

# Immune function in cigarette smokers who quit smoking for 31 days

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*A group of 28 healthy, white, male, light-to-moderate smokers, 21 to 35 years of age, were offered a financial inducement to abstain from smoking for 31 days. A matched control group of 11 smokers were paid to continue smoking during the same period. Nonspecific parameters of immune system function were monitored before and at various times after smoking abstinence. Abstinence increased natural killer cell cytotoxic activity but did not alter mitogen-induced T-lymphocyte proliferation as measured by responses to concanavalin A or phytohemagglutinin. Serum cortisol concentrations also decreased after smoking cessation; however, changes in immune function were not correlated with serum cortisol change, nor with indices of smoking such as plasma nicotine and cotinine levels. Responses to concanavalin A and phytohemagglutinin were positively correlated with change in self-reported alcohol ingestion during smoking abstinence. Results indicate that elevation in natural kill cell cytotoxic activity is detectable within 1 month of smoking cessation, even in light-to-moderate smokers. However, elevation in natural killer cell cytotoxic activity appears not to be directly related to cessation-induced reductions in plasma nicotine, cotinine, or circulating cortisol levels. (J ALLERGY CLIN IMMUNOL 1995;95:901-10.)*

**Key words:** Smoking, immunity, natural killer cell, Con A, PHA, cortisol, nicotine, cotinine, alcohol

Cigarette smoking is associated with reductions in serum immunoglobulin,<sup>1</sup> helper/suppressor T-cell ratios,<sup>2</sup> mitogen-induced lymphocyte transformation,<sup>3</sup> and natural killer cytotoxic activity (NKCA).<sup>4,5</sup> Although these findings imply a substantial health benefit of smoking abstinence, immunosuppression is not consistently found in all smokers,<sup>6</sup> and light-to-moderate smokers may not differ from nonsmokers in immune function.<sup>2,5,7</sup> Surprisingly, some studies even report enhanced immune function in smokers.<sup>8,9</sup> These discrepant results suggest a more complex relationship between smoking and immune function than is often appreciated.

The mechanism by which tobacco use may suppress immune function has not been established. A

## Abbreviations used

|            |                                          |
|------------|------------------------------------------|
| ANOVA:     | Analysis of variance                     |
| Con A:     | Concanavalin A                           |
| E:T ratio: | Effector:target ratio                    |
| NKCA:      | Natural killer cytotoxic activity        |
| PBMLs:     | Peripheral blood mononuclear lymphocytes |
| PBS:       | Phosphate-buffered saline                |
| PHA:       | Phytohemagglutinin                       |

direct role of smoking-induced nicotine exposure is suggested by in vitro suppression of NKCA by nicotine<sup>10</sup> and suppression of the proliferative response of blood lymphocytes to the T-cell mitogen concanavalin A (Con A) after acute nicotine administration in rats.<sup>11</sup> Alternately, smoking may depress immune function because nicotine stimulates the hypothalamic-pituitary-adrenal axis,<sup>12-14</sup> thereby elevating levels of endogenous glucocorticoids, which are powerful immunosuppressants in vivo<sup>15,16</sup> and in vitro.<sup>17-19</sup> The resulting elevation of plasma cortisol could then depress immune function.<sup>20</sup> Finally, tobacco-related polycyclic aromatic hydrocarbons, such as benzo[a]pyrene, have been

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implicated in suppression of B-cell lymphopoiesis<sup>21</sup> and cytotoxicity in lymphokine-activated killer cells.<sup>22</sup>

In a recent major study of lifestyle factors and health involving 2892 Japanese men and women, cigarette smoking was associated with decreased NKCA.<sup>7</sup> However, immune system functioning is influenced by other lifestyle variables, such as diet, alcohol consumption, daily workload, sleep, body weight, and psychologic stress.<sup>23-26</sup> Thus an improvement in immune function after smoking cessation could result from other lifestyle changes, which accompany smoking cessation (e.g., improved diet, increased rest, reduced alcohol and drug consumption).

Interpreting the results of smoking cessation studies is also confounded by self-selection and attrition problems. For example, 10% to 30% of participants in quit-smoking programs typically drop out within the first week, with fewer than 50% remaining abstinent for 1 month.<sup>27, 28</sup> Thus successful quitters constitute a nonrandom sample of smokers whose immunologic responses to smoking and smoking abstinence might not be representative of the population of smokers at large. Therefore there is a need to evaluate the consequences of smoking cessation in a representative sample that retains smokers who might normally drop out of conventional cessation programs. In addition, a matched group of smokers who continue to smoke would provide a highly desirable control for spontaneous immunologic changes that are unrelated to smoking abstinence.

