IMMUNOCHEMICAL IDENTIFICATION OF A THERMOSTABLE α-GLYCOPROTEIN IN BLOOD SERUM FROM PATIENTS WITH PATHOLOGY OF THE IMMUNE SYSTEM

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The method of extracorporeal hemosorption [1] is being used on an ever-increasing scale in modern clinical medicine, including in some diseases considered to have an autoimmune component (psoriasis, primary biliary cirrhosis, lupus erythematosus, etc.) [12]. As yet the concrete mechanism of the beneficial action of hemosorption on this category of patients has not been discovered.

The object of the present investigation was to look for and study pathological antigens in eluates from the charcoal used for hemosorption in these patients.

EXPERIMENTAL METHOD

IGI granulated charcoal from the column used for hemosorption on the patients with psoriasis and urticaria was carefully washed with physiological saline to remove blood, which was confirmed by immunodiffusion analysis for the principal plasma proteins. Elution was carried out with a 0.5 M solution of disodium hydrogen phosphate. The eluate was dialyzed against distilled water and concentrated by freeze-drying. To obtain antisera 12 rabbits were immunized with the resulting freeze-dried product in a dose of 50 mg per subcutaneous injection, using potassium alum as adjuvant. Schemes of immunization and also methods used to prepare the extracts and determine the physicochemical parameters of the proteins were described by the writers previously [3]. Immunodiffusion analysis was carried out with the use of a standard monospecific test system [4], with a sensitivity of 5 μg/ml. To neutralize antibodies against normal serum proteins, the resulting antisera were exhausted with mixed lyophilized plasma from 30 donors in a dose of 200 mg/ml.

EXPERIMENTAL RESULTS

As a result of immunization of 12 rabbits, antisera which, after neutralization of antibodies against normal serum proteins, continued to reveal an antigenic component, with the electrophoretic mobility of α_2-globulins and which withstood heating to 100°C for 1 h without any appreciable change in its antigenic and physicochemical characteristics, in a series of pathological blood samples, were successfully obtained from two rabbits; this component was a thermostable α-globulin (T-α-G). The physicochemical characteristics of this antigen were as follows: mol. wt. 90,000 ± 7000, relative electrophoretic mobility 0.77 ± 0.09, color test for glycoproteins positive, color test for lipoproteins negative, binds with concanavalin A-sepharose, thermostable (withstands heating to 100°C for 1 h), salted out with ammonium sulfate in 35-60° saturation, precipitated by 0.4% rivanol, with 0.3 M sulfosalicylic acid, and with 2% TCA, no change in electrophoretic mobility detectable under the influence of neuroaminidase.

T-α-G is thus a glycoprotein, as shown by the positive color test for glycoproteins and binding with concanavalin A-Sepharose. Meanwhile T-α-G, unlike some other glycoproteins, is precipitated by sulfosalicylic acid and TCA, and it does not contain appreciable quantities of sialic acids in the composition of its carbohydrate moiety, as shown by the test with neuraminidase. Attention also is drawn to the low immunogenicity of T-α-G, which creates

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TABLE 1. Immunodiffusion Determination of T-α-G in Patients' Blood Serum (M ± m)

Disease	Total No. of samples	No. of positive samples	Content of T-α-G, mg%
Psoriasis	14	9	0,8±0,1
Primary biliary cirrhosis	7	4	1,2±0,2
Rheumatic fever	25	20	7,3±0,9
Systemic lupus erythematosus	5	4	1,8±0,3
Familial hypercholesteremia	7	3	0,9±0,1
Urticaria	5	5	2,2±0,2
Primary carcinoma of the live	11	9	4,3±0,5
Carcinoma of large intestine	46	37	4,6±0,6
Carcinoma of kidney	40	17	1,4±0,2
	27	19	1,9±0,3
Healthy blood donors	51	3	—

TABLE 2. Immunodiffusion Determination of T-α-G in Extracts of Various Tissues and Biological Fluids (M ± m)

