Expression of CD95 on Peripheral Blood Lymphocytes in Patients with Autoimmune Diseases and Neoplasms

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CD95 expression on peripheral blood lymphocytes in patients with neoplasms was higher than in patients with autoimmune disorders. Apoptosis of T cells increased during tumor growth. The data suggest that neoplasms are accompanied by more severe immune dysfunction than autoimmune disorders.

Key Words: *immune deficiency; apoptosis; lymphocytes; neoplasms and autoimmune diseases*

Neoplasms and autoimmune diseases are the main causes of immune deficiency. Despite a large body of data, the mechanisms and reliable diagnostic criteria for immune deficiency in patients with neoplasms and autoimmune diseases are not established. Activation antigens attract much attention in evaluating immune status of patients with neoplasms and autoimmune diseases [5]. Inadequate expression of the activation marker CD95 plays the major role in the pathogenesis of neoplasms and autoimmune diseases [7,10].

CD95 (*Fas* or APO-1) belongs to the tumor necrosis factor receptor family and exists in transmembrane and soluble forms. *Fas* antigen is expressed on peripheral $CD4^+$, $CD8^+$, and B lymphocytes, granulocytes, monocytes, activated T and B lymphocytes, natural killer cells, and thymocytes, but not on plasma cells and erythrocytes. Being combined with *Fas* ligand (*Fas*-L), *Fas* antigen induces apoptosis of *Fas*-positive target cells [1].

CD95 expression on peripheral blood lymphocytes (PBL) and in various organs changes in some diseases. It was shown that the content of CD95 on tissue lymphocytes increases in malignant tumors [10]. However, there is no general agreement on the mechanisms of CD95 expression on PBL during various immune disorders. The number of CD95-positive lymphocytes increases in autoimmune thyroiditis [4] and uterine body cancer [3], while in renal carcinoma and ovarian cancer CD95 expression remains unchanged [3,6].

Here we studied CD95 expression on PBL in patients with autoimmune diseases and neoplasms and evaluated the possibility of using this parameter as a criterion for immune deficiency in these patients.

MATERIALS AND METHODS

Peripheral blood lymphocytes were obtained from 16 healthy individuals, 36 patients with various tumors, and 12 patients with autoimmune diseases (rheumatoid arthritis and autoimmune thyroiditis). The blood was taken during primary examinations; diagnoses were made before the start of therapy. The cells were isolated by centrifugation in the Ficoll-Verografin density gradient (1.077 g/ml). Lymphocyte subpopulations (CD3, CD4, CD8, CD16, CD19, CD71, CD95, and HLA-DR) was assayed by indirect immunofluorescence using monoclonal LT antibodies (Institute of Immunology, Russian Ministry of Health) [2]. We measured fluorescence of no less than 200 cells. Nonspecific binding of labeled serum was estimated in each experiment. The results were analyzed by Student's *t* test.

RESULTS

Nonspecific cytolysis by natural killer cells in patients with neoplasms was more intensive than in healthy

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individuals and patients with immune disorders. No significant quantitative changes in CD4 and CD8 subpopulations were found, but the number of B lymphocytes considerably increased in patients with neoplasms (Table 1).

Expression of activation markers on PBL markedly increased in patients with neoplasms (Fig. 1). Similar changes were found during lymphoproliferative disorders [1] and malignant tumors. Expression of CD71 and CD95 underwent most pronounced changes (15.83±2.38 and 12.41±2.92 *vs.* 4.73±2.55 and 3.73±2.43 in the control, respectively). During autoimmune disorders, expression of activation markers did not differ from the control.

Since these changes were most pronounced in patients with neoplasms, we analyzed expression of activation markers on PBL at various stages of tumor growth. Activation processes were markedly intensified in patients with stage III tumors compared to stages I and II. The number of HLA-DR$^+$ cells did not differ at various stages of tumor process (Fig. 2).

Since little is known on the dependence of activation marker expression on tumor localization, we studied expression of CD71, CD95, and HLA-DR in patients with lung, stomach, and breast cancers. Changes in CD71 and CD95 expression were most pronounced in patients with stomach cancer. The number of CD95-positive cells considerably increased in patients with breast cancer. Lung cancer was accompanied by less pronounced changes in these parameters (Fig. 3). HLA-DR expression did not differ in these patients (Fig. 3).

Thus, CD95 expression on PBL can serve as a diagnostic criterion for solid tumors of various localizations and, probably, reflects the severity of immune deficiency. Enhanced expression of C95 correlates with CD71 expression, which is consistent with published data [3].

TABLE 1. Lymphocyte Subpopulations (arb. units) in Patients with Neoplasms and Autoimmune Diseases (*M*±*m*)

Subpopulations	Control (*n*=16)	Patients	
		neoplasms (*n*=36)	autoimmune diseases (*n*=12)
CD3	55.27±3.21	57.10±3.14	57.40±4.36
CD4	27.60±2.24	27.77±2.61	26.93±3.09
CD8	26.60±3.76	30.10±3.37	28.23±4.54
CD16	13.47±2.61	22.40±2.41*	13.66±5.82
CD19	9.87±2.96	20.14±2.90*	13.56±4.57

Note. *$p<0.05$ compared to the control.

