

OKT3, a murine monoclonal antibody specific to the human CD3 complex, induces immunosuppression by depletion of T cells. Administration of OKT3 results in significant release of proinflammatory cytokines, such as TNF α and IL1 β . Liver recipients who experience rejection within 3 weeks after transplantation with OKT3 prophylaxis recover their T cells by postoperative day 10 despite complete initial clearance.

We sought to analyze the role of proinflammatory and Th-1 cytokines in T cell recovery and rejection after liver transplantation with OKT3 prophylaxis. In plasma samples from 32 patients, we measured TNF α , IL1 β and IL6 (before transplant and on postoperative days 1, 2 and 3) and IL2, IFN γ , sIL2R and sICAM (postoperative days 5, 7 and 10) and examined possible correlations with T-cell recovery and occurrence of rejection within 3 weeks.

TNF α , IL1 β , and IL6 did not correlate with T-cell recovery. In patients who rejected, IL2 and IFN γ on postoperative days 5 and 7 correlated with degree of T-cell recovery by day 10; a significant rise in sIL2R over time also correlated with T-cell recovery in this group.

Our results emphasize the role of Th-1 cytokines in rejection following OKT3 induction and suggest that markers of T cell activation may predict risk.

Key words: Liver transplantation, Cytokines, OKT3, Rejection

Cytokine profiles in early rejection following OKT3 treatment in liver transplant patients

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Introduction

OKT3, a murine monoclonal antibody specific to the human CD3 complex, induces immunosuppression by attaching to the CD3 portion of T lymphocytes, leading to clearance of the entire antigen receptor complex and depletion of T cells. OKT3 has been considered a major advance in the prevention and treatment of allograft rejection. Although several studies have shown OKT3 to be effective for prophylaxis against rejection of liver allografts,¹ acute rejection has been documented in patients receiving this treatment.

Cytokines play a crucial role in acute rejection, which is primarily a cell-mediated process. Several studies have examined the relationship between various cytokines and rejection in attempts to predict failure of immunotherapy after OLT. TNF α and IL1 β have been shown to be elevated early after transplant and have been implicated in the up-regulation of adhesion molecules, such as ICAM1 and VCAM, in patients experiencing rejection.² The central role of IL2 in T cell activation and proliferation is well documented, while IFN γ has been shown to stimulate B cells, macrophages and expression of MHC class I and II molecules.³

Administration of OKT3 results in significant release of proinflammatory cytokines, such as TNF α and IL1 β . Circulating levels of the T-cell derived cytokines IFN γ and IL2 also rise following OKT3 injection.⁴ Both proinflammatory and Th-1 cytokines disappear from the circulation within 12–24 hours after injection.⁴ It is not clear, however, whether the extent of this cytokine release influences the occurrence of allograft rejection after OLT.

A recent study conducted at our institution demonstrated a 30.8% rate of rejection within 3 weeks of liver transplantation in patients who received OKT3-induced immunosuppression.⁵ The same study revealed that rejection in these patients is preceded by recovery of T cells by POD10 despite complete initial clearance and therapeutic blood levels of OKT3. In the present study, we sought to clarify the roles of pro-inflammatory cytokines and Th-1 markers in T cell recovery after OKT3 induction and determine their value as predictors of early rejection. For the present investigation, samples from the patients in our original study were assayed for TNF α , IL6 and IL1 β preoperatively and on the first 3 postoperative days. Samples from POD5 and later were analyzed for IFN γ and IL2 as well as sIL2R and sICAM.

Patients and methods

Study population

We studied 32 patients who underwent orthotopic liver transplantation for hepatitis C at The Mount Sinai Medical Center between May 1994 and September 1996 and who received OKT3 (Ortho Biotech, Raritan, NJ) to induce immunosuppression with azathioprine and steroids.

Immunosuppression

Patients received OKT3, 5 mg IV daily, for 8 to 12 days, with the first dose given intraoperatively after revascularization of the graft. Patients also received methylprednisolone, 500 mg IV, prior to each of the first two postoperative doses. Each subsequent dose of OKT3 was preceded by a tapered steroid dose. Before each of the first three doses of OKT3, patients were also premedicated with diphenhydramine, 50 mg IV, and acetaminophen. All patients were also given 1.5 mg/kg of azathioprine during OKT3 therapy. Treatment with cyclosporine or tacrolimus was initiated on POD4, and OKT3 was continued until therapeutic blood levels of these agents were achieved. All patients received at least 8 days of OKT3 therapy.

