

RAISED SERUM LEVELS OF CACHECTIN/TUMOR
NECROSIS FACTOR α IN RENAL ALLOGRAFT REJECTION

BY C. P. J. MAURY AND A.-M. TEPPÖ

From the Fourth Department of Medicine, University of Helsinki, Helsinki, Finland

Cachectin, or tumor necrosis factor (TNF) α is a macrophage-derived cytokine implicated in host defense against tumor cell growth and parasitic infection (1, 2). Experimental studies have suggested a role for TNF also in shock (3, 4) and in the metabolic processes that lead to cachexia (1, 5, 6). Raised serum levels of TNF were recently demonstrated in patients with severe septicaemia (7) and in patients with kala-azar and malaria (8).

The range of stimuli that induce the production of TNF is not known in detail. Infectious agents and their products have been identified as TNF inducers (1, 2), but the *in vivo* role of noninfectious factors is not clear. In this study, the serum levels of TNF were monitored using a sensitive radioimmunoassay in 10 renal transplant recipients. The results show that marked elevations of TNF occur during episodes of renal allograft rejection.

Materials and Methods

Serum samples were collected daily for 3–4 wk from 10 patients (2 women, 8 men; mean age 39.4 yr, range 23–62 yr) after cadaveric renal transplantation. The underlying renal disease was chronic glomerulonephritis in five patients, chronic interstitial nephritis in two patients, diabetic nephropathy in two patients, and secondary amyloidosis in one patient. The patients received azathioprine and methylprednisolone as basic immunosuppressive medication. Episodes of rejection were managed by increasing the dosage of methylprednisolone, and in four patients by changing azathioprine to cyclosporine. The effect of uremia and hemodialysis treatment on the TNF levels were studied in 10 patients with end-stage kidney disease, receiving no immunosuppressive medication (7 women, 3 men; mean age 38.1 yr, range 15–65 yr). 40 voluntary blood donors (14 women, 26 men; mean age 40.2 yr, range 26–55 yr) served as normal controls.

Rejections were diagnosed on the basis of clinical findings and the results of serial transplant fine-needle aspiration biopsies (9). The rejection diagnoses were made without knowledge of the results of the TNF assays. 10 rejections were documented; 1 of them was irreversible, 2 patients had no rejections, and 2 had two rejection episodes. Infections were documented by cultures, virus isolation, and specific antibody determinations.

Serum TNF was measured by a double-antibody radioimmunoassay, in which TNF in serum competes with a fixed amount of ^{125}I -labeled TNF for the binding sites of specific rabbit antibodies. In brief, 0.1 ml of standards or sera and 0.05 ml of 80,000-fold diluted rabbit antiserum to recombinant human TNF (a gift from Dr. G. R. Adolf, Ernst-Boehringer-Institut für Arzneimittelforschung, Wien, Austria) were mixed and incubated at room temperature overnight. 0.05 ml of 1,000-fold diluted tracer solution ($\sim 5,000$ pg/ml, 8,000 counts/min for 50 μl) was added and incubated for 5–6 h at room temperature. Bound and free TNF were separated by adding 0.5 ml of Decanting

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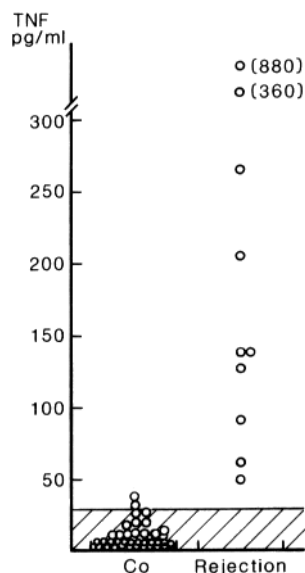


FIGURE 1. Serum immunoreactive TNF levels in blood donors (Co) and during 10 acute allograft rejections (peak levels) in 8 renal transplant recipients. The highest level was associated with an irreversible rejection. The hatched area represents the reference range (mean \pm 2 SD of controls).

