Mimicry of the *a* Determinant of Hepatitis B Surface Antigen by an Antiidiotypic Antibody. I. Evaluation in Hepatitis B Surface Antigen Responder and Nonresponder Strains

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Summary

B and T cell responses of several strains of mice, immunized with a monoclonal antiidiotype (anti-Id) that mimics the *a* determinant of hepatitis B surface antigen (HBsAg), were studied to determine if the immune response to the anti-Id was regulated by H-2-linked immune response genes as has been previously observed for HBsAg. We report that immunization with anti-Id could elicit HBsAg-specific antibodies in mice of the H-$2^{d,q,\text{ or }f}$ haplotype and in an outbred wild mouse strain (*Mus spretus*), thus circumventing the H-2 haplotype restriction pattern observed when immunizing with HBsAg in H-2^f mice. Purified lymph node T cells from mice of the H-$2^{d\text{ or }q}$ haplotype and *M. spretus* that were primed in vivo with HBsAg or anti-Id could be stimulated in vitro with either HBsAg or anti-Id but not with an irrelevant antibody of the same subclass as the anti-Id. However, purified lymph node T cells from H-2^f mice that were primed in vivo with the anti-Id could only be stimulated in vitro with anti-Id. No in vitro stimulation whatsoever was observed in H-2^f mice immunized with HBsAg. The effect of processing and presentation of the anti-Id by antigen-presenting cells (APC) was studied in mice of the H-2^d haplotype. Stimulation of purified lymph node T cells by HBsAg and anti-Id was shown to be strictly dependent on APC and restricted by major histocompatibility complex class II antigens at the I-A locus. Treatment of APC with paraformaldehyde or chloroquine abrogated the T cell response to all antigens except for a nine–amino acid synthetic peptide representing a partial analogue of the group *a* determinant of HBsAg S(139–147). The significance of these results is discussed in the context of understanding the mechanism of mimicry elicited by the anti-Id.

Analogous to the situation with HIV, there is no inbred animal model for hepatitis B virus (HBV)[1] infection and therefore most studies have evaluated HBV proteins as immunogens rather than as infectious agents. Extensive work by Milich (1) and others has established that the murine immune response to the group-specific *a* determinant of hepatitis B surface antigen (HBsAg) is H-2 restricted and shows a hierarchy of responsiveness at the B and T cell level. The hierarchy of the humoral response to HBsAg is as follows: high responders, H-$2^{q,d}$; intermediate responders, H-2^a > H-2^b > H-2^k; and nonresponders, H-$2^{s,f}$. The T cell proliferative response to HBsAg generally paralleled the same H-2 restriction observed for the humoral response.

For the study of immune responses to HBV, we produced anti-Ids against a mouse mAb, designated H3F5 (2) (Id, Ab1), which recognizes the protective *a* determinant epitope on HBsAg. The fusion of spleen cells obtained from BALB/c mice immunized with H3F5 resulted in six monoclonal anti-Ids. All of them inhibited the binding of HBsAg to the H3F5 Id, however, only two of these anti-Ids (designated as 2F10 and 4D4) recognized an interspecies crossreactive Id, as demonstrated by the reaction of these hybridomas with polyclonal anti-HBs sera from a variety of species (3, 4). Since the *a* determinant on HBsAg is important in conferring protection (5), we therefore wanted to determine whether our anti-Ids carried an internal image of the *a* determinant. To test this, anti-HBs sera (*ad* or *ay* specific) were adsorbed with HBsAg *ad* or *ay*. Only adsorption of antibodies against the *a* determinant abolished reactivity of the polyclonal sera with the anti-Ids (3, 4). 2F10 and 4D4 were therefore tentatively classified as "internal image" anti-Ids (Ab2β), and one of these anti-Ids (2F10) was further studied to determine if it could mimic HBsAg and generate specific B and T cell responses. We report here that immunization with the anti-Id 2F10 could

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

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elicit HBsAg-specific B and T cell responses that, unlike the antigen it mimics, are not restricted by a known HBsAg nonresponder haplotype.

Materials and Methods

Animals. BALB/c mice (H-2^d) were obtained from West Seneca Labs (West Seneca, NY). B10.M(H-2^f) and SWR(H-2^q) were obtained from The Jackson Laboratory (Bar Harbor, ME). Wild mice, *Mus spretus*, were a kind gift from Dr. Verne Chapman (Roswell Park Cancer Institute). All mice were female and 6–8 wk old at the start of the study.

