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Practical Considerations in Converting from Plasma-Derived to Recombinant Hepatitis B Vaccines

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Abstract

Plasma-derived and recombinant vaccines have been developed to prevent hepatitis B virus infections. Both types of vaccine perform very well with respect to safety, immunogenicity and protective efficacy. The protection afforded by both types of vaccine is satisfactory for at least 5 to 10 years after vaccination, and a further booster dose is not necessary during this period. However, the plasma-derived vaccine is costly to produce and there is an unjustified but prevalent fear that it may be contaminated by potential pathogens. The supply of human plasma for production of the plasma-derived vaccine cannot be assured once use of hepatitis B vaccines becomes universal. It is therefore inevitable that the recombinant vaccine will replace the plasma-derived vaccine. If necessary, both vaccines can be used in combination.

Future directions for hepatitis B vaccine development include: (i) determination of the need for incorporation of pre-S gene products to enhance immunoge-

nicity; (ii) defining a practical strategy to combat the problem of escape mutants after vaccination; and (iii) development of combination vaccines containing other inactivated antigens to allow complete immunisation against several diseases with a minimal number of injections.

1. Hepatitis B

The hepatitis B virus (HBV) is a member of a distinct family of hepatotropic DNA viruses called the hepadnaviruses. It is a double-shelled spherical particle with a diameter of 42nm. Its outer shell is composed of the hepatitis B surface antigen (HBsAg). Within its core the virus contains HBV DNA, the hepatitis core antigen and a nonstructural antigen called the hepatitis B e antigen (HBeAg).[1] The latter is a marker of active replication of HBV, and sera positive for HBeAg usually contain higher levels of HBV DNA.[2,3]

HBV DNA is a circular, partially double-stranded DNA molecule with a genome length of approximately 3200 nucleotides.[2] Four open reading frames have been identified, the *S*, *C*, *X* and *P* genes. The *S* gene and the pre-S region upstream from the *S* gene encode 3 surface proteins of unequal size but with identical C-terminal sequences: pre-S1 antigen (389 amino acids), pre-S2 antigen (281 amino acids) and S antigen (226 amino acids).[4,5] The *C* gene and the pre-C region upstream from the *C* gene encode hepatitis B core antigen and HBeAg. The *X* gene codes for a protein that has transcriptional transactivating properties. The *P* gene codes for the DNA polymerase. Like retroviruses, HBV uses reverse transcription as part of its replicative mechanism. HBV DNA is produced from an RNA precursor.[2]

At least 5 antigenic specificities are found on HBsAg particles. A group-specific determinant ‘a’ is shared by all HBsAg preparations and by two pairs of subtype determinants (*d*,*y* and *w*,*r*) which are mutually exclusive and which thus usually behave as alleles.[6] HBsAg subtypes are unevenly distributed around the world.[6]

2. Global Importance of Hepatitis B

HBV infection is a global health problem. The prevalence of HBV infection varies in different parts of the world. It is hyperendemic in many countries of Asia and Africa, with the prevalence of HBV infection ranging from 30 to 100%. Up to 20% of the population in these areas may experience chronic hepatitis B infection. Intermediate levels of HBV infection are characteristic of most countries in eastern Europe, the Middle East and Central and South America. The US, Canada, Australia and most countries of western Europe are areas of low endemicity for HBV infection, with carrier rates lower than 1% and the lifetime risk of infection often less than 10%.[7]

The patterns of HBV transmission differ in various parts of the world. In highly endemic areas, the dominant modes of HBV transmission are from infected mothers to infants in the perinatal period and to young children from infected members of the household or community.[8] Between 65 to 93% of infants born to HBeAg-positive carrier mothers become chronic HBsAg carriers.[9-16] Multiple modes of virus transmission are characteristic of areas with lower incidence of HBV infection. Most HBV infections occur in adolescent and adult populations and are acquired via sexual contact, intravenous drug abuse or other routes of horizontal transmission.[10,17]

Although a chronic carrier of HBV may be asymptomatic, chronic hepatitis, liver cirrhosis and hepatocellular carcinoma may develop in later life.[3,18,19] The importance of hepatitis B is stressed by the fact that more than 40% of persistently infected persons who reach adulthood will die of the consequences of the disease. More than 1 million fatalities occur annually due to serious sequelae of hepatitis B.[20]

3. Hepatitis B Vaccine

3.1 Vaccine Development

Epidemiological studies of natural HBV infec-

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tion have shown that development of hepatitis B surface antibody (anti-HBs) conferred protection against subsequent infection. Consequently, HBsAg purified from the plasma of healthy HBV carriers was used to develop hepatitis B vaccines during the 1970s.[7,21] The plasma-derived vaccine (PDV) was composed of noninfectious 22nm HBsAg particles purified by physicochemical methods. In many of these vaccines the HBsAg was treated by heat and chemicals to ensure that infectious virus did not persist.

