DNA-Hydrolyzing Antibodies in Lymphoproliferative Diseases

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Natural catalytic antibodies are known to be autoantibodies and are found in patients with different autoimmune diseases. The production of catalytic autoantibodies against DNA is demonstrated for a number of lymphoproliferative diseases. The statistical analyses performed show that this production is related to the chronic proliferation of mature B lymphocytes and correlates with the corresponding autoimmune disorders.

Key Words: *natural catalytic antibodies; autoantibodies; lymphoproliferative diseases*

Catalytic antibodies are a topic of current interest with regard to their substrate specificity, structure, and catalytic mechanism. Antibodies described previously have been artificially produced on analogs of transitional states of substrates in reactions catalyzed by corresponding enzymes [8].

For the first time natural antibodies with hydrolase activity vis-a vis vasoactive intestinal peptide have been isolated from the blood of asthmatic patients [5]. These antibodies have not been found in healthy individuals, suggesting their autoimmune origin.

This prompted a search for other catalytic antibodies in the blood of patients with various autoimmune disorders. Anti-DNA antibodies are one of the widespread autoantibody types in systemic autoimmune diseases. Investigations showed the presence of DNA-hydrolyzing antibodies in blood sampled from patients with all kinds of systemic autoimmune diseases (systemic lupus erythematosus (SLE), rheumatoid arthritis, scleroderma) [6]. Such antibodies are absent in the blood of healthy donors, whereas they are most frequent in SLE patients and are used as one of the generally accepted SLE markers. The hypothesis of an autoimmune origin of catalytic autoantibodies is thus confirmed.

The idea that catalytic autoantibodies can form as antiidiotypes to enzymes [1] was illustrated by antiidiotypic antibodies against acetylcholinesterase [4].

A number of lymphoproliferative processes are associated with autoantibodies appearing in the patient's blood. For instance, chronic lymphoid leukemia, a tumor consisting of CD5-positive B lymphocytes, is characterized by a high proportion of cross-specific idiotypes and the production of autoantibodies [2,3], particularly against DNA [7]. The data set forth here concern the detection and distribution of catalytic anti-DNA autoantibodies in the blood of patients with B- and T-mature-cell lymphoproliferation (including chronic lymphoid leukemia), and T- and B-cell lymphosarcomas, as well as an analysis of the correlation between the presence of catalytic anti-DNA autoantibodies and different nosologic forms and corresponding autoimmune disorders.

MATERIALS AND METHODS

The blood was sampled by venopuncture. Heparin was added to the blood samples at 8 μg/ml (as an anticoagulant).

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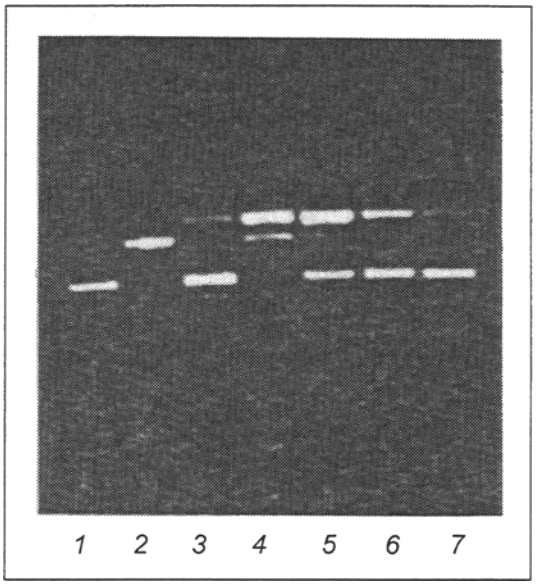

Fig. 1. Hydrolysis of supertwist plasmid pUC19 by catalytic autoantibodies against DNA (IgG fraction) isolated from the blood of patients with lymphoproliferative diseases. *1*) control (supertwist plasmid); *2*) control (linear plasmid); *3*) large-cell lymphosarcoma; *4*) lymphoma of pallial zone; *5*) chronic lymphoid leukemia; *6*) lymphocytic lymphoma; *7*) Waldenström's macroglobulinemia.

