Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Addictive Behaviors 32 (2007) 1526–1531
ADDICTIVE BEHAVIORS

Short communication

Relationship between cumulative effects of smoking and memory CD4+ T lymphocyte subpopulations

Akinori Nakata [a,b,*], Masaya Takahashi [a], Masahiro Irie [c], Yosei Fujioka [d], Takashi Haratani [a], Shunichi Araki [a]

[a] *National Institute of Occupational Safety and Health, Japan*
[b] *National Institute for Occupational Safety and Health, Cincinnati, USA*
[c] *Institute of Health Science, Kyushu University, Fukuoka, Japan*
[d] *Department of Public Health, Graduate School of Medicine, University of Tokyo, Tokyo, Japan*

Abstract

Previous studies have found that smoking is a strong factor that increases peripheral blood CD4+ T lymphocytes. However, most studies did not assess the cumulative long-life exposure of smoking on differential lymphocyte populations. In this study, to clarify the association of smoking habits and circulating lymphocytes, we conducted a cross-sectional study of 60 male current smokers. Smoking status was estimated by number of cigarettes smoked per day, smoking years, and Brinkman Index (BI) as calculated by multiplying the number of cigarettes smoked per day by the smoking years. Counts of CD4+CD45RO+CD69+ T and CD4+CD45RO+ T lymphocytes were strongly and positively correlated with BI and remained highly significant after controlling for alcohol drinking, leisure-time physical activity, and caffeine intake ($r_p > .465$, $p < .001$). These lymphocytes were also significantly correlated with the number of cigarettes smoked per day and smoking years, but the association was weaker than the BI. The findings suggest that the CD4+CD45RO+CD69+ T and CD4+CD45RO+ T lymphocytes are sensitive to cumulative effect of smoking, and may serve as a potential immuno-biomarker for active smoking.

Keywords: Smoking; CD4+CD45RO+ T lymphocyte; Brinkman Index; Cumulative effect

* Corresponding author. National Institute for Occupational Safety and Health, MS-C24, 4676 Columbia Parkway, Cincinnati, OH 45226, USA. Tel.: +1 513 533 8628; fax: +1 513 533 8596.
E-mail address: nakataa-tky@umin.ac.jp (A. Nakata).

0306-4603/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.addbeh.2006.11.007

1. Introduction

Many studies reported that cigarette smoking is a strong factor that increases total leukocyte count in the blood, and an elevated leukocyte count is a well-known risk factor for atherosclerotic vascular disease (e.g. Danesh, Collins, Appleby, & Peto, 1998). Among lymphocytes, circulating CD4+ T lymphocytes has been suggested as a major target cell of smoking and is increased by 15–50% in smokers compared to non-smokers. In particular, memory CD4+(CD4+CD45RO+) T lymphocytes were found to be selectively and dose dependently increased by smoking (e.g. Chavance, Perrot, & Annesi, 1993; Nakata, Tanigawa, Araki, Sakurai, & Iso, 2004).

Relatively little attention has been paid on the effect of cumulative long-life exposure to smoking on the lymphocyte subpopulations. Most previous research assessed current smoking status including number of cigarettes smoked per day or smoking years but not the combined effect. One indicator for measuring the combined effect is to multiply the number of cigarettes smoked per day by the smoking years, namely the Brinkman Index (BI). In this study, we used 3 indicators of smoking habits, i.e., BI, number of cigarettes smoked per day, and smoking years, to know the relationship between smoking and lymphocyte subpopulations in more detail.