Evaluation of early graft function

Prothrombin time (PT), AST, and ALT were recorded daily until discharge or death. Poor early graft function (PEGF) was defined as PT on POD 2 >18 sec and peak AST or ALT >2500 during the first three postoperative days.⁶

CD3 cell counts and OKT3 levels

Absolute CD3 counts (cells/ml) were measured from blood samples taken preoperatively and at three time points during OKT3 induction: early (POD2-4), middle (POD5-7) and late (POD8-10). CD3 cells were labeled using an anti-CD3 (IgG1) FITC-conjugated monoclonal antibody (Coulter Immunology, Hialeah, FL) and counted using flow cytometry. The percentage recovery of baseline was calculated for each patient at each time point.

Rejection

Rejections that occurred within the first 21 days after transplant were considered early rejections; the remaining patients were placed in the no-rejection group. Rejection was diagnosed on the basis of elevated LFTs and biopsy showing bile duct damage, mixed portal inflammatory infiltration, eosinophils

and endophlebitis. Rejection episodes were treated with methylprednisolone, 1 gm IV, for 2 days. None of the patients developed steroid-resistant rejection requiring further antilymphocyte therapy.

Sample collection

Blood was drawn preoperatively and on POD1, 2, 3, 5, 7 and 10. Samples were immediately centrifuged at 3600 rpm for 10 minutes, after which the plasma or serum was separated and stored at -80 C until assayed.

Cytokine, ICAM1 and sIL2R levels

Preoperative samples and those from POD1, 2 and 3 were assayed for TNF α , IL1 β and IL6. Samples from POD5, 7 and 10 were assayed for IFN γ , IL2, sIL2R, and sICAM1. Commercial ELISA kits were used and samples were run in duplicate in all cases. Kits for TNF α , IL6, IL1 β , IL2, IFN γ , and sIL2R were obtained from Immunotech (Marseille, France) and the ELISA assay for sICAM1 was purchased from R&D Systems (Minneapolis, MN). Optical densities were read using Bio-Kinetic reader model EL312e (Bio-Tek Instruments, Winsooski, VT).

Data Analysis

Student's *t*-test assuming unequal variance was used to compare results between the early rejection and no-rejection groups. ANOVA with repeated measures was used to compare data from different postoperative days within both the early rejection or no rejection groups. Chi-square test was applied to compare the incidence of PEGF between study groups. A *p* value of less than 0.05 was considered significant for both tests. Correlation was used to compare data within each group, and an *R*² value of greater than 0.55 was considered significant.

Results

Six patients were excluded (one for death on POD11 from sepsis, one for retransplantation on POD14 for hepatic artery thrombosis, three for early discontinuation of OKT3 due to adverse effects, and one for discharge home on POD8 prior to the last blood sample being drawn). Final analysis was performed on samples from 26 patients. Rejection episodes (one mild, six moderate and one severe) were diagnosed in eight patients; the results from the remaining 18 patients were analyzed in the no-rejection group. Although patients with no rejection demonstrated somewhat more profound IRI, the differences in the extent of IRI or in the incidence of PEGF did not reach statistical significance (Table 1).

Table 1. Early graft function in liver transplant patients

| | Rejecting patients | Non-rejecting patients | <i>p</i> value |
|------------|--------------------|------------------------|----------------|
| peak AST | 560±219 | 1038±1358 | NS |
| peak ALT | 536±259 | 739±838 | NS |
| PT on POD2 | 13.9±0.88 | 15.8±2.9 | NS |
| PEGF | 0/8 | 2/18 | NS |

The average onset of rejection was POD 14.25±3.3 (range: POD10–20). T-cell counts are listed in Table 2.

