T helper/inducer ($CD4^+$) and T suppressor/cytotoxic ($CD8^+$) subsets, respectively. Serum neopterin was measured by radioimmunoassay (Neopterin RIAcid, Henning-Berlin, Berlin, F.R.G.). Serum β_2-microglobulin (B2M) levels were measured using a double antibody radioimmunoassay technique (Beta-2-micro RIA, Pharmacia, Uppsala, Sweden).

To standardize the T-lymphocyte subset data for year-to-year laboratory variations, individual values were adjusted by dividing by the mean value for all seronegatives (who remained seronegative throughout the observation period) tested in the same year (26). We compared the percentage rather than the absolute number of $CD4^+$ and $CD8^+$ lymphocytes because this parameter has been shown to be more stable over time (27–28). No such adjustment was required for B2M and neopterin levels, however, as these levels were measured in a single batch on previously frozen sera. Changes over time in the percentages of $CD4^+$ (% $CD4^+$) and $CD8^+$ (% $CD8^+$) lymphocytes and in B2M and neopterin levels were evaluated both including and excluding AIDS cases. However, to avoid distortion in the trend in % $CD4^+$ due to the abrupt decline seen in this marker shortly before the onset of AIDS (29–30), only the data from AIDS-free subjects are presented.

Differences between means were assessed by standard paired-sample t tests. Cumulative group differences were assessed by applying the Kruskal–Wallis test to the total area under the curve and/or the area under the curve from baseline obtained for each individual. This method takes into account the variable length of follow-up for different individuals. Stratified analyses were performed using the Mantel–Haenszel and maximum likelihood χ^2 tests. AIDS-free survival was evaluated by Kaplan–Meier curves and Cox's test for life table data (31–32).

RESULTS

A total of 202 men met the criteria of this study, including 84 who were seropositive at the start of the study, 47 who seroconverted during the observation period, and 71 who remained HIV-1 antibody negative and were still in follow-up at the end of the observation period. The mean age and age range of these three groups—prevalent positives, seroconverters, and seronegatives—were similar (Table 1).

Thirty-nine percent of seronegatives were ciga-

TABLE 1. *Age and proportion of active cigarette smokers at study enrollment by HIV status*

HIV antibody status	n	Mean age (range) (years)	Proportion actively smoking cigarettes in 1982	p[a]
Seronegatives[b]	71	34.3 (23–59)	0.39	—
Prevalent positives[c]	84	34.6 (23–53)	0.44	0.31
Non-AIDS[d]	44		0.43	
PWA[e]	40		0.45	
Seroconverters[f]	47	33.7 (24–65)	0.60	0.03
Non-AIDS	39		0.56	
PWA	8		0.75	
Total	202	34.3 (23–65)	0.46	—

[a] p values were obtained by χ^2 test in separate comparisons with seronegatives.
[b] Persons who were still in follow-up and who remained HIV-1 antibody negative through 1988.
[c] Persons HIV-1 antibody positive at the onset of the study in 1982.
[d] Persons who had not developed any AIDS-defining illnesses through 1988.
[e] Persons in whom AIDS had been diagnosed during the observation period.
[f] Persons who became HIV-1 antibody positive after the onset of the study.

rette smokers at the onset of the study, compared with 44 percent of prevalent positives (p = 0.31) and 60 percent of seroconverters (p = 0.03, Table 1). We therefore examined whether smoking was associated with known risk factors for the acquisition of HIV infection among homosexual men. As previously reported in part (18), both the number of homosexual partners and the frequency of receptive anal intercourse at the start of the study in 1982 were strongly associated with prevalent positive or seroconverter status versus seronegative status ($p < 0.01$ for all comparisons, data not shown). Seroconverters continued to have more partners than seronegatives through 1983 ($p = 0.02$) and more frequent receptive anal intercourse through 1986 ($p < 0.02$). Among the men who were seronegative and practicing above-average receptive anal intercourse in 1982 (i.e., >19 times in the previous year, the median for seronegatives and subsequent seroconverters), 20 (65%) of 31 smokers seroconverted compared with 11 (42%) of 26 nonsmokers ($p = 0.09$). Among those practicing less frequent receptive anal intercourse there was no suggestion that smoking status was associated with seroconversion [6 (26%) of 23 smokers versus 8 (24%) of 33 nonsmokers, $p = 0.88$]. Compared with nonsmokers, smokers who remained seronegative through 1988 had an approximately threefold increased number of partners ($p < 0.06$ for each year except 1982 and 1988) but no increased frequency of receptive anal intercourse ($p \geq 0.25$ for all years, data not shown).