With the development of recombinant DNA technology, a new hepatitis B vaccine was produced in mid-1980.[7,21] The *S* gene of the HBV genome was inserted into an appropriate plasmid that could express HBsAg in the yeast.[7,21] The recombinant vaccine (RV) was made by purifying yeast-derived HBsAg with conventional biochemical techniques.

3.2 Vaccine Characteristics

Both PDV and RV are inactivated subunit vaccines that contain only HBsAg and no infectious virions are present. The HBsAg in both types of vaccines is adsorbed to aluminum hydroxide and thiomersol is added as a preservative. There are subtle chemical differences between the two types of vaccine: (i) the lipid moiety of PDV and RV is characteristic of plasma and yeast, respectively; and (ii) approximately 25% of the plasma-derived HBsAg is glycosylated, whereas the recombinant HBsAg is nonglycosylated.[7,21,22]

PDV, being derived from pooled plasma of carriers, may contain different subtypes of HBsAg according to the source of plasma.[23] Products of the pre-S region are stripped off in the production process of PDV.[24] However, small amounts of pre-S2 antigen may be present in some PDV.[25] RV usually contain only 1 subtype of HBsAg. However, all subtypes share the ‘a’ determinant of HBsAg, and antibody to this region confers protection against all subtypes.[26,27] In a natural infection with HBV, most antibodies (approximately 90%) are raised against the ‘a’ region of HBsAg.[28] Immunodominant B and T cell epitopes have been demonstrated in this region of HBsAg.[29] As observed with the PDV, the RV (subtype *adw*) protected against HBV challenges of subtypes *adr* or *ayw* in chimpanzees, thus highlighting the *in vivo* protective effect of anti-‘a’ antibodies.[30]

3.3 Safety of Vaccines

Both types of hepatitis B vaccine are very well tolerated and few adverse reactions have been reported. The most frequent adverse effects of hepatitis B vaccines are injection site soreness and mild fever.[21,31,32] The number of symptoms reported by the parents of vaccinated children was considerably lower than the reactions reported by vaccinated adults.[33] In placebo-controlled studies of PDV, adverse reactions were reported at similar frequencies among vaccine recipients and among those given placebo.[34,35] Studies that compared the reactogenicity of PDV and RV showed no significant differences in reported adverse effects.[36] Although there are some case reports of severe adverse reactions in the literature, these events have not been shown to occur at increased rates in vaccinated persons.[21]

Contaminating infectious agent has been a concern in the development of PDV. Efforts have been made to inactivate potential contaminating infectious agents, including the human immunodeficiency virus (HIV).[37] Vaccine-associated infection has not been observed in spite of worldwide use of PDV.

Concern has also been expressed about RV that contain minute quantities of yeast proteins. In all studies, no proven yeast hypersensitivity was observed.[33] In addition, no significant changes in specific anti-yeast antibodies were detected in most vaccine recipients.[38] In those who experienced increases of anti-yeast antibodies, there was no correlation with clinical symptoms.[31,36,39]

3.4 Protective Efficacy

The protective efficacy of hepatitis B vaccines for infants born to HBeAg-positive carrier mothers has been evaluated in many studies. In studies giving hepatitis B immune globulin (HBIg) at birth

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Table I. Protective efficacy of hepatitis B vaccine and hepatitis B immune globulin in infants of HBeAg-positive mothers

Investigator	Vaccine	Dose (μg)	Schedule (month)	HBIg (month)	n	HBsAg carriers (%)	Protective efficacy (%)
Stevens et al.[40]	PDV	10/20	0–1–6	0	78	15.4	80.0
	PDV	10/20	1–2–6	0	232	11.2	85.5
Lo et al.[16]	PDV	5	0.5–1.5–2.5	0	35	11.4	85.5
	PDV	2.5	0.5–1.5–2.5	0	40	10.0	87.2
Beasley et al.[12]	PDV	20	1–2–7	0	58	8.6	88.4
	PDV	20	0.25–1–6	0	50	6.0	91.9
	PDV	20	3–4–9	0-3	51	2.0	97.3
Xu et al.[15]	PDV	20	0–1–6	0	16	6.2	90.5
Wong et al.[14]	PDV	3	0–1–2–6	0	33	6.1	91.0
Hwang et al.[41]	PDV	10	0–1–6	0	52	7.7	91.3
Pongpipat et al.[42]	RV	5	0–1–6	0	20	10.0	89.2
Stevens et al.[43]	RV	5	0–1–6	0	351	5.4	92.3
Poovorawan et al.[44]	RV	10	0–1–2–12	0	65	1.5	97.6
	RV	10	0–1–6	0	60	0	>97.4
Lee et al.[45]	RV	20	0–1–2–12	0	54	7.4	91.6
	RV	10	0–1–2–12	0	56	1.8	98.0
	RV	20	0–1–2–12	0	60	3.3	96.3
Lee et al.[46]	RV	10	0–1.5–5.5	0	55	7.3	91.7

