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## Review Article

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# A Delayed Type Hypersensitivity (DTH) Skin Reaction to Hepatitis B Surface Antigen (HBsAg) and Intradermal Hepatitis B Vaccination

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**Abstract** The significance of a delayed type hypersensitivity skin reaction to hepatitis B surface antigen (HBsAg) (HBs-DTH) in type B viral hepatitis (VHB) and in intradermal hepatitis B (HB) vaccination is reviewed. HBs-DTH could be developed by the intradermal injection of HB vaccine in anti-HBs positive people and also in persons immunized with HB vaccine. Thus, HBs-DTH could serve as a useful marker for the acquisition of an active Th1 type immunoreactivity to HBsAg. HBs-DTH was absent in patients with chronic VHB. In contrast, HBs-DTH developed early in the convalescent phase of the acute VHB, whereas the production of anti-HBs was significantly delayed, thus suggesting that HBs-DTH may be involved in the recovery mechanisms of acute VHB. Intradermal HB vaccination is useful not only in lowering the cost, but also in the rapid development of anti-HBs, reversing non-responsiveness, improving postexposure prophylaxis and in immunizing immunosuppressed people. A similar vaccination strategy should prove to be useful in prevention and control of not only other infectious diseases but also malignant neoplasms.

Human hepatitis B virus (HBV) infection causes an acute, chronic, and persistent infection in humans. Acute infection in infancy usually causes a subclinical infection, thus establishing a high probability of developing into a chronic carrier state, and those infected infants become a reservoir of HBV capable of transmitting the virus and also resulting in chronic liver disease. When the virus infects adults, they usually induce acute hepatitis with a subsequent complete recovery from the infection. Thereafter, 5–10 % of such individuals proceed to the chronic state and only a few

exceptional deaths occur due to fulminant hepatitis. In contrast, the chronic carrier state is often associated with chronic hepatitis, which later develops liver cirrhosis and / or hepatocarcinoma 15–30 years after the initial infection. There are approximately 350 million carriers of HBV in the world, and the estimated deaths, which number more than 1 million annually, are attributable to the serious sequelae of chronic HBV infection<sup>1)–4)</sup>. At present, it is still impossible to eradicate HBV after a chronic carrier state has been established<sup>1)–4)</sup>.

The pathogenesis of HBV persistence,

hepatitis, and the recovery mechanism are still not fully understood. Although the probability of developing a carrier state following HBV infection is the greatest in early life and thereafter diminishes with increasing age, the mechanisms of the immunologic tolerance has not been elucidated<sup>2)~4)</sup>. It has been postulated that a low antigenicity of HBsAg and weak Th1 type immunoreactivity of infants may explain the high tolerance induction rate<sup>4)~7)</sup>. At least, the long-lasting presence (more than 6 months) of HBsAg indicates viral persistence, while the presence of anti-HBs clearly demonstrates a past history of recovery from an HBV infection and / or resistance to an HB virus infection. The presence of HBeAg correlates well with either high infectivity or possible active hepatitis, while anti-HBe correlates with low viral replication and mild hepatitis<sup>2)~4)</sup>. Assessment of DNA polymerase activity and assay of the HBV-DNA level has been shown to clearly demonstrate the replication of HBV. Emergence of mutated HBV causes exceptional events in patients with anti-HBe, leading either to severe or fulminant hepatitis, and thus resulting in the high mortality of patients<sup>7)8)</sup>. In addition, the HBV genome was also detected long after the recovery from acute type B viral hepatitis and multiple mutations were observed<sup>9)10)</sup>.

Nevertheless, the sequential events leading to either the termination of HBV infection or the eradication of HBV remain to be elucidated. Indeed, cellular immune responses rather than humoral immunity are believed to play a major role in the recovery from cell-associated persistent viral infection<sup>2)~4)</sup>. Cytotoxic T cell responses and / or antibody dependent cell mediated cytotoxicity are known to play a critical role in killing the HBV infected

hepatocytes. In contrast, cytotoxic T cells directed against HBcAg mediate the degree of hepatocyte damage, which is associated with chronic active hepatitis, but is not able to contribute to resolve the HBV infection<sup>11)12)</sup>. Such complicated, sometimes controversial observations have thus resulted in the pathogenesis of HB viral hepatitis remaining obscure.

