# MONOCLONAL ANTIBODIES TO VARIOUS CUTANEOUS EPITHELIAL ANTIGENS OBTAINED BY IMMUNIZING MICE WITH STREPTOCOCCAL GROUP A ANTIGENS

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UDC 616.98:579.861.2]-07:616.153.96-097-078.73

KEY WORDS: monoclonal antibodies; autoantibodies; streptococcal antigens; cutaneous epithelium.

The use of monoclonal antibodies (MCAB) to study cross-reacting antigens (CRAG) of microorganisms and tissues is the most promising method of discovery of hitherto unstudied CRAG and of confirming the specificity of cross-reactions. Data on the presence of CRAG common to the M substance of the group A streptococcus and myocardial muscle fiber antigen have been confirmed by MCAB [6]. It has been shown that the polysaccharide of the group A streptococcus is a CRAG that is common with the antigen of epithelial cells of the thymus and skin [9]. It has been confirmed as a result of obtaining MCAB that antibodies responding with cells of the basal epithelium of the skin and with the epithelium of the thymus are aimed at one determinant of the A polysaccharide and are autoantibodies.

The aim of this investigation was to study the possibility of obtaining MCAB reacting with epithelial cells in animals immunized with protein cell-wall antigens of group A streptococcus.

## EXPERIMENTAL METHOD

BALB/c mice weighing 16-20 g were immunized with a fraction containing nontype-specific protein cell wall antigens of group A streptococcus, type 5, obtained as a result of treatment of whole bacterial cells with KCNS [4]. The fraction was injected intraperitoneally in a dose of 20 μg 4 times with intervals of 7 days. The first dose of the fraction was injected together with Freund's complete adjuvant — FCA (expt. No. 5). By a similar scheme mice were immunized in experiment No. 12 with a culture of group A streptococcus, killed with formalin, in a dose of $10^{10}$ bacterial cells. The mice were immunized intraperitoneally with the corresponding antigens in the same dose 3-4 days before removal of the spleen. In experiment No. 10 the KCNS fraction was injected directly into the mouse spleen in a dose of 20 μg 3 days before hybridization [10]. Splenocytes of a nonimmune mouse were used as the control (expt. No. 13).

Mouse plasmacytoma cells (SP-2/0 and NP) were cultured on medium RPMI-1640 (Flow Laboratories, England) with the addition of 10% embryonic serum and of the components indicated below. For cell fusion the usual method [8] with 50% polyethylene-glycol (mol. wt. 4000 daltons, from Serva, West Germany) was used. The cells were transferred 48 h later to selective HAT medium (hypoxanthine, aminopterin, thymidine). Incubation was carried out at 37°C in an atmosphere with 7% $CO_2$ on 96-well panels (Linbro, England), followed by transfer of cells to HT medium (hypoxanthine and thymidine) in 24-well panels (Nunc, Denmark). Mouse peritoneal macrophages were added as feeder cells at all stages of culture. Cloning was carried out by the limiting dilutions method. The selected monoclones were kept at —70°C in culture medium with the addition of 10% dimethyl sulfoxide.

The supernatants were screened by the ELISA method with KCNS fractions [1] and also with cultures of group A streptococcus, types 5 and 29, treated with formalin. The MCAB thus obtained were studied with the same antigens and also with pepsin-treated streptococci of group A and other groups (C, E, L, A-variant), whose polysaccharides have common antigenic determinants with the polysaccharide of group A streptococcus [2, 7]. Primary screening of the supernatants and the study of MCAB were done in parallel tests on sections of human fetal joint tissues, the skin of BALB/c mice, and sections of human and bovine heart tissue. The indirect immunofluorescence method [9] was used with labeled antibodies to mouse immunoglobu-

N. F. Gamaleya Scientific-Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR P. A. Vershilova.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 8, pp. 197-200, August, 1986. Original article submitted June 14, 1985.

