This investigation thus showed that ICO-11 MAB block the response of patients' lymphocytes to tumor tissue extract in the LAI test in vitro, but have no effect under these circumstances on the response of patients' lymphocytes to normal tissue extract, or on spontaneous adhesion of normal human lymphocytes. Consequently, the results indicate that ICO-11 MAB block the binding of T cells, producing the LAI factor, with tumor-associated antigen. The ability of ICO-11 MAB to block activity of different subpopulations of immunocompetent cells was described previously in other immunologic tests. ICO-11 MAB blocked activity of natural killer cells of healthy blood donors, aimed against K-562 and Molt-4-4 target cells, and also depressed activity of cytotoxic T lymphocytes, induced in a 7-day mixed lymphocyte culture [2]. It has also been shown that ICO-11 MAB have a dose-dependent inhibitory influence on the lymphocyte blast transformation response to phytohemagglutinin and that they inhibited E_H-rosette formation in healthy blood donors [2].

Determination of the molecular weight of the antigens by immunoblotting showed that ICO-11 MAB revealed a polypeptide with mol. wt. of 180 kilodaltons from lysates of human blood mononuclear cells and thymocytes [2]. The spectrum of cells expressing the antigen revealed by ICO-11 MAB, the blocking of natural killer-cell activity [1] and of the lymphocyte blast transformation reaction, and the molecular weight of the antigen revealed indicate that ICO-11 MAB are aimed against the α-chain of a functionally-associated lymphocyte antigen [2]. Blocking of the reaction of ICO-11 MAB in the LAI test thus indicates a possible role of the α-chain of the functionally associated antigen in binding of the tumor-associated antigen by T cells in the phase of induction of the lymphocyte adhesion inhibition reaction.

LITERATURE CITED

1. A. Yu. Baryshnikov, L. P. Trubcheninova, E. V. Savel'eva, et al., Éksp. Onkol., No. 3, 34 (1985).
2. A. Yu. Baryshnikov, I. V. Dubinkin, E. V. Savel'eva, et al., Immunologiya, No. 5 (1987).
3. D. D. Kharkevich, D. Z. Tabagari, and A. Yu. Savinov, Vopr. Onkol., No. 7, 17 (1985).
4. D. D. Kharkevich, Yu. A. Sablikova, E. B. Polevaya, et al., Lab. Delo, No. 6, 374 (1985).
5. D. D. Kharkevich, E. B. Polevaya, N. A. Derevnina, et al., Éksp. Onkol., No. 6, 69 (1985).
6. A. Boyum, Scand. J. Immunol., 5, Suppl. 5, 9 (1986).
7. J. E. K. Hildreth and J. T. August, J. Immunol., 135, No. 5, 3272 (1985).
8. J. D. Keizer, I. Borst, C. J. Figdor, et al., Eur. J. Immunol., 15, No. 11, 1142 (1985).
9. A. M. Krensky, F. Sanchez-Madrid, E. Robbins, et al., J. Immunol., 131, No. 2, 611 (1983).

DYNAMICS OF ENDOGENOUS DEOXYRIBONUCLEASE ACTIVITY OF MOUSE THYMUS AND SPLENIC LYMPHOCYTE NUCLEI DURING THE IMMUNE RESPONSE

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Cell nuclei of animals and man contain various nucleases, among them endogeneous deoxyribonucleases (endo-DNases) [12]. The functions of these enzymes in higher eukaryotes have not yet been explained. However, data obtained on prokaryotes show that endonucleases take part in various genetic processes, mainly DNA recombination and repair. Most probably the nucleases of the cell nucleus must also perform similar functions. For endonucleases of one type, associated with chromatin, namely apurine/apyrimidine endonucleases, their participation

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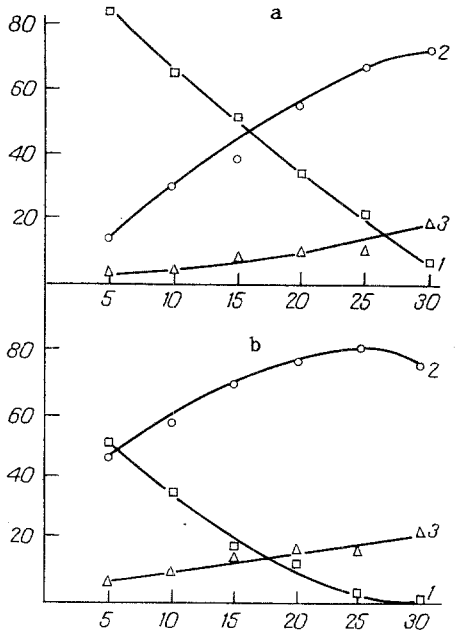

