

## Daily Serum Inflammatory Cytokine (Tumor Necrosis Factor- $\alpha$ , Interleukin-6) Monitoring in Liver Transplantation Focusing on Allograft Rejection: A Five-Case Report

Y. Kita, Y. Iwaki, K. Noguchi, A.G. Tzakis, S. Todo, J.J. Fung, and T.E. Starzl

**L**IVER allograft rejection is diagnosed largely based on liver function tests indicating graft damage associated with rejection, but histopathological diagnosis using biopsy is still necessary. Since it can be difficult to distinguish rejection from other causes of graft damage in the absence of liver histopathology, detection of a marker for immune activation and a mediator of inflammation could provide a noninvasive method of diagnosing rejection.

Several studies have shown that the postoperative course of inflammatory cytokines such as tumor necrosis factor (TNF)- $\alpha$ <sup>1</sup> is predictive of rejection after human orthotopic liver transplantation. We have also previously shown that daily monitoring of serum or plasma interleukin (IL)-6 levels can be useful in predicting long-term graft prognosis.<sup>2</sup> However, few reports compare cytokine profiles after liver transplantation.

Therefore, in this study, we monitored plasma and serum levels of cytokines in five transplant recipients who eventually lost liver grafts because of relapsing rejection episodes.

### SUBJECTS AND METHODS

FK 506 or cyclosporine (CyA), to which variable doses of methylprednisolone were added, were used in baseline immunosuppression; OKT3 was given as indicated. Using ELISA, we monitored serum levels of IL-2, IL-6, TNF- $\alpha$ , and IL-1 $\beta$  in three liver transplant recipients and two abdominal organ-cluster transplant recipients who had experienced allograft rejection and eventually lost their liver allografts. ELISA involved a two-step sandwich enzyme immunoassay using Quantikine (Research and Diagnostic Systems Inc. Minneapolis, Minn) as described elsewhere.<sup>2</sup> Daily monitored cytokine levels were evaluated and compared to clinical courses and the histopathological diagnosis of rejection. Serum and plasma levels were basically monitored daily around biopsy-proven rejection episodes.

### RESULTS

Serum levels of IL-2, IL-1 $\beta$ , and TNF- $\alpha$  in 30 healthy volunteers were all under detection. However, serum levels of IL-6 of 30 healthy volunteers were  $18 \pm 34$  pg/mL (mean  $\pm$  SD). As shown elsewhere,<sup>2</sup> serum and plasma levels of IL-6 decreased after liver transplantation, regardless of pretransplantation values. Patients with infection subsequently developed continuously high IL-6 levels. In patients without infection, significantly higher levels of IL-6 were usually found prior to the histopathological diagnosis of rejection. IL-6 elevations were spike-shaped, correlated poorly with the histological grade of rejection, and were highly responsive to augmented immunosuppression.

In the five cases of rejection episodes we saw, serum IL-2

and IL-1 $\beta$  levels were not detected during the entire monitoring period. The elevations of serum TNF- $\alpha$  levels were similar to those for IL-6. Clinical courses and evaluations of serum IL-6 and TNF- $\alpha$  levels in five cases were shown as follows.

#### Case 1

A 62-year-old man with alcoholic cirrhosis underwent orthotopic liver transplantation (Fig 1). His pretransplant plasma IL-6 level was high, but it was decreased after liver transplantation. The serum total bilirubin (TB) level also decreased as liver function improved. Liver biopsy on postoperative day (POD) 8 did not show acute cellular rejection (ACR). POD 16 to 17, showing that his liver function had recovered. Serum TB levels increased on POD 18, however, which indicate liver dysfunction due to ACR or some other cause. Liver biopsy performed on POD 19 showed moderate ACR. Soon after the diagnosis, intravenous administration of methylprednisolone ( $1 \text{ g} \times 5$ ) was given. ACR was partially responsive to augmented immunosuppressive therapy, the graft was eventually lost. Concerning serum inflammatory cytokine profiles, spike-shaped elevation of IL-6 ( $>100$  pg/mL) was observed on POD 15. This pattern of elevation is characteristic of daily IL-6 monitoring. Interestingly, an elevation in TNF- $\alpha$  was also observed on POD 12.

