

Biological parameters influencing the immunological response to plasma derived and recombinant hepatitis B vaccines

Maria Cristina HONORATI (a), Erminia MARIANI (b), Paolo DOLZANI (a)
and Andrea FACCHINI (b) (*)

(a) *Laboratorio di Immunologia e Genetica, Istituto di Ricerca Codivilla Putti,
Istituti Ortopedici Rizzoli, Bologna, Italy*

(b) *Istituto di Clinica Medica e Gastroenterologia, Università degli Studi, Bologna, Italy*

Summary. - Personnel (1856 subjects) belonging to local health units (medical and paramedical staff) that have been vaccinated since 1984 against hepatitis B virus (HBV) with HBsAg plasma purified preparations (Hevac-B and H-B-Vax) or recombinant DNA preparation (Engerix-B) were followed in plasma anti-HBs antibody levels. At the end of the protocols, different seroconversion percentages and different anti-HBs levels were reached: the best results were obtained with Engerix-B. Sex and principally age influenced the antibody production: women generally reached highest protective antibody levels and the 21-30 year group was more responsive than other groups. The injection of a supplementary 4th or 5th dose in low or non-responders could restore the specific immunity in the majority of the subjects and increase the anti-HBs level. The time course after the immunization of antibody levels depended on the level reached at the end of vaccination schedule. These data suggest that different antibody level monitorings of vaccinated subjects, planned on the basis of the antibody level reached at the end of vaccination, could prevent a loss of protection against the HBV infection.

Key words: hepatitis B, vaccine schedule, antibodies.

Riassunto (*Parametri biologici che influenzano la risposta immunitaria nei confronti di vaccini contro l'epatite B derivati da plasma e ricombinanti*). - Fra tutti i soggetti vaccinati contro il virus B dell'epatite (HBV) con HBsAg ottenuto per purificazione da plasma (Hevac-B e H-B-Vax) o secondo la metodica del DNA ricombinante (Engerix-B) e appartenenti al personale sanitario operante presso le unità sanitarie locali, 1856 sono stati controllati durante il protocollo di vaccinazione riguardo ai livelli plasmatici di anticorpi anti-HBs. Al termine del protocollo di vaccinazione sono state raggiunte diverse percentuali di sieroconversione e diversi livelli di anticorpi anti-HBs: i migliori risultati si sono ottenuti con il vaccino Engerix-B. Il sesso e soprattutto l'età influenzano la produzione di anticorpi: il gruppo delle femmine ha raggiunto generalmente i livelli anticorpali più elevati e il gruppo di soggetti di età compresa fra 21 e 30 anni è risultato il più responsivo. L'inoculo di una quarta o quinta dose supplementare di vaccino nei soggetti non o iporesponsivi ha indotto una risposta specifica nella maggioranza dei soggetti o aumentato i livelli plasmatici di anticorpi. Inoltre il decadimento dei livelli anticorpali dopo l'immunizzazione è dipendente dal livello raggiunto al termine della vaccinazione. Questi dati suggeriscono che monitoraggi differenti dei livelli anticorpali dei soggetti vaccinati, programmati in base al livello anticorpale raggiunto al termine della vaccinazione, possono prevenire la perdita della protezione nei confronti dell'infezione da HBV.

Parole chiave: epatite B, protocolli di vaccinazione, anticorpi.

Introduction

It has been estimated that more than 300 million people are infected by hepatitis B virus (HBV) all over the world. Of particular importance is the finding that among the clinical manifestations of HBV infection 5-10% of people become chronic carriers of the virus and about 25% of them will die from cirrhosis or hepatocellular carcinoma [1].

The introduction of safe and effective HBV vaccine in the early 1980s provided a mechanism to control the disease. The first generation vaccines consisted of HBsAg particles purified from HBV carriers [2]. Even though this vaccine was safe and effective in inducing protection, the large number of doses needed to vaccinate the subjects at risk and the unit price have prevented widescale vaccination in many developing countries [3]. Furthermore, a general fear developed that plasma-

(*) *In collaborazione con:* R. Ansaloni, L. Bonazzi, A. Giamperoli, G. Guerra, M. Martuzzi, M. Monti, M.C. Pirazzini, M. Tangenti (USL 27, Bologna); M. Baraldini, C.A. Busachi, M. Capelli, R. Da Re, G. Finzi, F. Giorgetti, M. Magnani, R. Miniero, E. Pisi, G. Sprovieri, U. Volta, M. Zanetti (USL 28, Bologna); G. Giuliani Piccari (USL 29, Bologna); A. Accogli, A.M. Borrelli, P. Cacciari, A. Pizzoferrato, A. Ragazzi, G. Risi (Istituti Ortopedici Rizzoli, Bologna).

derived vaccine may transmit human immunodeficiency virus (HIV) even though there was no solid evidence of this and the manufacturing procedures to produce HBV vaccine effectively inactivated the HIV virus. More recently DNA recombinant technology was applied to develop a second generation HBV vaccine which contains the peptide sequence of natural HBsAg [4]. Recombinant vaccines are safe and as immunogenic as plasma-derived products, without qualitative and quantitative differences in specific antibody production [5, 6].