HBeAg = hepatitis B e antigen; **HBIg** = hepatitis B immune globulin; **HBsAg** = hepatitis B surface antigen; **n** = number of recipients; **PDV** = plasma-derived vaccine; **RV** = recombinant vaccine.

concurrently with the first dose of vaccine, the protective efficacy has been between 80 and 100%, irrespective of vaccine type, study site or schedule (table I).[12,14-16,40-46] When used alone, the protective efficacy of hepatitis B vaccine was somewhat lower and ranged between 46 and 75%.[14-16,36,41,44] When only HBIg was given, a study in Taiwan showed that the protective efficacy was 42% for infants receiving a single 1ml dose of HBIg at birth and was 71% for infants receiving 0.5ml of HBIg at birth, 3 months and 6 months.[13] In a review of hepatitis B vaccine studies, it was shown that simultaneous use of HBIg was more important to elicit good protection when hepatitis B vaccines were given at lower dosages than at higher dosages.[47]

In one study, the protective efficacy was significantly better with the RV regimen than with the regimen that included PDV.[43] No biological explanation for this association was apparent. In another limited study of 40 infants, the protective efficacy was not significantly different between PDV and RV.[42] When interpreting these data, it must be recognised that the level of viraemia varies remarkably in HBeAg-positive carrier mothers. Perinatal exposure to high levels of maternal HBV DNA was shown to be the most important determinant of infection outcome in the infant.[48] On the other hand, protective efficacy of hepatitis B vaccines is dose-related,[47,49] and different doses of PDV and RV may not be directly comparable in terms of their potency. These variables must be taken into consideration when comparing different types of hepatitis B vaccine. An overview of study results indicates that both PDV and RV are highly effective in preventing perinatal transmission of HBV.

3.5 Immunogenicity

The minimum protective level following hepatitis B vaccination has been set in earlier protective efficacy studies at ≥10 mIU/ml of anti-HBs.[35,50] Some studies have demonstrated that the risk of HBV infection increases as anti-HBs levels decline to 10 mIU/ml.[51-53] Although it is under debate, the minimum protective level of anti-HBs is usually set at 10 mIU/ml and a nonresponsiveness to hep-

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atitis B vaccine is usually defined as a failure to mount an anti-HBs response above this level after vaccination.[54]

By using either PDV or RV, a protective level of anti-HBs may be achieved in >90% of healthy vaccine recipients.[43,45,46,55-57] Concurrent administration of HBIg at birth does not interfere with the infant's immune response to hepatitis B vaccine.[14,15] Similar anti-HBs responses were observed in infants born to mothers positive or negative for HBsAg and HBeAg.[36,46,58] The anti-HBs response to hepatitis B vaccine is reduced in people >40 years of age and in those who are immunocompromised.[59-62] Gluteal injection results in poor immunogenicity, probably reflecting failure to achieve intramuscular delivery, and should be avoided.[61,62]

A protective level of anti-HBs cannot be induced in 5 to 10% of vaccinees by either PDV or RV.[54] No response to HBsAg in immunocompetent vaccine recipients appears to be genetically determined, as supported by the identification of specific extended haplotypes in nonresponders.[63-65] A higher frequency of HLA-DR3 and HLA-DR7 was found among the Caucasian nonresponders.[64] A recent study in a Caucasian population showed that the majority of nonresponders expressed the phenotypes B8; DRB1*0301; DQB1*0201 or B44; DRB1*0701; DQB1*0201.[54] On the other hand, an association of HLA-DR14-DR52 with nonresponsiveness to HBsAg was demonstrated in a Chinese population.[65] The reasons for the association between different HLA-DR alleles and the poor immune response to HBsAg in different ethnic groups are not well understood. Since there is much polymorphism within the HLA-DR loci and diverse distribution of HLA typing in different ethnic populations, immunogenic peptides from the HBsAg vaccine may fail to bind appropriately to specific DR (for example, DR3 in Caucasians, DR4 in Japanese or DR14 in Chinese) associated class II molecules and this may lead to the lack of specific immune response to HBsAg.[65] When those who have no response to hepatitis B vaccine acquire natural infection with HBV, they mount a normal immune response to all HBV antigens and acquire anti-HBs. This observation may be related to the demonstration that T cell response to hepatitis B core antigen provides help for antibody production against HBsAg.[66,67]

