A SURROGATE HEPATITIS B VIRUS ANTIGENIC EPITOPE REPRESENTED BY A SYNTHETIC PEPTIDE AND AN INTERNAL IMAGE ANTIIDIOTYPE ANTIBODY

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There is considerable current interest in the development of techniques for the precise manipulation of the immune response resulting in either enhancement or suppression. In the context of responses to infectious agents, the use of molecules representing single defined epitopes able to substitute for antigen (i.e., surrogate antigens) would provide considerable advantages over the use of the native antigen (1–5). Two types of reagent could potentially fulfill this role, the first is a synthetic peptide antigen, and the second an internal image antiidiotype mAb. Because of the development of the technique of solid-phase peptide synthesis and methods for the prediction of potential antigenic determinants of complex antigens, it is now possible to synthesize peptides representing these antigenic determinants. An internal image antiidiotype is the hypervariable region of an antiidiotype that behaves as a surface determinant on the antigen with respect to recognition by antibody. A number of such polyclonal reagents have been developed, including rabbit antiidiotypic antibodies specific for an interspecies crossreactive idiotype on antibodies to hepatitis B surface antigen (6), but monoclonal internal image antiidiotypes appear to offer considerable advantages (7–13).

Over 200 million individuals are persistently infected with the hepatitis B virus (HBV),[1] presenting a major risk of transmission via contaminated blood and blood products. In addition exposure to HBV is associated with the development of hepatocellular carcinoma late in life. Hepatitis B is a particularly appropriate system in which to investigate the potential of surrogate antigenic epitopes for immunization. Vaccine development has been hindered however, as the virus cannot be grown in vitro, and standard virological techniques of detecting neutralizing antibody are not available. The currently licensed hepatitis B vaccines are therefore derived by extensive purification of the outer surface antigen of the virus (HBsAg), which is present in excess in the plasma of human HBV carriers (14). New, alternative sources of HBsAg include antigen expressed in yeast by recombinant DNA technology (15). It is clearly established (16) that

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[1] *Abbreviations used in this paper:* HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus.

antibody against the *a* group determinant, which occurs on all HBV isolates regardless of subtype and on the HBsAg vaccine, is protective against infection. Because of the wide availability of sera from both infected individuals and recipients of the licensed vaccine, as well as the availability of a number of monoclonal and polyclonal antibodies, we have used these antibodies to assess the extent to which synthetic peptide antigens and antiidiotypes mimic antigens expressed by the virus and recognized by the immune system. Our strategy includes measuring the affinity of the antibody-antigen reaction, which gives a valuable estimate of the closeness of fit of the antibody hypervariable regions to the antigenic epitope (17–18). Such measurements therefore give an indication of the similarity of the surrogate antigenic epitope to that on the whole antigen that induced the antibody response in vivo.

Herein, we have investigated the immunochemical characteristics of these two types of surrogate HBsAg antigenic epitopes consisting of (*a*) synthetic peptides representing amino acid residues 139–147, a hydrophilic region corresponding to a part of the *a* determinant present on the 226–amino acid major polypeptide component of HBsAg, and (*b*) a panel of monoclonal antiidiotypes raised against anti-HBs mAb, two of which behave as an internal image of an *a* determinant (19). We demonstrate that these surrogate antigens show concordance, in that the internal image antiidiotypes inhibit the binding of both monoclonal and polyclonal anti-HBs antisera to the cyclical and linear peptide.

Materials and Methods

Antiidiotype mAbs. The generation and maintenance of these antibodies has been described in detail elsewhere (19). DE52-purified antibodies were radiolabelled by the chloramine T method (20), and their antibody activities were assessed after radiolabelling by binding to idiotype-coated microtiter wells.

