IMMUNOFLUORESCENCE STUDY OF KERATIN OF HASSALL'S CORPUSCLES AND EPIDERMIS OF THE HUMAN SKIN

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The sera of patients with various autoimmune diseases (ulcerative colitis, multiple sclerosis, myasthenia gravis, rheumatic fever), containing antibodies against the keratin of human skin, if tested by the immunofluorescence method react with the cytoplasm of the cells and material from the chambers of Hassall's corpuscles of the human thymus. Adsorption of the sera with a suspension of the epidermis of the human skin abolishes the fluorescence of keratin in the skin and Hassall's corpuscles. These observations show that the cells of Hassall's corpuscles synthesize material antigenically identical with skin keratin.

The epithelial tissue of the thymus is known to contain myoid cells similar in their structure and antigenic composition to muscle fibers of the skeletal muscles and heart [1, 2, 8, 10-12, 18]. On this basis it was postulated that the epithelial cells of the thymus contain antigens of several organs and tissues and that their presence is essential for the provision of an antigenic environment in which immunological maturation of the lymphocytes takes place [1, 6, 11, 12, 18]. There is evidence to show that the epithelial cells of the thymus contain structures characteristic of the cells of other organs and tissues [10, 13, 14]. The cells of Hassall's corpuscles, for example, are known to synthesize material which, in its staining properties, resembles the keratohyalin of the epidermis of the skin [3, 10]. However, because of their morphological character, these and many other facts can only indirectly confirm the presence of hetero-organ antigens in the thymus. The problem can be studied more adequately by immunological methods, especially the immunofluorescence method. The sera of patients with various autoimmune diseases, containing antibodies against organospecific antigens, can be used in such investigations as the source of antibodies [5, 9, 16, 19]. An attempt was accordingly made to compare the reaction of sera of patients with ulcerative colitis, multiple sclerosis, myasthenia gravis, and rheumatic fever with the epithelial cells of the thymus and antigens of other organs, using the immunofluorescence method.

This paper describes the results of a comparative study of the antigenic properties of material from Hassall's corpuscles and the keratin of the skin, using antibodies found in the sera of patients with the diseases listed above.

EXPERIMENTAL METHOD

The indirect immunofluorescence method was used with pure antibodies against human γ globulin labeled with fluorescein isothiocyanate [4]. The reaction of sera from patients with ulcerative colitis (20 sera), myasthenia gravis (30 sera), multiple sclerosis (36 sera), and rheumatic fever (20 sera) and also the sera of clinically healthy persons (30 sera) with Hassall's corpuscles and the epidermis of the skin from persons dying from accident aged 11-25 years was studied. Tissue sections were treated with the serum for 40 min at room temperature, washed for 15 min with buffered physiological saline, pH 7.0, and then

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TABLE 1. Comparative Study of Reaction of Sera of Patients with Various Autoimmune Diseases with Keratin of Human Skin and with Human Hassall's Corpuscles

Disease	Number of sera studied	Number of sera giving positive reaction	
		with skin keratin	with Hassall's corpuscles
Ulcerative colitis	20	20	20
Multiple sclerosis	36	24	24
Myasthenia gravis	30	26	26
Rheumatic fever	20	12	12
Healthy donors	30	12	12

incubated for 30 min with labeled antibodies against human γ globulin. To identify the antigen of the Hassall's corpuscles and epidermis of the skin with which the sera reacted they were adsorbed with a suspension of keratinizing epidermis of the human skin and a homogenate of human heart, liver, brain, and spleen tissue. After removal from the skin surface the epidermis was washed three times with physiological saline, ground in a mortar, and then washed again. The method used to prepare tissue homogenates of other organs and the method of adsorption were as suggested by Kaplan [15]. The ratio between the volumes of the test serum and tissue homogenate was 1:2.