Subjects were categorized according to their reported smoking behavior in 1982, because this was the best indicator of smoking status throughout the observation period as well as at the time of seroconversion. We could identify only one of the 47 subjects who seroconverted during the study period who reported a different smoking status during the year of seroconversion. This subject reported smoking during the year of seroconversion and at the time of one of two subsequent visits but not at the start of the study. Eighteen of the 47 seroconverters reported the same smoking status during the year of seroconversion as at the start of the study. Smoking status was not available for the remainder of the subjects during the year of seroconversion, but 25 (all of whom seroconverted in 1983, a year in which only approximately 20% of subjects completed questionnaires) reported the same status 1 year before and 1 year after seroconversion, and the three remaining seroconverters reported the same status in all of the at least 3 years in which this information was available, including 1 year before seroconversion.

During the observation period, there was an overall decrease in the proportion of prevalent positives and seroconverters who smoked (0.44 to 0.39 and 0.60 to 0.52, respectively), but there was virtually no change among seronegatives (0.39 to 0.38). Among prevalent positives, this change appeared to be due to discontinuation of smoking alone, whereas among seroconverters there was also a twofold greater loss to follow-up among smokers than nonsmokers. The mean number of cigarettes smoked per day decreased among prevalent positives (from 22 to 16) and seronegatives (20–15) but not among seroconverters (16, no change). However, none of these interesting possible trends, all of which largely occurred after 1984, were statistically significant, and no meaningful comparisons with changes in immunologic parameters could be made. Similarly, we were unable to meaningfully assess whether physician and/or patient awareness of HIV-1 antibody results (beginning in 1985) or AIDS diagnosis had any significant influence on smoking behavior.

The proportion of $CD4^+$ lymphocytes (% $CD4^+$) among seronegative cigarette smokers was greater than that of nonsmokers each year of the observation period except 1983 (see Fig. 1A), but the difference was not significant when the data were

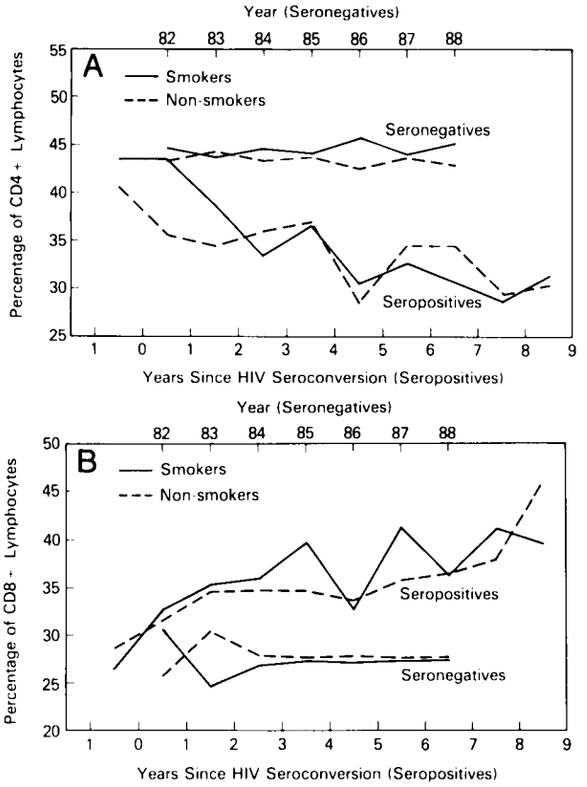

FIG. 1. Lymphocyte subset proportions in HIV-1-seropositive cigarette smokers and nonsmokers in relation to the estimated time of their seroconversion (see text) and year-by-year values for subjects seronegative through 1988. Subjects who developed AIDS were excluded. The number of seropositive subjects for whom these data were available varied from year to year: Year −0.5, 14 nonsmokers/17 smokers; Year 0.5, 11/12; Year 1.5, 28/32; Year 2.5, 26/23; Year 3.5, 27/31; Year 4.5, 25/26; Year 5.5, 20/18; Year 6.5, 18/14; Year 7.5, 15/10; Year 8.5, 8/3. Data were available for ≥40 seronegative nonsmokers and ≥23 seronegative smokers for each year except 1983, when data for only eight nonsmokers and seven smokers were available. **A:** Percentage of $CD4^+$ lymphocytes. **B:** Percentage of $CD8^+$ lymphocytes.

combined over the entire observation period (0.45 for smokers versus 0.43 for nonsmokers, $p = 0.48$ by Kruskal–Wallis test). Among AIDS-free seroconverters, however, the % $CD4^+$ of smokers was significantly higher than the % $CD4^+$ of nonsmokers 6 months after seroconversion ($p = 0.016$; Fig. 1A). At 18 months after seroconversion a borderline difference persisted ($p = 0.07$), but thereafter the % $CD4^+$ values of AIDS-free seropositive cigarette smokers and nonsmokers were indistinguishable ($p = 0.12$–0.9; Fig. 1A), and the overall change from baseline was not detectably different for the two groups. No statistically significant dose effect for the number of cigarettes per day on the % $CD4^+$ could be identified.