In Vivo and In Vitro Stimuli. 2F10 is a mouse monoclonal internal image anti-Id of the IgG1 subclass that has been shown to mimic the group-specific *a* determinant of HBsAg. 2F10 ascites was purified by 45% ammonium sulphate precipitation followed by passage over a protein A column. 2F10 Fab was prepared by digestion of the purified anti-Id with immobilized papain followed by a protein A–Sepharose column to remove Fc fragments and any undigested IgG, according to manufacturer's directions (Pierce Chemical Co., Rockford, IL). Separation of anti-Id 2F10 into H and L chains was performed essentially as described elsewhere (6), except dithiothreitol was used as the reducing agent. The murine mAb 2C3 (specific for the hapten phthalate) has the same subclass as 2F10 and was used as a control throughout this study (kindly provided by Dr. S. Ghosh, Indiana State University, Terre Haute, IN). HBsAg is the outer envelope protein of the hepatitis B virus. rHBsAg and rHBsAg-alum devoid of pre-S proteins were generously provided by Dr. W. F. Miller (Merck, Sharp and Dohme Research Laboratories, Westpoint, PA). A nine–amino acid synthetic peptide (CTKPTDGNC) that represents a partial analogue of the group-specific *a* determinant located in the S region (139–147) of HBsAg (7) was used as a positive control in antigen processing experiments. The peptide was synthesized as a COOH-terminal amide, and the purity (>80%) was assessed by HPLC using a Vydac C-18 column (Multiple Peptide Systems, San Diego, CA). The control peptide used in these experiments was either a variant of the S(139–147) peptide (CTKPSDRNC; this peptide was previously shown to abrogate T cell proliferative responses to murine T cells that were primed with rHBsAg [8]) or a 15–amino acid peptide from a sequence of yellow fever virus (GAMRVTKDTNDNNLY).

Induction of Anti-HBs Antibodies. Mice (five/group) were immunized intraperitoneally on days 0, 7, and 14 with either rHBsAg, 2F10 anti-Id, or control antibody 2C3. rHBsAg was administered as an alum-adsorbed precipitate at a dose of 0.5 μg/animal per injection. 2F10 anti-Id or control mAb was administered at 100 μg/animal per injection in CFA, IFA, and saline, respectively. Mice were bled retro-orbitally and the sera evaluated for anti-HBs-specific antibodies using a commercially available ELISA kit (AUSAB; Abbott Diagnostic Laboratories, Chicago, IL).

Isotype Distribution of the Anti-HBs Response. The isotype distribution of the anti-HBs response was determined using the Mouse Typer sub-isotyping kit (Bio-Rad Laboratories, Richmond, CA). Sera were diluted in PBS-0.05% Tween 20 and incubated on HBsAg-coated beads for 2 h. The beads were washed and a 1:2 dilution of rabbit anti–mouse subclass-specific antiserum was added for 1 h. The beads were washed again and a 1:3,000 dilution of goat anti–rabbit horseradish peroxidase conjugate was added. After 1 h the beads were washed and developed in a peroxidase substrate solution (2,2′-amino-di[3-ethyl-benzthiazoline sulfonate] and H_2O_2) for 30 min and results read on an automated EIA reader at 405 nm. The entire assay was performed at room temperature and every wash step consisted of five washes in PBS-Tween 20.

In Vitro Proliferation of Mouse Lymph Node T Cells. Mice (five/group) were immunized in the hind foot pads with either rHBsAg, anti-Id 2F10, or control monoclonal 2C3. Amount of antigen administered and schedule of immunization are the same as described above for the induction of anti-HBs antibodies. It may seem at first that our method of immunization for the induction of T cell responses is quite unorthodox (immunizing three times in the footpad vs. a single injection). We, however, only use 0.5 μg of rHBsAg per injection. This is substantially below the doses that are reported in the literature, where a single injection of 16 μg of HBsAg was administered and T cells collected 8–10 d later (9, 10). 1 wk after the third injection the animals were killed and the popliteal lymph nodes were collected, teased apart, and the cells washed twice in RPMI 1640 supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 50 μg/ml gentamicin, 0.2 mM nonessential amino acids, 11 μg/ml sodium pyruvate, 0.02 M Hepes, and 5×10^{-5} 2-ME (incomplete media), and once in complete media (incomplete media supplemented with 10% heat-inactivated FCS). T cell enrichment was performed at 10^8 cells/0.6 g of packed nylon wool (Cellular Products, Buffalo, NY). After enrichment, T cells were >97% pure as measured by staining with anti-Thy-1.2 antibody conjugated to FITC. The enriched T cells were adjusted to a concentration of 2.5×10^5 cells/well. 100 μl of cells was plated in 96-well flat-bottomed plates along with 5×10^5 irradiated syngeneic spleen cells as a source of APC. Appropriate concentrations of the different stimuli diluted in complete media (100 μl/well) were added to the cells in triplicate cultures. The stimuli were: rHBsAg (0.1 or 0.5 μg/well), 2F10 anti-Id and isotype control antibody (20 or 50 μg/well), synthetic peptide S(139–147) and control peptide (0.05 or 0.10 μg/well), Con A (1 μg/well), or media alone. The cells were then cultured for 120 h in a 5.5% CO_2 incubator at 37°C. [^{3}H]TdR (1 μCi/well) was added to each well 18 h before the end of the culture period. The cells were harvested onto glass fiber filters using an automatic cell harvester (Scatron, Sterling, VA). Proliferation, as measured by [^{3}H]TdR incorporation, was determined by liquid scintillation spectroscopy. Results are expressed as the mean cpm of [^{3}H]TdR incorporation of triplicate wells.

