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Immunology

Molecular mimicry of hepatitis B surface antigen by an anti-idiotype-derived synthetic peptide

(epitope mimicry/"a" determinant HBsAg/mRNA sequencing/molecular modeling/immune response)

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ABSTRACT **Monoclonal antibody 2F10 is an "internal-image" anti-idiotype (anti-id) antibody capable of mimicking the group-specific "a" determinant of human hepatitis B surface antigen (HBsAg). By mRNA sequencing and computer-assisted molecular modeling of monoclonal antibody 2F10, we identified a 15-amino acid region of the heavy-chain hypervariable region that has partial residue homology with sequences of the "a" determinant epitopes of HBsAg. We have established that a linear 15-mer peptide from a contiguous region on the anti-id antibody can (*i*) generate anti-HBsAg-specific antibodies when injected into mice, (*ii*) prime murine lymph node cells for *in vitro* HBsAg-specific T-cell proliferative responses, and (*iii*) stimulate *in vitro* human CD4+ T cells that were primed *in vivo* to HBsAg by natural infection with hepatitis B virus or vaccination with a commercially available HBsAg vaccine. Significantly, this peptide could also stimulate CD4+ T cells of human hepatitis B virus carriers. We conclude that a 15-mer peptide derived from the anti-id sequence can duplicate the B- and T-cell stimulatory activity of the intact anti-id antibody and the antigen that is mimicked, HBsAg.**

Infection with human hepatitis B virus (HBV) results in a gamut of clinical symptoms ranging from minor flu-like symptoms to death. The wide spectrum of responses is believed to be accounted for by the host's immune response to the virus because HBV is not directly cytopathic for hepatocytes (1). The specific serologic marker of HBV infection is the envelope protein, hepatitis B surface antigen (HBsAg), which contains three related proteins designated S, M (S plus pre-S2), and L (M plus pre-S1). All of these proteins share the 226-amino acid sequence of the S protein (in this paper, HBsAg will refer to the S protein). HBsAg possesses a common group-specific **a** determinant and two sets of mutually exclusive subtype-specific determinants **d/y** and **w/r**. Because antibodies directed toward the **a** determinant confer protection against HBV infection, regardless of subtype (2), any vaccine developed to prevent HBV infection should elicit immunity to the **a** determinant.

The immune network theory proposed by Jerne (3) predicts the appearance of several types of anti-idiotype (anti-id) antibodies during the immune response to a given antigen. The subset of "internal-image" anti-id antibodies (termed Ab2β) has been proposed to be anti-paratopic and to mimic the molecular features of the original antigen (4, 5). This working hypothesis is based on the concept that certain homologous or analogous molecular motifs of the anti-id sequence can mimic specific immunogenic epitopes of the infectious organism, thereby inducing a protective immune response (4, 5). Such anti-id antibodies have been used in various experimental systems as surrogate vaccines against specific bacterial, viral, and parasitic organisms (for review, see refs. 6 and 7).

We produced six monoclonal anti-id antibodies against a monoclonal antibody (mAb), designated H3F5 (id) (8), which recognizes the protective **a** determinant epitope on HBsAg [residues S-(139–147)]. All the anti-id antibodies inhibited the binding of HBsAg to the H3F5 id; however, only two of these anti-ids (2F10 and 4D4) were tentatively classified as internal-image anti-id antibodies by described criteria (9). The anti-id 2F10 was further studied to determine whether it could mimic HBsAg and generate specific B- and T-cell responses. Anti-id 2F10 could elicit, in several mouse strains, anti-HBsAg antibodies and prime T cells *in vivo* that could subsequently be stimulated *in vitro* by HBsAg (43); 2F10 could also stimulate *in vitro* CD4+ T cells from human donors that had been primed *in vivo* to HBsAg as a result of vaccination or HBV infection (10).