There were no significant differences in the proportions of $CD8^+$ lymphocytes with respect to cigarette smoking among either HIV-seronegative or -seropositive groups (Fig. 1B).

β_2-Microglobulin (B2M) levels increased markedly during the year of seroconversion among smokers but not nonsmokers ($p = 0.02$, Fig. 2A). Subsequently, B2M levels quickly merged and remained indistinguishable thereafter. The pattern was similar for neopterin, but the difference seen shortly after seroconversion was not significant ($p = 0.31$; Fig. 2B). The overall change from baseline was not detectably different for either measure.

Twenty-seven (56%) of the 48 subjects who developed AIDS during the observation period had *Pneymocystis carinii* pneumonia (PCP) as their initial AIDS-defining illness. There was no difference in the development of PCP versus other AIDS-defining illnesses with regard to smoking status ($p = 0.77$, data not shown), nor was there any differ-

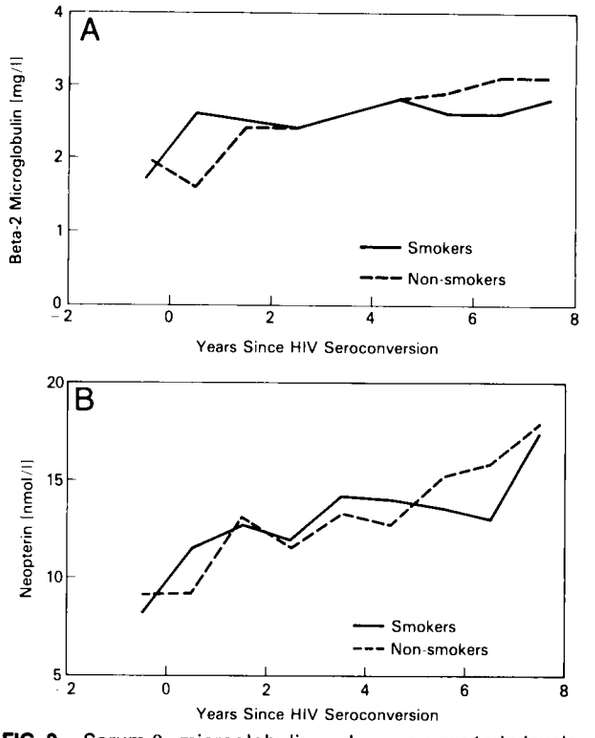

FIG. 2. Serum β_2-microglobulin and serum neopterin levels in HIV-1-seropositive cigarette smokers and nonsmokers in relation to the estimated time of their seroconversion (see text). Subjects who developed AIDS were excluded. The number of subjects for whom these data were available varied from year to year: Year −0.5, four nonsmokers/seven smokers; Year 0.5, 11/10; Year 1.5, 13/21; Year 2.5, 15/14; Year 3.5, 28/29; Year 4.5, 26/26; Year 5.5, 22/18; Year 6.5, 18/13; Year 7.5, 15/10. **A:** Serum β_2-microglobulin levels. **B:** Serum neopterin levels.

ence in the mean proportion of $CD4^+$ lymphocytes at the time that PCP was diagnosed in smokers versus nonsmokers ($p = 0.44$), although the data became sparse for this comparison (n = 10 smokers, six nonsmokers). Finally, there was no difference between the Kaplan–Meier AIDS-free survival curves of cigarette smokers and nonsmokers ($p = 0.31$; Fig. 3).