MHC Class II Restriction. To determine the MHC class II restriction in our system, we treated BALB/c (H-2^d) APC with mAbs MK-D6 or 34-1-4S, which are specific for I-A^d and I-E^d, respectively. The MK-D6 and 34-1-4S cell lines were obtained from American Type Culture Collection (Rockville, MD). 2×10^7 APC were incubated with 500 μl of either MK-D6 or 34-1-4S culture supernatant for 1 h at 4°C with shaking. The cells were then washed three times in incomplete media. These APC were immediately used in in vitro T cell experiments as described previously.

Chloroquine and Paraformaldehyde Treatment of APC. In experiments directed towards studying the role of antigen processing, spleen cells (as a source of APC) were treated essentially according to the procedure of Kovac and Schwartz (11). 3×10^7 APC were incubated for 20 min with 2 ml of either 0.3 M chloroquine or 0.5% (wt/vol) paraformaldehyde. The cells treated with chloroquine were washed four times in incomplete media and the cells treated with paraformaldehyde were washed five times in cold complete media. After this treatment the APC were set up in in vitro T cell experiments as described above.

Results

Anti-HBsAg Production by rHBsAg or Anti-Id. As shown in Fig. 1, *A* and *B*, both SWR/J (H-2^q) and BALB/c (H-2^d)

mice made a strong antibody response when injected with rHBsAg-alum, however, the B10.M strain (H-2^f) did not (Fig. 1 *C*), thus confirming earlier reports by other investigators on the classification of the B10.M strain as a nonresponder to HBsAg (12). The outbred wild mice, *M. spretus*, also made a strong anti-HBs response when injected with rHBsAg-alum (data not shown). Importantly, in all the above mouse strains (BALB/c, SWR/J, B10.M, and *M. spretus*), an anti-HBs response was elicited when the mice were injected with the monoclonal internal image anti-Id 2F10 (Fig. 1, *A–C*). Thus, our anti-Id was successful in circumventing nonresponsiveness to the *a* determinant of HBsAg in the B10.M mouse strain.

Isotype Distribution of Anti-HBs Responses. An analysis of the isotype distribution of the anti-HBs response showed that both anti-Id and rHBsAg induced responses in the BALB/c mice that were predominantly of the IgG1 subclass (Table 1). Additionally, the rHBsAg-immunized BALB/c mice produced a substantial amount of IgG2b and lesser amounts of IgG2a and IgM anti-HBs-specific antibodies. In sera from 2F10-immunized BALB/c mice collected at a later time point (3–5 wk after the third immunization), we also detected small amounts of IgG2b and IgM anti-HBs-specific antibodies. In contrast to the responses observed in BALB/c mice immunized with the anti-Id 2F10, B10.M mice produced predominantly anti-HBs antibodies of the IgG2b subclass and some IgG1 antibody.

In Vitro Proliferation of HBsAg- or Anti-Id-primed T Cells. Purified T cells obtained from the popliteal lymph nodes of mice primed in vivo with rHBsAg were stimulated in vitro for 120 h with varying concentrations of anti-Id, control antibody, rHBsAg, a synthetic peptide corresponding to residues 139–147 of the S region protein of HBsAg, control peptide, or media alone (Table 2). As previously shown by others, T cells from B10.M mice injected with HBsAg do not respond in vitro to HBsAg (13), and as seen in our experiments, these T cells also do not respond in vitro to the anti-Id. T cells obtained from the two other strains (BALB/c and SWR/J) primed with rHBsAg responded in vitro to both rHBsAg and anti-Id 2F10 (Table 2). Thus, the anti-Id is capable of efficiently stimulating in a specific manner T cells from rHBsAg-primed mice. Good proliferative responses were also observed in vitro with a synthetic nine–amino acid peptide (S[139–147]) that corresponds to *a* determinant residues of HBsAg.

