FLOW CYTOMETRIC ANALYSIS OF LYMPHOCYTES AND NONLYMPHOID CELLS IN NASAL LAVAGE OF HEALTHY PERSONS

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ABSTRACT

Objective: The nasal mucosal lymphocytes and nonlymphoid cells play a key role in the immune defence of the entire respiratory tract.

The purpose of this study is to detect lymphocytes and nonlymphoid cells in the nasal lavage of 25 healthy adult by flow cytometric analysis and compare them with the cells in the peripheral blood.

Methods: We studied 25 healthy volunteer students who did not have any upper respiratory diseases. Nasal Lavage was performed by instilling 8 ml of 0.9% sterile saline solution, 4 ml in each nostril with the subject head tilted 30° backwards. Then, lymphocytes and nonlymphoid cells obtained from the nasal lavages were analysed flow cytometrically.

Results: The flow cytometric analyses of nasal lavages were as follows: T helper cell/T suppressor cell ratio 2:1, total monoclear cell/monocyte ratio 11:1, T cell/B cell ratio 3.7:1, T cell/Natural killer cell ratio 5:1, T cell/active T cell ratio 5.8:1. The mean ratios were similar to the ratios of the peripheral blood ($p<0.05$).

Conclusion : Lymphoid and nonlymphoid cells which are present in the nasal mucosa play an important role in the inflammatory response and nasal lavage analysis reflects the immunoregulation of both nasal mucosa and peripheral blood. Therefore flow cytometric analysis of lymphocytes and nonlymphoid cells in nasal lavage is a very practical and an efficent technique in clinical practise.

Key Words: Flow cytometry, nasal lavages, lymphocytes

INTRODUCTION

The nasal mucosal lymphocytes and nonlymphoid cells are regulatory and effector cells in the complex process of inflammatory responses (1). Also nonlymphoid cells, such as HLA -DR^+ macrophages, could possibly play a role in immunoregulation by antigen uptake and its presentation to T lymphocytes (2,3). Moreover, HLA-DR^+ epithelial cells, present in the nasal mucosa, may activate CD_8^+ T lymphocytes (4).

The availability of monoclonal antibodies againts lymphocyte surface antigens makes it possible to identify and quantify lymphocyte subpopulations and nonlymphoid cells by immunohistochemical methods in the nasal mucosa (5).

Nasal lavage has been shown to be a useful tool in the study of inflammatory cells and their mediators in diseases affecting the nose (6,7). Several research groups are using nasal lavage to investigate the role of cells and mediators in allergic and nonallergic airway diseases.Different methods have been proposed to perform nasal lavage and a detailed morphologic identification of obtained cells has been described (8,9). Mucosal infiltration by T cells has been demonstrated in the upper airways of patients with rhinitis (1,10,11) but these cells were not detected in nasal lavage in the previous studies (6,7). In our study, lymphocytes and nonlymphoid cells obtained from nasal lavage of 25 healthy adults were analysed flow cytometrically. T helper cell to T suppressor cell ratio, T cell to B cell ratio, T cell to HLA-DR^+ ratio, T cell to Natural killer cell ratio were calculated and these ratios were compared with the ratios of the cells in the peripheral blood. A profile of lymphoid and nonlymphoid cells of nasal lavage in healthy persons were obtained.

MATERIALS AND METHODS

Subjects: We studied 25 healthy volunteer students (aged 18 to 24 years; mean age 20 years, 18 men and 7 women) who did not have any upper respiratory diseases such as nasal allergy, chronic sinusitis and common cold.

Nasal Lavage: Nasal lavage was performed by instilling 8 ml of 0.9% sterile saline solution, 4 ml in each nostril with the subject head tilted 30° backwards. Subjects were instucted neither to breathe nor to swallow for approximately 10 seconds during a valsalva maneuver and then to bend the head forward in order to collect the mixture of saline and nasal secretions. The liquid obtained was centrifuged (500g at 4° for 10 min) and a pipette was used to dissolve mucus plugs. Total cell counts were obtained by flow cytometric analysis as follows:

1- Supernatant was taken out; 20 µl of monoclonal anticors (double coloured coulter make) of CD3 / CD19, CD4 / CD8, CD3 / HLA-DR$^+$, CD3 / CD16+56, were added into each tube. Than 250 µl of nasal secretion sample was added.

2- Incubating for 20 minutes at room temperature, the tubes were made ready for analyses by Coulter-Multi-Q-prep instrument.

3- Three different solutions were automatically added to each tube while analysing through Coulter-Multi-Q-prep instrument.