 0007-4888/86/0008-1096 $12.50 

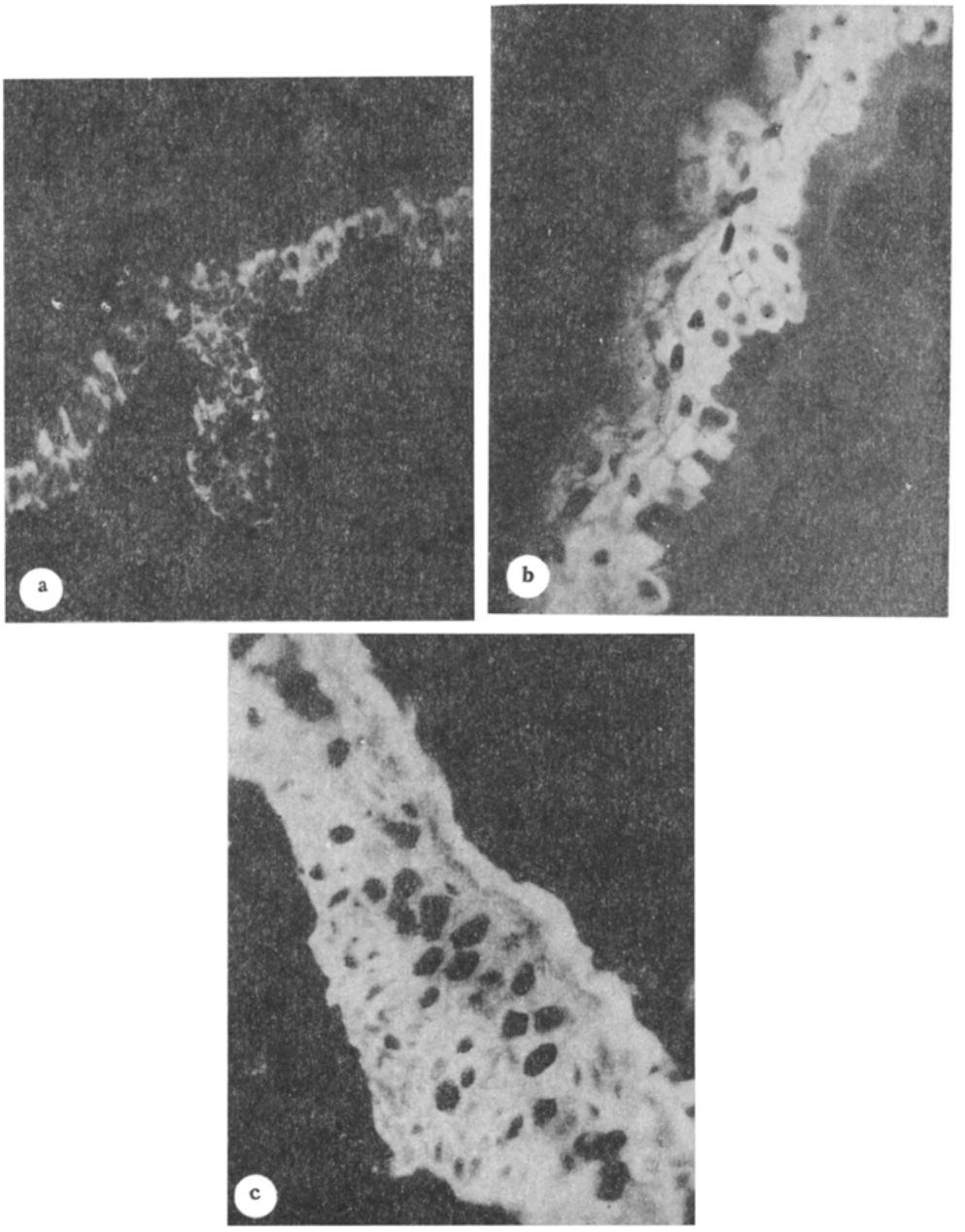


Fig. 1. Reaction of MCAB with various epithelial structures: a) reaction with basal layer; b) with superbasal layers; c) with all layers of epidermis. Magnification: objective 40, ocular – homal 3.

lins. The reactions were read with an ML-2 luminescence microscope with 40× objective. The homal 3 lens was used for photography. To inhibit reactions of MCAB with tissues, KCNS-fractions, a culture of group A streptococcus, treated with formalin or pepsin, and BCG (equivalent to 80 mg/ml protein or of dried weighed samples) was used.

## EXPERIMENTAL RESULTS

On preliminary screening (ELISA) of the supernatant of clones growing in 24-well panels in experiment No. 5 positive reactions with the KCNS fraction were determined in four cases, and with whole bacterial cells in 6 of 18 cases. No positive reactions were found in experiment Nos. 10, 12, and 13. Meanwhile, clones producing antibodies which, on immunofluorescence testing, reacted with cutaneous epithelial cells were found in three experiments.