The immunogenicity of PDV and RV has been compared in several studies and the results were inconsistent. Some authors suggested that the mean level of anti-HBs induced by RV was higher than that by PDV.[68,69] Some reports stated that RV appears to induce somewhat lower mean levels of anti-HBs when compared with PDV.[70-72] Other authors concluded that PDV and RV were equivalent in immunogenicity.[33,57]

It has been shown that the peak anti-HBs level after vaccination with PDV or RV correlated directly with the dose of vaccine administered.[43] In children who failed to respond to initial vaccination with PDV, supplementary doses of either PDV or RV could elicit a protective level of anti-HBs in a substantial proportion of children.[73,74] In addition, the immunogenicity of hepatitis B vaccines depends critically on the tertiary and quaternary structural properties of multimolecular HBsAg aggregates, which in turn may differ from product to product in the density and presentation of immunising epitopes.[7] Therefore, the potency of PDV and RV may not be directly comparable. Both types of vaccine are highly immunogenic.

3.6 Antibody Persistence

Typically, anti-HBs levels decline rapidly over the first 6 to 12 months after the initial vaccination series is completed, with a more gradual decline in subsequent years.[23,44,51,75-77] Several studies have been carried out to assess the persistence of anti-HBs after vaccination and yielded a wide range of seropositive rates for anti-HBs at the end of follow-up (table II). It is generally agreed that the persistence of anti-HBs correlates with the initial peak antibody level,[23,43,52,77,86] which in turn correlates with the dose of vaccine administered.[43,86] Differences in results of antibody persistence may thus be accounted for by different doses of hepatitis B

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Table II. Antibody persistence after hepatitis B vaccination

Study group	Vaccine	Dose (μg)	Schedule (month)	n	Follow-up duration (years)	Follow-up anti-HBs ≥10 mIU/ml (%)
Infants						
Lo et al.[76]	PDV	2.5/5	0–1–2–12	101[a]	5	97.0
Resti et al.[78]	PDV	5	0.7–2–12	72	5	95.8
Stevens et al.[43]	PDV	10/20	0–1–6/1–2–6	70	4–9	95.7
Chan et al.[79]	PDV	5	0–1–2–12	88[a]	4	95.5
	PDV	2	0–1–2–12	78[a]	4	92.3
	PDV	1	0–1–2–12	85[a]	4	95.3
Lee et al.[80]	PDV	20	0–1–6	411[a]	4	95.9
	PDV	10	0–1–6	253[a]	4	92.5
	PDV	5	0–1–6	93[a]	4	83.9
	PDV	2.5	0–1–6	54[a]	4	64.8
Gonzalez et al.[81]	PDV	10	0–1–6	56	7	87.5
Delage et al.[82]	PDV	10	4 schedules	127[a]	5	86
Moyes et al.[83]	PDV	2	0–1.5–5	54[a]	4	83.3
		1	0–1.5–5	16[a]	4	62.5
Yvonnet et al.[23]	PDV	5	0–1–2–12	73	6	78.1
Coursaget et al.[84]	PDV	5	0–1–2–12	31	11–12	67.7
Ding et al.[85]	PDV	30	0–1–6	74	9	51.4
Poovorawan et al.[44]	RV	10	0–1–2–12	91[a]	3–4	100
Stevens et al.[43]	RV	5	0–1–6/0–1–9	34	4–9	97.1
Lee et al.[86]	RV	20	0–1–2–12	43	5	93.0
	RV	10	0–1–2–12	44	5	86.4
	RV	20	0–1–6	48	5	70.8
Children						
Whittle et al.[87]	PDV	20	0–2–4	61	4	95.1
Lai et al.[57]	PDV	10	0–1–6	64	5	84.3
Zhang et al.[88]	PDV	10	0–1–2	95	5	72.6
Lai et al.[57]	RV	5	0–1–6	63	5	87.3
	RV	5	0–1	72	5	75.0
Goh et al.[89]	RV	5	0–1–6	31	4	87.0
	RV	2.5	0–1–6	30	4	80.8
	RV	1.25	0–1–6	30	4	73.0
	RV	0.6	0–1–6	31	4	70.3
Adults						
Trivello et al.[90]	PDV	?	0–1–2–14	653	6	93.9
	PDV	?	0–1–6	302	6	67.2
Jilg et al.[77]	PDV	20	0–1–6	177[a]	5	69.9
Homosexual men						
Hadler et al.[52]	PDV	20	0–1–6	94[a]	5	58.5

a Only seroconverters to initial vaccination were included in follow-up studies.