In reciprocal experiments, T cells were obtained from mice primed in vivo with 2F10 anti-Id and stimulated in vitro with various stimuli. Table 3 shows that T cells obtained from BALB/c, SWR/J, B10.M, and *M. spretus* primed in vivo with anti-Id can respond in vitro to the anti-Id. More importantly, T cells primed in vivo by the anti-Id do proliferate in vitro to rHBsAg (except the B10.M strain). This result is significant if this anti-Id were to be used as an alternative vaccine to HBsAg. The lack of proliferation observed in cultures of T cells obtained from B10.M mice immunized with the anti-Id is not likely a reflection of an attenuated B cell response in these animals. Though the results of a longitudinal study of anti-HBsAg responses in three strains of mice (Fig. 1) suggests that B10.M mice make a weaker antibody response, we in fact have additional results (data not shown), wherein, B10.M mice immunized in the footpad with the anti-Id do make equivalent antibody response to those seen in BALB/c mice. However, even when using cells from these animals, no T cell response to rHBsAg could be elicited.

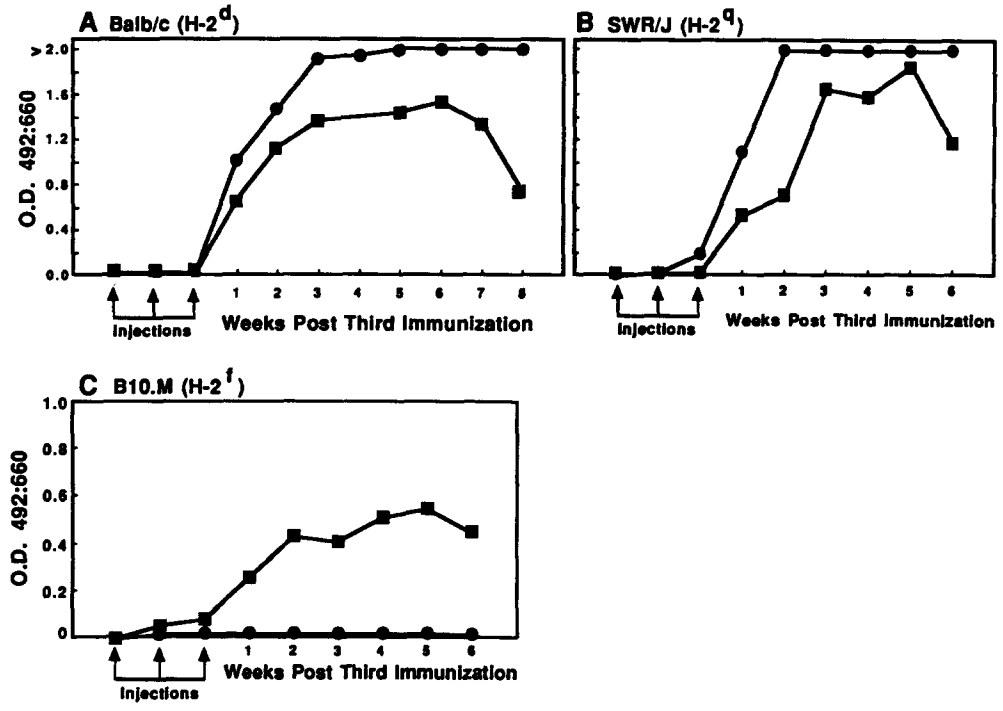

Figure 1. Comparison of anti-HBs antibody responses elicited by immunization with anti-Id (■) or rHBsAg (●) in (*A*) BALB/c (H-2^d), (*B*) SWR/J (H-2^q), or (*C*) B10.M (H-2^f). Results are expressed as OD 492:660 of the average of five mice per group at either a 1:20 dilution (SWR/J and BALB/c) or 1:5 dilution (B10.M) of their sera.

