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**Studies on Hepatitis B Vaccination and Factors Associated with  
the Vaccine Response**

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## ***Abstract***

Hepatitis B virus causes liver disease and up to 2 billion people have been in contact with the virus world wide. It can cause both acute and chronic disease. The routes for transmission are through blood, mother to infant at time of delivery and sexually. Chronic hepatitis B infection is a risk factor for development of liver cirrhosis and hepatocellular carcinoma. Prevention of hepatitis B virus infection is highly desirable. Since the early 1980s hepatitis B vaccine has been available. It can effectively prevent the disease and has been found to be safe. The World Health Organisation, WHO, has recommended all countries to implement the vaccine in their children's vaccination programmes and many countries have followed this recommendation. In Sweden so far the recommendation is vaccination of identified risk groups for hepatitis B. Health care workers who are at risk of having blood contact in their work is one such risk group.

In a large study on health care workers who were intradermally vaccinated with the hepatitis B vaccine, 960/1406 (68.3%) developed protective levels of antibodies to HBsAg (anti-HBs; defined as  $\geq 10$  mIU/mL) after three doses. After administering of an additional fourth dose to non-responders the response rate was 1187/1335 (88.9%). Risk factors for non-response were smoking and age above 40 years. Also, the vaccine response rates improved during the study and a risk of giving a too small dose with intradermal administration was also identified. This suggests that intradermal administration is dependent on well trained personnel.

A genetic factor which has been proposed to be associated with a non-responder status to HBV vaccination is the HLA haplotype of the host. In a study in on 69 responders and 53 non-responders the haplotypes were therefore determined. It was found that [DQB1\*0602; DQA1\*0102; DR15] and [DQB1\*0603; DQA1\*0103; DRB1\*1301] were more likely to be found in responders ( $p < 0.025$  and  $p < 0.05$  respectively). In non-responders the haplotype [DQB1\*0604; DQA1\*0102; DRB1\*1302] was found more frequently ( $p < 0.005$ ). This study supports that the HLA class II of the host is involved in the ability to respond to the HBV vaccination.

To further test the genetic link between the HLA of the host and a non-responder status, relatives to known intradermal non-responders with known haplotypes for DQA1, DQB1 and DRB1 were vaccinated in the same way, intradermally. The response rate in the relatives was 15/26 (58%) which is lower than expected suggesting a genetic influence on the vaccine response. In this study 5/6 with the haplotype [DQB1\*0604; DQA1\*0102; DRB1\*1302] were non-responders which is in line with the previous data that this haplotype is correlated to hepatitis B vaccine non-response.

Finally, to test a strategy by which we could induce an effective anti-HBs seroconversion in non-responders we revaccinated these with the combined hepatitis A and B vaccine intramuscularly at a double dose. Already after the first revaccination dose 26/44 (60%) responded with protective antibodies compared to 2/20 (10%) in a vaccine naïve reference group, suggesting an anamnestic response. After three doses 42/44 (95%) responded in the non-responder group and 20/20 (100%) in the reference group. All participants in the study responded to the hepatitis A antigen.

In conclusion these studies show that intradermal vaccine administration can be used and is effective, and that the ability to respond is influenced by several, including genetic, factors. Importantly a non-responder status to hepatitis B vaccination is not absolute, a double dose of the combined HAV and HBV vaccine effectively overcomes this non-response in most individuals.

## ***List of Papers***

I. Cardell K, Frydén A, Normann B. Intradermal Hepatitis B Vaccination in Health Care Workers. Response Rate and Experiences from Vaccination in Clinical Practise. Scand J Infect Dis 1999; 31: 197-200,

II. Langö-Warensjö A, Cardell K, Lindblom B. Haplotypes comprising subtypes of the DQB1\*06 allele direct the antibody response after immunisation with hepatitis B surface antigen. Tissue Antigens 1998; 52(4):374-80.

III. Cardell K, Lindblom B, Frydén A. Hepatitis B vaccination in relatives to known non-responders. A family study. Submitted.

IV. Cardell K, Åkerlind B, Sällberg M, Frydén A. Excellent response rate to a double dose of the combined hepatitis A and B vaccine in previous nonresponders to hepatitis B vaccine. J Infect Dis. 2008; 198(3): 299-304.

## *Abbreviations*

ALT	alanine aminotransferase
Anti-HAV	antibody against hepatitis A virus
Anti-HBc	antibody against hepatitis B core antigen
Anti-HBe	antibody against hepatitis B e antigen
Anti-HBs	antibody against hepatitis B surface antigen
APC	antigen presenting cell
BMI	body mass index (weight/length <sup>2</sup> )
cccDNA	covalently circular closed DNA
DNA	deoxyribo nucleoid acid
dsDNA	double stranded DNA
HAV	hepatitis A virus
HBIG	hepatitis B immunoglobulin
HBV	hepatitis B virus
HBcAg	hepatitis B core antigen
HBeAg	hepatitis B e antigen
HBsAg	hepatitis B surface antigen
HCC	hepato cellular carcinoma
HLA	human leukocyte antigen
IFN	interferon
Il	interleukin
Ig	immunoglobulin
IU	international units
MHC	major histocompatibility complex
OR	odds ratio
ORF	open reading frame
PCR	polymerase chain reaction
PHA	phytohemaglutinine
PBMC	peripheral blood mononuclear cell
RNA	ribo nucleoid acid
S/N	sample to negative
TCR	T cell receptor
Th	T-helper cell
TT	tetanus toxoid
WHO	World Health Organisation

## ***Introduction***

Viral hepatitis is a common disease that is seen in all countries, worldwide. There are five hitherto identified viruses that primarily cause clinical hepatitis. Hepatitis B virus was the first to be discovered, and it can cause both acute and chronic disease. The global burden of hepatitis B is severe, with more than 350 million people chronically infected. Hepatitis B virus is transmitted by blood, from mother to infant, and sexually. The hepatitis C virus can also cause chronic liver disease and is transmitted in the same way as hepatitis B, although mother-infant and sexual transmission are far less common. About 170 million people are estimated to have chronic hepatitis C virus infection. Hepatitis D, or delta hepatitis, is caused by an incomplete virus that requires hepatitis B virus to replicate. It is therefore only seen in association with hepatitis B virus infection. Hepatitis A and E are both transmitted through the faecal-oral route. These viruses cause an acute illness with symptoms of varying severity but which are self-limiting in the vast majority of cases. Chronic hepatitis is never seen after hepatitis A or E infection, and result in subsequent lifelong immunity. Some of the viral hepatitis can be prevented by vaccination, and vaccines against hepatitis A and hepatitis B have been available for about 20 years. Hepatitis D can also be prevented using the hepatitis B vaccine. There are no available vaccines against hepatitis C or E.

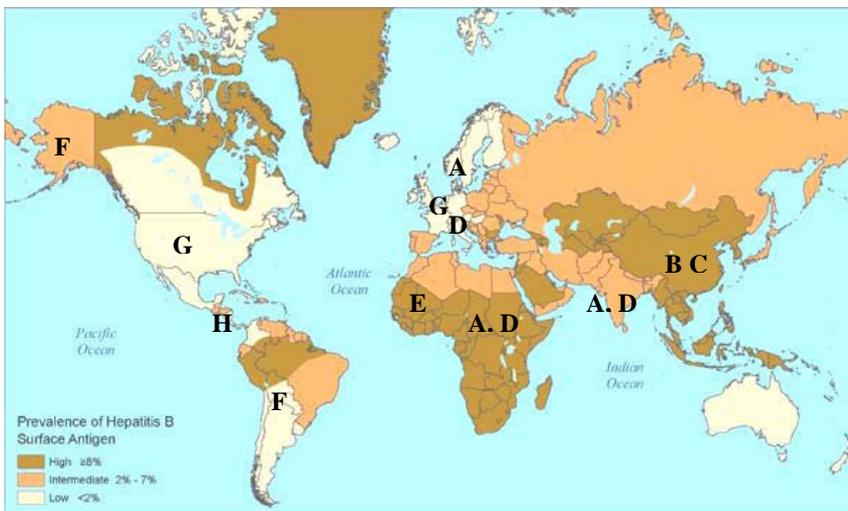
Hepatitis B is today effectively prevented by vaccination, which is now recommended by most health authorities. In health care, transmission of blood-borne viruses poses a threat to both patients and staff. Health care workers, especially those who are in frequent contact with blood, are at high risk of contracting these diseases. Hepatitis B vaccination provides excellent protection in most people, but some do not respond to the vaccine. Whether some of these non-responders are protected or not is not fully understood. The aim of these studies were to further investigate hepatitis B vaccination in healthy adults, to identify factors to vaccine non-response, and to find ways of managing those individuals who fail to respond to the standard vaccination schedule.

## ***Hepatitis B***

### *Epidemiology*

Hepatitis B virus infection was first recognised in 1965 when Blumberg and co-workers found the hepatitis B surface antigen, originally termed the *Australia antigen*, since it was first found in serum from an Australian patient.<sup>1-3</sup> Dr Baruch Samuel Blumberg was awarded the 1976 Nobel Prize in Physiology or Medicine for this discovery. The virus was fully described in the 1970s.<sup>4</sup> It is estimated that about 2 billions of the world's population has been infected with hepatitis B virus and more than 350 million people are chronic carriers. The virus is highly endemic in some regions, e.g. in South-East Asia and Sub-Saharan Africa, where up to 20% of the populations are chronic HBsAg carriers. In northern Europe and in Scandinavia in particular, the carrier rate is less than 1%, and it is estimated to be around 0.05% in Sweden.<sup>5</sup>

<sup>6</sup> There are 8 known subtypes of the hepatitis B virus designated A to H.<sup>7</sup> Different subtypes predominate in different parts of the world. For instance, subtypes B and C are most common in Asia, subtype D is found in the Mediterranean countries, and subtype A is found in the Nordic countries (Figure 1). The incidence of hepatitis B has declined in those countries that have implemented strategies for preventing hepatitis B, such as the screening of blood products, vaccination of risk groups, and including hepatitis B vaccination in the national childhood vaccination programme.<sup>8,9</sup> It has been shown that universal vaccination programmes reduce the rate of liver-related morbidity and mortality in high-endemicity countries.<sup>10</sup> In Sweden, around 1 500 cases of hepatitis B were notified to the health authorities in 2008. Most are chronic carriers and members of immigrant families from endemic areas. After a drop in incidence in the 1990s, there was an increase in notified cases of acute hepatitis B in around 2000 due to an epidemic among drug abusers and their sexual contacts. In recent years there has been a slight increase in the number of both acute and chronic disease, and acute hepatitis B represented about 13% of the total number of notified cases of hepatitis B.<sup>11</sup>



**Genotypes A and D** Africa, Europe and India

**Genotypes B and C** Asia

**Genotype E** West Africa

**Genotype F** Central and South America

**Genotype G** France, Germany and the USA

**Genotype H** Central America

*Figure 1. Map showing geographical distribution of chronic hepatitis B carriers and the predominating genotype in each region (Map modified from CDC, Atlanta, GA, USA, 2009.)*

The virus is transmitted in three ways: by blood, mother-infant and sexually. The most common route of transmission globally causing chronic infections is from mother to infant at delivery or, far less commonly, by intrauterine transmission. Mother to infant transmission is more common in Asia, while transmission by blood from child to child is more common in Africa where children are infected in early childhood.<sup>6,9</sup> In Sweden, and the rest of the world, intravenous drug abuse and sexual activity, both homosexual and heterosexual, are the main modes of transmission causing acute HBV infections. According to data from the Swedish Institute for Infectious Disease Control, there has not been a case of occupationally-acquired hepatitis B infection in Sweden since 1999.<sup>11</sup> Hepatitis B used to be a complication to blood transfusion before the introduction of blood product screening. Screening of blood donors and blood components has virtually eliminated this mode of transmission. Nevertheless, hepatitis B transmission is still a concern for health care organisations around the world. Transmission of hepatitis B from patients to health care workers and from health care workers to patients has been described.<sup>12-14</sup> Universally applied hygiene procedures and vaccination policies are therefore vital elements in the effort to eliminate hepatitis B transmission from health care.<sup>15</sup>

### *The Hepatitis B virus*

The virus is a small DNA virus that belongs to the hepadnavirus family and it can only infect humans and chimpanzees. Other viruses from the same family infect other vertebrates, e.g. the woodchuck and duck viruses that have been used as animal models of the disease. The hepatitis B virus genome is a partly double-stranded DNA molecule 3 200 nucleotides long. There are four open reading frames (ORF) which partly overlap and which code for the viral proteins. These proteins are the three surface antigens (pre-S1, pre-S2 and S), the core proteins (pre-C and C), the transactivating X-protein (X) and DNA polymerase (P).<sup>6,16</sup> The primary site for hepatitis B virus replication is the hepatocyte, but it has been suggested to also occur in peripheral blood mononuclear cells. The latter is still uncertain. The virus appears in serum in three different forms. The Dane particle is 42 nanometres in size and has an outer lipoprotein envelope that contains the HBsAg. An inner nucleocapsid layer is composed of HBcAg and encloses the HBV-DNA. The two other forms of hepatitis B virus consist of a sphere and a rod. Both of these are 22 nanometres in size and contain HBsAg but not HBcAg or HBV-DNA. The S region of the genome is divided into pre-S1, pre-S2 and S. The small (S) or major HBsAg is coded for by the S gene, the middle (M) HBsAg is coded for by the S and pre-S2, and the large (L) HBsAg is coded for by the S, pre-S2 and pre-S1 (Figure 2). The membrane of the virus contains a mix of all these three proteins. The S antigen can be divided into four different subtypes or serotypes, adw, adr, ayw and ayr. All subtypes include the dominant *a* antigen, which is involved in the protective properties of the antibodies to HBsAg. HBeAg is also a secreted non-structural protein which appears in serum and is believed to play a role in the immune tolerance induced by the virus. Peptides derived from HBcAg and HBeAg associated with HLA class I molecules are presented on the surface of hepatocytes and are believed to be a key targets for the cellular immune response and is therefore an important factor in the eradication of infected hepatocytes.<sup>17</sup>

