

*Rapid communications***Reduced protection against hepatitis B virus following vaccination in patients with Type 1 (insulin-dependent) diabetes**

P. Pozzilli<sup>1, 4</sup>, P. Arduini<sup>1</sup>, N. Visalli<sup>1</sup>, J. Sutherland<sup>1</sup>, M. Pezzella<sup>2</sup>, C. Galli<sup>2</sup>, S. G. Corradini<sup>2</sup>, L. Biasio<sup>3</sup>, E. A. M. Gale<sup>4</sup> and D. Andreani<sup>1</sup>

<sup>1</sup> Endocrinologia (I) and <sup>2</sup> Istituto Malattie Infettive, University of Rome "La Sapienza" and <sup>3</sup> Institute Merieux, Rome, Italy;

<sup>4</sup> Department of Diabetes and Immunogenetics, St Bartholomew's Hospital, London, UK

**Summary.** Twenty patients with well controlled Type 1 (insulin-dependent) diabetes of at least 10 years duration and 47 control subjects were vaccinated against the hepatitis B virus using the Hevac B vaccine. The vaccine was administered into the deltoid region on three occasions at intervals of 1 month. Thereafter a fourth dose was given to subjects still negative for antibody to hepatitis B surface antigen (HbsAb). The median rise of HbsAb titres was 230 mIU/ml in normal subjects and 50 mIU/ml in diabetic patients ( $p < 0.001$ ). Eight patients (40%) failed to reach HbsAb titres above 30 mIU/ml, the level considered to give optimal protection against the infection, whereas only one normal control subject failed to reach this level. Five patients (25%) showed no

response despite a fourth dose of the vaccine. There was an increased frequency of HLA-DR7 in low responders and a decreased ( $< 1.5$ ) helper/suppressor lymphocyte ratio. Diabetic patients are thus less likely to mount a protective antibody response following vaccination against hepatitis. Since hepatitis B surface antigen is reported to be considerably more common in diabetic patients than control subjects, infection with hepatitis B virus may have a greater risk of chronicity in diabetes.

**Key words:** Immune response, B-hepatitis, Type 1 (insulin-dependent) diabetes, vaccination.

There is increasing evidence that multiple immunological abnormalities occur in patients with Type 1 (insulin-dependent) diabetes of long duration [1]. The number of circulating helper (CD4) cells is reduced [2], the helper/suppressor (CD4/CD8) lymphocyte ratio is decreased [3, 4], phagocytic activity is impaired [5] and lymphocyte blastogenesis is reduced [6]. These are amongst the most consistent alterations reported in vitro and may well account for the greater susceptibility to infection in patients with poor metabolic control [7].

Antigenic challenges in vivo using the trivalent influenza vaccine have shown normal antibody titres [8] or slightly impaired response in patients with high levels of glycosylated haemoglobin [9]; furthermore, an intradermal test using various antigens to evaluate the delayed hypersensitivity response was normal in the majority of diabetic patients [10]. In vivo studies of the immune response may therefore give a better indication as to whether secondary immunodeficiency is a feature of patients with diabetes of long duration. The response to a viral infection may be reduced and it is worth noting that hepatitis B surface antigen (HbsAg) has been detected to be 11-fold more frequent in diabetic patients than normal subjects [11] and a greater

frequency of B hepatitis has also been reported [12]. It is estimated that 10% of subjects positive for HbsAg may evolve to chronic hepatitis [13].

In light of these observations diabetic patients may be considered at increased risk if they come in contact with the hepatitis B virus and vaccination should be considered. We report the data obtained following the administration of the hepatitis B vaccine in patients with Type 1 diabetes of long duration.

**Subjects and methods**

Twenty patients with Type 1 diabetes (12 males and 8 females, mean age  $34 \pm 13$ ) with a mean duration of diabetes of  $13 \pm 3$  years were investigated. Forty-seven normal hospital personnel (28 males and 19 females, mean age  $32 \pm 10$ ) were included as age- and sex-matched control subjects.