Anti-HBs = anti–hepatitis B surface antibody; **n** = number of recipients; **PDV** = plasma-derived vaccine; **RV** = recombinant vaccine.

vaccine used and by different durations of follow-up.

The rate of decrease in the anti-HBs level is not significantly affected by different types of vaccine and by different study areas. Antibody persistence has been examined after 4 years in adults administered either PDV or RV. Four years after vaccination, anti-HBs titres in groups receiving PDV or RV

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were of similar magnitude, indicating that the decrease in antibody titre with time is similar for the 2 types of vaccine.[33]

In a follow-up study of children vaccinated with PDV, Coursaget et al.[91] proposed a regression model to describe the changes of anti-HBs level over time:

$$\log_e(\text{anti-HBs}) = A + [B_1 \times \log_e(\text{peak anti-HBs after vaccination})] + [B_2 \times \log_e(\text{days since booster})]$$

They demonstrated that all the values of B_2 in different studies were equal to –1. In another study of children vaccinated with RV, the decline of anti-HBs level during a 5-year follow-up period also fitted well with the regression equation with the same value of –1 for B_2.[86] These results indicate that putting peak anti-HBs levels into a regression model may make different study results comparable. The most important factor of antibody persistence is the peak anti-HBs attained after vaccination.

3.7 Long term Protection

Several reports give insight to vaccine-induced long term protection in high-risk infants born to HBsAg carrier mothers (table III). If not infected, maternal anti-HBc in these infants will disappear after 2 years of age.[86] Therefore a positive anti-HBc beyond 2 years of age may be regarded as evidence of a previous HBV infection. In these follow-up studies, the incidence of acquiring natural infection in children previously responsive to hepatitis B vaccine ranged from 0 to 11.7%. Natural infections have also been identified in other study participants with vaccination.[43,52,87,88,93] These infections were usually manifest only by anti-HBc positivity and were infrequently accompanied by the expression of HBsAg.[51,52,78,80,87,94] The risk of acquiring natural infection after vaccination was shown to correlate with several factors, including:

- low antibody response to vaccination[52,75,77,87]
- incomplete immunisation[94]
- maternal HBsAg positivity[75,87]
- local endemicity[87]
- sexual activity in homosexual men.[52]

In some reports, a rise of anti-HBs during follow-up was regarded as evidence of natural infection. However, criteria for such a ‘natural booster’ have differed among studies.[51,52,57,79] Some factors other than natural infection may also contribute to the fluctuations of anti-HBs level, such as the natural occurrence of mild fluctuation, technical errors in assays and nonspecific increase due to concurrent illness.[57] In a follow-up study of vaccinated children with yearly visits, there were only 2 significant increases of anti-HBs level among 11 children with natural infection beyond 1 year of age.[86] The rates of decrease of anti-HBs were sim-

Table III. Late hepatitis B virus infections in immunised infants born to hepatitis B carrier mothers

Investigator	Vaccine	Maternal HBeAg	n	Follow-up (years)	Late infections [number (%)]	
					Anti-HBc+ only	HBsAg+
Stevens et al.[43]	PDV	+	70	5–10	6 (8.6)	0
Grosheide et al.[92]	PDV	+	72	5	8 (11.1)	0
Lo et al.[76]	PDV	+	199	3–5	NA	0
Lee et al.[80]	PDV	+	667	4	NA	4 (0.6)
Resti et al.[78]	PDV	+/–	494	5	NA	8 (1.6)
Gonzalez et al.[81]	PDV	+/–	79	7	NA	0
Ding et al.[85]	PDV	+/–	74	9	7 (9.5)	0
Delage et al.[82]	PDV	+/–	146	5	5 (3.4)	0
Poovorawan et al.[44]	RV	+	91	3–4	NA	0
Stevens et al.[43]	RV	+	34	5–10	1 (2.9)	0
Lee et al.[86]	RV	+	163	5	19 (11.7)	0

Anti-HBc = anti–hepatitis B core antibody; **HBeAg** = hepatitis B e antigen; **HBsAg** = hepatitis B surface antigen; **n** = number of recipients; **NA** = not available; **PDV** = plasma-derived vaccine; **RV** = recombinant vaccine.

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ilar in nonendemic and endemic areas.[86,91] Therefore, natural HBV infections contribute little, if anything, to maintenance of immunity.