The mechanism by which the virus attaches itself to the hepatocyte is not fully understood but it is thought to bind to a hitherto unidentified surface receptor. It is thought that proteins coded by the S-region, pre-S1 and pre-S2, play a role in this process. According to one theory the host cell receptor is thought to belong to the annexins, which are calcium binding proteins that interact with membrane phospholipids.<sup>18-20</sup> The virus nucleocapsid containing the HBV-DNA is then transported to the nucleus where replication takes place. After the genome has reached the nucleus, the single stranded DNA changes to form covalently closed circular DNA (cccDNA). An RNA polymerase from the host cell then transcribes DNA to RNA species, of which some are transported to the cytoplasm together with the HBV polymerase for encapsidation by HBcAg. These RNA pregenomes are reversely transcribed in to the mature partially double stranded DNA, dsDNA, inside the capsid. The formation of new virions is completed by the budding of HBcAg through the endoplasmic reticulum membrane and the virion leaves the cell by exocytosis. The genomic organization of HBV with overlapping reading frames limits the virus ability to undergo mutations. However, the mutations do appear, in particular during therapy, and have been described in all parts of the HBV genome.<sup>21</sup>

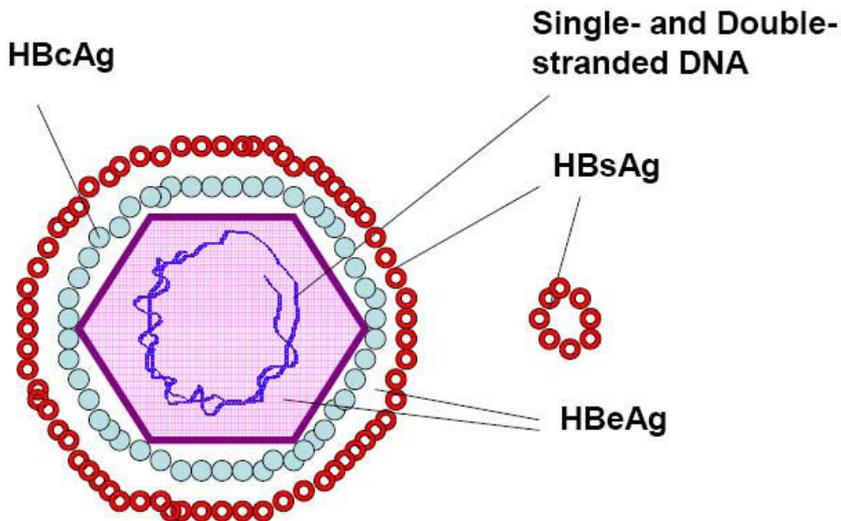


Figure 2. Schematic presentation of the hepatitis B virus.

### *Clinical aspects*

Hepatitis B virus infection can cause an acute infection as well as a chronic disease. Following an incubation period of one to six months, depending on the inoculation dose, some individuals present clinical signs of acute hepatitis. The initial phase is asymptomatic or sub-clinical in many cases, especially in children. Acute hepatitis B is a self-limiting disease in the vast majority of cases, but a few patients develop acute liver failure which can be fatal without liver transplantation. The presence of symptoms in the acute phase has been associated with a strong immune response and control of the virus, while patients with a sub-clinical acute infection have a greater risk of becoming chronic carriers. Those infected at birth or early childhood, when the immune system is not fully developed, have the highest risk of developing chronic hepatitis B infection. About 90% of children infected with hepatitis B virus at birth will become chronically infected, while only about 5–10% of newly-infected adults develop chronic disease.

Chronic hepatitis B infection is defined as HBsAg positivity that persists for more than six months. Chronic carriers of HBsAg often have no symptoms, and many individuals are not aware that they are infected. However, some patients will, years later, develop complications such as liver cirrhosis and/or hepatocellular carcinoma. It is estimated from WHO that 1–1.5 million people die each year due to complications of hepatitis B virus infection. Male chronic hepatitis B carriers, who are at the highest risk, were found to have a more than 20% lifetime risk of developing hepatocellular carcinoma.<sup>16, 22</sup> Chronic hepatitis B is also associated with immunological diseases such as polyarteritis nodosa, membranous or membranoproliferative glomerulonephritis, leukocytoclastic vasculitis, erythema nodosum, arthrititis and serum sickness (usually acute HBV infection), Raynaud's phenomenon and polyneuropathy (mononeuritis multiplex, usually with polyarteritis nodosa).<sup>23</sup>

Chronic hepatitis B virus infection is divided into four different phases. The first, often referred to as the *immune tolerance phase* is characterised by a high viral replication, low or minimal liver damage and normal ALT levels. The duration of the immune tolerance phase varies from a couple of months in adults to decades in individuals infected at birth or in early childhood. Most individuals will, however, enter the next phase, the *immune activation phase*, when the immune system tries to eliminate the virus. In this phase, the viral level replication decreases and the ALT level increases as a marker of increased inflammation in the liver. The histology of liver biopsies taken at this stage generally confirms a higher degree of inflammation. The virus itself is non-cytopathic to the host cell and the liver damage is most probably caused by an immunological reaction directed against the virus and infected cells. In most cases, immune activation leads to the next phase, the *immune surveillance phase*, with conversion from HBeAg to anti-HBe, low or undetectable HBV-DNA levels in serum, normal ALT levels and an improved histological picture. Seroconversion from HBeAg to anti-HBe seems to occur in approximately 5–10% of patients annually, and 80% of infected children show e-antigen seroconversion before reaching adult age. A fourth phase, the *reactivation phase*, has been recently described in some patients. It is characterised by increasing viral

load and increasing ALT levels in patients who had been in the immune surveillance phase (Figure 3).

An almost total clearance of virus and elimination of HBsAg from serum with conversion to anti-HBs occurs in up to 3–5% of patients in the surveillance phase annually. However, HBV-DNA remains in the hepatocytes even after seroconversion to anti-HBs, and reactivation of hepatitis B in spite of HBsAg and presence of anti-HBs can occur if the individual is undergoing severe medical immune suppression e.g. following stem cell or solid organ transplantation or during treatment with potent cytostatic drugs.<sup>24</sup>

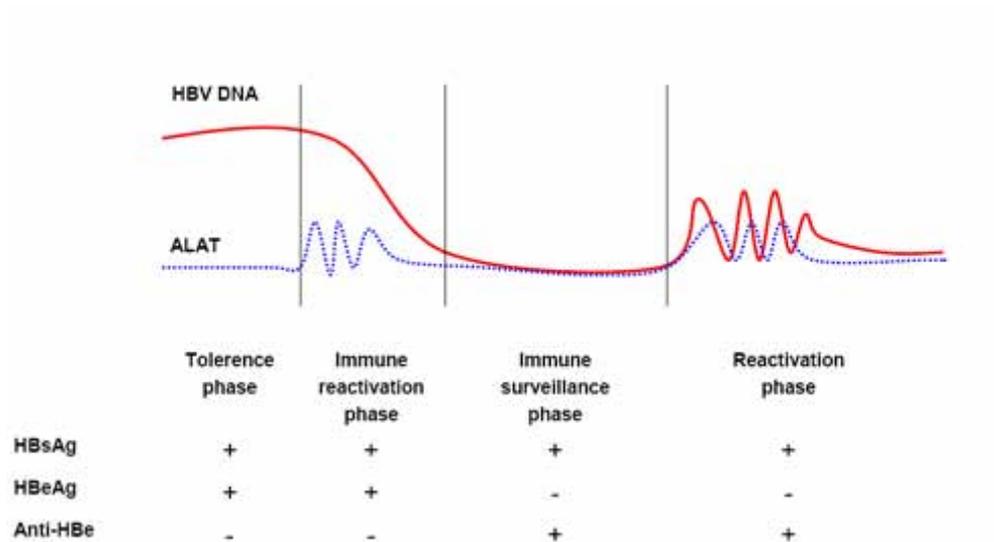


Figure 3. This diagram depicts the four stages of chronic hepatitis B, and the HBV-DNA and ALT levels during these phases.

### Diagnostics

Hepatitis B virus infection is diagnosed by the measurement of serum viral antigens and their corresponding serum antibodies. Several virus-specific antigens are measured routinely in clinical practice. HBsAg is a marker for hepatitis B carrier state and is present both in acute and chronic infection. HBsAg is, together with HBV-DNA, the first marker to be positive in early infection, followed by HBeAg. This correlates with high viral load and indicates that the patient is highly contagious. HBcAg is not a secreted protein and appears in serum soon after

exposure. It is not used in clinical practice. IgM antibodies to HBcAg, anti-HBc IgM, are markers for acute hepatitis B infection and persist for about 6 months after the onset of symptoms. Anti-HBc IgM could in some cases also be positive also in reactivation phases of the disease. The presence of hepatitis B virus can be determined by the detection of HBV-DNA by polymerase chain reaction (PCR) and this can be seen within weeks of exposure. The viral load can be determined by measuring the quantity of HBV-DNA in serum. Chronic carriers are defined by the presence of HBsAg for more than 6 months with or without HBeAg positivity. Anti-HBc IgG persists indefinitely and is therefore a marker of previous or ongoing hepatitis B infection. As the infection clears, the first antigen to disappear is HBeAg which results in the appearance of anti-HBe in serum. This is followed by loss of HBsAg and finally, in most cases, development of anti-HBs. The presence of antibodies to HBsAg, anti-HBs, achieved by natural infection or vaccination, is a marker of immunity.

Some individuals are infected with a mutant virus termed the *pre-core mutant*. Such viruses have a stop-codon in the genome in nucleotide 1896 in the pre-C region of the genome which renders it unable to express HBeAg. Patients with this virus are therefore HBeAg negative but anti-HBe positive. Those infected with strains carrying pre-core mutations can have a high viral load in the absence of serum HBeAg, and they have also been reported to have a higher risk of more aggressive disease. The number of people becoming infected with this precore mutant is increasing, and it is now the predominant viral strain in those infected in Southern Europe.<sup>25, 26</sup>

### *Treatment*

There is no established treatment for acute hepatitis B. Treatment is available for chronic hepatitis B in the immunoreactive and reactivation phases of the disease. The aim of treatment is to induce an host mediated immunological control of the infection, evidenced by a seroconversion in the e-antigen system from HBeAg positivity to anti-HBe or, in HBeAg negative patients, to reduce HBV-DNA levels. The ultimate goal with treatment is loss of HBsAg although this is an infrequent event after all treatments available so far. Successful treatment has been shown to reduce histological inflammation and disease progression with liver complications. There are two treatment options – interferon, which seems to enhance the ability of the host's immune system to clear the virus – and nucleoside/nucleotide analogues which have an anti-viral effect.<sup>27, 28</sup> Interferon is most likely to succeed in younger patients without cirrhosis and with a favourable genotype. Interferon alpha was the first available therapy for hepatitis B. When given subcutaneously at a dose of 5–10 million international units three times a week for 4–6 months, it induces seroconversion from HBeAg to anti-HBe in about 30% of cases compared to the spontaneous 5–10%. Standard interferon alpha has now been superseded by pegylated interferon alpha 2a and 2b, which have more favourable pharmacokinetic properties and which can be administered weekly. The currently recommended duration of interferon treatment is 48 weeks. Interferon treatment has many adverse effects and is most effective in certain genotypes (A-B).<sup>29</sup> It is not an option for

patients with cirrhosis as it can provoke liver failure in this particular group. Combination treatment with pegylated interferon and nucleoside/nucleotide analogues does not seem to provide any further benefit.<sup>27, 28, 30, 31</sup>

There are six nucleoside/nucleotide analogues that can be considered for inclusion in international and national treatment guidelines for chronic hepatitis B infection<sup>27, 28</sup> Lamivudine was the first of these, and it has shown good antiviral effect with few adverse effects. It does, however, induce resistance mutants in the YMDD-motif of the HBV polymerase in a high proportion of patients, and around 60% of treated patients will develop resistance after 5 years of treatment. For this reason, lamivudine is no longer recommended as first-line therapy in chronic hepatitis B. Entecavir and tenofovir, which have potent antiviral effects, have shown encouragingly low rates of resistance and are now recommended as first-line treatment in most patients when nucleoside/nucleotide analogues are indicated.<sup>32, 33</sup> Entecavir and lamivudine partly share the same resistance mechanism and therefore patients with established lamivudine resistance should not be treated with entecavir. Telbivudine is another antiviral drug that can be used, and although resistance rates are lower than those of lamivudine, they are higher than those of entecavir or tenofovir. Telbivudine shows cross-resistance with lamivudine.<sup>34</sup> Adefovir was the second drug to be approved for hepatitis B treatment. In current guidelines it is mainly recommended as add-on therapy to lamivudine when lamivudine resistance has developed. Nucleoside and nucleotide analogues are often well tolerated and they have been shown to improve histological and clinical parameters. A reduction of complications such as cirrhosis and hepatocellular carcinoma has been observed in treated patients. Several new therapeutic drugs are undergoing clinical trials and they are expected to be registered within the next few years.

Treatment should continue in HBeAg positive patients for at least six months following seroconversion to anti-HBe. In anti-HBe positive patients the optimal duration of treatment with nucleoside/nucleotide analogues is not known, and many patients are offered lifelong treatment.<sup>35</sup> The development of resistance remains a threat even with the newer drugs. Most people infected with hepatitis B live in developing countries, where mass treatment is not an available option. Treatment is also complex, and has many disadvantages.

The risk of cirrhosis and hepatocellular carcinoma is high, and screening for these complications is recommended for Asian carriers aged 40 or older and even earlier for African carriers. Prevention of hepatitis B infection with vaccination programmes is the only realistic way to deal with the infection worldwide.<sup>36-39</sup>

## *Vaccines against viral hepatitis*

### *Hepatitis B vaccine*

Hepatitis B can be effectively prevented by vaccination.<sup>36, 37, 39</sup> The World Health Organisation recommends that hepatitis B vaccination should be included in childhood vaccination programmes in all countries. Most countries have followed this recommendation but the Nordic countries, the Netherlands, Ireland and the United Kingdom have not yet started vaccinating all children. Instead, these countries have chosen to focus on identifying and vaccinating specific risk groups. The inclusion of hepatitis B vaccination in the universal childhood vaccination programme in Sweden is currently being discussed but no decision has yet been taken.<sup>40</sup> The prevalence of hepatitis B is low in Sweden, and so is the risk of being infected. The risk of chronicity is low if not infected in early childhood. This, together with the high cost of the vaccine, has so far been the main argument for restricting vaccination to risk groups. The Swedish National Board of Health and Welfare currently recommends that vaccination should be offered to those at risk of hepatitis B.<sup>41, 42</sup> These include children of hepatitis B infected mothers, partners of hepatitis B infected people, family members of hepatitis B virus infected persons, haemodialysis patients and health care workers at a risk of blood exposure as well as long-term travellers outside the Nordic countries. By this recommendation it is estimated that about 15-20% of all children in each age group will be vaccinated against hepatitis B in Sweden.