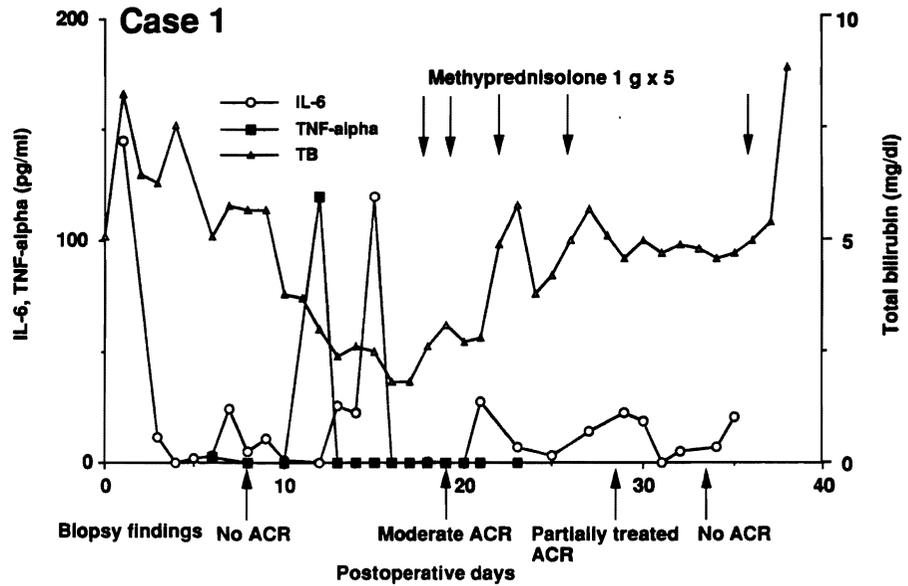
#### Case 2

A 48-year-old man with cryptogenic cirrhosis underwent orthotopic liver transplantation (Fig 2). His preoperative IL-6 level were also high but had normalized about 10 days after transplantation. Serum TB levels increased temporarily after transplantation, possibly due to ischemic injury shown by biopsy on POD 5. Serum TB levels normalized on POD 20. A biopsy undertaken on POD 21 showed mild ACR, and a bolus methylprednisolone (1 g) was administered. Serum TB levels did not increase until POD 30. Biopsy on POD 29 showed severe ACR and soon after the diagnosis, OKT3 monoclonal antibody (10 mg/d) was ad-

From the Department of Surgery (Y.K., A.G.T., S.T., J.J.F., T.E.S.) and Pathology (Y.I., A.J.D., K.N.), University of Pittsburgh, Pittsburgh, Pennsylvania.

Address reprint requests to Thomas E. Starzl, MD, PhD, Department of Surgery, University of Pittsburgh, 3601 Fifth Ave, Pittsburgh, PA 15213.

© 1996 by Appleton & Lange  
0041-1345/96/\$3.00/+0



**Fig 1.** A 62-year-old man with alcoholic cirrhosis underwent liver transplantation. A moderate ACR episode occurred 2 weeks after transplantation.

ministered for 1 week. Eventually, however, the liver graft was lost due to other complications. Regarding the dynamic changes in IL-6 and TNF- $\alpha$ , as in case 1, an IL-6 spike was observed the same day the liver biopsy showed severe rejection. It is to be noted that the TNF- $\alpha$  spike appeared 3 days before the IL-6 spike, as shown in Fig 2.

