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DRAFT ANNUAL PROGRESS REPORT
January 31, 1984

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CONTROL OF HEPATITIS VIRUS INFECTIONS BY NEW METHODS

DRAFT ANNUAL PROGRESS REPORT

by

Joseph L. Melnick, Ph.D.

January 31, 1984

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
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17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Please see page 3.

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DD Form 1473, Item 20: Abstract

During the past contract year, we have continued our investigations with anti-idiotype reagents that recognize a common idiotypic determinant on human anti-HBs molecules. Several important immunochemical parameters were established: (1) Injection of anti-idiotype antibodies prior to HBsAg inoculation enhanced the anti-HBs response when compared to groups of mice given control antibodies before HBsAg. (2) Primarily IgG anti-HBs was induced if the anti-idiotype antibodies were given as an alum precipitate, whereas IgM anti-HBs was generated if the anti-idiotype antibodies were administered in a soluble form. (3) The anti-HBs response also was increased when anti-idiotype antibodies were given in conjugation with a cyclic synthetic HBsAg peptide (SP1) which contains amino acid sequences 122-137. This anti-HBs response was comparable to a single injection of HBsAg.

We also have induced anti-HBs by injecting anti-idiotype antibodies alone. The anti-idiotype-induced anti-HBs expressed an interspecies idiotype that is shared by human anti-HBs produced by natural infection with HBV. In addition, the anti-HBs recognized the group-specific a determinant of HBsAg. These data suggest the potential use of anti-idiotypes as vaccines and immunopotentiators of the anti-HBs response.

Studies also have been performed on the use of an HBsAg polypeptide vaccine in young adults. It was demonstrated that on a weight basis the polypeptide vaccine was superior to Heptavax in generating an anti-HBs response. These studies reveal that critical antigenic determinants for inducing an anti-HBs response are associated with the low-molecular-weight polypeptides of HBsAg.

Finally, we have made a firm commitment from Baylor funds to continue our investigations with HBsAg synthetic peptides by purchasing an amino acid synthesizer. Previously, all peptides were generated in the laboratory of Dr. James Sparrow, at Baylor College of Medicine. However, with only one synthesizer available in his laboratory, too little time was allocated for the synthesis of HBsAg peptides, as Dr. Sparrow's primary interest and support is in the area of apolipoproteins. In addition, the quantities of the hepatitis peptides being produced were very limited. Since a synthesizer will now be available for our exclusive use, large quantities of HBsAg can be continuously synthesized and tested for their ability to induce protective immunity against HBV.

Foreword:

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.

TABLE OF CONTENTS

A. Enhancement of the Anti-HBs Immune Response by Prior Injection of Anti-Idiotype Antibodies

We have studied the modulating effects, prior to antigenic challenge, of in vivo administration of anti-idiotype reagents that recognize the common anti-HBs idiotype and have confirmed our previous studies conducted at the serological level. Injection of alum-precipitated anti-idiotype produced higher anti-HBs titers when compared to groups of mice receiving soluble anti-idiotype before HBsAg stimulation (Table 1). These data suggested that alum precipitation produced a more immunogenic form of the anti-idiotype for enhancing the anti-HBs response. It was noteworthy that primarily IgM anti-HBs was detected by an IgM type-specific radioimmunoassay in the group of mice receiving the soluble anti-idiotype preparation (Table 1). This was similar to our previous observation that the number of direct IgM anti-HBs plaque-forming units was enhanced by prior injection of soluble anti-idiotype.

In another set of experiments, we determined the optimal time interval for enhancing the anti-HBs response to be 14 days between primary injection of alum-precipitated anti-idiotype and a subsequent HBsAg inoculation (Table 2). Control groups of mice received IgG antibodies purified from the anti-idiotype-producing rabbit prior to injection of the anti-HBs idiotype. These control groups had a lower mean anti-HBs titer when compared to each of the anti-idiotype-treated groups. In addition, we determined the optimal anti-idiotype dose to be 50 µg for enhancement of the anti-HBs response (Table 3).