To identify the epitopes responsible for this observed mimicry, we began an extensive structural analysis of anti-id 2F10. We show that the mimicry of HBsAg by anti-id 2F10 is associated with a 15-amino acid sequence on its heavy-chain (H chain) hypervariable region. This sequence is partially homologous with **a** determinant epitopes of HBsAg, and a synthetic peptide corresponding to this sequence can duplicate the B- and T-cell stimulatory activity of the intact anti-id and HBsAg.

MATERIALS AND METHODS

***In Vivo* and *in Vitro* Stimuli.** H3F5 is mouse mAb (IgG2b) that is specific for the protective **a** determinant epitope S-(139–147) on HBsAg (8) and is the id that anti-id 2F10 recognizes. 2F10 is a mouse internal-image anti-id mAb of the IgG1 subclass that mimics the group-specific **a** determinant of HBsAg (9). mAbs 2F10 and H3F5 were purified from ascites fluid by 45% ammonium sulfate precipitation and then passage over a protein A column. The murine mAb 2C3 (anti-phthalate, IgG1) was used as an isotype control (S. Ghosh, Indiana State University). Soluble recombinant HBsAg (rHBsAg) and rHBsAg–alum, devoid of pre-S proteins, were provided by W. F. Miller (Merck Sharp & Dohme). 2F10 peptide was synthesized by solid-phase methodology (Multiple Peptide Systems, San Diego) and was conjugated to keyhole limpet hemocyanin (KLH) via glutaraldehyde.

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Abbreviations: HBsAg, hepatitis B surface antigen; rHBsAg, recombinant HBsAg; HBV, human hepatitis B virus; mAb, monoclonal antibody; id, idiotype; H chain, heavy chain; L chain, light chain; V_H and V_L, variable regions of H chain and L chain, respectively; CDR, complementarity-determining region; KLH, keyhole limpet hemocyanin.

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Gel Electrophoresis and Immunoblotting. One-dimensional, discontinuous SDS/12% PAGE analysis (Mini-Protean II, Bio-Rad) as described by Laemmli (11) was done on mAb 2F10 and control mAb 2C3 under reducing and nonreducing conditions. After electrophoresis, gels were prepared for immunoblotting and transferred electrophoretically from SDS gels to nitrocellulose paper (12). The blot was probed with ^{125}I-labeled H3F5 (id) (100,000 cpm/ml) for 2 hr, washed, and exposed to Kodak XAR-5 film with a Cronex intensification screen for 16–18 hr at −70°C.

Primer Extension and Sequencing of mAb 2F10. Primer extension and sequencing of mAb 2F10 RNA was done essentially as described by Geliebter (13) by using the primer 5′-CTCACTGGATGGTGGGAAGATGGATACAGT-3′ for the κ chain and the primer 5′-CAGGGGCCAGTGGATAGAC-3′ for γ1 H chain. The sequence information was analyzed by the IBI/Pustell DNA sequence-analysis system of Pustell and Kafatos (14). For comparing mAb 2F10 light-chain (L) and H-chain amino acid sequences with the HBsAg amino acid sequence, we used the GAP algorithm of Needleman and Wunsch (15).

Modeling of mAb 2F10. Computer-assisted modeling of the Fv region involved interactive graphics modeling based on canonical structures (16, 17); the parent structures (REI, HyHEL-5, McPC603) used for the initial modeling have been solved by x-ray crystallography, and coordinates were obtained from the Brookhaven Protein Data Bank. Residue homology comparison, canonical scaffolding-building, energy minimization, conformational searches (18), and dynamics simulation techniques were all used to construct the model; use of these procedures has been described (19).

Induction of Anti-HBsAg Antibodies. BALB/c mice (five per group) were immunized i.p. on days 0, 7, and 14 with either rHBsAg, anti-id 2F10, or the 15-mer 2F10 peptide. rHBsAg was administered as an alum-adsorbed precipitate at a dose of 0.5 μg per injection. Anti-id 2F10, carrier-free 15-mer 2F10 peptide, or 15-mer 2F10 peptide coupled to KLH (52% peptide by weight) was administered at 100 μg per injection in complete Freund's adjuvant, incomplete Freund's adjuvant, and saline, respectively. Mice were bled retroorbitally before and after immunization, and the sera were evaluated for anti-HBsAg-specific antibodies using an ELISA kit (AUSAB-enzyme immunoassay).

