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Th_1/Th_2 cytokines and ICAM–1 levels post-liver transplant do not predict early rejection

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TH_1 DERIVED cytokines IFN-γ and IL–2, Th_2 cytokine IL–4, and ICAM–1 have been implicated in liver allograft rejection. In order to determine whether monitoring of cytokine profiles during the first days post-liver transplant can predict early rejection we measured IFN-gg, IL–2, sIL–2 receptor, IL–4 and ICAM–1 in 22 patients, in plasma samples obtained within 4 h after liver perfusion (baseline) and between postoperative days (POD) 3–6. ICAM–1 and sIL–2R levels at POD 3–6 were significantly higher than at baseline but did not differ in presence or absence of rejection. Mean percentage increase of ICAM–1 levels was significantly lower in patients with Muromonab-C_3 Orthoclone OKT_3 (J.C. Health Care) (OKT_3) whereas percentage increase of sIL–2R levels was higher in OKT_3-treated patients. IFN-γ levels at POD 3–6 increased from baseline while IL–4 levels were unchanged. Levels of IFN-γ, IL–4 and their ratios did not correlate with rejection or immunosuppressive therapy. Thus, Th_1/Th_2 cytokine monitoring during the first week post-transplant does not predict early rejection and immunosuppressive therapy is the predominant factor affecting ICAM and sIL–2R levels after liver transplantation.

Key words: Th_1/Th_2 cytokines, ICAM–1, Immunosuppression, Liver transplant, Rejection

Introduction

Cytokines play a pivotal role in modulation of the immune response following solid organ transplantation. A distinct array of cytokines produced by two subsets of CD4+ helper T cells, Th_1 and Th_2, dictates induction and regulation of cellular and humoral immunity. The Th_1 subset secretes IL–2, IL–3, IFN-γ and TNF, which subsequently also induce expression of adhesion molecules, whereas Th_2 cells produce IL–4, IL–5, IL–6 and IL–10.[1,2] The Th_1/Th_2 paradigm is based on the hypothesis that a Th_1 immune response is detected in hosts undergoing rejection whereas an immune pattern that deviates towards a Th_2 response is associated with allograft tolerance.[2] Increased plasma TNF levels have been shown to precede rejection in both liver and kidney transplant patients and IL–2 and IFN-(have been consistently detected in hosts undergoing unmodified acute rejection.[3–6] IL–4 has been noted to be preferentially associated with allograft engraftment.[7–9] Yet, elevated IL–4 levels are also evident in rejecting patients, most notably in those with spontaneously resolving rejection.[10,11] This increase in IL–4 levels occurs at a later time point than the increase in IL–2 levels, likely indicating amelioration of the rejection episode. Further studies have suggested that rather than absolute levels of individual cytokines it is the ratio of Th_1/Th_2 cytokines which serves in determining allograft rejection or tolerance.[9]

In order to further elucidate the efficacy of cytokine monitoring in predicting rejection we serially monitored Th_1 cytokines IL–2 and IFN-γ, the Th_2 cytokine IL–4 and ICAM–1 levels in liver transplant patients during the first week post-transplant.

Materials and Methods

Patients

Twenty-two patients undergoing an orthotopic liver transplant were studied. Plasma samples were obtained from each patient within 4 h after liver perfusion and on postoperative days (POD) 3 and 6.

Data analysed included: primary immunosuppression, early graft function (poor early graft function was defined as prothrombin time (PT) >18 s on POD 2 and peak aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >2500 units during the first 3 postoperative days), evidence of infection (based on cultures of blood, sputum, urine, wounds and drains, cytomegalovirus (CMV) serology and CMV cultures) and histologically proven rejection during the first 2 weeks post-transplant.

Cytokine assays

Levels of IL–2, soluble IL–2 receptor (sIL–2R), IFNγ, IL–4 and ICAM–1 were determined (in duplicate aliquots) for each plasma sample, by commercial ELISA kits (R+D Systems, Minneapolis, MN). The absolute cytokine level was calculated based on a standard curve provided by the manufacturer.

