3. A. N. Mayanskii and M. E. Viksman, Byull. Éksp. Biol. Med., No. 2, 214 (1980).
4. A. N. Mayanskii and D. N. Mayanskii, Essays on the Neutrophil and Macrophage [in Russian], Novosibirsk (1983).
5. D. N. Mayanskii and N. P. Voronina, Byull. Éksp. Biol. Med., No. 4, 408 (1984).
6. D. N. Mayanskii, N. P. Voronina, and A.Yu. Voronin, Byull. Éksp. Biol. Med., No. 9, 324 (1985).
7. A. A. Pokrovskii, A. I. Archakov, and T. V. Alenicheva, Tsitologiya, No. 11, 1467 (1968).
8. V. A. Razvorotnev, Immunologiya, No. 3, 35 (1980).
9. M. Kh. Tnimov, A. V. Semenyuk, G. I. Nepomnyashchikh, et al., Byull. Éksp. Biol. Med., No. 3, 365 (1985).
10. B. M. Altura, Adv. Microcirculat., 9, 252 (1980).
11. F. Keller, M. T. Wild, and A. Kirn, Abstracts of the 3rd International Kupffer Cell Symposium, Amsterdam (1985), p. 60.
12. O. H. Lowry, N. J.Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
13. Q. Myrvik, E. S. Leake, and B. Farris, J. Immunol., 86, 128 (1961).
14. J. P. Nolan, Yale J. Biol. Med., 52, 127 (1979).
15. T. M. Saba, Surv. Immunol. Res., 2, 262 (1983).

PRODUCTION OF MONOCLONAL ANTIBODIES TO MAMMALIAN CELL NUCLEAR DNA BY IMMUNIZATION WITH STREPTOCOCCAL GROUP A POLYSACCHARIDE CONJUGATED WITH SYNTHETIC POLYELECTROLYTES

É. I. Drobyshevskaya, E. Yu. Pyt'eva,
E. V. Ryzhikova, N. A. Borodiyuk, I. M. Lyampert,
A. V. Nekrasov, and R. M. Khaitov

UDC 615.373.03:57.083.33/.012

KEY WORDS: monoclonal antibodies; DNA; streptococcal group A polysaccharide; synthetic polyelectrolytes

Previous investigations showed the presence of antibodies reacting with DNA or nuclei of mammalian cells in the sera of animals immunized with various microorganisms, including group A streptococcus. It was suggested that this depends on the presence of cross-reaction between mammalian and microbial DNA [10]. It was shown at the same time that autoantibodies to DNA can be obtained by injection of a lipopolysaccharide (LPS), capable of inducing polyclonal activation of B-cells, into animals [8]. There is some evidence that antibodies to DNA are highly varied [12]. It has been shown by the use of monoclonal antibodies (McAb) that some of them can react specifically with individual antigenic determinants of DNA [15], and in other cases cross reactions have been found between DNA and cardiolipin, tubulin, and thyroglobulin [6, 7]. Hence the importance of obtaining McAb to antigens of cell nuclei by immunization with various microbial antigens.

It was shown previously that the polysaccharide (PS) from group A streptococcus (A-PS) contains a cross-reacting determinant, antibodies to which are autoantibodies and react with epithelium of thymus and skin [11]. It has been suggested that injury to the epithelium of the thymus may be the cause of immunoregulatory disturbances leading to the development of an autoimmune process [11].

A-PS is known to be a nonimmunogenic hapten. It was shown previously that conjugates of haptens with synthetic polyelectrolytes (PEL) induce marked production of hapten-specific antibodies [3, 4]. As a result of immunization with A-PS, conjugated with synthetic PEL, an immune response was obtained to A-PS, and McAb cross-reacting with the epithelium of the stratum basale of the skin and thymus were isolated [2]. By long-term immunization with the

N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR. Institute of Immunology, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR P. A. Vershilova.) Translated from Byulleten Éksperimental'noi Biologiya i Meditsiny, Vol. 104, No. 8, pp. 210-212, August, 1987. Original article submitted June 20, 1986.