suspension 3^R (Micro Sepharose-anti-rabbit IgG; Pharmacia Fine Chemicals, Uppsala, Sweden) and by incubating at room temperature for 30 min followed by centrifugation. The radioactivity of the pellet was then measured. The determinations were done in duplicate. In each series, nine standard solutions of recombinant human TNF (a gift from Dr. Adolf) ranging from 15 to 10,000 pg/ml were included. The sensitivity of the assay is 7 pg/ml and the method can be used to measure concentrations up to 10,000 pg/ml. The intraassay coefficients of variation are 8.2 and 6.3% at concentrations of 15 pg/ml ($n = 12$) and 92 pg/ml ($n = 10$), respectively, and the interassay coefficients of variation are 9.4 and 7.0% at the same concentrations ($n = 9$).

Results

The serum levels of immunoreactive TNF were low (median <10 pg/ml) in healthy subjects (Fig. 1). During acute renal allograft rejections, marked elevations of TNF occurred (median peak level, 140 pg/ml) (Fig. 1). The highest level (880 pg/ml) was found in a patient experiencing a febrile irreversible rejection (Fig. 2D). The results of the daily monitoring of TNF levels in two patients with reversible rejection episodes are illustrated in Fig. 2, B and C. Peak levels of TNF were usually found at the time of the clinical diagnosis of rejection, although raised levels were observed already 1 or 2 d earlier. Fig. 2A shows the TNF curves in two renal transplant recipients experiencing no rejections. After a moderate initial rise in TNF on postoperative days 2 and 3, which was probably induced by the operation itself, TNF remained close to baseline levels (<10–50 pg/ml). The levels of TNF were <40 pg/ml in nontransplanted uremic patients on regular haemodialysis treatment, and dialysis did not significantly influence the level.

Taking a cut-off serum level of 100 pg/ml, there were three negative rejections and eight elevations of TNF \geq 100 pg/ml that were not associated with rejection. Three of the latter were predictable, occurring on postoperative days 2 or 3. Analysis of the remaining five elevations \geq 100 pg/ml showed that two were associated with signs of immunoactivation in concurrent transplant aspiration

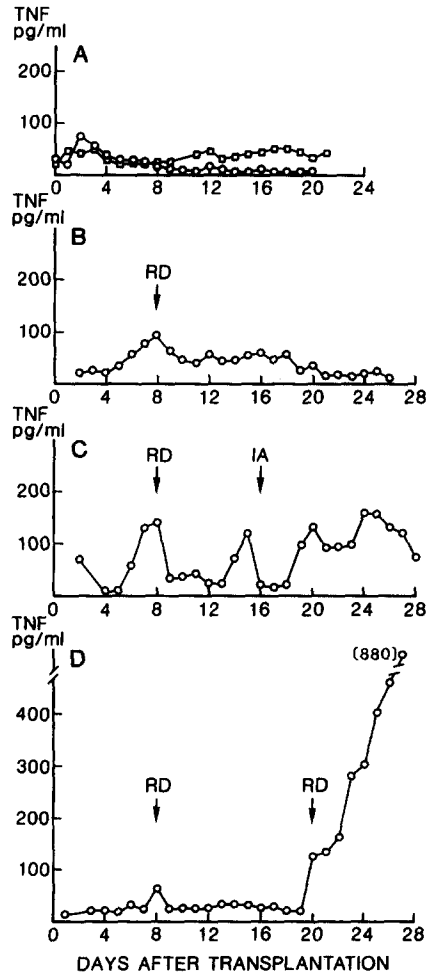


FIGURE 2. Results of daily monitoring of serum immunoreactive TNF levels in five renal transplant recipients. (A) TNF levels in two recipients experiencing no rejection episodes. (B) TNF levels during a febrile rejection episode clinically and cytologically diagnosed (RD) on posttransplant day 8. (C) TNF levels in a patient with a febrile rejection episode diagnosed on day 8 (RD). The TNF elevation peaking on posttransplant day 15 was associated with signs of immunoinactivation (IA) in a transplant aspiration biopsy specimen taken on day 16. The episode was not treated and was not associated with fever. The cause of the TNF elevation during days 19–28 was unclear; fever occurred during days 21–23. (D) TNF levels in a patient with irreversible febrile rejection diagnosed on posttransplant day 20. A mild rejection episode was diagnosed on posttransplant day 8.

