Anti-HBs Cellular Immune Response in Kidney Recipients before and 4 Months after Transplantation

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Patients with renal failure represent a population at risk for hepatitis B, since only 50 to 60% of them develop protective humoral responses after vaccination. As this could be due to an altered regulation of cellular immune responses, the objectives of the present study were to evaluate the proliferative abilities of lymphocytes from patients with chronic renal failure after stimulation in vitro with a mitogen (pokeweed mitogen [PWM]) or HBsAg. In order to differentiate between the immunodeficiency associated with renal failure and that due to immunosuppression posttransplantation, the same subjects were tested before and 4 months after kidney transplantation. The lymphoproliferation assay used was performed by flow cytometry, which is based on sequential analysis of the cell cycle and which allows analysis of cytokine production. Serologically, the group of 36 patients tested comprised 22% nonresponders, 30% poor responders, and 48% responders. Lymphocyte growth was observed for all patients after stimulation with PWM, indicating that these cells had the capacity to proliferate in vitro. The level of lymphoproliferation in response to PWM was significantly reduced after transplantation, yet both before and after transplantation, all serologic nonresponders developed cellular responses to at least two vaccines. No correlation between humoral and cellular responses was shown.

Proliferating cells were lymphocytes, which mostly secreted interleukin 4 (IL-4) and IL-10 for the three serologic groups. This study suggests that even when repeated vaccination fails to induce significant antibody levels in patients with renal failure, specific HBs cellular responses develop, and these may prove to be efficient in protecting these patients against hepatitis B.

Chronic renal failure (CRF) is a risk factor for chronic hepatitis B. Before vaccination was available, 3 to 10% of chronic hemodialyzed patients developed this disease (21). Blood contamination also occurred when transfusions were commonly given to these patients before the emergence of thorough screening of blood products and, later, generalized use of recombinant erythropoietin (17).

Since the 1980s, several vaccines against hepatitis B virus (HBV) have been available. Three recombinant vaccines, Engerix B, HBVax, and GenHevac, have been produced by genetic engineering (14). These vaccines are extensively used to protect patients with renal failure, yet only 50 to 60% of these patients develop a protective humoral response, evaluated by measuring plasma anti-HBs antibody levels after vaccination. This proportion is significantly lower than that observed in the general population, in which more than 90% of vaccinated people develop protective antibodies (12). Since 1982, patients diagnosed with renal failure have been vaccinated as early as possible. The schedule for hemodialysis patients’ vaccination includes two or three intramuscular injections at 0, 1, and 6 months (15). A number of patients, however, still fail to develop specific antibodies and are submitted to repeated vaccination attempts.

Since antibody production is T-cell dependent, the absence of antibody responses after vaccination against HBV could result from deficient humoral or cellular immune responses (6). The immunosuppressed status related to renal failure could be involved in the low rates of response of hemodialyzed patients (19). It is of importance, however, that since vaccination programs have been initiated with renal failure patients, the incidence of HBV disease has decreased considerably. Moreover, HBV disease does not develop either when such patients receive transplanted kidneys or, thus, when they receive immunosuppressive drugs. Yet, the status of humoral nonresponse usually persists after kidney transplantation. These data suggest that although humoral protective responses are difficult and sometimes impossible to achieve in this population, enough T-cell responses might be induced by the vaccination program to yield cellular protection.

To test this hypothesis, the study reported here was designed to evaluate proliferative cellular immune responses in vitro after stimulation with a mitogen or HBsAg in patients with CRF before and 4 months after kidney transplantation.

MATERIALS AND METHODS

Patients. Thirty-six consecutive kidney transplant recipients (25 males, 11 females; mean age, 44 ± 11 years; age range, 19 to 64 years) were included in a prospective fashion in the study on the day of transplantation. The causes of CRF varied (9 patients had immunoglobulin A [IgA] nephropathies, 9 had congenital nephropathies, 5 had autoimmune diseases, 2 had polycystic kidney diseases, and 11 had nondefined nephropathies). Twenty-five patients were on hemodialysis (mean duration, 46 ± 69 months) and eight were on peritoneal dialysis (mean duration, 35 ± 46 months) before kidney transplantation. Each patient was his or her own control, as a first test was performed with peripheral blood obtained just...
prior to transplantation and a second one was performed with blood obtained 4 months later.

Thirty patients were primary transplant recipients, and six received transplants for the second time.

