Phenotype of Peripheral Blood Lymphocytes and Serum Immunoglobulin Concentration in Patients with Early Rheumatoid Arthritis

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We studied the phenotype of peripheral blood lymphocytes and serum immunoglobulin concentration in patients with early rheumatoid arthritis and rheumatoid arthritis of more than 12 months duration. Subpopulations of $CDIgM^+$, $CD25^+$, and $HLA\text{-}DR^+$ lymphocytes and IgA concentration differed in these groups of patients with rheumatoid arthritis. The count of lymphocytes carrying $CDIgM^+$ and $HLA\text{-}DR^+$ receptors correlated with activity of rheumatoid arthritis.

Key Words: *early rheumatoid arthritis; immune status; lymphocyte phenotype; immunoglobulins*

Diagnosis of the early stage of rheumatoid arthritis (RA) allows us to perform adequate and timely therapy and prevent progression of the disease [5]. X-Ray signs of pathological changes are observed in the early stage of active RA.

Immune dysfunction in RA patients includes changes in cellular and humoral immunity [6]. Study of these changes can be informative to make early diagnosis and evaluate activity of the disease [7,8]. It is important to identify CD receptors on the subpopulation of lymphocytes that play a role in the development of immunoinflammatory reactions [6]. Some CD antigens ($CD3^+$, $CD4^+$, $CD8^+$, $CD16^+$, and $CD20^+$) were analyzed quantitatively in patients with RA [1,2,4]. Little is known about other pathogenetically important surface molecules. It should be emphasized that previous studies were conducted on patients with clearly defined clinical manifestations of the disease. Single experiments were performed to determine several parameters of lymphocyte phenotype in the early stage of RA [12].

We studied the phenotype of peripheral blood lymphocytes and concentration of serum immunoglobulins in patients with early RA. The relationship between immunological parameters and clinical signs was evaluated.

MATERIALS AND METHODS

We examined 98 patients with RA. There were 76 patients (50±13 years) with early RA of not more than 12 months duration. Twenty-two patients (48±14 years) had clearly defined clinical manifestations of RA with disease duration of more than 12 months. The patients of these groups had similar clinical signs of RA. The control group included 50 healthy donors of comparable sex and age. The diagnosis of early RA was made according to the algorithm proposed by American and European rheumatologists in 2002 [5]. The diagnosis of RA with disease duration of more than 12 months was made by ARA criteria developed in 1987. Activity of RA was determined taking into account the number of

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painful and swollen joints (examination of 28 joints), degree of morning stiffness, erythrocyte sedimentation rate (ESR), general health (visual analog scale), and disease activity score (DAS28). The content of rheumatoid factor in the blood was measured by the method of latex agglutination.

Lymphocytes were isolated from heparinized peripheral blood on a Ficoll-Verografin gradient (1.077 g/liter). The blood was diluted with medium 199 (ratio 1:1), layered on the gradient, and centrifuged at 1000 rpm for 30-40 min. The lymphocyte suspension was washed 2 times with the same medium (centrifugation for 7-10 min). The suspension of lymphocytes (15-16 μl, (5-8)×10^6 cells) was placed on microscope glass slides pretreated with 0.1% poly-L-lysine (dilution 1:20). The suspension was incubated in a thermostat at 37°C for 30 min, thoroughly rinsed with phosphate buffered saline, dried with filter paper, treated with 7 μl monoclonal serum (Sorbent), and maintained an a moist chamber at 4°C. It was washed in phosphate buffered saline, stained with labeled FITC antiserum against IgG, incubated at 4°C for 30 min, rinsed, covered with a mount fluid and a cover glass. The count of FITC-labeled lymphocytes was estimated under a MIK MED-2 luminescence microscope (LOMO). We estimated the absolute and relative number of $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, $CD23^+$, $CD38^+$, $CD16^+$, $CD56^+$, $CD54^+$, $CD25^+$, $CD71^+$, $CD95^+$, $CD10^+$, HLA-DR^+, and $CDIgM^+$ cells.

Serum immunoglobulin concentration was measured in Reafarm plates by the method of Manchini. Blood plasma samples were diluted by 32, 8, and 2 times with phosphate buffered saline to study IgG, IgA, and IgM, respectively. The test samples were maintained in wells of a plate until disappearance of a meniscus lens shade. The reaction was conducted at 4°C. The diameter of precipitate was measured with a special ruler (accuracy 0.1 mm). The measurements were performed 2 days after filling of wells. The concentrations of IgG, IgA, and IgM were determined using a special table.

The results were analyzed by Statistica 6.0 software (StatSoft, Inc.). The data are expressed as median values and interquartile ranges. Intergroup differences were estimated by Kruskal—Wallis test. Pairwise comparison involved Mann—Whitney test. The Spearman rank correlation coefficients were calculated. The differences were significant at $p<0.05$.

RESULTS

Intergroup differences in study parameters were statistically significant (Table 1). The patients with early RA and RA of more than 12 months duration significantly differed by the following parameters: $CDIgM^+$ ($p=0.05$), $CD25^+$ ($p=0.006$), and IgA ($p=0.009$). Intergroup differences in $CD19^+$ ($p=0.192$), $CD38^+$ ($p=0.239$), HLA-DR^+ ($p=0.095$), $CD95^+$ ($p=0.125$), $CD10^+$ ($p=0.374$), IgG ($p=0.113$), and IgM ($p=0.151$) were close to significant. The number of $CD3^+$, $CD16^+$, and $CD23^+$ did not differ in patients with early RA and RA of more than 12 months duration.