4- The first solution contained formic acid and the second solution was composed of sodium chloride, sodium carbonate, sodium sulphate which were used as stabilizers. But the third solution made up of paraformaldehyde was used as a buffer solution.

5- Following the treatment by Coulter-Multi-Q-prep instrument each tube was kept for 20 minutes at room temperature.

6- Than the solutions in each tube were flow cytometrically analysed and 10.000 cells were counted (Fig.1).

Peripheral Blood Lymphocytes: Mononuclear cells and nonlymphoid cells obtained from the peripheral blood of subjects were purified by ficoll hypaque density gradient and analyzed by flow cytometry.

Statistical Analysis: All data were expressed as mean value standard deviation. The significance of the differences was determined by Mann-Whitney-U tests. A p value of less than 0.05 was considered to be significant. A comparison of lymphocytes and nonlymphoid cells in nasal lavage was made with the cells in the peripheral blood. Statgraf 5.0 version statistical program was used in data analysis.

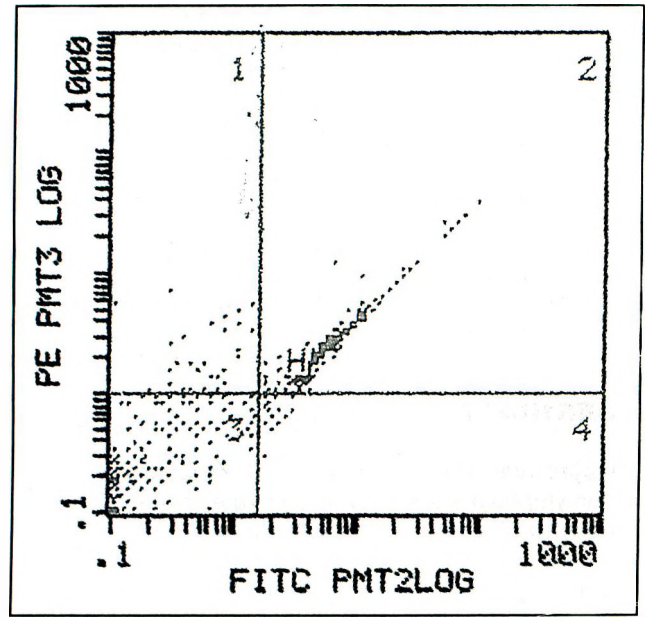

Fig.1: A histogram of gated lymphoid cells. The x- and y axes represent cell labeling with antibodies directed towards the CD8 (suppressor T lymphocyte) and CD4 (helper T lymphocyte) antigens, respectively. The fluorescent compounds attached to the anti-CD and anti-CD8 antibodies emit lights of different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

RESULTS

All participants had a normal distribution of lymphocyte subsets and their ratio in peripheral blood (Table I and Table II).

The mean ratio between helper and suppressor T lymphocytes in the nasal lavage were 2:1, this value was similar to the mean ratio of the cells in the peripheral blood. Number and distribution of lymphocyte and nonlymphoid cells and their ratio in the nasal lavage have been shown in Table III and IV.

Table I. Number and distribution of peripheral blood lymphocytes

	No	Total cell	B cell (CD_{19})	T cell (CD_3)	T helper (CD_4)	T suppressor (CD_8)	Natural killer cell (CD_{16+56})
Test subjects	25	10.000	12.2±4.2	78.3±6.1	48.3±6.4	25.3±5.2	15.3±5.2
Normal values	.....	.....	7.0±25.0	61.0±85.0	28.0±58.0	19.0±48.0	6.0±29.0

Table II. Lymphocyte subsets ratio in normal peripheral bood

	T cell/Natural killer cell (CD_3/CD_{16+56})	T helper cell/ T suppressor cell (CD_4/CD_8)	T cell/B cell (CD_3/CD_{19})	T cell/active T cell ($CD_3/HLADR^+$)	Total mononuclear cell/monocyte (CD_{45}/CD_{19})
Test subjects	6.2±2.2	1.9±0.6	4.9±1.8	5.4±1.8	10.2±2.4
Normal values	5.0±2.0	1.7±0.5	5.0±2.0	6.0±2.0	10.0±2.0

Table III. Number and distribution of lymphocytes and nonlymphoid cells in nasal lavage

	No	Total cell	B cell (CD_{19})	T cell (CD_3)	T helper (CD_4)	T suppressor (CD_8)	Natural killer cell (CD_{16+56})
Test subjects	25	10.000	20.8±5.1	65.5±7.1	30.5±6.2	16.2±2.2	17.6±4.2