HBsAg-related Peptides. A soluble complex from HBsAg was prepared by dissociation of native HBsAg particles with 2% Triton X-100 and separation by Con A–Sepharose chromatography (21). This complex contains all the antigenic determinants present on the 25,000 mol wt (p25) and 30,000 mol wt (gp30) HBsAg polypeptides. Two synthetic peptides representing amino acids 124–137 and 139–147, regions of hydrophilicity in the HBsAg, were prepared by chemical synthesis. The sequences of the peptides are as follows. Peptide 124–137: H_2N-cys-met-thr-thr-ala-gln-gly-thr-ser-met-tyr-pro-ser-cys-tyr-COOH; peptide 139–147: H_2N-cys-thr-lys-pro-thr-asp-gly-asn-cys-tyr-COOH. These peptides were produced in both linear and cyclical forms, the latter by the formation of a disulfide bond between the two terminal cysteine residues. The introduction of a tyrosine residue at the *C*-terminus facilitated radiolabelling of the peptides. Both peptides reacted with standard reagents defining the *a* region specificity, but 139–147 was bound by human anti-HBs antibodies with considerably higher affinity when in the cyclized form (17, 18).

Anti-HBs Antibodies. A panel of mAbs and polyclonal human, goat, rabbit, guinea pig, and swine anti-HBs antisera were used. H3F5, a mouse IgG2b anti-HBs mAb was the idiotype used for the generation of the antiidiotype mAbs. This antibody recognizes a highly conserved epitope on every isolate of HBsAg studied. It may thus be considered to be directed to an *a* determinant (22). H3F5 binds both the gp30 p25 complex and the cyclical form of peptide 139–147, but with low affinity (1.3×10^5 and 1.5×10^5/M, respectively), but showed no reactivity with peptide 124–137.

Fluid-phase Inhibition Assay. Appropriate dilutions of anti-HBs antibodies giving 50% binding of a constant amount of ^{125}I-labelled linear or cyclical peptide 139–147 were incubated with 0.2–5.0 μg of the purified antiidiotype mAbs. After incubation at 4°C for 1 h, antibody-bound ^{125}I-labelled peptide was determined by precipitation of the antibody-peptide complex with 50% saturated ammonium sulfate. The results are expressed as

percent inhibition of binding of the labelled peptide by the anti-HBs antibody in the presence of the monoclonal antiidiotype.

Antibody Affinity Measurements. The affinities of the anti-HBs antibody for the monoclonal antiidiotypes and the affinities of the monoclonal antiidiotypes for the monoclonal anti-HBs idiotype were determined using ^{125}I-labelled antibodies by modifications of the previously published method (23). Complexes of anti-HBs and ^{125}I-labelled antiidiotype and of monoclonal antiiodiotype and ^{125}I-labelled monoclonal anti-HBs idiotype were precipitated by PEG at a final concentration of 5%. The equilibrium constant or affinity (K) and the total antibody-combining site concentration were calculated from a modification of the Langmuir equation. Antibody affinity assays were performed in conditions of antigen excess, such that complexes composed of two antigens per antibody were preferentially formed. Under these conditions, antibody affinity rather than avidity is measured, despite the divalency of the antiidiotype (24).

Results

Inhibition of Binding of Anti-HBs Antibodies to Synthetic Peptide 139–147 and the gp30 p25 Complex by Antiidiotype mAbs

The four antiidiotype mAbs (2F10, 4D4, 2E7, and 3H1) were used in assays to assess their ability to inhibit the binding of ^{125}I-labelled C139–147 (cyclical 139–147) by a panel anti-HBs mAbs and a range of polyclonal anti-HBs antisera from a variety of species (Fig. 1). Inhibition of binding of C139–147 by the antibodies tested was obtained with both the 2F10 and 4D4 monoclonal antiidiotypes. Previous studies using an immunofluorescence assay had also shown that these two antibodies both behave as internal images of an *a* determinant of HBsAg (19). Internal image monoclonal antiidiotype 4D4 was, however, unable to inhibit the binding of C139–147 by the mouse anti-HBs mAbs H29.4 and 1329.2.3, or by a pool of anti-HBs Ig from human convalescents. Antiidiotype 2E7 was only able to inhibit the binding of C139–147 by the human and rabbit anti-HBs antisera. Antiidiotype 3Hl did not inhibit the binding of any of the anti-HBs antibodies tested.