Mean TNF α and IL6 levels were highly variable among patients in both groups, and no clear trends developed from the preoperative sample point to POD3. We found higher mean TNF α and IL6 levels in the no-rejection patients on all 4 days measured, but this difference reached significance only for IL6 on POD3 (Table 3). TNF α and IL6 did not correlate with efficacy of OKT3 therapy, as measured by clearance of T cells, in either group or with eventual recovery of T cells in the rejection group. TNF α levels strongly correlated with IL6 levels on POD2 and POD3 in patients with rejection ($R^2=0.8739$ and 0.9794 respectively) but not in the no-rejection group. IL1 β was detectable in only two of the samples analyzed.

The rejection group experienced a rise in mean IL2 levels from POD5 to POD10, while levels fell in the no-rejection group (Table 4). These changes from POD5 to POD10 were not statistically significant, and neither were the differences between the two groups. IL2 levels on POD5 and POD7 were found to correlate with eventual recovery of T cells on POD10 in the rejection group only ($R^2=0.7923$ and 0.7079 respectively) (Fig. 1).

Mean IFN γ levels were higher in the rejection group compared with the no-rejection group on all 3 days measured, but these differences never reached significance. There was also no clear trend over time for mean IFN γ levels in either group. IFN γ levels on

POD5 correlated well with degree of T-cell recovery by POD10 in the rejection group ($R^2 = 0.6387$) (Table 4).

Mean levels of sIL2R were higher in the no-rejection group on all 3 days measured. There was a statistically significant difference between the groups on POD5 and POD7 (Table 4). The two groups of patients displayed different patterns over time; mean levels rose with marginal significance from POD5 to POD10 in the rejection group ($p = 0.05$) but fell significantly in the no-rejection group ($p = 0.03$). Changes in sIL2R from POD5 to POD7 correlated very strongly with eventual recovery of T cells on POD10 (Fig. 2). The difference in sIL2R from POD5 to POD7 also correlated with IL2 and IFN γ levels on POD5 ($R^2 = 0.6509$ and 0.7857). These correlations were true only of the rejection group.

Average levels of sICAM1 were significantly higher in the no-rejection group on POD5 (Table 4). Like IL2, the values for sICAM1 fell after POD5 in the no-rejection group and rose in the rejection group, but these changes were not statistically significant. There was a strong correlation between sICAM1 and sIL2R levels on POD5 in both the rejection and no-rejection groups ($R^2 = 0.7433$ and 0.5914 , respectively).

Discussion

Induction with OKT3 after OLT has been used by many centers in attempts to decrease early cyclosporine-related renal dysfunction as well as the incidence

Table 2. T-cell recovery during OKT3 induction in liver transplant patients

| | Rejecting patients | Non-rejecting patients | <i>p</i> value |
|-------------------------------|--------------------|------------------------|----------------|
| Absolute CD3 count (cells/ml) | | | |
| Baseline | 266.1±196.3 | 529.7±378.2 | NS |
| POD2–4 | 7.9±14.2 | 4.4±4.7 | NS |
| POD5–7 | 20.1±25.3 | 19.9±50.5 | NS |
| POD8–10 | 213.3±184.9 | 22.7±32.4 | <0.001 |
| % recovery of baseline | | | |
| POD2–4 | 3.3±3.2 | 1.4±2.6 | NS |
| POD5–7 | 7.6±12.9 | 3.6±8.4 | NS |
| POD8–10 | 107.9±85.7 | 6.7±7.9 | <0.001 |

Table 3. Release of pro-inflammatory cytokines during the early postoperative period

| Cytokine | Rejecting patients | Non-rejecting patients | p value |
|---------------------------------------|--------------------|------------------------|---------|
| TNFα (pg/ml) | | | |
| Pre-op | 16.9 \pm 22.9 | 37.1 \pm 34.1 | 0.054 |
| POD1 | 27.6 \pm 22.1 | 53.5 \pm 28.3 | 0.079 |
| POD2 | 16.1 \pm 11.3 | 131.9 \pm 220.9 | 0.104 |
| POD3 | 27.1 \pm 32.5 | 47.9 \pm 51.1 | 0.123 |
| IL6 (pg/ml) | | | |
| Pre-op | 15.1 \pm 16.9 | 91.2 \pm 221.8 | 0.089 |
| POD1 | 67.4 \pm 47.5 | 345.1 \pm 359.8 | 0.074 |
| POD2 | 4.5 \pm 2.5 | 135.4 \pm 199.2 | 0.063 |
| POD3 | 14.3 \pm 18.3 | 147.9 \pm 217.5 | 0.011 |
| IL1β (pg/ml) | | | |
| Pre-op | – | 3.83 \pm 12.1 | NS |
| POD1 | – | – | NS |
| POD2 | – | – | NS |
| POD3 | – | – | NS |

of acute rejection. Studies have shown, however, that acute rejection with OKT3 prophylaxis still occurs in 26% to 33% of patients.¹ The mechanisms that contribute to rejection in the presence of OKT3 prophylaxis are not well understood, and no reliable methods for prediction of rejection in this circumstance are available.

