COMPARATIVE STUDY OF Ca,Mg-DEPENDENT ENDONUCLEASE IN CELL NUCLEI BY THE USE OF MONOCLONAL ANTIBODIES

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The enzyme Ca,Mg-dependent endonuclease is found in cell nuclei of organs and tissues of many species of animals [7]. The distribution of this enzyme raises the question of the conservatism, i.e., the similarity of its structure in animals of different species. Variations in the activity and, perhaps, the properties of Ca,Mg-dependent endonucleases in different organs [3] likewise suggests that the existence of isozymes may be one explanation of this phenomenon.

An approach to the solution of these problems may be provided by immunochemical methods of analysis of Ca,Mg-dependent endonuclease in the cell nuclei of certain organs and tissues of animals and man by the aid of monoclonal antibodies (MCAB).

Lymphocytes from various human, murine, and bovine lymphoid tissues and also rat hepatocytes, as cells possessing marked Ca,Mg-dependent enconuclease activity, and which have been used to obtain purified preparations of the enzyme, both by the present writers and in other laboratories [9, 10], were chosen as the test object.

EXPERIMENTAL METHODS

Cell nuclei were obtained from lymphocytes and hepatocytes by a modified method [2]. The nuclei were extracted with the aid of 0.3 M KCl and 0.5% Triton X-100 solution for 1 h in the cold with continuous mixing.

We used MCAB of two independent lines of hybridomas, producing antibodies belonging to the IgG class, and which did not inactivate the endo-DNases in the composition of the antigen–antibody complex [2]. The lines of hybridomas used were designated N and S. The source of the antibodies was ascites fluid from BALB/c mice.

Extracts were tested in 96-well panels by the following method. Antibodies fixed on protein A (Pharmacia, Sweden) were incubated with extracts of cell nuclei overnight at 0-4°C (5-10 µl of antibodies to 10 µg protein). After washing four times with 10 mM Tris, pH 8.0, pBR322 plasmid DNA was added to the complex thus formed in an amount of 0.5 µg and the mixture was incubated in solution containing 10 mM Tris, 5 mM $MgCl_2$, and 2 mM $CaCl_2$ (IB) for 2 h at 37°C.

Degradation products of the plasmid DNA were separated in horizontal blocks of 1.2% argarose gels, and the negatives were photographed and subjected to densitometry as described previously [3].

Binding of monoclonal antibodies with endo-DNases of the extracts was estimated by determining the loss of substrate (the superhelical form of the plasmid). The value A, the difference between the quantity of superhelical DNA in the control and experimental samples, expressed as a percentage of the content of superhelical DNA in the control samples, was used as the measure of activity of endonucleases adsorbed on the antibodies.

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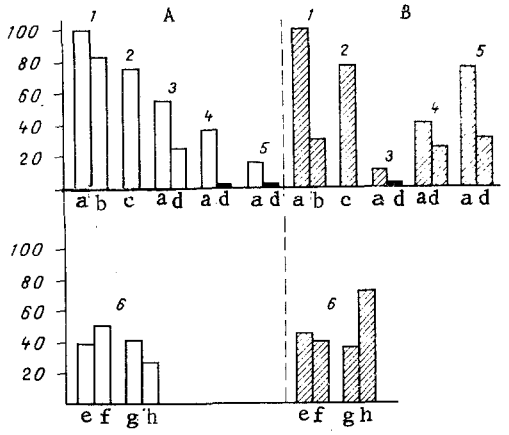

Fig. 1. Ca,Mg-dependent endonuclease actvity in cell nuclei of various animals and man, fixed on N MCAB (A) and on S MCAB (B). Ordinate, enzyme activity (in %). 1) Human; 2) rat; 3) BALB/c; 4) C57B1/6; 5) CBA; 6) bovine. a) Spleen, b) peripheral blood lymphocytes; c) liver; d) thymus; e) spleen (normal); f) spleen (leukemia); g) lymphocytes (normal); h) lymphocytes (leukemia).

EXPERIMENTAL RESULTS

Data on binding of N and S MCAB with Ca,Mg-dependent endonuclease contained in extracts of cell nuclei of human and bovine peripheral blood lymphocytes, CBA, C57B1/6, and BALB/c mouse spleen and thymus, and rat hepatocytes, are given in Fig. 1. The presence of cross reactions was discovered, evidence of the structural similarity of the antigenic determinants of Ca,Mg-dependent endonuclease in the cell nuclei of these two evolutionarily widely distant species of animals.

Meanwhile, comparison of cross reactions of N MCAB and S MCAB revealed definite differences. The N MCAB reacted with thymus extracts from BALB/c mice but did not react with thymus extracts from C57B1/6 and CBA mice. The opposite picture was observed with respect to S antibodies: reactions were found with thymus extracts from C57B1/6 and CBA mice, but no binding was found with thymus extracts of BALB/c mice. The character of response thus observed is evidence that antibodies of the N and S lines evidently specific for different antigenic determinants of Ca,Mg-dependent endonuclease.