Three groups were defined according to the patients’ levels of anti-HBs antibodies at the time of transplantation: a group of 17 serologic responders (group R) with anti-HBs antibody levels greater than 100 mIU/ml, a group of 11 poor responders (group PR) with anti-HBs antibody levels between 10 and 100 mIU/ml, and a group of 8 nonresponders (group NR) with anti-HBs antibody levels less than 10 mIU/ml.

Immunosuppression was induced with polyclonal antilymphocyte antibodies (rabbit thymoglobulin) for 17 patients or anti-interleukin 2 (anti-IL-2) receptor (Daclizumab or Zenapax) for 6 patients. At the time of immunosuppression induction, 13 patients were enrolled in a clinical trial without induction therapy. The patients received the following immunosuppressive regimens after induction: 5 patients received cyclosporine (Neoral) and corticosteroids; 7 patients received cyclosporine, motefil mycophenolate (Cellcept), and corticosteroids; 5 patients received tacrolimus (Prograf) and corticosteroids; 15 patients received tacrolimus, motefil mycophenolate, and corticosteroids; and 4 patients received tacrolimus and motefil mycophenolate.

All patients were vaccinated with HBs at the onset of dialysis (from 1 to 282 months before transplantation). Good humoral responders received only one round of vaccination, while up to 17 doses of vaccine were recorded for the others.

**Lymphocyte activation.** Sterile peripheral blood mononuclear cell suspensions were prepared from blood samples collected on EDTA by density gradient centrifugation on Ficoll (Lymphoprep; Nycomed Pharma, Oslo, Norway) after dilution of the blood sample with sterile RPMI 1640 medium (Sigma Chemical Co., St. Louis, Mo.). After 30 min of centrifugation at 400 × g, lymphocytes and part of the supernatant were harvested and washed in RPMI 1640 medium in order to obtain a peripheral blood mononuclear cell suspension with enough monocytes liable to act as antigen-presenting cells. The cells were resuspended at a concentration of 1 × 10^6 ± 0.5 × 10^6 cells/ml in complete medium composed of RPMI 1640 medium supplemented with 1% HEPES (Gibco BRL, Cergy Pontoise, France), 1% Ultroser (Gibco BRL), and 0.5% of a mixture of antibiotics (penicillin, 10,000 U/ml; streptomycin, 10,000 μg/ml; amphotericin B [Fun- gizone], 25 μg/ml; Gibco BRL). The cells were then distributed at 500-μl aliquots in a series of individual microwells (Nunclon Surface; Nunc, Roskilde, Denmark) that already contained 500 μl of culture medium alone; culture medium with 1 μg of pokeweed mitogen (PWM); a positive control for proliferation after mitogen stimulation (Sigma) per ml; or culture medium with 1% Tetavax anatoxin (anti-goat immunoglobulin antibody; Pasteur), GenHevac, or Engerix for 1 h at room temperature with 25 ml of goat anti-mouse immunoglobulin antibody (Dako) diluted 1:3,000 in PBS–TWEEN 20–1% BSA overnight at room temperature with gentle agitation. After a series of thorough washes with PBS–TWEEN 20, the membranes were further incubated for 1 h at 37°C. The DNA in the membranes was finally revealed by the enhancement of DNA exposed to DNAase digestion. Finally, the DNA was visualized by the exposure of the membranes to a solution of biotin-conjugated streptavidin-digoxigenin-alkaline phosphatase and 1.25 ng/ml of the relevant cytokine specific antibody (anti-IL-2, anti-IL-4, anti-IL-5, anti-IL-10, anti-IFN-γ, anti-IL-12, anti-IL-15, anti-IL-18, anti-IFN-α, anti-IFN-β, anti-IFN-κ, anti-IFN-ξ, anti-IL-18, anti-IL-21, and anti-IL-22) in 1% BSA in PBS. After 30 min of incubation at room temperature, the membranes were washed three times with PBS–TWEEN 20 and incubated for 10 min at room temperature with 25 ml of a solution of biotin-conjugated anti-goat immunoglobulin antibody (Amplified Opti-4CN Substrate kit; Bio-Rad). After a series of thorough washes with PBS–TWEEN 20, the membranes were incubated for 30 min at room temperature with 20 ml of a solution of horseradish peroxidase-conjugated streptavidin diluted 1:1,000 in PBS–TWEEN 20–1% BSA. Finally, after another series of washes with PBS–TWEEN 20, the membranes were incubated for a third time for 10 min at room temperature with 25 ml of a solution of biotin-conjugated anti-goat immunoglobulin antibody (Amplified Opti-4CN Substrate kit; Bio-Rad). After a series of washes with PBS–TWEEN 20–dimethyl sulfoxide and with PBS–TWEEN 20, the membranes were incubated for 30 min at room temperature with 20 ml of a solution of horseradish peroxidase-conjugated streptavidin diluted 1:1,000 in PBS–TWEEN 20–1% BSA (Amplified Opti-4CN Substrate kit; Bio-Rad). The membranes were washed in PBS–TWEEN 20 and finally revealed by immersion in 20 ml of a solution of Opti-4CN substrate (Bio-Rad) containing a chromogen and hydrogen peroxide. The reaction was stopped in distilled water, and the membranes were left to dry. The color intensity of each dot was then measured by refractometer with Quantity One quantitation software (Bio-Rad). Quantitation curves were drawn for each cytokine and used to calculate individual results. The latter were expressed in nanograms per milliliter and further adjusted to the number of cells in each pool.