Our current results demonstrate that proinflammatory cytokine profiles evaluated preoperatively and during the first 3 postoperative days did not correlate with the efficacy of OKT3 treatment as indicated by T-cell clearance. Additionally, elevated TNF α , IL1 β and IL6 levels in the first 3 days were not predictive of early rejection. Interestingly, levels of these cytokines were higher in the no-rejection group on all the days measured. It is difficult to assess the biological significance of this finding, as there was such wide

variation in the observed values. On the other hand, the strong correlation between TNF α and IL6 on POD2 and 3, observed in rejecting patients only, suggests the presence of an acute inflammatory response in this group. Other investigators have shown early elevations in serum levels of TNF α , IL1 β and IL6 in patients experiencing acute rejection episodes.^{7,8} Our data, which show higher mean levels of TNF α and IL6 in patients without early rejection, seems to contradict these findings. Previous studies, however, did not use OKT3 for induction therapy. The type of immunosuppression, among other factors, may therefore have an effect on early proinflammatory cytokine levels.

The extent of ischemia/reperfusion injury (IRI), which is considered to be the major determinant of early postoperative graft function,^{6,9,10} may also affect

Table 4. Th-1 cytokines and markers in liver transplant patients during OKT3 induction

| Cytokine | Rejecting patients | Non-rejecting patients | p value |
|---------------------------------------|---------------------|------------------------|---------|
| IL2(pg/ml) | | | |
| POD5 | 28.88 \pm 23.82 | 53.78 \pm 62.41 | 0.085 |
| POD7 | 27.75 \pm 35.72 | 28.81 \pm 30.76 | 0.473 |
| POD10 | 62.10 \pm 55.23 | 23.78 \pm 19.81 | 0.071 |
| IFNγ (IU/ml) | | | |
| POD5 | 1.42 \pm 1.62 | 0.66 \pm 0.70 | 0.134 |
| POD7 | 1.05 \pm 1.31 | 0.64 \pm 0.97 | 0.221 |
| POD10 | 1.45 \pm 2.59 | 0.53 \pm 0.73 | 0.201 |
| sIL2R(pM) | | | |
| POD5 | 135.63 \pm 69.87 | 223.33 \pm 75.62 | 0.01 |
| POD7 | 143.13 \pm 58.74 | 207.5 \pm 73.34 | 0.021 |
| POD10 | 175.29 \pm 53.82 | 205.17 \pm 73.45 | 0.154 |
| sICAM1(ng/ml) | | | |
| POD5 | 612.75 \pm 165.47 | 806.1 \pm 309.57 | 0.03 |
| POD7 | 649.50 \pm 197.82 | 664.5 \pm 267.81 | 0.49 |
| POD10 | 695.14 \pm 180.63 | 674.94 \pm 281.76 | 0.423 |