B. Enhancement of the Anti-HBs Response by Using Anti-Idiotype Antibodies in Conjunction with Synthetic Cyclic Peptide 1

We have also studied the effects of in vivo administration of anti-idiotype antibodies prior to a single injection of syntnetic cyclic peptide 1. Based on the data shown in Table 4, mice treated with anti-idiotype antibodies prior to injection of cyclic peptide 1 generated a higher mean anti-HBs titer (38.6 as opposed to 4) when compared to mice injected with control antibodies. In confirmation of our previous work, mice treated with anti-idiotype antibodies prior to HBsAg produced a higher mean anti-HBs titer when compared with mice receiving pre-IgG prior to HBsAg. It is noteworthy that mice given anti-idiotype antibodies and cyclic peptide 1 had comparable anti-HBs titers with mice receiving pre-IgG and complete HBsAg (38.6 compared to 34.0). These data indicate that anti-idiotype antibodies in conjunction with cyclic peptide 1 can induce anti-HBs titers comparable to a single injection of whole HBsAg particles.

C. Injection of Anti-Idiotype Antibodies Alone Induces Anti-HBs

We have previously shown that injection of anti-idiotype antibodies without HBsAg injection produced IgG anti-HBs plaque-forming cells. These data indicated that anti-idiotype antibodies alone can induce an anti-HBs response. Injection of anti-idiotype antibodies alone into mice produced a significant anti-HBs response when compared to mice given a similar injection of control antibodies (Table 5). This anti-idiotype anti-HBs expressed the interspecies idiotype, since inhibition values obtained with a group of 6 sera ranged from 27 to 54%. Conversely, less than 11% inhibition of the idiotype–anti-idiotype reaction was obtained with the non-anti-HBs-containing sera from the 6 mice injected with control antibodies. The specificity of the anti-HBs produced by anti-idiotype injection was determined by inhibition of binding to HBsAg subtype ayw by HBsAg subtypes adw, ayw and adr. Each of the 6 mouse antisera were inhibited from binding to HBsAg subtype ayw by the 3 HBsAg subtypes, indicating that the anti-idiotype anti-HBs was directed to the a determinant. Therefore, anti-idiotype antibodies can induce anti-

HBs that recognizes the a determinant of HBsAg, which has been shown to induce protective immunity to hepatitis B virus (HBV) in humans. In addition, this anti-HBs expresses an idiotype that is shared by anti-HBs produced in humans naturally infected with HBV.

The above series of experiments indicates that manipulation of the immune response to HBV by anti-idiotypes has been fruitful. These reagents may be useful as primers or potentiators of the immune response, thus decreasing the number of injections of an HBsAg vaccine necessary to elicit an immune response. Alternatively, anti-idiotype antibodies may have a potential use as a vaccine against HBV.

D. Response to HBsAg Polypeptide Vaccine in a Young Adult Population

An HBsAg, subtype adw, polypeptide (PP) vaccine (mol. wt. 25,000-30,000) was prepared from intact 22-nm HBsAg particles, packaged in a micellar configuration and alum-adsorbed. Three concentrations of this vaccine (0.8, 5 or 20 µg) were compared to 40 µg of the original starting material. Vaccine was administered at 0, 1 and 6 months to 52 recipients, and data were evaluated through 7 months. Local and systemic reactions were clinically nonsignificant. The anti-HBs seroconversion rates at 4 weeks for the 20 µg PP vaccine group (84%) and the 40 µg particle vaccine group (70%) were comparable. By 12 weeks, all vaccine recipients in the 5 and 20 µg PP groups had seroconverted versus 50% of the 0.8 µg group ($p < 0.02$). The latter group reached 100% seroconversion by month 7. Throughout the follow-up period, geometric mean (GM) anti-HBs levels (mIU/ml) were significantly higher in the 20 µg PP group than in the other groups. At 1 month, the GM anti-HBs level for the 20 µg PP group was 8.9, whereas the 40 µg particle vaccine group had a GM antibody level of 5.2. By 3 months, the levels were 202.4 vs. 90.0, respectively, and by 7 months the respective levels were 8910 and 3450. The 5 µg PP vaccinees have anti-HBs responses comparable to 20 µg Heptavax-B recipients. In summary, the alum-adsorbed, micellar PP vaccine has produced superior anti-HBs responses in humans when compared on a weight-to-weight basis with a 22-nm HBsAg vaccine from which it was derived. These studies confirm our previous findings in chimpanzees that critical antigenic determinants are associated with these low-molecular-weight polypeptides and provide a link to future vaccine studies using synthetic HBsAg macromolecules.

