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Original Article

The Relationship between the Frequency of the Common Cold and the Activities of Natural Killer Cells

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Abstract

We investigated the relationship between the frequency of the common cold and the conditions of the body's immune system among members of a generally healthy population. Self-reporting questionnaires on the frequency of the common cold and on fatigue and stress conditions were administered to a total of 67 healthy individuals aged 22 to 50. The activities of natural killer (NK) cells, which were determined by Eu^{3+}-DTPA release assay, and of NK cell members in the peripheral blood of the subjects were phenotypically ($CD3^-CD16^+CD56^+$) analyzed with three-color flow cytometry. The results showed that the frequency of the common cold was significantly correlated with NK activity and NK subset ($CD3^-CD16^+CD56^+$) frequency ($r=-0.34$ and -0.47 respectively, $P<0.01$). After adjusting for age, the mean NK subset ($CD3^-CD16^+CD56^+$) significantly differed ($F=3.384$, $P<0.05$) among the four frequency groups for the common cold, and the frequencies of the common cold were significantly different among the four stress/fatigue groups ($F=8.016$, $P<0.001$) for the males, as evaluated by ANCOVA. These results indicate that conditions of high stress and fatigue may increase the chance of catching the common cold due to a decrease in activities of NK cells.

Key words: NK activity, NK subset, frequency of the common cold, fatigue, stress

Introduction

It is known that the common cold, probably the most pervasive illness affecting humankind, is an infection that is caused by more than 200 different viruses or atypical bacteria. Clinical symptoms of the common cold vary according to the serotype of virus and bacteria, making it difficult to successfully prevent, diagnose, and treat the common cold[1]. Based on current estimates, the average adult in the U.S. is thought to have an average of five common colds per year[1]. Natural killer (NK) cells are lymphocytes that can kill certain types of tumor cells and virally infected cells without prior sensitization or restriction by the major histocompatibility complex[2-4]. NK cells appear to play a role of immune surveillance by acting as a primary defense mechanism against cancer and viral diseases, and the activity of NK cells is an integral component of the host defense system[5-16]. It has been reported that the immune response in vivo in humans was improved and can protect against influenza and the common cold[17]. Research has shown that psychological stress is associated with an increased risk of acute infectious respiratory illness[18-22]. However, the relationship between a host's natural immunity status and the frequency of the common cold in a generally healthy population has yet to be studied in detail from the standpoint of preventive medicine. Accordingly, this study elucidates the relationship between the frequency of the common cold and activities of NK cells in a sample of generally healthy subjects.

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Subjects and Methods

Subjects

The subjects of this study were recruited from 91 staff members working for two departments (Immunology and Public Health) of the Juntendo University School of Medicine in Tokyo on either a full-time or a part-time basis from April 1 to May 30, 1994. In order to obtain healthy subjects, those who had had acute or chronic illnesses or who were on medication at the time the blood samples were taken were excluded from the 84 persons who had submitted informed consents to cooperate in this study. A total of 67 subjects, comprising 26 medical doctors, 26 research associates, 8 graduate students, and 7 laboratory staff members, were finally selected for this study. They included 24 females and 43 males, and their ages ranged from 22 to 50 years old (mean age of 32.0).

Frequency of the common cold and fatigue and stress

Data on the frequency of the common cold and on fatigue and stress levels was obtained from a self-reporting questionnaire survey conducted at the time the blood samples were taken. The frequency of the common cold for each person was estimated as the average number of times he/she had had the illness over the past few years. Fatigue and stress levels were evaluated separately by a set of yes/no questions. The survey questions are presented in translation in the appendix.

Activities of NK cells

NK cells are a population of $CD3^-$ T-cell receptor lymphocytes. It has been reported that NK cells have $CD16^+$, the receptor for Fc portions of immunoglobulin molecules, and $CD56^+$, which is specific to large granular lymphocytes on their cell surfaces. Moreover, the $CD3^-CD16^+CD56^+$ subset is a cell that figures prominently in NK activity[29-32]. For these reasons, we decided to measure NK activity and NK subset ($CD3^-CD16^+CD56^+$) in this study.