Table 1
Relationship between Brinkman Index, number of cigarettes smoked per day, or smoking years and leukocyte and lymphocyte subpopulations in 60 smokers

Leukocyte subpopulations	Brinkman Index [a]		Number of cigarettes smoked per day		Smoking years	
	Pearson's correlation coefficients	Partial correlation coefficients [b]	Pearson's correlation coefficients	Partial correlation coefficients [c]	Pearson's correlation coefficients	Partial correlation coefficients [b]
Total leukocytes	.324*	.363**	.366**	.336*	.161	.190
Neutrophils	.290*	.335*	.313*	.283*	.120	.164
Eosinophils	.118	.157	.296*	.289*	.083	.111
Basophils	.242	.223	.162	.089	.235	.175
Monocytes	.117	.171	.132	.119	.072	.114
Total lymphocytes	.280*	.285*	.325*	.301*	.177	.162
Total CD3+ T lymphocytes	.173	.169	.221	.214	.125	.084
Total CD4+ T lymphocytes	.249	.243	.227	.179	.234	.182
CD4+CD45RO+ T lymphocytes	.445***	.465***	.387**	.315*	.382**	.391**
CD4+CD45RO+CD69+ lymphocytes	.439***	.497***	.379**	.322*	.348**	.401**
CD4+CD45RO+CD69− lymphocytes	.319*	.302*	.278*	.203	.299*	.268*
CD4+CD45RA+ T lymphocytes	.176	.145	.094	.024	.183	.123
CD4+CD69+ T lymphocytes	.391**	.410**	.285*	.189	.335**	.335**
CD8+ T lymphocytes	.093	.102	.265*	.294*	−.035	−.035
B (CD19+) lymphocytes	.184	.270*	.174	.181	.142	.239
Natural killer (CD3−CD56+) cells	.099	.079	.149	.133	.019	.001

*$p<0.05$, **$p<0.01$, ***$p<0.001$.
[a] Number of cigarettes smoked per day multiplied by smoking years.
[b] Partial correlation coefficient controlling for alcohol drinking, leisure-time physical activity, and caffeine intake.
[c] Partial correlation coefficient controlling for age, alcohol drinking, leisure-time physical activity, and caffeine intake.

2. Methods

2.1. Subjects

A total of 73 male smokers at a commercial company underwent an annual health check-up in April 2002. Subjects were all white-collar daytime workers aged 23 to 65 (mean 40) years. Eight subjects with health problems and 5 subjects with missing responses were excluded. Finally, we analyzed data from 60 current smokers. The subjects were not exposed to hazardous chemicals nor had signs or symptoms of infection. This study was carried out with the informed consent of all workers, and the study protocol was reviewed and approved by the Ethical Committee of the Kyushu University and the National Institute of Occupational Safety and Health, Japan.

2.2. Smoking status

Smoking status in current smokers was assessed by the following question: How many cigarettes a day do you smoke and how many years have you been smoking?

2.3. Covariates

Covariates included age, alcohol drinking (number of alcoholic drinks consumed per day, with one drink estimated as about 11.5 g of pure ethanol), caffeine intake (cups of tea or coffee/day), and physical activity (number of physical exercise per week). Daily alcohol consumption was categorized into 4 groups (nondrinker, less than 2, 2 to 3, 4 or more). Daily caffeine intake (cups of coffee or tea/day) was also divided into 4 groups (almost none, 1 to 2, 3 to 5, 6 or more). Leisure-time physical activity was categorized as no exercise, 1 to 2 times, and 3 times or more.

2.4. Blood sampling and flow cytometric analysis

Fasting blood samples were collected between 9 to12 a.m. from subjects, and immunologic analysis was conducted within 12 h of blood collection. Immune parameters were analyzed by standard techniques (Nakata et al., 2004). The following sets of monoclonal antibodies were used to perform four or three-color direct immunofluorescence surface-marker analysis: anti-CD45-FITC/anti-CD56-RD1/anti-CD19-ECD/anti-CD3-PC5, anti-CD45-FITC/anti-CD4-RD1/anti-CD8-ECD/anti-CD3-PC5, anti-CD45RA-FITC/anti-CD45RO-PE/anti-CD4-PC5, anti-CD4-FITC/anti-CD45RO-PE/anti-CD69-PC5, and mouse IgG1-FITC/Mouse IgG1-RD1/Mouse IgG1-PC5 (negative control). Monoclonal antibodies of anti-CD45RO-PE and anti-CD4-FITC were purchased from PharMingen (San Diego, California, USA) and all other antibodies were purchased from Beckman Coulter Inc, USA.

