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Non-Responsiveness to Hepatitis-B Vaccination: Revaccination and Immunogenetic Typing

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Summary. The variation in immune responses to standard inoculation of the hepatitis-B virus vaccine suggest that host factors influence response in ways that are not presently understood. We studied 25 low/nonresponding health care workers (anti-HBs titer <50 IU/l) after the third inoculation of an experimental hepatitis-B vaccine to determine their immune status (through lymphocyte phenotypes) and HLA type. After application of a fourth inoculation, the seroconverting subjects showed only low anti-HBs levels; three male subjects remained anti-HBs negative. Twelve months after the fourth inoculation only 9 of 25 subjects (36%) maintained anti-HBs titer >10 IU/l. Almost all subjects had normal B-cell and CD-4 and CD-8 counts and ratios. Relative to other European populations HLA-A-10 ($P<0.05$), B-12 ($P<0.025$), CW-5 ($P<0.05$), DR-3 ($P<0.025$), and DR-5 ($P<0.025$) were increased, whereas DR-2 ($P<0.05$) was decreased. However, after correction of the P-values for the number of HLA antigens determined, these differences were no longer significant. Furthermore, these HLA types were not the same as those reported in other studies (except for DR-3). We suggest that larger sample sizes or even not yet available immunogenetic markers will be required to prove an "immunogenetic background" in low/nonresponders, if it exists.

Key words: Genetics – Hepatitis-B virus – Immunogenetics – Vaccination

HBV infection with its complications – fulminant course, chronicity, and development of hepatocellular carcinoma – remains a major public health problem even in areas of low prevalence, such as Europe and the United States. To minimize this risk, HBV vaccination has been recommended for groups at high risk, such as health care workers. However, the 3%–5% of healthy persons who fail to respond to active immunization against HBV infection offer a challenge for research [6, 24].

The lack of immune response to HBsAg may be related to genetic factors, since female vaccine recipients usually develop higher antibody levels against HBsAg than do males [13]. On the other hand, the immune defect of hemodialysis patients and other immunocompromised patients may be a more general one, causing seronegative rates of up to 40% [3, 7, 22, 10].

Is there an "immunogenetic background" for nonresponsiveness in active immunization against hepatitis-B virus (HBV) infection?

Better understanding of the immune response regulation towards a complex peptide antigen (HBsAg) in man may provide new strategies for the preparation of a synthetic vaccine that circumvents nonresponsiveness.

Abbreviations: anti-HBc = antibody to hepatitis-B core antigen; anti-HBs = antibody to hepatitis-B surface antigen; HBsAg = hepatitis-B surface antigen

Methods

Participants

Between October 1982 and July 1983, 217 health care workers were inoculated three times with an experimental hepatitis-B vaccine. The vaccination schedule was: first inoculation at 0, second inoculation at 1 month after the first inoculation, and third inoculation 5 months after the first inoculation. Each subject was given a total of 120 µg HBsAG in the deltoid region. All participants denied prior hepatitis, were serologically negative for HBsAg, anti-HBs, and/or anti-HBc prior to first inoculation and all had normal transaminase levels.

Four percent of the test subjects (6 males and 3 females) did not seroconvert, i.e., they developed anti-HBs titers below 3 IU/l (nonresponders). Fourteen percent of the subjects (22 males and 9 females) developed anti-HBs titers above 3 IU/l and below 50 IU/l (low responders). The immune response showed a definite dependence on the sex and age of the vaccinated subjects. Males and older persons produced significantly lower anti-HBs titers. These data have been published previously [13].

Of the 40 subjects with anti-HBs titers below 50 IU/l after the third inoculation, 25 (6 nonresponders, 4 male and 2 female; and 19 low responders, 13 male and 6 female) were located 1 year later and are the subjects of this report. The median age of the 17 males was 36 years (range, 30–53 years), and the median age of the 8 females was 31 years (range, 23–46 years). A comparison of different characteristics revealed that the low/nonresponders consisted of significantly more males than the responders ($P=0.046$, Fisher's Exact Test, two-tailed).

Revaccination and Anti-HBs Titer

These 25 low/nonresponders were revaccinated a fourth time with the same experimental hepatitis-B vaccine (40 µg HBs Ag per dose) intramuscularly in the deltoid region approximately 1 year after the third inoculation. At 3 weeks and at 1 year after the fourth inoculation, sera were obtained for anti-HBs titers, as determined by radioimmunoassay (AUSAB, Abbott Laboratories, North Chicago, Ill.). International units per liter were calculated according to the formulas developed by Hollinger [9].

Immunologic and Genetic Methods

B lymphocytes and T_3-, T_4-, and T_8-lymphocyte populations were determined as has been previously described [8]. From these data, T_4/T_8 ratios were calculated.