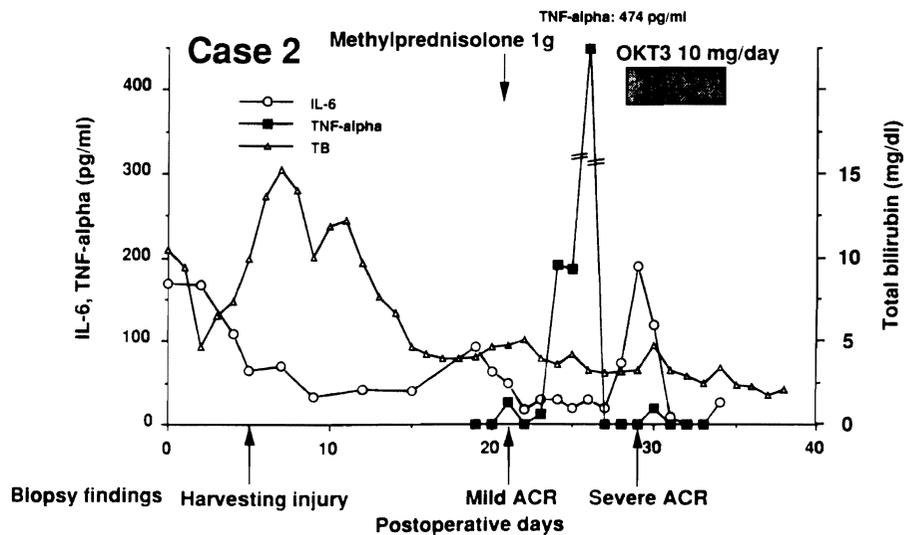
**Case 3**

A 36-year-old man with alcoholic cirrhosis underwent orthotopic liver transplantation (Fig 3). He experienced an early-phase moderate ACR, which was proved by liver biopsy on POD 4. On POD 5, an IL-6 spike was observed and bolus methylprednisolone (1 g) was administered. Since this rejection was treatment-resistant, OKT3 and steroid recycle therapy were tried. Biopsy on POD 19 still showed

mild to moderate rejection. Plasma IL-6 levels were high and the pattern paralleled that of serum TB level. In this case, the TNF- $\alpha$  level was not determined in the earlier phase of rejection, but a very high level of TNF- $\alpha$  was observed after POD 20. Eventually, the graft was lost.

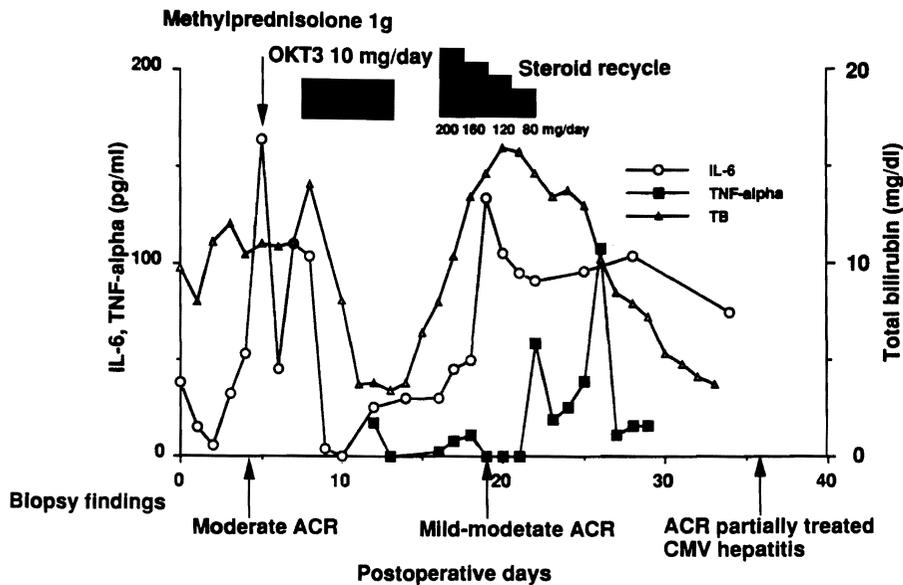
**Case 4**

A 39-year-old woman with somatostatinoma of the pancreas with liver metastasis underwent abdominal organ cluster transplantation (Fig 4). As early as POD 5, rejection was diagnosed clinically due to a progressive rise in serum TB (biopsy not performed) and a methylprednisolone bolus and OKT3 was initiated. This regimen was effective, but serum TB rose again from POD 11. An IL-6 spike was observed on POD 12 when steroid and OKT3 therapy was



**Fig 2.** A 48-year-old man with cryptogenic cirrhosis underwent liver transplantation. He experienced severe late-phase ACR episodes.

### Case 3



**Fig 3.** A 36-year-old man with alcoholic cirrhosis underwent liver transplantation. He had experienced relapsing ACR episodes.

restarted. This rejection was confirmed by biopsy subsequently on POD 13 indicating moderate ACR. TNF- $\alpha$  spikes were observed on POD 4 and 9. TNF- $\alpha$  spike on POD 4 is 1 day before the initiation of augmented antirejection therapy.

