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PATHOGENETIC AND FUNCTIONAL CHARACTERIZATION OF LYMPHOCYTES IN INFLUENZA PATIENTS

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Summary. — The kinetics of acrocentric chromosome associations and chromosome aberrations in peripheral blood and pleural exudate lymphocytes has been studied in 25 influenza patients and 7 exudative pleurisy patients. Lymphocytes without associations and with 2 associated acrocentric chromosomes were activated in the body, since their frequency appeared to be positively correlated with the immunoresponsiveness indices and with clinical symptoms. The number of these lymphocytes in pleural exudate was 2.5 times higher than in the peripheral blood. When comparing the frequency of chromosome aberrations in the patients' lymphocytes to the level of immunity, cytogenetic changes corresponded to the indices of cellular rather than humoral immunity.

Key words: influenza; chromosomes; chromosome aberrations; immunoresponsiveness; lymphocytes

Introduction

Cytogenetic method has been used for the study of the frequency of chromosome aberrations in blood lymphocytes of influenza patients (Frolov *et al.*, 1971; Ilyinskikh, 1982). Chromosome modifications of lymphocytes as immunocompetent cells may be indicative of their participation in immune responses. In this paper cytogenetic indices of patients were analysed and related to the activity of the disease and individual immune responsiveness. In addition to the frequency of chromosome aberrations, the frequency of associations of acrocentric chromosomes (AAC) has been studied. The latter was based on our observation of correlation of the frequency of AAC in blood lymphocytes with their previous proliferation and migration activity in the body (Frolov and Frolov, 1983; Frolov, 1984).

By cytogenetic method it is possible to study the actual functional activity of lymphocytes during immunogenesis, while other presently applied methods of evaluation of the functional activity of lymphocytes, as lymphocyte blast transformation (LBT) in response to mitogens or to specific antigens, are essentially in vitro tests.

Materials and Methods

Altogether 25 influenza patients aged from 18 to 46 years were examined during an influenza epidemic in winter 1979—1980. The diagnosis was confirmed by virus isolation in chick embryos and serologically by haemagglutination inhibition (HI) test. A 4-fold or higher increase of the titres was considered significant.

Lymphocytes. Blood was taken during acute phase of the disease (day 1 to 3) and during early convalescence (days 10 to 14 from the onset of the disease). Twenty healthy subjects of the same age served as controls. Lymphocytes were cultivated without separation (Bach, Hirschorn, 1963). For this purpose gelatine solution at a final concentration of 1% was added to the blood and after incubation at 37 °C for 20—30 min, the plasma with leukocytes was collected. After removal by low-speed centrifugation, the nutrient medium was added to the pellet containing blood cells to reach a final leukocyte concentration of 2×10^6/ml. The nutrient consisted of medium 199, 25% human serum of Rh-positive group IV and antibiotics (the mixture of 100 units/ml of penicillin and streptomycine). The prepared cell suspension was poured into 3 ml penicillin flasks. Depending on the purpose of experiment, 25 μg/ml of phytohemagglutinin (PHA) "Difco" or inactivated influenza vaccine (IIV) from A/Texas/1/77 strain (H3N2) (Leningrad Research Institute of Vaccines and Sera) at a final concentration of 5 μg/ml of protein were added. The cells were cultivated with PHA for 52 to 54 hr and with IIV for 6 days. LBT was morphologically assessed by analysing 1000 mononuclear cells in each preparation.

Chromosome preparations were obtained from a portion of the culture with PHA using conventional technique (Hungerford, 1965). The frequency of chromosome aberrations and the associating capacity of acrocentric chromosomes was tested in not less than 100 cells. Of the chromosome aberrations fragments, gaps and translocations were considered. Association was defined as an arrangement of acrocentric chromosomes facing each other with their short shoulders, the distance between them being shorter than the length of the 21—22 group chromosomes (Zang and Back, 1968).