CD4+ T lymphocytes have been functionally classified into either memory (CD4+CD45RO+) or non-memory (CD4+CD45RA+) (naïve) T cells (Sanders, Makgoba, & Shaw, 1988). The CD69 antigen has been identified as the earliest activation marker on the surfaces of antigen- or allergen-specific activated lymphocytes in vitro (Testi, D'Ambrosio, De Maria, & Santoni, 1994). We calculated the number in each lymphocyte subpopulation by multiplying lymphocyte counts by the percentage of positive cells in each category, as determined by flow cytometer (EPICS XL, Beckman Coulter Inc, California, USA).

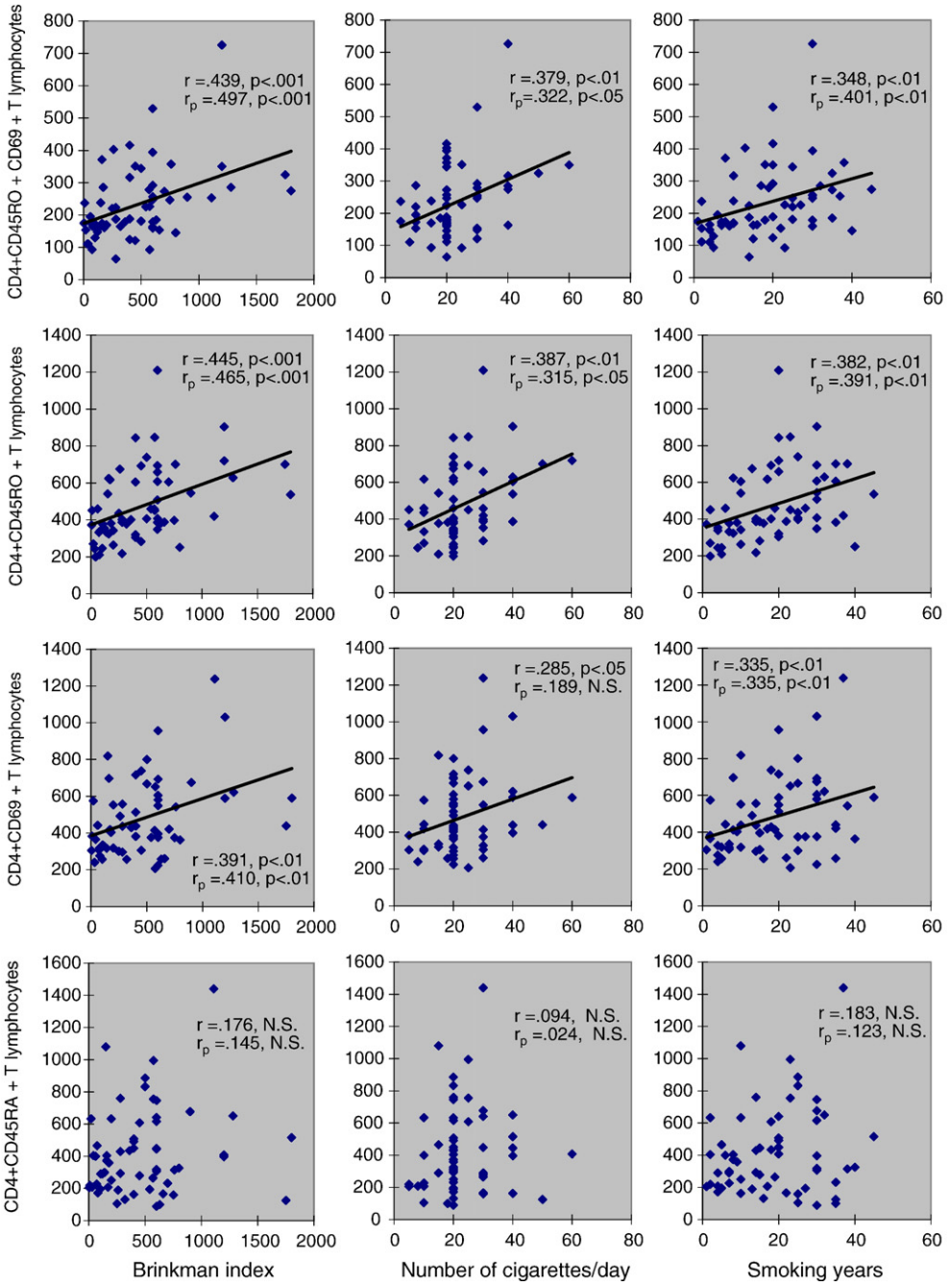

Fig. 1. Relationships between BI, number of cigarettes smoked per day, or smoking years and CD4+ T lymphocyte subpopulations. The r and r_p indicate the Pearson's correlation coefficient and partial correlation coefficient controlling for alcohol drinking, leisure-time physical activity, caffeine intake, and/or age, respectively. N.S. Not significant.

2.5. Statistical analysis

Relationships between smoking indicators and immune parameters were examined by the Pearson's correlation analysis together with partial correlation analysis controlling for confounders. The significance level for all statistical analyses was $p<0.05$ (two-tailed test). All data were analyzed using the Statistical Package for the Social Sciences version 11.5 (SPSS Inc., Chicago, USA).

3. Results

Average number of cigarettes/day in current smokers were 22.8 (SD, 10.2; range, 5–60), and had smoked for 1 to 45 (mean 18.5, SD, 11.4) years. The range of BI was 5 to 1800 (mean 467; SD, 399).