***In Vitro* Proliferation of Mouse Lymph Node T Cells.** Mice were immunized in the hind foot pads with either rHBsAg, anti-id 2F10, or the 15-mer 2F10 peptide–KLH. Amount of antigen and schedule of immunization are as described above. One week after the third injection, the animals were sacrificed, and the popliteal lymph nodes were collected and teased apart, the cells were purified as described elsewhere (20). The enriched T cells were adjusted to a concentration of 2.5×10^5 cells per well. One hundred microliters of cells was plated in 96-well flat-bottomed plates along with 5×10^5 irradiated syngeneic spleen cells as a source of antigen-presenting cells. Stimuli diluted in complete medium were added to the cells in triplicate cultures. The stimuli are as follows: rHBsAg (0.5 μg per well), anti-id 2F10 and isotype control antibody (50 μg per well), 2F10 peptide and control peptide (0.25 μg per well), Con A (1 μg per well), or medium alone. The cells were then cultured for 120 hr as described (20). Proliferation, as measured by [^{3}H]thymidine incorporation, was determined by liquid scintillation spectroscopy. Results are expressed as stimulation index.

Preparation of Human CD4$^+$ T cells. Peripheral blood mononuclear cells were prepared as described (20). The technique of Mage *et al.* (21) as modified by Wysocki and Sato (22) was used to further purify the peripheral blood mononuclear cells into a CD4$^+$ subset by using the following antibodies: anti-Leu2a (mouse mAb that recognizes the CD8 cluster on human T cells; predominantly cytotoxic T/suppressor T cells), anti-21.147 (mouse mAb that recognizes the CD11b/18 cluster and has reactivity against suppressor T cells, natural killer cells, monocytes, and granulocytes) and anti-26.263 (mouse mAb that reacts with the β chain of HLA-DR, -DP, and -DQ). All panning reagents were provided by Robert Evans (Roswell Park Cancer Institute).

CD4$^+$ T-Cell Proliferation Assay. CD4$^+$ cells were cultured in 96-well flat-bottom plates along with autologous adherent cells (source of antigen-presenting cells) at a ratio of 2.5×10^5 CD4$^+$ cells/1.25×10^4 adherent cells per well. All cells were cultured for 7 days in RPMI 1640 medium supplemented as above (no fetal calf serum), containing 10% heat-inactivated human AB$^+$ serum. Stimuli used are described above for mouse T-cell assays.

RESULTS AND DISCUSSION

Determination of Epitopes on Anti-id 2F10 Responsible for Mimicking the a Determinant of HBsAg. Immunoglobulins carry antigenic determinants (ids), and id expression may or may not depend upon the combination of the H and L chains of the antibody. Reducing SDS/PAGE, followed by immunoblot analysis revealed that expression of the internal-image epitope on anti-id 2F10 did not depend upon the combination of H and L chains. The H chain of anti-id 2F10 was strongly detected with the ^{125}I-labeled id (H3F5), whereas the L chain was only marginally detectable (Fig. 1*A*). Our earlier studies had established that T cells obtained from mice primed *in vivo* with the intact anti-id, responded vigorously *in vitro* to purified H chain but only minimally to purified L chain of anti-id 2F10 (43).

