COMPARATIVE PHYSICOCHEMICAL AND IMMUNOCHEMICAL STUDY OF THREE SOLUBLE ANTIGENS OF HUMAN LEUKOCYTES

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Leukocytes play a very important role in immunologic reactions. It is now firmly established that various biologically active substances, including lymphokines, chalones, and enzymes, which are protein in nature, are synthesized by these cells [1]. A tendency is now being observed for the functions of leukocytes to be studied at the molecular level, by identification and investigation of individual substances characteristic of leukocytes. However, most research into this problem has so far been undertaken by tests of biological activity which, as a rule, do not permit the factors discovered to be adequately characterized physicochemically, and for that reason, do not permit preparations of acceptable purity for biological testing to be obtained from them. Moreover, many workers are still unable to give a firm answer to the question whether the "factors" of leukocytic origin which they have described are the same substance, possessing several different functions, or completely different substances from the chemical point of view [1].

A reliable method of overcoming these difficulties is by primary immunochemical identification of immunogenic substances synthesized by leukocytes, followed by further studies of their physicochemical characteristics, purification, and biological testing under the control of immunochemical analysis at the individual antigen level.

The object of this investigation was a systematic immunochemical analysis of water-soluble antigens of human leukocytes (data on three of them are given).

EXPERIMENTAL METHOD

Leukocytes were isolated from whole venous blood of healthy blood donors, from which erythrocytes were removed by precipitation with phytohemagglutinin [4]. A lysate of leukocytes was obtained by freezing and thawing once.

The scheme of immunization of rabbits and also the methods of preparation of the extracts and determination of the physicochemical parameters of the proteins were described by the writers previously [3]. Monospecific antisera against soluble leukocyte antigen SLA-3 were obtained by immunizing rabbits with a rivanol filtrate of human milk, and exhaustion of the resulting immune serum with human seminal plasma and donors' blood serum. To obtain specific antiserum against SLA-4 the material for immunization consisted of the fraction of leukocyte lysate adsorbed on con-A-sepharose, and salted out in a semisaturated solution of ammonium sulfate; to remove contaminating antibodies against ballast proteins, exhaustion with liver and heart extracts was used. Monospecific antiserum against SLA-5 was obtained by immunizing rabbits with thermostable fraction of the rivanol filtrate from lysate of leukocytes from donors' blood and exhaustion of the resulting antiserum with heart extract.

Immunodiffusion analysis was carried out with a standard monospecific test system [5], whose sensitivity was taken to be 0.1 mg% when an enlarged well for the sample for analysis was used.

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TABLE 1. Comparative Physicochemical Characteristics of Three Human SLA

Parameter tested	SLA-3	SLA-4	SLA-5
mol. wt., daltons	35 000 ± 3 000	80 000 ± 6 000	40 000 ± 5 000
Relative electrophoretic mobility	0.59 ± 0.04	0.56 ± 0.03	0.72 ± 0.06
Staining for glycoproteins	Positive	Positive	Positive
Staining for lipoproteins	Negative	Negative	Negative
Binding with con-A-sepharose	Does not bind	Binds	Binds
Thermolability	Thermolabile	Thermolabile	Partially thermostable
Salting out with ammonium sulfate, % saturation	35–55	30–50	30–60
Precipitation with rivanol	Not precipitated	0.4%	Not precipitated
Precipitation by sulfosalicylic acid, M	0.5	0.3	0.5
Precipitation by trichloroacetic acid, %	2	2	2
Sialic acids	Not determined	Not determined	Not determined
Behavior toward enzymes:			
Trypsin	Destroyed	Destroyed	Resistant
Papain	Resistant	"	"
DNase	"	Resistant	"
RNase	"	"	"
Hyaluronidase	"	"	"

EXPERIMENTAL RESULTS

Three soluble leukocyte antigens (SLA) were identified in the composition of the leukocyte lysates by means of corresponding monospecific antisera; the results of comparative physicochemical analysis of these antigens are given in Table 1. The β_1-glycoprotein with mol. wt. 35,000 daltons was called SLA-3, the β_1-globulin with mol. wt. of 80,000 daltons SLA-4, and the α_2-glycoprotein with mol. wt. of 40,000 daltons SLA-5, for the α_2-macroglycoprotein and the thermostable α_2-globulin discovered and characterized previously by the writers [3], which also are leukocytic in nature, can best be named within the limits of a single classification as SLA-2 and SLA-1 respectively.