- *History: The first plasma-derived HBV vaccine*

Vaccination against hepatitis B has been available since the beginning of the 1980s.<sup>8</sup> The first vaccine on the market was licensed in the USA in 1982 and contained purified 22 nm HBsAg derived from the plasma of chronic hepatitis B carriers. Although the virus was inactivated, the risk of transmission of hepatitis B by this plasma-derived vaccine was an initial concern. However, the vaccine turned out to be safe in this regard. Aluminium hydroxide was added as an adjuvant and the vaccine was preserved with thiomersal. It produced good protective antibody levels in most individuals, although about 5–10% failed to achieve an adequate antibody response.<sup>43-46</sup> The amount of vaccine produced this way was limited and by far not enough for the need.

- *Recombinant HBV vaccine*

Yeast cell derived, recombinant vaccine with HBsAg has been available since 1989.<sup>8, 47</sup> The recombinant vaccine is produced by recombinant DNA technology from the yeast species *Saccharomyces cerevisiae* and is adsorbed onto aluminium hydroxide and preserved with

thiomersal. By using yeast the HBsAg becomes glycosylated similar to the native HBsAg produced in hepatocytes. The vaccine should not be frozen but is heat stable, which is an advantage in distribution. HBsAg produced with recombinant technique is equivalent to the 22 nm HBsAg particles produced by the virus. This production technique enables the production of large quantities of vaccine at reduced cost. Studies of recombinant vaccine show that intradermal injection produces a similar response to intramuscular injection, although with lower mean antibody titres.<sup>45</sup>

- New HBV vaccines

A number of new variants of the traditional HBsAg-containing vaccine have been developed.<sup>48</sup> Early studies in mice suggested that genetic non-responders to the vaccine may exist<sup>49</sup> and that this could be partly cured by the inclusion of pre-S1 and pre-S2 sequences in the vaccine.<sup>50</sup> This has prompted the use of pre-S sequences in some of the new vaccines.<sup>48</sup> However, the effects of these additions have not been as successful as initially hoped. The possibly best new HBV vaccine is the combined HAV and HBV vaccine which seems to offer a high seroconversion rate for both HAV and HBV.<sup>51</sup>

- Vaccine recommendations

Different vaccination schedules have been tested but the standard recommended regime is three intramuscular doses at 0, 1 and 6 months.<sup>8, 9, 42, 52, 53</sup> The intramuscular administration should be given in the deltoid muscle since inoculation in the buttocks has shown lower response rates.<sup>54-56</sup> The explanation behind this lower response rate with administration in the buttocks is that the deposition of vaccine is uncertain to be in the muscle and could instead be deposited in the subcutaneous tissue. Subcutaneous administration is not recommended as it produces a poor antibody response.<sup>55</sup> After three doses of vaccine, HBsAg antibody levels (anti-HBs) should be tested, and a level of 10 mIU/mL or greater is considered protective in otherwise healthy adults.<sup>8, 43, 46, 57, 58</sup> Booster doses were previously recommended at different times according to the level of anti-HBs taken 1–2 months after the third vaccination,<sup>52, 59</sup> but currently a level of 10 mIU/mL or greater is considered protective and booster doses are no longer recommended once this level has been achieved.<sup>60, 61</sup> Booster doses given at various time interval after primary vaccination results in an immediate and rapid increase in anti-HBs level both in children and in adults.<sup>62-65</sup> This is also shown in younger adults where a booster dose administered four years after one single dose of vaccine gave high seroconversion rate suggesting a persisting immune memory.<sup>66</sup> In immunocompromised patients at high risk of hepatitis B infection, booster doses are still recommended.<sup>53, 60</sup> Because of the high cost of vaccination and because studies showed that intramuscular vaccination failed to induce a protective antibody level in 5–10% of recipients, studies were initiated to evaluate the effectiveness of intradermal vaccination.<sup>45, 64, 67-75</sup> It was thought that this route of administration might provide an equivalent antibody response with smaller doses of vaccine.

Several studies showed that the two routes of administration produced similar immune responses to plasma-derived vaccine, as measured by anti-HBs levels.<sup>45</sup>

Post-exposure prophylaxis with hepatitis B vaccine is a possible option following known or suspected exposure to the virus. This is used in newborn babies of known hepatitis B positive mothers<sup>76</sup>, and also in others following accidental exposure to infected blood. Both vaccine and specific antibodies, hepatitis B immunoglobulins (HBIG), are given if the carrier is highly infectious, e.g. HBeAg positive with high HBV-DNA levels. In other cases, vaccine alone is considered sufficient for protection. Post-exposure prophylaxis should be given as soon as possible after exposure, preferably within 48 hours.<sup>42, 77</sup> For high viraemic mothers treatment with lamivudine is recommended by some authorities to furthermore minimize the risk of hepatitis B transmission to the child.<sup>78, 79</sup>

- Vaccine non-response

Irrespective of the type of vaccine and of the dose or route of administration, about 5–10% of all individuals fail to seroconvert to anti-HBs  $\geq 10$  mIU/mL with the standard vaccination scheme. The risk factors for non-response identified by several studies are; smoking, increasing age, overweight, male gender, impaired immune reactivity and some genetic factors.

Smoking is a risk factor in poor response to hepatitis B vaccine.<sup>71, 80, 81</sup> It is also known to lower the response to vaccines in general.<sup>82</sup> The initial response to influenza vaccination is equal in smokers and non-smokers.<sup>83, 84</sup> The antibody decline after influenza vaccination is reported to be faster in smokers than in non-smokers<sup>85</sup> but this has not been confirmed by others.<sup>84</sup> Most studies show that the rate of response to hepatitis B vaccine is reduced in smokers, and the difference is more pronounced if the vaccine is given intradermally.<sup>71</sup>

Vaccine effectiveness in the elderly has been reported to be lower than in children and young adults, although this association has not been demonstrated in all studies.<sup>71, 86, 87</sup> In a meta-analysis of 24 published trials of vaccination in adults, increasing age from as low as 30 years was associated with lower hepatitis B vaccine response.<sup>88</sup>

Overweight, expressed in most studies as body mass index (BMI = body weight in kilograms/height in meters squared), has been suggested as a risk factor for vaccine failure. This effect is mainly seen in the very obese, e.g. a BMI over 35, as shown in one study (overweight = BMI >25, and obesity = BMI >30).<sup>69</sup>

The effect of gender is not entirely clear. However, many authors have found a poorer response in males than in females. This effect seems to be more pronounced with intradermal vaccination. It has been suggested that the effect of gender could be explained by the greater

weight of men<sup>89</sup> but others have shown that males have a lower response rate even after correcting for weight.<sup>71</sup>

Diseases associated with a reduced immune response are known to impair the vaccine response. Patients with end-stage kidney disease, irrespective of whether they are on dialysis or not, have a poorer immune response to hepatitis B vaccine.<sup>90</sup> Dialysis patients, who are at high risk of acquiring hepatitis B infection, have a 50–60% response rate.<sup>91</sup> Doubling the dose of vaccine raises the response rate to 80% and is recommended in these patients.<sup>92, 93</sup> Response rates are similar in both haemodialysis and peritoneal dialysis patients.<sup>94</sup> The response rate to the vaccine is reported to be reduced in people infected with human immunodeficiency virus (HIV). The vaccine response in HIV-infected individuals is related to the CD4+ cell count, which correlates with the immune response and also to the HIV-RNA level in these patients. Those with a low CD4+ count and high HIV-RNA levels have a poorer response.<sup>95</sup> Doubling the standard dose in these patients will increase the response rate to the vaccine if the CD4+ count is over 350 and the HIV-RNA level is low.<sup>96</sup> The vaccine response in other types of chronic liver disease has not been investigated on a large scale, but some data suggest a reduced response.<sup>97</sup> Patients with cirrhosis of the liver are reported to have a lower response rate.<sup>97, 98</sup> Some data suggest that patients with chronic hepatitis C have a significantly lower response than healthy controls, one study showing a response rate of 70% after three intramuscular doses.<sup>99</sup> This low response rate was also seen in a study of patients treated for hepatitis C. In this study the finding was not related to the severity of the cirrhosis.<sup>100</sup> Some studies suggest that there is a lower response rate in people who consume large amounts of alcohol. These studies were made in people with a high daily intake of alcohol and without obvious cirrhosis.<sup>100, 101</sup> In one study of such subjects, doubling the standard dose increased the response rate from 46% to 75%.<sup>102</sup>

Genetic factors within and outside the Major Histocompatibility Complex (MHC) are associated with different vaccine responses.<sup>103-105</sup> Vaccine non-response had initially been thought to be an isolated phenomenon in otherwise healthy adults. In an early study with plasma-derived hepatitis B vaccine, responders and non-responders had the same response to another vaccine with a different antigen, tetanus toxoid<sup>103</sup> and in another study in twins the immune response to HBsAg and hepatitis A virus seemed to be different.<sup>105</sup> However, an association with some other diseases has been suggested. In one study in neonates, poor responders to hepatitis B vaccine were more likely to have a non-responder allele that also predisposed to immunological disorders such as diabetes and coeliac disease.<sup>106</sup> Adult patients with coeliac disease have also been shown to have a poor immune response to hepatitis B vaccine. One suggested reason for this is that patients with coeliac disease have a particular HLA genotype that has also been associated with poor vaccine response.<sup>107</sup>

Children respond better to hepatitis B vaccine than adults.<sup>108, 109</sup> This is true even in the newborn although the response improves within the first years after birth.<sup>110, 111</sup> Combination vaccines containing recombinant HBsAg and other antigens have been developed and are used in childhood vaccination programmes with good protective effect.<sup>112-114</sup> However, there

are rare cases of protection failure after successful hepatitis B vaccination with confirmed presence of hepatitis B markers in serum.<sup>36, 112, 115</sup> Recent studies reporting on the 15-20-year follow up from the start of the neonatal vaccination programme in Taiwan in 1984 have indicated an approximately 90% efficiency of the vaccination programme.<sup>9</sup>

Several mutations in the *a* determinant of the S antigen have been characterised.<sup>116</sup> These mutants have the potential to cause vaccine failure and are known as *vaccine escape mutants*.<sup>117</sup> The first and best described mutation is the one located at codon 145, in which glycine is changed to arginine. This mutant was first seen in Italy but has also been reported in children in South Asia.<sup>116</sup> The widespread use of the vaccine raised concerns about these escape mutants which are, in fact, increasingly being reported in vaccinated children.<sup>118</sup> In a recent study from Taiwan 10% of vaccinated children were diagnosed with occult hepatitis B.<sup>119</sup> Despite the fact that more than 150 countries have now implemented the vaccine in childhood vaccination programmes, available data show that the spread of these mutants does not yet represent a clinical problem.<sup>21</sup>

- *Side effects*

Side effects associated with recombinant hepatitis B vaccine are usually mild.<sup>8, 38, 71, 72</sup> It is considered to be one of the safest available vaccines, and the only contraindication to its use is known allergy to any of the vaccine constituents. The most commonly reported adverse effects relate to the site of injection, typically tenderness, swelling and redness of the skin. These effects are seen following about 22% of intramuscular vaccinations but they usually resolve spontaneously within a few days. Severe adverse effects such as allergic reactions have been described but they are very rare. Febrile and other systemic reactions have also been described but they are also unusual. Associations between the vaccine and neurological disorders, such as demyelinating disorders, have been suggested. A suggestion that Guillian-Barré syndrome was linked to the vaccine has not been confirmed in large analyses. A suspected link between hepatitis B vaccine and multiple sclerosis has not been confirmed in studies.<sup>120, 121</sup> A recent report from Swedish authorities on children's vaccinations addressing the literature found 8 studies without any relation found between hepatitis B vaccination and multiple sclerosis while one study found such a relation.<sup>122</sup> The side effects of intradermal and intramuscular vaccination do not differ. Itching and pain around the site of injection, and hypopigmentation have though been more frequently reported with the intradermal administration.<sup>75</sup>

- *Investigational vaccines and re-vaccination of non-responders*

As already mentioned recombinant vaccines produced in yeast or in mammalian cell lines, containing not only HBsAg but also Pre-S1 and/or Pre-S2, have been developed in an attempt to enhance the immune response, especially in non-responders.<sup>123-126</sup> The response rate to

these vaccines among previous non-responders is reported to be 60-80%.<sup>123</sup> There have also been studies on a vaccine derived from a *Salmonella* sp. bacterium intended primarily for oral use, and DNA vaccines.<sup>127</sup> The possible role of these vaccines is not clear.

Several other ways of trying to improve the response have been tested. For example, there are many studies of additional doses in a variety of combinations. Re-vaccination of non-responders by a different route to the initial schedule has been evaluated both for the intramuscular and intradermal route in both health care workers and immunocompromised patients.<sup>128, 129</sup> In one small Swedish study the response rate following two additional intramuscular booster doses was 60% in subjects who had failed to respond to three primary intradermal or intramuscular doses of vaccine.<sup>128</sup> In a study on health care workers, non-responders to three intradermal doses responded to intramuscular administration, with 60% responding after one dose and 89% after three.<sup>130</sup> There are also studies on non-responders to intramuscular vaccination. In one of these, 88% of subjects who had failed to respond to five intramuscular vaccinations responded to intradermal administration of recombinant vaccine (5 µg) fortnightly until a total of four doses had been given.<sup>131</sup> Similar results were seen in another study in which 94% of intramuscular non-responders responded to four doses of intradermal vaccination.<sup>132</sup> The addition of adjuvants such as GM-CSF has also been studied, but no definite enhancement of the immune response was reported.<sup>133-135</sup> Another adjuvant to recombinant hepatitis B vaccine, AS04, containing a lipid A and alum preparation, has been tested. This vaccine is registered for use in patients with end-stage kidney disease and dialytic patients where it has shown good immunological properties.<sup>136</sup> The results in non-responders were 98% after three doses compared to 68% in those receiving the standard recombinant vaccine.<sup>137</sup> This vaccine has also been studied in patients with liver failure waiting for liver transplantation with higher response rates compared to standard vaccine.<sup>138</sup> However, the adjuvant-containing vaccine used in this study also contained twice the amount of HBsAg (40 µg versus 20 µg) and this could have affected the result. In conclusion, response rates in previous non-responders to different revaccination regimes vary between 50-90%.<sup>139</sup> There is a wide range in the definition of non-responders, and the re-vaccination schedules that have been tested use different antigen doses, vaccination intervals and numbers of vaccinations. They are therefore not easily compared.

