

REACTION OF SERA OF PATIENTS WITH PEMPHIGUS
VULGARIS WITH ANTIGENS OF THE CEMENTING
SUBSTANCE FROM THE EPITHELIUM OF HASSALL'S
CORPUSCLES OF THE HUMAN AND ANIMAL THYMUS

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The sera of patients with pemphigus vulgaris, containing antibodies against the cementing substance of certain epithelia of ectodermal origin, when tested by the indirect immunofluorescence method were shown to react with antigens of the cementing substance of the epithelium of Hassall's corpuscles of the human and animal (guinea pig, rabbit) thymus. These observations, together with evidence obtained by other workers that the thymus contains antigens of heteroorganic nature, confirm the view that the thymus participates in the formation of natural immunologic tolerance to the body's own antigens.

Investigations have shown that the sera of patients with myasthenia gravis and rheumatic fever, containing antibodies against components of the muscle fibers of the skeletal musculature and heart, when tested by the indirect immunofluorescence method react with cytoplasmic antigens of the myoid cells of the thymus [1, 11, 19, 20]. Besides the myoid cells or sarcocytes, other structures of heteroorganic nature have been identified in the human and animal thymus. They include cells similar to the cells of ciliated epithelium, with the intestinal goblet cells, with the cells of the epidermis of the skin, etc. [3, 4, 10, 12, 14, 16]. It has now been shown that some of these structures have not only morphological, but also antigenic similarity with the cells of other organs [5, 19, 21]. On the basis of these factors several workers have postulated that the presence of heteroorganic antigens in the thymus is concerned with immunologic control, i.e., with the function of preventing the synthesis of autoantibodies [1, 2, 13, 14, 18]. Considerable morphological changes are found in diseases accompanied by marked autoimmune reactions, for example, in systemic lupus erythematosus, myasthenia gravis, rheumatic fever, and pemphigus vulgaris [2, 15, 18, 23], and these direct attention to this organ. Investigations into the antigenic composition of tissue elements of the thymus could be particularly interesting in connection with the study of the mechanisms of development of autoimmune processes. For this purpose sera obtained experimentally by immunization of animals and the sera of patients with various diseases, containing antibodies against particular tissue antigens, can be used for this purpose. The serum of patients with pemphigus vulgaris is known to contain high titers of antibodies against the cementing substance of the epithelium of the skin, the esophagus, and certain mucous membranes of ectodermal origin, reflecting the marked autoimmune reactions in this disease [9, 25].

The object of the present investigation was to study the sera of patients with pemphigus vulgaris with the components of the epithelial structures of the human and animal thymus by the use of the immunofluorescence method.

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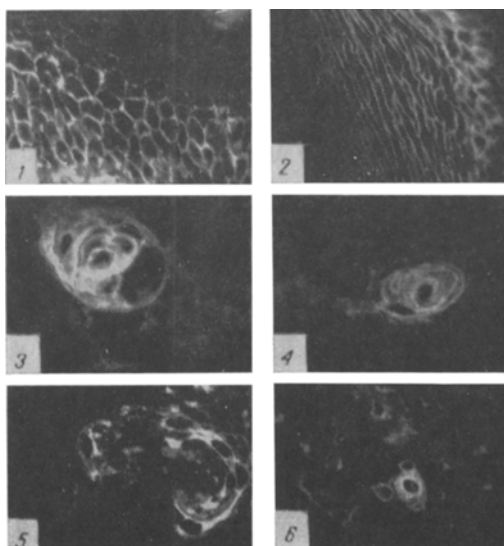


Fig. 1. Reaction of sera of patients with pemphigus vulgaris with the cementing substance of the epithelium of Hassall's corpuscles and certain other ectodermal epithelia. Luminescence of cementing substance (indirect immunofluorescence method; object 40, homal $\times 3$, ML-2 microscope): 1) human skin; 2) rabbit esophagus; 3 and 4) human embryonic thymus: Hassall's corpuscle; 5) guinea pig thymus: Hassall's corpuscle; 6) adult human thymus: formed Hassall's corpuscle.

EXPERIMENTAL METHOD

Pure antibodies against human γ globulin, conjugated with fluorescein isothiocyanate, were used for the immunofluorescence test. Antibodies were isolated from the serum of rabbits immunized with human serum globulins by the method of Avrameas and Ternynck [6].

The sera from six patients with pemphigus vulgaris, 35 sera from patients with other diseases (myasthenia gravis, rheumatic fever, systemic lupus erythematosus, lupus discoides, schizophrenia, ulcerative colitis, burns), and five sera from clinically healthy persons were tested.

To avoid nonspecific reactions the sera were first adsorbed with mouse liver powder or were used in high dilutions (1:128-1:1024) without preliminary adsorption.

Tissue sections 5 μ in thickness were cut in a cryostat (-20°C) from frozen unfixed tissue of the thymus, skin, esophagus, and other organs of a human embryo (14-16 weeks), of persons dying accidentally at the age of 20-25 years, and of guinea pigs and rabbits, and were incubated with the serum for 16 h at 4°C . In some experiments the reaction was carried out at room temperature (22°C) for 1 h.