The results expressed in Fig. 1 represent the inhibition of binding of C139–147 obtained with 5 μg of the purified antiidiotype antibody. Curves for the inhibition of binding by a range of antiidiotype 2F10 concentrations of the

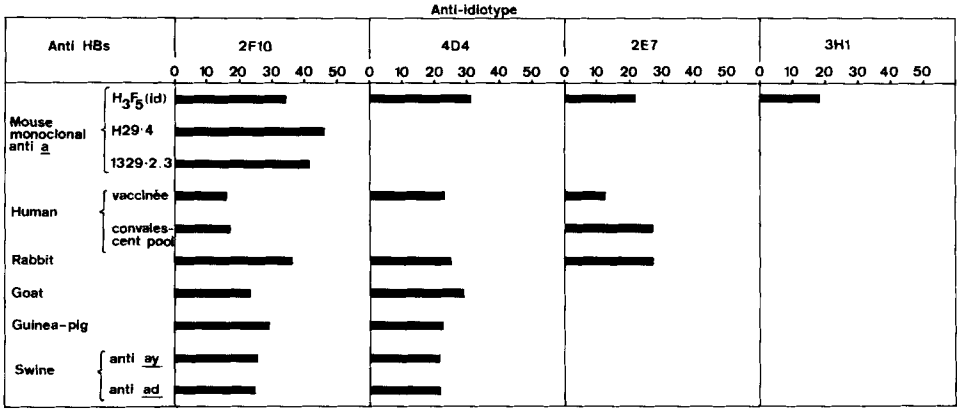

FIGURE 1. Inhibition of binding of a panel of monoclonal and polyclonal anti-HBs antisera to the cyclical peptide 139–147 by approximately 5 μg of monoclonal antiidiotypes.

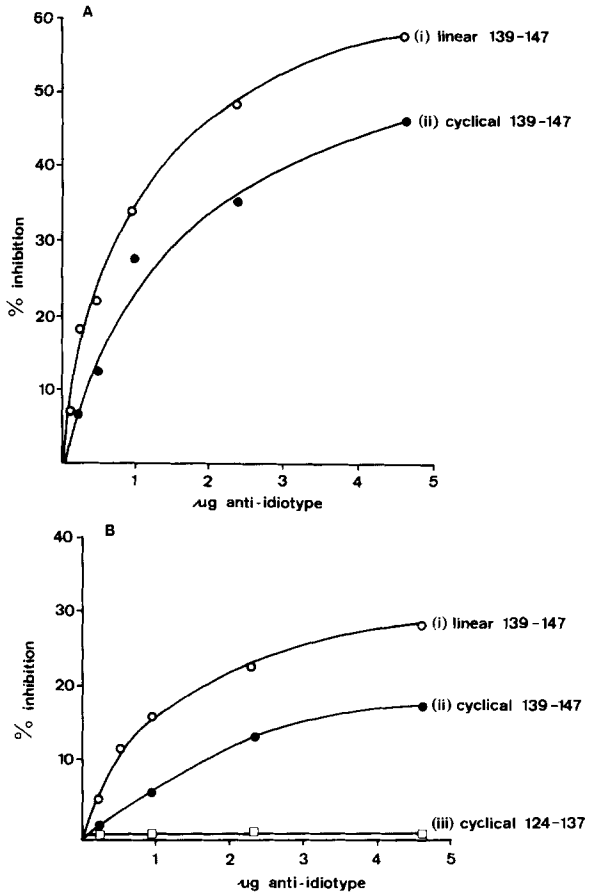

FIGURE 2. Inhibition of binding of (*A*) monoclonal anti-HBs H29.4 and (*B*) polyclonal human anti-HBs antibody to (*i*) the linear and (*ii*) the cyclical peptide 139–147 by the monoclonal internal image antiidiotype 2F10.

