The Role of Tumor Necrosis Factor in Allograft Rejection

Zheng-Gang ZHU1,2), Toshimasa ASAHARA1), Hiroshi YAHATA1), Eiji ONO1), Seiji MARUBAYASHI1), Hiroshi WATANABE1), Yasuhiro FUKUDA1) and Kiyohiko DOHI1)

1) The Second Department of Surgery, Hiroshima University, School of Medicine, Kasumi 1-2-3, Minami-ku, Hiroshima 734, Japan
2) The Department of Surgery, Rui Jin Hospital, Shanghai Second Medical University, Shanghai 200025, China

Key words: Tumor necrosis factor (TNF), Allograft rejection

Despite the many achievements in allotransplantation, the major problems are still the lack of early and reliable markers as well as the shortage of effective immunosuppressive agents for graft rejection. Up to now, allograft rejection is usually diagnosed by relatively nonspecific markers of inflammation or on the basis of organ function tests. The definitive standard remains histological examination of tissue from the biopsy of graft. However, increased knowledge of the cytokines and the development of specific and accurate assays have recently led to the possibility of analyzing immune rejection using less invasive procedures. Cytokines, as major components of both specific cellular and humoral immune responses, are mediators of cellular communication. The research of cytokines has been a field of extraordinary development in organ transplantation during the last decade. Recent studies have revealed that Tumor Necrosis Factors (TNFs), one kind of major cytokines, are not only limited to necrosing tumor cells, but play a very important role in the initiation and regulation of immunoreaction on allograft.1,4,15,22).

Bioimmunochemically, TNFα / cachectin and TNFβ / lymphotoxin represent two closely linked cytokines with cytostatic and cytotoxic activity in mediating the pathogenesis of acute rejection during the course of post-transplantation. TNFs mediate beneficial or deleterious biological effects depending on the quantity produced, the duration of release, and biochemical milieu of the responding cells.39). TNFα is mainly synthesized from activated monocytes or macrophages while TNFβ is produced from stimulated T-lymphocytes and some transformed B-lymphoblastoid cells. These molecules share 36% identity and 51% homology in their overall aminoacid sequences. They also share common receptor binding domains as well as exert similar immunologic effects. The major difference in these two compounds is that TNFβ is a glycoprotein while TNFα contains no carbohydrate. Gene structure analysis demonstrates that both these molecules were derived from a similar ancestral gene and they may be highly conserved across species.7,11,21,39). A large number of papers reported that elevated TNFs level could be found in serum, urine and graft tissue before or during rejection episodes in recipients following organ allotransplantation. On the other hand, a significant effect following anti-TNFs therapy alone or a synergistic response in combination with immunosuppressive agents have been successfully applied as new approaches to immunosuppression for allotransplantation. In this article, the authors will review the beneficial effectiveness of predicting rejection by measuring TNFs level and the therapeutic potential of anti-TNFs treatment in allotransplantation.

I. Determination of TNFs levels in early diagnosis of allograft rejection

1. For the hepatic allotransplantation

Imagawa DK et al prospectively studied 50 adult patients following orthotopic liver transplantation, serial plasma concentrations of TNFs were determined by using a micro ELISA technique. TNFα levels were elevated 1–2 days before the clinical diagnosis of rejection, although its peak levels occurred at the time of rejection. The mean plasma TNFα level was significantly higher in patients experiencing a rejection episode (942+/-83 pg/ml), as a control; samples taken from nonrejecting liver transplants were 240+/-6 pg/ml only (p=0.0001). The usefulness of first-week peak TNFα levels was also examined in predicting ultimate graft outcome, twenty-two patients with normal liver function had peak levels of 581+/-93 pg/ml during the first postoperative week. In the 9 patients with graft failure had peak TNFα levels of 2146+/-766 pg/ml (p=0.004). In addition, using the logistic regression of first-week peak TNFα levels, the probability of graft loss could be precisely predicted18). Sankary H's address for correspondence: Kiyohiko Dohi, M.D., Ph.D. Professor of the Second Department of Surgery, Hiroshima University School Medicine, Kasumi 1–2–3, Minami-ku, Hiroshima 734, Japan
study demonstrated that serial determination of peripheral blood 
TNFβ may also aid the diagnosis of hepatic allograft rejection early on. In a 
univariate analysis, comparisons between changes in 
serum TNFβ levels and values of other param-
eters including total leukocyte count, total biliru-
bil, alkaline phosphatase and AST showed that the 
elevation of serum TNFβ level preceded the 
clinical syndrome by at least 1 day. There was 
less change of TNFβ in patients not experiencing a 
rejection episode (0.77+/−0.03, n=301) as 
compared with those patients with rejection 
(4.09+/−1.3, n=12) (p<0.001). All other parameters 
were of no significance for diagnosing early 
rejection. Another clinical study suggested that 
an intraoperative elevation of TNFα could be of 
predictive value in early rejection reaction. Preopera-
tive TNFα values were not different in pa-
tients developing rejection (n=8) compared with 
those without rejection (n=20). After re-establish-
ment of circulation in the graft, the median TNF-
α level in the rejection group was 18.0 pg/ml, 
which rose to a median of 100 pg/ml at the end of 
surgery, patients who did not develop any re-
jection revealed a median TNFα level at the end of 
surgery of 11.5 pg/ml (p=0.004). With a cutoff 
value of 100 pg/ml for TNFα, development of re-
jection could be predicted in 23/28 (82%), an error 
in 5 of 28 (18%)22.