Table 1. *Isotype Distribution of Anti-HBs Responses*

Mouse	Immunogen	IgG1	IgG2a	IgG2b	IgG3	IgM
BALB/c	None*	0.042	0.035	0.041	0.048	0.047
(H-2^d)	2F10‡	**1.289**	0.069	0.020	0.075	0.097
	2F10§	**0.919**	0.080	**0.180**	0.033	**0.126**
	2C3‖	0.064	0.041	0.058	0.069	0.064
	HBsAg¶	**1.686**	**0.219**	**0.814**	0.095	**0.238**
B10.M	None*	0.027	0.004	0.045	0.008	0.032
(H-2^f)	2F10‡	**0.245**	0.026	**0.892**	0.089	0.096
	2C3**	0.048	0.022	0.032	0.065	0.052
	HBsAg‖	0.040	0.039	0.028	0.035	0.027

Comparison of the isotype distribution of the anti-HBs response in BALB/c and B10.M mice immunized with anti-Id 2F10, isotype control antibody 2C3, or rHBsAg. Sera were collected 1 wk after the third injection (unless indicated otherwise) and tested at a 1:50 dilution. Results are expressed as OD at 405 nm.

* Pooled normal sera. Values are the average of four separate experiments.

‡ Values represent the average of six mice.

§ Values represent the average of sera from one mouse bled 3, 4, and 5 wk after the third injection.

‖ Values represent the average of two mice.

¶ Values represent the average of sera from two mice bled 3, 4, and 5 wk after the third injection.

** Values represent the average of three mice.

The nine–amino acid synthetic peptide S(139–147) was also able to elicit a response in vitro in BALB/c, SWR/J, and *M. spretus* (Table 3), again confirming the *a* determinant specificity of the response that our anti-Id is able to generate.

Table 2. *In Vitro Proliferation of HBsAg-primed T Cells*

Stimulus	Concentration	BALB/c (H-2^d)	SWR/J (H-2^q)	B10.M (H-2^f)
	μg/well			
2F10	20	43,842	54,172	908
	50	66,066	86,121	746
2F10 Fab	20	ND	54,648	ND
2F10 H chain	20	43,812	45,283	ND
2F10 L chain	20	4,089	ND	ND
2C3	20	1,238	296	593
	50	1,248	305	773
rHBsAg	0.1	49,628	72,044	717
	0.5	41,533	109,513	762
Peptide	0.05	21,799	45,190	762
S(139–147)	0.1	27,107	17,307	559
Control	0.05	408	113	602
Peptide	0.1	340	469	816
Media		761	276	823

HBsAg and 2F10 anti-Id can stimulate in vitro T cells from mice of different H-2 haplotypes (except B10.M) that were primed in vivo with HBsAg. Proliferation was assessed as described in Materials and Methods. Results are expressed as the average cpm of [^{3}H]TdR incorporation of either four (BALB/c), two (B10.M), or one (SWR/J) separate experiment.

The antigenic specificity of the proliferative responses shown in both Tables 2 and 3 is controlled by the lack of response in the absence of an in vitro stimulus (media alone), but more importantly, no response is observed in cultures stimulated with an isotype-matched control antibody or a control synthetic peptide. Additional specificity control was achieved by immunizing mice with our isotype-matched control antibody 2C3 and performing similar T cell assays as discussed earlier. The results in Table 4 show that no stimulation was observed with any in vitro stimuli except for the in vivo immunizing antibody 2C3.

To establish that our anti-Id was not stimulating these primed T cells nonspecifically by TCR crosslinking, we digested the anti-Id into either monovalent Fab fragments or separate H and L chains. As seen in Tables 2 and 3, both the anti-Id Fab fragments and H chains can stimulate T cells that were primed with either HBsAg or anti-Id. It is interesting to note that the H chain of the anti-Id stimulates these primed T cells very efficiently and probably accounts for most of the stimulation seen with the intact anti-Id.

Processing and Presentation of HBsAg and Anti-Id. APC are necessary for the uptake of complex antigen, degradation of the antigen in endosomal compartments into peptide fragments, and subsequent association of these peptides with MHC class II antigens on the surface of the APC. T cells specific for a given antigen then recognize this peptide-MHC class II complex and respond in in vitro proliferation assays by secreting IL-2 and proliferating. Proliferation can therefore be blocked by: (*a*) blocking the uptake of antigen into the APC (paraformaldehyde treatment of APC); (*b*) allowing uptake of the antigen into the APC but blocking degradation of the antigen into appropriate peptide fragments (chloroquine treatment of APC); or (*c*) blocking the association of

Table 3. *In Vitro Proliferation of Anti-Id-Primed T Cells*

Stimulus	Concentration	BALB/c ($H\text{-}2^d$)	SWR/J ($H\text{-}2^q$)	B10.M ($H\text{-}2^f$)	*M. spretus*
	μg/well				
2F10	20	160,495	46,669	35,695	49,068
	50	161,504	54,413	48,494	75,005
2F10 Fab	20	ND	46,340	ND	54,660
2F10 H chain	20	ND	34,233	ND	44,746
2C3	20	2,167	739	441	357
	50	2,483	543	358	645
rHBsAg	0.1	43,682	42,995	791	46,406
	0.5	52,557	53,953	771	61,225
Peptide	0.05	21,668	44,840	408	36,548
S(139–147)	0.1	26,296	32,984	368	22,825
Control	0.05	1,707	973	560	539
Peptide	0.1	1,836	699	527	601
Media		2,255	1,039	420	554