monoclonal anti-HBs and pooled human anti-HBs antibodies to both the linear and cyclical forms of 139–147 are shown in Fig. 2. The 2F10 was able to inhibit the binding of the mouse mAb to both linear and cyclical 139–147 (Fig. 2*a*). Furthermore, the antiidiotype inhibited the binding of the linear peptide more effectively than that of the cyclical peptide. The affinity of the monoclonal anti-HBs for the linear peptide was 2.1×10^6/M; and for the cyclical peptide, 5.0×10^6/M. The inhibition of the binding of the pooled human anti-HBs to the linear and cyclical forms of 139–147 by 2F10 (Fig. 2*b*) was lower than that observed with the mouse monoclonal anti-HBs, and was no higher than 28%. Again, the binding of the linear peptide was inhibited to a greater extent than the cyclical peptide. The affinity of the human anti-HBs for the linear peptide was 9.3×10^6/M; and for the cyclical peptide, 9.1×10^7/M. The binding of the pooled human anti-HBs Ig to the cyclical form of a peptide representing amino acids 124–137 of the *y* subtype of HBsAg could not be inhibited by antiidiotype mAb 2F10 (Fig. 2*b*) or by 4D4 or 2E7 (data not shown). The binding of peptide 124–

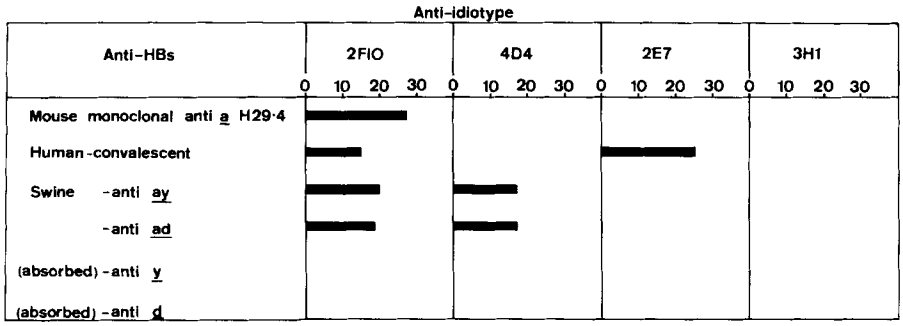

FIGURE 3. Inhibition of binding of anti-HBs antisera to the gp30p25 complex by four monoclonal antiidiotypes.

TABLE I

Affinities of Monoclonal Antiidiotypes for Anti-HBs Idiotype H3F5

Antiidiotype	Affinity (K) for H3F5 idiotype
2F10	3.2×10^8
4D4	5.0×10^7
2E7	1.4×10^8
3H1	2.6×10^8
H29.4 (irrelevant idiotype)	—

137 by a mouse monoclonal anti-HBs (192.75) was not inhibited by any of the antiidiotypes (data not shown).

The gp30 p25 polypeptide complex expresses all the antigenic repertoire of HBsAg. Both antiidiotypes 2F10 and 4D4 inhibited the binding of the gp30 p25 complex by mouse, human, and swine anti-HBs antibodies (Fig. 3), but the levels of inhibition were slightly lower than those seen when the synthetic peptides were used as antigens. The binding of swine anti-HBs *ad* and *ay* antibodies to the gp30 p25 complex was inhibited by both 2F10 and 4D4. However, these antiidiotypes did not inhibit the binding of the polypeptide complex by swine anti-*y* and anti-*d* antibodies, confirming the similarity of the antiidiotypes and C139–147 to an *a* determinant(s) of HBsAg. Antiidiotype 2E7 was only able to inhibit the binding of gp30 p25 by the human anti-HBs. Antiidiotype 3H1 was unable to inhibit the binding of the polypeptide complex by any of the antibodies used.

Affinity Measurements

Affinity of the monoclonal antiidiotypes for the H3F5 anti-HBs idiotype. The four monoclonal antiidiotypes all bound the H3F5 idiotype (to which they are directed) with affinities ranging from 5×10^7 to 3.2×10^8/M (Table I), and differences between the four antibodies were small.

