IMMUNOCHEMICAL IDENTIFICATION AND PHYSICOCHEMICAL CHARACTERISTICS OF HUMAN LEUKOCYTIC β_2-SIALOGLOBULIN (SLA-6)

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Great importance is nowadays attached to evaluation of immunologic status, which is essential for elucidation of the pathogenesis of several diseases and also for the planning of properly oriented methods of immunocorrection [1].

Determination of the functional state of the leukocytes is a very important step in the assessment of the state of cellular immunity; in recent years there has been a tendency for leukocyte function to be studied at the molecular level by identification and study of individual substances synthesized by these cells.

The aim of this investigation was an immunochemical analysis of the sixth human soluble leukocytic antigen (SLA-6). Five antigens were described previously by the writers [3-5].

EXPERIMENTAL METHOD

Leukocytes were isolated from whole venous blood from normal blood donors after separation of the erythrocytes by precipitation with phytohemagglutinin [6]. The leukocytes were lysed by a single freezing and thawing.

The scheme of immunization of the rabbits and methods of preparation of extracts and of determination of the physicochemical parameters of the proteins were described previously [2]. Monospecific antisera against SLA-6 were obtained by immunizing rabbits with a semipurified preparation of this antigen with the addition of potassium alum as adjuvant and exhaustion of the resulting immune serum with normal human plasma. The method of preparing the semipurified SLA-6 for immunization was as follows. Lysed leukocytes from blood donors were treated with a 0.4% solution of rivanol. The supernatant (after preliminary precipitation of the rivanol with 5% sodium chloride) was treated with 0.6 M sulfosalicylic acid to precipitate ballast proteins. The protein fraction resistant to sulfosalicylic acid was precipitated with 2% phosphotungstic acid. The residue obtained after centrifugation at 5,000g for 20 min was dissolved in phosphate buffer (pH 8.2) and dialyzed for 6 days against distilled water. The fraction thus isolated was concentrated by lyophilization.

Immunodiffusion analysis was carried out with a standard monospecific test system [7] the sensitivity of which, if a well of increased width was used for the sample to be analyzed, was taken to be 0.1 mg%.

EXPERIMENTAL RESULTS

A leukocytic sialoglobulin with electrophoretic mobility of β_2-globulins was identified in the composition of the lysed leukocytes from normal human venous blood with the aid of monospecific antiserum against SLA-6. The physicochemical characteristics of this antigen are given in Table 1, which shows that SLA-6 is a glycoprotein, containing sialic acid, as shown by the positive neuraminidase test, leading to changes in electrophoretic mobility. Meanwhile, SLA-6, unlike several other glycoproteins, does not bind with con A-sepharose and is precipitated by TCA. The definite heterogeneity of this protein relative to sulfosalicylic acid, which precipitates 75% of the antigenic activity of SLA-6, and to exposure to the temperature factor (after heating the lysed leukocytes at 100°C for 1 h 10% of its antigen activity is

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TABLE 1. Physicochemical Characteristics of SLA-6

Parameter	Property
Molecular weight, daltons	70,000 ± 6000
Relative electrophoretic mobility	0.34 ± 0.03
Staining for glycoproteins	Positive
Staining for lipoproteins	Negative
Binding with con A-sepharose	Does not bind
Thermolability	Partially thermostable
Salting out with ammonium sulfate, percent saturation	40 - 65
Precipitation with 0.4% rivanol	Not precipitated
Precipitation with sulfosalicylic acid (0.6 M)	Partially resistant
Precipitation with 2% TCA	Precipitated
Change in electrophoretic mobility after treatment with neuraminidase	Decrease in anodal electrophoretic mobility to that of γ-globulins
Behavior with enzymes:	
Trypsin	Resistant
Papain	"
DNase	"
RNase	"
Hyaluronidase	"

TABLE 2. Immunodiffusion Determination of SLA-6 in Extracts of Various Human Tissues and in Human Biological Fluids

Tissue extract and biological fluid	Number of samples	Content of SLA-6, mg% (M ± m)
Hemolysate	23	1,1±0,1
Lysed leukocytes	12	1,3±0,1
Lysed lymphocytes	6	—
Pus	9	6,3±0,9
Spleen	11	3,8±0,2
Lung	6	—
Liver	9	—
Kidney	1	—
Heart	6	—
Brain	6	—
Thymus	4	—
Blood serum	23	—
Saliva	14	0,8±0,1
Sperm	8	—
Milk	8	—
Urine	7	—

still preserved), is noteworthy; this may be due both to the intrinsic molecular heterogeneity of SLA-6 and also to the formation of a complex between this antigen and certain other substances possessing, in particular, the property of thermostability.

Comparative immunodiffusion analysis showed that SLA-6 is not immunochemically identical with SLA-1 [4], SLA-2 [3], SLA-3, SLA-4, or SLA-5, and also with α-fetoprotein, carcino-embryonic antigen, and NCA-I.

It must be noted that SLA-6 was determined in virtually identical quantities in lysed leukocytes and hemolysates, whereas it could not be found in lysed lymphocytes (Table 2). This factor, as well as the high SLA-6 content in extracts of pus of varied origin, is definite evidence in support of the granulocytic origin of this glycoprotein. The high resistance of SLA-6 to the action of various enzymes (Table 1) evidently facilitates realization of the function of this leukocytic protein in foci of inflammation and destruction, despite saturation of these areas with lysosomal enzymes.

The SLA-6 content in hemolysates in acute hemocytoblastosis and lymphatic leukemia was reduced, whereas in cases of chronic myeloid leukemia, the level of this antigen was many times higher. A decrease in the content of SLA-6 in hemolysates in chronic diseases with an autoimmune component (rheumatoid arthritis, psoriasis), and also its secretion into the blood serum during exacerbation of chronic salpingo-oophoritis also were established. A special study is required to elucidate the functions of SLA-6 and for clinical evaluation of the test for this antigen.

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