To further delineate this anti-id epitope, we sequenced the H- and L-chain variable regions (V_H and V_L, respectively) of mAb 2F10 by using standard mRNA sequencing techniques (13) and deduced the amino acid sequence. The primary amino acid sequences predicted for the V_H and V_L segments of mAb 2F10 are closely associated with the V_H of the J558 family and the κ chain of the variable region of MOPC-149. We then compared amino acid sequences of the anti-id H and L chains with the known sequence of HBsAg by using the GAP algorithm (15). We first compared amino acid sequences of the anti-id antibody with a 13-amino acid sequence on HBsAg [residues S-(135–146)], a region that represents a partial analogue of the group-specific a determinant (25). This alignment showed a 58% similarity and 25% identity between HBsAg-(135–146) and a region encompassing the third complementarity-determining region (CDR3) and part of the adjacent framework (FR3) of the 2F10 H chain (H:88–99; Fig. 1*B*). We also searched for alignment between amino acids in the FR3 and CDR3 regions on the anti-id H chain and the complete HBsAg sequence (226 residues). We obtained values of 60% similarity and 30% identity for a sequence of 11 residues (124–134) of HBsAg that were adjacent to the 13 residues used in the previous alignment. This region of HBsAg is also believed to contain a partial group a determinant epitope of HBsAg (26). Fig. 1*B* shows the alignment obtained. No sequence homology was observed between the V region of the 2F10 L chain and HBsAg.

Genetic variants of HBV (arising from immunological pressure) have been defined, which correspond to amino acid substitutions within the protective a determinant region of HBsAg [S-(139–147)] (27, 28). These reports demonstrated an identical amino acid substitution of Gly-145 → Arg in the S protein of HBV. Recent studies using synthetic peptides corresponding to residues S-(139–147) of HBsAg, with a Gly-145 → Arg substitution demonstrated a decrease in antigenicity and abrogation of *in vitro* proliferative responses of both human and mouse T cells primed *in vivo* to HBsAg (29). It is important to note that this glycine residue is conserved within the sequence of the anti-id 2F10 H chain that we have identified as homologous to the a determinant region of