Test object	Total No. of samples	No. of positive samples	T-α-G content, mg%
Spleen	10	10	34,2±4,1
Lung	8	8	23,7±1,8
Thymus	6	6	9,4±1,3
Lymph node	5	5	8,2±0,9
Liver	9	9	2,3±0,1
Kidney	11	11	2,1±0,2
Prostate	5	5	2,0±0,2
Thyroid gland	5	5	0,9±0,1
Heart	6	5	0,8±0,1
Muscle	5	5	0,7±0,1
Brain	6	0	—
Skin	5	0	—
Adrenal gland	4	0	—
Stomach	5	1	—
Large intestine	4	0	—
Sperm	8	8	28,6±3,4
Saliva	14	14	1,6±0,2
Urine	12	0	—
Milk	2	0	—

definite difficulties in the obtaining of rabbit antisera against this antigen. Accordingly, in the next stage of the investigation we used a semipurified preparation of T-α-G, prepared with allowance for its thermostability, for reimmunization. The low immunogenicity of T-α-G and also its thermostability are evidently due to the specific character of the carbohydrate component of T-α-G.

On the basis of the monospecific antiserum a standard test system for T-α-G was isolated and used to study its content in pathological blood samples. As Table 1 shows, T-α-G was found in a certain percentage of cases in some diseases in whose pathogenesis a disturbance of the immune status is postulated. The highest titers of this antigen were recorded in certain samples of serum from patients with rheumatic fever and with carcinoma of the stomach and liver (up to 64 mg%). Meanwhile some pathological samples were negative for T-α-G. T-α-G was detected at the limit of sensitivity of the test system in only three of the 51 sera from blood donors. However, the use of a more sensitive modification of immunodiffusion analysis (by widening the well for the test sample), so that it was possible to detect T-α-G in quantities down to 0.1 mg%, this antigen was discovered in the blood sera of another 27 donors. This is evidence that there is a definite "subthreshold" T-α-G level in blood serum from healthy blood donors.

As Table 2 shows, the highest concentrations of T-α-G in the healthy subjects were found in extracts of organs rich in lymphoid tissue and also in sperm. Since lymphoid tissue cells are present in many organs, it can be postulated that the main source of T-α-G is lymphoid tissue.

T-α-G is thus a tissue antigen linked primarily with lymphoid tissue and which, in certain pathological states when the immune status of the patient is disturbed, is found in increased quantities in the blood.

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SOME ASPECTS OF THE REACTION OF HEALTHY HUMAN BLOOD SERUM WITH EPIDERMIS

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In some forms of pathology of the skin antibodies against certain antigens of the epidermis characteristic of the particular disease appear in the patients' blood. For instance, in lupus vulgaris a high titer of antibodies against antigens of intercellular adhesive substance is observed [7], in bullous pemphigoid there is a high titer of antibodies against antigens of the basement membrane [13], and the sera of patients with burns react with antigens of cells of the stratum basale of the epidermis [5]. Meanwhile there have been only isolated reports of the reaction of healthy human serum with antigens of cells of the epidermis [5]. Accordingly, the object of the present investigation was to study by the indirect immunofluorescence method the reaction of healthy human serum with the epithelium of the skin.

EXPERIMENTAL METHOD

The reaction of the sera of 70 clinically healthy subjects (blood donors) with sections of fetal and adult (aged 20-25 years) human skin was studied by the indirect immunofluorescence methods. Pieces of skin tissue taken from the region of the chest were freed from subcutaneous cellular tissue and frozen in petroleum ether, cooled in a mixture of acetone and dry ice to −76°C. Unfixed frozen sections were treated with serum (dilution from 1:4 to 1:20) for 18 h at 4°C. After rinsing for 20 min in running buffered physiological saline, pH 7.2, the sections were incubated for 45 min with fluorescein isothiocyanate-labeled antibodies against human IgG. Antibodies were isolated from donkey antiserum by means of an immunosorbent containing human IgG treated with glutaraldehyde. In control experiments, skin sections were treated with human IgG (concentration 500 μg/ml) for 18 h at 4°C, after which the sections were incubated with luminescent antibodies against human IgG. To study the organ specificity of the reaction the sera were first absorbed with a suspension of epithelial cells from human fetal skin, a suspension of keratinized epidermal cells, and a homogenate of tissues of certain organs (liver, kidney, heart, brain, spleen). The suspension of fetal epidermal cells was obtained by treating skin with 1% trypsin solution for 3 h at 37°C. The suspension of keratinized cells from adult human epidermis was obtained by mechanical stripping of the surface layers of the epithelium. The serum and suspension of epidermal cells or homog-

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