HBsAg and 2F10 anti-Id can stimulate in vitro T cells from mice of different H-2 haplotypes that were primed in vivo with 2F10 anti-Id. In every strain tested 2F10 anti-Id can induce an anti-HBs response, however, it cannot prime T cells in the B10.M($H\text{-}2^f$) strain that can be recalled in vitro by HBsAg. Proliferation was assessed as described in Materials and Methods. Results are expressed as the average cpm of [^{3}H]TdR incorporation of either two (B10.M) or one (BALB/c, SWR/J, *M. spretus*) separate experiment.

the MHC class II antigen-peptide fragment complex to primed T cells with antibodies directed towards MHC class II antigens (anti-I-A or anti-I-E antibody treatment of APC).

It is known that HBsAg is a T-dependent antigen (14) and that the in vitro T cell proliferative responses to HBsAg are dependent upon APC. We therefore set out to determine if processing of the anti-Id was necessary before its presentation and recognition by primed T cells. Treatment of the APC with either chloroquine or paraformaldehyde completely abrogated the in vitro T cell proliferative responses that were induced by priming in vivo with either HBsAg or anti-Id (Fig. 2). The response induced by the synthetic nine–amino acid peptide S(139–147), however, was not affected by this treatment and served as a positive control. Although results are only shown for T cells obtained from BALB/c mice injected with rHBsAg, similar results were obtained using anti-Id-primed BALB/c T cells.

Table 4. *In Vitro Proliferation of Isotype Control Antibody-primed T Cells*

Stimulus	Concentration	BALB/c ($H\text{-}2^d$)
	μg/well	
2F10	20	548
	50	987
2C3	20	11,066
	50	15,566
rHBsAg	0.1	40
	0.5	547
Peptide	0.05	937
S(139–147)	0.1	1,073
Control	0.05	1,113
Peptide	0.1	1,167
Media		757

Specificity control for anti-Id 2F10. BALB/c mice were primed in vivo with isotype-matched control mAb 2C3 (IgG1,k). Primed T cells from these mice cannot be stimulated in vitro by either anti-Id or HBsAg. Proliferation was assessed as described in Materials and Methods. Results are expressed as the average cpm of [^{3}H]TdR incorporation of two separate experiments.

Data in Table 5 are from experiments where BALB/c APC ($H\text{-}2^d$) were treated with either mAbs MK-D6 (anti-I-A^d) or 34–1–4S (anti-I-E^d). The results clearly show that the restriction for both anti-Id and HBsAg is imposed by the I-A^d locus, as complete abrogation of the response is seen in cultures where the APC were treated with the anti-I-A^d-specific antibody. APC treated with anti-I-E^d antibodies were able to present HBsAg/anti-Id similar to untreated cultures. Data from other laboratories have shown that both I-A^d- and I-E^d-encoded Ia molecules can present HBsAg in BALB/c mice (9). The presence of APC was critical in our in vitro proliferation assay as no stimulation whatsoever was observed using rHBsAg/anti-Id–primed T cells stimulated by either antigen or anti-Id in the absence of APC (Fig. 2).

Discussion

The surface envelope of HBV contains three related proteins designated S, M (S + preS2), and L (M + preS1). All

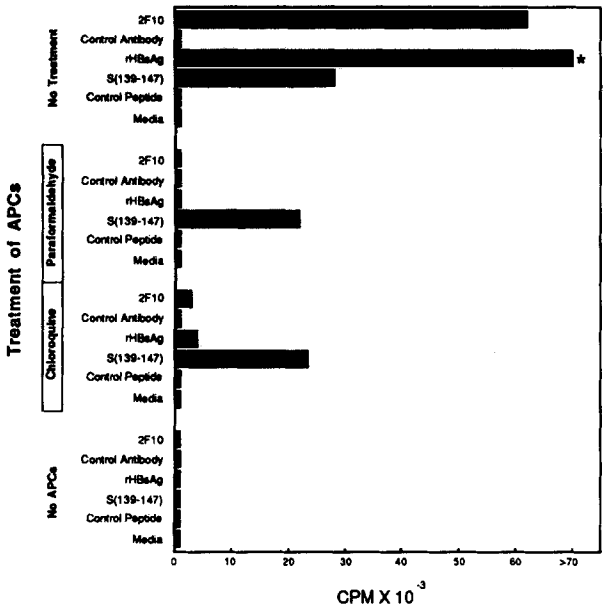

Figure 2. T cells were obtained from BALB/c mice that were injected with rHBsAg and set up in in vitro proliferation assays with the various stimuli shown along the *x*-axis. APC were treated with either chloroquine or paraformaldehyde. As can be seen, both these protocols abrogate the in vitro proliferative responses to all stimuli except S(139–147) peptide. Results are expressed as the average cpm of [^{3}H]TdR incorporation of one experiment. In the absence of APC no response is observed.