**Statistical analysis.** The data were fed into a computer and analyzed by Myositis software (GraphPad, San Diego, Calif.) using analysis of variance (ANOVA), the chi-square test, and Student’s t test after checking for normality by the Kolmogorov-Smirnov test. Paired tests were performed with Prism software (GraphPad, San Diego, Calif.). Statistical significance was retained for P values lower than 0.05.

**RESULTS**

**Cellular immune responses before transplantation.** Among the whole group of patients studied, 92% displayed lymphocyte proliferation after stimulation with PWM, with a significant (P < 0.001, ANOVA) increase in the mean percentage of cells in S phase between day 3 (10.2% ± 0.9%) and day 6 (12.5% ± 1.2%), with maximal proliferation detected on day 5 (19.4% ± 1.2%). Fifty-six percent of the patients also displayed significant lymphocyte proliferation in response to the positive control, HBVAX.

Fifty-eight percent of the patients developed lymphoproliferation after stimulation with Engerix, 67% developed lymphoproliferation after stimulation with GenHevac, 36% developed lymphoproliferation after stimulation with HBVAX, and 67% developed lymphoproliferation after stimulation with the pre-S antigen (P = 0.03, chi-square test) (Fig. 1). Data on the mean percentage of cells in S phase on each day for each serologic group and for each antigen are provided in Table 1. Less than half of the patients in group R developed cellular responses after stimulation with Engerix (47%), HBVAX (29%), or the
pre-S antigen (47%), while 59% developed lymphocyte proliferation after stimulation with GenHevac.

By contrast, the patients in group NR responded strongly to the four vaccines studied: Engerix, 88%; GenHevac, 100%; HBVax, 63%; and pre-S antigen, 88%.

Cells from patients in group PR proliferated mostly after stimulation with pre-S antigen (82%) but only poorly after HBVax, 63%; and pre-S antigen, 88%.

The mean CPI for group NR was significantly higher than those for groups PR and R (P = 0.02, ANOVA) (Fig. 2). Nine patients in group PR or R had a CPI equal to 0; i.e., they did not respond to any of the vaccine antigens tested.

The influences of various parameters liable to influence immune responses were examined (Table 2). No significant difference was observed between the three groups by sex or age whether sex and age were considered globally or for each group. Similarly, no difference was observed between hemodialyzed and peritoneally dialyzed patients or according to the administration of immunosuppressive treatments before transplantation, i.e., in IgA nephropathy patients and subjects with autoimmune disease.

Finally, as three HLA haplotypes (HLA-DR3, HLA-DR7, and HLA-DQ2) have been reported to be implicated in humoral responsiveness after vaccination against HBV, we examined the information relative to donor-recipient matching. Among the 12 patients who had one of these haplotypes, 75% indeed had anti-HBs antibody levels lower than 100 mIU/ml and were in the NR or PR group (P = 0.05, chi-square test). However, no significant difference in specific cellular responses was observed between HLADR3- and HLADR7-positive or HLA-DQ2-positive patients (mean CPI, 2.4 ± 0.4) and patients lacking these major histocompatibility complex specificities (mean CPI, 2.3 ± 0.3).

**Cellular responses after transplantation.** After transplantation lymphocyte proliferation was demonstrated for 97% of the subjects when their cells were stimulated with PWM, with a significant (P = 0.025, ANOVA) increase in the mean percentage of cells in S phase between day 3 (8.50% ± 1.0%) and day 6 (10.90% ± 1.30%) and maximal proliferation on day 4 (14.40% ± 1.90%) (P = 0.025), while significant proliferation was demonstrated for only 49% of the subjects after stimulation with Tetavax.