To accomplish this, we offered smokers a financial inducement to quit or continue smoking for 31 days. The primary goal of these studies was to characterize the changes in NKCA and in mitogen-stimulated proliferation of lymphocytes by Con A and by phytohemagglutinin (PHA) resulting from smoking cessation. Experimental conditions were designed to maximize compliance with and completion of the program. A second goal was to assess the degree to which postcessation changes in immune function indices were related to changes in serum cortisol levels, alcohol consumption, and plasma concentrations of nicotine and cotinine, a nicotine metabolite of which the plasma concentration provides an index of nicotine consumption.<sup>29</sup>

## METHODS

The research protocol was approved by the Carbon-dale Committee for Research Involving Human Subjects.

## Subjects

Participants were screened from a pool of white men who responded to a newspaper advertisement offering to pay cigarette smokers to quit smoking. Individuals who reported chronic diseases or health problems that might interfere with their participation or current use of prescription or nonprescription psychoactive drugs were eliminated from the pool, as were those who reported regular consumption of more than 15 alcoholic drinks per week. A total of 48 white men, aged 21 to 35 years, were selected. All participants reported habitually smoking at least 10 cigarettes per day with a nicotine delivery of 0.6 to 1.2 mg per cigarette, as estimated by the Federal Trade Commission, for at least 2 years.

## Procedure

Potential participants were invited to the laboratory for an interview during which the study requirements were explained. Only those prospective participants who expressed a strong desire to abstain from smoking and who were willing to deposit a check for \$50.00, which was to be forfeited unless all study requirements were successfully completed, were invited to participate. (Checks were returned to all participants who completed the 31-day program and whose plasma nicotine and cotinine levels indicated that they had complied with the smoking abstinence requirements.) Informed consent was obtained from those who qualified and elected to participate. Participants were offered the additional inducement of \$400.00 on completion of all requirements, which included participation in laboratory data collection sessions (described below) and visits to the laboratory every other day for monitoring of breath carbon monoxide (measured with a Mini CO Carbon Monoxide Breath Analyzer [model 1000; Catalyst Research Corp., Baltimore, Md.]). Quitters whose expired carbon monoxide levels exceeded 8 ppm were reminded of penalties for smoking during the abstinence period, and saliva samples were collected for later assays of salivary nicotine as a means of detecting unauthorized smoking.

After the orientation session, participants attended seven laboratory data collection sessions: 1 to 2, baseline; 3 to 7 Post-Quit or Continue-Smoking. Data collection sessions were scheduled at 1, 3, 10, 17, and 31 days after the cessation of smoking for the Quit group and at the same times for the Smoker (continue smoking control) group. At the start of each of these sessions, subjects completed a questionnaire indicating their cigarette and alcohol consumption during the previous week. An alcoholic drink was defined as 12 ounces of beer, 5 ounces of wine, or 1 ounce of liquor. One week after the second baseline session, all participants were required to abstain from smoking, starting at 11:59 PM of the night before the test session, to be conducted at either 1:00 or 3:00 PM on the next day. This session was designated "cessation day 1" (C1). Subjects were randomly assigned to the Quitter or Smoker groups after the C1 session. Smoker group subjects were required to

resume smoking at their previous levels for the remainder of the study, at which time they were instructed in methods of quitting smoking as described by the American Lung Association. To maintain a schedule of testing at weekly intervals, Quitters were required to resume smoking until 11:59 PM of the third night before the next weekly laboratory session, "cessation day 3" (C3), when they were required to cease smoking for the remainder of the study. However, they were allowed some latitude regarding lapses from total abstinence, as long as they smoked no more than 3 cigarettes in a single day, no more than 10 cigarettes during the total abstinence period, and no cigarettes at all on any experiment test day. Each subject was tested at the same time for the remaining four laboratory sessions, designated C3, C10, C17, and C31. An indwelling catheter was inserted into a medial antecubital vein at the start of each laboratory session. To verify smoking abstinence, blood samples were taken 1 hour after catheter insertion for subsequent determinations of plasma nicotine and cotinine levels. Blood samples for assays of cortisol were also taken at the same time of day as the nicotine and cotinine samples: at 2:00 PM for half the subjects and at 4:00 PM for the other half. Samples were centrifuged, and plasma and serum fractions were frozen at  $-90^{\circ}\text{C}$  for later assay. Nicotine and cotinine concentrations were determined in the laboratory of Dr. Neal Benowitz, University of California at San Francisco, by gas chromatography with nitrogen-phosphorus detection<sup>30</sup>; 5-methylnicotine and 1-methyl-5-(2-pyridyl)-pyrrolidine-2-one ("ortho-cotinine") were used as internal standards. This method has been modified for simultaneous extraction of nicotine, cotinine, and caffeine with the use of capillary gas chromatography.<sup>31</sup> Serum cortisol was determined by radioimmunoassay (Diagnostic Products Corp., Los Angeles, Calif.) at the American Health Foundation, Valhalla, New York.