Numbers of total leukocytes, neutrophils, total lymphocytes, CD4+CD45RO+ T, CD4+CD45RO+ CD69+ T, CD4+CD45RO+CD69− T and CD4+CD69+ T lymphocytes were significantly and positively correlated with BI and remained significant after controlling for confounders. Numbers of total leukocytes, neutrophils, eosinophils, lymphocytes, CD4+CD45RO+ T, CD4+CD45RO+CD69+ T, and CD4+CD69+ T lymphocytes were also significantly and positively correlated with number of cigarettes/day (Table 1, Fig. 1). Numbers of CD4+CD45RO+ T, CD4+CD45RO+CD69+ T, CD4+CD45RO+ CD69− T, and CD4+CD69+ T lymphocytes were significantly correlated with smoking years.

4. Discussion

The main finding of this study was that CD4+CD45RO+ T and CD4+CD45RO+CD69+ T lymphocytes were strongly and positively correlated with BI. These lymphocytes were also significantly correlated with the number of cigarettes/day and smoking years, but the association was weaker than the BI. The findings suggest that these lymphocytes are sensitive to cumulative effects of smoking, and may serve as a potential immuno-biomarker for active smokers.

One of the reasons why CD4+CD45RO+ T lymphocytes increase with amount and duration of smoking may be explained as follows. Smoking may induce the naïve CD4+ T lymphocytes to shift towards memory CD4+ T lymphocytes by exposure to recurring antigen (tobacco particulates). Memory CD4+ T lymphocytes proliferate repeatedly in response to exposure to smoking, which in turn leads to accelerate ageing of lymphocytes. A recent study reported that the cumulative exposure to cigarette smoking enhances telomere shortening in circulating lymphocytes (Valdes et al., 2005), suggesting pro-ageing effect of smoking. Smokers may have difficulties in response to recall antigens due to repetitive proliferation of antigen-specific memory T lymphocytes, which may account for smokers to have higher incidence of infectious diseases compared to non-smokers (Arcavi & Benowitz, 2004).