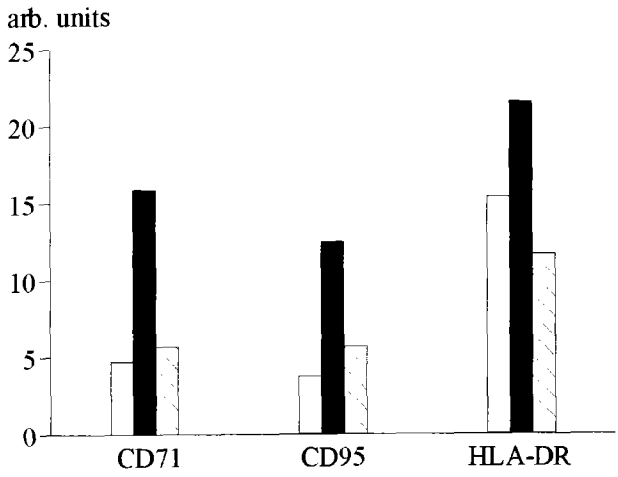

Fig. 1. Expression of activation markers on lymphocytes from healthy individuals (light bars) and patients with neoplasms (dark bars) and autoimmune (dashed bars) diseases.

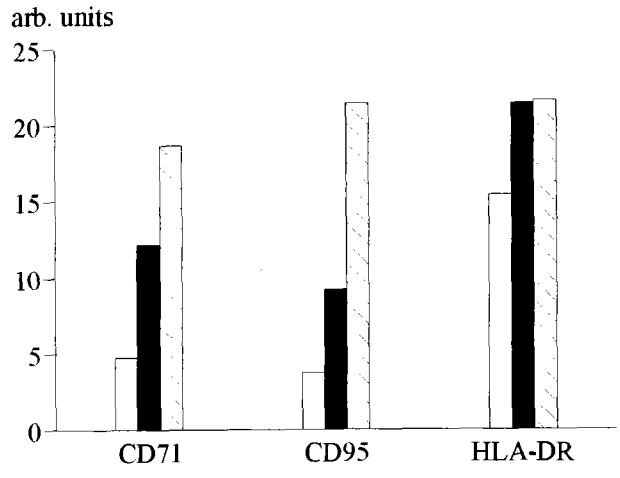

Fig. 2. Expression of activation markers at various stages of tumor growth: control (light bars), stage I-II (dark bars), and stage III (shaded bars).

Our findings demonstrate high expression of CD71 and CD95 in patients with neoplasms. Similar results were reported previously [3]. In patients with ovarian cancer, CD95 expression correlated with CD71 expression. In patients with Sjogren's syndrome, the content of CD95$^+$CD4$^+$ PBL considerably increases [8]. High expression of CD95 on PBL probably indicates the activation of T lymphocyte apoptosis. These processes become more intensive with progression of tumor growth, because CD95 expression is higher at the late stage of disease. The number of HLA-DR-positive cells increases in patients with neoplasms. Similar changes were observed in ovarian cancer and blood tumors [9,11]. The absence of significant changes in CD95 expression in patients with autoimmune disorders can be explained by the fact that all patients were examined at the early stage of disease.

Hence, high expression of CD95 on PBL indicates stimulation of T lymphocyte apoptosis, which

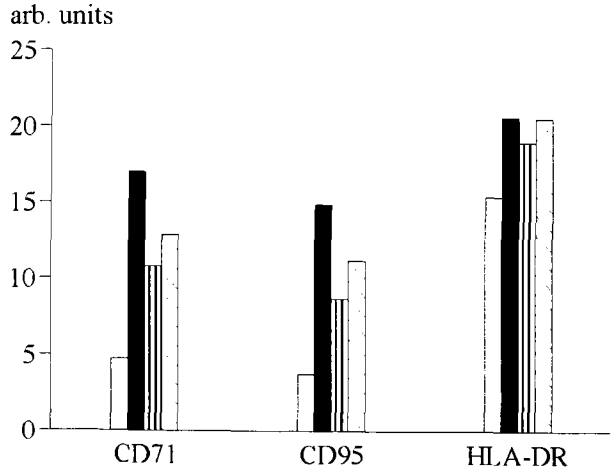

Fig. 3. Expression of activation markers in patients with tumors of various localizations: control (light bars), stomach cancer (dark bars), lung cancer (vertical shading), and breast cancer (slant shading).

impairs immunologic reactivity of the body. Expression of activation markers (CD71, CD95, and HLA-DR) in patients with neoplasms surpasses that in patients with autoimmune disorders, which suggests that tumor growth is accompanied by more severe immune disturbances. CD95 expression on peripheral blood T lymphocytes increases with progression of tumor growth.

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