

IMMUNE DEFECT IN THE EARLY STAGE OF CHRONIC RENAL FAILURE

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Summary

In the uraemic state both cellular and humoral immune defects can be observed [1]. However, little is known about the development of this secondary immunodeficiency in the course of chronic renal failure. Thirty-five patients with chronic renal failure were immunized with hepatitis B vaccine. Mean serum creatinine was 4.9mg/dl. Only 19 patients developed anti-HBs following vaccination (54%), compared with 94 per cent of healthy controls. A subgroup of 11 patients with a mean serum creatinine of 2.4mg/dl was investigated in vitro with regard to their lymphocyte function. Mitogenic (PHA, ConA, PWM) and allogeneic stimulation of peripheral lymphocytes were performed. There was a marked difference between patients and healthy controls, indicating that the secondary immune defect known in end-stage renal disease starts very early in the development of chronic renal failure.

Introduction

Patients with terminal renal failure are at high risk of acquiring hepatitis B virus infection [2]. Only 60 per cent of uraemic patients develop anti-HBs antibodies following vaccination with hepatitis B vaccine [3] as compared to over 90 per cent in high risk groups with normal renal function (hospital staff) [4]. Therefore vaccination in the early course of chronic renal failure has been suggested. To investigate the immune response following hepatitis B vaccination in the early stage of chronic renal failure 35 patients with chronic renal disease and varying degrees of renal insufficiency were immunized with hepatitis B vaccine. The immunization rate was compared with healthy controls. In addition, a subgroup of 11 patients with a mean serum creatinine of 2.4mg/dl was investigated by in vitro lymphocyte proliferation assays.

Patients and methods

Thirty-five patients with chronic renal failure and 19 healthy controls (medical staff) were vaccinated with hepatitis B vaccine (H-B Vax, MSD). Three intramuscular injections were given at 0, 1 and 6 months. All patients and the controls received 20 μ g at each interval. The mean serum creatinine was 4.9mg/dl (range 1.5 to 9.1mg/dl) for the patient group. No patient received drugs known to interfere with the immune response. Anti-HBs was determined by commercially available test systems (Ausab, Abbott Laboratories, Chicago, Illinois, USA). Based on serum creatinine levels patients were divided into two groups. The serum creatinine of group I (n=20) ranged between 1.5 and 3.5mg/dl (\bar{X} =2.9mg/dl), of group II (n=15) between 3.6 and 9.1mg/dl (\bar{X} =6.9mg/dl).

In vitro tests Human peripheral blood mononuclear cells (PBL) were isolated by Ficoll Hypaque density gradient centrifugation (Pharmacia Fine Chemical, Uppsala, Sweden). Mononuclear cells were washed three times in RPMI 1640 containing 2% FCS, 1% penicillin-streptomycin and 200mM L-Glutamin. Proliferative assays with different mitogens were performed as follows. PBL were resuspended in final culture medium RPMI 1640 with 10% fetal calf serum (FCS); 10⁵ PBL/well in a total volume of 200 μ l were cultured in round-bottom microtitre plates (Costar) in the presence of 5 μ g/ml phytohaemagglutinin (PHA), 12.5 μ g/ml Concanavalin A (Con A) and Pokeweed mitogen (PWM) (final dilution 1:100). PBL in final culture medium without mitogens served as controls.

In a mixed lymphocyte culture (MLC) 10⁵ PBL were stimulated with 5000 rad-irradiated Epstein-Barr virus transformed B cells (10⁵ cells) in round-bottom microtitre plates.

After 48 hours in culture at 37°C, 5% CO₂ in a humidified atmosphere cells were pulsed with 1 μ Ci ³H-thymidine (³H-TdR). Sixteen hours later cultures were harvested on filter papers using a Titertek cell harvester. Filters were dried and counted in a liquid scintillation spectrometer (Packard). Stimulation Index (SI) was obtained by dividing cpm ³H-TdR uptake of stimulated cultures by medium controls.

In vitro production of IgG [5] 10⁵ unfractionated PBL were cultured in triplicate in round-bottom microtitre plates (Costar) at 37°C, 5% CO₂ in a humidified atmosphere for seven days. Final culture medium was RPMI 1640 supplemented with 1% penicillin-streptomycin, 200mM L-Glutamin and 10% FCS. Each well contained a total volume of 200 μ l. PBL were stimulated with PWM at a final dilution of 1:100. After seven days lymphocytes were pelleted and supernatants harvested. IgG concentration of supernatants was measured by an ELISA technique.

Results

Thirty-five patients were immunized with hepatitis B vaccine. Nineteen (54%) patients developed anti-HBs antibody following the vaccination schedule (responder). Their mean serum creatinine and age was 4.3mg/dl and 39.1 years

compared to 4.9mg/dl and 33.2 years in the non-responder group, respectively. Between group I (serum creatinine 1.5–3.5mg/dl) and group II (serum creatinine 3.6–9.1mg/dl) there was no difference in the immunization rate. Eleven patients of group I (55%) compared with eight patients of group II (53%) developed anti-HBs after active vaccination with hepatitis B vaccine. In contrast, 94 per cent of controls showed an immune response after vaccination.

In addition there was a marked difference in the amount of specific antibody production to HBs antigen between responders in the patient group and responders in the control group. Patients showed a delayed and weaker immune response than controls (Figure 1).

