Antigen CD38 thus is widespread and is not limited to a particular line, but characterizes proliferating cells.

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MONOCLONAL AUTOANTIBODIES TO EPITHELIAL STRUCTURES OF THE THYMUS OBTAINED BY IMMUNIZATION WITH GROUP A STREPTOCOCCAL ANTIGENS

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UDC 615.371:579.862.1].015.46.07

KEY WORDS: thymus, epithelium, streptococcal antigens

The epithelial tissue of the thymus constitutes a microenvironment in which T lymphocytes undergo maturation and differentiation. Direct contact between lymphocytes and epithelium and synthesis of hormones and other soluble factors are important [10]. It has been shown with the aid of monoclonal antibodies (MCA) that the epithelium of the thymus is heterogeneous, and different microenvironments are found in the human thymus [9]. This may probably have an important role in maturation of different T-lymphocyte subpopulations.

It was shown previously that the polysaccharide of group A streptococcus (A-PSC) is a cross-reacting antigen. Antibodies to A-PSC are autoantibodies and react with the epithelium of the stratum basale of the skin and with epithelium of the cortical and medullary zones of the thymus [14]. These data have been confirmed by the obtaining of MCA to A-PSC [5]. Autoantibodies to the same epithelial structures of the thymus and skin have been found in rheumatic fever and other autoimmune processes in man [7, 13] and also in the early stage of the autoimmune process in New Zealand mice [6].

It has been shown with the aid of MCA to various keratins that antibodies reacting with the basal epithelium of the skin are directed toward the endocrine epithelium of the thymus [10]. On the basis of the data given above it has been suggested that the main cause of immunoregulatory disturbances during autoimmune processes is damage to the endocrine epithelium of the thymus by autoantibodies [4]. In some immunopathological processes autoantibodies have been found to other epithelial structures of the thymus [10].

It is accordingly interesting to look for autoantibodies to epithelial structures of the thymus in connection with immunization by various microbial antigens.

As a result of immunization of BALB/c mice with nontype-specific (NTS) protein cell wall antigens of group A streptococcus, MCA reacting with various epithelial structures of the skin were obtained previously [1]. It was shown that the MCA are autoantibodies arising as a result of polyclonal activation of lymphocytes by NTS streptococcal antigens and they do not interact with antigens of group A streptococcus [1].

The greater part of the epithelium of the thymus consists of cells of epidermal genesis [10]. However, no clear evidence has yet been obtained to show against which epithelial struc-

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(Presented by Academician of the Academy of Medical Sciences of the USSR S. V. Prozorovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsny, Vol. 107, No. 1, pp. 77-80, January, 1989. Original article submitted March 25, 1988.