The frequency of acrocentric chromosome associations in blood lymphocytes was estimated by the mean number of AAC per cell (mAAC/cell). In addition, the lymphocytes were classified into groups depending on the number of AAC which ranged between 0 and 10. Lymphocyte classes without associations and with two associated acrocentrics (LC_{0+2}) were chosen. They were assumed to be activated already in the body, since they were produced as a result of consecutive mitotic divisions in peripheral lymphoid organs (Frolov and Frolov, 1983).

Humoral immunity was assessed by HI test based on an increase in serum virus-specific antibody titres as well as on the quantitation of serum immunoglobulins (Mancini *et al.*, 1965). HI antibody levels were expressed in natural log units and, moreover, values of mean geometric titres (MGT) were calculated. The number of T-lymphocytes and B-lymphocytes, respectively, was determined using the method of spontaneous rosette formation with sheep red blood cells (Mendes *et al.*, 1974) and with mouse red blood cells (Dobozy *et al.*, 1976). As clinical signs of the disease the degree of fever and intoxication symptoms (headache, giddiness, loss of consciousness, pain in the eyes, muscles and joints) were considered. Moreover, to estimate the migration activity of T-lymphocytes in the organism, the frequency of AAC was studied in the lymphocytes of peripheral blood and pleural fluid in 7 patients with acute exudative pleurisy, since in their immune response as well as in influenza patients topographically similar local peripheral lymphoid organs are involved (Ogra, 1979). The data obtained were treated by the analysis of variance using computer-assisted (EC-1022) correlation regression analysis.

Results

The number of blood cells and the immunological indices in acute and convalescent phase of the disease are shown in Table 1. In influenza patients a total leukocyte count was reduced in either phase of the disease. In acute phase the amount of lymphocytes was also reduced at the expense of T-cells, whereas the number of B-lymphocytes remained unchanged and by the end of the disease it was higher than in control healthy subjects. The responsiveness of T-lymphocytes to PHA, which is indicative of their nonspecific antigenic

Table 1. Blood cell counts and immunological indices of influenza patients in acute and convalescent phase of the disease

Indices tested	Healthy subjects	Patients in the disease phase	
		acute	convalescent
Number of cells[a]			
Leukocytes, 10^9/l	5.41 ± 0.3	4.32 ± 0.38*	3.83 ± 0.32*
%	29.4 ± 2.2	26.6 ± 2.6	36.5 ± 3.4
Lymphocytes, 10^9/l	1.59 ± 0.07	1.15 ± 0.12*	1.41 ± 0.11
%	69.9 ± 1.9	63.2 ± 2.3*	72.3 ± 2.5
T-lymphocytes, 10^9/l	1.18 ± 0.11	0.72 ± 0.08*	1.02 ± 0.15
%	5.2 ± 0.6	7.0 ± 0.8	7.5 ± 0.9*
B-lymphocytes, 10^9/l	0.08 ± 0.01	0.08 ± 0.02	0.11 ± 0.03*
LBT values[b]			
C	2.1 ± 0.4	3.8 ± 0.5	8.2 ± 0.9**
IIV	4.8 ± 0.5	7.8 ± 0.5*	23.2 ± 2.0**
PHA	78.4 ± 2.0	52.0 ± 3.7**	77.0 ± 3.5
Antibody levels[c]			
HI ln	1 : 1.77 ± 0.26	1 : 2.06 ± 0.31	1 : 3.43 ± 0.22**
abs	1 : 5.9	1 : 7.8	1 : 30.9*
IgG	9.35 ± 0.55	7.28 ± 0.38*	7.36 ± 0.46*
IgA	1.67 ± 0.14	1.58 ± 0.19	1.51 ± 0.14
IgM	0.90 ± 0.04	1.63 ± 0.2*	1.87 ± 0.21**

[a] Absolute (in 10^9/l) and relative (in %) values.
[b] Obtained with control (C) lymphocytes or lymphocytes to which IIV or PHA were added.
[c] HI antibody levels expressed in natural log (ln) units or MGT values.
Note: In this as well as in Table 2, values significantly different from controls (healthy subjects) at $p < 0.05$ and $p < 0.01$ are designated by one and two asterisks, respectively.

stimulation potential (Petrov, 1976), was decreased only in acute phase of the disease; in convalescent it was restored. Specific LBT in response to IIV as well as spontaneous LBT (in lymphocyte cultures which neither antigen nor mitogen were added to) were enhanced during convalescence indicating activation of cellular immunity. Antibody titres in acute phase of the disease were low but reached a significant level in convalescence. Throughout the investigation low contents of IgG and a rise of IgM were observed.

