Expression of the Fas/APO-1/CD95 Antigen Mediating Apoptosis in Human Leukemic Cells

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The Fas/APO-1 antigen is not detected on peripheral blood leukocytes of patients with chronic myelocytic leukemia in the chronic phase, but is expressed on blast cells in 50% of patients with chronic myelocytic leukemia in blast crisis. The expression of Fas/APO-1 correlates with that of leukocytic CD34 antigen. In patients with lymphomas, acute lymphoblastic leukemia, or acute myeloblastic leukemia some tumor cells carry Fas/APO-1 antigen.

Key Words: *Fas/APO-1/CD95 antigen; apoptosis; leukemic cells*

Cell death is an important component of the overall biological process. Recently, a Fas/APO-1/CD95 molecule mediating programmed cell death (apoptosis) was identified on the surface of lymphoid cells. This antigen is a cysteine-rich transmembrane glycoprotein with Mr 48 kD belonging to the superfamily of receptors for nerve growth factors and tumor necrosis factor. It is recognized by IPO-4, APO-1, and anti-Fas monoclonal antibodies (MAB) [1,4,13]. Human Fas/APO-1 gene is located in the 23rd chromosome. The Fas/APO-1 antigen is expressed on cortical thymocytes, various lymphoblastoid cell lines, and activated T and B cells [2,3, 12]. It was detected in some epithelia and mesenchymal cells. The binding of MAB APO-1 to this antigen induces apoptosis in apoptosis-sensitive APO-1$^+$ cells *in vitro* [6]. Presumably, Fas molecules are involved in the differentiation of cortical thymocytes. In the presence of excessive amounts of Fas/APO-1 antigen, Fas-positive thymocytes undergo apoptosis [5,14]. In thymectomized mice, anti-APO-1 MAB induces regression of human BJAB B-cell lymphoma by apoptosis [6].

In this study we examined the expression of Fas/APO-1/CD95 antigen on peripheral blood cells from patients with different forms of leukemia.

MATERIALS AND METHODS

Fas/APO-1 antigen was identified by indirect immunofluorescence assay on freshly isolated cells and cells stored in liquid nitrogen. Blood was collected from the cubital vein, and cells were isolated by precipitation in a 1% gelatin solution. All cells were washed three times, after which 5×10^6 cells were incubated with 50 μl MAB for 30 min at room temperature. The cells were then washed with phosphate buffer and incubated with 50 μl FITC-conjugated sheep antiserum against murine immunoglobulins (MedBioSpektr). They were washed again and analyzed in a FACScan flow cytofluorimeter (Beckton Dickenson). Samples in which the antigen was expressed by more than 10% cells were considered as antigen-positive. The following MAB were used: IKO (Oncology Research Center, Moscow), IPO-4 (provided by Prof. D. F. Gluzman), My10 anti-CD34 MAB

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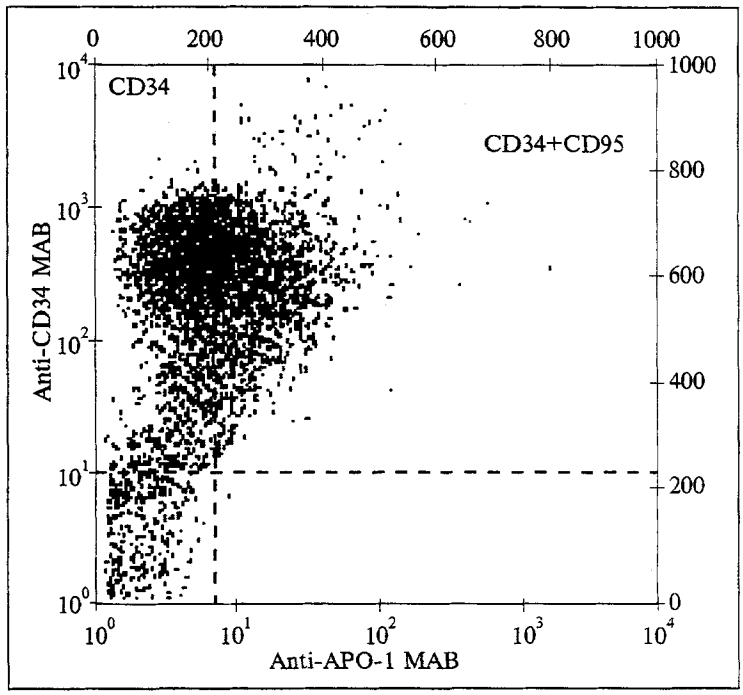

Fig. 1. Coexpression of CD34 and Fas/APO-1/CD95 antigens. Double staining technique. MAB = monoclonal antibody.