## Reagents and chemicals

Reagents and chemicals used in this study were American Chemical Society-certified Tissue Culture Grade or Molecular Biology grade. They were purchased from Sigma Chemical (St. Louis, Mo.) or Fisher Scientific (Pittsburgh, Pa.), unless otherwise noted.

## Immune assays

One hour after catheter insertion, venous blood was collected in vacutainers containing acid citrate dextrose solution for NKCA and mitogen proliferation assays. Assays were performed by a researcher who was blinded to the status of the participants. Peripheral blood mononuclear lymphocytes (PBMLs) were isolated by gradient centrifugation on lymphocyte separation medium (Histopaque 1077, Sigma), washed three times with phosphate-buffered saline (PBS), and resuspended in complete media before assay. RPMI 1640 tissue culture medium (Sigma) was supplemented with 10% heat-inactivated fetal calf serum (Cell Culture Laboratories,

Cleveland, Ohio), penicillin (100 U/ml) and streptomycin (100  $\mu\text{g}/\text{ml}$ ), glutamine (2 mmol/L), sodium pyruvate (1 mmol/L), Fungizone (2.5  $\mu\text{g}/\text{ml}$ ), and minimum essential media nonessential amino acids (0.1  $\mu\text{mol}/\text{L}$ ; Gibco-BRL, Gaithersburg, Md.). A human erythroleukemic cell line, K562, was maintained by biweekly splitting and feeding in complete medium to maintain logarithmic growth. Cells were washed twice with PBS before use in assays.

PBMLs were tested in triplicate for NKCA in a 4-hour chromium 51-release assay.<sup>32</sup> K562 target cells,  $1 \times 10^7/\text{ml}$ , were washed twice and incubated at  $37^{\circ}\text{C}$  for 1 hour with 100  $\mu\text{Ci}$   $\text{Na}_2^{51}\text{CrO}_4$ , specific activity 390 mCi/mg Cr (Amersham Corp., Arlington Heights, Ill.). Cells were then washed four times in complete medium and adjusted to a concentration of  $5 \times 10^5$  cells/ml. PBMLs were washed twice and adjusted to a concentration of  $2 \times 10^6$  cells/ml. With the use of a V-bottomed microtiter plate (Nunc Intermed, Batavia, N.Y.), 100  $\mu\text{l}$  of the target cell suspension was added to each well to produce final effector-to-target (E:T) ratios of 40:1, 20:1, 10:1, and 5:1. Complete lysis was determined by target cells incubated with 100  $\mu\text{l}$  of 1% Nonidet-P40, and spontaneous release was determined by incubating target cells with 100  $\mu\text{l}$  of complete medium. Plates were incubated at  $37^{\circ}\text{C}$  for 4 hours and centrifuged at 400 g for 10 minutes. One hundred microliters of supernatant was removed from each well, placed in a  $12 \times 75$  mm polypropylene tube, and counted on a Gamma 500 Spectrophotometer (Beckman Instruments, Irvine, Calif.). The median disintegrations per minute of the triplicate assays performed on each subject was determined, and percent lysis was calculated:

% lysis =

$$\frac{\text{dpm (experimental)} - \text{dpm (spontaneous)}}{\text{dpm (total release)} - \text{dpm (spontaneous)}} \times 100$$

Lymphocyte proliferation was determined by a tritiated thymidine uptake assay.<sup>33</sup> Washed PBMLs were adjusted to a concentration of  $1 \times 10^6$  cells/ml in complete medium. In flat-bottomed microtiter plates, 100  $\mu\text{l}$  of PBMLs was added to 100  $\mu\text{l}$  of Con A (0.2 mg/ml; Difco, Detroit, Mich.) or PHA (0.2  $\mu\text{g}/\text{ml}$ ; Difco). Control wells contained 100  $\mu\text{l}$  of PBMLs with 100  $\mu\text{l}$  complete medium. All assays were done in triplicate. Plates were incubated at  $35^{\circ}\text{C}$  for 48 hours. Twenty microliters of tritiated-thymidine, 50  $\mu\text{Ci}/\text{ml}$  with specific activity of 45 Ci/mmol/L (Amersham Corp.), was added to each well, and the plates were incubated at  $35^{\circ}\text{C}$  for an additional 24 hours. Cells were harvested on an M-24R Cell Harvester (Brandel, Inc., Gaithersburg, Md.), suspended in 5 ml of scintillation fluid (Ecolite; ICN Biomedicals, Irvine, Calif.), and counted for 1 minute with an LS 6800 Counter (Beckman Instruments). Results are expressed as median disintegrations per minute minus control disintegrations per minute.