Significant changes of the AAC frequency were found in influenza patients (Table 2). Thus, for instance, mAAC/cell in the total population of blood lymphocytes of influenza patients was higher in acute phase of the disease than in healthy subjects and it was significantly decreased during convalescence. As mAAC/cell in the lymphocyte population is inversely proportional to the frequency of LC_{0+2}, the letter occurred more often in convalescence than in acute phase of the disease.

The frequency of lymphocytes with chromosome aberrations was higher in influenza patients than in healthy subjects. In addition to fragments and gaps the patients' lymphocytes also had breaks in the centromere and translocations.

Table 2. Cytogenetic indices of lymphocytes in influenza and acute exudative pleurisy patients (M ± SD)

Groups examined	mAAC	Frequency of LC_{0+2} (%)	Number of cells with chromosome aberrations (%)
Healthy subjects	3.93 ± 0.11	27.9 ± 1.7	1.8 ± 0.5
Patients with influenza	4.27 ± 0.11*	23.2 ± 1.9*	5.6 ± 0.9**
	3.53 ± 0.12*	36.9 ± 2.6**	6.4 ± 1.0**
acute exudative pleurisy	4.34 ± 0.22**	17.4 ± 4.8**	2.4 ± 0.5
	3.35 ± 0.22**	42.4 ± 2.8**	3.6 ± 0.9

Note: For influenza patients the numerator indicates the acute and the denominator the convalescent phase of the disease; for acute exudative pleurisy patients the numerator characterizes the values in peripheral blood and the denominator those in pleural exudate.

The frequency of AAC in lymphocytes from blood and pleural fluid showed dramatic differences in acute exudative pleurisy patients. The mAAC/cell in T-lymphocytes from pleural fluid was significantly lower than in the blood, and accordingly, the frequency of LC_{0+2} has increased 2.5 times. It is worth to note that the frequency of AAC in T-lymphocytes of these patients significantly differed from that of healthy subjects and was similar to the corresponding indices in influenza patients. It should be noted, however, that in Table 2 the denominator for influenza patients presents mAAC/cell values in the blood lymphocytes during convalescence and for pleurisy patients — in the pleural exudate in acute phase of the disease.

Positive correlation was revealed between the frequency of LC_{0+2} and LBT in response to IIV (X_1) as well as the number of T-lymphocytes in the

Table 3. Correlation coefficients (r) in acute disease and in convalescence

Phase of influenza	Cytogenetic indices	LBT in response to IIV X_1	Antibody titres X_2	Number of T-lymphocytes X_3	Degree of fever X_4	Intoxication signs X_5
Acute	LC_{0+2}	0.75*	−0.27	0.65*	−0.17	−0.18
	Chromosome aberrations	0.09	0.02	−0.32	−0.27	−0.16
Convalescent	LC_{0+2}	0.70*	−0.52*	0.71*	−0.41*	−0.38*
	Chromosome aberrations	0.76*	−0.30	−0.32	−0.38*	−0.16

Note: LC_{0+2} — lymphocyte class without associations and with two associating acrocentrics; asterisk indicates r values surpassing the critical ones at p = 0.05.

peripheral blood (X_3) in influenza patients at both acute and convalescent phase of the disease observation (Table 3). The correlation between the frequency of LS_{0+2} and the antibody titres (X_2), the degree of the fever (X_4) as well as the intoxication signs (X_5) was medium and negative only during convalescence. The number of cells with chromosome aberrations was highly correlated to LBT in response to IIV also only during convalescence, whereas negative correlation with other indices at that period was low and reached a significant level only in patients with high fever.