- *Therapeutic vaccines*

Immune therapy with vaccines for the treatment of hepatitis B has also been evaluated.<sup>140-144</sup> Recombinant vaccines have been studied in chronic HBsAg carriers with chronic liver disease and have shown a higher seroconversion rate in the HBeAg system during follow-up compared to non-vaccinated individuals (8–15% versus 0%), but the studies are small.<sup>141</sup> T-cell vaccines which aim to eliminate infected hepatocytes by activating cytotoxic lymphocytes have been studied. DNA vaccines containing viral DNA have also been tested in an attempt to enhance the immune response and eradicate the virus.<sup>142</sup> These studies remain experimental, and therapeutic vaccination will probably not be generally available for many years.

### *Hepatitis A vaccine*

The hepatitis A virus is a picornavirus. This RNA virus was discovered in 1973. Following an incubation period of two to six weeks, it may cause acute symptomatic hepatitis, although sub-clinical infection is also common, especially in children. Acute infection never results in chronic disease, and infection confers lifelong immunity. Hepatitis A is widely spread throughout the world with the exception of Western Europe, North-America and the Australian continent. Hepatitis A is linked to the standards of hygiene in the society. Vaccination against hepatitis A has been available since 1992. It has proved very effective in preventing the disease and is recommended for all travellers outside the areas mentioned above.<sup>8, 145</sup> Some countries have included hepatitis A vaccination in the childhood vaccination programme. The vaccine consists of inactivated hepatitis A virus, and protection against hepatitis A is mediated through the production of a specific IgG antibody, anti-HAV IgG. The primary vaccination schedule consists of two vaccinations given at least six months apart. The need for booster doses is a subject of some debate, but protection is generally considered to last for at least 20 years.<sup>146</sup> The assumed level of protective anti-HAV is > 20 mIU/mL. Non-response to hepatitis A vaccine does occur, but appears rare and is certainly far less common than that seen with hepatitis B vaccination.<sup>147</sup> Intradermal administration of hepatitis A vaccine has been tested in a few studies, with varying results.<sup>70, 148, 149</sup>

### *Combined hepatitis A and B vaccine*

A combined hepatitis A and B vaccine has been available since 1997.<sup>150</sup> It has shown great immunological effect for both the hepatitis A and B components, producing similar results to monovalent vaccines given separately.<sup>151</sup> Its constituent components are essentially the same as the monovalent vaccines. The hepatitis B component of the combined vaccine contains the same amount of antigen as its monovalent counterpart, i.e. 20 µg, whilst the hepatitis A component has half the amount of inactivated virus compared to the monovalent vaccine, i.e. 770 as opposed to 1440 Elisa units/ml. The standard vaccination schedule for this combined vaccine is 1.0 ml intramuscularly at 0, 1 and 6 months. Checking anti-HBs titres in healthy individuals after immunisation with the combined vaccine is recommended in risk groups for hepatitis B infection and in groups with a expected low response rate, even though it seems to be even more effective in inducing an immune response than the monovalent vaccines.<sup>152-154</sup>

## *Aspects of immune response*

### *HLA-linkage*

The human leukocyte antigens (HLA) are coded by the major histocompatibility complex (MHC) group of genes located on chromosome six in the human genome (Figure 4). The HLA molecules are divided into three classes, of which HLA class I and II are involved in the immune response to pathogens. HLA class I molecules are present on all nucleated cells in the body and their function is to present peptides that have been synthesised or modified by the host cells. The HLA class I antigens play an important role in the host's defence against intracellular pathogens. The HLA class II molecules are present on the surface of antigen-presenting cells such as macrophages, B-lymphocytes and dendritic cells. Class II antigens are made up of two chains,  $\alpha$  and  $\beta$ , both of which have two domains. The binding groove consists of one domain from each chain. There are three pairs of HLA class II loci: HLA-DP, HLA-DQ and HLA-DR. Each individual has two alleles, one from the father and one from the mother. Some genotypes of HLA-DR have an extra  $\beta$ -chain gene. Consequently, these three MHC class II genes can result in four types of HLA class II molecules. Each individual presents at least six different HLA class II molecules on the surface of the antigen-presenting cells if none of the alleles are homozygous. The alleles in the MHC region are polymorphic and a high number of alleles exist within the population. Genes encoding for these antigens are strongly linked in the genome and are therefore in most cases inherited together. Linked alleles on one chromosome are called haplotypes. This linkage makes it possible to predict haplotypes without knowing all the alleles at different loci in the genome. The alleles and haplotypes differ between individuals with different ethnicity.<sup>155-157</sup> Some of the genes in the MHC region are pseudogenes which means that they have lost their protein-coding ability.

Both HLA class I and class II molecules are involved in the immune response to hepatitis B antigen.<sup>103, 158, 159</sup> An association with HLA class I and non-response has been shown for HLA B8 and B44.<sup>160, 161</sup> Homozygotes for the haplotype HLA-B8, SC0, DR3 were originally identified as being associated with lower response than heterozygotes for the same haplotype.<sup>104</sup> The conclusion drawn from these studies was that it seemed that response was inherited in a dominant fashion and non-response consequently was inherited in a recessive fashion.<sup>104, 162</sup> For HLA class II, non-response is associated with the HLA-DRB1 alleles \*0301 \*1302, \*0701, DR 14 and with DQB1\*02 and DPB1\*1101.<sup>159, 163, 164</sup> In contrast the alleles DRB1\*0101, DRB1\*11, DRB1\*1501, DQB1\*0501, DQB1\*0602 and DPB1\*1101 are associated with vaccine response.<sup>159, 163, 165</sup> As different HLA genes are linked and inherited together, an association exists between response and certain haplotypes. An observed association between a gene and the response is not necessarily an indicator of a direct effect of the proteins coded by the genes. The gene could be just a marker for the response pattern while the effect on the immune response is located in another gene in the inherited haplotype. One gene could affect the immune response only in combination with another certain gene.<sup>105</sup>

The results of some studies also suggest that a lack of complement 4A, associated with certain HLA class III subtypes, is associated with reduced vaccine response.<sup>161, 166, 167</sup> The complement proteins are important for B cell activation and the development of B cell memory. Lack of C4A could therefore impair the humoral immune response.

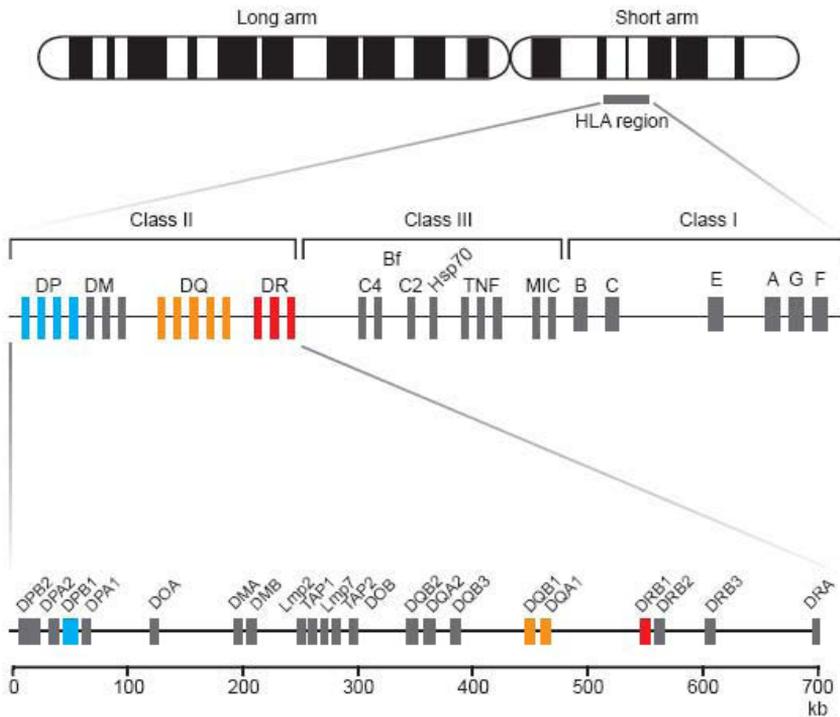


Figure 4. Schematic presentation of the HLA region on the chromosome 6. The genes studied in this project are coloured in the figure.

### Antigen presentation

The antigen-presenting cells (APCs) ingest foreign protein in this case HBsAg and then degrade it to peptides of 12-15 amino acids. HLA class II molecules within the cell associated

with degraded viral peptides and these complexes are transported and presented on the surface of the APCs. In the lymph nodes the peptide-loaded HLA class II molecule on the surface of the APC is then recognized by T cell receptor (TCR) of HBsAg-specific CD4+ cells (Figure 5). Through a simultaneous ligation of additional co-stimulatory molecules the CD4+ T cells then become activated. These now activated T cells can in turn activate HBsAg-specific B-cells, presenting the same HLA class II peptide on its surface. These B cells then mature and undergo both a switch in heavy chain expression of the secreted immunoglobulin, as well as somatic mutations that result in an increased affinity for the antigen. The mature B cells, or plasma cells, now proliferate and produce IgG subclasses in blood and also IgG produced after vaccination with HBsAg is IgG1.<sup>168-170</sup> Thus priming HBsAg-specific cytotoxic T cells (CTLs) by vaccination with recombinant HBsAg has been shown in mice<sup>171</sup> but this is less well documented in humans. T helper, T memory and B memory cells are involved in the immune memory following hepatitis B vaccination and natural hepatitis B infection.<sup>172</sup>

Resident APCs, such as macrophages and dendritic cells, are more frequent in skin than in an untreated muscle.<sup>173, 174</sup> The APCs of the skin consist of two kinds of dendritic cells. The Langerhans cells are located in the inner part of the epidermis layer, whereas dendritic cells present in the dermis are called dermis dendritic cells. These are not identical with the Langerhans cells of the epidermis. The dendritic cells are potent antigen presenting cells for induction of immunity against incoming antigens and have the origin in the bone marrow. Intradermal vaccination is targeted to the dermis.<sup>173</sup> Regarding hepatitis B vaccination, it has been shown that intradermal administration induces a stronger B cell and T cell response.<sup>175</sup> Theoretically, intradermal vaccination offers the possibility of equally effective vaccination response using lower vaccine doses. It might also offer a means of enhancing the immune response in previous non-responders to intramuscular vaccination.

Apart from hepatitis B vaccine, intradermal vaccination has been tested for other pathogens, including influenza and rabies. The reported response rate for intradermal influenza vaccine was good, except in people aged over 60 where the response was reported to be lower.<sup>176</sup> Recently the first influenza vaccine with a novel microinjection system for intradermal use was registered in Sweden and this vaccine has shown very good response rates in all age groups.<sup>177, 178</sup> For rabies vaccine, intradermal and intramuscular administration produced equal responses and the intradermal route is now used widely for this vaccine due to a simpler administration.<sup>179</sup> Hepatitis B vaccination is, however, the most widely investigated vaccine as regards intradermal administration.

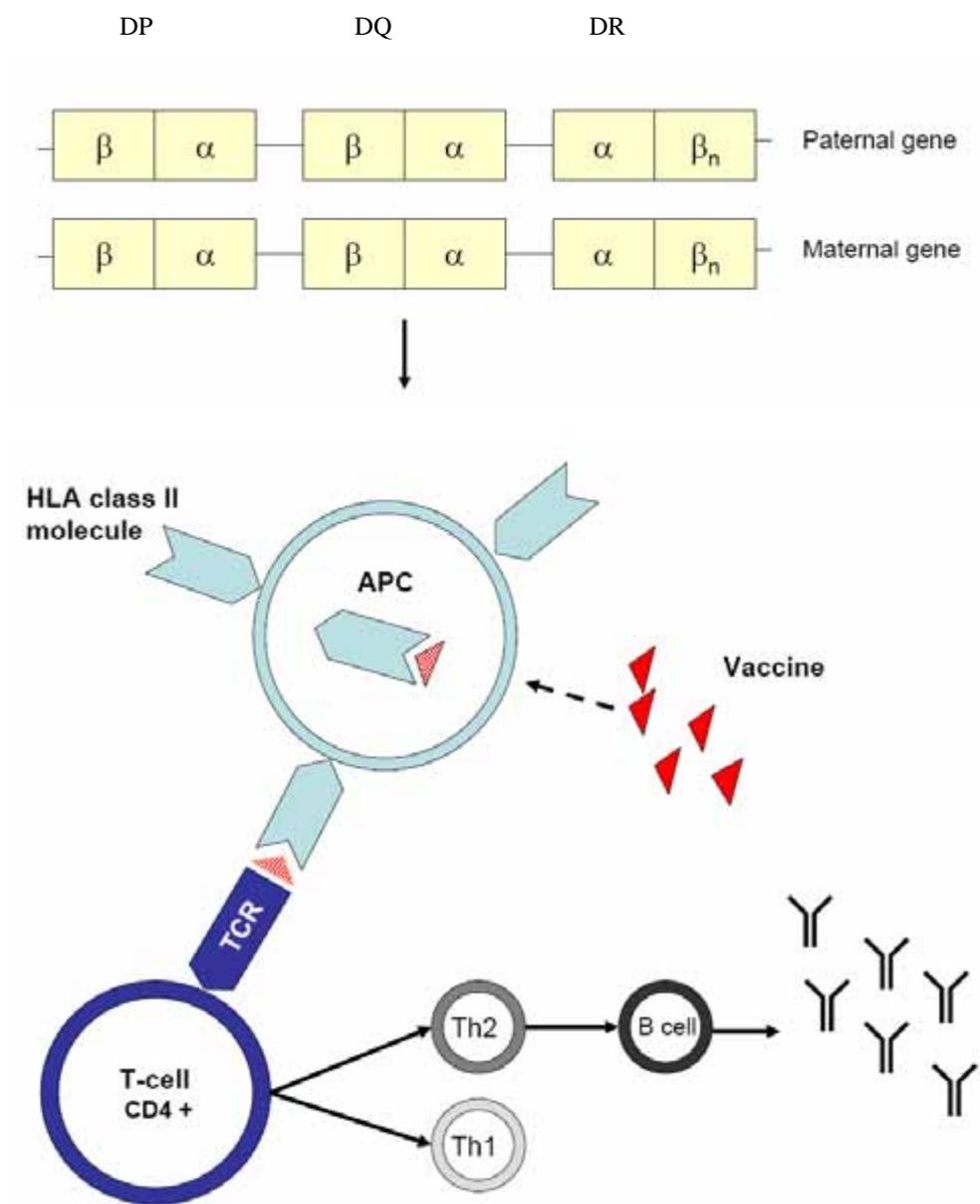


Figure 5. Diagram depicting the HLA class II gene and antigen presentation of HLA class II antigen-peptide complex.