EXPERIMENTAL RESULTS

When unadsorbed serum of patients with pemphigus vulgaris in a dilution of 1:128-1:1024 was layered above tissue sections of the human, rabbit, and guinea pig skin, esophagus, and mucous membrane of the mouth, bright luminescence of the cementing substance of the epithelium of these organs was observed (Fig. 1: 1, 2). The use of sera in such high dilutions (differing for different sera) completely ruled out the possibility of reaction with other tissue structures. Serum P-3 which reacted with the cementing substance in a dilution of 1:1024 was the most active. After twofold dilution of this serum (1:2048) the luminescence of the cementing substance was no longer continuous, as usually, but consisted of discrete deposits.

Application of the sera in dilutions of 1:128-1:1024 to tissue sections of human embryonic and adult guinea pig thymus was followed by intense luminescence of the cementing substance of the epithelium of Hassall's corpuscles (Fig. 1: 3, 4, 5). After application of the sera to tissue sections of adult human and rabbit thymus luminescence was observed on the surface of the epithelial cells located in the immediate

vicinity of the thymic corpuscles or attached to the corpuscles during their formation (Fig. 1: 6). The reaction of the cementing substance of the Hassall's corpuscles with sera preliminarily adsorbed by mouse liver powder was similar in character, but under these circumstances the sera had to be used in smaller dilutions (1:32-1:256).

Application of the unadsorbed sera from patients with pemphigus vulgaris in low dilutions (1:32-1:128) to the sections was followed by luminescence of several elements: the myoid cells of the thymus, the epidermal cells of the skin, discs of the muscle fibers or the skeletal musculature, and the myelin sheaths of the medullary cords of the nerve fibers.

In the control experiments when the sera were applied to tissue sections of other organs of epithelial origin (liver, kidney, intestinal wall, salivary gland) no reaction with the cementing substance of the epithelium of these organs could be found. Treatment of the sections of the thymus and skin, after application of the serum of a patient with pemphigus to them, with unlabeled antigens against human γ globulin prevented the reaction with the labeled antibodies. Sera from donors and patients with other diseases (systemic lupus erythematosus, lupus discoides, rheumatic fever, myasthenia, ulcerative colitis, schizophrenia, burns) did not react in any single case with the cementing substance from the epithelium of the thymus, skin, esophagus, and mucous membrane of the mouth. They contained antibodies against antigens of other structures of these various organs: myoid cells of the thymus, cell nuclei, the ectoplasmic and endoplasmic zones of the cytoplasm of the epidermal cells of the skin, and Hassall's corpuscles of the thymus. The serum from patients with burns and lupus discoides contained antibodies against the cytoplasm and nuclei of the epithelial cells but antibodies against the cementing substance were completely absent.

The study of the sera of the patients with pemphigus vulgaris by the indirect immunofluorescence method thus showed that the cementing substance of the cells of stratified squamous epithelium and of the epithelium of Hassall's corpuscles of the thymus contains common antigens.

In diseases accompanied by autoimmune reactions the patients' sera can contain antibodies against antigens from different organs simultaneously: in myasthenia, for example - antibodies against antigens from components of skeletal musculature, nuclear material, and cytoplasm of thyroid gland cells [17, 22]; in pemphigus - antibodies against antigens of the cementing substance of certain epithelia of ectodermal origin and against antigens of the skeletal musculature [8]; in lupus - against antigens of nuclear material of cells from different organs and cytoplasmic antigens of various cells [2]. When patients' sera are used to study antibodies against one of the antigens of a particular tissue or organ, either preliminary adsorption must be carried out with a tissue homogenate of the corresponding organ or sera must be used which contain antibodies against the studied antigen in titers considerably higher than antibodies against the antigens of other organs [7]. In the present investigation one of the sera studied from patients with pemphigus vulgaris reacted with the components of the skeletal musculature and the other tissues only to a dilution of 1:128, but with the cementing substance of Hassall's corpuscles to a dilution of 1:1024. On the application of this serum to thymus sections in low dilutions (1:128) a reaction was observed both with the cementing substance and with the cytoplasm of the cells of Hassall's corpuscles. It was impossible under these conditions to differentiate between the two components of the thymic corpuscles because of the high intensity of their luminescence. When sera in higher dilutions were used (1:1024) luminescence only of the cementing substance between the cells of the Hassall's corpuscles was observed. These findings show that the sera of patients with pemphigus vulgaris contain antibodies not only against antigens of the cementing substance, but also against antigens of the cell cytoplasm of the Hassall's corpuscles. However, since antibodies against these antigens were present in the sera in different amounts, the reactions with these antigens could be differentiated by diluting the sera and in this way the localization of the cementing substance of the cells of the Hassall's corpuscles could be demonstrated.

Several antigens have been determined by the immunofluorescence method in the composition of Hassall's corpuscles: the S-factor, γ globulin, albumin, and a substance antigenically similar to skin keratin [5, 21]. The data showing that the epithelial tissue of the thymus contains antigens similar to antigens of muscle fibers of the skeletal musculature and heart [1, 11, 19, 21], the keratin of the skin epidermis [5], the mucin secreted by the cells of many organs of ectodermal origin [21], and the cementing substance of the epithelium of the skin and mucous membranes, i.e., heteroorganic antigens, confirm the hypothesis that the thymus is the site of formation of natural immunologic tolerance to the organism's own antigens.

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