NOTES

Specificities of Albumin Receptors and Albumin Antibodies

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Receptor sites for polymerized human serum albumin (pHSA) on the envelope of hepatitis B virus (hepatitis B surface antigen) and antibodies to pHSA in sera of patients with liver disease were differentiated by an indirect fluorescent antibody method. Analysis of the specificities of pHSA receptors and antibodies to pHSA by hemagglutination inhibition suggested that pHSA receptors reacted with a species-specific site on pHSA, whereas "autoantibodies" to pHSA cross-reacted with pHSA from different species. These human antibodies inhibited the binding of hepatitis B surface antigen-associated receptors to pHSA.

Several studies, including our own, have demonstrated receptor sites for polymerized human serum albumin (pHSA) on the envelope (hepatitis B surface antigen [HBsAg]) of hepatitis B virus (1, 4, 12, 13, 15, 16). Receptors for pHSA have also been observed on the surface of rabbit hepatocytes (9). On the basis of these and other observations, the hypothesis was formulated that pHSA mediates the attachment of hepatitis B virus to hepatocytes during infection (4). We suggested that antibodies to pHSA, which are frequently found in sera of patients with various liver diseases (2, 5-8, 11, 16), may interfere with the attachment of hepatitis B virus to hepatocytes and reduce infectivity. Therefore, we attempted to differentiate pHSA receptors from antibodies to pHSA and to determine the sites on the pHSA molecule that bind to the receptor or the antibody, or both.

Total albumin-binding activity in serum was measured by passive hemagglutination of pHSA-coated erythrocytes (RBCs) by the method of Imai et al. (4) with minor modifications (16). Twelve sera with high titers of albumin-binding activity were selected: four HBsAg-positive sera from patients with chronic active hepatitis (three of these were HBeAg positive); one serum each from patients with HBsAg-negative chronic active hepatitis, primary biliary cirrhosis, and alcoholic liver disease; two sera from a chimpanzee with acute hepatitis B, followed 15 months later by acute hepatitis A (sera obtained before inoculation with hepatitis B, before inoculation with hepatitis A, and during acute hepatitis type non-A/non-B did not show albumin-binding activity); two rabbit antisera to human albumin; and one rabbit antiserum to rat albumin (Cappel Laboratories, Cochranville, Pa., and Behring Diagnostics, Somerville, N.J.). All sera were stored at -20°C. HBsAg and HBeAg were determined by radioimmunoassay (Abbott Diagnostics, North Chicago, Ill.). The human sera exhibited pHSA-binding activity at titers of 1:32 and higher. The chimpanzee sera obtained during acute viral hepatitis B and acute viral hepatitis A had titers of 1:1,024 and 1:256, respectively. The titer of the xenogeneic antiserum to albumin was 1:50,000, 1:250,000 for rabbit anti-human and 1:500,000 for rabbit anti-rat albumin antiserum.

Indirect fluorescent antibody staining of pHSA-coated RBCs after incubation with the sera facilitated the distinction of immunoglobulin-mediated binding from receptor binding to pHSA (16). The staining patterns for bound HBsAg and immunoglobulins were strikingly different. HBsAg attached to the surface of pHSA-coated RBCs as numerous fine granules (Fig. 1), whereas immunoglobulin binding resulted in a smooth linear surface staining (Fig. 2). Four HBsAg-positive human sera showed HBsAg-associated binding sites for pHSA. In two of these, and in the HBsAg-negative sera from patients with primary biliary cirrhosis, chronic active hepatitis, and alcoholic liver disease, binding of immunoglobulin G (IgG), IgM, and, rarely, IgA to pHSA was detected. In this test system, cross-inhibition experiments with sera containing HBsAg-associated pHSA receptors or antibodies to pHSA (at endpoint dilution) were performed. The binding of HBsAg to pHSA-coated RBCs was completely blocked by human antibodies to pHSA, but not by xenogeneic antisera, whereas HBsAg did not interfere.
with the reaction of antibodies with pHSA-coated RBCs.