#### Case 5

A 55-year-old man with pancreatic carcinoma and liver metastasis underwent abdominal organ cluster transplantation. The clinical course was complicated by relapsing ACR and subsequent sepsis (Fig 5). In this case, the graft was eventually lost. In the earlier periods (POD 10 and 20), two IL-6 spikes (POD 15 and 19) and one TNF- $\alpha$  spike (POD

17) were observed. These elevation of cytokines seem very closely related to serum TB and biopsy findings (POD 15: moderate ACR, POD 22: mild to moderate ACR, POD 29: moderate ACR).

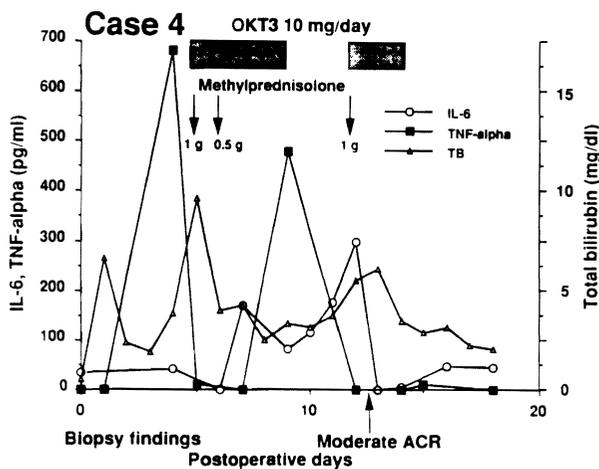
#### DISCUSSION

In this study we have shown that inflammatory cytokines (TNF- $\alpha$ , IL-6) are induced in liver allograft rejection. Daily monitored serum levels of these cytokines are potentially a good premonitor of allograft rejection.

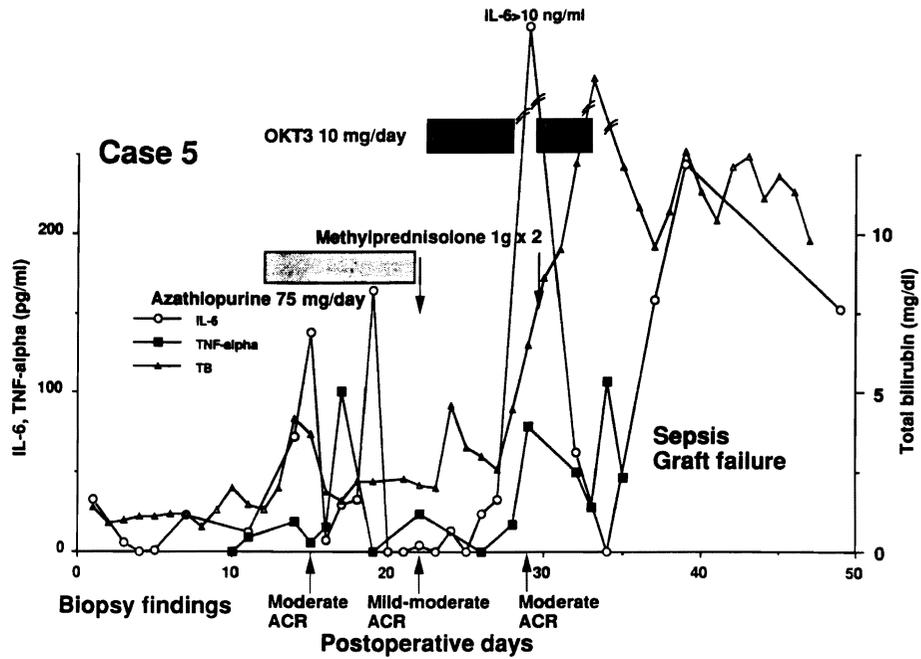
Liver allograft rejection is characterized by cellular infiltration consisting mainly of monocytes/macrophages and activated T lymphocytes. Inflammatory cytokines such as TNF- $\alpha$  and IL-6 are mainly secreted by these cells. Kupffer cells and vascular endothelial cells in the liver may also secrete these cytokines. Therefore, both infiltrating host immune cells or cells of the allograft (activated Kupffer cells and endothelial cells) conceivably play a role in producing these cytokines.<sup>3</sup>

