Effects of recombinant interleukin-2 and revaccination for hepatitis B in previously vaccinated, non-responder, chronic uraemic patients

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Abstract

**Background.** Growing evidence suggests that it is possible to seroconvert chronic renal failure patients who are absolute non-responders to hepatitis B vaccine by means of either additional booster vaccine doses or associated IL-2 administration or both. We have studied the possibilities of hepatitis B seroconversion by revaccination and its dependence on vaccine dose, and the effects of a concurrent low-dose rHuIL-2 regime.

**Methods.** Forty known absolute non-responders with chronic renal failure were entered into a complete revaccination protocol. Patients were randomly assigned to two dosage groups of either 20 or 40 μg hepatitis B vaccine administered at 0, 1, 2 and 6 months. Further randomly selected patients from each dosage group were given 500 000 U of rHuIL-2 in the same deltotoid area 4 h after vaccine administration.

**Results.** Sixty-seven per cent of patients revaccinated with 40 μg attained antibody protecting levels compared to only 20% of those receiving doses of 20 μg (P < 0.025). When compared with initial values, the Th-CD4/CD25 cell count was significantly reduced immediately after HuR-IL2 administration (P < 0.0003) and significantly increased 1 month after the last dose was given (P < 0.0003). A definite rHuIL-2 effect on HBV antibody synthesis could not be demonstrated, nor was erythropoietin found to enhance seroconversion.

**Conclusions.** From these results we suggest that more intense and frequent antigenic stimulation as obtained by revaccination using four doses of 40 μg may effectively reduce the pool of hepatitis B vaccine non-responders in chronic renal failure patients.

**Key words:** chronic renal failure; hepatitis B revaccination; interleukin-2

Introduction

Hepatitis B vaccination is commonly carried out in most uraemic patients, whether haemodialysed or not. However, only 60% develop protective antibody levels [1–3]. This failure of uraemic patients to produce an adequate antibody response after hepatitis B vaccination compared to healthy individuals [4] has been considered to be of multifactorial origin. Defects of monocyte function [5] and underexpression of the TCR/CD3 antigen receptor by Th-1 cells [6] have been proposed, among others, as factors leading to low interleukin-2 (IL-2) secretion. This would also account for defects in progression and proliferation within the Th-1 cell-cycle, resulting in an impaired or absent response by antibody-forming cells.

The Th-1 high-affinity IL-2 receptor (IL-2R) has been found to be overexpressed in fresh T-cells from uraemic patients, independent of TCR/CD3 expression and in inverse correlation to T-cell IL-2 secretion [7]. This finding and the observation of enhanced T-cell proliferation in vaccinated non-responder chronic renal failure patients, when exposed to low concentrations of exogenous IL-2, has suggested that its simultaneous administration with the hepatitis B vaccine may induce an adequate anti-HBS antibody response [8].

Vaccinated non-responder chronic renal failure patients are currently considered as definite non-responders. The question arises as to whether the above patients are in fact true non-responders. Hepatitis B prevention in these patients is currently based on both HBsAg surveillance and the isolation of HBsAg carriers. It has been suggested that both
the vaccine dose and frequency of administration employed in uraemic patients should be higher than those usually administered to the normal population, but a homogeneous policy has not yet been attained. Thus we subsequently encounter wide differences in vaccination methodology between authors in this field [9,10].

The aims of this prospective study carried out on non-responder chronic renal patients were to evaluate the effects of vaccine dose and of simultaneously administered rHuIL-2.

Subjects and methods

Forty-five non-responder patients gave their written consent and were entered in the study. 12 patients had been previously vaccinated with three or more doses of 20 μg. The remaining 33 patients had received three or more doses of 40 μg, as indicated.

Those who had been vaccinated with doses of 20 μg before entering the present study were revaccinated with four doses of 20 μg, while those who had previously received doses of 40 μg were revaccinated with four doses of 40 μg. S-HuR-HB vaccine (Engerix-B) was given in the deltoid region, scheduled at 0, 1, 2 and 6 months.

IL-2 was administered in randomly selected patients from both the above dosage groups. Des-alanine-L, serine-125 non-glycosylated rHuIL-2 (Proleukin) was administered at a dose of 500 000 U intramuscularly, 4 h after each vaccine dose had been given and as close as possible to its injection site by using the same needle track.

