fitness modifiers against the severe form of β thalassemia.

The mechanisms responsible for the increased $^{A}\gamma$ globin synthesis on this $\delta\beta^0$ thalassemia chromosome have not been defined. It has been postulated that nondeletion HPFH and δβ thalassemia are caused by defects in a putative switch sequence (*12*), one candidate for which is the Alu repeat region upstream from the δ globin gene (*13*). This study indicates that the δβ thalassemias can also arise from multiple mutational events. It will be interesting to see whether any of the other chromosomes associated with nondeletion HPFH and δβ thalassemia also harbor β^0 or β^+ thalassemia genes.

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Antibody to Hepatitis B Virus Induced by Injecting Antibodies to the Idiotype

Abstract. *Anti-idiotype reagents that recognize a common idiotype associated with antibody to hepatitis B surface antigen (anti-HBs) were used to induce anti-HBs in mice. The anti-idiotype–induced anti-HBs was found to recognize the group-specific* a *determinant of hepatitis B surface antigen and to express an interspecies idiotype. These findings suggest that anti-idiotypes may be useful as vaccines or vaccine primers.*

Alternative approaches are being sought for the preparation of a vaccine for hepatitis B virus (HBV). At present the vaccine is made from the plasma of persons chronically infected with HBV by purifying the hepatitis B surface antigen (HBsAg) and treating the particles with disinfecting agents (*1, 2*). Although this vaccine has proven to be safe and effective, its high cost and limited availability preclude its use in developing countries where hepatitis B and its sequelae constitute a major health problem (*3*). Two alternative approaches are under active investigation for the preparation of well-defined, HBsAg-specific vaccines. The first involves the use of DNA fragments derived from HBV cloned into suitable vectors (*4, 5*). The second involves synthetic peptides that contain amino acid sequences analogous to those associated with the major native protein component of HBsAg (*6–10*).

Jerne (*11*) proposed that the immune response to an antigen can be regulated through an idiotype–anti-idiotype network. Idiotypes, located on or close to the antigen-binding site of both antibody molecules and lymphocyte antigen receptors, are components of this network. We recently characterized an idiotype shared by human antibodies to HBsAg (anti-HBs) (*12–14*). This common idiotype was also expressed on anti-HBs produced in BALB/c mice and in six other species, indicating an interspecies idiotypic cross-reaction (*15*). We also found that prior injection of antibodies to the idiotype (hereafter referred to as idiotype antibodies) into mice markedly increased the number of spleen cells secreting immunoglobulin M (IgM) anti-HBs when the mice were subsequently inoculated with HBsAg (*16*). The anti-HBs response in the serum of mice was enhanced by prior treatment with idiotype antibodies (*17*). Anti-HBs–secreting cells could be induced solely by injecting idiotype antibodies, and this anti-HBs expressed the interspecies idiotype (*16, 17*). These findings are supported and extended by the study reported here, in which we induced anti-HBs in the serum of mice receiving idiotype antibodies alone and analyzed the specificity of the induced anti-HBs.

The preparation of affinity-purified rabbit idiotype antibodies and their specificity have been described elsewhere (*13–18*). These preparations neither contain nor bind HBsAg. An immunoglobulin G (IgG) fraction obtained from the serum of the rabbit before injection of the idiotype served as a control antibody preparation. All antibody preparations were adsorbed to alumina gel at

Table 1. Anti-HBs response expressed as the reciprocal dilution of antiserum that bound HBsAg subtype *ayw* and the percentage inhibition of binding a constant dilution of mouse antiserum to HBsAg subtype *ayw* by 5 μg of HBsAg subtypes *ayw*, *adw*, and *adr*. Each group of six mice was given 50 μg of alum-precipitated idiotype antibody (IA) or IgG from an unimmunized animal (pre-IgG) on day 0, followed by the same injection on day 14, all by the intraperitoneal route. Serum was obtained on day 26, and the end point anti-HBs titer and S/N ratios for binding the three serotypes of HBsAg were determined. The ability of these mouse sera to inhibit the human idiotype–anti-idiotype reaction was examined at a 1:10 dilution. N.D., not determined.

First injection	Second injection	Anti-HBs titer (*ayw*)	Inhibition (percent)			
			adw	*ayw*	*adr*	Idiotype–anti-idiotype reaction
IA	IA	750	85	85	70	38
IA	IA	1000	88	86	78	46
IA	IA	1000	96	92	84	51
IA	IA	1250	96	96	90	54
IA	IA	250	90	84	76	27
IA	IA	750	80	86	71	34
Pre-IgG*	Pre-IgG	< 5	N.D.	N.D.	N.D.	0 to 11

*All six mouse antisera were negative at the dilution tested.

*p*H 7.0 to increase immunogenicity (*19*). Antibody concentrations were determined in a spectrophotometer at 280 nm, with an extinction coefficient of 15 being used for a 1 percent preparation.