(1) Isolation of human lymphocytes: All the blood samples were collected between 9:00 am and 12:00 at noon. Within two hours after the blood samples were taken, mononuclear cells were isolated from 10 mℓ of a heparinized peripheral blood sample by density gradient centrifugation on a Ficoll-Hypaque mixture, washed twice, and then suspended in RPMI 1640 medium for assay of NK activity and phenotypic analysis of NK cells.

(2) Assay of NK activity: NK activity was determined by the Eu^{3+}-DTPA release assay[25-28]. Target cells consisted of K562, a human erythroleukemic cell line, and were labeled with Eu^{3+}-DTPA for 20 minutes at 4℃ on ice. The labeled target cells (1×10^5/mℓ) were suspended along with the effector cells (2×10^6/mℓ) in a standard medium (RPMI 1640 plus 10% FCS). Effector cells in 100 μl of the medium were added to target cells in 100 μl of the medium in each well of a set of round-bottomed microplates (effector-to-target ratio of 5:1 to 20:1) and incubated for two hours at 37℃ with 5% carbon dioxide. The spontaneous release was determined by incubating target cells containing the complete medium only, and the maximum release was determined by lysing the target cells with 10 μl of 10% Triton X-100. The percentage of specific lysis was calculated according to a standard formula, as follows :

$$(\%)\text{NK activity} = \frac{\text{experimental release - spontaneous release}}{\text{maximal release - spontaneous release}} \times 100\%$$

(3) Phenotype of NK cells: During the phenotypical analysis, 1×10^6 cells were suspended in phosphate-buffered saline (PBS), stained with fluorescein (FITC)-leu11a (Anti-CD16) (Phermingen, USA), phycoerythrin (PE)-NKH-1 (Anti-CD56)(Coulter, USA) and PerCP-Leu-4 (Anti-CD3)(Becton Dickinson, USA) monoclonal antibodies, and incubated for 15 minutes at 4℃. The stained cells were washed twice with PBS and resuspended in PBS for three-color flow cytometry analysis in a FACScan (Becton Dickinson).

Statistical analysis

One-way analysis of variance (ANOVA) was used to compare the frequency of the common cold, NK activity, and NK subset among age groups. The comparisons of these variables between females and males were conducted by the Student's t-test. Correlations among four variables (age, NK activity, NK subset, and frequency of the common cold) were examined by using Pearson's correlation coefficients. Subjects were divided into four groups according to frequency of the common cold, i.e 0 or 1, 2 or 3, 4 or 5, and 6 or more. Adjusting for the effects of age, analysis of covariance (ANCOVA) was used to identify significant differences in NK activity and NK subset among the four groups. Subjects were also categorized into four groups based on stress and fatigue levels, i.e stress (+) and fatigue (+), stress (+) and fatigue (−), stress (−) and fatigue (+) , and stress (−) and fatigue (−) groups. Differences in the frequency of the common cold, NK activity and NK subset among these four groups were also compared using ANCOVA. The data was analyzed using the computer software package Numerical Analysis Program (NAP)[36].

Results

Table 1 shows the frequency of the common cold and of immunological functions by age and sex. The average frequency of the common cold over the previous year in the entire population under observation was 2.9. The frequency of the common cold, NK activity and NK subset were not significantly