DISCUSSION

The accumulating evidence that cigarette smoking alters the immune system suggests the possibility that it could modify the host response to infection with HIV-1. In the normal host, cigarette smoking has been associated with reversible increases in the absolute lymphocyte, monocyte, neutrophil, and total leukocyte counts (3–5); an increase in the number of chromosomally injured peripheral blood lymphocytes (6–8); a decrease in the number and proportion of circulating natural killer (NK) cells (9,10); and an increase in the proportion of $CD4^+$ lymphocytes (5,11,12). In the pulmonary compartment, cigarette smoking has been linked to an increased number and proportion of bronchoalveolar lavage fluid macrophage/monocytes (13,14); a decreased proportion of $CD4^+$ lymphocytes and an increased proportion of $CD8^+$ lymphocytes in lavage fluid (13); a decrease in the activity of lavage fluid NK cells (14); increased activation but reduced function of lavage fluid macrophages (15,16); and an increased number of parenchymal dendritic and Langerhans cells, with a marked shift to a higher proportion of the more highly differentiated Langerhans cells (17). Clinically, in addition to its well-known relationships with various malignancies and with increased respiratory tract bacterial infections in specific populations, cigarette smoking has been shown to be associated with a decreased response to hepatitis B vaccine (33), an increased risk of acquiring epidemic influenza (34,35), and an apparent increased risk of varicella pneumonia when primary infection occurs during adulthood (36).

In this study, we did not detect any effect of cigarette smoking on the risk of AIDS in HIV-infected individuals, either in the previous (21) or in the current analysis. If such an effect does exist, it is too subtle for us to identify with the limitations of the power of our study, the length of our follow-up period, the decline in the proportion of smokers during the observation period, and/or the potential biases (whose magnitude and direction cannot be reliably estimated from the available data) introduced by loss to follow-up and in other ways. Considering power by itself, with an estimated cumulative AIDS

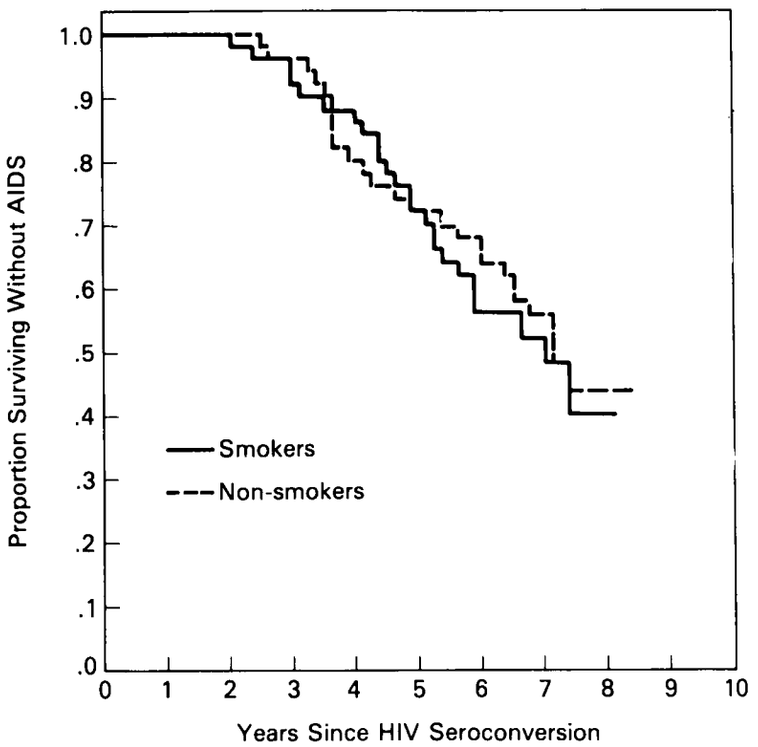

FIG. 3. Proportion of AIDS-free survivors among HIV-1 seropositive cigarette smokers and nonsmokers in relation to the estimated time of their seroconversion.

incidence of 0.25 among unexposed (nonsmoking) subjects, our study had an approximately 80% likelihood of detecting a relative risk of 1.9 or greater with a 5% risk of making a type I error (i.e., at $p = 0.05$).

However, we have observed some interesting and possibly important alterations in the immunologic parameters of HIV-1-infected persons with respect to smoking. Although these changes only occurred at one of many points in time for each parameter, suggesting that random variation may be responsible, their overlap (i.e., the occurrence of all of the changes during the same time period) argues that their possible significance be considered more seriously.

The increase in B2M levels at the time of seroconversion that we observed among smokers versus nonsmokers suggests an increased immune response to initial infection with HIV-1. B2M, the smaller, nonpolymorphic peptide component of the heterodimer class I histocompatibility antigens that is found on the surface of virtually all nucleated cells, and neopterin, a guanosine triphosphate metabolite thought to largely originate from activated macrophages and monocytes, have both been shown to be increased in a variety of inflammatory processes (37–39). Several investigators have shown that the progressive elevation of these substances during the latent period of HIV-1 infection is associated with a substantially increased risk of AIDS (40–44). More recently, it has also been shown that significant elevations in these parameters as early as 1–2 years after seroconversion can reliably predict the development of AIDS (25). We would therefore expect that if the B2M levels of smokers remained elevated relative to nonsmokers, the rate of development of AIDS would be increased among smokers. What we observed, however, was that within 2 years of seroconversion the mean B2M levels for the two groups had virtually merged, and they remained indistinguishable thereafter. The overall pattern was the same for neopterin, but the initial increase among smokers was not statistically significant.