### *T cell response*

HLA class II molecules associated with viral peptides present the complex on the surface of APCs to CD4+ T helper (T<sub>h</sub>) cells. This results in activation of these T cells with cytokine production, leading to differentiation into effector cells and memory cells. Several epitopes of the S-region of the hepatitis B virus genome have been identified as being important for T cell activation. It has been suggested that non- or low responders have a defect in HBsAg presentation by the antigen-presenting cells but this does not seem to be the case.<sup>180</sup> The T helper cell response can be divided depending on their cytokine production in T<sub>h</sub> 1 and T<sub>h</sub> 2 response. The T<sub>h</sub> 2 like cells promotes the activation of B cells and production of antibodies to the presented antigen. Early studies suggested that non-responders might have a deficit T<sub>h</sub> 2 response.<sup>181</sup> In another study high responders had a T<sub>h</sub> 1 like cytokine profile whereas non-responders failed to produce cytokines.<sup>182</sup> More recent studies have suggested that non-response is due to inadequate secretion of both T<sub>h</sub> 1 and T<sub>h</sub> 2 cytokines.<sup>183, 184</sup> Some studies have reported a cell-mediated response despite a lack of protective antibodies.<sup>185, 186</sup> The T<sub>h</sub>-cell response is polyclonal and has been reported to be more diverse in responders than in non-responders.<sup>187-189</sup> Thus, this implies that there may be degrees of response rather than just the presence or absence of a response to the vaccine.

## *Aims*

Aims of this study were to determine

- the efficiency of intradermal vaccination with recombinant hepatitis B vaccine in healthy adults in clinical practise
- factors associated with response and non-response to the hepatitis B vaccine
- if certain HLA class II haplotypes (DPB1, DQB1, DQAI and DRB1) were associated with hepatitis B vaccine response or non-response
- the response to hepatitis B vaccine in relatives to known non-responders
- a possible correlation between certain HLA class II antigen haplotypes in relatives to non-responders with special reference to amino acid nr 86 in the DRB1 allele
- to what extent a non-responder status to hepatitis B vaccinations is absolute
- the response to hepatitis B surface antigen in previous non-responders immunised with high-dose of the combined hepatitis B surface antigen and hepatitis A virus vaccine
- the ability of hepatitis B vaccine non-responders to produce hepatitis A antibodies

## ***Materials and Methods***

### *Subjects*

The cohort in this study included health care workers who were offered intradermal vaccination with the recombinant hepatitis B vaccine between 1991 and 1994 at the Department of Infectious Diseases, Linköping University Hospital (Figure 6). A total of 2 247 subjects started the vaccination programme and 1 800 completed the vaccination schedule with 3 or 4 doses followed by anti-HBs testing. Of these, 1 406 subjects also completed a questionnaire on lifestyle factors and these are the original subjects of this thesis (paper I). Before each dose the participants were asked about any side effects with the previous doses and any reported adverse effects were recorded.

Non-responders, defined as having anti-HBs levels of <10 mIU/mL and responders with anti-HBs >100 mIU/mL from the study in paper I were recruited for studies on the genetic influence of HLA class II antigens on response and non-responders. Smoking individuals were excluded in this study. Haplotypes for DPB1, DQA1, DQB1 and DRB1 were analysed in these responders and non-responders (paper II).

Non-responders with known haplotypes from the genetic study (proband) were then selected for a family study in which relatives were vaccinated using the same strategy. The probands were chosen from non-responders in the genetic study and were selected by their particular haplotypes and also for having relatives living within reasonable reach of the University Hospital of Linköping (paper III). A proband is a member of a family that is the index individual for a family tree in genetic studies.

Non-responders from both of the described studies (paper I and III) together with some non-responders identified during routine clinical vaccination were asked to participate in a revaccination study. Non-response in this study was defined as anti-HBs <10 mIU/mL after at least four intradermal doses of hepatitis B vaccine. A total of 48 non-responders were included. Twenty previously unvaccinated subjects, mainly health care students and workers, served as the reference group (paper IV).

The studies were approved by the Ethics Committee at the Health University, Linköping. The revaccination study (paper IV) was approved by the Swedish Medical Products Agency.

## Study subjects

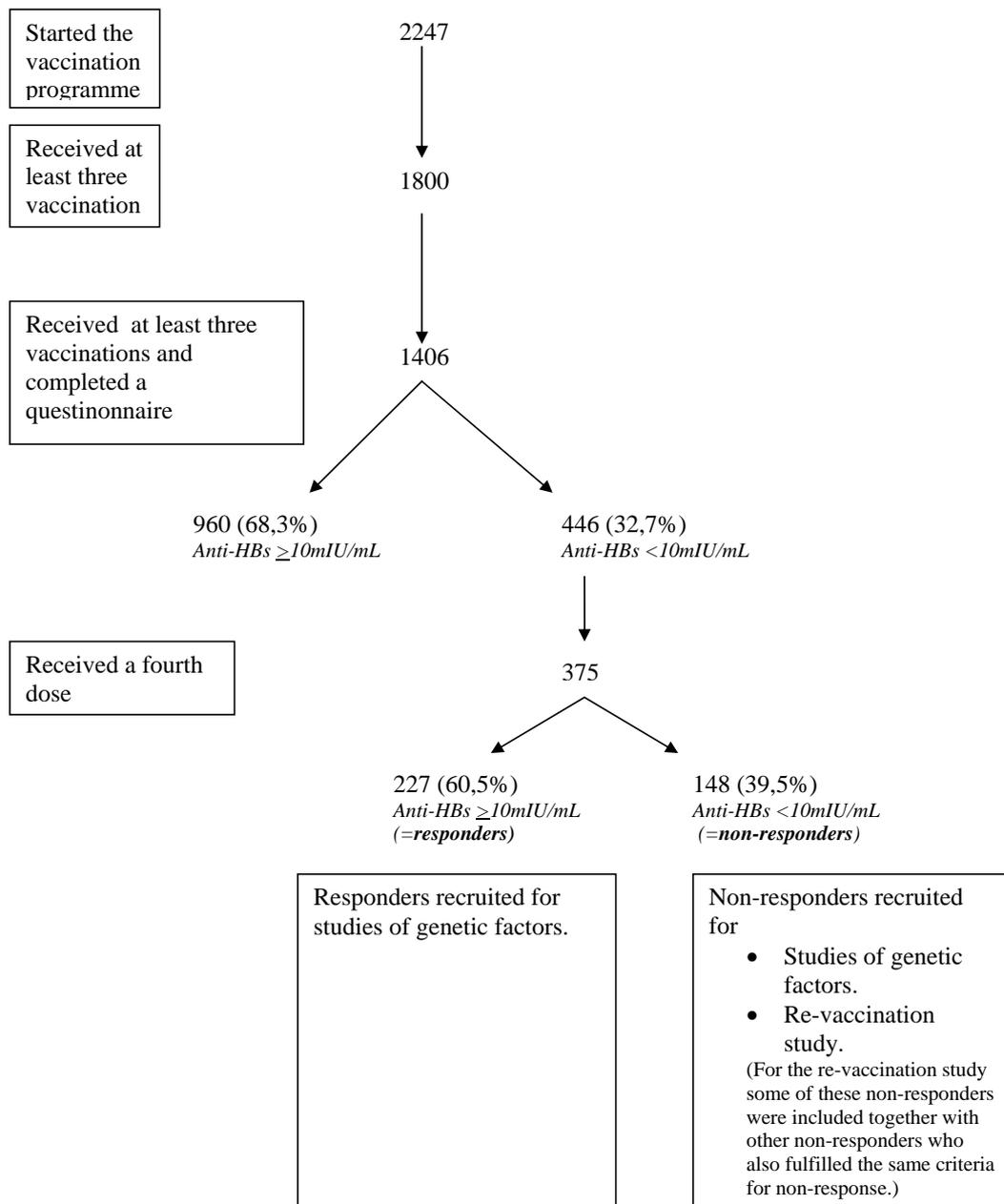
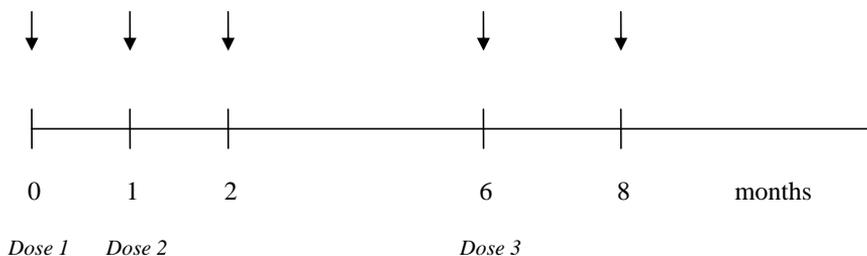


Figure 6. Study design.

## Vaccination

Primary immunisation with hepatitis B vaccine was carried out using the recombinant hepatitis B vaccine, (Engerix-B® 20 µg/ml, GlaxoSmithKline) given intradermally, except for the reference group in the revaccination study. A vaccine dose of 0.1 ml (2 µg) was administered intradermally at 0, 1 and 6 months. After a further 1-2 months, anti-HBs were estimated and those with levels below 10 mIU/mL were given a fourth dose of 0.1 ml by the same route. After four doses, vaccinees were defined as responders if the anti-HBs level was  $\geq 10$  mIU/mL and non-responders if it was  $< 10$  mIU/mL. All participants were vaccinated by one of three highly experienced nurses at the Vaccination Centre of the Department of Infectious Diseases, Linköping. They were instructed to look for a blister after each intradermal injection (papers I-III).

In the revaccination study (paper IV), all participants received the combined hepatitis A and B vaccine (Twinrix® 20 µg HBsAg/770 inactivated HAV Elisa units/mL, GlaxoSmithKline) at a dose of 2.0 ml intramuscularly at 0, 1 and 6 months. A total of 1.0 ml was given in each deltoid muscle. The study group consisted of non-responders who had all received primary vaccination according to the above schedule, and all had received at least one booster dose making a total of not less than four administered intradermal vaccinations. The reference group was vaccinated again with the combined vaccine using the same doses and time intervals as described above. All subjects in the revaccination study tested negative for HBsAg, anti-HBc, anti-HBs and anti-HAV before inclusion. Blood samples were collected on five occasions during the study; before each dose, two months after the first dose and 1-2 months after the third dose (Figure 7).



*Figure 7. Diagrammatic representation of the revaccination study (paper IV). Vaccine doses were administered at 0, 1 and 6 months. The blood samples were collected immediately before each vaccination. The arrows mark occasions when blood samples were collected for determination of humoral and cell mediated immune response.*

## *Data collection*

- *Serological testing*

All testing of HBV markers was performed at the Clinical Microbiology Laboratory at Linköping University Hospital. Measurements of the anti-HBs levels were carried out using Organon Hepanostika anti-HBs (Organon Teknika, Boxtel, The Netherlands) and Abbott IMX Ausab (Abbott, North Carolina, IL, USA.) in paper I. In the remaining papers AxSYM AUSAB (ABBOTT Laboratories, North Chicago, Illinois, US) was used for all serological tests.

Assessment of the exact anti-HBs and anti-HAV titres was done in paper IV as follows: Samples with anti-HBs concentrations reported as greater than 1 000 mIU/mL were diluted using the Automated Dilution Protocol. Samples with anti-HBs concentrations reported as greater than 25 000 mIU/mL by the Automated Dilution Protocol were diluted using a manual dilution of 1:25. The amount of anti-HBs in samples was determined using a calibration curve. Measurement of the anti-HAV levels was carried out using AxSYM HAVAB 2.0 Quantitative (ABBOTT Laboratories, North Chicago, Illinois, US). The dynamic range of this assay is 0-100 mIU/mL. For anti-HAV  $\geq 100$  mIU/mL a standard dilution protocol described by the manufacture was used. For anti-HAV  $\geq 20\ 000$  mIU/mL no further dilution protocols existed. Therefore no further dilution was carried out. These titres are referred to as 20 000 mIU/mL in the statistical analysis.

- *Side effects*

In the clinical study (papers I-III), subjects were asked about any adverse effects following the previous vaccination before each dose was given, and any reported adverse effects were recorded in the vaccination file according to normal departmental procedures. In the revaccination study (paper IV), the participants were asked about adverse effects in the same way but they were also asked to complete a questionnaire about adverse effect after each dose.

- *HLA class II typings*

DNA was extracted from 5 ml blood drawn in Vacutainer Tubes containing EDTA from each subject in the study. The extraction of DNA was done according to the SDS-UREA method.<sup>190</sup>

DPBI polymorphism was detected by sequence specific oligonucleotides (SSOs) in dot-blot hybridizations after polymerase chain reaction (PCR) amplification of DNA with generic primers according to the XI<sup>th</sup> Histocompatibility Workshop protocol.<sup>191</sup> With this procedure a total of 19 different alleles could be identified.

DQA1 and DQB1 polymorphisms were analysed by PCR amplifications with allele specific primers according to the supplier's recommendation (Dynal, Oslo). To distinguish DQB1\*0302 from DQB1\*0305 in heterozygotes with DQB1\*02, the kit DQB1 2<sup>nd</sup> set was used (Dynal, Oslo). The amplification products were detected in agarose gels and photographed. Approximately 12 DQA1 alleles and 12 DQB1 alleles could be identified by this technique.