However, owing to recent advances in modern medicine, HBV infection is now preventable by vaccination. Recent programs in the HBV vaccination program has contributed not only to the prevention of mother to child transmission, but also helps prevent sexual transmission (related to both homosexual and heterosexual activity), and transmission among drug abusers, high risk adults including health-care workers, firemen, police officers, and accidental infection<sup>13)15)</sup>. Nevertheless, the high cost of the HB vaccine has limited its worldwide application, especially in the endemic areas of Southeast Asia, the western Pacific and Africa, where the routine vaccination of infants and children is considered to be imperative to avoid horizontal transmission<sup>13)14)</sup>. In addition, problems regarding the presence of non-responders, incomplete protection against accidental postexposure prophylaxis, the need for the rapid immunization of travellers to endemic areas, occupational high risk individuals, the poor immune response to HB vaccine in hemodialysis patients and persons infected with human immunodeficiency virus (HIV) all still remain to be solved.

The development of a clinically applicable HB vaccine, the first practical subunit vaccine, made it feasible to prevent HBV infection<sup>13)~15)</sup>. In addition, the intradermal application of the vaccine also provided a novel clinical clue to identifying cellular

immune responsiveness as an observable skin reaction of the delayed type hypersensitivity skin reaction to HBsAg (HBs-DTH)<sup>15)~17)</sup>. This reaction should be regarded as the clinically detectable Th1 type immunoreactivity at the effector level *in vivo*. Furthermore, HBs-DTH developed in the early convalescent phase of the acute type B viral hepatitis whereas the production of anti-HBs was significantly delayed, which thus suggests that this reaction may be involved in the recovery mechanism from acute type B viral hepatitis<sup>18)19)</sup>. In addition, an intradermal HB vaccination has been shown to be useful not only in the reduction of cost, but also in the rapid development of anti-HBs, the reversal of non-responders, improving postexposure prophylaxis, and in immunizing immunosuppressed people.<sup>20)~34)</sup>

We have made a review to show the significance of HBs-DTH in patients with type B acute and chronic hepatitis and also demonstrate the clinical usefulness of intradermal HB vaccination which was conducted with HBs-DTH as a marker to acquire positive Th1 type immunoreactivity to HBsAg.

#### **A Delayed Type Hypersensitivity Skin Reaction to HBsAg (HBs-DTH)**

A delayed type hypersensitivity skin reaction to HBsAg (HBs-DTH) was observed to develop in naturally anti-HBs positive individuals as the development of redness and/or an induration measuring more than 5mm in diameter 48 hours after being intradermally inoculated with the HB vaccine<sup>15)18)</sup>. The HBs-DTH could also be induced in individuals immunized with the HB vaccine intradermally<sup>16)17)</sup>. When HB naive people were immunized with HB vaccine intradermally every two weeks until HBs-DTH

developed, they develop HBs-DTH at  $4.8 \pm 1.0$  weeks, and anti-HBs was produced at  $8.4 \pm 0.9$  weeks post vaccination, thus suggesting that the development of HBs-DTH was a convincing marker for the later production of anti-HBs<sup>18)</sup>. The development of a reaction was dose dependent and more than  $5\mu\text{g}$  of plasma derived HBsAg was required to achieve a reliable and stable positive reaction<sup>19)</sup>. This dose was relatively larger than the dose used in previous studies using an intradermal clinical application of the HB vaccine. A similar degree of dose dependent DTH skin reactivity was also observed in recombinant yeast derived HBsAg. Presence of immunopotentiator alum contained in the HB vaccine preparation, did not enhance the mean-diameter of skin reactivity<sup>19)</sup>, however, the alum did enhance the development of induration. A histological examination of the reactive area obtained by a biopsy revealed the presence of mononuclear cell infiltrations around the injected vaccine, while a few histiocytic infiltrations were also observed. An immunohistochemical study revealed that the infiltrated mononuclear cells were predominantly CD4 positive T cells while only a few CD8 positive cells were admixed<sup>19)</sup>. CD20 positive B lymphocytes were scarcely seen<sup>19)</sup>. These histological observations were consistent with delayed type hypersensitivity. Although a skin reaction to HBsAg has heretofore been regarded as an adverse reaction following intradermal HB vaccination<sup>22)~41)</sup>, this reaction is considered to be clinically useful in identifying positive immunoreactivity to HBsAg. It should be noted, however, that people with an allergic background might also develop skin reactions which can not be discriminated by this reaction<sup>15)</sup>. Therefore, when people have

an allergic background, the skin reactivity to HBsAg must be analyzed with even more care. Consequently, the overall specificity of HBs-DTH in HB immune individuals was 96.0 % and sensitivity was 94.3 %, thus indicating that HBs-DTH could be a useful and reliable marker for the acquisition of positive cellular immunity to HBsAg<sup>16)~18)</sup>.

