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Abstract: Stress activates the hypothalamic-pituitary-adrenal axis, increases circulating glucocorticoids, and is associated with alterations in immune functions. Nasal mucosa is richly innervated by sympathetic nerve fiber and nasal patency changes in response to various sympathetic stimuli. These fibres form close neuroeffector junctions with lymphocytes and macrophages.

Nasal lavage and peripheral blood samples were collected from healthy medical students one hour before their exams and a week later, after the declaration of results of the exams were announced. We determined and compared the distribution of T lymphocyte subsets in students under psychological stress

The T cell: natural killer cell, T helper cell: T suppressor cell, and T cell: active T cell ratios from nasal lavage and peripheral blood prior to the exams were significantly lower ($P<0.05$) than a week after the exams. The changes in the T lymphocyte subset ratios in peripheral blood were similar to those in nasal lavage. It was found in this study that stress causes a decrease in the ratios of T cell: natural killer cell, T helper cell: T suppressor cell and T cell: active T cell in peripheral blood. These changes are also reflected in nasal lavage, indicating the presence of an immune response in nasal mucosa against stress.

Key Words: Psychological stress, nasal immunity, lymphocytes

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Introduction

The immune system has idiosyncratic, self regulatory properties and functions, as does the nervous system and the endocrine system. Immunoregulatory processes are part of an integrated defence system. This integration is reflected in the development of psychoneuroimmunology (1).

Nasal mucosa is richly innervated by sympathetic nerve fiber and nasal patency changes readily in response to various sympathetic stimuli (2). There are several transmitters such as noradrenaline and substance P, in specific nerve fibres associated with primary and secondary lymphoid tissue. These transmitters interact with immune cell in the lymphoid tissue and affect immunoregulation (3,4).

Some inflammatory diseases of the human respiratory mucosa may be related to T lymphocyte abnormalities in peripheral blood (5).

In this study we determined and compared the distribution of T lymphocyte subsets in the peripheral blood and nasal lavage of students under psychological stress using flow cytometric analyses.

Materials and Methods

Subjects: 25 healthy volunteer students (aged 18 to 24 years; mean age 20 years, 18 men and 7 women) participated in this study. These subjects were free of upper respiratory disease such as nasal allergy, chronic sinusitis or common cold. The nasal lavage and peripheral blood samples were collected from the medical students one hour before their exams and one week later, after the declaration of the results of the exams.

Nasal Lavage: Nasal lavage was performed by administering 8 ml of 0.9 % sterile saline solution, 4 ml in each nostril, with the head tilted 30°C backwards, as reported previously (6). Subjects were instructed not to

breathe or swallow for approximately 10 seconds during a valsalva maneuver and were then told to bend the head forward so that the mixture of saline and nasal secretions could be collected. The liquid obtained was centrifuged (500g at 4°C for 10 min) the supernatant was removed and the sediment pipetted to dissolve mucus plugs. Flow cytometric analysis was performed as follows:

1. 20 µl of monoclonal antibodies (double coloured coulter) of CD₃/CD₁₉, CD₄/CD₈, CD₃/HLA-DR+, CD₃/CD₁₆₊₅₆, were added to each tube. Than 250 µl of the nasal secretion sample was added.

2. After incubation for 20 minutes at room temperature, the tubes were ready for analysis with a by Coulter-Multi-Q-prep instrument.

3. Three different solutions were automatically added to each tube during analysis with the Coulter-Multi-Q-prep instrument.

4. The first solution, containing formic acid, and the second solution composed of sodium chloride, sodium carbonate and sodium sulphate, were used as stabilizers. The third solution, paraformaldehyde, was used as a buffer solution.

5. After analysis with the Coulter-Multi-Q-prep instrument, each tube was kept for 20 minutes at room temperature.

6. The solutions in each tube were analysed using flow cytometry and 10,000 cells were counted (Figure 1).

Peripheral Blood Lymphocytes: Mononuclear cells obtained by ficoll hypaque density from the peripheral blood were analyzed by flow cytometry, using the method described for the nasal secretion samples.