DRB1 polymorphism was identified by restriction fragment length polymorphism (RFLP) technique in Southern blots. This method does not detect any subtypes and a total of 13 alleles could be distinguished. The Southern blot procedure was performed as previously described.<sup>192</sup>

Subtyping of the DRB1\*13 allele was done using allele-specific primers from a DRB1\*13 kit (Dynal, Oslo). Twenty-five subtypes of the DRB1\*13 allele could be detected by this method.

DNA sequencing of the DQB1-0604/0609 alleles – Amplification of the samples was performed according to the SB Typer™ DQB HLA class II sequencing kit (Pharmacia Biotech).

- Statistics

The response rates in different subgroups were compared with multiple logistic regression (paper I and III).

The allele frequency distributions of the non-responders and responders in paper II for DPB1, DQA1, DQB1 and DRB1 were compared in a chi-square  $\chi^2$  test of homogeneity. Haplotypes were designated according to the most probable combination. Comparisons of single allele and haplotype frequencies between the non-responders and the responders were performed in a  $\chi^2$  test with Yate's correction. Significance was considered at  $p < 0.05$ . P-values were not corrected for the number of alleles that could be detected in each of the HLA loci.

In paper IV, differences in demographic data between the groups were analyzed by Student's t test. For comparing titres after each dose, the Mann-Whitney rank-sum test was used and response rates after each dose were compared with Fisher's exact test. The influence of cofactors was analysed using a general linear model. For anti-HBs, in this paper, we choose to do the calculations with logarithmic data because of the wide range of titre levels and because the effect seemed to be multiplicative rather than additional for anti-HBs. We used numeric values for anti-HAV. Correlation between log anti-HBs and anti-HAV titres after three doses was calculated. The revaccination study was designed as an open trial. We estimated that, with a study group of about 50 non-responders and a reference group of 20, we would be able to show differences between the groups but not similarities (paper IV).

## Results

### *Response rates after intradermal vaccination (paper I)*

The overall response rate to the hepatitis B vaccine in the clinical study in healthy health care workers after three intradermal doses was 68.3% (960/1406), and 88.9% (1187/1335) after giving a fourth intradermal dose to those not responding after the third dose (Table 1, Figure 8). Using a logistic regression model for evaluation of factors affecting the response, no statistical difference was observed in this study between men and women, or with respect to the Body Mass Index (BMI). Subjects older than 40 years had a significantly lower response rate than those aged less than 25 years (87.0% and 97.8% respectively,  $p = 0.05$ ). Smokers were commoner among non-responders (Figure 8) and the negative effect of smoking on the response rate increased with increasing tobacco consumption. The response rates for all smokers was 55.7% ( $p = 0.025$ ) and in heavy smokers (defined as smoking more than 20 cigarettes per day) the response rate was 50.6% ( $p < 0.001$ ).

*Table 1. Seroconversion in different subgroups after 3 or 4 doses of intradermal hepatitis B vaccine. BMI is defined as weight in kg/height in metres squared.*

	<i>n</i> after 3 doses	<i>n</i> with anti-HBs $\geq$ 10 after 3 doses (%)	<i>n</i> after 3 or 4 doses resp.	<i>n</i> with anti-HBs $\geq$ 10 after 3 or 4 doses resp. (%)
Total	1406	960 (68.3)	1335	1187 (88.9)
Men	239	164 (68.6)	219	194 (88.6)
Women	1167	796 (68.2)	1116	993 (89.0)
Smoking	305	170 (55.7)	284	229 (80.6)
Non-smoking	1101	790 (71.8)	1051	958 (91.2)
Age $\leq$ 25	94	73 (77.7)	89	87 (97.8)
Age 26–39	669	476 (71.2)	632	566 (89.6)
Age $\geq$ 40	643	411 (63.9)	614	534 (87.0)
BMI* $<$ 25 ( $<$ 24)	963	656 (68.1)	906	812 (89.6)
BMI* $\geq$ 25 ( $\geq$ 24)	443	304 (68.6)	429	375 (87.4)

\* See BMI definitions for men and women in text.

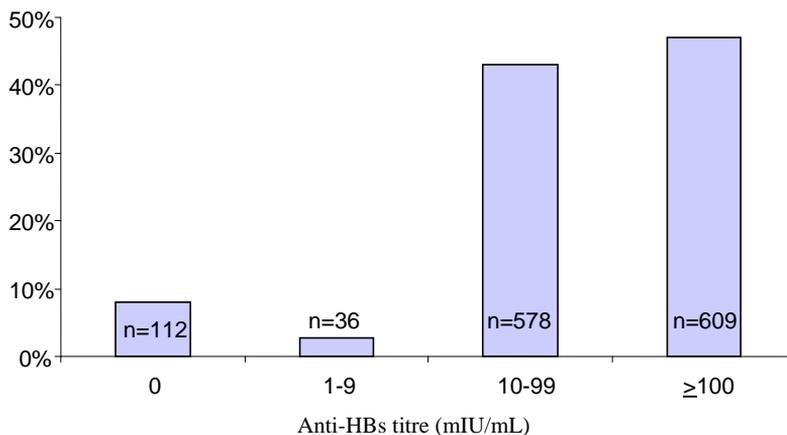


Figure 8. Proportion of subjects in each response group as defined by anti-HBs titres, following 3 or 4 doses.

There was an association between response rates with time from study start, suggesting that skill of injection was a determining factor (Table 2). When subjects were divided into three groups, depending on the date of first vaccination, the response rate showed a sequential rise from 54% to 72% and 81%. No serious adverse events were reported by the vaccinees during or after the study period.

Table 2. Seroconversion rate (anti-HBs  $\geq 10$  mIU/mL) in relation to date of first vaccination.

	Sept. 91 –Nov. 91	Dec. 91 –Mar. 92	Mar. 92 –May 93
Total	532	357	517
Men	48	71	120
Women	484	286	397
Anti-HBs $\geq 10$ (%)	54	72	81

Vaccination cost comparisons for the initial presentation of the study were made using contemporary (1998) vaccine prices in Swedish Crowns and assuming a response rate of 85% to three intradermal and 95% to three intramuscular doses of vaccine (Table 3). We also showed that, at the assumed response rates, the cost of intradermal vaccination was about 27% of that of intramuscular vaccination if the latter also included a booster dose. The costs of intradermal vaccination are shown at the response rate level of 80%, which was achieved with three doses in the latter part of the study, and at 85% which is a prior estimate of the response rate in our study group based on the results of other studies. Calculations are also shown for an intramuscular schedule without a fourth dose as the response rate of three intramuscular doses is equal to that of four intradermal doses. New calculations using 2008 prices (vaccine costs and costs of serological testing) show that, assuming a response rate of 85% for intradermal administration, the cost of the intradermal schedule is 34% of the intramuscular schedule (with booster dose included). The difference in relative costs between 1998 and 2008 is mainly due to the higher costs of serological testing.

*Table 3. Cost calculations (1998) for intradermal and intramuscular administration of vaccine (SEK).*

Vaccination schedule	Estimated response rate after 3 doses	Costs for initial doses <sup>a</sup>	Cost for serological testing <sup>b</sup>	Costs for additional doses <sup>a</sup>	Costs for additional serological testing <sup>b</sup>	Total cost
3 doses id (0.1 ml)	80%	100x3x24=7200	100x71=7100	20x24=480	20x71=1420	16200
3 doses id (0.1 ml)	85%	100x3x24=7200	100x71=7100	15x24=360	15x71=1065	15725
3 doses im (1.0 ml)	95%	100x3x165=49500	100x71=7100	5x165=825	5x71=355	57780
3 doses im (1.0 ml)	95%	100x3x165=49500	100x71=7100			56600

<sup>a</sup> Seven 0.1 ml intradermal doses are obtainable from 1.0 ml of vaccine. (165 SEK/7)

<sup>b</sup> Analysis cost for anti-HBs in 1998 at Department of Microbiology, Linköping University Hospital, Sweden (71 SEK).

In conclusion, the study presented in paper I shows that intradermal hepatitis B vaccination is technically difficult but could be used safely in healthy adults if post-vaccination estimation of anti-HBs is done. It is cost-effective in the context of mass vaccination, bearing in mind that smoking and age over 40 years are factors associated with lower response rates.

#### *Studies on genetic factors (paper II)*

From the participants described in paper I non-responders with anti-HBs <10 mIU/mL and responders with anti-HBs >100 mIU/mL were included in a study on HLA class II alleles. Smokers were excluded in this study. There were no significant differences in the age, gender and BMI distribution between the groups. A total of 53 non-responders and 69 responders were included. Of these 39 of the non-responders and 65 of the responders were typed for the DPB1 locus. For the DPB1 locus no significant difference was found between responders and non-responders and therefore this gene was not included in the further analysis. Of the non-responders 52 were typed for DQA1 and 53 for DQB1 and DRB1. For the responders 68 were typed for DRB1 and all 69 were typed for DQB1 and DQA1. There was a significant difference between non-responders and responders in the allele frequency distribution for DQB1 ( $p<0.001$ ), DQA1 ( $p>0.01$ ) and DRB1 ( $p<0.001$ ). For the DQB1 allele the subtypes DQB1\*0602 and DQB1\*0603 were less frequently found in non-responders than responders 0.11 versus 0.33 ( $p<0.01$ ) and 0.06 versus 0.22 ( $p<0.01$ ) respectively. DQB1\*0604 was predominately found in non-responders 0.21 versus 0.03 ( $p<0.005$ ). For DQA1 only one allele differed significantly between non-responders and responders where DQA1\*0103 were less common in non-responders 0.04 versus 0.21 ( $p<0.025$ ). DRB1\*1301 non-responders had a lower frequency than responders 0.06 versus 0.22 ( $p<0.05$ ) and for DRB1\*1302 the result was the opposite 0.25 versus 0.06 ( $p<0.01$ ). DR 15 showed difference in frequency between the groups where non-responders had this allele less frequently 0.11 versus 0.34 ( $p<0.01$ ). Significant differences of the subtypes are shown in table 4. The frequencies for all other subtypes analysed, did not differ between non-responders and responders.

Table 4. Significant differences in frequencies of the DQA1, DQB1 and DRB1 alleles in non-responders and responders.

Allele	Non-responders	Responders	Significance
DQB1*0602	0.11	0.33	P<0.01
DQB1*0603	0.06	0.22	P<0.05
DQB1*0604	0.21	0.03	P<0.005
DQA1*0103	0.04	0.22	P<0.025
DRB1*1301	0.06	0.22	P<0.05
DRB1*1302	0.25	0.06	P<0.01
DR15	0.11	0.34	P<0.01

Haplotypes were determined according to the most probable combinations.<sup>155</sup> The haplotypes HLA [DQB1\*0602; DQA1\*0102; DR15] and [DQB1\*0603; DQA1\*0103; DRB1\*1301] were less frequently found in non-responders ( $p<0.025$  and  $p<0.05$  respectively) (Table 5). In non-responders the haplotype [DQB1\*0604; DQA1\*0102; DRB1\*1302] was found more frequently ( $p<0.005$ ). Sixteen different HLA class II genotypes were found in both 32 responders and 25 non-responders. Thus, there seem to be some genetic linkage in the HLA region to a non-, or slow response to HBsAg vaccination.

Table 5. Haplotype frequency differences in 52 non-responders and 68 responders.

HLA haplotype	NR (n=52)		R (n=68)		P
	n	frequency	n	frequency	
<i>DQB1*02; DQA1*0501; DR3</i>	20/52	0.38	15/68	0.12	-
<i>DQB1*02; DQA1*0201; DR7</i>	13/52	0.25	9/68	0.13	-
<i>DQB1*0602; DQA1*0102; DR15</i>	6/52	0.12	23/68	0.34	<0.025
<i>DQB1*0603; DQA1*0103; DRB1*1301</i>	3/52	0.06	15/68	0.22	<0.05
<i>DQB1*0604; DQA1*0102; DRB1*1302</i>	11/52	0.21	2/68	0.03	<0.005

HLA haplotypes in italics have previously been reported to be more frequent in nonresponders.

### *Family study (paper III)*

Data for analysis was obtained from a total of 8 probands with at least one family member that had been vaccinated according to the study protocol. The families with their vaccine responses are shown in figure 9. Among 26 relatives to known non-responders to hepatitis B vaccine, 58% (15/26) responded to three or four intradermal doses of hepatitis B vaccine and achieved anti-HBs levels  $\geq 10$  mIU/mL. A total of 11 responders achieved an anti-HBs level of  $> 100$  mIU/mL, while four subjects had an anti-HBs level of 10-100 mIU/mL. Five of the relatives were smokers, two of which responded to immunisation and three of which did not. In the statistical analysis, age was significantly associated with non-response ( $p = 0.021$  OR: 0.92 with 95% confidence interval 0.86-0.99). Gender, smoking, BMI and amino acid in position 86 of the DRB1 gene had no influence on the results. The haplotype [DQB1\*0604; DQA1\*0102; DRB1\*1302] were found in 6 individuals and of these 5 were non-responders in all participants. Among the relatives 3 of 4 with this haplotype were non-responders ( $p < 0.042$  OR: 0.79 med 95% confidence interval 0.00-0.86).

In conclusion, the response rate of relatives to known non-responders in the family study was lower than expected, indicating that genetic factors played a role in the immune response to the vaccine. The immune response is dependent on genetic factors both within and outside the HLA class II region. The amino acid in position 86 of the DRB1 gene seemed not to account for the response differences, as had been previously suggested but this needs to be clarified in larger studies.