**TABLE I.** Characteristics of participants in Smoker and Quitter groups at baseline, before cessation of smoking

| Variable             | Smokers |        | Complete Quitters |        | Dropout Quitters |        |
|----------------------|---------|--------|-------------------|--------|------------------|--------|
|                      | Mean    | SD     | Mean              | SD     | Mean             | SD     |
| <i>n</i>             | 11      | —      | 28                | —      | 6                | —      |
| Cigarettes/day*      | 23.7    | 7.3    | 20.5              | 6.3    | 28.7             | 10.8†  |
| Plasma nicotine      | 15.5    | 6.0    | 13.6              | 3.8    | 16.4             | 6.0    |
| Plasma cotinine      | 264.7   | 137.4  | 246.1             | 83.8   | 341.1            | 83.1†  |
| Alcoholic drinks/wk* | 5.5     | 4.6    | 7.7               | 6.6    | 13.7             | 6.5†   |
| Serum cortisol       | 8.8     | 3.0    | 10.3              | 3.3    | 10.9             | 2.5    |
| NKCA (20:1)          | 40.1    | 8.8    | 34.2              | 15.0   | 38.8             | 16.6   |
| NKCA (10:1)          | 27.4    | 5.8    | 24.4              | 11.6   | 28.7             | 13.7   |
| Con A (dpm)          | 69,130  | 45,872 | 65,614            | 40,906 | 54,222           | 28,480 |
| PHA (dpm)            | 107,684 | 53,468 | 140,490           | 63,660 | 109,728          | 39,167 |

\*Self-reported.

†Difference between Complete Quitter and Dropout Quitter:  $p < 0.05$ .

### Dependent measures and statistical analyses

Mean baseline measures were determined by averaging the two precessation baseline values for each dependent variable. Differences between Quitter and Smoker groups at baseline were evaluated with individual  $t$  tests for independent samples. Pre- and postcessation nicotine, cotinine, and cortisol concentrations were analyzed with separate, "mixed," Smoker Status (Quitter vs Smoker)  $\times$  Quit Day (baseline vs C1 vs C3 vs C10 vs C17 vs C31) analyses of variance (ANOVAs), with the Geisser-Greenhouse correction for sphericity of repeated measures on the Quit Day factor. For NKCA analyses, postcessation changes from baseline were computed by subtracting mean baseline values from raw NKCA values for each E:T ratio on each postcessation Quit Day. These change scores were analyzed with a "mixed," Smoker Status (Quitter vs Smoker)  $\times$  E:T Ratio (40:1 vs 20:1 vs 10:1 vs 5:1)  $\times$  Quit Day (C3 vs C10 vs C17 vs C31) ANOVA, with Geisser-Greenhouse correction for sphericity of repeated measures on the E:T ratio and Quit Day factors. In addition to estimates of main and interaction effects, this ANOVA also indicated whether the average change scores within E:T ratios exceeded zero. Analyses of simple main effects were performed on significant ( $p < 0.05$ ) interactions. Comparable ANOVAs were performed on changes from baseline in mitogen-induced proliferative responses to Con A and PHA.

## RESULTS

### Participant compliance

Two Smoker group and six Quitter group participants withdrew before the end of the 31-day data collection period. Data from one Quitter were excluded because his plasma nicotine and cotinine levels indicated that he had continued to smoke

during the cessation phase. Among the remaining 28 Quitters, three were judged to have had temporary smoking lapses on the basis of occasional slight elevations in their plasma nicotine and cotinine concentrations. Their data were retained because they appeared to have otherwise complied with the main abstinence criteria and had smoked fewer than 10 cigarettes during the 31-day cessation period. Thus complete data sets were obtained from 28 of 35 (80%) of the Quitters and 11 of 13 (85%) of the Smokers who participated. Table I shows that Smokers and Quitters who successfully completed the program were comparable in indices of smoking, alcohol consumption, and immune function before the cessation phase (all  $p$  values  $> 0.05$ ). However, dropouts from the Quitter Group smoked and drank somewhat more heavily, exceeding Complete Quitters in mean baseline cotinine level ( $t[31] = 2.37$ ,  $p < 0.05$ ); number of cigarettes smoked per day ( $t[32] = 2.52$ ,  $p < 0.05$ ); and number of alcoholic drinks consumed per week ( $t[32] = 2.03$ ,  $p < 0.05$ ).