This study analyzes retrospectively the level of protection induced by different types of plasma derived and recombinant HBV vaccines. The study was conducted on medical and paramedical staff at local health units 27, 28, 29 and Istituti Ortopedici Rizzoli (IOR) Bologna, Italy.

The vaccination campaign started in 1984 and the majority of health workers at risk for infection agreed to be vaccinated. From 1984 to about 1990 mostly plasma-derived vaccines were used. After 1990 the large majority was vaccinated using recombinant vaccines. Seroconversion and protective anti-HBs serum levels were monitored during and at the end of the vaccination schedule. The time course decrease of specific antibody levels was followed and utilized to monitor the percentages of subjects that 24 and 60 months after the end of the vaccination schedule were at risk for infection due to the fall in their specific antibody level to non protective values.

Materials and methods

Vaccinated population

3361 health workers in local health units 27, 28, 29 and Istituti Ortopedici Rizzoli, Bologna, agreed to be vaccinated against HBV. All subjects had normal blood and biochemical screening tests and no markers of HBV infection (HBsAg, HBeAg, HBsAb, HBeAb, HBcAb) as evaluated by RIA or EIA (Sorin, Biomedica Spa, Saluggia, VC, Italy). The age interval of vaccinees was 17-62 years.

Type of vaccines and schedule

Three different commercial preparations were used:

1) two "first generation" vaccines were plasma purified preparations: H-B-Vax (Merck Sharp & Dohme, Zurich, Switzerland) containing 20 µg HBsAg/dose and Hevac-B Pasteur (Pasteur Institute, Paris, France) containing 5 µg HBsAg/dose;

2) one "second generation" vaccine was a recombinant DNA preparation: Engerix-B (Smith Kline-Rit s.a., Rixensard, Belgium), containing 20 µg HBsAg/dose.

All the vaccines were administered by intramuscular route: Hevac-B and Engerix-B were injected in the deltoid region, H-B-Vax schedule included the two

alternative injection sites: at the beginning of the study the gluteus region was chosen and later substituted by the deltoid region because of a better seroconversion. Hevac-B vaccination schedule was 4 doses at 0, 1, 2, 14 months; H-B-Vax and Engerix-B schedules were 3 doses at 0, 1, 6 months, with an additional fourth dose at 12 months for low or non responders.

Specific antibody production

Serum samples were collected one month after the second dose for the Hevac-B vaccine, immediately before the inoculum of the third dose and 30-60 days after the third and fourth doses for the Hevac-B, the second, third and fourth doses for the H-B-Vax and Engerix-B vaccines. Anti-HBsAg antibody level was evaluated by EIA (Sorin Biomedica, Saluggia, Italy) and compared to the WHO standard. The low sensitivity of this test was 2 IU/l. The subjects presenting an antibody level over 2 IU/l were considered responders. An antibody level ≥ 10 IU/l was considered protective [7, 8].

Statistical analysis

For statistical analysis all subjects were divided into two groups: protected subjects (antibody level ≥ 10 IU/l) and not protected subjects (antibody level < 10 IU/l).

All the data concerning the antibody levels reached by the study group were collected in a data base for further statistical analysis using an SPSS package. Due to non parametric distribution of data, statistical analysis was performed after logarithmic transformation of data.

Means, standard deviation and 95% confidence limits of transformed data were calculated for each group and for each considered schedule time. The anti-logarithm of these data represents the geometric mean titre (GMT) and a skew 95% confidence limit compared to the original scale. Mann-Whitney, χ^2 and Kendall tests were used to analyze the different groups.

Results

Degree of seroconversion during and at the end of the vaccination schedule

Only 1856 out of 3361 subjects that started the immunization in 1984 were considered for statistical analysis. These subjects were divided into 4 groups depending on the vaccine type received and on the site of administration (Table 1).