3.8 Booster Dose after Primary Vaccination

Because vaccine-induced antibodies wane over time, some investigators have suggested that a further booster dose of hepatitis B vaccine may be needed at some time after primary vaccination.[23,51,81,83,91] It was shown that hepatitis B vaccination may prevent not only the risk of becoming a carrier but also subclinical infections manifested by anti-HBc positivity.[51,86] A report from Senegal demonstrated that the annual rate of HBV infection was higher in unvaccinated (11.5%) than in vaccinated children (1.5%).[51] A serological survey in Taiwan showed the risk of HBV infection decreased among both the vaccinated and the unvaccinated cohorts of children, 5 years after a mass hepatitis B vaccination programme.[95] These observations indicate that hepatitis B vaccination may lower the overall risk of natural infection not only because of the immunity elicited by the vaccine but also because of a reduced risk of horizontal transmission after a mass vaccination programme including health education. For children in hyperendemic areas of HBV infection, the risk of becoming a carrier after acquiring infection is low because those infected by horizontal transmission of HBV at older ages are at lower risk of becoming carriers.[8]

When a booster dose of hepatitis B vaccine was given 4 to 6 years after primary vaccination, a dramatic increase of anti-HBs was observed in most vaccine recipients whether or not they retained a protective level of anti-HBs (≥10 mIU/ml).[23,78,81,83,90] Therefore, the decrease of anti-HBs levels below 10 mIU/ml in previously responder study participants does not necessarily mean a loss of immunological memory. The *in vivo* humoral immune response to HBsAg has been correlated with the *in vitro* cellular immune response to HBsAg in vaccinees.[96] Since cell-mediated immunity lasts longer than humoral immunity, it may be responsible for the good antibody responses to booster vaccine.

Many follow-up studies have found that the protection afforded by hepatitis B vaccine is satisfactory for at least 5 years after vaccination.[52,76,78,79,83,86-89,92,93] A few studies with a longer duration of follow-up also found that the protection is also satisfactory for 7 to 10 years after vaccination.[43,81,85] In a 9- to 12-year follow-up study of vaccinated children, a booster dose at school age did not significantly increase the protection against HBV infections manifested either by HBsAg positivity or by anti-HBc positivity.[84] Therefore, it can be concluded that the protection afforded by hepatitis B vaccines is satisfactory within 5 to 10 years after vaccination and a further booster dose is not necessary during this period. On the other hand, a further decline of anti-HBs level is to be expected 10 years after vaccination. For children reaching puberty, HBV transmission through sexual contact may be a concern.[97] Therefore, a booster dose of hepatitis B vaccine may be needed in later life.

Two options may be considered for maintaining protective immunity against hepatitis B infection. Anti-HBs levels may be tested 1 month after the first booster and the next booster administered before the minimum protective level is reached. A protective level of 100 mIU/ml is suggested to be appropriate.[54] This strategy may be preferred for high-risk individuals, such as healthcare workers. However, routine testing of anti-HBs is difficult to perform in universal vaccination programmes. The other strategy is to provide booster vaccination to all vaccinated individuals at regular intervals without determination of anti-HBs.[54] Although nonresponders may not be detected, this strategy appears to be cost-saving and easily applicable in universal vaccination programmes. Continued assessment of long term protection against HBV infection is needed to determine the optimal time interval to administer a further booster dose of hepatitis B vaccine in later life.

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4. Converting from Plasma-Derived to Recombinant Vaccines

4.1 Disadvantages of Plasma-Derived Vaccines

Although both PDV and RV are highly efficacious and safe, it is inevitable that RV will replace PDV. Although PDV has been shown to be safe, there was an unjustified but prevalent fear that potential pathogenic live organisms would escape inactivation and remain in the final vaccine product. The emergence of HIV infections has intensified such fears, although the manufacturing process has been shown to be capable of eliminating any infectious HIV.[37] Many people in need of vaccinations perceived the PDV as risky and declined to receive it. Such concerns interfered with successful large-scale vaccination programmes.[98]

The production of PDV is relatively expensive and the supply of the required HBsAg-positive serum cannot be assured when a universal hepatitis B vaccination programme is in place. For instance, the carrier rate for HBV in the Taiwanese general population has been 10 to 20%.[8,9] A mass hepatitis B vaccination programme was launched in Taiwan in 1984.[99] A recent serological survey in Taipei showed that the overall prevalence rate of HBsAg in children under 12 years old had dropped from 9.8% in 1984 to 1.3% in 1994.[100] Mass hepatitis B vaccination has proven to be a powerful strategy for combating HBV-associated disease, and the source for production of PDV has thus become limited.