TABLE 1

Comparison of Alum-Precipitated and Soluble Anti-Idiotype for Induction of Anti-HBs[a]

First injection	Second injection	No. of mice	Anti-HBs response (mean ± SEM)[b]	Micro-SPRIA	
				Anti-HBs IgG[c]	Anti-HBs IgM[d]
Anti-idiotype alum. ppt.	HBsAg	4	487.5 ± 315.0	4938	1250
Anti-idiotype soluble	HBsAg	4	72.5 ± 50.0	86	1000
Pre-rabbit IgG alum. ppt.	HBsAg	4	< 5.0[e]	<5.0	<5.0
Pre-rabbit IgG soluble	HBsAg	4	6.25 ± 1.08	30	50

[a]Each group of mice received 40 µg of anti-idiotype or pre-IgG on day 0, followed by 6 µg of HBsAg on day 14, all by the intraperitoneal route. Mice were bled on day 26.

[b]The values are the reciprocal dilution of antisera which gave an endpoint S/N of 2.1 as measured by AUSAB.

[c]The mean value of reciprocal dilution of antisera which gave an endpoint S/N of 2.1 as measured by solid-phase RIA using ^{125}I-labeled goat anti-mouse γ-chain specific antiserum.

[d]The mean value of reciprocal dilution of antisera which gave an endpoint S/N of 2.1 as measured by solid-phase RIA using ^{125}I-labeled rabbit anti-mouse µ-chain specific antiserum.

[e]All mice were negative for anti-HBs at a serum dilution of 1:5.

TABLE 2

Optimal Time Interval Between Injection of Anti-Idiotype and HBsAg for Induction of Anti-HBs[a]

First injection	HBsAg injection (no. of days after primary inoculation)	Anti-HBs response[b] (mean ± SEM)
Anti-idiotype	7	130 ± 32.1
	14	4222 ± 1825.7
	21	200 ± 64.6
Pre-IgG	7	5 ± 5.0
	14	< 5.0[c]
	21	30 ± 11.5

[a]Each group of 4 mice received 40 µg of alum-precipitated anti-idiotype or pre-IgG on day 0, followed by 6 µg of HBsAg on the days specified, all by the intraperitoneal route. Mice were bled 12 days after injection with HBsAg.

[b]The values are the reciprocal dilution of antiserum which gave an endpoint S/N of 2.1 as measured by the IgG anti-HBs solid-phase radioimmunoassay.

[c]All mice were negative for anti-HBs at a serum dilution of 1:5.

TABLE 3

Effect of Dose of Alum-Precipitated Anti-Idiotype for Induction of IgG Anti-HBs Using Micro-SPRIA[a]

First injection	Second injection	Reciprocal arithmetic mean titer	Log_{10} of the reciprocal arithmetic mean titer	Standard deviation[b]	
500 ng anti-Id[c]	HBsAg	583	2.76	0.77	
500 ng pre-IgG	HBsAg	86	1.93	0.26	($p > 0.2$)[d]
5 µg anti-Id	HBsAg	4938	3.69	0.32	
5 µg pre-IgG	HBsAg	66	1.82	0.88	($p < 0.02$)
50 µg anti-Id	HBsAg	9378	3.97	0.10	
50 µg pre-IgG	HBsAg	6	0.78	0.18	($p < 0.001$)
200 µg anti-Id	HBsAg	4344	3.64	0.62	
200 µg pre-IgG	HBsAg	30	1.48	0.25	($p < 0.01$)

[a]Each group of 4 mice received various concentrations of alum-precipitated antibodies on day 0, followed by 6 µg of HBsAg on day 14. Mice were bled on day 26.

[b]The standard deviation of the log_{10} of the reciprocal arithmetic mean titer.

[c]Several of the mouse antisera were negative at the dilution tested. To facilitate the computations the reciprocal of the titer was considered 0.5, assuming that greater than 2-fold concentration of the sample would give a positive result.