Discussion

Analysis of the data obtained indicates that cytogenetic changes in T-lymphocytes in the blood on influenza patients are closely related to the development of immune response. Variations of the quantity of AAC and LC_{0+2} in the lymphocytes can be explained in the following way: It is known that antigen-sensitive lymphocytes are activated after antigen contact and by 3 to 6 days they are transformed into blast cells in the peripheral lymphoid organs; then they successively divide 6 to 10 times to produce an effector lymphocyte clone. Taking into account that during a mitotic cycle the number of AAC is decreased to the mean of 20 to 25% (Zhdanova, 1974; Sigmund *et al.*, 1979), it can be assumed that these lymphocytes will have either no associations or not more than 2 associating acrocentrics resulting from their reassociation within a short period between divisions, i.e. they will constitute the lymphocyte class LC_{0+2}. Later on these lymphocytes migrate into the blood, are attracted to the recirculating pool and become available for analysis when introduced into culture. Thus, LC_{0+2} consists of lymphocytes which are activated by antigens in the human organism and the lymphocytes with higher frequency of associating chromosomes are in the state of recirculation of different duration without antigenic stimulation. This was confirmed by correlation analysis data. Thus, for instance, a high correlation was found between the proportion of LC_{0+2} and cellular immune responses (LBT in response to IIV). This correlation is due to the appearance of antigen-sensitive lymphocytes and memory cells in the blood responding by LBT to virus antigens in the culture (Süss, 1980). The lymphocytes recently underwent a series of mitoses in response to antigenic stimulation in patients and a part of them will constitute LC_{0+2}.

The above suggests that the rise of mAAC in the blood lymphocytes resulting from a decrease of the frequency of LC_{0+2} observed in acute phase of the disease is mediated, among others, by the immunosuppressing effect of the influenza virus which causes a reduction of the proliferative activity of the lymphocytes during immunogenesis. This is evident from the decrease of LBT in response to PHA, the number of leukocytes and the level of immunoglobulins. However, the withdrawal of activated T-lymphocytes from the recirculation pool and their deposition in the sites of antigen location is another cause of the decrease of LC_{0+2} frequency in the patients blood in this period. It has been demonstrated that the lymphocytes release mediators

in the sites of the development of specific immune response. These mediators inhibit the migration of activated lymphocytes; the latter are, therefore, temporarily deposited (Medunitsyn *et al.*, 1980; Ilanto *et al.*, 1982). The studies in the patients with acute exudative pleurisy provide evidence for this conclusion. Thus, for instance, the frequency of LC_{0+2} in the blood of these patients was low, whereas their quantity in the pleural fluid was 2.5 times higher. This is a direct evidence for the deposition of activated lymphocytes in the sites of antigen location.

The development of immune responses in influenza patients reaches its maximum during convalescence. The respiratory tract is sanated and previously deposited activated lymphocytes start free recirculation. This can account for the increased amount of LC_{0+2} in the blood as well as the enhancement of their relationship with cellular and humoral immune responses and clinical indices.

Chromosome aberrations appear in the lymphocytes of influenza patients owing to the toxic effect of the virus and its metabolites on the cell. It should be noted that the number of chromosome aberrations in T-lymphocytes of convalescents was maintained at a high level. A correlation between the frequency of T-lymphocytes with chromosome aberrations and the activity of cellular and humoral responses as well as several clinical indices was observed only in acute phase of the disease. This seems to be related to prolonged life time of lymphocyte subpopulations as well as to the return of effector lymphocytes, previously deposed at damaged foci, to the circulation at this time.

Thus, cytogenetic indices of blood lymphocytes in influenza patients are closely related to the individual immune responsiveness and to the phase of the infection process. The data obtained have laid the basis for the application of cytogenetic method in combination with immunological methods for evaluation of the functional activity and migration capacity of different lymphocyte populations in the patient's organism. The number of AAC in the lymphocytes appears to be the most instructive cytogenetic parameter in this respect.

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