Data analysis

For each cytokine mean patients' absolute levels at 4 h post-perfusion (baseline levels) were compared with mean absolute levels at 3 and 6 POD. Data were also analysed by calculating the mean change in cytokine levels, e.g. each patient's baseline levels at 4 h post-perfusion were expressed as 100%, and the patient's cytokine levels at 3 and 6 POD expressed as percentage of baseline. The percentage increase from baseline to levels at POD 3 and 6 was compared among patients with and without rejection and among those receiving cyclosporine A (CsA) or OKT_3 as primary immunosuppression. Results were compared by the Mann–Whitney test and by one-way analysis of variance (ANOVA).

Results

Of the patients studied there were 11 males and 11 females, with an age range of 49.4 ± 8.9 (mean ± SD). Indication for transplantation was hepatitis C virus (HCV) cirrhosis in 13 patients, hepatitis B virus (HBV) in two, cryptogenic cirrhosis in one, primary biliary cirrhosis in two, autoimmune liver disease in two, fulminant liver failure in one, and primary sclerosing cholangitis in one. This diversity precluded data analysis according to primary liver disease. The primary immunosuppressive drug was cyclosporine in 15 patients and OKT_3 in seven patients.

In nine patients no rejection episodes were documented within the first 12 days post-transplant. In 13 patients rejection was diagnosed during the first 12 days post-transplant (in six patients rejection was diagnosed during days 6–8 post-transplant.

Cytokines

IL–2 levels were non-detectable in all plasma samples studied. sIL–2R levels increased from 74.8 ± 11.8 pmol/ml (mean ± standard error) at 4 h post-perfusion to 171.4 ± 23 pmol/ml at POD 3–6, $P < 0.001$. The mean percentage increase from baseline levels was significantly higher in patients with OKT_3 induction compared with cyclosporine treated patients: 531.5% ± 140.8 vs. 237.7% ± 32.3, $P < 0.001$. Mean percentage increase from baseline did not differ in the presence or absence of rejection (305.3% ± 70.7 vs. 366.6% ± 104.4).

Mean levels of IFN-γ at POD 3–6 were significantly increased compared with post-perfusion levels; from 111 ± 28 pg/ml post-perfusion to 163 ± 39 pg/ml at POD 3–6, $P < 0.05$. Mean percentage increase from baseline did not correlate with rejection episodes (rejection vs. no-rejection: 181.1% ± 50.1 vs. 206.4% ± 68.7) or immunosuppressive therapy (CsA 231.2% ± 47.8 vs. OKT_3 227.6% ± 85.4).

ICAM–1 levels at 3–6 days post-transplant were significantly higher than baseline post-perfusion levels; 431 ± 38.6 pg/ml vs. 223 ± 44.0 pg/ml, $P < 0.001$. Percentage increase in ICAM–1 levels (baseline levels expressed as 100%) did not differ in the presence or absence of rejection (272.9% ± 45.0 vs. 295.1% ± 85.8, mean ± standard error). Mean percentage increase from baseline was significantly lower in patients with OKT_3 induction compared with CsA-treated patients; 216.4% ± 33.4 vs. 312.7% ± 63.6, $P < 0.01$.

No correlation was observed between percentage increase in IFN-γ levels and the increase in ICAM–1 levels (for all patients $R^2 = 0.09$, in patients with rejection $R^2 = 0.02$).

IL–4 levels were unchanged during the time period studied. Mean baseline levels 678.5 ± 46.8 pgm/ml vs. 650.5 ± 50.1 pg/ml at POD 3–6. Levels were similar in the presence or absence of rejection and in OKT_3 and CsA-treated patients.

The ratios of IL–4/IFN-γ remained unchanged over time; 14.3 ± 3.7 post-perfusion vs. 13.1 ± 5.6 at POD 3–6. Although the ratio of IL–4/IFN-γ at both baseline and POD 3–6 was lower in patients experiencing early rejection compared with patients without rejection, differences were not statistically significant.

Discussion

In post-liver transplant patients serial monitoring of plasma Th_1 cytokines IL–2 and IFN-γ, Th_2 cytokine IL–4 and the adhesion molecule ICAM–1 during the first week post-transplant did not enable prediction of early rejection.