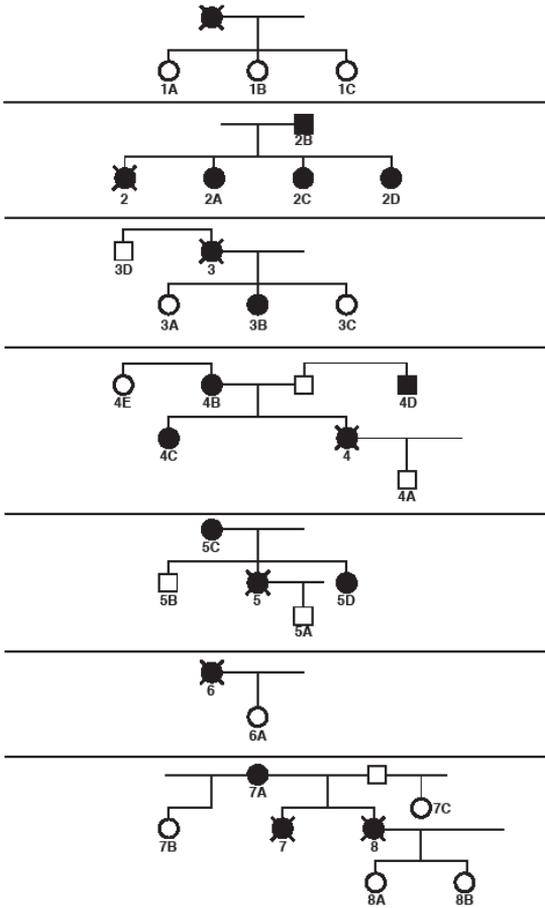
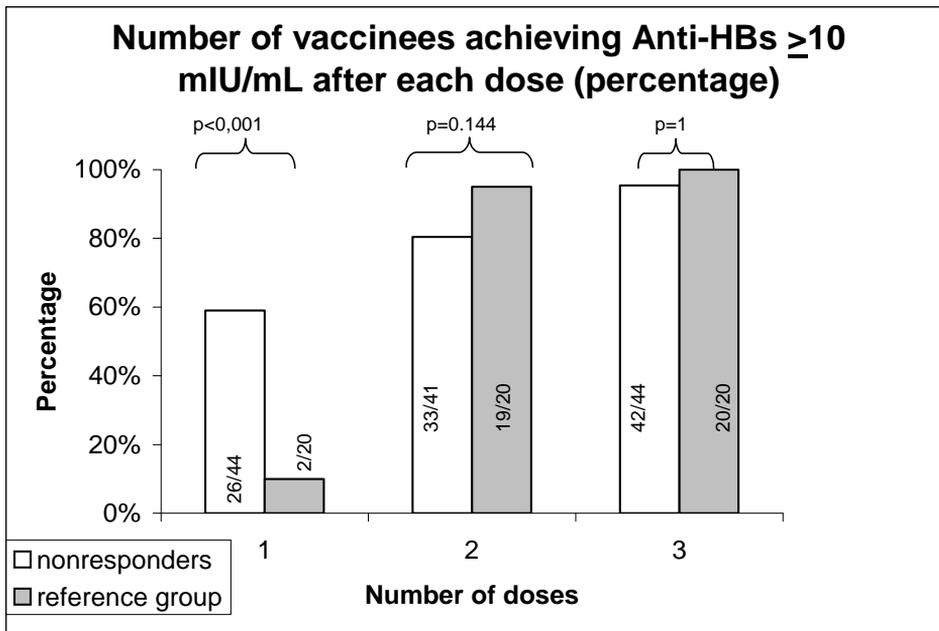


Figure 9. Diagram representing the family tree in the study. Probands are marked with the symbol . Women are represented as circles and men as squares. The filled symbols represent non-responders.

*Revaccination study (paper IV)*

The response rate for hepatitis B after three double doses (2.0 ml Twinrix<sup>R</sup>, Glaxo SmithKline) given by the intramuscular route of combined hepatitis A and B vaccine was 42/44 (95%) in previous non-responders and 20/20 (100%) in unvaccinated individuals (Figure 10). Already after one dose of the combined vaccine 59% (26/44) of non-responders had anti-HBs  $\geq 10$  mIU/mL indicating that they were primed to HBsAg from the initial vaccination schedule. After the first dose the response rate in the non-responder group was significantly higher than in the reference group where only 2/20 (10%) had an anti-HBs  $\geq 10$  mIU/mL ( $p < 0.001$ , Fisher's exact test). No significant differences in response rates were seen after second and third dose. Importantly, the anti-HBs levels were after three doses significantly lower in previous non-responders ( $p < 0.001$ ) compared to primary responders. (Figure 11)



*Figure 10. Proportion of individuals in the revaccination study achieving anti-HBs  $\geq 10$  mIU/mL after each dose. The significance of the difference between the groups is shown. (Fisher's exact test).*

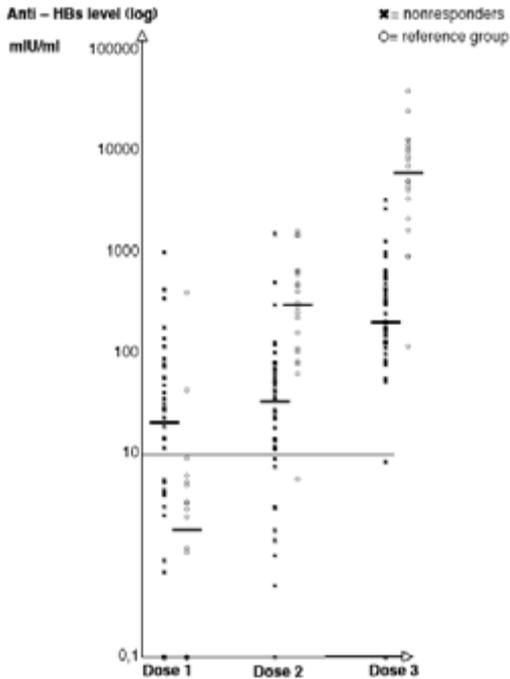


Figure 11. Logarithmic scale of Anti-HBs titres following each dose. The median titre in each group is shown with a bold horizontal line.

All vaccinees in the revaccination study responded to the hepatitis A component of the vaccine with anti-HAV levels of  $\geq 20$  mIU/mL which is considered to represent immunity. The median anti-HAV level after three doses of the combined hepatitis A and B vaccine, was significantly lower ( $p = 0.001$ ) in the primary non-responders than in the reference group (Figure 12). Overall, the study showed a correlation between anti-HAV and anti-HBs levels in vaccinated individuals. A correlation coefficient of 0.648 for log anti-HBs and anti-HAV was found after three doses ( $p < 0.001$ ).

One individual in the non-responder group developed extreme fatigue after the first dose and a notification about the reaction to the Swedish Medical Products Agency was done. No further vaccination was given to this individual and the Swedish Medical Products Agency considered the reaction to be a possible adverse effect of the vaccine. Apart from this, no major adverse effects were reported. Less than 10% of participants reported a minor adverse event which included a reversible local reaction at the site of injection, headache and tiredness in the days following the vaccination.

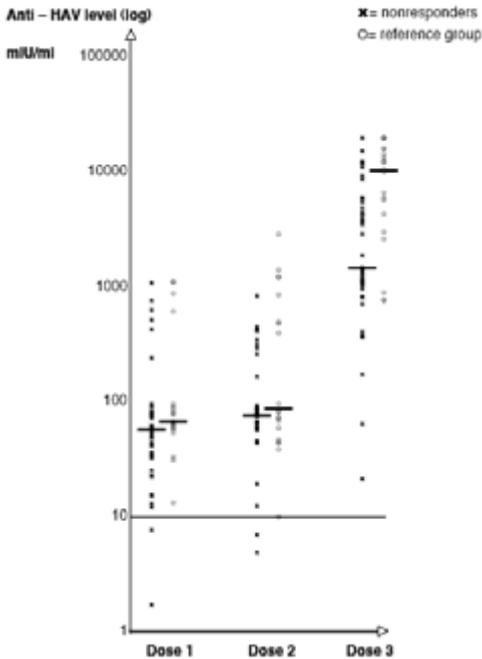


Figure 12. Anti-HAV titre after each dose with the median marked as a bold horizontal line.

In conclusion, the re-vaccination study showed that the combined hepatitis A and B vaccine was highly effective in inducing hepatitis B vaccine response in previous non-responders to at least four intradermal doses. This was also confirmed with respect to HBsAg-specific T cell responses where a majority of both the previous non-responders and the naïve individuals developed in vitro detectable proliferative and/or cytokine responses in peripheral blood. (Nyström J, Cardell K, Frydén A, Sällberg M. Vaccine 2008).<sup>193</sup> This strongly suggests that a true non-response to hepatitis B vaccine was very rare and there were no non-responders to the HAV part of this study. There was a correlation between the responses to HBsAg and HAV in both groups. At least some non-responders seem to be primed to the HBsAg since they showed a humoral response already after the first revaccination dose. A similar observation was made when analysing the T cell responses to HBsAg at the same time point.<sup>193</sup> We conclude that non-responders are most often slow-responders.

## ***Discussion***

There are two main reasons for choosing the intradermal route of administration. Firstly, it is less expensive and secondly, the skin is known to have a large number of antigen presenting cells which are thought to enhance the immune response to the vaccine. Our large clinical study concluded that intradermal recombinant hepatitis B vaccination can be safely administered and that response rates are satisfactory, at least in people aged 40 yrs or less and in non-smokers.

It is generally agreed that precise administration is essential and this requires well-trained nurses. In the clinical study (paper I), a high vaccine failure rate was observed in the first part of the study. Analysis revealed that the doses given were sometimes too small, implying that imprecise dosage is a risk associated with this route of administration. Filling the syringe to the 0.1 ml mark resulted in only 0.07 ml being administered to the vaccinee. It is necessary, therefore, to fill the syringe to 0.13 ml to ensure that the correct 0.1 ml dose is given. It is also important to make sure a blister is produced, to confirm that the vaccine has been given intradermally and not subcutaneously. Subcutaneous administration has been shown to have a much lower response rate.<sup>55</sup> The response rate increased from 51% to 81% after administration procedures were improved. No association could be found between response and any specific batches of vaccine.

The laboratory changed its method of measuring anti-HBs during the course of the study. This change was made as part of reorganisation of virology diagnostics following a directive from the Swedish National Board of Health and Welfare that aimed to put all virological testing under the supervision of a clinical virologist. The need for cost reductions and efficiency improvements also demanded greater automation. The two methods used in the study are validated and certified. The Organon Hepanostika anti-HBs test was, at the time, the leading test in Sweden. The Abbot IMX Ausab was validated by standard departmental procedures before the change in method was made. In addition to clinical validation, 99 samples from the study (paper I) were analysed and the results compared with those obtained by a reference method at the Swedish Institute for Infectious Disease Control, showing good agreement. In conclusion, the difference in response rate between the earlier and latter parts of the study could not be explained by the change in method of anti-HBs analysis.

It is important to measure the antibody level after completed intradermal vaccination to establish whether a response has been achieved or not. In this study, the anti-HBs titres were given within intervals and not as exact titres. However, other studies report that mean titres are generally lower after intradermal administration than after intramuscular administration. This is probably unimportant in the clinical setting as there is now international consensus that booster doses are not needed in healthy adults once an anti-HBs level of  $\geq 10$  mIU/mL has been achieved.

It is known that the anti-HBs level drops in the years after vaccination and that a third of all those vaccinated will have anti-HBs levels of <10 mIU/mL after 5 years. The time to titre <10 mIU/mL depends on the initial titre measured 1-2 months after the last dose.<sup>43, 58</sup> Until recently, there had been no reported case of acute symptomatic hepatitis B following successful vaccination that had produced anti-HBs titres >10 mIU/mL, but now there is a case report of where a patient developed acute symptomatic hepatitis B despite previous successful vaccination has been published.<sup>194</sup> The authors of this case report concluded that the infection was due to waning of the vaccine-induced immune response. Millions of individuals have however been vaccinated with excellent protection and the recommendations will certainly not change after a single reported case.

No major adverse effects were reported to the nurses during the study. Serious adverse effects would almost certainly have come to our attention. The main adverse effect noticed was a local reaction. Over 2 000 individuals started the vaccination schedule but 447 did not complete it. Many of those who did not receive all the doses probably failed to do so because they changed their employment or moved out of the area, although we have no definite proof that this was the case. Vaccinations given during the study were funded by the employer. It has not been possible to further characterise those who failed to complete the full course of vaccination but there is nothing to suggest a systematic drop out. Studies of hepatitis B vaccination in health care workers have also shown that they lack awareness of the benefits of vaccination and that there is need to promote the benefits and encourage vaccination.<sup>195</sup>

Intradermal vaccination is not recommended by the manufacturers but according to our study and others it could be used safely. A recent meta-analysis of intradermal hepatitis B vaccination suggested that it should be preferentially used in groups known to have good response rates, such as women and children. Administration of the vaccine by this route requires a certain level of skill and attempts are currently being made to develop intradermal administration systems that are easier and safer to use. This could make it easier to widen the use of this route of administration which was shown to be cost-effective in the context of mass vaccination at the time the study was carried out. Recalculation of the cost figures in 2008 confirmed these cost benefits, although the cost differential had diminished since 1998, mainly because of the rising costs of serological testing. In 1998 the costs of the intradermal schedule were estimated to be 27% of the intramuscular schedule. The same figure for 2008 was 34% and even after taking into account the lower initial response rate (80%), the intradermal route remains more cost-effective. This is also true even if a fourth dose is not given to non-responders in the intramuscular group. The cost benefit of intradermal administration followed by serological testing and administration of additional doses (intradermal or intramuscular) has been shown by another Swedish study.<sup>196</sup>

Smoking is strongly associated with a poor response rate to hepatitis B vaccine as shown by this study and others.<sup>71, 75, 80</sup> The exact reason for this is not fully understood. The immunoglobulins are reported to be lower in smokers than in non-smokers.<sup>197, 198</sup> Smoking is known to impair dendritic cell function.<sup>199, 200</sup> Nicotine has been found to reduce the antigen

uptake by the dendritic cells and it also reduces the capacity of naïve T cells to differentiate into T<sub>h</sub>1 cells. Proliferation of T cells after non-specific stimulation is impaired in smokers.<sup>201</sup> However, the impaired immune response to hepatitis B vaccine in smokers may not be part of a general poor response to vaccines since other vaccines produces the same responses in smokers as well as in non-smokers. Influenza vaccination has also been studied intensively, and no difference in antibody response is seen in smokers and non-smokers.<sup>84</sup>

In our studies, we did not find any gender differences in response rates. A difference has been found in other studies which suggest that men have lower response rates than women.<sup>71</sup> This difference seems to be more pronounced when the intradermal route is used. One author has suggested that the lower response in men<sup>89</sup> is related to their higher body weight but this has not been confirmed by other studies. The explanation in our study could not be due to the number of participating men though this is dealt with in the statistical analysis.