The anti-HBs antibody concentration was assayed at months 0, 1, 2, 3 and 7 of the revaccination schedule by routine method using a commercially available test (IMx USAB, Abbot) Revaccination was considered successful when a HBs antibody titre higher than 10 U/l was detected during the revaccination schedule up to month 7.

CD3 (anti-Leu 4, clon SK7), CD4 (Multi-Clone Anti-Leu 3a + 3b, Clones SK3 + SK4), CD8 (Anti-Leu 2a, Clon SK1), CD9 (Anti-Leu 12, Clon 4G7), and CD25 (Anti-interleukin-2 receptor, Clon 2A3) antigen expression was investigated by flow cytometry (FACSCAN, Becton Dickinson, Mountain View, USA) using commercially available monoclonal antibodies (Becton Dickinson), quantitative analysis of CD3, CD4, CD8, CD9, CD4/CD25, and CD8/CD25 membrane antigen expression was carried out on 1,500–2,000 lymphoid cells, obtained at three stages of the vaccination schedule: at basal state before the first vaccine dose, four h after the first dose of IL-2 and one month after the last IL-2 dose was given.

Repeated measurements analysis was performed with Friedman and Wilcoxon tests. The χ² test was used for analysis of association between two categorical variables, using Yates’ correction or Fisher exact test when appropriate. Multivariable logistic regression analyses were performed to study the correlations between revaccination response and vaccine dose, IL-2 administration, age, and sex. Statistical analysis was performed by using the BMDP software package.

Results

Five patients were excluded from analysis: three patients were transplanted and two others died. The data from the remaining 40 patients entered in the present study have been evaluated. No side-effects to IL-2 were encountered throughout the duration of the study.

Twenty-two patients (55%) had HBs antibody titres greater than 10 U/l at the end of the revaccination programme. Revaccination was more frequently successful in the group receiving 40-μg doses than in the group receiving 20-μg doses (67 vs 20%, P=0.025) (Table 1).

Analysis of the effect of IL-2 administration in successful responders revealed a higher number of responses in the group of IL-2-treated patients, but a statistically significant difference could not be established (55 vs 45%, P=0.056). On multivariate analysis, the independent correlate of response to vaccination was dose of vaccine (OR, 3.3 (95% CI, 1.22 to 8.83)) (P=0.0182). Administration of IL-2 (OR, 2.1 (95% CI, 0.95 to 4.6)) (P=0.06) sex, or age were not statistically significant.

When HCV seropositives and erythropoietin-receiving patients, both IL-2 treated and untreated cases were compared (6 patients with positive hepatitis C virus serology, 4 responders vs 2 non-responders, P=0.67; and 14 patients with erythropoietin treatment, 7 responders vs 7 non-responders, P=0.64) no differences in terms of antibody response could be observed. The expression of T-cell antigens CD3, CD4, and CD8 did not change during the study. CD19 antigen expression increased 4 h after the administration of IL-2 (9 ± 2% vs 31 ± 11%, P=0.0003) but differences between basal and final levels were not observed. Th-CD4/CD25 counts declined after rHuIL-2 administration. On the other hand, Th-CD4/CD25 expression was found to increase 1 month after the last dose of IL-2 had been given (Table 2).

Discussion

When given to dialysed and non-dialysed patients with chronic renal failure, and in spite of its widespread use, hepatitis B vaccination has not led to successful prevention of HBV infection in this group of patients. It is currently agreed that only 50–60% of such patients attain protective antibody levels to HBV of 10 U/l or higher. Until recently no novel strategies had been advanced which might produce protective antibody titres in the 30–40% of remaining patients commonly

| Table 1. Anti-HBs response at month 7 in patients who underwent either the 20- or 40-μg hepatitis B vaccine schedule |
|-------------------------------------------------|----------------|----------------|----------------|
| 40 μg (n=30) | 20 μg (n=10) | P |
| Seroconversion (≥ 2 mU/ml) | 26 (87%) | 5 (50%) | 0.028 |
| Seroprotection (≥ 10 mU/ml) | 20 (67%) | 2 (20%) | 0.025 |
| High response (≥ 50 mU/ml) | 18 (60%) | 1 (10%) | 0.009 |

Values are expressed as cases (percentage).
considered as permanently unresponsive [1–3]. In these patients, the prevention of hepatitis B virus infection is based on both HBsAg surveillance and the isolation of HBsAg carriers.