Affinities of anti-HBs antisera for the surrogate antigens. The affinities of human, rabbit, and goat polyclonal anti-HBs antisera for the surrogate antigens (antiidiotypes and peptide 139–147) were assessed and compared with their affinities for the gp30 p25 complex (Table II). The affinities of these three polyclonal

TABLE II

Affinities of Various Polyclonal Anti-HBs Antisera for Surrogate Antigens and gp30 p25 Complex

Antigen	Anti-HBs antiserum					
	Human convalescent pool		Rabbit		Goat	
	Antibody-combining sites (pmol per 10 μl serum)	*K*	Antibody-combining sites	*K*	Antibody-combining sites	*K*
gp30 p25	198	3.0×10^7	172	1.2×10^7	279	3.5×10^7
Cyclical 139–147	97	7.2×10^7	110	3.3×10^7	160	1.4×10^7
Linear 139–147	119	8.0×10^6	106	9.0×10^6	189	5.9×10^6
2F10 antiidiotype	7.7	1.4×10^8	7.2	9.0×10^8	12	8.4×10^8
4D4 antiidiotype	—		6.7	2.0×10^8	11.8	6.6×10^8
2E7 antiidiotype	5.4	5.8×10^8	12.1	3.5×10^8	—	
3H1 antiidiotype	—		—		—	

antibodies for the monoclonal antiidiotypes 2F10, 4D4, and 2E7 ranged from 1.4×10^8 to 9×10^8/M. These values represent high-affinity binding, and are significantly higher than those for the binding of the 139–147 peptide and the gp30 p25 polypeptide complex. However, the levels of such high-affinity antibodies in the sera (expressed as pmol of binding sites) are considerably lower than the levels of the antibodies able to bind the peptide antigens. The results of these binding studies are consistent with the data derived from the inhibition experiments (Fig. 1). Thus the human anti-HBs failed to bind 4D4, the goat anti-HBs failed to bind 2E7, and antiidiotype 3H1 was not bound by any of the anti-HBs antisera.

Discussion

The studies reported here show that the monoclonal antiidiotypes, shown by immunofluorescence to act as internal images of an HBsAg group *a* determinant, compete in inhibition RIA with synthetic peptides representing the same determinant. This is true not only with binding to monoclonal idiotypes derived from the mouse, but also with a variety of polyclonal anti-HBs sera from human and several animal species. The competition by antiidiotype is restricted to the 139–147 peptide representing part of the a group determinant, and there is no influence on the binding of a peptide corresponding with amino acid residues 124–137 of the *y* subtype version of HBsAg. This confirms the results of previous studies, in which swine antisera specific for both *ay* and *ad* determinants lost their ability to react with internal image antiidiotypes when antibody to the *a* but not to the *d* or *y* determinants were absorbed out (25). It is fascinating that antiidiotypes and peptides produced as surrogate antigens using different anti-*a* reagents should show convergence in having this property of mutual inhibition.

The binding of the linear 139–147 peptide by the monoclonal and polyclonal anti-HBs was more readily inhibited by antiidiotype than was the binding of the cyclical peptide, and this is consistent with a lower affinity of the anti-HBs sera

for the linear form. This indicates that the cyclized peptide has a reasonable similarity to the conformation of the native determinant on HBsAg, and that there is a significant loss of entropy in adapting the linear peptide to fit the corresponding antibodies.

Analysis of the binding curves revealed that the internal image antiidiotypes were bound with higher affinity by the various mAb and heteroantisera than were the peptides. In addition, one might envisage that the intact divalent antiidiotype molecule would be bound by the B cell surface receptors in vivo with a higher avidity than would be expected with the presumably monovalent peptide.

Of particular interest was the finding that levels of antibody, expressed as picomoles of antigen-combining sites, of polyclonal sera for the peptides was greater than that for the antiidiotype (Table II). This indicates that the peptide can combine with a wider spectrum of the antibodies in a polyclonal anti-HBs serum than can the antiidiotype, which is therefore more restricted in its simulation of the original HBsAg. Further support for this view comes from the studies on competitive binding for polyclonal antibodies, in which the antiidiotype fails to produce more than a relatively minor degree of inhibition of peptide binding, with inhibition reaching a plateau at ~20%. The implication of these observations for the use of these surrogate antigens for potential vaccines is that, while antiidiotypes may be capable of stimulating B cells of higher affinity, they would react with a more restricted range of B cell specificities than would the peptides.