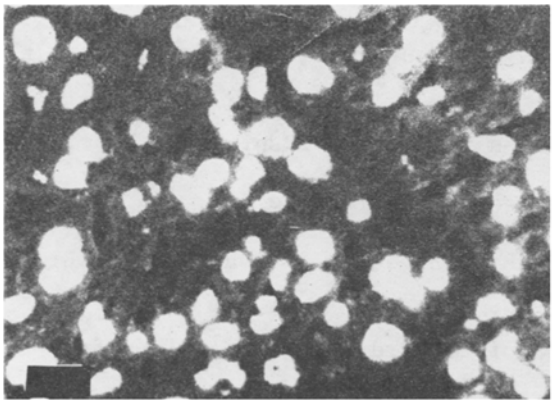

Fig. 1

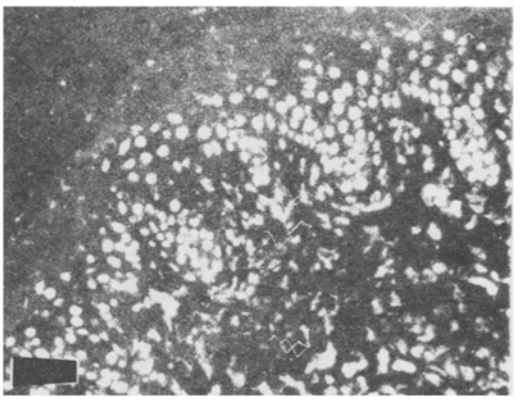

Fig. 2

Fig. 1. Reaction of McAb of clone B1/2 with cell nuclei on liver section from BALB/c mouse. Here and in Figs. 2 and 3: IIFM with antibodies to fluorescein-labeled mouse immunoglobulins (1:16). Magnification: objective 40, ocular, homal 3.

Fig. 2. Reaction of McAb of clone B1/2 with cell nuclei from epidermis and dermis in section of human embryonic skin.

conjugate, besides antibodies reacting with epithelium, autoantibodies reacting with human and animal cell nuclei also were found. Antibodies of this kind are not found if PEL alone are injected into mice [5].

The aim of this investigation was to obtain McAb reacting with cell nuclei from various human and animal tissues by immunization with A-PS conjugated with synthetic PEL.

EXPERIMENTAL METHOD

BALB/c mice weighing 16-18 g were immunized with purified A-PS conjugated with synthetic PEL [5]. The quantity of PS was determined by Dische's rhamnose method. Immunization was carried out by four intraperitoneal injections at intervals of 7 days. The dose of PS was 20-50 μg. A conjugate containing 50 μg of PS was injected into the mice 3 days before hybridization. For comparison, long-term immunization of mice with group A streptococcus (strain J-17A4), treated with pepsin [11], was carried out. Immunization proceeded by the same scheme (number of microbial cells containing from 20 to 50 μg of PS) in two cycles, with an interval of 3 months between them. Hybridomas were obtained [9], and monoclones and ascites fluids containing McAb prepared as described previously [1]. Plasma cytoma NP cells ($2.6 \cdot 10^7$) and splenocytes of mice immunized with the conjugate of A-PS and PEL ($2 \cdot 10^8$) together with 50% polyethylene-glycol with mol. wt. of 4 kilodaltons ("Serva," West Germany), were used for fusion. In the control experimental splenocytes of an unimmunized BALB/c mouse were fused with the plasmacytoma cells. The clones were screened and the McAb and whole sera tested by the indirect immunofluorescence method (IIFM) as described previously [11], with fluorescein-labeled antibodies to mouse immunoglobulins. The IIFM tests were carried out on frozen sections through the liver of BALB/c mice, human liver, and human embryonic skin from the region of a joint, and on a monolayer culture of human fibroblasts (cultures of fibroblasts were generously provided by A. F. Panasyuk, Institute of Rheumatology, Academy of Medical Sciences of the USSR). The reactions were read with an ML-2 microscope with 40× objective. A homal 3 ocular was used for photography. Reactions with nuclei were inhibited by the use of purified A-PS in a dose of 2 mg to 0.2 ml or 100 mg of pepsin-treated streptococcus to 1 ml of culture medium or hybridomas containing McAb. Inhibition also was carried out with native and denatured calf thymus DNA (from BDH, England). After addition of 2 mg DNA to 0.2 ml of supernatant containing McAb, the sample was incubated for 2 h at 37°C and then for 18 h at 4°C. The McAb contained in ascites fluid or culture medium of the clones, and also whole sera, were analyzed by enzyme immunoassay (EIA) [14] on a pepsin-treated culture of streptococcus [1] and with native and denatured DNA (10 μg/ml). Paraphenyleneiamine was used as the substrate. McAb were tested in Ouchterlony's immunodiffusion test with sera to mouse IgG and IgM (Miles Laboratories).