Currently the normal population is immunized against HBV infection with 20 µg of vaccine administered three times [4]. This method is still employed to vaccinate uraemic patients, despite the low rates of protective antibody levels obtained. For the purposes of this study we have accepted the currently agreed criteria and evaluated as unresponsive all patient lacking an adequate antibody response level after receiving three vaccine doses of 20 µg each, administered at 0, 1 and 6 months. Nevertheless, the possibility of an improved response by using four vaccine doses has been suggested for these patients [9,10]. Notable seroconversion rates have been documented in previous non-responders after a booster revaccination dose is given [9,11]. These findings may raise the possibility of wrongly classifying some patients as non-responders who could be classified as responders were they exposed to a larger amount of antigen [12]. Unfortunately an agreed policy with regards to a more effective HBV vaccination programme for chronic uraemic patients is yet to be established.

Unresponsiveness to the HBV vaccine has been ascribed to the immunodeficient state associated with uraemia, which is considered multifactorial in aetiology. Dysfunction of monocyte-related and Th-1 helper cell mechanisms that would result in impaired signalling to memory and antibody-forming cells have been postulated [5,6]. The present study may suggest that defective monocyte antigen presentation associated with low Th-1 cell TCR/CD3 antigen receptor recognition could participate in the HBV vaccine unresponsiveness eventually seen in many patients with chronic renal failure. It may also explain the higher response rate observed when four doses of a HBV vaccine each of 40 µg are implemented. Therefore our results indicate that revaccination may be effective and dose dependent even in those patients who had received up to four doses of 40 µg in their initial immunization schedule.

As a corollary, this data may indicate that classifying patients as unresponsive may require the observation of antibody titres lower than 10/I after revaccination with four doses of 40 µg.

Another study has previously shown that the expression of an otherwise functionally normal, high-efficiency Th-1 cell IL-2 receptor may be upregulated. This upregulated expression was also found to be inversely correlated to the IL-2-secreting capacity of the T-helper cell [3]. In the immunostatic state, reduced IL-2 secretion may be responsible for inadequate progression and proliferation within the T-cell cycle. This is suggested by the increased proliferative capacity observed in fresh T-cells taken from uraemic subjects after the addition of low concentrations of exogenous IL-2 ‘in vitro’ [7]. These findings suggest a potentially beneficial effect imparted by IL-2 on the HBV antibody response of uraemic patients when administered in association with the HBV vaccine.

In a separate pilot study, six of only 10 non-responder patients appeared to respond to concurrent IL-2 administration and HBV revaccination [8]. These encouraging results have not been confirmed by other groups [11], and appreciable differences in the methods applied make them non-comparable to our present study. Our results may not demonstrate an enhanced IL-2-mediated HBV antibody response, although cellular changes strongly indicative of a biological effect due to rHuIL-2 are detected. The method we have used is similar but not identical to that described in the preliminary report mentioned above, as highlighted below. We have given four (rather than two) doses of HBV vaccine at two different strengths, as well as IL-2. Furthermore, it is known that HBV vaccines vary in immunogenicity according to the preparation [13], as do the biological effects of IL-2 [14]. Thus, a difference in the administered IL-2 effect, influencing the HBV antibody response rate, cannot be excluded between these two studies.

It has been suggested that erythropoietin therapy may enhance the HBV antibody response induced by vaccination [15]. However, our results do not point towards any such associated erythropoietin effect.

Until new data are available, our results strongly suggest that HBV revaccination alone is effective in seroconverting many previously unresponsive uraemic patients, and that stimulation with a larger HBV vaccine antigen dose may procure the highest HBV antibody response rates.

### References


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**Table 2.** CD4/CD25 and CD8/CD25 membrane antigen expression in revaccinated patients receiving IL-2 (n = 20)

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<th>Basal</th>
<th>At 4 h of 1st IL-2 dose</th>
<th>At 7 months</th>
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| CD4/CD25 (%)   | 12.8 ± 7.1* | 8.2 ± 4.8**             | 18.7 ± 8.8*** | <0.0001
| CD8/CD25 (%)   | 1.43 ± 0.7  | 1 ± 0.6                 | 2.1 ± 2     | 0.1054

*P* = 0.0003 between basal and 4-h values; **P** = 0.00001 between 7-months and 4-h values; ***P** = 0.0003 between basal and 7-month values.


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