#### **A Delayed Type Hypersensitivity Skin Reaction to HBsAg in Type B Viral Hepatitis**

A delayed type hypersensitivity skin reaction to HBsAg was not observed in type B chronic hepatitis or carriers who were positive for HBsAg irrespective of the presence of HBeAg or anti-HBe, thus indicating the immunologic tolerance to HBsAg<sup>18)</sup>. In contrast, in patients with acute type B viral hepatitis, HBs-DTH developed early in the convalescent phase of the disease along with the disappearance of HBsAg<sup>18)19)</sup>. Interestingly, in patients with type B acute viral hepatitis HBs-DTH developed as early as age- and sex- matched controls who received the HB vaccine intradermally in the same regimen<sup>19)</sup>. In contrast, the development of anti-HBs was significantly delayed in patients with acute type B viral hepatitis compared with the vaccinated controls<sup>19)</sup>. This finding clearly demonstrates that an active suppression exists for the production of anti-HBs in patients with acute type B viral hepatitis in the convalescent phase of the disease. This may be due to the Th1 dominant immunoreactivity to HBsAg in patients with type B acute viral hepatitis who successfully recovered from the infection. These observations also provide an immunologic clue for the eradication of HB virus in patients with type B viral hepatitis.

#### **Intradermal Hepatitis B Vaccination**

Although the effectiveness of intradermal vaccination has been extensively studied, there is disagreement regarding the usefulness of intradermal HB vaccination. Intradermal HB vaccination has been reported to be as good as intramuscular HB vaccination while it costs substantially less<sup>22)~34)</sup>. However, other reports have indicated a lower anti-HBs level, a shorter anti-HBs persistence, a lower effectiveness than intramuscular vaccination, and a lower response rate in males and older people<sup>35)~41)</sup>. These controversial results from previous studies may possibly be due to either the vaccine preparation, the vaccine dose, or technical problems; successful intradermal HB vaccination is difficult because of the appropriate injection technique required; i.e., an unsuccessful intradermal HB vaccination causes the injection of HB vaccine into a subcutaneous area where the vaccination effect is even lower than in the intramuscular route. In addition, an unsuccessful intradermal injection may result in the loss of the vaccine from the inoculated area. Moreover, previous studies have shown a lower effectiveness when using one tenth the dose of the HB vaccine used in intramuscular vaccination<sup>35)~41)</sup>. Regarding the dose dependence of the development of HBs-DTH, it was shown that 5 $\mu$ g was required which is one fourth of standard vaccine dose<sup>17)</sup>. Therefore, the one tenth dose of the HB vaccine may be too low to achieve a satisfactory immune response to HBsAg. To determine the cost effectiveness of intradermal HB vaccination, further studies to assess the appropriate vaccine dose, cost, and anti-HBs persistence are required in a mass study. At least, when the relatively larger

dose is used in the intradermal vaccination, both a good anti-HBs response and anti-HBs persistence could be achieved in a Japanese study<sup>30)31)</sup>. When the appropriate vaccine dose is used, intradermal vaccination is superior in cost-effectiveness, and should be considered for worldwide use, especially in developing countries where HBV is endemic and financial problems play an important role in medical decisions.

### Specific Usefulness of Intradermal HB vaccination

Intradermal HB vaccination is useful not only in increasing cost effectiveness but also in reversing either a low response rate or non-responsiveness to HBsAg, while it also allows for rapid immunization and postexposure prophylaxis, and is better for patients receiving hemodialysis<sup>20)21)42)</sup> and patients receiving renal transplants<sup>43)</sup>. The most significant advantage of intradermal HB vaccination is the recognition of the development of DTH skin reaction to HBsAg as a useful marker for the acquisition of positive immune responsiveness to HBsAg. In order to reverse the rate of non-responsiveness, intradermal HB vaccinations were done every two weeks until HBs-DTH developed. A mean of 2.3 additional vaccinations were required, thus resulting in a reversal rate of up to 94 %<sup>22)</sup>. One year later, anti-HBs was positive in 74 %, which thus suggested the persistence of anti-HBs to be good<sup>21)</sup>. This rapid vaccination protocol could also be effectively applied to patients on hemodialysis who are high risk patients and known to be poor responders to the vaccine<sup>42)</sup>. This regimen is also useful in people who are exposed to highly contagious HBeAg positive body fluids and/or blood and those who have received hyperimmune HB globulin<sup>21)</sup>. In