While this pattern suggests that there is a more marked initial immune response to HIV infection among smokers, it also indicates that this alteration is transient. Whether this altered immune response, including its transient nature, is specific to HIV infection or follows exposure to a variety of acute and chronic infectious agents and other antigenic stimuli is unclear. Silverman et al. (45) reported an increased in vitro lymphocyte response to phytohemagglutinin in cigarette smokers <40 years of age, but a subsequent study failed to confirm this finding (46).

As with B2M levels, the $CD4^+$ lymphocyte levels of seropositive smokers were initially elevated relative to nonsmokers, but this difference also became undetectable within 2 years after seroconversion. As noted previously, cigarette smokers as a group have been reported to have higher $CD4^+$ lymphocyte levels than nonsmokers (5,11,12), although this has not been confirmed in all studies (13,47,48). Among our HIV-seronegative men, and among our seroconverters prior to seroconversion, smokers tended to have higher proportions of $CD4^+$ lymphocytes than nonsmokers, but the differences we observed could have been due to chance. If cigarette smokers do have higher $CD4^+$ lymphocyte levels, our data indicate that when they become infected with HIV-1 it takes up to 2 years for viral-related depletion of $CD4^+$ cells to overwhelm the smoking effect. Recently, Royce and Winkelstein reported similar findings for the San Francisco Men's Health Study cohort, although their longitudinal data only extended to approximately 1 year after seroconversion (49). If these observations are correct, they suggest that particularly among smokers, HIV-1 is not entirely latent after the period of acute infection is completed (30) but continues to be slowly destructive during the first 2 years after seroconversion. Our findings also indicate that later on in the course of infection, it is probably not necesary to take smoking behavior into account when $CD4^+$ lymphocyte levels are used to determine the appropriateness of initiating antiretroviral therapy, as Royce and Winkelstein have suggested it might be (49).

If cigarette smokers do have increased $CD4^+$ lymphocyte levels, the elevated B2M levels we observed among smokers at the time of seroconversion could simply be due to a more widespread initial infection among the expanded pool of $CD4^+$ cells. Alternatively, this increase in B2M levels could primarily be due to more active initial replication of the virus, which might be related to qualitative rather than quantitative effects of smoking on $CD4^+$ cells. HIV-1 replication has been shown to be increased in activated $CD4^+$ lymphocytes (50,51), and although similar studies comparing cigarette smokers with nonsmokers have not been reported, it may be relevant that the lymphocytes of smokers have been shown to replicate in vitro more

rapidly than the lymphocytes of nonsmokers (8). Analysis of frequent, prospectively measured p24 antigen levels or direct measurement of $CD4^+$ lymphocyte activation and HIV replication in smoking versus nonsmoking seroconverters might allow an assessment of this possible mechanism. If increased initial replication does occur, it might be expected to result in a larger pool of HIV-infected cells and an accelerated pre-AIDS phase when latency breaks down. Any such positive effects on the progression of HIV-1 infection could be opposed, however, by other, possibly stimulatory effects of smoking on the immune system associated with the reported increase in $CD4^+$ lymphocytes. Multiple effects by multiple mechanisms would not be surprising given the more than 48 major volatile and 51 major particulate constituents of cigarette smoke (52). The resulting overall effect on the progression of HIV-1 infection could be positive, negative, indeterminate, or variable among individual smokers.

There are many possible explanations for our finding that seroconverters are more likely than seronegatives to be cigarette smokers, but it seems most likely that smoking is a surrogate marker for increased risk-taking behavior that we have not measured, e.g., frequency of unprotected sexual contacts, anonymous partners, and partners with unknown HIV status (53). This is supported somewhat by our finding that among persons who practiced above-average receptive anal intercourse, smokers may have been more likely to become HIV-1 antibody positive.

In summary, although we have been unable to detect an effect of cigarette smoking on the overall risk of *Pneumocystis carinii* pneumonia or AIDS in HIV-1-infected homosexual men, we have observed several findings that suggest that smoking may in fact alter the immune response to HIV-1 infection. Further study is required to confirm these findings and to determine whether they have clinical significance.

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