Table 1 Frequency of the common cold, NK activity, and NK subset by age and sex

		Frequency of the common cold	NK activity (%)				NK subset (%)
	n	Occurrences per year	E/T: 5:1	E/T: 10:1	E/T: 20:1	n	$CD3^-CD56^+CD16^+$
Age(years)							
20-29	26	3.6 ± 0.6	19.0 ± 2.2	30.6 ± 3.0	46.1 ± 3.9	18	7.9 ± 1.7
		(0 ~ 12)	(3.1 ~ 56.1)	(6.2 ~ 69.6)	(10.1 ~ 88.7)		(0.4 ~ 32.4)
30-39	36	2.6 ± 0.3	21.8 ± 1.7	36.4 ± 2.7	53.1 ± 3.1	23	10.3 ± 1.4
		(0 ~ 7)	(6.3 ~ 49.0)	(9.2 ~ 74.4)	(18.5 ~ 83.5)		(0.9 ~ 24.3)
40-49	5	2.1 ± 0.8	23.4 ± 7.3	37.6 ± 8.6	51.3 ± 10.8	3	7.5 ± 3.7
		(0 ~ 4)	(7.0 ~ 49.8)	(10.1 ~ 56.3)	(20.0 ~ 81.7)		(0.2 ~ 12.1)
Sex							
Female	24	3.3 ± 0.6	18.9 ± 2.2	30.6 ± 3.1	47.2 ± 4.1	16	7.6 ± 0.9
		(0 ~ 12)	(3.1 ~ 49.8)	(6.2 ~ 56.3)	(10.1 ~ 81.7)		(3.0 ~ 14.2)
Male	43	2.7 ± 0.3	21.9 ± 1.7	36.2 ± 2.4	51.2 ± 2.9	28	10.1 ± 1.5
		(0 ~ 10)	(6.3 ~ 56.1)	(9.2 ~ 74.7)	(14.1 ~ 88.7)		(0.2 ~ 32.4)
Total	67	2.9 ± 0.3	20.8 ± 1.3	34.2 ± 2.0	50.2 ± 2.4	44	9.2 ± 1.0
		(0 ~ 12)	(3.1 ~ 56.1)	(6.2 ~ 74.4)	(10.1 ~ 88.7)		(0.2 ~ 32.4)

Differences in frequency of the common cold, NK activity, and NK subset among age groups (ANOVA) or between females and males (Student's t-test) were not significant (p>0.05).

Values represent the mean ± SEM. Ranges are shown in parentheses.

different among the age groups or between males and females. The frequency of the common cold was significantly negatively correlated with NK activity, and NK subset ($r=-0.34$ and -0.47, respectively, $p<0.01$), but was nonegatively correlated with age ($r=-0.16$, $p>0.05$).

In males, NK subset decreased with the increase of the frequency of the common cold (Figure 1). The overall age-adjusted mean NK subset significantly differed among the four groups of the common cold ($F=3.384$, $P<0.05$) in a comparison using ANCOVA. NK activity tended to decrease as the frequency of the common cold increased, but after adjusting for age, NK activity among the four groups did not show significant differences ($F=1.755$, $P>0.05$).

In females, NK activity and NK subset tended decrease with the increases in frequency of the common cold. Overall age-adjusted mean of NK subset and NK activity among the four frequency groups did not show significant differences ($F=1.745$ and 2.386, respectively, $P>0.05$) according to ANCOVA.

Table 2 shows the frequency of the common cold, NK subset and NK activity among the four stress/fatigue groups, by gender. After adjusting for age, the frequency of the common cold was significantly different in males among the four stress/fatigue groups. The overall age-adjusted NK activity and NK subset means among the four stress/fatigue groups did not show significant differences in males. Age-adjusted means of the frequency of the common cold, NK activity and NK subset among the four stress/fatigue groups did not show significant differences in females, according to ANCOVA.

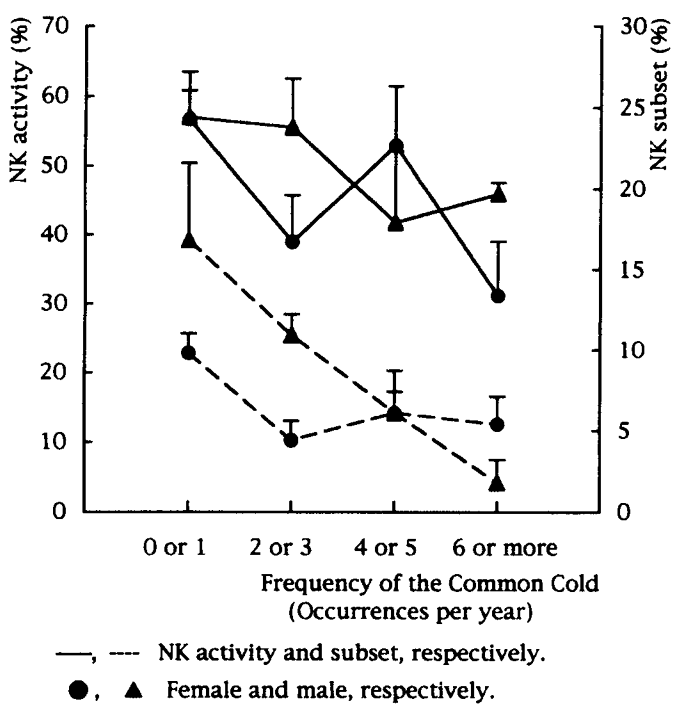

Fig. 1 Comparisons of NK activity (E/T: 20:1) and NK subset ($CD3^-$ $CD16^+CD56^+$) among four groups according to the frequency of the common cold using ANCOVA adjusting for age.
Values represent the mean ± SEM.