The antibody IgG-fraction was isolated according to a method described elsewhere [5,6]. Tris-HCl buffer was added in two-stages (25 and 50%) to the cell-free plasma in a final concentration of 20 mM, pH 7.5. An antibody fraction sedimented with 50% ammonium sulfate was loaded on a HiTrap PrG-Sepharose (Supelco) affine column in buffer containing 20 mM Tris-HCl, pH 7.5, and 50 mM NaCl. Bound antibodies (IgG fraction) were eluted with 100 mM glycine-HCl solution, pH 2.6. Purified antibodies underwent primary screening for DNA-hydrolyzing activity. For this, 20 μg of the antibody fraction were incubated with 1 μg of supertwist plasmid pUC19 at 37°C with 10 mM Mg^{2+} overnight. Control supertwist plasmid DNA was incubated under analogous conditions without the addition of antibodies. Plasmid DNA was deproteinized and fractionated by electrophoresis in 1% agarose gel. The activity of catalytic autoantibodies against DNA was determined by the degree of relaxation of supertwist plasmid.

Active antibody preparations were placed in buffer containing 20 mM Tris-HCl, pH 9, and fractionated on an anion-exchange column (Mono-Q, Pharmacia). Elution was performed for 40 min with an NaCl concentration gradient from 0 to 500 mM.

The catalytic anti-DNA autoantibodies-enriched fraction was concentrated with a change of buffer (20 mM Tris-HCl, pH 7.5, 50 mM NaCl). Antibody preparations were stored in 50% glycerin at -20°C.