We excluded 1505 subjects from the statistical analysis because of an irregular serological control or incomplete vaccination schedule. The large majority of these subjects were protected against infection but their antibody levels may be not representative of typical seroconversion obtained with the standard protocol.

Table 1. - Subjects immunized with different HBV vaccines

Vaccine	Hevac-B	H-B-Vax		Engerix-B
Inoculation site	deltoid	gluteus	deltoid	deltoid
Subject number	198	207	122	1329

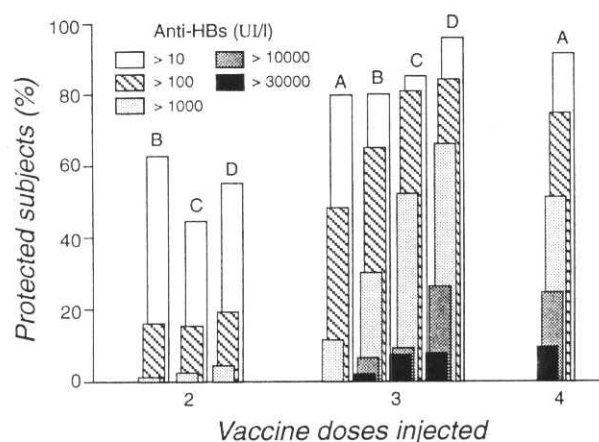
**Fig.1.** - Distribution of total immunized subjects for different seroconversion levels. All vaccines were compared: A) Hevac-B (4 doses); B) H-B-Vax gluteus (3 doses); C) H-B-Vax deltoid (3 doses); D) Engerix-B (3 doses).

Fig. 1 reports the comparison between anti-HBs levels reached during the vaccination schedule for the different HBV vaccines. The degree of seroconversion was influenced by the different type of vaccine and by the site of injection. Using H-B-Vax at the end of the vaccination protocol the group injected in the deltoid region presented a similar number of seroconverted

subjects, compared with the group injected in the gluteus. The overall number of non protected (anti-HBs < 10 IU/l) was 15% (deltoid) and 20% (gluteus). 52% of subjects injected in the deltoid presented an antibody level over 1000 IU/l ($p < 0.01$ deltoid vs gluteus inoculation site) and this level was even more elevated (> 30.000 IU/l) in 7% of subjects ($p < 0.01$). These levels were reached in a low percentage of the subjects vaccinated in the gluteus region. At the end of the vaccination schedule with Hevac-B and Engerix-B vaccine preparations a progressively high number of subjects was protected ($p < 0.05$): 91% and 96% respectively ($p < 0.05$ and $p < 0.01$ compared with H-B-Vax). The highest anti-HBs levels were reached with Engerix-B (3 doses) and Hevac-B (4 doses).

Using a 3 dose schedule, the percentage of subjects which reached a protective level (> 10 IU/l) or presented high antibody levels (> 1000 IU/l) was obtained by the different types of vaccine in this sequence: Engerix-B, H-B-Vax (deltoid), H-B-Vax (gluteus) ($p < 0.01$). The percentage of subjects with a protective level (≥ 10 IU/l) or high antibody levels (> 1000 IU/l) increased also after the fourth Hevac-B dose but they were lower than values obtained with Engerix-B at the end of vaccination schedule ($p < 0.01$).

Sex and age influence on protective anti-HBs levels

No difference in protective levels was observed when male and female percentages were compared for all types of vaccines and/or site of injection (Table 2). On the contrary, females presented higher levels of anti-HBs ($p < 0.05$ at least) after H-B-Vax (deltoid) and Engerix-B.

Fig. 2 shows the influence of the age of the subjects vaccinated in terms of anti-HBs antibody levels reached at the end of the vaccination schedules. The age of the subjects has a marked effect on the specific antibody production at the end of vaccination. The highest antibody

Table 2. - Sex influence on immune specific response

Vaccine	Sex	Protected subjects (%) (> 10 IU/l)	GMT	95% confidence limits
Hevac-B	Male	90	1023	416 - 2449
	Female	91	1748	760 - 4014
H-B-Vax (gluteus)	Male	81	501	289 - 858
	Female	81	588	389 - 912
H-B-Vax (deltoid)	Male	82	741	267 - 1823
	Female	87	1318 (*)	623 - 2507
Engerix-B	Male	92	1413	1096 - 1789
	Female	97	2884 (**)	2454 - 3339

Males vs females: (*) $p < 0.05$; (**) $p < 0.01$.