people who were exposed to HBeAg positive specimens, even after the transfer of hepatitis B immunoglobulin<sup>20)</sup>, to 60 % acquired hepatitis B virus infection and 6 % developed clinical hepatitis<sup>44)45)</sup>. Therefore, a combination of passive and active immunization was attempted with an improved vaccination effect, however, complete protection was not achieved<sup>46)~48)</sup>. In contrast, the use of intradermal vaccination in combination with passive anti-HBs globulin transfer, complete protection was provided<sup>21)</sup>. Fifteen persons who had all been accidentally exposed to specimens positive for HBeAg received anti-HBs globulin, and also intradermal vaccinations every two weeks until HBs-DTH developed. In the presence of passively transferred anti-HBs, initial intradermal HB vaccination did not induce DTH skin reactivity 48 hours after inoculation, since they were not actively immune to HBsAg. However, after an average of 3.1 vaccinations, they developed HBs-DTH, thus indicating the development of positive immunoreactivity to HBsAg<sup>21)</sup>. When the HBs-DTH developed, they were relieved to know that they had successfully acquired immunity to the virus, and such individuals were thus able to evade from HB virus infection. By this regimen, the protection was complete and no acute type B viral hepatitis nor HB virus infection occurred<sup>21)</sup>.

The immunological mechanisms of effective intradermal vaccination still remain to be elucidated especially regarding the reversal of non-responders. The immunoglobulin subclass of the anti-HBsAg antibodies produced in nonresponders was mainly IgG1, which was the same that observed in individuals who were naturally positive for anti-HBs as well as in individuals who were positive for anti-HBs after vaccination<sup>21)</sup>,

thus suggesting that no alternate immunoglobulin pathway was activated in nonresponders who received intradermal revaccinations. In addition, since many Langerhans cells, which are very able antigen-presenting cells, are present in the epidermal area and because antigen remains for a long time in the epidermis during intradermal immunization, pertinent antigen presentation and/or antigen persistence may therefore stimulate the immune system to produce anti-HBs effectively.

#### **Adverse Reactions of Intradermal Hepatitis B Vaccination**

Since the intradermal vaccination protocol involved the skin reactions as a marker for the development of HBs-DTH, all individuals who developed active immunity had skin reactions with redness and indurations sometimes associated with itching. The skin reactions remain for weeks or months, but thereafter subsided<sup>21)23)28)</sup>. In intramuscular immunization, the development of acute posterior multifocal placid pigment epitheliopathy (APMPPE) and eosinophilia has been reported following intramuscular booster immunization<sup>49)</sup>. Although eosinophilia gradually decreased, the APMPPE improved while the sequelae persisted<sup>49)</sup>. In the intradermal vaccination regimen, systemic adverse reactions were rare, however, there was one case in which eosinophilia developed with fever and general weakness<sup>50)</sup>. Fortunately, after removing the vaccinated skin area by a biopsy, the eosinophilia disappeared<sup>50)</sup>. Thus, intradermal vaccination has another advantage; i.e., when an unexpected adverse reaction does occur, the causal antigen can be easily removed by a biopsy.

#### **Conclusions**

When 5  $\mu$ g of HB vaccine, which was approximately one fourth dose of standard subcutaneous or intramuscular vaccination, was injected intradermally, a delayed type hypersensitivity skin reaction to hepatitis B surface antigen (HBsAg) (HBs-DTH) could be developed in anti-HBs positive people and also in persons successfully immunized with HB vaccine. Thus, HBs-DTH could serve as a useful marker for the acquisition of an active cellular immunity to HBsAg, which could be also regarded as the clinically detectable Th1 type immunoreactivity to HBsAg at the effector level *in vivo*. HBs-DTH was absent in patients with type B chronic hepatitis and carriers. In contrast, HBs-DTH developed early in the convalescent phase of the acute type B viral hepatitis, whereas the production of anti-HBs was significantly delayed, thus suggesting that HBs-DTH may be involved in the recovery mechanisms from type B acute viral hepatitis. In addition, intradermal HB vaccination is useful not only in lowering the cost, but also in the rapid development of anti-HBs, reversing non-responsiveness, improving postexposure prophylaxis and in immunizing immunosuppressed people. In the future, a similar vaccination strategy should also prove to be useful in the prevention and control of not only other infectious diseases but also malignant neoplasms<sup>51)~55)</sup>.

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