Criteria for vaccination were as follows: (a) insulin dependency of at least 10 years; (b) negativity for HbsAg, HbsAb and HbcAb; (c) absence of proliferative retinopathy and other severe diabetic complications; (d) absence of any active infection at the time of entry; (e) HbA<sub>1c</sub> less than 9% (normal range 4–7%). Subjects were vaccinated with the Hevac B vaccine (Pasteur Vaccins, Paris, France), which is prepared from plasmas of healthy asymptomatic carriers negative for HBe antigen and for anti-HIV antibodies. Its manufac-

turing process ensures a highly purified and inactivated HBsAg preparation. The vaccine (5 µg in 1 ml solution) was injected subcutaneously into the deltoid region on three occasions at intervals of 1 month. If subjects were still negative for anti-HBsAg (HbsAb) after the third injection, a fourth injection was given 3 months later.

In diabetic patients before and after the third dose of vaccine, an *in vivo* evaluation of cell mediated immunity was performed by using the Multitest, a device by which simultaneously different antigens (streptococcus, tetanus, diphtheria, proteus, candida, trichophyton and glycerine as control) are administered subcutaneously in the forearm; the immune reaction is measured at 48 h and a mean score calculated [10].

Venous blood samples were collected before and after the third dose of vaccine for measurement of HbsAb titres using a commercially available radioimmunoassay kit (Abbott). The following measurements were also made in diabetic patients: (a) blood glucose, urea, creatinine, total protein, transaminase, bilirubin and HbA<sub>1c</sub>. (b) lymphocyte phenotypes using monoclonal antibodies defining total T (CD3), helper (CD4), suppressor/cytotoxic (CD8) lymphocytes and activated cells with receptors for interleukin-2 (anti-TAC). (c) HLA phenotyping, including the DR-DQ specificities.

## Results

No side effects were recorded in either group following the three doses of the Hevac B vaccine; biochemical parameters did not change thereafter in the control group (data not shown). Normal subjects showed a median titre of HbsAb of 230 mIU/ml after the third injection; only 3 of them (6%) did not respond after the third dose and required a fourth injection which

then induced a normal HbsAb titre. According to WHO criteria, HbsAb titres between 10 and 30 mIU/ml, even if low, may still induce protection from B hepatitis infection; in our control group optimal protection (>30 mIU/ml) was obtained in 98% of subjects.

Data of the diabetic patients are shown in Table 1. A low median HbsAb titre (50 mIU/ml) and a reduced CD4/CD8 lymphocyte ratio (the latter is a frequent feature in diabetic patients with disease of long duration) were the most striking features despite an apparently normal generation of TAC positive cells. Patients were well controlled throughout the period of investigation (mean HbA<sub>1c</sub> was 7%) and the Multitest was normal (a reduced score following a specific challenge is expected even in normal subjects). In total only 12 patients (60%) developed HbsAb titres above 30 mIU/ml; out of 8 patients (40%) with titres below 30 mIU/ml, 5 (25%) did not show any response after the third injection of vaccine and therefore received a fourth dose; even so, the HbsAb titre was still undetectable. The characteristics of these 5 subjects are shown in Table 2. These patients did not differ much in terms of their immune response from those who responded to the virus, except that activated T cells appeared not to be generated following vaccination; three of the 5 non-responsive patients were HLA DR7 positive versus 2 in the other 15 patients, and this phenotype is known to be associated with low HbsAb titre following B hepatitis vaccination [14].

**Table 1.** Laboratory data of patients with Type 1 (insulin-dependent) diabetes following vaccination with Hevac B vaccine against the hepatitis B virus (values are mean ± SD)

	Before vaccination	After the 3rd dose
%CD4+ve cells <sup>a</sup>	40 ± 4	37 ± 6
%CD8+ve cells <sup>a</sup>	25 ± 4	25 ± 4
CD4/CD8 ratio <sup>a</sup>	1.6 ± 0.4	1.5 ± 0.4
%TAC+ve cells <sup>a</sup>	1.5 ± 3	3.1 ± 2
Multitest (score) <sup>a</sup>	8.3 ± 8	4.1 ± 3.5
%HbA <sub>1c</sub>	7.2 ± 1.4	6.9 ± 1.3
HbsAb titre (mIU/ml) (median)	-	50.4