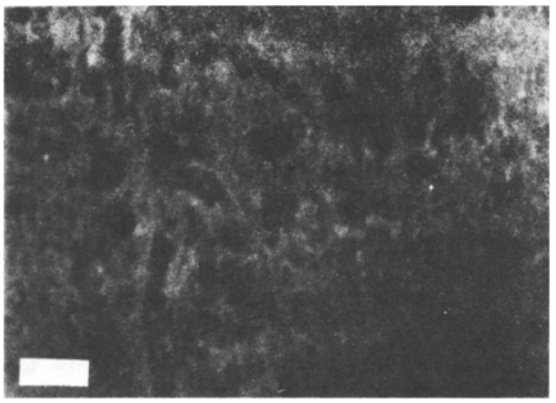

Fig. 3. Inhibition of reaction of B1/2 McAb with cell nuclei by double-helical DNA on liver section from BALB/c mouse.

EXPERIMENTAL RESULTS

Investigation of seven mouse sera by the IIFM after long-term immunization with a pepsin-treated culture of group A streptococcus revealed antibodies reacting with liver cell nuclei in all these animals, and also in 23 of 30 sera obtained by immunization of mice with the conjugate of A-PS with PEL. Homogeneous fluorescence of the cell nuclei was observed with the sera of most animals, although in a few cases perinuclear fluorescence also was found. None of 16 sera of unimmunized mice reacted with nuclei. All sera were tested in dilutions of 1:2 to 1:20.

During screening of 47 supernatants of hybrid clones by the IIFM a reaction with tissues was found in seven cases: five clones reacted with the stratum basale of the epidermis of the human skin and two with cell nuclei. Clones B1/2 and A5/2, whose McAb reacted with cell nuclei, were selected for detailed study. Cloning of these clones by the limiting dilution method showed that in 66% of cases B1/2 monoclones and in 70% of cases A5/2 monoclones continued to synthesize antibodies reacting with nuclei. Tests on 44 supernatants of repeatedly recloned monoclones containing McAb on tissue sections of liver and of human embryonic skin revealed homogeneous fluorescence of the nuclei (Figs. 1 and 2). The character of the reactions on a monolayer culture of human fibroblasts (six supernatants containing McAb were tested) was similar. The McAb were autoantibodies, because they reacted in a syngeneic system to tissues of BALB/c mice. Tests on ascites fluids showed that both monoclones (B1/2 and A5/2) produced antibodies which reacted to the test tissues in an optimal dilution of 1:320 (no further dilutions of the ascites fluids were tested).

The results of inhibition are evidence that the McAb were evidently active against double-helical DNA, for complete inhibition of the reaction was observed with native DNA (Fig. 3). Denatured DNA did not inhibit the reaction with nuclei. Meanwhile, tests of McAb by EIA revealed a reaction with native and denatured DNA, although the antibody titers in the reaction with native DNA were considerably higher (to 1:25,000). McAb reacting with nuclei were shown not to react when tested in EIA with a pepsin-treated culture of group A streptococcus, containing A-PS on the surface. When five monoclonal antibodies reacting with other tissue substances, and obtained in this experiment, were tested no reactions with nuclei were found. Precipitating McAb A3/2, which reacted with A- and L-PS of group A streptococci and were obtained by immunization of mice with pepsin-treated group A streptococcus [1], likewise did not react with cell nuclei. Inhibition of the reaction of McAb B1/2 and A5/2 with nuclei was not found with the aid of A-PS or during adsorption with a pepsin-treated culture of streptococcus. The precipitation test showed that McAb produced by these monoclones reacted with anti-IgM-serum.