Significant lymphocyte proliferative responses were noted for the cells from 60% of the patients stimulated with Engerix, 57% of the patients stimulated with GenHevac, 51% of the patients stimulated with HBVax, and 60% of the patients stimulated with pre-S antigen. This time, no statistically significant difference was noted between the vaccines studied or the day of culture (chi-square test) (Fig. 1 and Table 3).

Cells from all patients in groups NR and R displayed significant proliferative responses after stimulation with PWM, while the cells from only 90% of those in group PR did. No significant difference in CPIs was noted among the three groups. Only two patients, one in group R and one in group PR, had a CPI equal to 0.

After transplantation, five patients in group PR had become nonresponders and five patients in group R had become poor responders. However, this was not correlated to the cell proliferation status, as five of these patients had increased CPIs and five had decreased CPIs compared to their pretransplan-

### Table 1. Percentages of cells in S phase per day (days 3 to 6) for each of the three groups of patients and for each vaccine studied before transplantation

| Patient group and vaccine | Mean ± SE % of cells in S phase on day: | 3 | 4 | 5 | 6 |
|---------------------------|------------------------------------------|---|---|---|---|
| **NR (n = 8)**            |                                          |   |   |   |   |
| Engerix                   | 2.6 ± 1.1                                | 5.7 ± 3.5 | 4.1 ± 3.6 | 3.6 ± 2.8 |
| GenHevac                  | 4.0 ± 2.2                                | 6.1 ± 7.0 | 5.1 ± 2.8 | 4.9 ± 5.0 |
| HBVax                     | 2.7 ± 1.8                                | 4.3 ± 3.0 | 3.8 ± 3.1 | 3.2 ± 2.0 |
| Pre-S antigen             | 3.8 ± 2.7                                | 7.9 ± 5.2 | 3.7 ± 3.0 | 3.4 ± 3.0 |
| **PR (n = 11)**           |                                          |   |   |   |   |
| Engerix                   | 1.9 ± 1.8                                | 2.6 ± 2.5 | 4.1 ± 3.3 | 3.5 ± 2.2 |
| GenHevac                  | 3.2 ± 1.7                                | 4.6 ± 3.2 | 3.5 ± 2.5 | 4.1 ± 3.8 |
| HBVax                     | 2.0 ± 1.0                                | 1.9 ± 1.8 | 2.0 ± 1.5 | 2.6 ± 1.9 |
| Pre-S antigen             | 3.4 ± 2.7                                | 4.2 ± 2.8 | 6.2 ± 7   | 3.5 ± 1.6 |
| **R (n = 17)**            |                                          |   |   |   |   |
| Engerix                   | 2.5 ± 2.5                                | 3.0 ± 3.2 | 4.9 ± 5.4 | 3.4 ± 3.6 |
| GenHevac                  | 4.1 ± 2.7                                | 4.4 ± 3.3 | 4.9 ± 3.9 | 4.8 ± 4.5 |
| HBVax                     | 2.8 ± 3.6                                | 2.0 ± 3.9 | 3.5 ± 3.5 | 2.9 ± 3.3 |
| Pre-S antigen             | 3.4 ± 3.0                                | 4.1 ± 4.5 | 4.3 ± 4.5 | 4.6 ± 5.4 |

FIG. 1. Percentage of cellular responders to the four vaccines tested before (black bars) and 4 months after (white bars) kidney transplantation (P = 0.03, chi-square test).

FIG. 2. Mean and standard error (bars) CPIs for each serologic group before (black bars) and 4 months after (white bars) kidney transplantation. As each significant proliferative response is scored as 1, an individual patient’s score varies between 0 (no proliferation) and 4 (significant proliferation in response to the four antigens tested).
tation status. This difference was not related to the type of induction of immunosuppression.

The immunological characteristics of the six patients who received a second transplantation did not differ from those of the other patients.

There was no significant difference between patients receiving triple therapy for immunosuppression and the other patients.

Comparison before and after transplantation. After transplantation, a significant decrease in the mean percentage of cells in S phase compared to the pretransplantation values was observed after stimulation with PWM ($P = 0.02$), GenHevac ($P = 0.02$), and pre-S antigen ($P = 0.05$) (Student’s $t$ test).

Similarly, CPIs were slightly lower after transplantation than before transplantation for groups NR and PR, yet they increased in group R (Fig. 2), without statistical significance, however.

