

EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

The Sera of Patients with Acquired Immunodeficiency Syndrome Contain Specific Antibodies in a Latent State

L. P. Sizyakina, V. M. Orlova, and A. M. Poverennyi

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Latent antibodies reacting with antigens of human immunodeficiency virus are detected in patients with acquired immunodeficiency syndrome. Antibodies are detected by ion-exchange chromatography on QAE-Sephadex. A relationship is noted between the disease stage and the level of latent antibodies.

Key Words: *acquired immunodeficiency syndrome (AIDS); natural antibodies; enzyme immunoassays for AIDS diagnosis*

Scientists earlier isolated the agent of immunodeficiency from a number of biological fluids containing no cells, including blood serum and plasma [2].

Later antigen p24 was detected. The level of the antigen in the plasma correlated with its infectivity and the disease severity [3]. Further studies revealed that this antigen can react with various components of the plasma, specifically with the components of the complement system [4], acute phase proteins [5], and fibronectin [1].

Certain polyanions are known to inhibit virus-cell interactions [7] and the process of virus replication.

Previously we showed that blood sera contain rather high titers of latent natural antibodies capable of reacting with DNA and other polyanions [6]. These antibodies were detected using ion-exchange chromatography on QAE-Sephadex [6].

The data gave us grounds to assume that some of the antibodies to human immunodeficiency virus (HIV) in patient sera may be latent, the level of latent antibodies depending on the clinical status. This hypothesis was experimentally verified.

Medical Radiology Research Center, Russian Academy of Medical Sciences, Obninsk; Rostov Medical Institute, Rostov-on-Don (Presented by A. F. Tsyb, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

Blood sera of 20 children aged 4 to 7 years suffering from acquired immunodeficiency syndrome (AIDS) were tested. The disease stages were classified as stage IIb (generalized lymphadenopathy and hepatosplenomegalia) and IIIa (same as IIb concomitant with candidiasis, pneumocystic pneumonias, etc.)

HIV antibodies were detected in enzyme immunoassay (EIA) using the Vektor test system. Immunoblotting was carried out using Pasteur WB.

The sera were fractionated on QAE-Sephadex A-50 (Pharmacia) as described elsewhere [6]. The procedure consisted in the following: the serum was equilibrated with phosphate buffer, pH 7.3, and passed through a column packed with QAE-Sephadex (fraction I). The column was washed and eluted with the same buffer containing 0.5 M NaCl (fraction II). The initial serum and fractions I and II were brought to 1:100 dilution with phosphate buffer, pH 7.3, and titration was carried out.

RESULTS

Table 1 presents the results of titration of antibodies reacting with HIV antigens in the sera and fractions

TABLE 1. Results of EIA with the Sera of AIDS Patients (Stages IIb and IIIa) before and after Ion-Exchange Chromatography

Serum No.	Optical density			
	whole serum	fraction I	fraction II	increase of density
<i>Stage IIb</i>				
1	0.485	0.563	0.502	2.1
2	0.931	0.964	0.924	1.99
3	0.571	0.384	0.417	1.4
4	1.184	0.906	1.116	1.7
5	0.562	0.193	0.375	1.01
6	0.8	0.456	0.411	1.08
7	1.005	0.48	0.45	0.83
8	1.113	0.79	0.842	1.46
9	0.443	0.257	0.383	1.45
10	0.736	0.384	0.346	0.99
<i>Stage IIIa</i>				
11	1.271	1.019	1.103	1.67
12	0.634	0.605	0.7	2.05
13	0.763	0.675	0.767	1.9
14	1.091	1.057	1.018	1.9
15	0.669	0.703	0.613	1.97
16	0.464	0.297	0.307	1.3
17	0.505	0.544	0.372	1.81
18	0.826	0.805	0.756	1.88
19	0.858	0.753	0.741	1.74
20	1.491	1.501	1.332	1.9

after fractionation of the sera of children with stage IIb and IIIa AIDS on QAE-Sephadex. It is noteworthy that ion-exchange chromatography boosts the titer of antibodies, as can be seen from the results of EIA. The table demonstrates that the total optical density of fractions I and II in patients with stage IIIa is 1.7 to 2.1 times higher than the optical density of the initial serum (mean density 1.88). Immunoblotting revealed antigens tested in the initial sera in the fractions.

A different picture is seen in patients with stage IIb. The optical density in these patients is only 1.4 times higher, and is virtually unchanged in half of the patients (sera Nos. 3, 5, 6, 7, and 10).

Previously it was demonstrated [6] that ion-exchange chromatography reveals antibodies present in the sera in a latent state, probably in complexes with

various components of the serum. As the transition to stage IIIa set in, latent antibodies to HIV antigens were detected in high titers in all patients. Detailed laboratory and clinical studies will no doubt make it possible to assess the significance of this phenomenon.

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