Cytokine and Cytokine-Receptor Profiles After Liver and Heart Transplantation

P. Fraunberger, M. Pfeiffer, M. Haller, R.M. Hoffmann, F.M. Zwiebel, P. Überfuhr, K.W. Jauch, D. Nagel, A.K. Walli, and D. Seidel

ONE of the early events in graft rejection is activation of immune cells, followed by the release of cytokines. Tumor necrosis factor (TNF) and interleukin-1 (IL-1) released by monocyte-derived macrophages lead to activation of endothelial cells with subsequent expression of adhesion molecules and MHC antigens.[1] Together with T-lymphocyte–derived cytokines such as interferon-gamma (IFN-gamma) and interleukin-2 (IL-2), these cytokines are thought to mediate important steps for leukocyte infiltration and tissue inflammation.[2] Depending on the degree of the immune reaction, they are also released into the blood circulation, but the physiological significance of this release is not fully understood.

The most common diagnostic tool used to establish graft rejection is still tissue biopsy and histologic grading.[3] However, this is an uncomfortable procedure and may not always distinguish between acute episodes of rejection and other complications such as viral reactivation. Even though clinical chemical parameters such as transaminases, bilirubin, and liver function parameters (coagulation factors) show alterations in their serum levels during early or late phases of rejection, they are not very specific and sensitive.[4–6] Levels of cytokines have been measured in patients after heart, liver, kidney, and bone marrow transplantation. Although serum levels of cytokines such as TNF, IL-2, interleukin-6 (IL-6), and interleukin-2-receptor (IL-2-R) show alterations during acute transplant rejection in renal and liver transplant patients, only IL-2-R serum levels have been useful in early detection of graft rejection.[7–13]

TNF is a key mediator of the immune reaction and has been demonstrated to be involved in the transplant reaction,[1,2,14] but the significance of TNF serum levels during transplant rejection is unclear. Whereas some studies report a correlation between TNF levels and graft rejection,[15,16] others are less conclusive.[7,17] This may be due to the short biological half-life of TNF. Soluble TNF receptors (TNF-R p55 and p75), which are derived from the cell surface by proteolytic cleavage, reflect the response of the organism to a TNF stimulus.[18,19] Because these receptors circulate much longer in the serum than TNF itself,[20] TNF-R levels in serum may be better markers for the immune reaction. This has been reported in other diseases like malaria and cancer.[21,22]

Because patients undergoing organ transplantation are maintained on immune suppressive therapy, infections are not uncommon. The clinical picture of bacterial or viral infection is often indistinguishable from the situation observed during acute graft rejection. At present, no specific immune markers are available to distinguish between these clinical situations. However, several studies report increased serum cytokine levels during infection. Endotoxins are strong stimuli for TNF, which then can induce IL-6 production,[23,24] one of the key mediators of acute phase response.[25] High serum levels of IL-6 have been described as markers for infection in liver-transplant recipients.[26] In addition, IFN-gamma is believed to play a role in the immune response of the host-to-viral infections because of its antiviral activity.[27]

Although cytokines play a crucial role in immune defense, the diagnostic value of serum cytokine levels has not been established. To examine the usefulness of serum cytokine measurements for distinguishing infectious and noninfectious graft complications, we followed serum levels of TNF; IL-6; IFN-gamma; and the cytokine receptors TNF-R p55, TNF-R p75, and IL-2-R in patients after liver and heart allograft transplantation.

PATIENTS AND METHODS

Sera from 25 liver- and 27 heart-transplant patients were collected daily during their hospitalization and stored at −30°C. Serum levels of TNF, IL-6, and IFN-gamma were measured by enzyme-linked immunosorbent assay (ELISA, Medgenix GmbH, Ratingen, FRG), and IL-2-R levels by a sandwich enzyme-immunoassay (EIA, T-Cell Diagnostics Inc., Cambridge, Mass USA). Soluble TNF-R p55 and p75 were measured by Cobas Core (Hoffmann La Roche, Basel, Switzerland) with enzyme-linked immunological assays (ELIBA), which were kindly provided by Hoffmann La Roche.[21]