The importance of age in vaccine response has been shown for several vaccines, including hepatitis B. This age effect is not absolute and this negative influence on vaccine response is apparent from as early as 30 years of age, according to some authors.<sup>88</sup> In our intradermal vaccination study, we found a correlation between age and response rate in subjects aged over 40 years. Theoretically this age effect could be due to a decrease in lymphocyte proliferation activity as has been suggested by some authors. In one study comparing intramuscular administration of recombinant vaccine in elderly subjects above 59 years of age the only statistical factor associated with non-response was use of any medication at the time of vaccination.<sup>202</sup> The authors' conclusion was that this finding was a part of general health problem and a reduced immune response followed by that.

We did not find that Body Mass Index (BMI) affected vaccination results. Others have shown that BMI, especially above 30, is associated with a lower response rate. Few subjects in our study had a BMI greater than 30 and this low number could explain why we were unable to demonstrate any effect. In paper I, our statistical analysis used the WHO classification of BMI that was applicable at the time which defined overweight differently in men and women (>25 for men and >24 for women). It is unlikely that the use of the current BMI classification, where the definition of overweight is the same for men and women (>25), would have changed the result.

Our lifestyle questionnaire did not contain questions about alcohol consumption. Previously published studies suggest that alcoholics have a lower response to hepatitis B vaccination and that this difference cannot be attributed to other factors e.g. compliance. The reason for the low response rate in this group of patients remains unclear. People with liver cirrhosis also have a lower response rate and this complication of alcohol abuse is a possible explanation for the association between low response and high alcohol consumption seen in some, but not all, studies. It is not known whether low or moderate alcohol consumption affects the response rate as no adequate studies have been carried out.

In addition to the properties of the actual vaccine, the route of administration, the dose used and other environmental factors such as the lifestyle factors mentioned above, genetics play a significant role in the immune response to hepatitis vaccination. In our study of relatives of hepatitis B vaccine non-responders, the response rate of 58%, (paper III) was lower than that seen in a non-select group 88.9% (paper I). Extensive research has failed to identify the precise genes involved or clarify how they affect the response. There is clearly a relation to HLA class II antigen because certain haplotypes predominate in responders and others in non-responders. From the study on the genetic influence on vaccine response including responders and non-responders recruited from the study described in paper I, haplotypes for HLA class II antigen were determined for 122 individuals, 53 non-responders and 69 responders. A correlation between HLA [DQB1\*0604; DQA1\*0102; DRB1\*1302] and non-response ( $p < 0.005$ ) and HLA [DQB1\*0602; DQA1\*0102; DRB1\*15] and HLA [DQB1\*0603; DQA1\*0103; DRB1\*1301] and response ( $p < 0.025$  and  $p < 0.05$  respectively) were seen. DQA1\*0102 is present in both responders and non-responders and it is therefore unlikely to be of importance for vaccine response (paper II). For the allele DRB1\*1301 and DRB1\*1302, the only difference in the amino acids is in position 86, (valine in DRB1\*1301 and glycine in DRB1\*1302). The amino acid in position 86 is located in the peptide-binding groove of the molecule.<sup>203</sup> For DR 15, which is correlated in the this study as well as in others to vaccine response,<sup>165, 204, 205</sup> the amino acid in this position is valine. The theory of the importance of the particular amino acid in position 86 is supported by the fact that the two responder alleles DQB1\*0602 and DQB1\*0603 differ from the non-responder allele DQB1\*0604 in this amino acid.<sup>203</sup> In the family study the haplotype HLA [DQB1\*0604; DQA1\*0102; DRB1\*1302] was seen in 5 out of 6 non-responders and this is consistent with the published data on non-responders (paper III). However, no statistical correlation between the amino acid in position 86 of the DRB1 and vaccine response could be demonstrated. This study is though to be considered as a pilot study and the sample size is too small to answer this question. Thus, it does not rule out that this specific amino acid could play a role in the vaccine response. Although small, this study confirms that the peptide binding involved in vaccine response is not simply related to a certain gene or haplotype. Several epitopes of the HBsAg can bind to the receptor and the binding capacity is not reliant on one single gene. Some data support the theory that the reduced capacity to produce enough amount of anti-HBs is not due to defect antigen presentation nor antigen binding but a defect in T cell reactivity.<sup>162, 206, 207</sup> Sixteen haplotypes were equal in both responders and non-responders. Our findings suggest and support results from other studies that there are genes both within and outside the MHC locus involved in the immune response to hepatitis B vaccine.<sup>105, 208</sup>

Non-response to hepatitis B vaccine is not absolute in the vast majority of cases, but should rather be seen as degrees of responses. In the revaccination study, 95% of subjects (42/44) achieved an anti-HBs level above 10 mIU/mL. Importantly, after the first dose the non-responders had an anamnestic response with respect to both anti-HBs and HBsAg-specific T cells (paper IV and Nyström et al).<sup>193</sup> This supports that the first course of vaccination failed to effectively prime these responses in the non-responders but after an improved immunization schedule also these responded well. Mean titres in previous non-responders

were however, lower than in vaccine-naïve subjects. This suggests that unfavourable factors, including inheritance and lifestyle factors can be overcome by an increase in dose and by changing the initial route of administration.

Adding adjuvants has been tested in several new vaccines, some of which include Pre-S1 and Pre-S2, and there have been reports of increased response rates in non-responders. We added another antigen, inactivated hepatitis A virus, and this may have acted as an adjuvant. This could therefore be a way of improving hepatitis B vaccine response. However we cannot conclude from this study whether the inclusion of the HAV antigen the increasing dose of hepatitis B vaccine alone, or both contributed to the beneficial effect.

A clear observation from our study is that non-responders and low-responders to hepatitis B vaccination also have a lower response to hepatitis A vaccine than high responders to hepatitis B vaccination. A correlation between the titres of the two antigens has been observed in healthy vaccinees in a few studies but investigations in non-responders have not, to our knowledge, been carried out systematically. The correlation between the titers could be due to a low response to antigens in general in these non-responders, although in earlier studies on other antigens such as tetanus toxoid, non-responders and responders to hepatitis B vaccine had similar responses to tetanus toxoid antigen. Hepatitis B vaccine response could therefore be a marker of immune response in general. Another alternative may be that the clearly impaired response to HBsAg in the non-responders impairs the response to HAV when the antigens are administered at the same site. This hypothesis could be tested by comparing the anti-HAV responses in responders and non-responders using the monovalent HAV vaccine.

As regards the cell-mediated immune response we have reported that 9% (3/33) of the previous non-responders in the revaccination study, proliferated in peripheral blood mononuclear cell (PBMC) stimulated with HBsAg or had  $\text{ifn-}\gamma$  production before the first revaccination dose was given which was not seen in any individual in the reference group consisted of vaccine naïve patients.<sup>193</sup> This indicates that, at least in some cases, protection against hepatitis B after vaccination could exist in the absence of an anti-HBs level of  $> 10$  mIU/mL. This is further supported by the finding that 60% of earlier non-responders responded with anti-HBs production to one double dose of the combined hepatitis A and B vaccine. In the study mentioned above by our group of the cell-mediated immune response there was an increase in PBMC proliferation in both groups over time but there was no difference between the groups. There was a weak association between anti-HBs levels 1-2 months after completion of the revaccination schedule and the magnitude of HBsAg induced PBMC proliferation in all participants but this did not differ between the groups. Furthermore analysis of the cytokines induced by HBsAg stimulation in this study did not clarify whether  $T_h 1$  or  $T_h 2$  stimulation was predominant.<sup>193</sup>

In this revaccination study, two subjects were unable to produce a humoral response with anti-HBs  $\geq 10$  mIU/mL and they both had received a total of four intradermal doses of 0.1 ml and three doses of combined hepatitis A and B vaccine at a double dose i.e. 2.0 ml. One of these

subjects had undetectable anti-HBs indicating that true non-response to hepatitis B vaccine occurs but is rare. The other subject had an anti-HBs titre of 8 mIU/mL after the revaccination study and is therefore defined as non-responder but has a weak response to the antigen. Whether or not these individuals could be helped by another vaccine that includes Pre-S1 and or Pre-S2 as well, or by one of the new DNA vaccines, cannot be established without further investigations.

## ***Conclusion***

Hepatitis B vaccination is safe and can be administered intradermally with adequate response rates in healthy adults, but the intradermal administration route is technically difficult and can only be recommended in centres with suitably trained nurses.

Many factors can influence the response rate to hepatitis B vaccine. Smoking is strongly associated with non-response, and response rates fall with increasing tobacco consumption. Vaccine response also seems to deteriorate with increasing age.

It is essential that anti-HBs levels are analysed following the completed vaccination schedule.

Intradermal administration is more cost-effective than intramuscular administration in the context of mass vaccination, and is especially effective in young people and non-smokers.

Genetic factors are of importance in the immune response to hepatitis B vaccine. Different HLA class II haplotypes are linked to vaccine response where two identified haplotypes were found to be correlated to response and one such haplotype was correlated to non-response. However this is an incomplete genetic explanation as genes outside the MHC region also seem to play an important role. No certain conclusion of the role of the amino acid in position 86 of the DRB1 allele can be drawn from these studies.

Revaccination with a high dose HBsAg with an additional antigen (hepatitis A) induces adequate hepatitis B seroconversion in the vast majority of cases. True non-response seems to be very rare. Most non-responders are thus in fact rather slow-responders.

Some non-responders seem to have a cell-mediated immune reaction as demonstrated by the anamnestic response, and it is suggested that these individuals could be at least partially protected against hepatitis B infection despite anti-HBs levels remaining at <10 mIU/mL.

Non-responders and low-responders to hepatitis B vaccine have a poorer response to added hepatitis A virus antigen, suggesting that there could be a more universal dysfunction underlying the poor immune response to vaccines and other antigens.

## *Sammanfattning på svenska*

Infektion med hepatit B virus orsakar leversjukdom och drabbar många människor globalt. Man har uppskattat att 30-50% av världens befolkning någon gång haft hepatit B och att mer än 350 miljoner människor är kroniska bärare av sjukdomen. Hepatit B smittar via blod, sexuella kontakter och från mor till barn i samband med förlossningen. Kronisk hepatit B infektion är en riskfaktor för utvecklande av skrumplever och levercancer. Behandling av hepatit B finns sedan ca 20 år tillbaka men är besvärlig och inte alltid verksamt. Förebyggande av hepatit B är därför mycket viktigt. Sedan början av 1980-talet finns vaccin mot hepatit B. Detta vaccin har visat sig vara säkert när det gäller att förebygga hepatit B och har mycket få biverkningar. WHO har rekommenderat alla länder att införa hepatit B vaccination i det allmänna barnvaccinationsprogrammet. I Sverige har man hittills valt att inte göra detta utan i stället vaccinera riskgrupper. Sjukvårdspersonal som kan komma i kontakt med blod i sitt arbete utgör en sådan riskgrupp liksom anhöriga till personer med hepatit B. Emellertid svarar inte alla individer med skyddande nivåer av antikroppar efter vaccination. Vaccinet har utprovats för att ges i muskulaturen (intramuskulärt). För att reducera kostnader och för att undersöka vaccinsvaret har man även försökt ge vaccinet i huden (intradermalt).

I en stor studie där sjukvårdspersonal har vaccinerats i huden svarade 960/1406 (68%) med skyddande antikropps-nivåer efter tre doser och 1187/1335 (89%) efter tre eller fyra doser. Riskfaktorer för ett uteblivet vaccinationssvar var rökning och ålder över 40 år. Man såg också att andelen personer som svarade på vaccinationerna ökade under studiens gång. En risk att ge en för liten dos kunde identifieras när man använder sig av intradermal vaccination och att det är nödvändigt med välutbildad personal om man ska använda sig av detta vaccinationssätt. Även om vaccinationssvaret vid intradermal vaccination är något sämre än vid intramuskulär vaccination är detta administrationssätt kostnadseffektivt när många individer skall vaccineras mot hepatit B

Genetiska faktorer har inverkan på hur bra man svarar på hepatit B vaccin. Man har identifierat ett antal sådana genetiska faktorer bland HLA komplexet. Detta komplex kodar för proteiner som finns på cellers yta och har del i kroppens immunförsvar mot främmande ämnen. I en studie jämfördes personer som inte svarat på hepatit B-vaccination med dem som svarat bra. Man fann då i en del fall kopplingar mellan vissa så kallade HLA klass II-gener och vaccinationssvaret. I en uppföljande studie vaccinerades anhöriga till personer som tidigare inte svarat på hepatit B vaccin med känd uppsättning av vissa HLA-gener. Denna studie visade att 15/26 (58%) svarade på vaccination vilket är lägre än förväntat och tyder ytterligare på att genetiska faktorer spelar roll för vaccinationssvaret.

Personer som inte svarat på fyra intradermala doser hepatit B-vaccin (så kallade non-responders), vaccinerades intramuskulärt med ett kombinerat hepatit A- och B-vaccin i en hög dos vid tre tillfällen. Redan efter den första dosen svarade 26/44 (60%) med skyddande antikropps-nivåer jämfört med 2/20 (10%) i en grupp som tidigare aldrig vaccinerats mot

hepatit B. Detta talar för att de hade ett immunlogiskt minne mot vaccinet även om de inte lyckats producera skyddande antikroppar. Efter tre doser svarade 42/44 (95%) av tidigare non-responders och 20/20 (100%) av tidigare ovaccinerade. Alla i studien svarade på hepatit A delen av vaccinet. Man såg dock att de som tidigare inte svarat på hepatit B vaccin hade lägre antikropps nivåer även mot hepatit A. Det förelåg också en korrelation mellan antikropps nivåerna mot hepatit A och B efter vaccinationerna hos alla i studien.

Sammanfattningsvis visar studierna i denna avhandling att intradermal hepatit B vaccination kan användas på ett säkert sätt, att det är flera både ärftliga och omgivningsfaktorer som styr vaccinationssvaret hos en individ samt att de flesta kan fås att svara på vaccination med höga vaccindoser i kombination med hepatit A vaccin.

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