The specificity of the albumin-binding activity was tested by hemagglutination inhibition, using polymeric and monomeric albumins from different species for inhibition. Polymeric and monomeric albumins were prepared from crystallized human serum albumin (Nutritional Biochemical Corp., Cleveland, Ohio), crystallized bovine serum albumin (Miles Laboratories, Inc., Elkhart, Ind.), and crystallized rat serum albumin (Miles Laboratories) by cross-linking with glutaraldehyde, followed by chromatography on a Sephadex G-200 column (16). To assess the degree of polymerization, the optical densities of polymers and monomers at comparable protein concentrations were measured at 525 nm (3). The optical densities were as follows: pHSA, 0.033, monomeric human serum albumin, 0.003; polymeric bovine serum albumin, 0.024; monomeric bovine serum albumin, 0.002; polymeric rat serum albumin, 0.036; monomeric rat serum albumin, 0.003. Heat aggregation of human serum albumin was performed by the method of Onica et al. (14), followed by Sephadex G-200 chromatography as described above. For hemagglutination inhibition, endpoint dilutions of sera were incubated with the antigens in serial dilutions starting at a protein concentration of 1 mg/ml (10). The lowest protein concentration showing significant inhibition of hemagglutination was recorded. The results are summarized in Table 1. HBsAg-associated pHSA receptors in two human HBsAg-positive sera and in the serum of the chimpanzee during acute viral hepatitis B were inhibited by glutaraldehyde-polymerized human albumin only. Five human sera with antibodies to pHSA, including two HBsAg-positive sera, which also contained pHSA receptors and the chimpanzee serum obtained during acute viral hepatitis A, showed inhibition by glutaraldehyde-polymerized albumin from different species. There was no correlation between the protein concentrations of the inhibiting albumin polymers and the species of origin. The lowest inhibitory protein concentrations ranged from 250 µg/ml to 100 ng/ml, whereas monomeric and heat-treated albumins did not react at 1 mg/ml. In contrast, hemagglutination of pHSA-coated RBCs by rabbit anti-human serum albumin and rabbit anti-rat serum albumin was inhibited by the homologous polymeric and monomeric albumins at protein concentrations of less than 100 ng/ml, whereas higher protein concentrations were required for inhibition by heterologous polymeric and monomeric albumins and by heat-treated monomeric albumin.

![Fig. 1. HBsAg binds to the surface of pHSA-coated RBCs in a finely granular pattern (indirect immunofluorescence method [×1,500]).](image1)

![Fig. 2. Autoantibodies to polymerized albumin bind to pHSA-coated RBCs in a smooth pattern (indirect immunofluorescence method [×2,500]).](image2)

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<tr>
<th>Table 1. Specificities of albumin receptors and albumin antibodies as determined by hemagglutination inhibition</th>
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<td><strong>pHSA Receptor or Antibody</strong></td>
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<td><strong>Receptors</strong></td>
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<td>Xenogeneic antibodies</td>
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<td>* HSA, Human serum albumin; BSA, bovine serum albumin; RSA, rat serum albumin; Poly, polymeric; Mono, monomeric.</td>
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These three reaction patterns (Table 1) suggest that different antigenic determinants of the polymeric albumin molecule are involved. Receptors for pHSA appear to bind to a species-specific site, whereas "autoantibodies" to pHSA seem to react with a species nonspecific determinant on the pHSA molecule, and xenogenic antibodies to albumin with a determinant that is shared by polymeric and monomeric heat- and glutaraldehyde-treated albumins, as well as native albumin. These findings were confirmed by 0.6% agarose gel double immunodiffusion except for the reaction of HBsAg-associated receptors with pHSA. Immunodiffusion is either not sensitive enough or not suited to demonstrate this receptor-ligand interaction.

To date, no direct evidence has been reported to support the hypothesis that pHSA forms a link for the attachment of hepatitis B virus to hepatocytes. The species specificity of HBsAg-associated pHSA receptors would explain the restricted host range of hepatitis B virus (mainly humans and chimpanzees). Further studies are required to determine whether pHSA receptors are specific for hepatitis B virus and absent from hepatitis A virus and hepatitis non-A/non-B virus.

The results of the cross-inhibition experiments, i.e., inhibition of HBsAg receptor-mediated binding to pHSA-coated RBCs by human antibodies to pHSA, support our hypothesis that antibodies to pHSA in sera of patients with liver disease may interfere with the attachment of hepatitis B virus to hepatocytes and reduce infectivity.

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LITERATURE CITED