In a control experiment with fusion of splenocytes of an unimmunized BALB/c mouse with plasmacytoma cells, the supernatants of 30 clones were screened. Antibodies reacting with nuclei could not be isolated. This may be due to the limited number of screened supernatants. In some cases McAb to DNA were obtained by fusion not only of lymphocytes of mice of autoimmune lines, but also splenocytes of normal unimmunized animals with myeloma cells [12].

The character of fluorescence of the cell nuclei was studied with McAb to nuclear DNA on various tissues and transplantable cell lines [13]. The authors cited showed that the reaction detected in the IIFM on the tissues depended on the antibody concentration. In our own investigations no such relationship was found, for the cell nuclei fluoresced homogeneously with different concentrations of McAb.

By immunization with A-PS conjugated with synthetic PEL, McAb to nuclear DNA, which were autoantibodies of the IgM class, were thus obtained. It will be noted that antibodies reacting with nuclei could not be detected either in sera of normal BALB/c mice or on fusion of nonimmune splenocytes. As already stated, antibodies reacting with nuclei likewise were not found after injection of the animals only with the PEL used to obtain conjugates [5]. Meanwhile, long-term immunization with pepsin-treated streptococcus, without PEL, induced the production of antibodies reacting with nuclei. The study of these antibodies and their comparison with B1/2 and A5/2 McAb will be undertaken in the future.

The McAb obtained are not antibodies to cross-reacting determinants of the A-PS used for immunization. This is shown by the negative results obtained when McAb were tested by EIA with a pepsin-treated culture of group A streptococcus, containing A-PS on the surface, and also by experiments in which inhibition of antibodies was not obtained by means of A-PS or streptococcus. Meanwhile, as has already been pointed out, A-PS is a cross-reacting antigen common with antigen of thymus epithelium [11]. Consequently, the possibility cannot be ruled out that immunoregulatory disturbances may occur, which give rise to autoantibody production to other tissue antigens, including to cell nuclei [5]. It is interesting that the McAb produced belonged to the IgM class, for antibodies of the IgM class have been obtained by polyclonal activation by means of LPS [8]. The problem of the mechanisms determining the appearance of autoantibodies to DNA only after long-term immunization with A-PS conjugated with PEL or with pepsin-treated streptococcus requires further study.

LITERATURE CITED

1. É. I. Drobyshevskaya, N. A. Borodiyuk, V. Yu. Kolesnikova, and I. M. Lyampert, Byull. Éksp. Biol. Med., No. 3, 339 (1986).
2. I. M. Lyampert, A.V. Nekrasov, et al., Immunologiya, No. 1, 64 (1987).
3. R. V. Petrov, V. P. Evdakov, R. M. Khaitov, et al., Dokl. Akad. Nauk SSSR, 236, No. 5, 1260 (1977).
4. R. V. Petrov and R. M. Khaitov, Progress in Science and Technology: Series Immunology [in Russian], Vol. 7, Moscow (1978), pp. 223-244.
5. E. V. Ryzhikova, A. V. Nekrasov, I. M. Lyampert, et al., Byull. Éksp. Biol. Med., No. 6, 710 (1986).
6. S. G. Avrameas, G. Dighiero, P. Lymberi, and B. Guilbert, Ann. Immunol. (Paris), 134D, 103 (1983).
7. G. S. Eisenbarth, Anal. Biochem., 111, 1 (1981).
8. F. Fish and M. Ziff, J. Immunol., 128, 409 (1982).
9. G. Köhler and C. Milstein, Nature, 256, 495 (1975).
10. I. M. Lyampert and T. A. Danilova, Prog. Allergy, 18, 423 (1975).
11. I. M. Lyampert, L. V. Beletskaya, N. Borodiyuk (N. Borodijuk), et al., Immunology, 31, 47 (1976).
12. D. S. Pisetsky and S. A. Caster, Cell Immunol., 12, 294 (1982).
13. F. Tron, L. Jacob, M. A. Lety, and J. F. Bach, Clin. Immunol. Immunopath., 24, 351 (1982).
14. A. Voller, Enzyme Immunoassay, ed. by E. T. Maggio, Boca Raton, Florida (1980), pp. 181-196.
15. R. W. Warren, D. M. Sailstad, S. A. Caster, and D. S. Pisetsky, Arthr. Rheum., 27, 545 (1984).