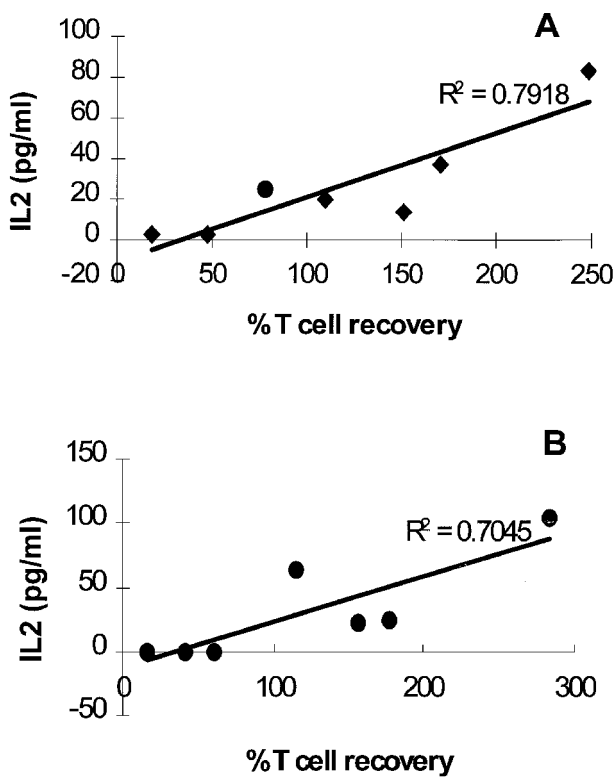


FIG. 1. Correlation between IL2 levels on POD5 (A) and POD7 (B) and T-cell recovery on POD10 in rejecting patients.

early postoperative cytokine levels. In the present study, however, early graft function was comparable among the patients in the study groups suggesting that the changes in cytokine profiles are more likely related to the onset of rejection.

OKT3 induction appears to have a similar effect on both sIL2R and sICAM levels. Mean values for sIL2r were significantly higher on POD5 and POD7 in the no-rejection group, and a similar difference was demonstrated for sICAM on POD5. Significant variations in sIL2R and sICAM levels have been shown depending on the type of immunosuppression, with the highest levels among patients receiving antilymphocytic therapy such as OKT3.^{8,11} This is not surprising if one considers that both IL2 receptor and ICAM are membrane-bound protein complexes found on lymphocytes. The correlation between sIL2R levels and sICAM1 levels on POD5 in both the early rejection and no-rejection groups supports a similar mechanism behind their release.

Mean levels of IL2 and IFN γ did not differ between the two patient groups at any time point. Additionally, changes in levels of these cytokines measured over time were not predictive of rejection. The strong correlations between levels of IL2 on POD5 and POD7, and IFN γ on POD5, with the degree of T-cell recovery by POD10, were specific to the early rejection group. These findings suggest that rejection occurring during OKT3 induction is mediated by Th1

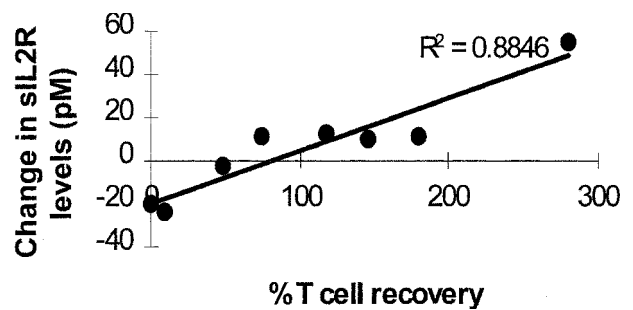


FIG. 2. Correlation between change in sIL2R levels from POD5 to POD7 and T-cell recovery on POD10 in rejecting patients.

cytokines. Further support for this mechanism is given by the significant rise in sIL2R levels from POD5 to POD10 in the same group and by the correlation between the increase in sIL2R levels from POD5 to POD7 and T-cell recovery.

Several pathways have been proposed as possible mechanisms of acute rejection occurring during OKT3 induction, including the development of anti-OKT3 antibodies and low OKT3 levels. Sutherland *et al.* demonstrated a clonal expansion of OKT3-resistant, allospecific T cells within the graft of a patient experiencing rejection during OKT3 induction.³ These cells maintained IL2 production *in vitro* despite binding of OKT3. This sustained IL2 production may be important for the escape phenomenon. While systemic IL2 levels in our study were not different between the two patient groups, they do not accurately reflect intragraft cytokine production. Although we did not measure *in vitro* IL2 production by T cells isolated from our patients, the fact that a correlation between IL2 levels and subsequent T cell recovery existed only among patients with rejection supports the central role of IL2 in rejection episodes occurring after OKT3 induction. Our data suggests that even though there is equivalent production of IL2 and IFN γ in both groups, only the T cells of the patients who will eventually experience rejection respond to these cytokines by T cell proliferation. Thus, there may be a difference in sensitivity to IL2 among the lymphocytes of rejecting and non-rejecting patients. Differential sensitivity of T cells to IL2 has been demonstrated in several studies and can serve as another possible mechanism for OKT3 resistance.¹²