Statistical Analysis: All data are expressed as mean value±standard deviation. Signigicant differences were determined by Mann-Whitney-U tests. A p value of less

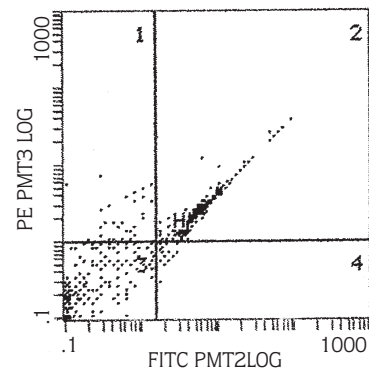


Figure 1. Histogram of gated lymphoid cells. The x and y axes represent cells labeled with antibodies directed towards the CD8 (suppressor T lymphocyte) and CD4 (helper T lymphocyte) antigens, respectively. The fluorescent compounds attached to the anti-CD4 and anti-CD8 antibodies emit lights of different wave length and color. Distinct populations of helper and suppossor T lymphocytes are identified in the upper left and lower right

than 0.05 was considered significant. A comparison was made of the lymphocyte subsets in the nasal lavage and the peripheral blood. A Statgraf 5.0 version statistical program was used in the data analysis.

Measurement of Stress Level:

In this study, and state- trait anxiety inventory (SAI) developed by Spielberger et al. (7) was used. The students were asked to evaluate themselves. The inventory consisted of 40 questions, the first 20 of which were used to determine the state of the student under psychological stress at that time. The remaining 20 questions were used to test the presence of any trait anxiety.

The test was performed one hour before the exam and a week after the declaration of the results. The average points obtained from the SAI were statistically compared using Mann-Whitney-U tests

	No	T cell/NK cell (CD ₃ /CD ₁₆₊₅₆)	T helper cell/ T suppressor cell (CD ₄ /CD ₈)	T cell/active T cell (CD ₃ /HLA-DR+)
Before examination	25	3.98±0.86	0.98±0.36	3.12±0.76
After examination	25	5.10±1.12	2.01±0.78	5.65±1.42
P value	P<0.05	P<0.05	P< 0.05	

Table 1. Lymphocyte subset ratios of nasal lavage before and after examination

		T cell / NK cell (CD ₃ /CD ₁₆₊₅₆)	T helper/ T suppressor cell (CD ₄ /CD ₈)	T cell / active T cell (CD ₃ /HLA-DR+)
Before examination	25	2.96±0.76	0.80±0.29	3.75±1.14
After examination	25	5.35±1.08	1.92±0.49	5.45±1.76
Normal values		5.00±2.00	1.70±0.50	6.00±2.00
P value		P< 0.05	P< 0.05	P< 0.05

Table 2. Lymphocyte subset ratios of peripheral blood before and after examination

Stress level	No	T cell / NK cell (CD3 / CD16+56)	T helper cell/ T suppressor cell (CD4 / CD8)	T cell/ active T cell (CD3/HLA-DR+)
20-30	12	3.96±0.76 p<0.05	0.99±0.42 p>0.05	3.18±0.86 p>0.05
30-40	10	4.12±0.94 p<0.05	1.12±0.86 p>0.05	3.84±1.75 p>0.05
40-50	18	4.98±1.10 p<0.05	1.46±0.75 p>0.05	3.94±1.82 p>0.05
50-60	10	5.32±1.20 p<0.05	1.92±0.72 p>0.05	4.02±1.96 p>0.05

Table 3. Changes in T lymphocyte subsets in nasal lavage related to the degree of stress.

Stress level	No	T cell / NK cell (CD3 / CD16+56)	T helper cell / T suppressor cell (CD4 / CD8)	T cell / active T cell (CD3/HLA-DR+)
20-30	12	2.54±0.84 p<0.05	0.72±0.42 p>0.05	3.60±1.04 p>0.05
30-40	10	3.45±0.14 p<0.05	0.94±0.86 p>0.05	4.90±1.12 p>0.05
40-50	18	4.40±1.02 p<0.05	1.2±0.96 p>0.05	4.98±1.20 p>0.05
50-60	10	5.74±1.04 p<0.05	1.10±0.75 p>0.05	5.28±0.84 p>0.05

Table 4. The changes in lymphocyte subsets of peripheral blood related to the stress level

Table 5. Mean SAI points prior to and after the exams.

	No	SAI point
Before examination	25	32.6±4.35
After examination	25	54.2±5.8
P value		P<0.05

Results

The T cell: natural killer cell (CD_3/CD_{16+56}), T helper cell: T suppressor cell (CD_4/CD_8), and T cell: active T cell ($CD_3/HLA-DR+$) ratios of the samples of nasal lavage and peripheral blood collected prior to the exams were significantly lower ($P<0.05$) than the ratios of the samples obtained one week after the exams (Table 1).