EXPERIMENTAL RESULTS

Examination of sections of the human thymus in the luminescence microscope in light with a wavelength of 400 nm revealed the yellow autofluorescence of the contents of the large cystic Hassall's corpuscles and of material lying freely in the tissue, sometimes in cross-section having a structure resembling mature hair. The intensity of fluorescence of the autofluorescent components of the Hassall's corpuscles and of the material lying freely in the tissue was not increased by the use of the direct and indirect immunofluorescence methods. Consequently, this material did not contain γ globulin and did not react with the patients' sera. The autofluorescent outer layer of keratin of the skin, which undergoes desquamation, possessed similar properties. Together with autofluorescent material, most mature Hassall's corpuscles also contained material whose fluorescence increased considerably after treatment of the section with labeled antibodies against human γ globulin. This is evidence that it contained γ globulin. The cytoplasm of the cells of Hassall's corpuscles did not react with antibodies against human γ globulin. Fluorescence of the absolute majority of thymic corpuscles (both the cells and the contents of the chambers) was observed only when the indirect immunofluorescence method was used, i.e., when the section was treated consecutively with the patient's serum and labeled antibodies against human γ globulin.

The results of the comparative study of the reaction of sera from patients with various autoimmune diseases with Hassall's corpuscles and with the epidermis of the human skin are given in Table 1; they show complete agreement between the frequency of reaction of the sera with the skin keratin and with the Hassall's corpuscles. If the sera did not contain antibodies against skin keratin they likewise did not react with

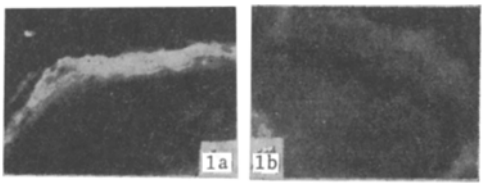

Fig. 1

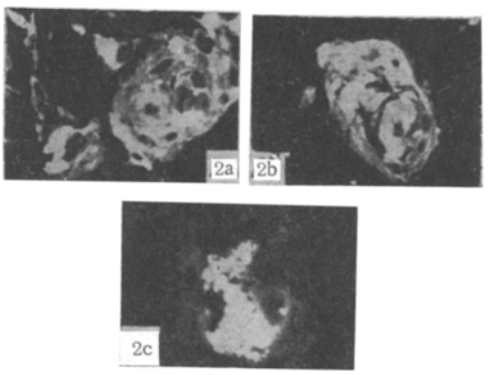

Fig. 2

Fig. 1. Section through human skin tissues: on treatment with the serum of a patient with ulcerative colitis fluorescence of the keratin of the zona pellucida of the epidermis (a) is seen; on treatment with a donor's serum (control) fluorescence is absent (b); magnification 40×, Homal, ×3.

Fig. 2. Section through tissues of the human thymus: in response to treatment with the serum of a patient with ulcerative colitis fluorescence of the cells and keratin in the chamber of Hassall's corpuscles is observed (a, b); on treatment with same serum of the patient with ulcerative colitis, but previously adsorbed with a suspension of the epidermis of the human skin, no fluorescence of the cells or of part of the keratin in the chamber of the thymic corpuscle was present but the autofluorescence of the mature keratin was preserved (c); magnification 40×, Homal, ×3.

Hassall's corpuscles. In sections through human skin the sera reacted chiefly with the keratin of the zona pellucida (Fig. 1a, b) and to a lesser degree with the zone of nuclear membranes and with the cytoplasm of cells of the zona granulosa. If these sera were applied in layers to sections of the human thymus intensive fluorescence of the cells and the contents of the chambers of many Hassall's corpuscles was observed in the medullary zone of the lobules (Fig. 2a, b). In the control experiments in which unlabeled and labeled antibodies against human γ globulin were layered consecutively above sections of the skin and thymus treated with serum, no fluorescence of the Hassall's corpuscles and epidermis of the skin could be found. Adsorption of the sera with a suspension of keratinizing epidermis of the human skin completely suppressed their reaction with the keratin of the skin and the Hassall's corpuscles and had no effect on fluorescence of their autofluorescent components (Fig. 2c). Consequently, the antibodies present in the sera are directed against the same component of the Hassall's corpuscles and epidermis of the skin, namely the keratin.

It can thus be taken as established that the thymus contains three hetero-organ antigens. These are antigens of the myoid cells, similar to the antigens of muscle tissue. Next to be mentioned is the S-factor found in Hassall's corpuscles, a structural component of the IgA molecule present in the mucus secreted by the cells of many epithelial organs [18]. Finally, the results of the present investigations show that Hassall's corpuscles produce a material antigenically identical with the keratin of the skin. The Hassall's corpuscles are possibly polypotent structures whose cells can synthesize several different antigens related to antigens not only of the skin, but also of other epithelial organs derived from the ectoderm and endoderm.

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