4.2 Use of Plasma-Derived and Recombinant Vaccines in Combination

When PDV is to be replaced by RV, it has to be determined whether these 2 types of vaccine can be used in combination. There have been 2 studies evaluating the immunogenicity and the protective efficacy of combined vaccination.[101,102] The first concluded that the booster effect of RV was inferior to that of PDV in healthcare workers who had received 2 doses of PDV. It was also suggested that there might be an accelerated decline in anti-HBs when different vaccines were used in combination.[101]

Another study demonstrated that a combined vaccination with PDV and RV was both protective and immunogenic in infants.[102] The protective efficacy of combined vaccination in high-risk infants ranged from 89 to 93%, similar to that in other reports using either PDV or RV (table I). Most of the children remained anti-HBs-positive at 30 months of age and there was no accelerated drop in anti-HBs level.[102]

As discussed previously, the potency of vaccine is related to its dosage but not to its type *per se*. PDV was used at a dose of 20μg in the former study,[101] while 5μg of PDV was used in the latter study.[102] Therefore, the above-mentioned differences in results can be attributed to different doses of vaccine used. A combined vaccination with PDV and RV can be used safely when necessary. Different products of RV are equally immunogenic and are interchangeable;[102] either can be used to complete an immunisation series begun with the other. If a further booster dose of hepatitis B vaccine is to be given at some time after primary vaccination, both PDV and RV may be used for those primed with either type of vaccine.[23,25,78,81,83,90]

5. Need for a New Recombinant Vaccine

5.1 Pre-S–Containing Recombinant Vaccine

Although available hepatitis B vaccines are safe and potent, hepatitis B vaccines could be improved in several respects. At least 3 doses of current hepatitis B vaccines are needed to induce good immunity. About 5 to 10% of fully immunocompetent healthy individuals do not mount a humoral antibody response to currently available hepatitis B vaccines. The nonresponse rate is higher in individuals with compromised immune systems.[66] It should be noted that nonresponders remain susceptible to infection with HBV. For example, a placebo-controlled study of PDV was carried out in France in an HBV 'high-risk' setting of staff, patients on maintenance haemodialysis and their

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relatives.[103] Follow-up of 73 patients and 191 staff showed that vaccinated individuals who did not respond to the vaccine by developing anti-HBs were infected at the same rate as the unvaccinated controls, i.e. nearly 50%, as indicated by anti-HBc production alone (5%), transient antigenaemia (15%) or prolonged antigenaemia (25%). To overcome the problem of nonresponsiveness, there have been some new recombinant hepatitis B vaccines that contain not only the S antigen, but also the product of the pre-S region.[24,25,59,104]

The pre-S1 and pre-S2 regions appear to have an important immunogenic role in terms of augmenting anti-HBs titres, eliciting antibodies effective in viral clearance and prevention of hepatocyte binding, stimulation of cellular responses and circumvention of genetic nonresponsiveness to the S antigen.[4,105-107] In inbred strains of mice, immune responsiveness to the products of the pre-S regions is controlled by different immune-response genes from those controlling the immune response to the product of the *S* gene.[108-110] In mice, the products of the pre-S region are more immunogenic than the product of the *S* gene.[106] Theoretically, a pre-S–containing hepatitis B vaccine might be more immunogenic and could enhance immune responsiveness in nonresponders. Indeed, Milich and colleagues[111] demonstrated in the murine model that the independence of major histocompatibility complex–linked gene regulation of immune responses to pre-S1, pre-S2 and S regions of HBsAg would assure fewer genetic nonresponders to a vaccine containing all 3 antigenic regions.

One new type of RV is derived from Chinese hamster ovary cells and contains the major S protein and the minor pre-S1 and pre-S2, in their glycosylated and non-glycosylated forms.[24] Among the 10μg dose recipients, a seroprotective level of anti-HBs was achieved in 96% after the second injection and in 100% after the third injection.[24] RV containing pre-S2 antigens has also been shown to be highly immunogenic in human trials.[25,104] A recent study evaluated the immunogenicity of an RV containing pre-S1, pre-S2 and S antigenic components of HBsAg in 100 health workers with persistent nonresponsiveness to conventional hepatitis B vaccines.[112] An overall seroconversion rate of 69% was achieved after a single dose of the new hepatitis B vaccine. Therefore, pre-S–containing vaccines may be one of the solutions to overcome the problem of genetic nonresponsiveness to conventional hepatitis B vaccines.