Increases of CD4+CD45RO+ T and CD4+CD5RO+CD69+ T lymphocytes may also play an important role in the initiation and progression of atherosclerosis. It has been reported that most of the leukocytes that accumulate at atherosclerotic lesions are monocyte-derived macrophages and CD4+ T lymphocytes (Zhou, 2003). Majority of CD4+ T lymphocytes at the lesions were also found to be cells expressing CD45RO+antigen (Stemme, Holm, & Hansson, 1992). Another study observed that expression of activated (CD69+) antigen on CD3+ T lymphocytes increased with the severity of the coronary syndrome (Hosono et al., 2003). A recent study demonstrated that the mean intima-media thickness of the common carotid artery were positively associated with circulating memory CD4+ T lymphocytes (Tanigawa et al.,

2003). Thus increase of memory CD4+ T lymphocyte subpopulations by smoking may promote atherosclerotic vascular disease.

In conclusion, despite the fact that the amount and duration of smoking were assessed by self-report, the present data suggest that memory CD4+CD45RO+ T and CD4+CD45RO+CD69+ T lymphocytes are sensitive to the cumulative effects of smoking. The finding underscores the need for further investigation whether increases in these lymphocytes predict intravascular tissue damage and atherosclerotic vascular disease.

References

Arcavi, L., & Benowitz, N. L. (2004). Cigarette smoking and infection. *Archives of Internal Medicine*, *164*, 2206−2216.

Chavance, M., Perrot, J. Y., & Annesi, I. (1993). Smoking, CD45R0+ (memory), and CD45RA+ (naive) CD4+ T cells. *American Review of Respiratory Diseases*, *148*, 237−240.

Danesh, J., Collins, R., Appleby, P., & Peto, R. (1998). Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease: meta-analyses of prospective studies. *Journal of the American Medical Association*, *279*, 1477−1482.

Hosono, M., de Boer, O. J., van der Wal, A. C., van der Loos, C. M., Teeling, P., Piek, J. J., et al. (2003). Increased expression of T cell activation markers (CD25, CD26, CD40L and CD69) in atherectomy specimens of patients with unstable angina and acute myocardial infarction. *Atherosclerosis*, *168*, 73−80.

Nakata, A., Tanigawa, T., Araki, S., Sakurai, S., & Iso, H. (2004). Lymphocyte subpopulations among passive smokers. *Journal of the American Medical Association*, *291*, 1699−1700.

Sanders, M. E., Makgoba, M. W., & Shaw, S. (1988). Human naive and memory T cells: Reinterpretation of helper-inducer and suppressor-inducer subsets. *Immunology Today*, *9*, 195−199.

Stemme, S., Holm, J., & Hansson, G. K. (1992). T lymphocytes in human atherosclerotic plaques are memory cells expressing CD45RO and the integrin VLA-1. *Arteriosclerosis and Thrombosis*, *12*, 206−211.

Tanigawa, T., Kitamura, A., Yamagishi, K., Sakurai, S., Nakata, A., Yamashita, H., et al. (2003). Relationships of differential leukocyte and lymphocyte subpopulations with carotid atherosclerosis in elderly men. *Journal of Clinical Immunology*, *23*, 469−476.

Testi, R. D., D'Ambrosio, R., De Maria, & Santoni, A. (1994). The CD69 receptor: A multipurpose cell-surface trigger for hematopoietic cells. *Immunology Today*, *15*, 479−483.

Valdes, A. M., Andrew, T., Gardner, J. P., Kimura, M., Oelsner, E., Cherkas, L. F., et al. (2005). Obesity, cigarette smoking, and telomere length in women. *Lancet*, *366*, 662−664.

Zhou, X. (2003). CD4+ T cells in atherosclerosis. *Biomedicine and Pharmacotherapy*, *57*, 287−291.