Fig. 1

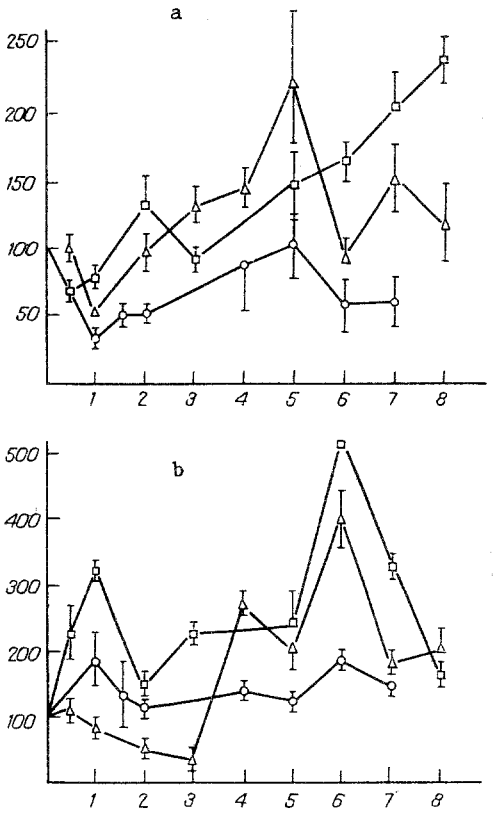

Fig. 2

Fig. 1. Kinetics of breakdown of supercoiled pBR 322 DNA under the influence of endo-DNases from thymus (a) and splenic (b) cell nuclei. Abscissa, time (in min); ordinate, DNA content (in %). 1-3) Supercoiled, open ring, and linear DNA.

Fig. 2. Dynamics of activity of endo-DNases from splenic (a) and thymus (b) cell nuclei during the immune response. Abscissa, time after immunization (in days); ordinate, endo-DNase activity (in %). Results of three experimental series are given: in each series activity of enzymes of the animals of the control group was taken as 100%.

in excision repair of DNA is determined by the substrate specificity of these enzymes and has been confirmed by experiments in vitro [9]. The functional role of the other endonucleases of the cell nucleus remains unexplained, although the participation of these enzymes in genome function seems sufficiently well founded.

Our understanding of the function of endo-DNases of cell nuclei can be improved by the study of changes in their activity and spectrum in various biological processes. Some observations have shown that these changes take place during differentiation, malignant transformation, and hormonal induction. An interesting model with which to study the functional role of the DNases of cell nuclei is activation and differentiation of lymphocytes during the immune response. Processes of reorganization of the genome, which lie at the basis of antigen-dependent and antigen-independent stages of lymphocyte differentiation have been proved, and they suggest that enzymes, either endo-DNases themselves or possessing endo-DNase activity, play a role in them [7].

The aim of this investigation was to study activity of chromatin-associated endo-DNases of lymphocytes during formation of the primary immune response in vivo.

EXPERIMENTAL METHOD

Male CBA mice weighing 20-25 g were used. The mice were immunized with sheep's red blood cells (SRBC), injected intraperitoneally in a dose of $5 \cdot 10^7$ cells per animal. At different times after immunization the mice were killed by cervical dislocation. Cell nuclei were isolated from thymus and splenic lymphocytes as described previously [3], except that the concentration of dense sucrose was 2 M and the pH of all solutions was 8.5.

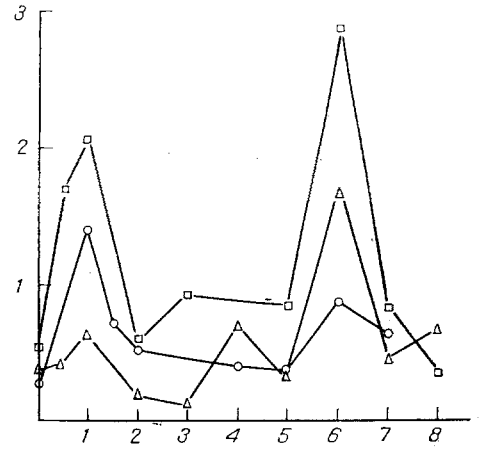

Fig. 3. Changes in ratio between activities of endo-DNases from thymus and splenic cell nuclei during the immune response. Results of three experimental series shown. Abscissa, time after immunization (in days).

The isolated cell nuclei were extracted with 0.3 M KCl solution in 0.075% Triton X-100 (pH 7.4) at 0-4°C for 30 min. The extracts were centrifuged at 12,000g and activity of the endo-DNases present in them was determined as follows.