Table IV. Lymphocyte subsets ratio in nasal lavage

	T cell / Natural killer cell (CD_3/CD_{16+56})	T helper cell / T suppressor cell (CD_4/CD_8)	T cell / B cell (CD_3/CD_{19})	T cell / Active T cell ($CD_3/HLADR^3$)	Total mononuclear cell/monocyte (CD_{45}/CD_{19})
Test subjects	5.1±1.3	2.0±0.8	3.7±1.2	5.8±1.5	11.3±2.1

DISCUSSION

The nose is the first line of defence of the airways and must be capable of excluding potentially damaging inhaled substances as quickly as possible. It is therefore understandable that neural reflexes, resulting in sneezing and rhinorrhoea, play a more important role in the nose than in the bronchi (12). Nasal discharge can have a multitude of sources, including nasal glands, goblet cells, plasma exudation, secretions from paranasal sinuses, tears, and condensed expired water. The glands are under the control of the parasympathetic nervous system, in contrast to the goblet cells in the surface epithelium which have a secretory mass estimated to be only 2% of the glands. Traditionally, the substance blown out of allergic noses has been called secretion. However, its origin from submucosal glands has been questioned because plasma exudation has been demonstrated as a consistent consequence of allergic airways during inflammation (12,13). Plasma products are found in nasal surface fluid.

Lymphocytes are a normal cell population in the airway mucosa from the nose to the alveoli (14). The nasal intraepithelial lymphocytes probably play a key role in the immune defense of the entire respiratory tract. T and B lymphocytes which are regulatory and effector cells in the complex process of inflammatory responses, are found in the nasal mucosa (1). Also nonlymphoid cells, such as HLA-DR$^+$ macrophages, have vital importance in the regulation of immune responses (3). Many HLA-DR$^+$ expressing cells, such as macrophages, Langerhans cells, and dentritic cells, are known to be directly involved (15). In the airway epithelium of HLA-DR$^+$ positive cells and T suppressor/cytotoxic cells and T helper / inducer cells in the surface epithelium could be a site for initiation of the immune response.

Such a hypothesis has been proposed by Brandtzaeg (16), who also showed HLA-DR$^+$ reactivity in human nasal epithelium. Poulter et al (17). have demonstrated that bronchoalveolar lavage may be an inappropriate sampling procedure for the investigation of T-cell infiltration in the bronchial mucosa of asthmatic patients.

Although mucosal infiltration by T cells has been demonstrated in the upper airways of patients with rhinitis, these cells were not detected in nasal lavage in the previous studies (18). Different methods have been proposed to perform nasal lavage and a detailed morphologic identification of obtained cells has been described (6-8). Some problems are present in the analysis of cells obtained by nasal lavage such as mucus plugs. Interfering, in the assessment of total and differential cell counts, or broken cells may cause misintrepretation of the results. May-Grünwald-Giemsa (MGG) stain is a method to identify cells obtained by nasal lavage, however, macrophages or monocytes could not be detected by this method of cell identification (8). In our present study T lymphocytes, B lymphocytes, active T lymphocytes, Natural killer cells and their ratios in nasal lavage in healthy persons were studied. We compared these values with the lymphoid cells and lymphoid subsets ratios of normal nasal mucosa which was described by monoclonal antibody in a study with avidinbiotin immunperoxidase technique that was performed by Winther et al (Table V).

In our study, T lymphocyte distribution in peripheral blood and in nasal mucosa was similar to the results of Drexhage et al. (19) and Knutsen et al (20). In contrast to the study of Prat et al. (8) our study concludes that T cell infiltration in nasal mucosa is reflected by nasal lavage. Using flow cytometric analysis in a previous study, we showed the effect of topical corticosteroids on the distribution of T lymphoid cells in nasal lavage (21).

Lymphoid and nonlymhoid cells which are present in nasal mucosa play a big role in inflammatory response and nasal lavage analysis reflects the immunregulation of both nasal mucosa and peripheral blood. We suggest that flow cytometric analysis of lymphocytes and nonlymphoid cells in nasal lavage is a very practical and an efficient technique in clinical practice.

Table V. Comparison of the lymphoid subsets ratios of normal nasal mucosa and nasal lavage

	T cell/Natural killer cell (CD_3/CD_{16+56})	T helper cell/ Tsuppressor cell (CD_4/CD_8)	T cell/B cell (CD_3/CD_{19})	Total mononuclear cell/monocyte (CD_{45}/CD_{19})
Nasal mucosa	4.8/1	2.5/1	3/1	10/1
Normal values	5.1±1.3	2.0±0.8	3.7±1.2	11.3±2.1

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