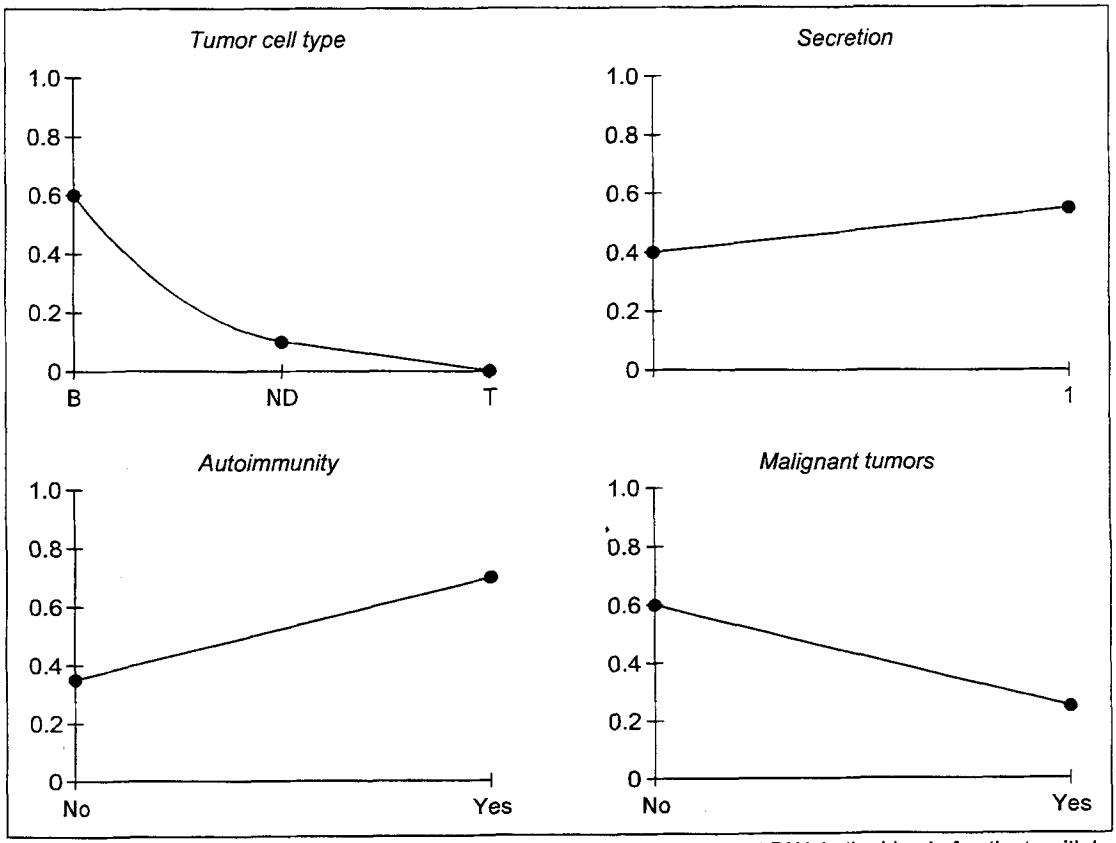

Fig. 2. Correlation between the presence of catalytic autoantibodies against DNA in the blood of patients with lymphoproliferative diseases and various diagnostic parameters. Ordinate: percent probability of existence of catalytic autoantibodies against DNA in patient blood calculated on the basis of this sample. B-cell (*B*) and T-cell (*T*) tumors. *ND* means undifferentiated types of tumors.

RESULTS

The antibody IgG fraction of blood serum from 67 patients with different lymphoproliferative diseases was studied. DNA-hydrolyzing activity was found in the purified IgG fraction in 30 patients.

Plasmid DNA hydrolysis proceeded till the formation of the circular, or, rarely, of the linear form (from 20 to 100% conversion into the curcular form, Fig. 1). This attests that the antibodies obtained produce mainly single-stranded breaks in DNA.

Correlation analysis was performed to study the presence of catalytic antibodies against DNA in the blood and various parameters characterizing the diseases. The latter were as follows: tumor cell phenotype (T- or B-cell), the degree of maturity of the lymphoproliferative process, and the presence or absence of monoclonal secretion of immunoglobulins and of autoimmune disorders.

The analysis was performed using Microsoft Excel software. The data are presented in Fig. 2.

The analysis showed that the production of catalytic antibodies correlates with the mature-cell nature of the tumor (r =33). Catalytic antibodies against DNA were found predominantly in the blood of patients with chronic lymphoid leukemia and other similar chronic lymphoproliferative diseases (lymphocytoma, lymphoplasmocytoid lymphoma, lymphoma of the pallial zone).

Although the sample of T-cell lymphoproliferation was too small to be representative (5 patients), it may be assumed that there is no catalytic activity against DNA in the case of the T-cell form of the disease.

A study of the correlation between the activity of catalytic anti-DNA autoantibodies and mono- or oligoclonal secretion of immunoglobulins testifies to the independence of these factors (r =20).

Most interesting was the analysis of the correlation between the DNA-hydrolyzing antibodies and autoimmune disorders (autoimmune hemolytic anemia, partial red-cell aplasia, various forms of dermatitis, glomerulonephritis, etc.) developing against the background of lymphoproliferative processes. This analysis revealed preferential anti-DNA autoantibody production against the background of autoimmune complications (r =40). However, there were a significant number of mature B-cell tumors accompanied by the production of catalytic autoantibodies against DNA but without autoimmune complications (12 persons).

Based on the analysis the following assumptions can be made:

1. Production of catalytic autoantibodies against DNA is found in the case of mature B-cell proliferation and is practically absent in malignant lymphatic and T-cell tumors.

2. There is evidently no correlation between the production of catalytic autoantibodies against DNA and tumor cell antibody secretion.

3. Autoimmune disorders accompanying lymphoproliferative diseases increase the likelihood of the presence of catalytic autoantibodies against DNA. Nevertheless, the existence of severe autoimmune disorders is not a necessary condition for the production of catalytic autoantibodies against DNA.

The presence of catalytic autoantibodies against DNA may be used in future as a tool in the differential diagnostics of various nosologic forms of lymphoproliferative diseases.

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