All incubations were done in buffer solution containing 10 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$, 1 mM $CaCl_2$, and 5 mM mercaptoethanol. The incubation samples contained, in a total volume of 50 µl: 0.25 µl protein of extracts (in a volume of 0.5-1 µl) and 0.5 µg DNA of plasmid pBR322 (in a volume of 0.5-1 µl in 10 mM Tris-HCl, pH 7.4). Incubation was carried out at 37°C for between 10 and 30 min, and was stopped by cooling the samples to 0°C. The samples were treated with 10 µl of 50% glycerol with bromphenol blue. DNA was fractionated by electrophoresis in horizontal blocks of 1.2% agarose (Bio-Rad, USA) in Tris-acetate buffer, under a voltage of 10 V/cm for 3 h. Gels were stained with ethidium bromide, photographed, and DNA determined quantitatively as described previously [3].

The unit of endo-DNase activity was taken to be that quantity of it which led to accumulation of 1 µg of the linear form of plasmid pBR 322 DNA during incubation for 10 min at 37°C in a buffer solution containing 5 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM mercaptoethanol, and 10 mM Tris (pH 7.4). The plasmid DNA was isolated as described previously [4] and strain Escherichia coli HB 101, maintaining stable replication of the plasmid in monomer form, was used as the source. Titers of antibodies to SRBC were determined by the direct hemagglutination test [2].

EXPERIMENTAL RESULTS

Extraction of the cell nuclei with solutions of salts (usually sodium chloride) with ionic strength of 0.1-0.4 M led to solubilization of most of the endo-DNases active at neutral pH values in the presence of divalent cations, and associated with chromatin [8]. An increase of ionic strength led to activation of the nucleases. To extract the lymphocyte nuclei, we used KCl in the presence of the nonpolar detergent Triton X-100. When their final concentration in the extracting solution were 0.3 M and 0.075%, respectively, affective solubilization of the lymphocyte endo-DNases, associated with chromatin, took place. Maximal activity of the resulting extracts was exhibited in the presence of Mg^{2+} and Ca^{2+}. Addition of EGTA in the presence of Mg^{2+} inhibited activity by more than 90%.

The main activity of solubilized endo-DNases is thus connected with Ca/Mg-dependent endonuclease. Similar data are to be found in the literature also [10].

The kinetics of degradation of plasmid pBR 322 DNA by extracts of lymphocyte nuclei from mouse spleen and thymus is shown in Fig. 1. In the presence of Ca and Mg, the covalently closed plasmid DNA was cleft with the formation of open-ring and linear DNA. Measurement of endo-DNases on the basis of their action on supercoiled circular DNA is a sensitive and precise method of detection of these enzymes, and accordingly it was used in the present investigation. The measure of activity was taken to be accumulation of linear DNA and not reduction of the quantity of substrate supercoiled DNA, for the extracts which we obtained might have contained enzymes interacting with supercoiled DNA, notably DNA topoisomerases [5].

It will be noted that under the experimental conditions used accumulation of the linear form of DNA under the influence of endo-DNases from splenocyte nuclei took place more rapidly

than when the enzymes from thymocyte nuclei were used, thus demonstrating the greater activity of endo-DNases from splenocyte nuclei.

After immunization of the mice the titer of antibodies to SRBC in the blood ($\log_2$; M ± m) was 0.3 ± 0.4 after 12 h, 1.0 ± 0.7 after 24 h, 4.5 ± 0.3 after 2 days, 5.8 ± 1.0 after 3 days, 8.2 ± 0.7 after 4 days, 8.3 ± 0.8 after 6 days, and 8.5 ± 0.6 after 7 days.

In the course of the immune response marked changes were observed in endo-DNase activity from nuclei of both splenocytes and thymocytes (Fig. 2). The data in Fig. 2 are shown separately for three experimental series, in each of which 25-35 animals were used. It will be clear from Fig. 2 that marked changes in endo-DNase activity in splenic and thymus cell nuclei were observed 12 h after immunization. In the spleen, during the 1st day, activity of the enzymes fell, but in the thymus, on the other hand, it rose, i.e., reciprocal changes were observed in endo-DNase activity in cell nuclei of splenocytes and thymocytes. After the 1st day endo-DNase activity in splenocyte nuclei as a whole began to increase during development of the immune response; in two of the three series activity reached a peak on the 5th day after immunization. In the thymus, in the period from the 2nd to the end of the 3rd day after immunization, activity fell or reverted to the control level. On the 6th day after immunization a marked increase in activity was observed.