Fig. 1 shows the decline from baseline in mean plasma nicotine and cotinine concentrations after overnight smoking abstinence (C1) in both the Quitter and Smoker groups ( $F_s[1,37] = 269.8$  and  $80.3$  for nicotine and cotinine, respectively; both  $p$  values  $< 0.001$ ). By the tenth day of abstinence (C10), Quitters' mean plasma nicotine and cotinine levels approached the limits of detectability of the assay (i.e., 1.0 ng/ml for nicotine and 10.0 ng/ml for cotinine). Inspection of individual values confirmed that Quitters' plasma cotinine levels were below 20 ng/ml in all but three of 28 subjects by

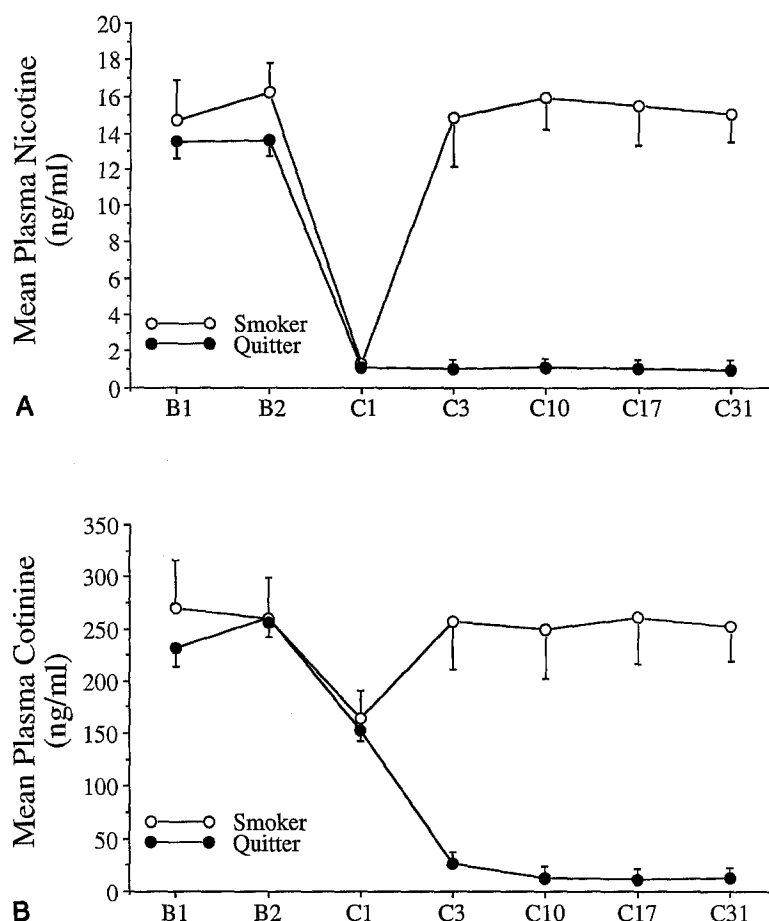


**FIG. 1.** Mean plasma nicotine (**A**) and cotinine (**B**) concentrations in Quitters ( $n = 28$ ) and Smokers ( $n = 11$ ) on two baseline days (B1 and B2) and on test days across 31 days during the smoking abstinence phase (C1 to C31). Vertical lines indicate standard errors.

C10, verifying compliance with the stop-smoking requirements.

#### Cessation effects on immune function and serum cortisol

The ANOVA on postcessation changes from baseline in NKCA revealed no significant main effect of Quit Day or E:T ratio (both  $p$  values  $> 0.05$ ), but a significant main effect for Smoker Status ( $F[1,37] = 5.01, p < 0.05$ ) and a significant Smoker Status  $\times$  E:T ratio interaction ( $F[3,111] = 4.10, p < 0.05$ ) were observed. Follow-up analyses of simple effects showed that the effects of quitting smoking were greatest with E:T ratios of 20:1 and 10:1. After smoking cessation, the NKCA of Quitters increased above baseline with the 20:1 E:T ratio ( $F[1,37] = 4.17, p < 0.05$ ) (Fig. 2). In contrast, Smokers' NKCA decreased below base-

line during the same period with the 20:1 E:T ratio ( $F[1,37] = 6.52, p < 0.05$ ); Smokers' NKCA was also marginally below baseline with the 10:1 E:T ratio ( $F[1,37] = 3.27, p < 0.08$ ). Consistent with these differences, NKCA changes were higher in Quitters than in Smokers with E:T ratios of 20:1 ( $F[1,37] = 10.55, p < 0.01$ ) and 10:1 ( $F[1,37] = 5.28, p < 0.05$ ). In contrast, analyses of raw or log-transformed changes from baseline indicated that Quitters and Smokers did not differ significantly in Con A- or PHA-induced T-cell activation at any time during the experiment.