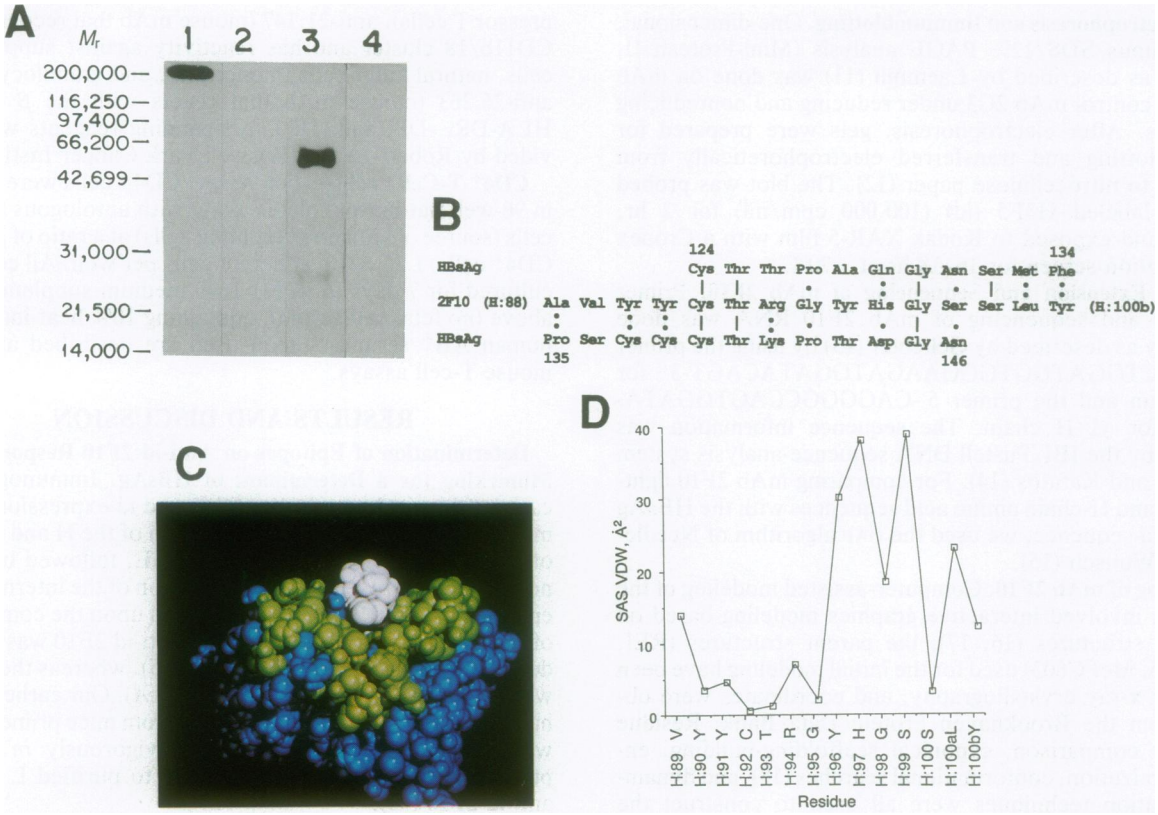

FIG. 1. Determination of epitopes on anti-id 2F10 mAb responsible for mimicking the a determinant of HBsAg. (*A*) Composite of SDS/PAGE with subsequent immunoblot analysis probed with ^{125}I-labeled H3F5. Lanes: 1 and 2, mAbs 2F10 and 2C3 nonreduced; 3 and 4, mAbs 2F10 and 2C3 reduced. (*B*) Comparison of the region of deduced amino acid sequence of mAb 2F10 H chain showing homology with the HBsAg S-region amino acid sequence. – –, Identity; ···, similarity. S-region amino acids of HBsAg use the numbering system in Valenzuela *et al.* (23). The numbering system of anti-id 2F10 is as in Fig. 1*D*. (*C*) Computer-simulated model of mAb 2F10 Fv region depicting the H chain CDR3 residues H:96 to H:100b (white), other H- and L-chain CDRs (green), and framework regions (blue). (*D*) The solvent-accessible surface (SAS) van der Waals area (VDW Å) of the CDRs was calculated according to the algorithm ACCESS (24); the SAS area of the H chain CDR3 is shown; residues H:96Y–H:100bY are highly exposed.

HBsAg. Some other important residues to consider correspond to positions that distinguish serological subtypes of HBV from each other and additionally some positions for which information is available on amino acid replacement and its effect on immunological reactivity of HBsAg. Lysine occupies position 141 in the HBsAg sequence, whereas arginine occupies that position in the anti-id sequence. However, we have previously shown, by deliberate amino acid replacement, that a Lys → Arg substitution at this position does not alter the ability of the peptide to elicit T-cell proliferation of human cells *in vitro* (29). On the other hand, at position 142 there is a proline in the HBsAg sequence and a glycine in the anti-id 2F10 sequence. The presence of a glycine or an isoleucine at this position has been reported to decrease the antigenicity of HBsAg (30). At position 143 there is either a serine or a threonine, depending on the HBV subtype; we observe a tyrosine at this position in the 2F10 peptide sequence. The effect of such a replacement at this position on the immunogenicity of HBsAg is presently unknown.

Threonine occupies position 126 in almost all HBV subtypes (except adr, which has an isoleucine); in the anti-id sequence there is an arginine at this position. However, from replacement set analysis we know that an arginine replacement does not alter *in vitro* proliferation of human T cells obtained from HBsAg-vaccinated donors (20). At position 131 there is a threonine instead of an asparagine, corresponding to subtypes ayw and adw, respectively; serine at this position in the anti-id sequence would relate the residue more closely to threonine than to asparagine. Additionally we know (20) that deliberate replacement of serine at this position is tolerated at the level of human T-cell proliferation. All subtypes except adw have methionine at position 133, and in the anti-id we report the presence of leucine. However, experiments with replacement set analysis have already established that Met/Lys → Leu is immunologically tolerated (20). Finally, subtype ayw has a Tyr-134, also present in the anti-id sequence. The presence of tyrosine rather than phenylalanine at position 134 was also reported by Charnay *et al.* (31) and Pasek *et al.* (32).