Cytokine assay. Detectable levels of the three cytokines tested were observed in all samples. The levels ranged from 25 to 130 ng/ml for IFN-$\gamma$, 90 to 2,500 ng/ml for IL-4, and 40 to 900 ng/ml for IL-10 (Table 4).

No significant difference was observed between the three serologic groups either before or after transplantation. However, IL-4 and IL-10 concentrations were the highest (0.5 to 1 log difference compared to the IFN-$\gamma$ concentrations), suggesting a preferential TH2-like profile both before and after transplantation.

Fours months after transplantation, the levels of production of IL-4 were slightly higher for patients in groups NR and R, as was the level of production of IL-10 for patients in group R.

### DISCUSSION

CRF is associated with a status of immunosuppression that results in an increased susceptibility to infections with bacteria or viruses, such as HBV. Moreover, anti-HBs antibody production after vaccination is altered, as only 50 to 60% of CRF patients develop a protective humoral response (20). This defect could be related to alterations in either B- or T-cell responses (18), but the results reported here suggest that strong and thus possibly protective cellular responses develop in vaccinated CRF patients and that these persist after transplantation.

The study reported here was designed to examine in vitro cellular immune responses after stimulation with HBV vaccine antigens in CRF patients before and 4 months after kidney transplantation, thus allowing appreciation of the respective effects of CRF-induced and drug-induced immunosuppression. Lymphocyte activation analysis after stimulation with PWM allowed us to show that nearly all patients had lymphocytes with functional capacities to proliferate in vitro before and after transplantation.

Among the antigens tested, lymphoproliferation was the most important with GenHevac and pre-S antigen, both of which contain the pre-S2 antigen, which is absent from the other vaccines. This suggests that the vaccine’s composition could influence cellular responses. Other studies have previously shown that GenHevac has superior immunogenicity, which was also attributed to the presence of the pre-S2 antigen sequence in this vaccine (9, 10). Interestingly, before transplantation, 100% of humoral nonresponders displayed in vitro cell proliferation after stimulation with GenHevac, confirming the efficacy of this antigen in triggering cellular immune responses.

No global association between age and immune responses was established in our study, while others have reported that older age could be related to decreased immune responses after vaccination of healthy subjects or hemodialysis patients (4, 16). In addition, no correlation between age, the mode of

### TABLE 2. Distribution of humoral and cellular responses by sex and mode of dialysis before kidney transplantation

| Characteristic | NR ($n = 8$) | PR ($n = 11$) | R ($n = 17$) | Mean ± SE* CPI |
|---------------|-------------|-------------|-------------|----------------|
| Age (yr [mean ± SE]) | 42.6 ± 2.9 | 47.4 ± 2 | 42.3 ± 3.4 | 2.26 ± 0.3 |
| No. (%) of men ($n = 27$) | 6 (22) | 9 (33) | 12 (45) | 3.36 ± 6.3 |
| No. (%) of women ($n = 9$) | 2 (22) | 2 (22) | 5 (56) | 2.5 ± 0.4 |
| No. (%) of patients on: | | | | |
| Hemodialysis ($n = 25$) | 6 (24) | 7 (28) | 12 (48) | 2.25 ± 0.7 |
| Peritoneal dialysis ($n = 8$) | 1 (12) | 2 (15) | 5 (63) | 2.36 ± 0.3 |

* SE, standard error.

b Three patients received transplants before the initiation of dialysis.

### TABLE 3. Percentages of cells in S phase per day (days 3 to 6) for each of the three groups of patients and for each vaccine studied 4 months after transplantation