SLA-3, SLA-4, and SLA-5 are glycoprotein in nature (positive stain and, in addition, in the case of SLA-4 and SLA-5, binding with con-A-sepharose), but they do not contain in their composition the carbohydrate moiety of sialic acids (within the limits of sensitivity of the neuroaminidase test for a decrease in anodal electrophoretic mobility). All three antigens are precipitated by sulfosalicylic and trichloroacetic acids, evidence of the small contribution of the carbohydrate component to the composition of these glycoproteins.

The identified antigens were sensitive to different degrees to the action of proteolytic enzymes: Whereas SLA-4 was easily destroyed by both trypsin and papain, SLA-3 was resistant to the action of papain but was degraded by trypsin. SLA-5 preserved its antigenic structure during contact with both enzymes.

By their behavior toward salting out with ammonium sulfate (precipitated within the saturation range from 30 to 60%), SLA-3, SLA-4, and SLA-5 can be characterized as globulins.

SLA-3 and SLA-4 are thermolabile (they lose their antigenic properties on heating to 70°C for 30 min), but SLA-5 is relatively thermostable (on heating to 100°C for 1 h 20% of the molecules of this glycoprotein completely preserve their antigenic structure). The heterogeneity of SLA-5 relative to the action of the temperature factor is evidence of the existence of at least two fractions of this antigen, but the possibility cannot be ruled out that some SLA-5 molecules become thermostable through the formation of complexes with certain other thermostable substances.

Comparative data on the content of the three identified leukocytic antigens in various human tissue extracts and biological fluids are given in Table 2. They show that, besides circulating leukocytes, SLA-3, SLA-4, and SLA-4 are contained mainly by extracts of organs rich in lymphoid tissue, especially the spleen. It must be pointed out that the results of determination of these antigens in organ extracts may be influenced to some degree by the presence of blood as an impurity, for it is practically impossible to remove all the blood from the tissue.

The high content of SLA-3 and SLA-4 in specimens of pus (obtained by opening carbuncles and abscesses) will be noted, and SLA-3 in addition is constantly discovered in samples of human milk, amniotic fluid, and saliva (Table 2).

The content of SLA-3, SLA-4, and SLA-5 in the leukocyte lysate prepared by freezing and thawing a leukocyte suspension once was virtually indistinguishable from that obtained by the use of a prolonged extraction pro-

TABLE 2. Comparative Immunodiffusion Determination of Three Leukocytic Antigens in Various Human Tissue Extracts and Biological Fluids (M ± m)

Test object	Total number of tests	Antigen content, mg%		
		SLA-3	SLA-4	SLA-5
Leukocyte lysate	18	1,4±0,2	6,0±0,7	0,4±0,1
Pus	7	24,8±3,2	2,0±0,3	1,8±0,2
Spleen	11	2,2±0,2	1,8±0,2	4,3±0,5
Lymph node	5	1,6±0,2	1,4±0,2	1,2±0,1
Lung	6	Traces	1,6±0,3	Traces
Kidney	11	1,0±0,1	1,2±0,2	»
Liver	9	Traces	—	»
Heart	6	—	—	»
Brain	6	—	—	—
Blood serum	26	Traces	—	—
Milk	8	2,6±0,2	—	—
Saliva	14	1,6±0,3	—	—
Amniotic fluid	16	0,7±0,1	—	—
Urine	7	—	—	—

Legend. Traces – antigen concentration does not exceed 0.2 mg%.

cedure, with Triton X-100 and Tween-80 as detergents [3]. This fact may be evidence that there is no close connection between the identified antigens and leukocyte membranes, by contrast, for example, with transplantation antigens. Transplantation antigens are often conventionally called leukocytic (HLA), but they are detected by methods quite different in principle, utilizing the property of isoantigenicity [1], whereas the SLA which we have identified do not behave as isoantigens.

The functional role of SLA-3, SLA-4, and SLA-5, their connection with concrete leukocyte populations, and their synthesis in different pathological states are matters for further study.

LITERATURE CITED

1. N. V. Medunitsyn, V. I. Litvinov, and A. M. Moroz, Mediators of Cellular Immunity and Intercellular Interaction [in Russian], Moscow (1980).
2. D. D. Petrunin, I. M. Gryaznova, Yu. A. Petrunina, et al., Byull. Éksp. Biol. Med., No. 5, 600 (1978).
3. D. D. Petrunin, Yu. M. Lopukhin, M. N. Molodenkov, et al., Byull. Éksp. Biol. Med., No. 4, 66 (1982).
4. H. Friemel (editor), Immunological Methods [Russian translation], Moscow (1979).
5. N. I. Khramkova and G. I. Abelev, Byull. Éksp. Biol. Med., No. 12, 107 (1961).