To visualize this epitope, computer-assisted molecular modeling of the H and L chains CDRs of mAb 2F10 was completed, using canonical immunoglobulin structure libraries, energy-minimization techniques, and molecular dynamics simulations (16, 17, 19, 33). Visual and computational inspection (Fig. 1 *C* and *D*) of the completed mAb 2F10 Fv model revealed that H-chain amino acids in positions H:96–H:100b (Fig. 1*D*) had considerable surface solvent accessibility [determined by the algorithm ACCESS (24)]. These data and the fact that secondary-structural analysis of this area revealed a β-turn, support the prediction that this region is probably antigenic (34, 35). Additionally, such CDR loop motifs have been shown to be highly antigenic as idiotypic determinants and have been identified as the determinants responsible for anti-id molecular mimicry in another system (36). Based on the sequence homology searches and examination of the computer-assisted molecular modeling, we elected to synthesize a 15-mer peptide (AVYYCTRGYHGSSLY), referred to as 15-mer 2F10 peptide. This peptide was tested to determine whether it could duplicate the *in vitro* and *in vivo* properties displayed by the intact anti-id 2F10 mAb.

Anti-id 15-mer Peptide Can Induce Antibodies to the Antigen That the Peptide Mimics. Fig. 2*A* shows that 15-mer 2F10

peptide covalently coupled to KLH could successfully elicit anti-HBsAg antibodies when administered to BALB/c mice, although the response was less than that seen after injection of rHBsAg or intact 2F10. Anti-HBsAg antibodies were also elicited when mice were immunized with the peptide alone (not conjugated to a protein carrier), providing the initial evidence that this 15-mer contained not only a B- but also a T-cell epitope [the antibody response to HBsAg is strictly T-cell dependent (37)].

Mouse T-Cell Stimulation. To provide further evidence that the 15-mer 2F10 peptide contained a T-cell stimulatory epitope, BALB/c mice were primed *in vivo* with the 15-mer 2F10 peptide, and the primed T cells were tested for their ability to proliferate *in vitro* to the appropriate stimuli. Fig. 2*B* shows that T cells obtained from mice primed *in vivo* with 15-mer 2F10 peptide can respond *in vitro* to 15-mer 2F10 peptide and intact 2F10. More importantly, proliferation also occurs after *in vitro* stimulation with HBsAg. This result has significance in considering the use of this anti-id peptide as an alternative vaccine. For comparison, mice were also immunized with rHBsAg or the intact anti-id. Primed T cells from each group could also be appropriately stimulated (Fig. 2*B*).

Human T-Cell Stimulation. To demonstrate the efficacy of our anti-id 15-mer peptide in a more clinically relevant system, human CD4+ T cells from individuals primed *in vivo* by vaccination or from HBV infection were cultured in *in vitro* proliferation assays with a panel of different stimuli. The results of Fig. 3 clearly establish that anti-id 2F10 15-mer peptide can stimulate *in vitro* human CD4+ T cells primed *in vivo* by vaccination with the licensed hepatitis B vaccine (i.e.,