| Patient group and vaccine | Mean ± SE % of cells in S phase on day: |
|---------------------------|-----------------------------------------|
|                           | 3 | 4 | 5 | 6 |
| NR ($n = 8$)              |   |   |   |   |
| Engerix                   | 2.1 ± 1.4 | 2.6 ± 1.5 | 2.9 ± 1.6 | 3.8 ± 2.4 |
| GenHevac                  | 2.4 ± 1.4 | 2.5 ± 1.3 | 3.2 ± 2.3 | 3.3 ± 3.2 |
| HBVax                     | 1.6 ± 1.4 | 2.8 ± 1.6 | 2.2 ± 2.5 | 2.9 ± 3.5 |
| Pre-S antigen             | 2.6 ± 2.0 | 2.9 ± 1.9 | 2.6 ± 1.4 | 3.0 ± 2.6 |
| PR ($n = 11$)             |   |   |   |   |
| Engerix                   | 2.5 ± 1.8 | 3.4 ± 2.3 | 2.0 ± 0.9 | 1.9 ± 1.6 |
| GenHevac                  | 2.4 ± 2.0 | 2.9 ± 2.7 | 1.9 ± 1.7 | 2.0 ± 1.0 |
| HBVax                     | 2.9 ± 1.4 | 3.0 ± 2.7 | 2.1 ± 2.6 | 2.6 ± 3.0 |
| Pre-S antigen             | 2.2 ± 1.5 | 3.3 ± 2.6 | 1.7 ± 1.1 | 2.1 ± 1.6 |
| R ($n = 17$)              |   |   |   |   |
| Engerix                   | 2.5 ± 2.0 | 3.9 ± 3.3 | 4.0 ± 3.8 | 3.7 ± 6.0 |
| GenHevac                  | 2.5 ± 1.8 | 2.4 ± 2.8 | 3.5 ± 3.8 | 2.7 ± 2.6 |
| HBVax                     | 2.2 ± 1.8 | 2.1 ± 1.5 | 1.9 ± 1.5 | 3.6 ± 6.6 |
| Pre-S antigen             | 1.8 ± 1.4 | 3.1 ± 2.1 | 3.6 ± 4.6 | 3.1 ± 1.8 |
dialysis, and antigen-specific cellular proliferation in vitro was observed for each of the three serologic groups of patients. No difference between patients with IgA nephropathies or autoimmune diseases and the other patients studied was seen in terms of the partition of patients into the good, poor, and no humoral response groups. HTLA typing analysis confirmed a genetic predisposition toward poor humoral responses after vaccination against hepatitis B (3). Indeed, haplotypes HTLA-DR3, HLADR7 (2), and HLADQ2 (13) were more frequently represented in those with poor and no humoral responses; but this was not related to altered cellular responses in our experience.

After kidney transplantation, the mean percentages of cells in S phase significantly decreased compared to the values pretransplantation. This indicates that immunosuppressive drugs have a stronger effect on cellular responses than CRF-induced immunosuppression. This effect was also observed for the humoral responses, as the serologic status of a few patients had changed 4 months after transplantation. However, no correlation between cellular and humoral responses was observed.

The group with no humoral response presented a specific in vitro proliferative response toward at least two of the antigens tested. In the group with humoral responses, the restoration of proper renal function after transplantation appeared to have a beneficial effect, since although the percentages of cells in S phase were lower, CPIs increased after transplantation for these patients. Since systematic vaccination of CRF patients has been encouraged, HBV infections have regressed in these patients, despite the continuation of mediocre humoral responses. Our data suggest that cellular protection is provided by vaccination for these patients. The presence of higher levels of sensitized cells in those with no humoral response compared to those in patients with humoral responses could be due to the increased attempts at vaccination performed for those subjects who failed to develop antibody levels considered protective or to the fact that these individuals preferentially develop T-cell responses. This would allow an increased frequency of HBsAg-specific memory cells and thus an increased probability that enough of them would be present in the peripheral blood to obtain significant growth in vitro. It is worth noting that as many as 17 vaccination attempts were made for some of the nonresponders, in some cases up to 9 months before the tests were run.

The results of our cytokine assays showed that cultured lymphocytes mostly secreted IL-4 and IL-10. IL-10, an immunosuppressive type TH2 cytokine, inhibits TH1 cytokines such as IFN-γ. Honorati et al. (8) have observed that TH0 and TH2 cells secreting IL-4 and IL-5 could be generated after HBV vaccination, while TH1 cells secreting IFN-γ were produced in those with chronic hepatitis. Chedid et al. (1) have proposed that responsiveness after vaccination could be related to the production of excessive IL-4, which inhibits IL-2 synthesis and which is responsible for the immunodepression observed in hemodialysis patients (7).

In conclusion, this study shows that before transplantation 75% of the CRF patients studied displayed significant lymphoproliferative responses toward HBV antigens in vitro, and this value rose to 95% after transplantation, despite the deleterious effect of iatrogenic immunosuppression on proliferation. More interestingly, all serologic nonresponders had such sensitized T cells. CRF patients, a population at risk for hepatitis B, who fail to produce antibody levels even after repeated vaccinations would thus develop specific HBs cellular responses which may prove efficient in protecting these patients against the disease.

The fact that the in vitro proliferative responses of cells toward HBV antigens remained detectable and significant 4 months after transplantation also demonstrates that this protection is durable, despite the immunosuppressive treatments related to transplantation.

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