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ANTIGEN-SPECIFIC DETERMINATION OF SERUM LEVELS OF HBsAg/IgM and HBsAg/IgG CIRCULATING IMMUNE COMPLEXES IN HBV-INFECTED PATIENTS BY ELISA

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During the recognition and elimination of hepatitis B virus its surface antigen (HBsAg) is present in patients' blood sera mainly in the form of infectious and noninfectious circulating immune complexes (HBsAg-containing CIC) [5, 10]. During the formation of these CIC, HBsAg may form complexes not only with specific antibodies to it (pre-S_1, pre-S_2, pre-S), but also with modified host proteins: polymerized human serum albumin (pHSA) [9], immunoglobulin M [11] and G [6], and with the corresponding autoantibodies to them.

Correspondingly, the qualitative and quantitative composition of HBsAg-containing CIC and, in particular, the ratio of virus-specific antibodies and autoantibodies to host proteins, are largely determined by the character and outcome of immunologic resolution of HBV infection (or HBV + HDV infection on account of HBsAg common to them).

It has recently been shown that injection of human HBsAg/anti-HBs CIC obtained in vitro (or monoclonal anti-HB "a" of class G with a preserved Fc-fragment) stimulates the more effective proliferative response of HBsAg-specific T lymphocytes and a more effective anti-HBs-response, requiring a 100-500 times lower concentration of HBsAg for this purpose [7, 8].

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The frequency of incorporation of virus-specific and/or autoantibodies to host proteins in the composition of natural HBsAg-containing CIC has received little study. All that is known is that if anti-HBs of class M are removed from the blood sera of patients with acute HBV infection, virtually 100% of the HBsAg is extracted. In this case, autoantibodies to polymerized human albumin (anti-pHSA-M), capable of mimicking anti-HBsM, may be included also in the composition of the HBsAg/IgM CIC [12]. From the diagnostic point of view, experience of working in the field of serodiagnosis of the various forms of human virus hepatitis has shown that reliability of detection of virus-specific structures can be substantially increased only by their parallel detection in the free and bound form in CIC.

This paper gives the results of development and clinical trials of diagnostic ELISA test systems for the antigen-specific detection of HBsAg/IgM and HBsAg/IgG CIC relative to the detection of free HBsAg and specific antibodies to hepatitis A, B, and D viruses in acute and chronic forms of virus hepatitis in adults and children.

EXPERIMENTAL METHOD

The antigen-specific determination of HBsAg/IgG CIC followed the procedure of indirect ELISA by the method of Pernice et al. [13, 14], with the following modifications: as the solid-phase antibodies of the carrier (polystyrene) we compared rabbit anti-HBs obtained from HBsAg/anti-HBs CIC prepared in vitro [1] and monoclonal anti-HB "a" (a commercial preparation from the D. I. Ivanovskii Institute of Virology, Academy of Medical Sciences of the USSR, Moscow). By analogy with the method of Pernice and Sedlaček [13], we developed a method of antigen-specific determination of HBsAg/IgM CIC, with the aim of eliminating false-negative results obtainable on detection of only HBsAg/IgG CIC. A parallel determination by ELISA was made of free HBsAg [2] and anti-HBs of the M and G classes, by indirect ELISA as developed by the writers previously, using pepsin-treated HBsAg as the solid phase antigen [3].

The principle of ELISA for antigen-specific determination of HBsAg-containing CIC [13] is based on binding of the free antigenic determinants (AD "a") of HBsAg incorporated into a CIC, with anti-HBs fixed to the solid phase of the carrier, followed by detection of HBsAg/IgM (or IgG) CIC – specific antibodies to the H-chains of human IgM or IgG, labeled with peroxidase (anti-H-IgM, Px and anti-H-IgG, Px, goat) (a commercial preparation from the N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow). The specificity of binding of HBsAg, included in the composition of the CIC recorded, was monitored in ELISA No. 2 (antigen-specific determination of IgM/HBsAg or IgG/HBsAG CIC with the aid of anti-IgM (or anti-IgG) of the solid phase of the carrier and anti-HBs, Px-conjugate). During parallel testing of the blood sera of patients with acute and chronic HBV infection (107 samples) 91% agreement of the results was obtained with some differences in titers. As the positive control (K^+) we used HBsAg/IgM and HBsAg/IgG CIC prepared in vitro, and as the negative control (K^-) we used a pool of donors' sera, negative for HBsAg-containing CIC. Altogether 202 blood sera from patients with acute HBV infection and with different degrees of severity (groups 1-4), from 53 patients with chronic HBV infection (group 5), and from 35 patients (children) with chronic forms of virus hepatitis, negative for HBsAg, but having antibodies to the delta-agent in 100% of cases (anti-HDB+, group 6), were studied.

In a separate study, the formation of HBsAg-containing CIC was monitored in children developing virus hepatitis in the Tadjik SSR (1987) during an epidemic, where, in the course of identifying the causes of the virus hepatitis epidemic we established the serologic diagnosis of epidemic hepatitis "both A and B" because of the simultaneous recording of antibodies of the M and G classes to hepatitis viruses A (anti-HAVM/G) and B (anti-HBcM/G and anti-HBsM/G) [4].