Median HbsAb in control subjects: 230 mIU/ml ( $p < 0.001$  Mann Whitney U test). <sup>a</sup> Normal value in control population: CD4=40-50%; CD8=20-30%; CD4/CD8 ratio=2-2.5; TAC=1-4; Multitest=7-10

## Discussion

We found that at least 40% of patients with well controlled Type 1 diabetes of long duration had a reduced immune response to hepatitis B virus following vaccination, implying inadequate protection against infection with the virus. The type and route of administration of the vaccine gave optimal HbsAb titres in the control group, suggesting that the deltoid region (and not the gluteal region in which a high prevalence of low responses has been observed [15]) should be the site of preference for the administration of B hepatitis vaccine.

**Table 2.** Individual data (pre- and post-vaccination) of 5 patients with no generation of HbsAb titres despite a fourth dose of Hevac B vaccine. (NA = data not available)

	DR-3/7 DQ-W2/W3		DR-3/7 DQ-W2		DR-5/7 DQ-W2/W3		DR-5/6 DQ-W1/W3		DR-4/6 DQ-W1/W2	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
%CD4	33	41	40	43	36	30	37	39	40	29
%CD8	30	30	NA	21	32	29	24	NA	29	22
ratio	1.1	1.4	NA	2	1.1	1.0	1.5	NA	1.4	1.3
%TAC	9	0	4	1.9	0	2.0	0	0	0	0
%HbA <sub>1c</sub>	6.3	6.5	7.0	7.7	5.1	5.3	7.5	8.7	7.5	7.3
Multitest	8	6	7	6	9	4	5	4	6	NA

Genetic factors may be involved but it is likely that immunological disturbance is mainly responsible for the impaired HbsAb titre observed in patients with Type 1 diabetes. An increased prevalence of HLA-DR7 in low responders and of HLA-DR3 in high responders to the B hepatitis virus has been reported in normal individuals [14]; it seems surprising that patients with Type 1 diabetes did not show higher HbsAb titres (the highest HbsAb titre observed was 170 mIU/ml compared to 850 mIU/ml in the control group) since this disease is associated with HLA-DR3. However we found HLA-DR7 to be present in 3 of 5 patients who had no response at all to the vaccine despite a fourth dose and this may explain the lack of HbsAb generation.

The immunological status of the patients, in particular an impaired CD4/CD8 lymphocyte ratio, may have contributed to the low HbsAb titres observed. The lymphocyte ratio, despite its poor specificity, is a simple and widely used test for evaluation of the cell mediated immune response [16] and is reported to be reduced in patients with a long duration of disease [3, 4]. Immunoregulatory lymphocyte subsets have a major role in the induction of a primary immune response (as in the case of the B hepatitis vaccine) and any abnormality of these cells in number and/or function may result in impaired generation of a specific cell mediated and humoral immune response. In the case of a secondary immune response (as might be the case with vaccination with influenza virus or a multiple antigenic challenge like the Multitest) a memory T cell is responsible for the expansion of a clone of sensitised lymphocytes and this process does not require, as does the primary response, a normal CD4/CD8 lymphocyte ratio [17]. This may explain our finding that in diabetic patients a normal antibody titre is generated following the influenza vaccine [9] but not the B hepatitis vaccine. Poor metabolic control may cause impaired immune responses in diabetic patients [18]; but this was not the case in our study since all patients were well controlled throughout the period of follow-up, suggesting that the primary defect is immunological.

In conclusion, previous observations that patients with Type 1 diabetes are more likely to be carriers of HbsAg [11] and have increased frequency of B hepatitis [12] and that low HbsAb titres are observed following vaccination with the hepatitis B virus, suggest that there may be a greater risk of chronic infection in these patients. Although there is currently no data to suggest this, adequate protection might be achieved by administering repeated doses of vaccine at 1 year after the first dose in addition to immunostimulatory drugs; studies in this direction are in progress in our hospital.

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Dr. P. Pozzilli  
Department of Diabetes and Immunogenetics  
St. Bartholomew's Hospital  
West Smithfield  
London EC1A 7BE  
UK