HLA-A, -B, -C, and -DR antigens were determined by the standard two-step microlymphocytotoxicity test according to Terasaki and McClelland [25] using the modification by van Rood [26]. A comparison was made with the results on more than 2500 Europeans [12] whose distribution of HLA antigens was very similar to that of healthy blood donors tested at this hospital.

Statistics

Statistical assessment of the frequency of HLA-antigens was done using the chi-square test and Yate's correction [1]. Adjustment of the P-values was done according to Svejgaard, which involved multiplying the P-values by the number of antigens tested [23].

Results

Revaccination

Three weeks after the fourth inoculation, three male participants (12%) had not produced an anti-HBs titer above 3 IU/l. The remaining 22 (88%) showed titers between 3.6 and 1231 IU/l (Fig. 1). Twelve months after the fourth inoculation the titer of anti-HBs had declined markedly (Fig. 2).

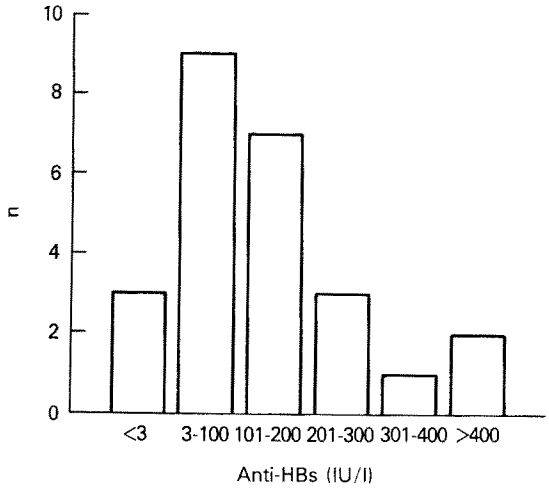

Fig. 1. Distribution of anti-HBs titers 3 weeks after the fourth inoculation. Note the relatively low anti-HBs levels. Three male participants still remained nonresponders (anti-HBs titers <3 IU/l). The highest titer was 1231 IU/l; n = number

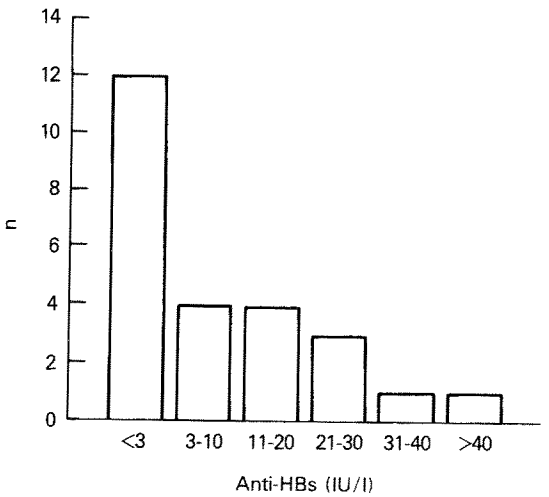

Fig. 2. Distribution of anti-HBs titers 12 months after the fourth inoculation. Note the marked titer decline. Sixteen participants had anti-HBs titers ≤ 10 IU/l; n = number

Table 1. HLA-DR frequencies of the low/nonresponders. DR3 and DR5 were found to be increased, whereas DR2 was decreased. Note, however, that significance did not hold up after the *P*-values were corrected by the number of determined HLA antigens (cp* = corrected *P*). Controls were taken from a European population analysis [12]; OR, odds ratio; n.s., not significant

Antigen	Low/nonresponders ($n=25$)		Controls ($n=2499$)		OR	*P*	cp*
	N	%	*n*	%	OR	*p*	cp*
DR1	4	16.7	332	13.3	1.30	>0.05	n.s.
DR2	2	8.3	627	25.1	0.27	<0.05	<2.35
DR3	10	41.7	509	20.4	2.80	<0.025	<1.8
DR4	6	25.0	457	18.3	1.49	>0.05	n.s.
DR5	10	41.7	487	19.5	2.95	<0.025	<1.8
DRw6	1	4.2	107	4.3	0.97	>0.05	n.s.
DR7	5	20.8	584	23.4	0.86	>0.05	n.s.
DRw8	1	4.2	135	5.4	0.76	>0.05	n.s.
DRw9	1	4.2	55	2.2	1.93	>0.05	n.s.
DRw10	0	0	35	1.4		>0.05	n.s.

Twelve subjects (48%) were now anti-HBs negative (anti-HBs less than 3 IU/l). Sixteen subjects (64%) showed anti-HBs titers ≤ 10 IU/l, which is felt to be the minimum effective titer against hepatitis-B virus infection. The anti-HBs titer of 8 other participants (32%) ranged from 11 IU/l to 40 IU/l. Only 1 subject showed a markedly high antibody titer of 155 IU/l. No subject with anti-HBs titers of ≥ 200 IU/l 3 weeks after the fourth inoculation decreased to <10 IU/l during the 12 months after that inoculation.