EXPERIMENTAL RESULTS

Table 1 gives the results of parallel detection of free HBsAg, HBsAg/IgM CIC, HBsAg/IgG CIC, and anti-HBs of class G in the blood sera of patients with acute and chronic forms of virus hepatitis. Table 1 clearly shows that the efficacy of formation of HBsAg-containing CIC accompanied by anti-HBsG is dominant in the mild form of the disease (groups 1 and 2). If the course of HBV infection is severe (group 4), on the other hand, discovery of free HBsAg (50.4%) is dominant with the lowest percentage of detection of anti-HBsG (11.9%), and is 2-3 times less effective than the formation of HBsAg-containing CIC. In adults with chronic HBV infection (group 5) discovery of HBsAg/IgG was dominant (62.2%) in only 15% of cases – accompanied by free HBsAg. In children with chronic HBV + HDV infection (group 7), negative for HBsAg but having antibodies to the delta-agent, detection of HBsAg/IgM CIC predominated over detection of HBsAg/IgG CIC, but their percentage detection was only half as high as when the disease followed a mild course. Together with

TABLE 1. ELISA. Diagnostic and Clinico-Pathogenetic Importance of Detection of HBsAg-Containing CIC (in % of number of samples tested) in Blood Sera of Patients with Acute and Chronic Forms of HBV Infection

Character of clinical course of virus hepatitis	HBsAg	Anti-HBsG	HBsAg/IgM CIC	HBsAg/IgG CIC	Free HBsAg + HBsAg/ CIC
Acute form					
Mild course: 1st day of icteric period	26 3,8	26 73	15 60,6	26 53,8	26 0
Mild course: 2-31 days	47 30	47 42,5	19 63,1	47 40,4	19 15,7
Moderately severe course	62 56,4	62 32,2	16 33,3	62 25,8	16 31
Severe course	109 50,4	109 11,9	35 17,1	109 32,1	35 31,4
Chronic HBV infection	53 22,6	53 28,6	33 30,3	53 62,2	33 15,1
Chronic HBV + HDV infection (children)	35 0	35 17,1	35 28,5	35 11,1	35 0

TABLE 2. ELISA. Efficacy of Formation of HBsAg-Containing CIC Depending on Degree of Severity of Clinical Course of Epidemic Virus Hepatitis "Both A and B" in Children in the Tadjik SSR (1987)

Character of clinical course of virus hepatitis	Day	Anti-HBV-M	Anti-HBV-G	HBsAg	HBsAg/ IgM CIC	HBsAg/ IgG CIC	Anti-HBcM	Anti-HBcG	Anti-HBsG	Billirubin
Virus hetapitis "both A and B"										
Mild course, 6 years 8 months	2	+—	—	—	—	+	+	+	+	76,8
	5	+	—	—	+	+	—	+	+	81,0
	8	—	+	—	+	—	+	++	+	71,8
	14	—	+	—	—	+	—	+	++	25,6
	19	+	+	—	+	+	—	+	++	16,8
Moderately severe course, 7 years	5	+	+	+	—	—	+	+	+	111,2
	9	+	+	+	—	+	+	+	+	25,6
	15	+	—	+	—	+	+	+	+	20,5
	24	+	+	+	—	+	+	+	+	16,8
Severe course, 2 years, 1 month	2	+	—	—	+	—	—	+	—	171,0
	5	—	+	—	+	++	—	+	—	121,0
	8	—	+	—	++	+	—	+	—	51,0
Spordiac virus hepatitis	23	—	+	—	+	+	—	+	—	20,5
Severe course, 11 months	9	—	—	—	++	—	—	—	—	383,0
	11	—	—	—	+++	—	—	—	—	
				(11 days – coma)						

detection of HBsAg/IgM CIC (28.5%), free anti-HBs of the M class were recorded in these children (27%), mainly as mutually exclusive markers.

Thus parallel detection of free HBsAg, HBsAg/IgM CIC, HBsAg/IgG CIC, and anti-HBs by ELISA in patients with acute and chronic forms of virus hepatitis is of significant diagnostic and clinico-pathogenetic importance. The reason is that it is only in 15-30% of cases that free HBsAg and HBsAg-containing CIC are recorded simultaneously (mainly in patients with a moderately severe and severe form of the infection). In the remaining cases, especially in the phase of immunologic resolution of the infection, they are present in blood sera as mutually incompatible markers. Table 2 gives the results of detection of HBsAg-containing CIC in the course of the disease in children who developed the illness during an epidemic of virus hepatitis in the Tadjik SSR (1987), and diagnosed serologically by us as virus hepatitis "both A and B" [4].

Table 2 shows that in the epidemic of "both A and B" virus hepatitis in children of the Tadjik SSR effective formation of HBsAg-containing CIC detectable along with anti-HBsG, takes place in the mild and moderately severe forms of the disease, but without the latter in the case of a severe course of the disease. It is also clear from Table 2 that free HBsAg is not a reliable serologic marker in epidemic and sporadic virus hepatitis and is mutually incompatible with the formation of effective concentrations of HBsAg/IgM CIC and HBsAg/IgG CIC.

Thus, parallel detection of HBsAg/IgM CIC and HBsAg/IgG CIC, if the appearance of free anti-HBsG is taken into account, is of significant diagnostic and clinicopathogenetic importance in sporadic and epidemic versions of human virus hepatitis, in which the host's immune response is associated with the surface antigen of hepatitis B virus.

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