JOURNAL OF VIROLOGY, May 1983, p. 653–655
0022-538X/83/050653-03$02.00/0

Vol. 46, No. 2

Inhibition of a Common Human Anti-Hepatitis B Surface Antigen Idiotype by a Cyclic Synthetic Peptide

RONALD C. KENNEDY,[1] GORDON R. DREESMAN,[1] JAMES T. SPARROW,[2] ALAN R. CULWELL,[2] YANUARIO SANCHEZ,[1] IRINA IONESCU-MATIU,[1] F. BLAINE HOLLINGER,[1] AND JOSEPH L. MELNICK[1]*

Department of Virology and Epidemiology[1] and Department of Medicine,[2] Baylor College of Medicine, Houston, Texas 77030

Received 27 September 1982/Accepted 24 January 1983

A common human anti-hepatitis B surface antigen idiotype-anti-idiotype reaction was partially inhibited by a cyclic synthetic hepatitis B surface antigen peptide. Reduction of the intrachain disulfide bond and subsequent alkylation destroyed its inhibitory activity, suggesting that a conformation-dependent group *a* epitope was associated with this cyclic peptide.

A number of early studies (reviewed in reference 11) demonstrated that antibody produced in response to the hepatitis B surface antigen (HBsAg), the surface or envelope material of the 42-nm hepatitis B virus (HBV) particle, is protective against infection, whereas antibody to the core antigen has no protective effect. Effective Formalin-inactivated HBV subunit vaccines have been produced (10) and licensed (6) which utilize purified HBsAg derived from the plasma of healthy persons chronically infected with HBV.

Two alternative approaches are available for the preparation of a well-defined, HBsAg-specific vaccine. The first became possible when the DNA fragments derived from HBV were cloned into bacterial plasmids (12, 17). The second alternative recently became possible when four laboratories generated synthetic peptides (2, 5, 9, 14, 18) that contained amino acid sequences analogous to those associated with the major building block of HBsAg, i.e., P25, a polypeptide with a molecular weight of 25,000 (13). P25 in conjunction with a glycosylated form with molecular weight of 30,000 (GP30) represents more than 65% of the protein mass of the 22-nm lipoprotein spheres associated with HBsAg (11). The presence of an immunogenic determinant in association with a P25-GP30 mixture has been demonstrated in that chimpanzees immunized with such material were solidly protected against challenge with infectious HBV (4).

Recently, a report from our laboratory described a common idiotype (ID) shared by human antibodies to HBsAg (anti-HBs) (7). The common ID was associated in part with the antibody-combining site, because HBsAg and nondenatured P25-GP30 partially inhibited the common ID binding its anti-idiotypic antiserum. Further characterization revealed that the induction of the common ID was by the group-specific *a* determinant of HBsAg and that conformation was important, as denatured HBsAg viral polypeptides virtually lost their inhibitor capacity when compared with native polypeptides (8). Based on these data, we decided to examine the possibility that two synthetic polypeptides (5) would also inhibit the common ID-anti-ID reaction.

Both peptides 1 and 2 were capable of partially inhibiting (30 to 33%) the ID-anti-ID reaction, whereas no inhibition was detected with similar concentrations of an unrelated synthetic peptide containing 18 amino acids (Fig. 1). On a weight basis the two peptides and intact HBsAg particles seemed equally efficient as inhibitors. The specificity of the ID-anti-ID reaction was demonstrated by the inability of human serum albumin, human immunoglobulin G, and hepatitis A virus to inhibit the reaction (Table 1). It has been shown that a native HBsAg-derived aggregate of P25-GP30 isolated as a micelle is more immunogenic than intact HBsAg particles (15, 20). Based on this observation, we used HBsAg-derived polypeptide micelle preparations as inhibitors of the ID-anti-ID reaction to establish whether this virus-specific polypeptide free of human serum components was a more efficient inhibitor on a weight basis than HBsAg particles. A much higher level of inhibition (65%) was noted with the micelle than with intact HBsAg (Table 1). By using an increased quantity (250 μg) of peptide 1 as an inhibitor, a similar degree of inhibition (63%) was obtained. However, on a molar basis peptide 1 was much less efficient at inhibiting the ID-anti-ID reaction (Table 1). Higher concentrations of peptide 1 were not available to test for increased inhibition values