The changes in the T lymphocyte subset ratios in peripheral blood were similar to those in nasal lavage (Table 2).

We found a statistically significant increase in T cell: natural killer cell ratio with increasing stress ($p<0.05$), but increases in the T helper cell: T suppressor cell and T cell: active T cell ratios were not significant ($p>0.05$, Table 3 and Table 4). Measurement with SAI indicated a statistically significant difference between the mean points obtained prior to and after the exams ($p<0.05$, Table 5).

Discussion

The anatomic structure of the nose provides evidence for the psychosomatic theory of vasomotor rhinitis. There is erectile tissue around the turbinates. This tissue is innervated by sympathetic fibres arising from sphenoidal and superior cervical ganglions. Pregnancy and menstruation cause minimal nasal hypersensitivity but when either occurs together with personal conflict, personality hypersensitivity increases and symptoms form.

Two pathways link the brain and the immune system. The autonomic nervous system and neuroendocrine flow out via the pituitary. Both routes provide biologically active molecules capable of interacting with the cells of the immune system. Primary and secondary lymphoid organs are innervated with noradrenergic post ganglionic

sympathetic nerve fibres (8). These fibres form close neuroeffector junctions with lymphocytes and macrophages. The neurotransmitters released from these nerves which are diffuse and act at distant sites, further extending the potential for neuroimmune interactions. Moreover, lymphocytes, monocytes/macrophages and granulocytes possess receptors for those neurotransmitters (9).

Noradrenaline interacts with β -adrenoreceptors on thymic lymphocytes to inhibit thymocyte mitogenesis and enhance the expression of cell surface differentiation antigens (10). Plasma noradrenaline usually originates in the endings of sympathetic post ganglionic fibers in response to sympathetic stimulation. The released noradrenaline partially combines with the sympathetic receptors over the effector cells in the nasal mucosa, but most of it is reincorporated into the sympathetic nerve endings and only a small percentage escapes into the peripheral circulation (2).

Stress-induced brain-mediated immunoregulation is affected by two pathways autonomic outflow and (neuro) endocrine outflow. Particular attention has been given to the interaction effects of chronic and acute stress (11).

Stress activates the hypothalamic-pituitary adrenal axis, increases circulating glucocorticoids and is associated with alterations in the immune function (14). Stress induced immunosuppression has been observed in adrenalectomised animals (13).

A stressful lifestyle affects immunological parameters such as natural killer cells, the number of T and B cells and mitogen response (14).

It was found in this study that stress causes a decrease in the ratios of T helper cells: T suppressor cells, T cells: active T cells and T cells: natural killer cells in peripheral blood. The decrease in the T helper cell: T suppressor cell ratio and natural killer cell population has been shown in many studies (15,16,17). Schedlowski et al.(18) showed that, after acute psychological stress, there was a significant increase in sympathetic adrenal hormones (adrenaline and noradrenaline). Lymphocyte subsets and the functional capacity of natural killer cells exhibited an increase immediately after a jump, followed by a decrease to significantly below the start values 1 hour later. This indicates the quick adaptation of the immune system to stress situations.

Nasal lavage has been shown to be useful in the study of inflammatory cells and their mediators in diseases affecting the nose (19,20). Although mucosal T cell infiltration was observed in the upper airways of patients

with rhinitis, these cells could not be detected in nasal lavage in previous studies (21,22). Using flow cytometric analysis in a previous study, the authors showed the effect of topical corticosteroids on the distribution of T lymphoid cells in nasal lavage (23). The same method was used in this study to measure the stress induced changes in nasal lavage and peripheral blood. The changes in T lymphocyte subset ratios in peripheral blood are similar to those in nasal lavage.

In addition, we found a statistically significant increase in the T cell: natural killer cell ratio with increasing stress levels ($p < 0.05$) but the increases in the T helper cell: T suppressor cell and T cell: active T cell ratios were not significant ($p > 0.05$). The natural killer cell activities, T helper cell numbers and interferon production capability of lymphocytes in the students during the exam period decreased and, as a result, there was an increase in the

incidence of upper respiratory tract infection. This is supported by the finding of decreased EBV antibody titers and the decreased frequency of EBV reactivation in students during summer holidays (14,15).

Nasal mucosa changes may play an important role in the pathogenesis of nasal inflammatory diseases. In this study, changes in the lymphoid subset ratio in nasal lavage indicated the immune response of nasal mucosa against stress.

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