Comparison of interorgan differences in binding of N MCAB and S MCAB with enzyme from the spleen and thymus showed that the degree of binding was similar for both lines. Within each line of mice (CBA, BALB/c, C57BL/6), N MCAB and S MCAB bound the enzyme from splenic cell nuclei by a greater degree than enzyme from cell nuclei of the thymus. Since these antibodies were obtained to Ca,Mg-dependent endonuclease of human splenic lymphocytes, the above reactions could be evidence of the existence of tissue-specific isozymes of the enzyme. The marked degree of interlinear differences in binding is noteworthy, for it suggests that these antibodies may be used as markers of the isozyme spectrum of the Ca,Mg-dependent endonuclease of animals of these lines. In connection with the possibility of detection of isozymes it was decided to study the degree of binding of these antibodies with enzyme from lymphocyte nuclei under normal and pathological conditions.

Previously the writers found changes in Ca,Mg-dependent endonuclease activity of human and bovine peripheral blood lymphocytes in cases of chronic lymphatic leukemia (CLL) [1, 4].

Data on binding of N and S MCAB with extracts of cell nuclei from bovine splenic and peripheral blood lymphocytes from normal animals and animals with CLL are given in Fig. 1. It will be clear that both lines of antibodies studied bind Ca,Mg-dependent endonuclease and are contained in extracts of bovine peripheral blood and splenic lymphocytes. The degree of binding in CLL differs from that observed with normal animals; a difference in the character of response is observed, moreover, for N MCAB and S MCAB.

N MCAB bind enzyme from splenic lymphocytes of animals with CLL by a greater degree than normal animals: enzyme from peripheral blood lymphocytes binds to a lesser degree with N MCAB from animals with CLL than from normal animals, whereas S MCAB binds to a greater degree.

In our opinion this is evidence in support of the view that Ca,Mg-dependent endonuclease isozymes are present in cell nuclei of lymphocytes recognized by the antibodies which were used, and that the relative proportions of these isozymes vary depending on the functional state of the lymphocytes and, in partiuclar, in CLL.

When these results are evaluated the following point must be noted. The method of detection of cross reactions of MCAB with Ca,Mg-dependent endonuclease (endonucleases) contained in extracts of lymphocyte nuclei is capable of estimating the presence or absence of binding of the enzyme with antibodies only qualitatively, and of revealing a tendency in the change of cross reactions with cells in different states and, in particular, in CLL. However, even a qualitative assessment of the degree of binding of MCAB of two independent lines with endonucleases from cell nuclei and the change in activity of enzymes of different origin (tissue, organ, and species), may be of essential importance. First, the data demonstrate the presence of similar antigenic determinants in Ca,Mg-dependent endonuclease from cell nuclei of animals of different species and of man. This suggests the structural similarity of the enzyme in all these species studied and also that the MCAB obtained as described above can be used to isolate and characterize Ca,Mg-dependent endonuclease not only from human splenic lymphocytes but also from other test objects.

Meanwhile the question remains of the number of corresponding determinants and their possible presence in other nuclear endonucleases than the Ca,Mg-dependent type. The most realistic basis for this possibility, in our view, is the existence of protein precursors, which are the same for several endonucleases differing in the character of their activity [7]. It will be evident that by the use of these MCAB it will be possible to obtain data in this interesting and important field of research.

Second, comparison of interaction between antibodies and enzymes from different organs and tissues, and also under normal and pathological (CLL) conditions, indicates sufficiently definitely the presence of tissue-specific isozymes of this enzyme. Similar data, also obtained by the use of MCAB, have been described for uracil-DNA-glyoxylase from cell nuclei of the human placenta [5].

LITERATURE CITED

1. A. K. Gozdarov, I. V. Chupyrina, G. F. Koromyslov, et al., Abstracts of Proceedings of the 5th All-Union Biochemical Congress [in Russian], Vol. 3, Moscow (1986), p. 312.
2. N. N. Khodarev, I. I. Votrin, A. G. Basnak'yan, and S. S. Debov, Biokhimiya, 44, 222 (1978).
3. N. N. Khodarev, I. A. Sokolova, and I. I. Votrin, Byull. Éksp. Biol. Med., No. 12, 40 (1982).
4. N. N. Khodarev, T. N. Ivanova, and I. I. Votrin, Dokl. Akad. Nauk SSSR, 268, No. 4, 1000 (1983).
5. P. Arenas and M. Sirover, Proc. Natl. Acad. Sci. USA, 80, 5822 (1983).
6. L. A. Burgoyne and D. R. Havish, Cell Nucl., 4, A48 (1978).
7. T. Y. Chow and M. J. Fraser, Can. J. Biochem., 57, 889 (1979).
8. T. Hashida, Y. Tanaka, N. Matsunami, et al., J. Biol. Chem., 257, 13,114 (1982).
9. W. H. Strätlung, J. Biol. Chem., 259, 5893 (1984).