In conventional clinical procedures, liver rejection is first determined through liver dysfunction, then histologically confirmed by biopsy, after which antirejection therapy is started. In this study, we observed spike-shaped TNF- $\alpha$  elevation followed by the elevation of IL-6 (Fig 6). It is well known that TNF- $\alpha$  induces IL-6 secretion, so, theoretically, TNF- $\alpha$  appears earlier in the bloodstream than IL-6. It is also well known that TNF- $\alpha$  is very difficult to detect in the blood. The five cases we presented all suffered from aggressive rejection, and therefore TNF- $\alpha$  could be detected, which is unusual under other circumstances. We attempted to measure TNF- $\alpha$  in other cases of rejection, but found it impossible (data not shown).

TNF- $\alpha$  may also play an important role in the pathophys-



**Fig 4.** A 39-year-old woman with pancreatic somatostatinoma and liver metastasis underwent abdominal organ cluster transplantation. She experienced moderate ACR.

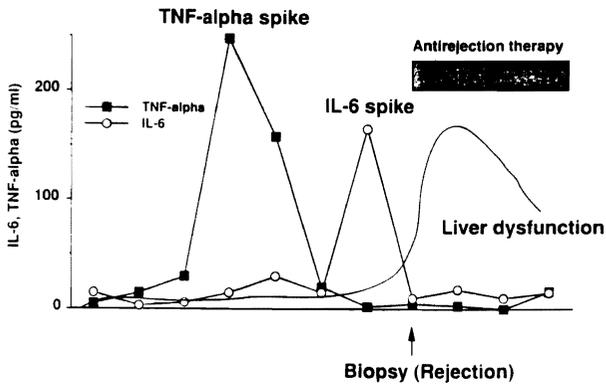


**Fig 5.** A 55-year-old man with pancreatic carcinoma and liver metastasis underwent abdominal organ cluster transplantation. He experienced relapsing ACR episodes 2 weeks after transplantation, and eventually suffered from sepsis and liver graft failure.

iology of reperfusion injury in liver transplantation. Takei et al<sup>4</sup> reported that Kupffer cells were activated following cold preservation, and inflammatory mediators were released from rat liver grafts after reperfusion. Goto et al<sup>5</sup> reported that the release of TNF- $\alpha$  by the rat liver graft depended on the period of preservation. Thus, the activation of Kupffer

cells plays an important pathophysiological role in graft damage after reperfusion.

The present results indicate that inflammatory cytokines such as TNF- $\alpha$  and IL-6 play an important role in the early phase of the rejection episodes. These cytokines are known to upregulate the expression of adhesion molecules such as ICAM-1, thereby facilitating leukocyte adhesion to sinusoidal endothelium and subsequently causing graft damage mainly due to circulatory failure.<sup>6,7</sup> As we previously noted, the more IL-6 spikes demonstrated in recipients, the higher the chance of graft loss.<sup>2</sup> Therefore, specific inactivation of cytokines or of their actions may prove to be a powerful tool in the prevention and treatment of allograft rejection and graft failure.



**Fig 6.** Schematic presentation of serum elevation of TNF- $\alpha$  and IL-6 in rejection episodes in liver allograft recipients. Usually, TNF- $\alpha$  and IL-6 spikes appeared prior to liver dysfunction. Clinically, when liver dysfunction appears, liver biopsy is performed. After histological confirmation, antirejection therapy is initiated.

REFERENCES

1. Imagawa DK, Millis JM, Olthoff KM, et al: *Transplantation* 50:219, 1990
2. Kita Y, Iwaki Y, Demetris AJ, et al: *Transplantation* 57:1037, 1994
3. Hoffmann MW, Wonigeit K, Steinhoff G, et al: *Transplantation* 55:329, 1993
4. Takei Y, Marzi I, Kaulfman FC, et al: *Transplantation* 50:14, 1990
5. Goto M, Takei Y, Kawano S, et al: *Hepatology* 16:487, 1992
6. Adams DH, Wang LF, Burnett D, et al: *Transplantation* 50: S6, 1990
7. Steinhoff G, Behrend M, Schrader B, et al: *Am J Pathol* 142:481, 1993