The plateau of inhibition with antiidiotype of the binding of cyclical peptide to polyclonal anti-HBs is lower than that obtained with the linear peptide. This suggests that, in addition to the affinity considerations discussed earlier, a fraction of the anti-HBs serum may crossreact with antiidiotype and linear peptide, but not with the cyclical peptide. Presumably, the constraints on conformation induced by cyclization preclude some but not all of the structures required to bind antibody, whereas the linear peptide is not subject to the same restrictions.

The various monoclonal antiidiotypes give different patterns of inhibition with the group of monoclonal and polyclonal antibodies used in these studies. It may be useful to evaluate these different patterns in relationship to the analysis of the molecular basis of internal image behavior (25). We postulated that an antiidiotype could resemble the antigen in terms of (*a*) mimickry of the contact residues used to bind antibody, (*b*) an obligatory set of residues on the antibody-combining site common to several different antibodies, or (*c*) an idiotype associated with but not part of the combining site. The latter seems to be excluded by the fact that all the antiidiotypes were completely inhibited in their binding to idiotype by antigen, i.e., they are binding-site related. This leaves us with models based upon contact residues, either on the antigen or on the antibody, as in (*a*) and (*b*) above. The present data do not allow us to differentiate between these two possibilities, but either model can account for the present findings on the basis of a minimum of three contact residues. In Table III, we have assumed that the antiidiotypes can mimic up to three contact residues on the peptide. The results can be accounted for by assuming that the various monoclonal antiidiotypes bear zero, one, two, or all three of the postulated antigenic contact residues on the

TABLE III

Model to Account for Internal Image Reactivities Based Upon Recognition of a Minimum of Three Contact Residues on HBsAg Peptides

Antibodies	Contact residues		
	R1	R2	R3
Anti-HBs	Residues bound by anti-HBs		
Monoclonal			
H3F5	+	+	+
H29.4	−	−	+
1329.2:3	−	−	+
Polyclonal			
Goat	−	+	+
Guinea pig	−	+	+
Swine	−	+	+
Human-vaccineé	+	+	+
-convalescent pool	+	−	+
Rabbit	+	+	+
Anti-Id mAb	Residues mimicked by anti-Id		
2F10	+	+	+
4D4	−	+	−
2E7	+	−	−
3H1	−	−	−

peptide. Clearly, the mAb (2F10) with the maximum resemblance to the antigen and cyclical peptide 139–147 would be likely to be a valuable surrogate to use in a vaccine.

It could be argued that the cluster of epitopes to which we loosely refer as the *a* antigenic determinant might best be simulated in a potential vaccine by a synthetic peptide such as 139–147 or an appropriate cluster of monoclonal internal image antiidiotypes.

Summary

The use of molecules that represent single, defined epitopes able to substitute for antigen (i.e. surrogate antigens) offers considerable advantages over the use of native antigen for the precise manipulation of the immune response. We have investigated the immunochemical characteristics of two types of surrogate hepatitis B surface antigen (HBsAg) epitopes: (*a*) linear and cyclical synthetic peptides representing amino acid residues 139–147, a hydrophilic region corresponding to part of the *a* determinant of the HBsAg, and (*b*) four monoclonal antiidiotypes raised against anti-HBs mAb, two of which behave as an internal image of an *a* determinant.

Polyclonal anti-HBs antisera bound the monoclonal antiidiotypes with affinities of the order of 10^8/M, and to the peptides with >10-fold lower affinities. However, the levels of antibody in the polyclonal antisera for the peptides was

greater than for the antiidiotypes. In inhibition RIA, the surrogate antigens show concordance in that the internal image antiidiotypes inhibit the binding of both monoclonal and polyclonal anti-HBs to the linear and cyclical 139–147 peptides.

These results imply that surrogate antigens could indeed be useful as potential hepatitis vaccines, but while the antiidiotypes may stimulate B cells of higher affinity, they would react with a more restricted range of B cell reactivities than would the peptides. A future HBV vaccine may thus comprise a synthetic peptide such as cyclical 139–147 or a cluster of monoclonal internal image antiidiotypes.

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