We studied the relationship between immunological parameters and activity of RA (Table 2). IgM concentration tended to correlate negatively

TABLE 1. Lymphocyte Phenotype and Serum Immunoglobulin Concentration in Examined Patients

Parameter	Control (n=50)	Early RA (n=76)	RA of more than 12 months duration (n=22)	p
$CD19^+$, %	14 (11-18)	31 (25.0-37.5)	25 (20.0-32.8)	<0.001
$CD4^+$, %	49 (44-56)	40 (33.1-48.0)	41 (38.5-53.0)	<0.001
$CD8^+$, %	28.7 (26-33)	20.4 (167.0-25.8)	22.5 (13.8-35.3)	<0.001
$CD4^+/CD8^+$, %	1.67 (1.55-1.87)	1.93 (1.34-2.56)	1.70 (1.29-3.42)	=0.231
$CD38^+$, %	16.5 (11-20)	24.2 (16.2-34.5)	31.0 (23.5-40.0)	<0.001
$CD54^+$, %	5.2 (4.3-6.2)	18.5 (12.5-28.0)	19.5 (15.3-25.0)	<0.001
$CDIgM^+$, %	5.6 (5.1-6.0)	25.0 (13.3-30.8)	30.0 (16.7-38.5)	<0.001
HLA-DR^+, %	17 (14-20)	22.2 (16.5-37.0)	32.0 (20.8-41.6)	<0.001
$CD25^+$, %	11 (8.5-14.0)	16.1 (12-23)	25 (16-36)	<0.001
$CD95^+$, %	6.8 (5-10)	18.0 (10.2-25.0)	24.0 (11.1-32.0)	<0.001
$CD10^+$, %	4 (1-7)	14 (5.5-24.0)	18.0 (13.5-26.9)	<0.001
IgG, g/liter	14.9 (13.6-16.3)	17.5 (10.8-26.0)	21.6 (17.7-29.0)	=0.011
IgA, g/liter	3.0 (2.3-3.8)	4.7 (3.8-6.6)	6.6 (5.3-7.9)	<0.001
IgM, g/liter	1.8 (1.4-2.2)	4.1 (2.2-8.7)	3.1 (2.1-5.0)	<0.001

TABLE 2. Correlations between Immunological Parameters and Disease Activity in RA Patients

Parameter	Number of swollen joints	Erythrocyte sedimentation rate
$CD19^+$	R=-0.07, p=0.634	R=-0.38, p=0.008
$CD38^+$	R=0.027, p=0.03	R=-0.24, p=0.049
$CDIgM^+$	R=0.32, p=0.043	R=-0.14, p=0.344
$HLA\text{-}DR^+$	R=0.45, p<0.001	R=-0.21, p=0.071
$CD95^+$	R=0.33, p=0.006	R=0.03, p=0.808
IgG	R=0.25, p=0.041	R=-0.12, p=0.317

with the duration of early RA (R=-0.24, p=0.052). Correlation coefficients were estimated in patients with more than 12 months history of RA. The ratio of $CD10^+$ and concentration of IgA correlated with the age of patients (R=-0.31, p=0.057; and R=0.65, p=0.006, respectively). The ratio of $CD38^+$ and $CD95^+$ correlated with the duration of RA (R=-0.41, p=0.059; and R=-0.34, p=0.076, respectively). The concentration of IgG and IgM correlated with the number of swollen joints (R=0.53, p=0.035; and R=-0.51, p=0.046, respectively). In healthy donors a correlation was revealed between the ratio of $HLA\text{-}DR^+$ and age (R=0.31, p=0.055).

As distinct from healthy donors, the immune status of RA patients was characterized by an increase in the ratio of $CD19^+$, $CD10^+$, $CD38^+$, and lymphocytes with receptors for IL-2 ($CD25^+$) and IgM ($CDIgM^+$). These patients had high number of apoptotic cells ($CD95^+$). The concentration of IgG fractions increased in patients with RA. The observed changes in RA patients reflect a strong immunoinflammatory reaction mediated by humoral and cellular immunity.

The number of CDIgM and concentration of IgA in patients with RA of more than 12 months duration were higher compared to patients with early RA. These characteristics reflect the initial phase of the immune response to antigenic stimulation. Moreover, we revealed a positive correlation between the number of swollen joints and ratio of $CD38^+$ and $CD95^+$. A negative correlation between the ratio of $CD38^+$ and ESR suggests that the early stage of RA is characterized by prevalence of cellular immune reactions.

The ratio of $CD54^+$ and concentration of IgA increased, while the number of immunoregulatory $CD8^+$ and $CD4^+$ cells decreased in patients with RA. The $CD4^+/CD8^+$ ratio remained unchanged in RA patients. These data indicate that helper cells and adhesion molecules are involved in immunoinflammatory reactions during the acute stage of RA. The count of lymphocytes with HLA-DR receptors increased in RA patients of both groups. Therefore, histocompatibility antigens stimulate the immunoinflammatory reaction.

Changes in the ratio between subpopulations of lymphocytes and increase in the number of immature cells reflect abnormal maturation and decelerated differentiation of T and B cells. These changes underlie intolerance to autoantigens, contribute to the development of autoimmune RA, and are particularly pronounced in patients with RA of more than 12 months duration [9,12]. It was probably associated with age-related involution of the thymus and immune dysregulation [10]. Specific leukocyte antigens are involved in antigen presentation and stimulate the autoimmune process [6].

Thymus dysfunction and variations in lymphocyte differentiation probably determine progression of the autoimmune reaction in RA patients. The increase in IgG concentration reflects unknown autoimmune reactions in the early stage of RA and sometime exists over 10 years before the appearance of clinical manifestations [3,11]. Immune dysfunction in patients with early RA correlates with the disease activity score. We conclude that receptors for CDIgM and HLA-DR can serve as an immunological marker of early RA in patients with active disease.

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