of these proteins share the 226-amino acid sequence of the S protein, which is the major protein of HBsAg. In a well-documented series of experiments, it has been determined that the murine B and T cell responses to HBsAg are H-2 haplotype restricted and under the control of complex MHC class II region genes (1). It was therefore of great interest to us to determine if this pattern of restriction also applied to the responses generated by an anti-Id that mimics the group-specific *a* determinant on HBsAg. The studies presented here show that the B and T cell responses induced by our anti-Id 2F10 are apparently not restricted by the MHC haplotypes that we have tested, in that all mice immunized with 2F10 anti-Id, including the HBsAg nonresponder B10.M strain (H-2^f), made anti-HBs responses. These responses were specific for the *a* determinant, and the anti-HBs antibodies carried the Id to which the immunizing anti-Id had been raised (data not shown). Thus, 2F10 anti-Id was successful in circumventing S region nonresponsiveness in B10.M mice. The magnitude of the anti-HBs response induced by our anti-Id varied, with SWR and BALB/c eliciting a higher anti-HBs titer than B10.M and *M. spretus*. It should be noted that in our experiments the anti-Id was not coupled to any carrier protein for the induction of anti-HBs antibodies. Therefore, our anti-Id must contain both B and T cells epitopes to achieve this response. Milich et al. (13) were also able to circumvent S region nonresponsiveness in B10.M mice by activation of the Th cell response to the pre S1 antigen, which could provide help not only to pre S1 specific but also to S-specific B cells with subsequent production of antibodies to the S region. This response, however, was not directed to the *a* determinant epitope.

Besides being able to induce an anti-HBs response, 2F10 anti-Id was also capable of priming in vivo T cells from SWR/J, BALB/c, and *M. spretus* that could subsequently proliferate in vitro to both anti-Id and HBsAg. The synthetic peptide S(139–147), which represents a partial *a* determinant epitope, could also stimulate anti-Id-primed T cells in vitro, further demonstrating the fidelity with which our anti-Id mimics the *a* determinant. It has been shown by others that

Table 5. *The Effect on T Cell Proliferation after Treatment of APC with Anti-Ia Reagents*

		APC treatment					
		rHBsAg-primed T cells			Anti-Id-primed T cells		
Stimulus	Concentration	No treatment	Anti-I-A^d	Anti-I-E^d	No treatment	Anti-I-A^d	Anti-I-E^d
	μg/well						
2F10	50	59,132	3,090	43,867	65,920	1,537	56,101
2C3	50	716	412	390	655	294	562
rHBsAg	0.1	82,405	2,505	81,327	54,228	396	35,678
Peptide S(139–147)	0.1	42,571	3,400	48,373	43,873	346	21,544
Control Peptide	0.1	ND	ND	ND	563	256	421
Media		408	346	627	189	343	461

In vitro proliferation of T cells primed with either rHBsAg or 2F10 anti-Id are restricted at the I-A^d locus in BALB/c mice. This is seen by the significant decrease of proliferation in cultures treated with MK-D6 (anti-I-A^d antibody) versus those treated with 34-1-4S (anti-I-E^d antibody) or no treatment. Proliferation was assessed as described in Materials and Methods. Results are expressed as the average cpm of [^{3}H]TdR incorporation of two separate experiments.

anti-Id antibodies can influence specific T cell reactivity (15–18). Thus, in the reovirus system, Sharpe et al. (16) have demonstrated that an internal image anti-Id that was raised against an antireovirus type 3 mAb could trigger T cell immunity (delayed type hypersensitivity and CTL responses) to reovirus type 3 in naive mice.