(provided by Dr. T. Trishman, USA), and APO-1 anti-Fas/APO-1 MAB (provided in the framework of the Fifth International Conference on Differentiation Antigens of Human Leukocytes).

RESULTS

The Fas/APO-1 antigen was expressed by blood cells from 14 patients with chronic myelocytic leukemia (CML) in the chronic phase, 14 patients with CML in blast crisis, and 4 patients with lymphoblastic lymphoma involving "leukemization" as well as by bone marrow cells from 13 patients with acute non-lymphoblastic leukemia (ANLL), and 8 patients with acute lymphoblastic leukemia (ALL). The results are summarized in Table 1.

The antigen was not expressed by polymorphonuclear leukocytes from patients with CML in chronic phase, but it was present on blast cells from 7 out of 14 patients with CML in the blast crisis phase. Blast cells from these 7 patients were examined 1-3 times during each aggravation of the disease and the emergence of blast cells in peripheral blood. In repeated tests, Fas antigen was expressed on cells from 5 out of 7 patients. The antigen expression was decreased in 3 cases and appeared in 2 cases.

Blast cells expressed CD34, CD33, CD13, CD14, CD15, CD11b, CD19, CD22, and HLA-DR antigens. The expression of Fas/Apo-1 strongly correlated with that of CD34 (r=0.89). There was no correlation between the expression of Fas/Apo-1 and that of other antigens.

As shown in Table 1, Fas/APO-1 antigen was expressed by bone marrow blast cells from 3 out of 13 patients with ANLL, from 1 out of 8 patients with ALL, and from 1 out of 4 patients with lymphoblastic lymphoma. The proportion of antigen-positive cells in the patients ranged from 10% to 80% and did not correlate with the proportion of blast cells.

Strong correlation between the levels of Fas/APO-1 and CD34 on blast cells from patients with CML suggests that these antigens were expressed by the same cells, which was confirmed by the double-staining technique (Fig. 1). Since CD34 is a marker of the polypotent stem cell, our findings indicate that the apoptosis-mediating Fas/APO-1 antigen is also expressed on the stem cell. Presumably, apoptosis in the Fas system represents a mechanism whereby the differentiation of the polypotent stem cell is regulated, and inactivation of this mechanism may cause the development of CML. In the present study we did not assess the degree of DNA fragmentation in Fas/APO-1-positive cells. Meanwhile, spontaneous apoptosis was detected in 0.1-16% of cells from patients with CML in the blast crisis [15].

There is evidence that apoptosis can be induced during terminal stages of the myeloid cell differentiation. Detailed investigation of apoptosis using promyelocytic HL-60 cells as a model [8,11] showed that programmed cell death is triggered by the loss of viability of differentiating leukemic myeloid cells after the removal of inducers [7]. Apoptosis is induced before the terminal stage of differentiation

TABLE 1. Expression of Fas/APO-1 Antigen on Cells from Patients with Leukemia

Form of leukemia	No. of patients	No. of positive cases	Expression rate, %
Chronic myelocytic leukemia:			
chronic phase	14	0	0
blast crisis phase	14	7	50
Acute nonlymphoblastic leukemia	13	3	23
Acute lymphoblastic leukemia	8	1	10
Lymphoblastic lymphoma	4	1	25

and is associated with the absence of the necessary growth factors [7]. Normal neutrophils undergo apoptosis during inflammation, after which they are recognized and phagocytized by macrophages [10]. Similarly to normal neutrophils, granulocytes of patients with CML in the chronic phase do not express Fas/APO-1.

In other leukemias, the occurrence of the Fas/APO-1 expression is lower than in CML during the blast crisis (Table 1). It was reported that Fas/APO-1 is expressed on cells from patients with mediastinal B-cell lymphoma originating from follicular centers and on cells from patients with hairy cell leukemia, but not on cells from patients with ALL, chronic lympholeukemia, or Burkitt's lymphoma [9]. In our study, lymphoblasts from only 1 out of 8 patients with ALL expressed Fas/APO-1. It can be suggested that the apoptosis-mediating molecule Fas/APO-1 is more frequently expressed on leukemic cells which are at the level of differentiation of normal cells and are capable of perceiving the physiological signal of programmed death (stem cell, cortical thymocytes, B cells of follicular centers) than on leukemic cells at other levels of differentiation.

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