Diagnosis of graft rejection in liver transplant patients was established by biochemical (transaminases, bilirubin, alkaline phosphatase, and eosinoplilia),[28] histologic (portal infiltration, bile duct lesions, and endotheliitis), and clinical findings (fever, malaise, neurological alterations). All patients with established graft rejection received steroid bolus therapy (500 mg methylprednisolone/d) for 3 to 5 days. Steroid-resistant rejection episodes were treated with antihuman CD3, OKT3 at 5 mg/d for 14 days. In patients with heart transplantation, routine tissue biopsy and echocardiography were performed to assess graft rejection. Histopathologic grading according to the International Society for Heart and Lung Trans-

From the Department of Clinical Chemistry (P.F., M.P., D.N., A.K.W., D.S.), Department of Anesthesiology (M.H.), Department of Internal Medicine II (R.M.H., F.M.Z.), Department of Heart Surgery (P.Ü.), Department of Surgery (K.W.J.), Großhadern Hospital, University of Munich, Germany.

Address reprint requests to Peter Fraunberger, Institut für Klinische Chemie, Klinikum Großhadern, Marchioninistr. 15, 81366 München, Germany.

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Table 1. Comparison of Serum Cytokine Levels of Patients After Liver Transplantation With Stable Graft, Graft Rejection, Infection Complications, and Rejection With Additional Infection

	Stable graft n = 8	Organ rejection n = 9	Infection n = 9	Rejection and infection n = 7
TNF [pg/mL]	19.6 ± 10.9 (5.0–39.1)	112.2 ± 59.7* (30.0–215.1)	42.6 ± 17.7*† (9.0–63.0)	82.9 ± 63.0* (26.0–217.0)
TNF-R p55 [ng/mL]	3.9 ± 1.9 (1.4–7.4)	12.6 ± 4.7* (5.6–19.6)	9.0 ± 7.2* (4.4–15.1)	17.6 ± 13.6* (6.2–43.2)
TNF-R p75 [ng/mL]	8.2 ± 3.0 (3.2–14.2)	38.3 ± 18.4* (19.8–73.4)	19.8 ± 5.9*† (6.0–27.4)	32.3 ± 13.8* (14.4–50.8)
IL-2-R [U/mL]	1402 ± 371 (825–2152)	6749 ± 2708* (3056–12000)	3784 ± 1583* (1758–7950)	7177 ± 2846* (4242–12000)
IL-6 [pg/mL]	23.4 ± 17.0 (1.0–65)	32.6 ± 25.9 (4.0–77.0)	210.5 ± 141.9*† (85.0–573)	221.6 ± 111.8*† (71.0–474.0)
IFN-gamma [U/mL]	0.04 ± 0.06 (0.0–0.2)	0.25 ± 0.30 (0.0–1.0)	0.19 ± 0.23 (0.0–0.9)	0.64 ± 1.37* (0.0–5.3)

n = Numbers of all episodes documented.
*Significantly different from stable graft, $P < .05$.
†Significantly different from organ rejection, $P < .05$.
Values in parentheses are ranges.

plantation criteria was used for the diagnosis of rejection. Heart-transplant patients with rejection grading score Ib and above were treated with steroids (500 mg/d methylprednisolone) for 3 days.

Criteria for infection were clinical symptoms (fever, malaise, tachycardia) as well as a documented septic focus and cultural growing of a pathogen. The following infections could be documented: *Pseudomonas aeruginosa, Staph. aureus, E. coli, Enterococcus facalis, Corynebacterium spec., Klebsiella pneumonia, Candida albicans,* and *cytomegalo virus.*

Cytokine measurements on the day of diagnosis were correlated retrospectively with the following clinical situations: stable grafts (n = 8 for liver transplant patients and n = 7 for heart transplant patients), rejection (n = 9 episodes in 9 liver transplant patients and n = 17 episodes in 10 heart transplant patients), infection (n = 9 in liver and n = 10 in heart transplant patients), and rejection-accompanied infection (n = 7 in liver transplant patients).

Statistics

Values for cytokine measurements are given as mean plus or minus the standard deviation. For statistical comparison, the Mann-Whitney-Wilcoxon-Test was used and a P value less than .05 was considered as significant.