In experiment No. 5 clones of this kind accounted for 2.7% of 72 clones tested, 13.9% of 36 in experiment No. 10, and 15.7% of 57 in experiment No. 12.

In the control test, during hybridization of splenocytes of an unimmunized mouse, no hybridomas synthesizing antibodies to epithelial cells or the other tissue tested, could be found.

Incidentally, in no case was agreement found between the reaction with the tissues and positive results in the ELISA test with the KCNS fraction or with whole cultures of streptococcus. On subsequent recloning, four monoclones producing MCAB to different layers of cutaneous epithelium were obtained. Two of them were obtained as a result of fusion of splenocytes with the myeloma line SP-2/0 and two with NP cells. It was found that monoclones A6/1 (expt. No. 5) and A3/2 (expt. No. 10) produce MCAB that react with cells of the basal layer of human embryonic cutaneous epithelium (Fig. 1a). Monoclone B5/1 (expt. No. 10) synthesizes MCAB which react with the superbasal layers of human cutaneous epithelium (Fig. 1b). MCAB obtained during culture of monoclone C6/2 (expt. No. 12) induce fluorescence of all layers of human embryonic skin (Fig. 1c). When MCAB were tested on sections of the skin of BALB/c mice, positive reactions were found with the corresponding structures with three types of MCAB (A6/1, B5/1, and C6/2). Meanwhile, positive reactions to mouse skin were not found with MCAB of type A3/2.

Negative results were obtained when all MCAR were tested by the ELISA method with the KCNS-fraction or with a formalin-treated streptococcal culture. The same results were obtained when MCAB were tested on cultures of different groups of streptococci, treated with pepsin. No inhibition of the reaction of all MCAB tested with epithelial cells as a result of the use of the KCNS fraction, of group A streptococcus treated with formalin or pepsin, and also of BCG, could be obtained.

The MCAB were thus not connected with streptococcal CRAG. These antibodies are evidently aimed at different epithelial antigens, for MCAB of types A6/1 and A3/2 react with basal cells of the cutaneous epithelium, those of type B5/1 with superbasal layers, and those of type C6/2 with all layers of the epidermis. It must be pointed out that in three cases the antibodies were evidently autoantibodies, for MCAB of types B5/1, C6/2, and A6/1 react with the same structures of cutaneous epithelium of BALB/c mice. Nonspecific reactions of MCAB with epithelial cells on account of FC-receptors must be ruled out. The basis for this is the negative results obtained with epithelial cells during testing of many immunoglobulins produced by hybridomas, and reacting positively in the ELISA test.

Incidentally, other investigators obtained MCAB which react with the same structures (basal, superbasal, or all layers of the epidermis) as a result of immunization of mice with keratin [11]. It was shown that MCAB are specific with respect to different classes of keratin with a particular molecular weight, but it has not been definitely shown that these MCAB are autoantibodies.

In other investigations, as a result of fusion of splenocytes of nonimmunized mice with myeloma cells, it has been shown that many of the resulting clones synthesize MCAB reacting with a number of tissue substances (tubulin, actin, myoglobin, etc.) [5]. The MCAB obtained are classed with the so-called "natural antibodies." Only in one case has it been shown that these MCAB are autoantibodies. The authors cited concluded that the cause of production of natural antibodies may be polyclonal activation of normally encountered autoreactive clones [5].

In our investigations with splenocytes of an unimmunized mouse no MCAB reacting with cutaneous epithelium or with heart tissues could be obtained. This may be because of the limited number of clones screened.

Previous investigations without the use of MCAB showed that the normal organism contains a set of B cells, potentially capable of reacting with different tissue antigens, including autologous antigens [3]. The cause of autoantibody production during immunization with streptococcal antigens, besides the appearance of autoantibodies to CRAG, may thus also be autoantibody production due to polyclonal activation under the influence of these antigens. Meanwhile, the obtaining of MCAB to tissue antigens in unimmunized mice [5] may evidently depend on preceding infection of these animals by viruses or microorganisms. A detailed study of autoantibodies arising to various tissue antigens as a result of polyclonal activation will shed some light on the potential ability of the normal organism to produce autoantibodies of varied specificity. MCAB reacting with different cutaneous epithelial antigens of man and animals, thus obtained, can be used to determine corresponding antigens in the epithelial tissue of the thymus and other organs. In addition, with the aid of these MCAB it will be possible to study expression of the corresponding antigens in normal tissues during embryogenesis, and also in epithelial tumors of varied origin.