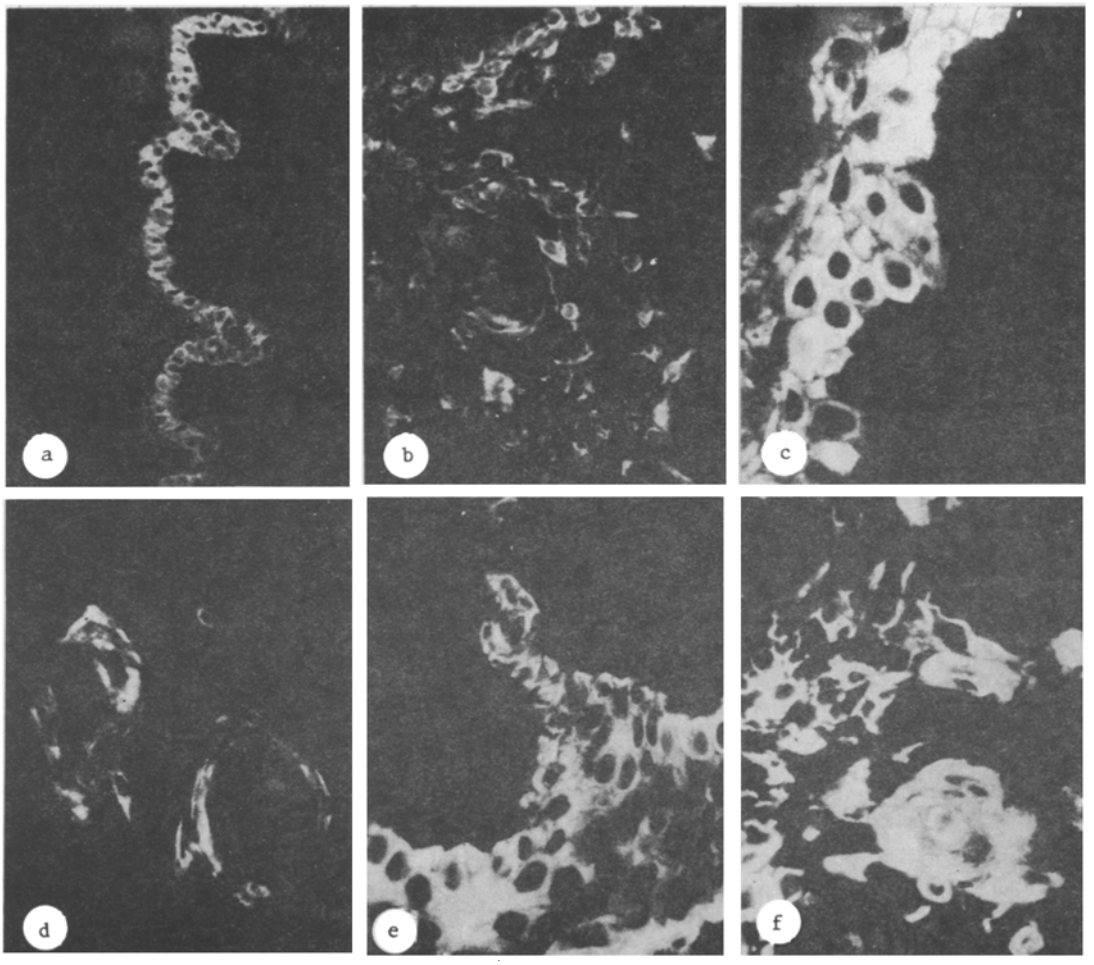

Fig. 1. Reaction of MCA A6/1 (a, b,), B5/1 (c, d), and C6/2 (e, f) with epithelium of human skin and thymus (IIFM). a) With stratum basale of human skin epithelium (embryonic joint); b) with epithelium of medullary and cortical zones of human thymus; c) with stratum superbasale of human skin epithelium (embryonic joint); d) with epithelium of human thymus around Hassall's corpuscles; e) with all layers of human skin epithelium (embryonic joint); f) with epithelium of cortical and medullary zones of human thymus and with Hassall's corpuscles.

tures of the thymus antibodies reacting not only with the basal epithelium of the skin, but also with other epidermal cells, are directed.

The aim of this investigation was to discover to which epithelial structures of the thymus MCA obtained by immunization with NTS antigens of group A streptococcus, and reacting with various epidermal antigens, are directed.

EXPERIMENTAL METHOD

Culture fluids containing MCA, obtained by immunization of BALB/c mice with the fraction containing NTS protein cell wall antigens of group A streptococcus, type 5, were used [1]. The following MCA were used: A6/1, reacting with the stratum basale of human skin epithelium, B5/1, directed toward the stratum superbasale of the human epidermis, and C6/2, interacting with all layers of the human epidermis. All these MCA are autoantibodies, for they react with analogous skin structures of BALB/c mice [1].

MCA A3/2, obtained by immunization with group A streptococci treated with pepsin, were used as the control. These MCA reacted during enzyme immunoassay with a determinant common for polysaccharides of streptococci of groups A and L, but did not induce fluorescence when tested by the indirect immunofluorescence method (IIFM) on various tissues [3]. Supernatants of MCA in a dilution of 1:16 were tested by IIFM as described previously on frozen sections of human and BALB/c mouse thymus tissues [14], with fluorescein-labeled rabbit antibodies to mouse immunoglobulins (N. F. Gamaleya Research Institute of Epidemiology and Microbiology).

In control experiments MCA were tested on sections of skin epithelium (the joint of a human embryo), and also on human and bovine myocardial sections by the method in [14]. The reaction was read by means of an ML-2 luminescence microscope with 40 × objective. A Homal 3 × was used for photography. To determine to which class of immunoglobulins the MCA belonged, the gel-diffusion test was used with rabbit sera to IgG and IgM ("Miles"). The MCA were subjected to the immunoblotting test with extract prepared from human skin epithelium, treated with citrate buffer (pH 2.8). Epithelial cells were isolated by treatment of human skin with a 0.25% solution of trypsin [2]. Vertical electrophoresis of the proteins was carried out with 10% polyacrylamide gel [12]. The molecular weights of the proteins were determined with the aid of a standard set of markers ("Pharmacia," Sweden). The proteins were transferred from the polyacrylamide gel to nitrocellulose paper ("Bio-Rad," USA) by the method in [15]. Peroxidase-labeled rabbit antibodies to mouse immunoglobulins (N. F. Gamaleya Institute) were used. The substrate was 4-chloro-1-naphthol ("Sigma," USA).