To analyze the specificity of the anti-HBs response in the serum of BALB/c mice, we performed a solid-phase radioimmunoassay (*20*). Briefly, 200 ng of purified HBsAg (subtype *ayw*) was coated onto the wells of a microtiter plate. Fivefold dilutions of mouse antiserum were added to the wells after postcoating and washing steps. The unbound serum protein was washed off and ^{125}I-labeled goat antiserum to the mouse γ chain was added. Residual radioactivity was removed, the wells were washed, and radioactivity was counted. Data were expressed as the anti-HBs end-point titer, where the ratio of positive (S) to negative (N) counts per minute was 2.1 (*20*). The negative value was obtained from serum from the respective mice before immunization. In addition, the presence of the interspecies idiotype on mouse anti-HBs was determined by a solid-phase radioimmunoassay (*15*).

Two groups of BALB/c mice were treated with two injections of alum-precipitated idiotype antibodies or IgG from nonimmune rabbits, and serum was obtained 12 days after the final injection. In the mice receiving two injections of idiotype antibodies, a significantly greater IgG anti-HBs response ($\log_{10}$ arithmetic mean titer, 2.9) was observed than in mice given control antibodies (0.3) ($P < 0.001$, two-tailed *t*-test). A mean anti-HBs titer of 1:833 was obtained for six mice receiving idiotype antibodies, whereas no anti-HBs was detected in six mice injected with control antibodies at a serum dilution of 1:5 (Table 1). These results confirm our previous findings that idiotype antibodies alone can produce IgG anti-HBs in BALB/c mice (*16, 17*).

Analysis of the anti-idiotype–induced anti-HBs demonstrated the expression of the interspecies idiotype (Table 1). Six sera containing anti-HBs produced by anti-idiotype injection inhibited the idiotype–anti-idiotype reaction 27 to 54 percent. Conversely, less than 11 percent inhibition was obtained with serum from six mice injected with control antibodies.

Serologically, HBsAg particles contain a group-specific determinant referred to as *a* and two sets of allelic subtype determinants: *d* or *y* and *w* or *r*. Their combination results in the four major serotypes of HBV: *adw, ayw, adr,* and *ayr* (*21, 22*). Since antibodies directed against the group-specific *a* determinant confer protective immunity against

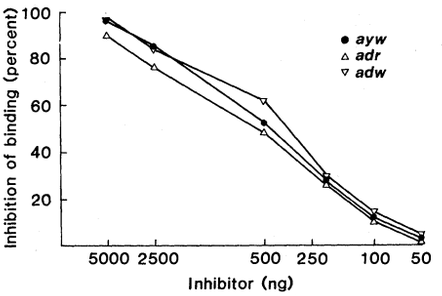

Fig. 1. Inhibition curves of a representative anti-idiotype–induced, anti-HBs–containing serum binding to HBsAg subtype *ayw*. Each point represents the mean of triplicate values. No inhibition was obtained with 5 μg of ovalbumin.

HBV (*23*), an alternative HBV vaccine should elicit immunity to the critical *a* antigenic determinant. Therefore, it was critical to characterize the specificity of the anti-HBs response in mice inoculated with idiotype antibodies. Initially, we showed that these antisera bound to antigen wells coated with three individual HBsAg subtypes (*adw, ayw,* and *adr*) at the same level. To measure this cross-reactivity more precisely, we tested each serum shown in Table 1 by a competitive solid-phase radioimmunoassay (*24*). Increasing concentrations of each of the three individual HBsAg subtype preparations were preincubated with a constant quantity of mouse antibody. This mixture was then tested for residual anti-HBs activity with HBsAg subtype *ayw*–coated wells. Inhibition curves for a representative anti-idiotype–induced anti-HBs serum are shown in Fig. 1. The antibody appears to be specific for the group *a* determinant since inhibition was obtained with all three subtypes and the slopes of the inhibition curves are similar. The percentage inhibition of each of the six antisera with 5 μg of each HBsAg subtype is shown in Table 1. These findings indicate that the anti-HBs immune response of these animals was chiefly directed to a common group-specific determinant.

Theoretical implications for anti-idiotype vaccines against infectious agents have been discussed by others (*25–27*). To our knowledge, the only infectious agent that idiotype antibodies have experimentally induced protective immunity against has been African trypanosomiasis in mice (*28, 29*). It is noteworthy that genetic restrictions were placed on the protection to trypanosomiasis. This is important since humans represent a genetically outbred population, which may severely limit the effectiveness of anti-idiotype vaccines. Also, it is not known whether idiotype antibodies would be able to boost preexisting antibody levels. However, the problems associated with using idiotype antibodies produced in a heterologous species for injection into humans may be circumvented by the use of human hybridoma antibodies (*30*). Our demonstration that anti-HBs induced by injecting idiotype antibodies recognizes a common immunogenic *a* determinant of HBsAg and expresses an idiotype shared by naturally occurring anti-HBs suggests that anti-idiotype vaccines may be useful against HBV. Research to develop such vaccines should be conducted in higher species, since only chimpanzees and humans can be infected with human HBV.

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