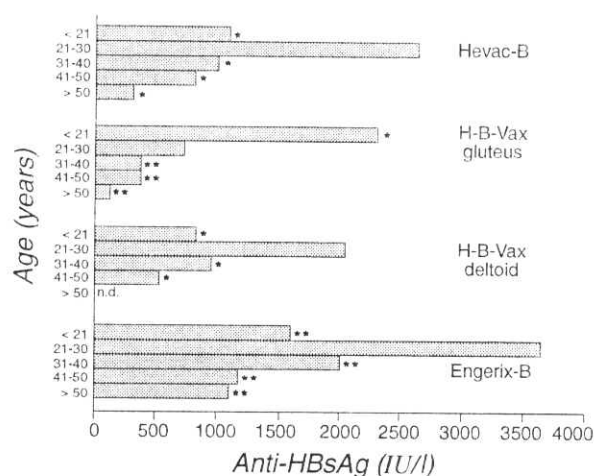


Fig. 2. - Distribution of total immunized subjects for different age intervals. The 21-30 year group was compared with each age interval for the same vaccine type: * $p < 0.05$; ** $p < 0.01$.

levels were reached by the 21-30 year group and progressively decreased in the following decades for all but one (H-B-Vax glutеus) vaccine preparations. Comparing the different types of vaccine, the highest antibody levels were reached with Engerix-B for the 21-30 decade ($p < 0.01$ vs H-B-Vax inoculated in glutеus; $p < 0.01$ vs Hevac-B).

Frequency distribution of anti-HBs levels

The analysis of distribution of serum levels of anti-HBsAg antibodies at the end of Engerix-B vaccination with recombinant HBsAg showed that the major frequency of distribution was in the interval between 11 and 2000 IU/l (Fig. 3). This interval contained also the major frequency of distribution for all the other vaccine preparations examined and for both sites of injection (data not shown). Mean age of subjects of this group was 32.8 ± 9.8 years (interval of age 17-59 years).

Low or non-responders

An antibody level < 160 IU/l was reached by 385 subjects at the end of the vaccination schedule of three doses (vaccinated with Engerix-B or H-B-Vax). They were considered low or non responders. This level was chosen in agreement with accepted values (10 - 100 IU/l) for low responders, also including 15 subjects with an antibody level of 101-160 IU/l because of a high risk of exposure to infection. They were injected with another dose at the twelfth month after the beginning of vaccination. 130 out of 385 subjects were followed after the fourth dose. This supplementary dose increased the number of subjects protected against HBV infection to values similar to those obtained with 3 doses and determined an increased level of seroconversion (Table 3). However, the antibody level in these subjects remained substantially low (GMT after standard 3 dose schedule: Engerix-B = 3370 ± 12 IU/l, $p < 0.01$; H-B-Vax = 1238 ± 9 IU/l, $p < 0.01$).

A subgroup of 40 low or non-responder subjects who underwent the 4 dose protocol with Engerix-B or H-B-Vax, were followed for the antibody level decrease and injected again with a 5th boost two years after the beginning of vaccination schedule.

After the fourth and fifth supplementary doses the GMT of the antibody level reached 440 and 4.680 IU/l respectively with a significant positive correlation ($p < 0.01$).

Time course decrease in anti-HBs antibodies

Twenty-four and 60 months after the end of the vaccination schedule, serum samples from 345 subjects belonging to the group vaccinated with recombinant vaccine Engerix B were analyzed for the specific antibody level decrease (GMT at the end of the vaccination: 3820 ± 6.5 IU/l; interval of age: 23-38 years; mean age: 29.4 ± 4.4). The parameters examined as sex and age did not significantly influence the decrease in antibody level, even if the age did affect the starting level of antibody. The time course decrease was dependent on the antibody

Table 3. - Effect of a 4th supplementary dose in 130 low or non responder subjects ($\leq 160,000$ IU/l)

Vaccine type	Response after 3 doses			Response after 4 doses			Protection reached after 4th dose % (*)
	Response (IU/l)	GMT	% (*)	Response (IU/l)	GMT	% (*)	
Engerix-B	protective (10 - 160)	39	69	protective (15 - 160,000)	320	69	88
	non protective (<10)	4	31	protective (32 - 204)	58	19	
				non protective (<10)	6	12	
H-B-Vax deltoid	protective (25 -136)	45	57	protective (148 - 3292)	694	57	85
	non protective (<10)	2	43	protective (30 - 198)	54	28	
				non protective (<10)	3	15	

(*) percentage on the total number of subjects receiving 4 doses.