biopsies, one was associated with a herpes simplex infection and signs of immunoinactivation in aspiration biopsy samples, one was associated with a febrile *Klebsiella pneumoniae* infection, and one with a fever episode of unknown etiology.

Discussion

The finding of increased circulating levels of TNF during episodes of acute allograft rejection has implications both with respect to the mechanisms of rejection as well as the mechanisms involved in the regulation of TNF production in general. Studies with monoclonal antibodies to cell surface antigens have shown that T cells and mononuclear phagocytes predominate in the rejecting graft (10, 11). It is thought that donor antigen-induced activation of the mononuclear phagocytes leads to the release of IL-1, which activates T cells to produce IL-2 and to express IL-2 receptors (12, 13). IL-2 is required for the clonal expansion of cytotoxic/suppressor T cells implicated in graft rejection. Our results suggest that not only IL-1 but also TNF is released by the activated

mononuclear phagocytes in the graft. This has impact on the inflammatory process, since recent studies show that TNF activates neutrophils (14, 15), enhances prostaglandin production (16), stimulates production of procoagulant activity (17, 18), stimulates fibroblast growth (19), and increases the surface expression of class I major histocompatibility complex antigens (20). Moreover, TNF is a potent pyrogen (21) and regulates the hepatic acute-phase protein gene expression (22). Thus, several of the local and systemic manifestations observed in patients with acute renal allograft rejection could be explained on the basis of TNF release. In fact, the question arises whether the release of TNF from the activated mononuclear phagocytes is a primary event, since under experimental conditions TNF production may amount to ~1% of the total secretory products of macrophages (5), and TNF has been shown to induce the release of IL-1 from monocytes (21) and endothelial cells (23).

Glucocorticoids are known to inhibit TNF gene transcription and prevent its synthesis (1, 24). The transplant patients received methylprednisolone and azathioprine as basic immunosuppression, and rejection episodes were managed by increasing the methylprednisolone dosage, so medication may have influenced the absolute levels of TNF. In a few patients, a rapid decline in TNF levels was noted after starting the high-dose steroid regimen, which could be explained by a direct suppression of TNF synthesis.

Two recent studies in man have demonstrated elevated levels of TNF in infectious diseases. Scuderi et al. (8), using an enzyme immunoassay, found a mean TNF level of 119 pg/ml in patients with kala-azar and malaria, and Waage et al. (7), using a bioassay, found raised TNF levels in patients with septic meningococcal disease. In the septicemic patients, serum TNF levels over 440 U/ml (corresponding to 100 pg/ml) were associated with a poor outcome. It is noteworthy that the peak levels of TNF during renal allograft rejection (median 140 pg/ml), as measured by radioimmunoassay, were in the same concentration range as seen during the infectious states. Thus the release of TNF into circulation in man is not confined to severe bacterial and parasitic infections, but occurs also as a result of noninfectious immunologic tissue injury. Our results are compatible with the view that TNF, like IL-1, is a regulatory monokine that has a basic function as a mediator of inflammation.

Summary

A sensitive radioimmunoassay was used for monitoring serum levels of endogenous cachectin/tumor necrosis factor α (TNF) in 10 renal transplant recipients. Acute allograft rejections were associated with marked elevations of circulating TNF. The peak levels of TNF (median 140 pg/ml) were in the same concentration range as previously reported in parasitic infections. The results show that the release of TNF into circulation is an early event in renal allograft rejection and that raised levels of TNF in man can also be induced by noninfectious stimuli.

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