EXPERIMENTAL RESULTS

MCA were studied by IIFM on sections of human skin epithelium (the joint of an embryo) and on epithelial tissues of the human and BALB/c mouse thymus. The following results were obtained.

MCA A6/1, reacting with cells of the stratum basale of the epidermis, reacted on sections of human thymus with epithelial cells of the cortical and medullary zones of the thymus (Fig. 1a, b). MCA B5/1, which reacted with the stratum superbasale of skin epithelium, induced fluorescence on sections of human thymus only of individual epithelial cells of the outer layer of Hassall's corpuscles (Fig. 1). MCA C6/2, which induced fluorescence of all layers of the epidermis on sections of skin epithelium, when tested on sections of human thymus, reacted with epithelial cells of the cortical and medullary zones, and also with Hassall's corpuscles themselves (Fig. 1e, f). Similar reactions were discovered on sections of BALB/c mouse skin and thymus. The intensity of the reaction of MCA C6/2 on epithelial tissues of BALB/c mouse thymus was much weaker, possibly due to the smaller amount of this determinant in the epithelium of the mouse thymus. No reaction was found on human and bovine heart tissues. Control MCA A3/2 did not induce fluorescence of human and BALB/c mouse thymus epithelium, or of other tissues tested. MCA A6/1, B5/1, and C6/2 were shown to be in the IgM class.

The results of polyacrylamide gel electrophoresis showed that proteins with different molecular weights were present in the extract of human skin epithelium. Testing with MCA A6/1, B5/1, and C6/2 by the immunoblotting method showed that only MCA A6/1 reacted with a protein with mol. wt. of 50 kD. No specific staining of bands was found with other MCA. The negative reactions with MCA may have been due to absence of the corresponding antigens in the extracts or to denaturation of individual proteins during their isolation. The MCA obtained, moreover, were IgM and may have been relatively weak-affinity antibodies. This is a matter for further study.

The results are evidence that autoantibodies to thymus endocrine epithelium and to the stratum basale of the skin may arise as a result of polyclonal activation of lymphocytes by streptococcal NTS antigens. Autoantibodies to these epithelial structures, found in patients with erysipelas and rheumatic fever [7], may therefore be connected in some cases with polyclonal activation of lymphocytes. Among the cell wall proteins of the streptococcus an antigen with a mitogenic action on T lymphocytes has been found [11]. It is therefore possible that the MCA studied were obtained as a result of polyclonal activation of T lymphocytes. This problem, and also the ability of microbial antigens related to polyclonal B-cell activators to induce production of autoantibodies to the above-mentioned epithelial structures, require further study.

Discovery of a protein component reacting with MCA A6/1 is interesting. In the future this must be isolated and purified and the reactions of the various autoantibodies found in man and New Zealand mice in autoimmune processes with this protein component must be compared.

In some investigations autoantibodies reacting with the stratum superbasale of skin epithelium have been found in all cases in the sera of clinically healthy persons [2]. Consequently, MCA B5/1, reacting with these epidermal structures [1] and with individual epithelial cells around Hassall's corpuscles, are evidently similar to antibodies found usually in healthy individuals. Such autoantibodies may perhaps arise during polyclonal activation of lymphocytes by various microbial antigens.

It has been suggested that autoantibodies reacting on thymus sections with Hassall's corpuscles may be found in certain immunopathological processes [4, 10]. In this connection discovery of the reaction of MCA C6/2 with epithelium of the cortical and medullary zones of the thymus and with Hassall's corpuscles is interesting. MCA obtained as a result of polyclonal activation by streptococcal antigens, incidentally, may perhaps not cause damage to the epithelial structures of the thymus. This can be explained on the grounds that all the MCA studied belong to the IgM class, and autoantibodies of the IgM class are "natural" autoantibodies, unable to damage tissue [8]. It is intended in future investigations to determine the cytotoxic action of MCA relative to the corresponding epithelial cells in vitro and the accessibility of these thymus antigens in the intact organism.

Data [9, 10] on heterogeneity of the thymus epithelium were thus confirmed. It was also shown that determination of autoantibodies on the skin of the joint of a human embryo is an indicator of the epithelial structures of the thymus against which these autoantibodies are directed.

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