The relatively higher sIL2R and sICAM1 levels in the no rejection group can be explained by the fact that for any given population of T cells, there will be a heterogeneous response to OKT3.³ sICAM1 has been shown to partially block adhesion of leukocytes and thereby modify the immune response.¹³ Rubin *et al.* have demonstrated that sIL2R efficiently binds IL2 without producing an immunostimulatory effect.¹⁴

In summary, our study failed to establish any correlation between increased levels of TNF α , IL6 and IL1 β and early rejection after OKT3 induction. Our previous study showed that early rejection after OKT3 induction is preceded by a recovery of T-cell count to baseline by post-transplant day 10. This T-cell recovery appears to be a Th-1 driven process, with signs of T-cell activation around POD5. Monitoring of peripheral T-cell numbers and sIL2R levels may help to identify patients who are likely to reject despite OKT3 prophylaxis.

References

- McDiarmid SV, Millis MJ, Terasaki PI, Ament ME, Busuttil RW. OKT3 prophylaxis in liver transplantation. *Dig Dis Sci* 1991; **36**:1418-26
- Platz KP, Mueller AR, Rossaint R *et al*. Cytokine pattern during rejection and infection after liver transplantation: improvements in postoperative monitoring? *Transplantation* 1996; **62**:1441-50
- Sutherland FR, Aboujaoude M, White MJ *et al*. Mechanisms of rejection during OKT3 therapy: propagation and characterization of CD3 resistant allospecific cytotoxic T cells from a rejecting liver allograft. *Clin Immunol Immunopathol* 1991; **60**:40-54
- Ferran C, Sheehan K, Dy M *et al*. Cytokine-related syndrome following injection of anti-CD3 monoclonal antibody: further evidence for transient *in vivo* T cell activation. *Eur J Immunol* 1990; **20**:509-15
- Sheiner PA, Guarrera JV, Grunstein E *et al*. Increased risk of early rejection correlates with recovery of CD3 cell count after liver transplant in patients receiving OKT3 induction. *Transplantation* 1997; **64**:1214-16
- Emre S, Schwartz ME, Mor E *et al*. Obviation of prereperfusion rinsing and decrease in preservation/reperfusion injury in liver transplantation by portal blood flushing. *Transplantation* 1994; **57**:789-803
- Tilg H, Vogel W, Aulitzky WE *et al*. Evaluation of cytokines and cytokine-induced secondary messengers in sera of patients after liver transplantation. *Transplantation* 1990; **49**:1074-80
- Hoffmann MW, Wonigeit K, Steinhoff G, Herzbeck H, Flad HD, Pichlmayr R. Production of cytokines (TNF-alpha, IL-1-beta) and endothelial cell activation in human liver allograft rejection. *Transplantation* 1993; **55**:329-35
- Greig PD, Woolf GM, Sinclair SB *et al*. Treatment of primary liver graft nonfunction with prostaglandin E1. *Transplantation* 1989; **46**:447-53
- Ploeg RJ, D'Alessandro AM, Knechtle SJ *et al*. Risk factors for primary dysfunction after liver transplantation: a multivariate analysis. *Transplantation* 1993; **55**:807-13
- Granot E, Tarcsafalvi A, Emre S *et al*. Th1/Th2 cytokines and ICAM-1 levels post-liver transplant do not predict early rejection. *Mediators of Inflammation* 2000; **9**:34-9
- Odum N, Kanner SB, Ledbetter JA, Svejgaard A. MHC class II molecules deliver costimulatory signals in human T cells through a functional linkage with IL-2-receptors. *J Immunol* 1993; **150**:5289-98
- Marlin SD, Staunton DE, Springer TA, Stratowa C, Sommergruber W, Merluzzi VJ. A soluble form of intercellular adhesion molecule-1 inhibits rhinovirus infection. *Nature* 1990; **344**:70-2
- Rubin LA, Jay G, Nelson DL. The released interleukin 2 Th1/Th2 cytokines and ICAM-1 levels post-liver transplant do not predict early rejection: receptor binds interleukin 2 efficiently. *J Immunol* 1986; **137**:3841-4

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