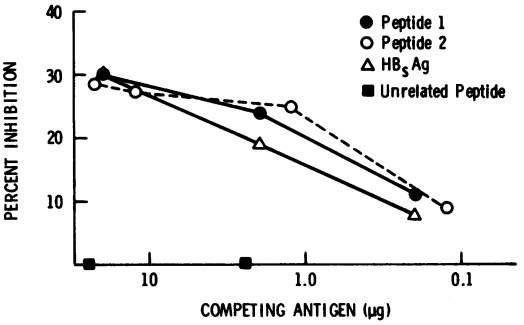

FIG. 1. Inhibition of binding of ^{125}I-labeled ID to its anti-idiotypic antiserum by various synthetic peptides and intact HBsAg particles as determined by radioimmunoassay. The unrelated synthetic peptide is an analog of apolipoprotein C-2, which contains the sequences from amino acids 60 through 78. Briefly, a limiting dilution of a rabbit anti-idiotypic antiserum was used to coat the wells of a polyvinyl, flat-bottom microtiter plate (Dynatech Laboratories, Alexandria, Va.) for 8 h at 4°C. After the addition of 1% bovine serum albumin to block nonspecific binding sites on the wells, equal volumes of the respective inhibitor and of ^{125}I-labeled, specifically purified human immunoglobulin G anti-HBs (ID) which had been preincubated for 4 h at 4°C were added to the anti-idiotypic antiserum-coated wells. After incubation for 1 h at 25°C, the excess radioactivity was removed, and the wells were washed and counted. Percent inhibition (% I) was calculated by using the following formula: % I = 100 × [1 −(cpm with inhibitor/cpm without inhibitor)].

in the ID-anti-ID reaction. Similar results have been obtained during the immunochemical analysis of other proteins. Different peptide fragments of sperm-whale myoglobin exhibited only a portion of the antigenic reactivity that was demonstrated by the intact protein (1). This decreased efficiency was dependent on both the size of the peptide and its location with respect to other amino acids that were responsible for stabilizing the three-dimensional conformation of myoglobin.

The role of disulfide bonds in maintaining the antigenicity and immunogenicity of HBsAg has been reported by several laboratories (3, 16, 19). Our synthetic peptides were cyclized by the formation of an intramolecular disulfide bond between cysteine residues at positions 124 and 137 (5). The importance of conformation was demonstrated since reduction of the intrachain disulfide bond and alkylation of the cysteine residues of peptide 1 completely destroyed its ability to inhibit the ID-anti-ID reaction (Table 1).

The observations that our synthetic polypeptides can inhibit the binding of a common ID with its anti-idiotypic antiserum and can also block the binding between a common anti-HBs ID and HBsAg provide direct evidence that the peptides represent a portion of HBsAg determinants which elicit antibodies produced during HBV infection in humans. Similar to the antigenic activity of native HBsAg, the antigenic determinants appear to be conformation dependent. The synthetic peptides produced by Lerner et al. (9), Prince et al. (14), and Bhatnagar et al. (2) induce antibody responses in rabbits or mice. In addition, Vyas (18) and Prince et al. (14) have reported that synthetic peptides containing immobilized P25 amino acid residues 134 to 146 and 138 to 149, respectively, bind human anti-HBs. Bhatnagar et al. (2) reported that amino acid residues 139 to 147 contain the group-specific *a* determinant, whereas the data presented in this report suggest that a second conformation-dependent *a* epitope may be associated with residues 119 to 137. It is noteworthy that on a molar basis peptide 1 was

TABLE 1. Percent inhibition of the common anti-HBs ID binding its anti-idiotypic antiserum with different concentrations of various inhibitors

Inhibitor	Concn (μg)	Inhibition (%)[a]
HBsAg	25	33
	2.5	22
Native HBsAg-derived polypeptide[b]	7.5	65
	0.75	22
	0.075	12.5
Peptide 1	250	63
	25	33
	2.5	30
Peptide 1, reduced and alkylated[c]	25	1
	2.5	0
Human immunoglobulin G	20	0
	5	1
Human serum albumin	20	0
	5	0
Hepatitis A virus	20	0
	5	0

[a] The inhibition determinations represent the means of triplicate values. Solid-phase radioimmunoassay was used to measure the percent inhibition of the ID-anti-ID reaction (7).

[b] Aggregate of P25-GP30 isolated as a micelle.

[c] Peptide 1 was reduced with a threefold molar excess of 2-mercaptoethanol and alkylated with a 10 M excess of iodoacetate under an N_2 atmosphere. The reduced peptide was desalted on a Bio-Gel P2 column equilibrated in bicarbonate buffer (pH 8.6). The concentration of peptide was determined by absorbance at 275 nm, using an extinction coefficient of 8.3 for a 1% solution.

approximately 10^3-fold less efficient than intact HBsAg in inhibiting the ID-anti-ID reaction, whereas the peptide of Bhatnagar et al. was 10^{10}-fold less efficient at binding a rabbit anti-peptide 139 to 147 when compared with intact HBsAg. Although no comparison can be made between techniques involving the inhibition of an ID-anti-ID reaction and direct binding, the inability of the respective peptides to compete equally with HBsAg on a molar basis indicates that neither peptide represents the complete *a* determinant and suggests that more than one *a* epitope is present.

Inhibition of a common anti-HBs ID-anti-ID reaction offers an alternative approach for immunoanalysis of synthetic peptide preparations. The inhibition by the two synthetic polypeptides in this report suggests that they are related to antigenic determinants responsible for eliciting a population of anti-HBs expressing a common ID.

This investigation was supported by research contract DAMD17-82C-2155 from the U.S. Army Medical Research and Development Command, by research grant Q-435 from the Welch Foundation, and by National Research Service Award CA 09197 from the National Institutes of Health. J.T.S. is an Established Investigator of the American Heart Association.