Serum cortisol decreased in Quitters from a mean of 10.3 at baseline to a mean of 7.8  $\mu\text{g/dl}$  after smoking cessation ( $F[1,37] = 15.50, p < 0.001$ ). The change in Smokers across 31 days, from a mean of 8.8 at baseline to a mean of 8.2  $\mu\text{g/dl}$ , was nonsignificant ( $p > 0.05$ ). Self-reported

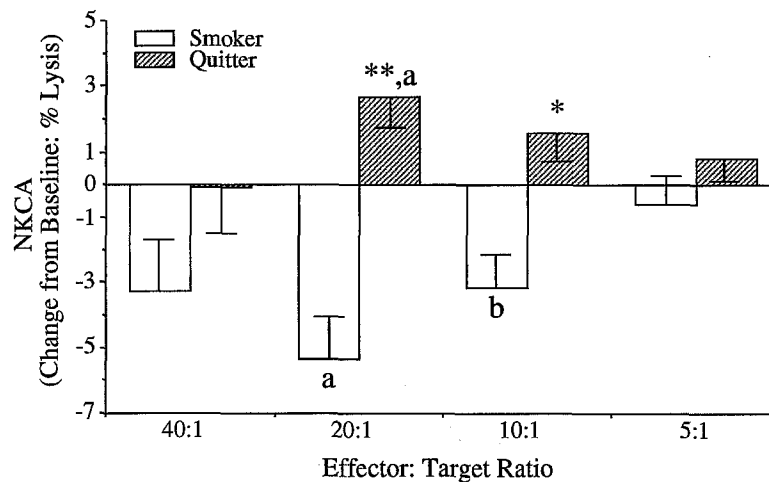


**FIG. 2.** Mean changes in NKCA (percent lysis) in Quitters ( $n = 28$ ) and Smokers ( $n = 11$ ) across 31 days of testing, at four E:T ratios. Vertical lines represent standard errors. Asterisks refer to significance of differences between Quitters and Smokers. \* $p < 0.05$ ; \*\* $p < 0.01$ . Letters refer to significance of differences from baseline <sup>a</sup> $p < 0.05$ ; <sup>b</sup> $p < 0.08$ .

**TABLE II.** Pearson correlations relating indices of immune function to measures of cigarette consumption, serum cortisol, and self-reported alcohol ingestion at baseline in 39 study participants

|                   | NKCA10:1 | Con A | PHA   | No. of cigarettes | Nicotine | Cotinine | Cortisol | Alcohol |
|-------------------|----------|-------|-------|-------------------|----------|----------|----------|---------|
| NKCA20:1          | 0.959*   | 0.210 | 0.133 | -0.226            | -0.047   | -0.170   | -0.070   | -0.088  |
| NKCA10:1          |          | 0.125 | 0.135 | -0.245            | -0.044   | -0.114   | -0.096   | -0.138  |
| Con A             |          |       | 0.140 | -0.319†           | -0.165   | -0.209   | 0.010    | -0.151  |
| PHA               |          |       |       | -0.028            | 0.042    | 0.155    | -0.096   | -0.256  |
| No. of cigarettes |          |       |       |                   | 0.403†   | 0.529*   | 0.011    | -0.019  |
| Nicotine          |          |       |       |                   |          | 0.859*   | -0.168   | -0.259  |
| Cotinine          |          |       |       |                   |          |          | -0.193   | -0.320† |
| Cortisol          |          |       |       |                   |          |          |          | 0.261   |

\* $p < 0.01$  (two-tailed).

† $p < 0.05$  (two-tailed).

alcohol consumption increased significantly during the cessation phase, but to approximately the same extent in both Quitter and Smoker groups, from a mean of 7.1 to a mean of 11.4 drinks per week, ( $F[1,74] = 6.46, p < 0.01$ ).