RESULTS

Liver Transplant Recipients

The data on serum levels of cytokines in patients after liver transplantation with stable grafts, acute graft rejection, infection, and rejection accompanied by infection are summarized in Table 1. Mean serum levels of TNF, TNF-Rs and IL-2-R were significantly increased during nine episodes of rejection and seven episodes of rejection and infection in comparison with values of patients with stable grafts (n = 8). Serum levels of IL-6 did not change in patients with graft rejection, but infection (n = 9) or rejection and infection increased these levels significantly. Although levels of TNF, TNF-R, and IL-2-R were also elevated significantly during infection, these increases were not as high as those noted in patients with graft rejection. Increases in IFN-gamma values were significant only in patients with organ rejection and infection.

A representative time course of changes in serum levels of IL-2-R, TNF, and TNF-R p55 and p75 of a patient with acute graft rejection after liver transplantation is shown in Fig 1A. All four parameters increased within a few days before the clinical diagnosis of acute rejection could be established by biopsy. TNF levels increased at the onset of rejection and returned to nearly normal values within 3 days. In contrast, IL-2-R, TNF-R p55, and p75 levels remained elevated for about 1 week and then declined slowly.

Heart Transplant Recipients

Although there were slight increases in mean values of cytokines and their receptors during 17 episodes of graft rejection in 10 patients after heart transplantation, these changes were not statistically significant when compared to seven patients with stable graft (n = 7) (Table 2). A typical pattern of TNF, TNF-Rs, and IL-2-R serum levels during graft rejection of a heart transplant patient is shown in Fig 1B.

During infection, serum levels of cytokines and their receptors were increased significantly, as has been seen during infection after liver transplantation. Although IL-2-R levels increased during infection, they were not significantly different from patients with graft rejection. This may be due to a small, but nonsignificant increase of IL-2-R during rejection, and to the great variation of IL-2-R levels during infection. IFN-gamma was also increased during infectious episodes, but these increases were not significant due to the great variation of IFN-gamma serum levels (Table 2).

