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...
Fig. 1. Reaction of McAb of clone BI/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 BI/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 monoclonal and ascites fluids containing McAb prepared as described previously [1]. Plasma cytopma NP cells (2.6·10^7) and splenocytes of mice immunized with the conjugate of A-PS and PEL (2·10^8) 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 immunized 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 40x 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). Paraphenyleneamidine was used as the substrate. McAb were tested in Ouchterlony's immunodiffusion test with sera to mouse IgG and IgM (Miles Laboratories).