[d]Determined by the two-tailed Student's t-test.

TABLE 4

Priming the Anti-HBs Response by Prior Injection of Anti-Idiotype Antibodies[a]

First injection	Second injection	No. of mice	Anti-HBs response (mean ± SEM)[b]
Pre-IgG	peptide 1	6	4.0 ± 1.9
Anti-idiotype	peptide 1	7	38.6 ± 9.6
Pre-IgG	HBsAg	5	26.0 ± 8.7
Anti-idiotype	HBsAg	6	10,416 ± 306

[a]All mice received 50 µg of either alum-precipitated anti-idiotype or control, preimmune rabbit IgG on day 0, followed by 50 µg of peptide 1 or 6 µg of HBsAg on day 14, all by the intraperitoneal route. Serum was obtained on day 30, and the reciprocal of the endpoint dilution which gave an arbitrary positive-to-negative cpm ratio of 2.1 was determined by radioimmunoassay as previously described.

[b]Mean of reciprocal endpoint titer (± standard error of the mean) in the responding animals.

TABLE 5

Anti-HBs Response in Mice Injected with Anti-Idiotype Antibodies[a]

First injection	Second injection	Anti-HBs titer (ayw)	% inhibition			% inhibition of idiotype–anti-idiotype reaction
			adw	ayw	adr	
Anti-idiotype	Anti-idiotype	750[b]	85	85	70	38
Anti-idiotype	Anti-idiotype	1000	88	86	78	46
Anti-idiotype	Anti-idiotype	1000	96	92	84	51
Anti-idiotype	Anti-idiotype	1250	96	96	90	54
Anti-idiotype	Anti-idiotype	250	90	84	76	27
Anti-idiotype	Anti-idiotype	750	80	86	71	34
Pre-IgG[c]	Pre-IgG	< 5	ND[d]	ND	ND	0-11

[a]Anti-HBs response expressed as the reciprocal dilution of antiserum which bound HBsAg, subtype ayw, and gave an arbitrary positive (S) to negative (N) counts per minute ratio of 2.1. In addition, 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 was determined. Each group of 6 mice was given 50 µg of alum-precipitated anti-idiotype or pre-IgG on day 0, followed by a similar injection on day 14, all by the intraperitoneal route. Serum was obtained on day 26, and the endpoint 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.

[b]Statistical method using two-tailed Student's t test was based on the $\log_{10}$ arithmetic mean titer.

[c]All 6 mouse antisera were negative at the dilution tested.

[d]Not determined.

Publications during the Past Year that Acknowledged Support of This Contract

Kennedy, R.C., Adler-Storthz, K., Sanchez, Y., Melnick, J.L. and Dreesman, G.R. Immune response to hepatitis B surface antigen: enhancement by prior injection of antibodies to the idiotype. Science 221:853-855, 1983.

Hollinger, F.B., Khan, N.C., Oefinger, P.E., Yawn, D.H., Schmulen, A.C., Dreesman, G.R. and Melnick, J.L. Posttransfusion hepatitis type A. J. Am. Med. Assoc. 250:2313-2317, 1983.

Kennedy, R.C. and Dreesman, G.R. Production and characterization of anti-idiotype reagents for the analysis of viral antigen systems. J. Virol. Meth. 7:103-115, 1983.

Dreesman, G.R. Polypeptide and synthetic peptide vaccines for hepatitis B virus. In Advances in Hepatitis Research (F.V. Chisari, ed.). Masson Publishing, New York, in press.

Kennedy, R.C. and Dreesman, G.R. Enhancement of the immune response to hepatitis B surface antigen: in vivo administration of anti-idiotype induces anti-HBs which expresses a similar idiotype. J. Exp. Med., vol. 159, March 1984.

Kennedy, R.C., Melnick, J.L. and Dreesman, G.R. Viral hepatitis B antibody (anti-HBs) induced by injecting anti-idiotype antibodies. Science, in press.

Kennedy, R.C., Sparrow, J.T., Sanchez, Y., Melnick, J.L. and Dreesman, G.R. Enhancement of a viral hepatitis B antibody (anti-HBs) response to a synthetic cyclic peptide by priming with anti-idiotype antibodies. Submitted for publication.

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