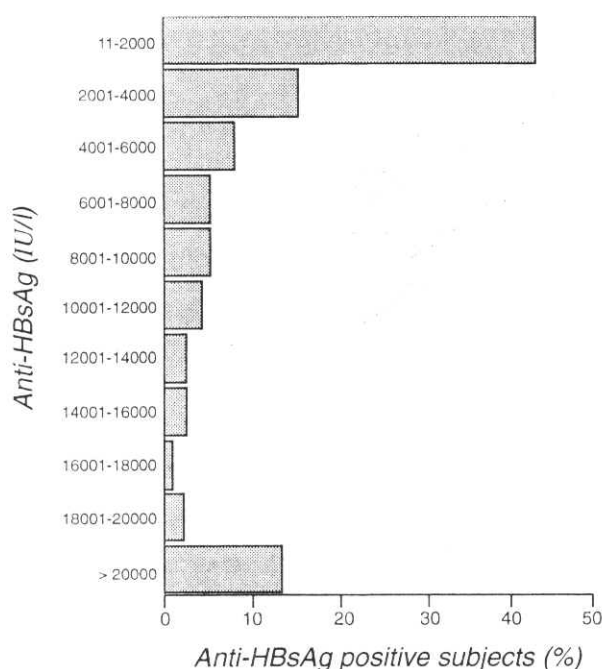


Fig. 3. - Distribution of Engerix-B immunized subjects for seroconversion level intervals.

level reached at the end of a complete vaccination schedule and was positively correlated with this variable ($p < 0.05$). Twenty-four months after the end of vaccination, 60% of subjects were protected with a GMT of 427 ± 3.1 IU/l, and after a further 36 months the percentage of protected subjects was 55% with a GMT of 337 ± 2.9 IU/l.

Discussion

Different types of vaccines, time schedules and sites of injection can induce a different distribution in the proportion of anti-HBs levels reached.

The highest antibody levels obtained with Engerix-B cannot be simply explained by the amount of antigen present in the vaccine preparation. The same amount (20 µg) of antigen is present in the H-B-Vax preparations which gave the lowest response in term of protective antibodies. The different immunogenicity of the vaccines may arise from the purification techniques used to obtain the final product. These data agree with previous data from other authors demonstrating an increased percentage of seroconversion and the development of a higher antibody level with recombinant vaccines than with plasma purified ones [9]. Furthermore, 96.3% of cases with seroconversion were found in a group of children immunized after the third month of life with a plasma purified vaccine; using the same protocol but with a recombinant vaccine, all of another group of children seroconverted [10].

It is possible that the "s" antigen obtained by recombinant DNA technology offers better immunogenicity than the antigen purified by plasma. This difference may be due to the glycosylation of "s" natural protein. The "s" recombinant antigen which is not glycosylated, could be easily presented and processed and since the weight is equal, non glycosylated recombinant antigen is constituted by more protein than the plasma purified form.

In agreement with others [11] we found highest protective antibody levels among women. These differences are evident only when the number of subjects is high, probably due to the broad variability of the data (as shown by the wide range of 95% confidence limits).

Although the efficiency in inducing anti-HBsAg response by the different vaccine preparations was high, a low number of low or non-responders was still present even using second generation recombinant vaccines. Antibody production against a specific antigen is influenced by a broad individual response variability. Part of this variability may be related to the MHC system as it was suggested that a low response to HBV vaccine is due to the lack of a dominant MHC gene and to homozygous haplotype for HLA-B8, SC01, DR3 antigens [12]. The highest antibody levels were obtained in the 21-30 year age group: the other older groups reached a progressive lower amount of protective antibodies. It is well known that aging is associated with a progressive impairment of the immune system, affecting mainly T lymphocyte activities. This alteration also affects the efficiency of specific antibody production to different environmental antigens or vaccines (e.g.: influenza vaccine) and the immunological "memory" to specific antigen [13].

We demonstrated that the time course decrease in antibody level depends on the values reached at the end of the vaccination schedule and that aging influences this value. A protective antibody level present 24 months after the end of the vaccination seems to be determinant for the maintenance of protection until at least 60 months after vaccination. It is also likely that the minimal protective antibody level will be lost sooner in the aged than in the young groups after the 60 months examined. A different monitoring schedule is recommended for *low responders*, especially those over forty years of age.

Acknowledgements

This work was partially supported by Ministero della Sanità, Progetto Finalizzato "Protezione dall'infezione da virus dell'epatite B in operatori ospedalieri: risposta immunitaria cellulare ed anticorpale e valutazione della memoria immunologica", IOR Ricerca Corrente and MURST ex quota 60%.

Received on 2 May 1995.

Accepted on 22 November 1995.