LITERATURE CITED

1. **Atassi, M. Z.** 1975. Antigenic structure of myoglobin: the complete immunochemical anatomy of a protein and conclusions relating to antigenic structure of proteins. Immunochemistry **12**:423–438.
2. **Bhatnagar, P. K., E. Papas, H. E. Blum, D. R. Milich, D. Nitecki, M. J. Karels, and G. N. Vyas.** 1982. Immune response to synthetic peptide analogues of hepatitis B surface antigen specific for the *a* determinant. Proc. Natl. Acad. Sci. U.S.A. **79**:4400–4404.
3. **Dreesman, G. R., F. B. Hollinger, R. M. McCombs, and J. L. Melnick.** 1973. Alteration of hepatitis B antigen (HB Ag) determinants by reduction and alkylation. J. Gen. Virol. **19**:129–134.
4. **Dreesman, G. R., F. B. Hollinger, Y. Sanchez, P. Oefinger, and J. L. Melnick.** 1981. Immunization of chimpanzees with hepatitis B virus-derived polypeptides. Infect. Immun. **32**:62–67.
5. **Dreesman, G. R., Y. Sanchez, I. Ionescu-Matiu, J. T. Sparrow, H. R. Six, D. L. Peterson, F. B. Hollinger, and J. L. Melnick.** 1982. Antibody to hepatitis B surface antigen after a single inoculation of uncoupled synthetic HBsAg peptides. Nature (London) **295**:158–160.
6. **Immunization Practices Advisory Committee.** 1982. Inactivated hepatitis B virus vaccine. Morbid. Mortal. Weekly Rep. **31**:317–328.
7. **Kennedy, R. C., and G. R. Dreesman.** 1983. Common idiotypic determinant associated with human antibodies to hepatitis B surface antigen. J. Immunol. **130**:385–389.
8. **Kennedy, R. C., Y. Sanchez, I. Ionescu-Matiu, J. L. Melnick, and G. R. Dreesman.** 1982. A common human anti-hepatitis B surface antigen idiotype is associated with the group *a* conformation-dependent antigenic determinant. Virology **122**:129–131.
9. **Lerner, R. A., N. Green, H. Alexander, F. T. Liu, J. G. Sutcliffe, and T. M. Shinnick.** 1981. Chemically synthesized peptides predicted from the nucleotide sequence of the hepatitis B virus genome elicit antibodies reactive with the native envelope protein of Dane particles. Proc. Natl. Acad. Sci. U.S.A. **78**:3403–3407.
10. **McAuliffe, V. J., R. H. Purcell, and J. L. Gerin.** 1980. Type B hepatitis: a review of current prospects for a safe and effective vaccine. Rev. Infect. Dis. **2**:470–492.
11. **Melnick, J. L., G. R. Dreesman, and F. B. Hollinger.** 1976. Approaching the control of viral hepatitis type B. J. Infect. Dis. **133**:210–229.
12. **Moriarty, A. M., B. H. Hoyer, J. W. K. Shih, J. L. Gerin, and D. H. Hamer.** 1981. Expression of the hepatitis B virus surface antigen gene in cell culture by using a simian virus 40 vector. Proc. Natl. Acad. Sci. U.S.A. **78**:2606–2610.
13. **Peterson, D. L.** 1981. Isolation and characterization of the major protein and glycoprotein of hepatitis B surface antigen. J. Biol. Chem. **256**:6975–6983.
14. **Prince, A. M., H. Ikram, and T. P. Hopp.** 1982. Hepatitis B virus vaccine: identification of HBsAg/a and HBsAg/d but not HBsAg/y subtype antigenic determinants on a synthetic immunogenic peptide. Proc. Natl. Acad. Sci. U.S.A. **79**:579–582.
15. **Sanchez, Y., I. Ionescu-Matiu, J. T. Sparrow, J. L. Melnick, and G. R. Dreesman.** 1982. Immunogenicity of conjugates and micelles of synthetic hepatitis B surface antigen peptides. Intervirology **18**:209–213.
16. **Sukeno, N., R. Shirachi, J. Yamaguchi, and N. Ishida.** 1972. Reduction and reoxidation of Australia antigen: loss and reconstitution of particle structure and antigenicity. J. Virol. **9**:182–183.
17. **Tiollais, P., P. Charnay, and G. N. Vyas.** 1981. Biology of hepatitis B virus. Science **213**:406–411.
18. **Vyas, G. N.** 1981. Molecular immunology of hepatitis B surface antigen (HBsAg), p. 227–237. *In* P. Maupas and P. Guesry (ed.), Hepatitis B vaccine. Elsevier/North-Holland Publishing Co., Amsterdam.
19. **Vyas, G. N., K. R. Rao, and A. B. Ibrahim.** 1972. Australia antigen (hepatitis B antigen): a conformation antigen dependent on disulfide bonds. Science **178**:1300–1301.
20. **Zuckerman, A. J., C. R. Howard, and J. Skelly.** 1981. Hepatitis B micelle vaccines, p. 251–262. *In* P. Maupas and P. Guesry (ed.), Hepatitis B vaccine. Elsevier/North-Holland Publishing Co., Amsterdam.