Immunologic Studies

Most subjects had normal T_3- (median 1260, range 470-3270/mm^3), T_4- (median 770, range 188-2240/mm^3), and T_8-lymphocyte (median 376, range 120-1470/mm^3) counts. Only 1 subject had a low T_4-cell count (188 cells/mm^3). The T_4/T_8 ratio was <1 in this and in another subject who had an increased T_8-cell count (1470/mm^3). Since antibody synthesis requires B cells, we also determined these quantitatively (median 175, range 42-590/mm^3). Only 1 subject, different from the above-mentioned ones, showed a reduced B-cell count (only 42/mm^3). Neither difference was statistically significant. Clinically and serologically, there was no evidence of acute or chronic infection, nor of a general immunodeficiency in these subjects.

Histocompatibility Testing

The 25 participants were examined for their HLA-A, -B, and -C antigens and 24 were examined for HLA-DR antigens. The DR typing was not successful in 1 subject. For controls, we used the data from a European population analysis in which more than 2500 European sera were typed [12].

A-10 ($P<0.05$), B-12 ($P<0.025$), and CW-5 ($P<0.05$) were found to be increased. DR-2 expression was decreased ($P<0.05$) and DR-3 ($P<0.025$) and DR-5 ($P<0.025$) were increased (Table 1). However, the *P*-values were no longer significant after correction for the number of HLA antigens determined.

Discussion

Hepatitis-B vaccine is made of purified HBsAg prepared from the plasma of chronic HBsAg carriers. Several factors are known to be associated with diminished antibody responsiveness to vaccination. The immune response is usually excellent in females and young recipients, but decreases with age. Males evidence significantly lower anti-HBs levels than females [13, 28]. Hemodialysis and immunosuppression negatively affect immune response to vaccination [3, 7, 22, 10].

We administered a fourth inoculation to 25 health care workers who had no or only a minor immune response after three inoculations. Three subjects failed to respond at all to the fourth inoculation. Only 9 persons (36%) remained protected (>10 IU/l anti-HBs) 12 months after the fourth inoculation. Similar findings among poor responders were also reported by Craven and coworkers using a different vaccine preparation [5]. On the other hand, among normal responders only 8.7% fell to unprotective levels over 12 months [11]. Generally, levels of anti-HBs following immunization predict levels 12 months later.

There were no significant changes of total lymphocytes and lymphocyte subpopulatons. Thus,

the immune defect in the low/nonresponders seems to be specific for HBsAg and does not indicate a general immunodeficiency.

Patients with chronic hepatitis-B virus infection are known to be hyporesponsive to HBsAg, as were these study participants. The low response has been attributed to decreased T_4/T_8-lymphocyte ratios due to elevated T_8-lymphocyte cell counts in chronic hepatitis-B. Activation of T_8 lymphocytes by HBsAg has therefore been postulated to be responsible for the absence of anti-HBs production [2, 14]. However, in our study T_8 levels were almost all in the normal range.

The immune response of HBsAg appears to be regulated by genetic factors. Milich and Neurath and co-workers have elucidated the complex regulatory mechanisms by studying high responder and low/nonresponder mice [16–21]. After crossbreeding experiments, it was possible to identify haplotype $H\text{-}2^{q,d}$ as high responders, haplotype $H\text{-}2^{a,b,k}$ as intermediate to low responders, and haplotype $H\text{-}2^{s,f}$ as nonresponders. Furthermore, immune responses are governed by two immune response (Ir) genes, one in the I-A subregion (Ir-HBs-1) and one in the I-C subregion (Ir-HBs-2) of the murine H-2 complex. Ir-HBs-1 regulates the primary responses to all HBsAg determinants, whereas the influence of Ir-HBs-2 is determinant-specific, affecting the responses to the d or y determinants.

Our results suggest evidence for an "immunogenetic background" regulating the immune response to HBsAg in man. In this study, A-10, B-12, CW-5, DR-3, and DR-5 were found to be increased in histocompatibility testing, whereas DR-2 was decreased. With regard to DR-3, this is in agreement with Craven et al. [5]. Paradoxically, an increased frequency of HLA-DR-3 was also seen in patients with autoimmune chronic active hepatitis [15]. Usually, one would expect patients with autoimmune diseases to be high responders instead of low responders. Our results differ from Craven et al. [5], Cooksley et al. [4], and Walker et al. [27] with regard to DR-7, which they found elevated.

However, these authors did not correct their *P*-values according to the number of determined HLA antigens [23]. After performing this correction, the above-mentioned differences in our study no longer remained significant. Thus, our results may only be suggestive of trend differences in the frequency of HLA antigens in nonresponders to HBsAg. The different findings in various studies may indicate that most associations were due to chance. Furthermore, the conflicts between the various reports suggest that better immunogenetic markers will be necessary for a clear indication of a genetic regulatory mechanism, such as is indicated in the mouse model.

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