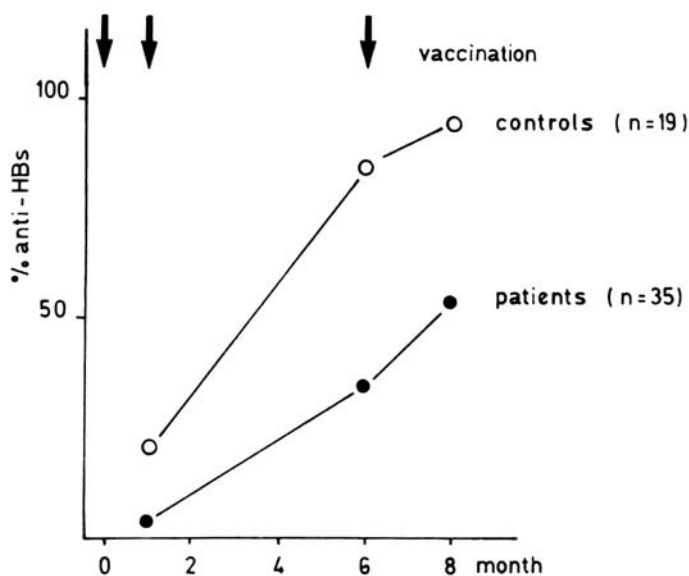


Figure 1. Immunization rate of healthy controls and patients after triple immunization

Mean time for development of anti-HBs was 3.6 months for patients compared to 1.8 months for controls. Mean geometric anti-HBs titres were 1:32 for the patient group as compared to 1:303 for the control group.

Eleven patients of group I with a mean serum creatinine of 2.4mg/dl (range 1.5 to 3.0mg/dl) were further investigated by *in vitro* studies. Proliferative responses of peripheral blood mononuclear cells following mitogenic and allogeneic stimulation were measured and compared to healthy controls. Unfractionated PBL were treated with Con A, PHA and PWM or stimulated with an allogeneic lymphoblastoid B cell line. Compared to the controls, patients with only a mild degree of renal insufficiency demonstrated a significantly reduced proliferative response to all systems employed (Figure 2).

No difference was found in IgG production following stimulation with PWM.

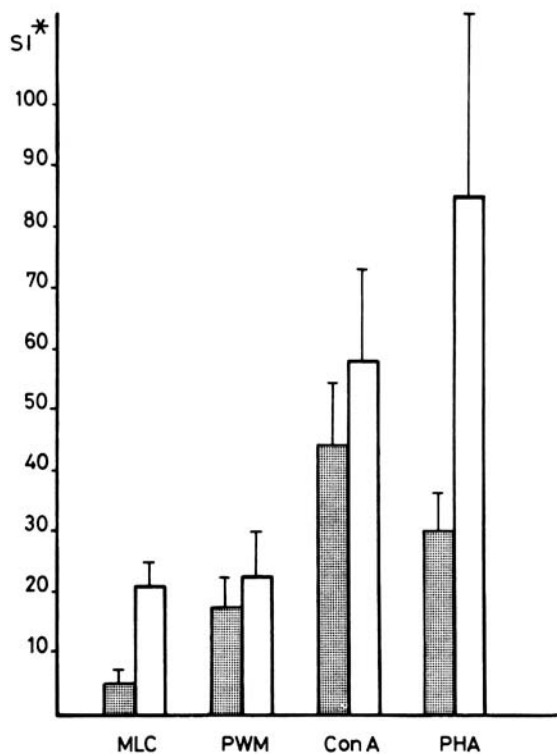


Figure 2. Proliferative response after mitogenic (Con A, PHA, PWM) and allogeneic (MLC) stimulation (mean \pm SD). *SI=Stimulation Index

Discussion

End-stage renal failure patients develop a secondary immune defect [6]. Nutritional deficiency, toxins (phenol, ULDL) [7] and serum inhibitory factors have been discussed as possible causes [8]. The severity of these changes increases with declining renal function. Few data exist about the immune status in the early stage of chronic renal disease. In this study patients with a mean serum creatinine of 4.9mg/dl had an immune response rate (54%) which was markedly reduced compared to healthy controls (94%), but which did not exceed the immunization rate of patients on chronic haemodialysis treatment (56%). Although patients with terminal renal failure had received 40 μ g of the vaccine at each interval compared to 20 μ g used in this study, the low immunization rate, especially in group I, was unexpected. Furthermore, the immunization rate did not differ between group I with better renal function (mean serum creatinine 2.9mg/dl) and group II (mean serum creatinine 6.9mg/dl).

In vitro studies of 11 patients from group I revealed a reduced proliferative response to all mitogens and alloantigens used compared to healthy controls.

Similar results have been reported for patients on chronic haemodialysis treatment. These results support the notion that an altered cellular immune response already exists at a very early stage of renal failure and seems to be an important factor for the development of the secondary immunodeficiency seen in patients with chronic renal diseases. In contrast to haemodialysis patients the in vitro production of IgG by peripheral blood lymphocytes in patients with a mild degree of renal insufficiency (serum creatinine 1.5–3.0mg/dl) was not impaired. Since the proliferative responses measured in vitro require a complex cellular collaboration (T-, B-cells, macrophages) a precise definition of the cell primarily involved is not possible at the present time. Further studies are necessary to characterize the immune defect seen in chronic renal failure. The impaired immune response described in patients on chronic haemodialysis treatment starts very early in the course of chronic renal failure indicated by the weak immunization rate after hepatitis B vaccination and by the impaired proliferative response of peripheral blood lymphocytes from these patients after mitogenic and allogeneic stimulation.

References

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