### Correlations among measures

To assess the degree to which indices of immune function were associated with various smoking parameters, with serum cortisol levels, and with self-reported alcohol ingestion, a series of Pearson and partial correlation coefficients were calculated. Table II shows that smoking indices (number of cigarettes smoked and plasma nicotine and cotinine levels) were moderately to highly intercorre-

lated at baseline. However, NKCA and response to PHA were not significantly correlated with plasma nicotine and cotinine concentrations, nor were they related to self-reported cigarette and alcohol consumption at baseline (all  $p$  values  $> 0.05$ ). Response to Con A was also unrelated to these parameters except for a modest negative correlation between response to Con A and self-reported cigarette consumption at baseline ( $r[37] = -0.319, p < 0.05$ ); thus, although increased number of cigarettes smoked was associated with decreased proliferative response to Con A at baseline, plasma nicotine and cotinine levels were not significantly correlated with response to Con A.

Partial correlations on raw scores and changes

from baseline, controlling for smoker status, indicated that NKCA, Con A, and PHA were not significantly correlated with raw scores or changes from baseline in plasma nicotine and cotinine concentrations, nor with self-reported cigarette and alcohol consumption, with one exception. Interestingly, changes from baseline in responses to Con A and to PHA were modestly positively correlated with changes in self-reported alcohol consumption ( $r[36] = 0.421$ ,  $p < 0.01$ ;  $r[36] = 0.339$ ,  $p < 0.05$ , respectively); that is, increased alcohol consumption during the abstinence phase was associated with increased lymphocyte proliferation in response to both Con A and PHA. Postcessation changes in Con A- and PHA-stimulated lymphocyte activity, summed across test days, were also modestly correlated with each other ( $r[36] = 0.357$ ,  $p < 0.01$ ). However, reanalysis of changes in both Con A- and PHA-stimulated lymphocyte activity by means of analysis of covariance with change in alcohol consumption as a covariate yielded nonsignificant effects of smoking cessation for both Con A and PHA stimulation of lymphocytes.

As expected, abstinence from cigarette smoking produced increases in NKCA, as well as decreases in serum cortisol levels. However, NKCA was not significantly correlated with self-reported cigarette consumption or blood nicotine, cotinine, or cortisol concentrations at baseline, nor were postcessation changes in NKCA correlated with these parameters at any time after smoking cessation. T-cell activation in response to Con A was modestly associated with number of cigarettes smoked before the smoking cessation phase. However, abstinence-induced changes in response to Con A and PHA were not consistently affected by smoking cessation but appeared to be modestly positively correlated with changes in alcohol consumption.

## DISCUSSION

Providing a substantial monetary incentive and frequent monitoring of compliance produced nearly complete abstinence among habitual smokers in this study. Plasma nicotine and cotinine concentrations decreased to marginal levels within 10 days of the start of abstinence, and a much higher rate (80%) of successful completion of the abstinence program was achieved than is normally obtained in smoking cessation studies.<sup>27, 28</sup> However, because the six Quitter group dropouts were somewhat higher in precessation cotinine, number of cigarettes smoked, and alcohol consumption

than those who completed all phases of the study, the results presented here may underestimate changes that occur in heavier smokers and drinkers. Thus these data are likely to be highly representative of the effects of quitting smoking in light-to-moderate smokers.

The most important finding of this study is that NKCA increased substantially in Quitters, relative to the matched control group that continued to smoke, within 31 days of smoking cessation. Hersey et al.<sup>34</sup> found an increase in NKCA by 3 months after smoking cessation, particularly in groups of quitters that included more subjects who smoked 30 to 40 or more cigarettes per day before quitting. Our findings support results of earlier studies implicating cigarette smoking in reduced NKCA<sup>4, 5, 7, 34</sup> but suggesting that NKCA increases within 1 month of smoking cessation, even in smokers who average only about 20 cigarettes per day.

We found a modest correlation ( $r = -0.319$ ) between number of cigarettes smoked and Con A-induced T-cell activation at baseline, which is consistent with an earlier report showing depressed responses to PHA and Con A in habitual smokers, relative to nonsmoking control subjects.<sup>3</sup> Nevertheless, in contrast to the NKCA findings, quitting smoking did not appear to enhance Con A- or PHA-stimulated lymphocyte proliferation. Although the reason for this discrepancy is not readily apparent, results of previous studies have been inconsistent; some studies report depression, some enhancement, and some no effect of smoking.<sup>3</sup> It is conceivable that changes in responses to Con A and PHA require longer than 1 month to develop after smoking cessation and that such changes are smaller and less readily detectable in lighter smokers, such as the ones we tested.