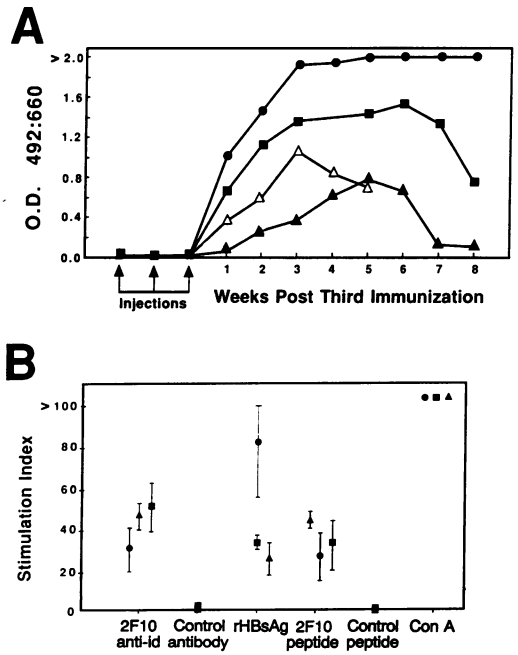

FIG. 2. 2F10 15-mer peptide can elicit anti-HBsAg antibodies *in vivo* and can prime T cells for an *in vitro* HBsAg-specific T-cell proliferative response in BALB/c mice. (*A*) Comparison of anti-HBsAg antibody responses elicited by immunization with rHBsAg–alum (●), anti-id 2F10 mAb (■), 2F10 15-mer peptide (△), or 2F10 15-mer peptide–KLH (▲). Results are expressed as OD 492:660 nm of the average of five mice per group at a 1:20 dilution. Sera from mice injected with unconjugated 2F10 15-mer peptide were assayed at a 1:2 dilution. (*B*) Results of proliferation assays with purified T cells from mice primed *in vivo* with rHBsAg–alum (●), anti-id 2F10 mAb (■), or 2F10 15-mer peptide–KLH (▲). Cells were stimulated in triplicate cultures with the following *in vitro* stimuli: rHBsAg (0.5 μg per well), anti-id 2F10 mAb and control mAb (50 μg per well), 2F10 15-mer peptide and control peptide (0.25 μg per well), Con A (1 μg per well), or medium alone.

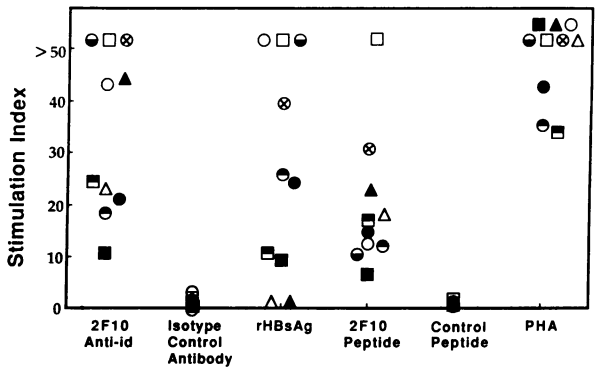

FIG. 3. Anti-id peptide can stimulate human CD4+ T cells *in vitro* that were primed *in vivo* as a result of vaccination with the licensed hepatitis B vaccine (RecombiVax, HB; ⊖, ⊗, ⊖, ○, ●); natural infection with the hepatitis B virus (⊟, □, ■), and hepatitis B carriers (△, ▲). *In vitro* stimuli are the same as for Fig. 2*B*, except phytohemagglutinin (PHA) was used instead of Con A. Each symbol represents one person.

individuals serologically HBsAg−, anti-HBsAg+, anti-HBcAg−). These findings agree with those of Fig. 2*B* with T cells from mice immunized with rHBsAg. Individuals primed *in vivo* to HBsAg from infection with HBV are distinguished serologically as HBsAg−, anti-HBsAg+, anti-HBcAg+ (naturally infected and recovered individuals) or as HBsAg+, anti-HBsAg−, anti-HBcAg+ (HBV carriers). In a manner analogous to that reported above for vaccinated individuals, CD4+ T cells from naturally infected and immune individuals could also be stimulated *in vitro* by anti-id 15-mer peptide, intact anti-id antibody, and rHBsAg. Thus, 2F10 anti-id 15-mer peptide could stimulate CD4+ T cells primed *in vivo* either as a result of vaccination or exposure to live virus.

Before synthesis of the 15-mer 2F10 peptide, we had demonstrated that the intact anti-id antibody could stimulate CD4+ T cells from 27 HBV carriers (M.W.P., A.T., S. Lall, P. Ogra, and Y.T., unpublished data). When the anti-id 2F10 15-mer peptide became available, we had the opportunity to test its usefulness in two additional HBV carrier patients (Fig. 3). It is significant to note that 2F10 15-mer peptide and intact 2F10, which both mimic HBsAg, can stimulate CD4+ T cells from HBV carriers, whereas the native antigen, HBsAg, cannot. It may be argued that this anti-id antibody is stimulating T cells nonspecifically by T-cell receptor crosslinking; however, the stimulation seen with the monovalent Fab fragment of the anti-id (data not shown) and the anti-id 2F10 15-mer peptide refutes this possibility. No *in vitro* responses were elicited with peripheral blood mononuclear cells from nonimmune individuals (HBsAg−, anti-HBsAg−, anti-HBcAg−), thus establishing specificity of the assay (data not shown).