In our patients IL–2 levels were non-detectable in all plasma samples studied. IL–2 was non-detectable even at baseline measurements within the first few hours post-liver perfusion denoting that the initial dose of immune suppressive therapy (CsA or OKT_3) is sufficient to abolish IL–2 release from activated lymphocytes. IL–2 levels were similarly nonmeasurable in the 13 patients in whom early rejection was diagnosed. An increase in IL–2 levels is frequently observed in transplant recipients experiencing episodes of rejection[10,12,13] but have not been found, in all studies, to be sufficiently reliable to diagnose or exclude rejection.[5] Indeed, Baan *et al.*[11] noted intragraft IL–2 mRNA expression in only 36% of post-liver transplant patients with rejection. In renal transplant recipients studied during the first 14 days

post-transplant, IL–2 levels, when measurable, were predictive of impending graft rejection and increased a mean 2.8 days prior to clinical diagnosis of rejection.[10] Although rejection was diagnosed in six of our patients as early as days 6–8 post-transplant, no elevation in IL–2 levels was evident.

Soluble IL–2 receptor levels were increased at POD 3–6 in all patients, both in the absence and presence of rejection. The highest levels were measured in patients whose primary immunosuppression was OKT_3, thereby likely denoting the marked degree of cytolysis which occurs following OKT_3 administration.

Monitoring of IFN-γ levels was also of no predictive value regarding early rejection. Levels increased to a similar degree in all patients during the first week post-transplant corroborating findings of a previous study in renal transplant patients in whom rises in IFN-γ levels during the first 2 weeks post-transplant was not associated with rejection.[14] IFN-γ is one of the major inducers of ICAM–1 but changes in IFN-γ levels did not correlate with changes in ICAM–1 levels (analysed both for all patients and for the rejection group). Although significant elevations in ICAM–1 levels have been reported in renal transplant patients 2–3 days prior to diagnosis of clinical rejection,[14] in our patients rejection was not accompanied by changes in ICAM–1 levels. Multiple factors appear to affect plasma ICAM–1 levels; preoperative ischaemic injury, reperfusion injury, graft dysfunction and infections.[15–17] Of the infectious pathogens, CMV is remarkable in its ability to upregulate expression of adhesion molecules on infected cells.[18] Among our patients, seven had infectious episodes diagnosed during the study period and although in none was CMV infection diagnosed three were CMV negative prior to transplantation from a CMV positive donor. Thus, the numerous variables affecting plasma ICAM–1 levels preclude the use of this parameter in predicting rejection. Our findings point to immunosuppressive therapy as a major determinant of ICAM–1 levels as these were significantly lower in patients receiving primary immunosuppression with OKT_3.

Levels of the Th_2 cytokine IL–4 remained relatively stable throughout the first week post-transplant in all patients studied, and did not correlate with the absence or presence of rejection. A previous study, in renal transplant recipients, reported that the highest IL–4 levels are detected late in the course of clinical rejection suggesting that the rise in IL–4 levels coincides with resolution of the rejection episode.[10] This observation is in accord with findings in liver transplant patients in whom intragraft IL–4 mRNA expression was detected in 70% of biopsies with histological evidence of rejection obtained from patients without clinical signs of rejection. In contrast, IL–4 mRNA expression was present in only 19% of biopsies without rejection and 18% of biopsies with histological evidence of rejection and concurrent graft dysfunction.[11] Thus, in our patients, as plasma IL–4 levels were determined not later than POD 6, a later rise in IL–4 may have been missed. Interestingly, Gorczynski *et al.*[19] observed equivalent transcription of IL–4 in peripheral blood lymphocytes and liver biopsies of all liver transplant patients, regardless of rejection status. Furthermore, levels of another Th_2 cytokine, IL–10, which are expected to rise in patients with uncomplicated transplants do not differ in rejecting and non-rejecting patients nor does intragraft expression of IL–10 mRNA differ between uncomplicated transplants, acute and chronic rejection or normal liver controls.[20]

The late rise in IL–4, compatible with a role for Th_2 cells in suppressing the Th_1 dependent immune response, has suggested that the Th_1/Th_2 balance may be more predictive of the immune response than individual cytokine levels. Yet, in post-liver transplant children the IFN-γ/IL–4 ratio could not discriminate between infectious episodes, other than CMV and rejection.[21] In our study, although in patients with rejection the IFN-γ/IL–4 ratio was higher than in non-rejecting patients, differences did not reach statistical significance.

In summary, our observations do not support a role for cytokine monitoring, during the first week post-OLT, in predicting early rejection. Plasma levels of sIL–2R, ICAM–1, IFN-γ, IL–4 and their ratios do not correlate with rejection. Notably, immunosuppressive therapy is the predominant factor affecting plasma sIL–2R and ICAM–1 levels after liver transplantation.

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