Although the role of smoking-induced glucocorticoid elevation in immunomodulation is a matter of considerable theoretic interest, the relationship between abstinence-induced changes in *in vivo* serum cortisol levels and immune function in smokers has not been reported previously. In this study, serum cortisol concentrations decreased reliably after smoking abstinence, consistent with earlier reports of smoking-induced elevation in blood cortisol.<sup>12-14</sup> However, although glucocorticoid elevation is known to be potentially immunosuppressive,<sup>15-19</sup> we found no significant correlations between baseline serum cortisol and NKCA or responses to Con A or PHA before smoking cessation; nor was postcessation cortisol correlated with changes in these indices of immune function.

Earlier studies, in which between-groups designs were used, document a relationship between in vivo cortisol levels and measures of immune function in clinical populations. For example, two studies<sup>35, 36</sup> report suppressed NKCA in clinically depressed and bereaved subjects whose mean serum cortisol concentrations exceeded those of normal control subjects (mean = 26.5 vs 9.1, and 15.4 vs 11.8  $\mu\text{g/dl}$ , respectively, in the two studies). Similarly, lymphocyte proliferation in response to Con A and PHA was suppressed in two studies<sup>37, 38</sup> of clinically depressed patients whose mean serum cortisol levels exceeded mean control levels (mean = 16.0 vs 11.7 and 14.0 vs 8.6  $\mu\text{g/dl}$ , respectively, in the two studies). Thus differences between groups in in vivo serum cortisol levels, which are comparable to but somewhat larger in magnitude than those we observed in the Quitter group at baseline and after smoking cessation (10.3 vs 7.8  $\mu\text{g/dl}$ ), have been associated with alterations in NKCA and mitogen-induced lymphocyte proliferation. Nevertheless, correlations between in vivo cortisol and immune function parameters have proved to be nonsignificant in a number of studies,<sup>18, 35, 36, 39</sup> as in this study. This suggests the lack of a direct causal relationship between in vivo cortisol secretion and NKCA or mitogen-induced lymphocyte proliferation.<sup>25</sup> However, as has been noted previously,<sup>39</sup> a single daytime measure of cortisol may not be sufficiently sensitive to detect subtle disturbances in hypothalamic-pituitary-adrenal function that may influence immune system function.

An unexpected finding was that greater alcohol consumption during the abstinence period was associated with greater mitogen-induced lymphocyte proliferation in response to Con A and PHA. To our knowledge, a positive relationship between alcohol consumption and mitogen-induced lymphocyte proliferation has not been reported previously. In fact, alcohol ingestion has been shown to suppress various measures of lymphocyte proliferation in human beings and laboratory animals.<sup>40-43</sup> Although the positive correlations we observed may be entirely fortuitous, it could also be the case that recently abstinent smokers respond differently to alcohol than habitual smokers or nonsmokers. Furthermore, the fact that our sample was drawn from a pool of carefully screened moderate alcohol users may also have contributed to this anomalous finding. Interestingly, although immunosuppression often accompanies long-term excessive

alcohol consumption,<sup>23, 44</sup> a positive relationship between moderate alcohol consumption and NKCA has been noted in some studies.<sup>7, 23, 45</sup>

Although NKCA clearly increased after smoking cessation, NKCA was not correlated with the precessation number of cigarettes smoked, plasma nicotine and cotinine levels, or postcessation changes in these parameters. The absence of a relationship between change in NKCA and measures of precessation smoking heaviness is somewhat surprising because earlier studies report greater suppression of NKCA in heavy smokers than in those who smoke less. A possible methodological explanation for this discrepancy might be that correlations were attenuated by restriction in the range of smoking heaviness among participants because fewer than half (12 of 28) reported smoking 21 or more cigarettes per day, whereas only three reported smoking 31 or more cigarettes per day before cessation.

Nevertheless, concerning possible mechanisms of suppression of NKCA, our results suggest that exposure to nicotine or its primary metabolite, cotinine, does not directly depress NKCA. A lack of suppression of NKCA by nicotine in vitro has been reported previously.<sup>22</sup> Thus exposure to other agents in cigarette smoke such as polycyclic aromatic hydrocarbons<sup>21, 22</sup> may be relatively more important in immunosuppression in smokers than nicotine or cotinine exposure. Interestingly, level of polycyclic aromatic hydrocarbon exposure has been reported to be uncorrelated with number of cigarettes smoked and plasma cotinine levels,<sup>46</sup> suggesting that indices of cigarette, nicotine, and cotinine exposure may not adequately reflect exposure to some tobacco-related immunosuppressive agents.

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