From the experiments presented in this report it appears that the anti-Id is activating T cells through the same mechanisms used by nominal antigen. That is, stimulation of HBsAg/anti-Id–primed T cells by either HBsAg or anti-Id is accessory cell dependent and needs to be processed and presented to the T cells by APC in the context of class II MHC molecules. The in vitro proliferation that is observed using monovalent Fab fragments or isolated H chains rules out the possibility that our anti-Id is causing proliferation of these primed cells by crosslinking receptors on the T cells, as has been described with the anti-CD3 antibody (19). This is consistent with other reports of anti-Id activating antigen-primed T cells (17, 18). Rees et al. (17) have shown that a rabbit anti-Id that mimics a 38-kD protein purified from *Mycobacterium tuberculosis* could stimulate human PBL obtained from either *M. tuberculosis* patients or BCG-vaccinated individuals. It was determined that the proliferation elicited by the anti-Id was MHC restricted and dependent on interaction with APC (18).

It is interesting to note that the separation of intact anti-Id 2F10 into separate H and L chains revealed that the T cell epitope was located on the H chain since T cell proliferation to isolated H chains but not L chains was found to be comparable to that elicited by intact anti-Id. It is possible that the T cell stimulatory epitope on the H chain of the anti-Id also represents the B cell epitope. The above observations of 2F10 anti-Id mimicking HBsAg-specific B and T cell responses correlate well with recent data from our laboratory. Reducing SDS-PAGE followed by Western blot analysis revealed that the expression of the internal image epitope on anti-Id 2F10 was mainly localized to the H chain of the anti-Id. mRNA sequencing and molecular modeling experiments revealed an area of homology between the H chain and protective *a* determinant epitopes of HBsAg. A synthetic peptide that represents this region of homology can duplicate the B and T cell stimulatory responses of the intact anti-Id and the antigen that is mimicked, HBsAg (19a). Additional support for T cell stimulatory epitopes also representing B cell epitopes comes from the influenza hemagglutinin system. It was observed that all of the hemagglutinin (HA1) synthetic peptides recognized by $CD4^+$ T cell clones (specific for the HA of X31 virus [H3N2 subtype]) corresponded to residues that were contained within the primary sequence of HA1 B cell epitopes identified as a result of their recognition by neutralizing antibody(20).

Somewhat surprisingly, although 2F10 anti-Id generated an anti-HBs response in B10.M mice, it could not prime T cells that can be stimulated in vitro with HBsAg. This inertness of HBsAg in B10.M may be explained by the fact that: (*a*) B10.M mice may have a limited T cell repertoire that is lacking recognition structures with reasonable affinity for HBsAg/MHC combination (T cell hole); or (*b*) APC of B10.M mice may not process or present HBsAg in such a way that it can be recognized by anti-Id-primed T cells. These issues are being further explored. Preliminary data from this laboratory indicate that the reason anti-Id-primed B10.M T cells are unable to respond in vitro to HBsAg may be due to low affinity binding of the *a* determinant peptide to MHC class II molecules on B10.M APC (21). An alternative view for the inability of HBsAg to stimulate in vitro 2F10 anti-Id-primed B10.M T cells may be found in the work done in the GL antigen system (22). The synthetic random copolymer L-glutamic acid L-lysine (GL) is reported to be weakly or nonimmunogenic in all inbred strains of mice. Upon immunizing mice with the antigen poly(L-Glu L-Lys L-Tyr) (GLT), no in vitro response was observed when these primed T cells were stimulated with the antigen GL. However, after cloning these GLT-primed T cells, GL-reactive cells could be isolated at a high frequency. The same may be true with anti-Id-primed B10.M T cells.

Although we do not envision the use of anti-Ids to replace conventional vaccines, there may, however, be certain niches where anti-Ids would be very useful, for example, in individuals who are nonresponders to the licensed HBV vaccine. According to some reports the immune response to the HBV vaccine in humans parallel those reported by Milich (1) and colleagues in mice, in that the response to HBsAg in both humans and mice are MHC linked. In mice, nonresponsiveness to the S protein of HBsAg is associated with the$H\text{-}2^{f,s}$ haplotype, while in humans it is associated with the extended haplotype HLA-B8,SC01,DR3 and HLA-B44,FC31,DR7 (23–25). The anti-HBs response that was elicited in the B10.M mouse strain by immunizing with anti-Id suggests that this anti-Id may be useful in nonresponder individuals. The fact that our anti-Id is able to elicit HBsAg-specific cellular and humoral responses in the outbred strain *M. spretus* is encouraging, since humans represent a genetically outbred population. An anti-Id vaccine consisting of an invariant T cell epitope and an appropriate B cell epitope might well induce specific cellular and humoral immunity in a genetically diverse human population. Further, the sharing of Ids of related specificity between T and B cells would permit the use of the same anti-Id to activate both B and T cells for an anamnestic recognition of similar epitopes on the infectious organism.

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