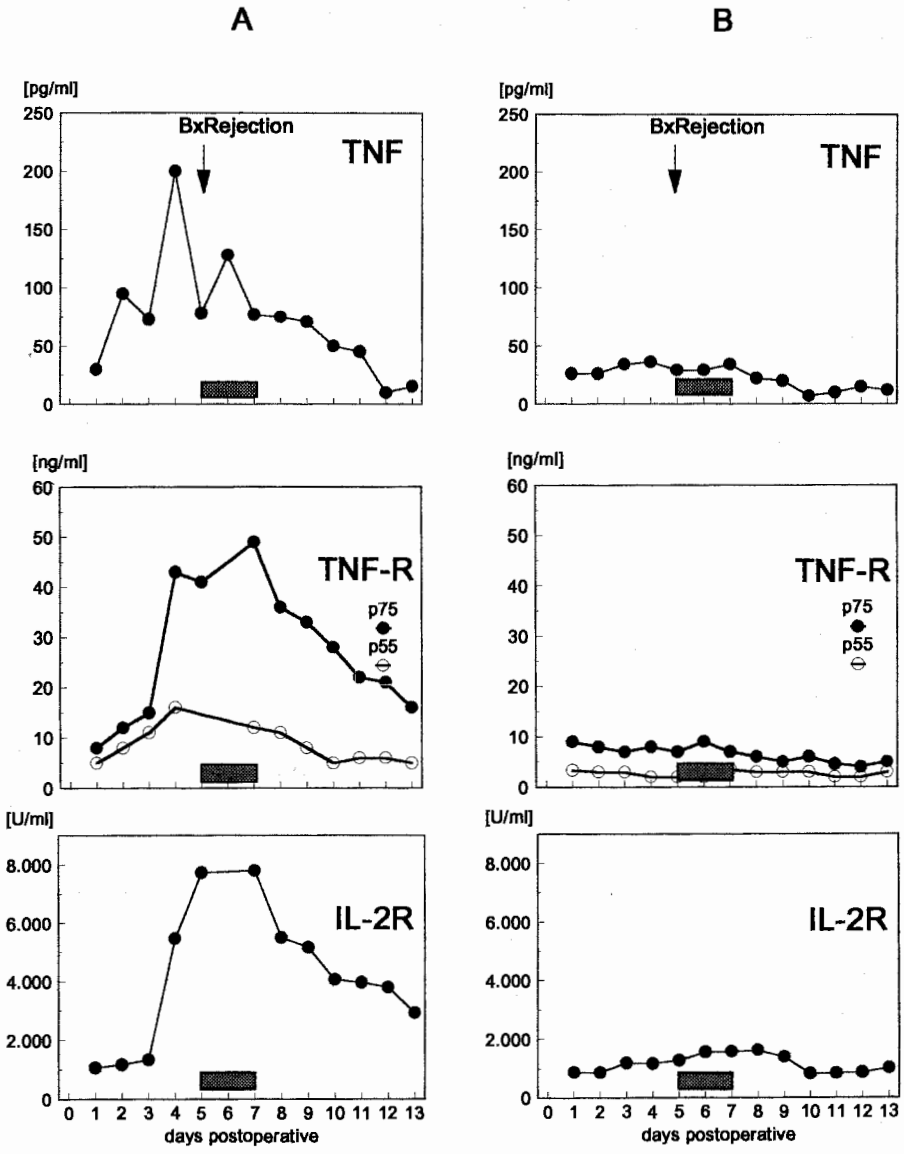

Fig 1. Follow up of TNF, TNF-R p55, and p75 and IL-2R serum levels of a patient after liver (Fig 1A) and a patient after heart (Fig 1B) transplantation during histologically diagnosed graft rejection (⟶ BxRejection). ▦ represents steroid bolus therapy (500 mg methylprednisolone/d for 3 days).

DISCUSSION

Activation of immune cells is an early event in allograft rejection.[29,30] Cytokines such as IL-1, IL-2, and TNF mediate differentiation and proliferation of T-cells and macrophages, which are responsible for graft rejection.[31] TNF induces a series of immunologic events, including expression of adhesion molecules, activation of T-lymphocytes, induction of further cytokine release, and neutrophil tissue infiltration.[2] Infiltration and activation of the target cytotoxic effector cells lead to subsequent vascular leakage and tissue destruction.

Increased serum levels of TNF have been reported in patients with endotoxinemia,[32] severe septecemia,[33] meningococcal disease,[34] and parasite infection.[35] Furthermore, effects of experimentally induced endotoxinemia and bacteraemia could be counteracted by antibodies to TNF in animal models.[36,37] Even though the involvement of TNF in graft rejection has been clearly demonstrated by immunohistochemical[1] and antibody studies,[14] measurements of its serum levels during rejection episodes are inconclusive in both liver-[7] and heart-transplant patients.[17,38] In agreement with earlier reports,[15] the present study showed a strong elevation of TNF levels in liver-transplant recipients, which reached a peak and diminished within the next 2 to 3 days (Fig 2). A cyclic release of TNF has been reported during experimental inflammation.[39] Furthermore, immunohistochemical studies showed no further increase in the number of TNF-positive cells with increasing severity of rejection.[1] The weak correlation between TNF serum levels after tissue rejection in some previous studies is in contrast to our

Table 2. Comparison of Serum Cytokine Levels of Patients After Heart Transplantation With Stable Graft, Graft Rejection, and Infection Complication

	Stable graft n = 7	Organ rejection n = 17	Infection n = 10
TNF [pg/mL]	15.0 ± 7.3 (3.0–27.0)	18.0 ± 11.3 (4.0–47.0)	69.1 ± 60.9† (14.0–182.0)
TNF-R p55 [ng/mL]	3.9 ± 1.1 (2.0–5.8)	3.8 ± 3.1 (1.0–8.8)	9.2 ± 5.9*† (3.0–21.6)
TNF-R p75 [ng/mL]	5.9 ± 2.1 (2.2–9.6)	7.4 ± 3.1 (3.2–17.0)	18.7 ± 10.8*† (7.0–36.8)
IL-2-R [U/mL]	827 ± 314 (375–1313)	1296 ± 484 (647–2569)	3437 ± 2937* (797–8841)
IL-6 [pg/mL]	21.3 ± 15.6 (3.0–54.0)	23.2 ± 20.1 (2.0–73.0)	265.1 ± 268.7*† (15.8–1150.0)
IFN-gamma [U/mL]	0.01 ± 0.04 (0.0–0.1)	0.15 ± 0.46 (0.0–2.3)	0.81 ± 1.01 (0.0–2.9)

n = Numbers of all episodes documented.
*Significantly different from stable graft, $P < .05$.
†Significantly different from organ rejection, $P < .05$.
Values in parentheses are ranges.

data. This may be due to the fact that TNF was not monitored continuously in these studies, and the TNF peak was missed.