5.2 Escape Mutants

All subtypes of HBV share the 24-amino-acid neutralisation epitope (the common ‘a’ determinant) within the HBsAg. The common ‘a’ determinant lies between amino acids 124 to 147 of HBsAg[113] and is believed to have a double loop conformation.[114] Antibodies to this antigenic epitope confer protection against all subtypes of HBV.[26,27] Mutations in this region may lead to a substantial change of antigenicity and a reduced cross-reactivity with polyclonal anti-HBs.[115,116]

HBV has a higher intrinsic mutation rate than other DNA viruses, probably because its replication requires an intermediate RNA molecule and reverse transcriptase.[2] Several vaccine-induced escape mutants of HBV with an altered ‘a’ determinant have been described since the first report by Carman and colleagues[117] in 1990. It was suggested that mutations in the ‘a’ determinant of HBsAg enable HBV to escape from neutralisation by either HBIg or vaccine-induced anti-HBs. To date, there has been a total of 39 missense mutations in the ‘a’ determinant from 33 carriers who failed to be protected by HBIg and/or hepatitis B vaccine.[117-123] The Gly-to-Arg mutation at amino acid 145 is the most commonly found mutation. The distribution of such escape mutants was apparently worldwide.

The ‘a’ determinant has been characterised in 12 mothers who gave birth to children with mutant HBV infections.[117-120,122,123] Only 2 mothers possessed the same dominant strain of mutant HBV as the child.[119,120] By sequencing surface gene clones, Okamoto and colleagues[118] demonstrated that wild-type and mutant HBV coexisted in the mother who gave birth to 2 children infected by mutant HBV. These observations indicate that mu-

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tants were selected from a quasi-species of HBV by immune pressure.

Large-scale screening for HBV mutant in carriers who failed to be protected by vaccination has been done in 2 studies.[119,120] In the study from Singapore, 18 of 43 (42%) carrier children were infected by mutant HBV.[119] Among 27 carrier children who had receive active + passive immunoprophylaxis at birth in Taiwan, 6 children (22%) were infected by mutant HBV.[120] These observations suggest there may be some geographic differences in the prevalence of HBV mutants.

With universal vaccination, there is concern that HBV surface gene mutants could become dominant strains and cause breakthrough infections in people with protective levels of anti-HBs. Mutations in the 'a' determinant have also been observed in carriers without vaccination.[124,125] Incorporation of HBsAg corresponding to the individual variants into the vaccine may be a possible solution to the problem.[118,122,126] However, this strategy may be hampered by the rising cost of vaccine and the difficulty to incorporate different types of mutant. Another proposal is to use vaccines containing the pre-S gene product that may also induce protective antibodies. Both strategies require a change of the presently used RV. However, chimpanzees have been successfully protected against a variant virus by previous immunisation with conventional RV.[127] Further studies are required to determine whether these variants represent a practical threat to vaccine efficacy.

5.3 Combination Vaccine

Currently recommended schedules for hepatitis B vaccine and other childhood vaccines do not coincide precisely. To reduce the number of visits for vaccination and to improve the compliance rate, some modified schedules have been proposed.[46,128] In a study in Taiwan, the second dose of hepatitis B vaccine was delayed for 2 weeks and the first dose of diphtheria-tetanus-pertussis (DTP) vaccine was given 2 weeks earlier to accomplish a simultaneous injection at 6 weeks of age.[46] The results showed that such a simplified schedule was protective and well tolerated.

Matching injection time to that of other vaccines, however, can subject infants to multiple injections during one visit. This problem can be circumvented by including hepatitis B vaccine in formulations with other vaccines such as DTP and *Haemophilus influenzae* type b (Hib) vaccines. Several combination vaccines have been developed and are now in clinical trials.[129] Compliance with universal vaccination may be enhanced by the development of these combination vaccines that allow complete immunisation against several antigens with a minimal number of injections.

6. Conclusions

Although the production process and some chemical characteristics differ between PDV and RV, both types of vaccine contain the same major component of HBsAg. Safety is excellent for both PDV and RV. Hepatitis B vaccination is safe and there is no report of vaccine-associated infection, although it can be reactogenic, with some severe adverse reactions reported. Some studies showed that the protective efficacy, the immunogenicity or the durability of antibody response was different between PDV and RV. However, PDV and RV may not be directly comparable in terms of potency. Both types of vaccine are highly protective and immunogenic. In long term follow-up studies, the protection afforded by hepatitis B vaccines is satisfactory within 5 to 10 years of vaccination. Further follow-up of these vaccinees is needed to determine whether a booster dose of hepatitis B vaccine is necessary after adolescence.

Although it is equally effective as RV, continued use of PDV is hampered by its higher cost, the fear of contaminating pathogens and the shortage of HBsAg-positive human plasma for production in the future. PDV and RV may be used in combination when necessary. Hepatitis B vaccine could possibly be made more immunogenic by incorporating the pre-S region and more convenient for administration by combining it with other inactivated vaccines. Escape mutants of HBV have

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emerged under the immune pressure exerted by HBIg and hepatitis B vaccines. The prevalence of these mutants should be monitored to determine whether a new vaccine policy is needed to overcome this problem.

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