As was pointed out above, endo-DNase activity in cell nuclei of splenic lymphocytes of the immunized animals was 2-4 times greater than that from thymocyte nuclei. Data showing changes in the ratio of activity of the thymus enzymes to activity of the splenic enzymes in the course of the immune response are given in Fig. 3. It will be clear from Fig. 3 that after immunization two clearly marked periods of reversal of activity were observed, i.e., activity of endo-DNases from thymocyte nuclei exceeded that of the enzyme from splenic nuclei by 2-3 times. These periods corresponded to the 1st and 6th days after immunization.

Immunization of the animals with thymus-dependent antigen (SRBC) thus led to marked changes in endo-DNase activity in the cell nuclei of splenic and thymus lymphocytes. These changes began in the early (inductive) phase of the immune response and were characterized by a definite phasic nature. The changes in endo-DNase activity discovered in the cell nuclei evidently characterize changes in the functional properties and structure of the lymphocyte populations of the spleen and thymus. In this respect they correlate well with known biochemical and cytologic data on the phasic character of proliferative and antibody-producing activity of lymphocytes in the course of the immune response [1]. Possibly endo-DNase activity of lymphocyte nuclei can be usefully regarded as an enzymic marker of immune processes.

Analysis of the mechanisms of specific and nonspecific lymphocyte stimulation shows that the primary changes in this case take place at the genome level and are connected with changes in the spectrum of expressed genes, the conformation of chromatin, and the character of methylation of DNA (as a rule, with lowering of the methylation level [7, 13]). In the course of the immune response, not only immunocompetent cells, but also a considerable proportion of the lymphocyte population become involved in proliferation and differentiation processes, so that detection of the corresponding biochemical changes is possible not only in vitro, but also in vivo. In particular, it has been shown that T killer cells with spliced C_μ-genes appear in the spleen [6]. Data in the literature are evidence on the whole that induction of the immune response leads to increased functional activity of the lymphocyte genome with respect to transcription, replication, and recombination. The corresponding processes are accompanied, or largely determined by changes in the chromatin. Endogenous nuclear endonucleases, primarily Ca/Mg-dependent, are distributed nonrandomly in the chromatin and they may be associated with functionally active regions of the genome [3, 11]; specifically cleaving DNA, they may lead under these circumstances to local and selective changes in chromatin conformation [14]. In addition, the writers showed previously that excessively methylated regions of DNA in the composition of the chromatin are distinguished by their hypersensitivity to endogenous Ca/Mg-dependent cell nuclear endonuclease [4], so that a role for this enzyme in demethylation of specific regions of the genome can be postulated [3].

It accordingly seems reasonable to suggest that the changes which we discovered in activity of cell nuclear endo-DNases are linked with a change in the functional status of the lymphocytes during the immune response, possibly on account of a change, under the influence of these enzymes, in the level of DNA methylation and in the conformation of chromatin regions activation of which is essential for the initiation of differentiation.

LITERATURE CITED

1. H. Friemel (ed.), Immunologic Methods [Russian translation], Moscow (1979), p. 109.
2. V. A. Trufakin, Immunomorphological Aspects of Autoimmune Processes [in Russian], Moscow (1983).
3. N. N. Khodarev and I. I. Votrin, Byull. Éksp. Biol. Med., No. 12, 37 (1982).
4. D. B. Clewell and D. R. Helinski, J. Bacteriol., 110, 1135 (1972).
5. A. Forster, M. Hobart, H. Hengurtner, and T. H. Rabbits, Nature, 286, 897 (1980).
6. T. Hsieh and D. Brutlag, Cell, 21, 115 (1980).
7. M. E. Koshland, J. Immunol., 131, 1 (1983).
8. G. O. Machrey and J. Bonner, Biochemistry, 20, 5466 (1981).
9. D. W. Mossbauch and S. Linn, J. Biol. Chem., 258, 108 (1983).
10. M. Nakamura, Y. Sakaki, N. Watanabe, and J. Takagi, J. Biochem. (Tokyo), 89, 143 (1981).
11. J. Paul and J. D. Duerksen, Biochem. Biophys. Res. Commun., 68, 97 (1976).
12. H. Sierakowska and D. Shugar, Prog. Nucleic Acid Res. Mol. Biol., 20, 59 (1977).
13. U. Storb, B. Arp, and R. Wilson, Nature, 294, 90 (1981).
14. J. N. Vanderbilt, K. S. Bloom, and J. N. Anderson, J. Biol. Chem., 257, 13,009-13,017 (1982).