In response to TNF release, soluble TNF-Rs are expressed and released from the cell surface of various cells, particularly macrophages and lymphocytes. Because soluble TNF-receptors inhibit the biological activity of TNF both in vivo and in vitro, release of these receptors may control the serum levels of active TNF.[18,19] In our study, serum TNF-R levels increased prior to confirmation of organ rejection histologically and decreased within about 1 week. In those patients in whom blood samples are drawn at a stage when serum TNF levels have already declined, TNF-R levels may serve as useful diagnostic parameters for rejection. The stronger increase of TNF-R p75 compared with TNF-R p55 is probably due to different expression of TNF-Rs by various cell types. While TNF-R p75 is primarily expressed by cells from myeloid origin, TNF-R p55 is mainly expressed by epithelial-like cells.[40] This is supported by the findings of Aderka et al[22] and Digel et al,[41] who found a stronger increase in TNF-R p75 serum levels in patients with solid tumors and leukemia, respectively. The similar increase of both receptors during endotoxinemia[42] and inflammation[20] suggests an involvement of both epithelial and immune cells. Higher elevation of TNF-R p75 serum levels than of TNF-R p55 during rejection in liver transplant patients in our study may suggest involvement of immune cells.

Because of low serum levels and short biological half-life, measurements of IL-1 and IL-2, which may also be released into the circulation during rejection, are of limited diagnostic value in transplanted patients.[7,17] However, serum concentrations of soluble IL-2-R, which are expressed and released by activated peripheral mononuclear cells, are significantly increased in liver-transplant patients with graft rejection (Table 1), as shown also in previous studies.[7,11–13]

In contrast to Chollet-Martin et al,[16] but in agreement with Sabokbar et al,[38] no correlation between serum cytokine levels and tissue biopsy in patients after heart transplant rejection was found in the present study. Furthermore, even measurements of cytokines in blood samples collected directly from the coronary sinus in heart transplant patients showed no further increase of cytokines during rejection episodes.[43] It is thus possible that the immune response during rejection of heart transplants is too small to cause systemic elevation in cytokine levels.

Because transplant patients are under immune-suppressive therapy, infections are a major clinical problem in addition to graft rejection in the postoperative phase. However, the discrimination between infection and graft rejection on the basis of only clinical symptoms is often difficult, and specific immune markers to discriminate between these two complications are not yet available. Although several cytokines such as TNF, IL-1, IL-2, IL-6, and IFN-gamma are involved in host response to infections, only IL-6 has been described as a marker for infectious complications in liver transplants.[26] Because no increase of IL-6 levels could be noted during rejection episodes, this cytokine may serve as a marker for distinguishing between rejection and infection in liver transplant recipients. However, in those cases with rejection and concomitant additional infection, IL-6 levels are also increased, and therefore our data also confirm these findings of serum levels of IL-6 have a limited diagnostic value in these patients.

In contrast, no definite pattern of cytokines was observed in patients who rejected heart transplants. Nevertheless, serum cytokine levels might be helpful for the exclusion of infection complications because increases during infection were significant.

Although TNF increase during rejection is much stronger than during infection (Table 1), and TNF is known as a strong stimulus for IL-6 production, no increase of IL-6 could be noted during rejection of liver transplants. Because steroids have been shown to inhibit IL-6 expression,[44] steroid bolus therapy after diagnosed rejection may contribute to the lack of IL-6 increase during rejection. In addition, release of certain inhibitory cytokines may be responsible for low IL-6 levels during rejection.[26] Because soluble TNF-Rs are greatly increased during rejection as compared with infection, the inhibitory function of TNF-Rs on TNF bioactivity[18,42] might be an additional explanation for low IL-6 levels during rejection.

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