Discussion

In this study, it was shown that the frequency of the common cold in healthy subjects was inversely correlated with NK activity and NK subset. The NK subset means among the four groups of the common cold significantly differed in males after adjusting for age. NK activity of both sexes and NK subset in females showed a decreasing tendency that coincided with an increase in frequency of the common cold, though not significant. These results indicate that the frequency of the common cold in healthy people is associated with the activities of NK cells; i.e. when the activity of NK cells is high, the frequency of the common cold is low.

This study suggests that stress and fatigue are associated with the frequency of the common cold. After adjusting for age, the frequency of the common cold was a significant factor among the four stress/fatigue groups in males. This finding is consistent with previous reports that stress or fatigue is associated with an increased risk of acute infectious respiratory illness[18-22]. This association may be mediated through NK activity, which functions as an intermediate variable between the frequency of the common cold and stress and fatigue levels, because NK activity has been shown to be associated with stress and fatigue in many studies. Low NK activity is also shown to be associated with chronic fatigue syndrome[23-24].

Because this study is designed to be cross-sectional, it is not appropriate to determine the causal relationship between any two variables under investigation. However, the results of our study indicate the possibility of a primary prevention of the common cold. If the stress or fatigue experienced by a person is reduced by some intervening activity, then his or her activities of NK cells may increase, which, in turn, serves to prevent the person from catching the common cold. The development of effective methods to reduce stress or fatigue thus becomes an important issue from the standpoint of public health.

The weaknesses of this study should also be noted. The frequency of the common cold, present health status, and

Table 2 Frequency of the common cold, NK activity, and NK subset among the four stress/fatigue groups: analysis of covariance, with age as the covariate

	Stress(+) Fatigue(+)	Stress(+) Fatigue(−)	Stress(−) Fatigue(+)	Stress(−) Fatigue(−)	F value	P value
Male						
Frequency of the common cold (Occurrences per year)	4.8 ± 0.8	3.3 ± 0.4	2.5 ± 0.6	1.8 ± 0.2	8.016	0.0003
NK activity (%)	44.6 ± 7.2	43.6 ± 8.7	48.8 ± 6.5	57.7 ± 3.8	1.618	0.201
NK subset (%)	2.5 ± 1.5	7.0 ± 1.0	9.4 ± 4.0	12.0 ± 2.0	2.668	0.072
Female						
Frequency of the common cold (Occurrences per year)	5.2 ± 1.6	3.3 ± 1.5	2.8 ± 0.8	2.1 ± 0.6	1.419	0.268
NK activity (%)	35.1 ± 6.5	53.3 ± 9.3	62.0 ± 8.6	48.0 ± 6.8	1.112	0.369
NK subset (%)	6.8 ± 1.9	10.0 ± 2.9	(−)	7.4 ± 1.1	0.437	0.655

Values represent the mean ± SEM.

medication for acute or chronic illnesses were measured respectively by a self-reporting questionnaire having only a single question for each issue. Stress and fatigue were also measured separately by single questions in the questionnaire. While this brief questionnaire was used so that even busy participants could respond, neither the reliability nor the validity of the questionnaire was examined, which constitutes the major weakness of this study. Regarding physical health status, however, the reliability and validity of a single question thus asked have been evaluated relatively high[33-35]. With regard to the evaluation of stress, a measurement tool whose reliability and validity had been ascertained beforehand should have been used.

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Appendix

Key questions in the self-reporting questionnaire.

1. How many times have you had the common cold over the past few years?	( )/Each year.
2. Do you suffer from fatigue in your daily lifestyle?	Yes / No
3. Do you suffer from stress in your daily lifestyle?	Yes / No
4. Do you feel ill now?	Yes / No
5. Do you currently take any medications?	Yes / No