CONCLUSIONS

Mimicry of external antigens by internal-image anti-id epitopes can occur by either analogous or cross-reactive homologous id mimicry. As in nature, where most mimicry is analogous, the same is true for anti-id mimicry, where elementary interactive components (electrostatic, H bonding, van der Waals, etc.) contribute to the formation of a three-dimensional region on the anti-id antibody responsible for mimicry of the external antigen. Thus, where anti-id epitopes mimic carbohydrate or lipid epitopes, mimicry is obviously established by a functional conformation rather than identity at the primary-amino acid-sequence level.

Although many investigators have reported the existence of anti-id antibodies and their relative usefulness as surrogate antigens, only limited reports testify to anti-id mimicry due to homologous cross-reactive id mimicry. Ollier *et al.* (38) have shown that the diversity region of the H-chain segment of an

internal-image anti-id mAb presents a Glu-Ala-Tyr (GAT)-like epitope. In the rabbit a1 allotype system, two anti-id mAbs were produced that show a1-like internal image. mRNA sequencing of these anti-id proteins revealed amino acid homology on the V_H (CDR2) with the nominal antigen, however, in reverse orientation. Synthetic peptides made from this a1-like region could inhibit the binding of a1 immunoglobulin to anti-a1 antibody (39). No *in vivo* B- or T-cell responses to either of these anti-id peptides have yet been reported. Clearly, the best-studied system to date of cross-reactive homologous epitope mimicry is the work in the reovirus type 3 system (36, 40, 41). By amino acid sequencing and computer-modeling procedures, homology between a portion of the hemagglutinin on the reovirus type 3 and the variable regions of an anti-id mAb has been shown (36). Synthetic peptides from these regions of homology on the anti-id (V_H and V_L) antibody show quite different biological effects (41). The V_L peptide defines a predominant B-cell epitope (which induces potent neutralizing antibodies) and an epitope that is important in delayed-type hypersensitivity responses to the reovirus type 3 hemagglutinin. However, the V_H peptide defines a major helper T-cell epitope on the anti-id that is recognized by reovirus type 3-induced helper T cells.

We have not only identified the homology between the anti-id region and the a determinant of HBsAg but have also demonstrated that a synthetic peptide representing this area of homology can mimic native HBsAg by eliciting specific B- and T-cell responses *in vivo* and *in vitro*. One of the limitations of most synthetic peptides is that they fail to produce a secondary protective immune response due to a lack of an anamnestic recognition of helper T-cell epitopes. Sharing of ids between T and B cells of related antigen specificity would permit use of the same anti-id/anti-id-derived peptide to activate both T and B cells for an anamnestic recognition of similar epitopes on the infectious organism. We have demonstrated that the anti-id 2F10 can stimulate antigen-specific B and T cells and that a 15-amino acid synthetic peptide can duplicate these responses. This aspect may circumvent the problem of using nonhuman antibody preparations (mouse monoclonal anti-id) in human subjects. Additionally, the responses seen using anti-id/anti-id-derived peptide in stimulating HBV carrier $CD4^+$ T cells are promising and may also have relevance in individuals who are nonresponders to the licensed HBV vaccine. The cellular mechanism for unresponsiveness to HBsAg at the T-cell level in HBV carriers has not been determined. Antigen-specific suppressor mechanisms and genetically determined nonrecognition have been implicated (42). The anti-id and anti-id peptide may overcome these limitations because they mimic only a limited portion of the HBsAg sequence, do not carry suppressor epitopes, and can probably be recognized by a wide range of human major histocompatibility complex haplotypes. These results strongly support the usefulness of the 2F10 15-mer peptide, not only for experimental animal models but also in a clinically relevant human system.

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