2. For the cardiac allotransplantation

Lowey and Blais first demonstrated the pres-
ence of TNFα in rejecting rat cardiac allograft in 
1988. By establishing heterotopic heart transplant 
model (WE to LEW), they used a potent 
neutralizing antiserum to recombinant murine 
TNFα (rMu TNFα), together with the recombi-
nant product itself, to measure quantitatively se-
rum TNFα activity of rejecting rat cardiac 
allografts and culture supernatant of graft infil-
trating cells respectively. The results strongly 
verified that TNFα is released by cells of mono-
cyte/macrophage lineage infiltrating in rejecting 
grafts24. From the clinical standpoint, cardiac 
transplantation is now an accepted therapy for 
end-stage heart disease, graft rejection remains a 
serious problem, the diagnosis of rejection is pres-
ently mainly based on endomyocardial biopsy (EMB). However, this procedure is invasive, and 
early findings in the rejection process are still dif-
cult. Chollet-Martin S et al recently reported a 
marked elevation of plasma TNF level during epi-
isodes of cardiac allograft rejection in human be-
ings. The plasma level of immunoreactive TNF 
level were always below 170 pg/ml (124.1+/−5.8 
pg/ml) in 34 healthy subjects. By contrast, in-
creased TNF plasma levels were observed during 
all 8 episodes of rejection in 6 heart transplant 
recipients. The mean of the peak TNF levels dur-
ing rejection was 607.0+/−42.0 pg/ml (210−1200 
pg/ml). In all of these patients, high TNF levels 
were concomitant with persistent abnormalities 
in EMB and echocardiography despite antirejec-
tion therapy8. In conclusion, increase TNF plasma 
levels occurred at an early stage of rejection 
and persisted for as long as abnormalities on 
EMB and echocardiography. To assay the plasma 
TNF level with noninvasive technique may be of 
considerable clinical interest in assisting with 
early detection and monitoring of heart trans-
plant rejection.

3. For the renal allotransplantation

In a cadaveric renal transplantation study, 
Maury and Teppo, using a sensitive double-anti-
body radioimmunoassay, demonstrated a median 
peak TNFα level of 140 pg/ml during 10 episodes 
of acute rejection in 8 renal transplant recipients. 
By comparison, healthy subjects had median lev-
els of <10 pg/ml. The elevation of TNFα levels 
after surgery was observed 1–2 days prior to the 
clinical diagnosis of rejection, and the highest lev-
el (880 pg/ml) was associated with an irreversible 
rejection25. Similarly, McLaughlin et al eval-
uated the sequential changes of plasma and uri-
inary TNFα levels in renal allograft recipients, 
found that most rejection episodes (24/37, 65%) 
were associated with detectable plasma TNFα on 
the day of initiation of treatment or during the 3 
previous days (mean level, x=135 pg/ml, range 
80–5000 pg/ml), and high urinary TNFα was also 
presented in almost half of these cases (18/37, 
49%; x=53 pg/ml, range 80–2000 pg/ml). In most 
cases, urinary TNFα was detected concomitantly 
with plasma TNFα. Contrast sharply with blood 
donors or patients prior to transplantation, not 
only a low incidence of detectable plasma TNFα 
(20%, x=62 pg/ml, range <80–625 pg/ml; and 
x=65 pg/ml, range <80–2000 pg/ml, respectively) 
but also a negative urinary TNFα was detected. 
In this study, systemic infection also gave rise to 
a high circulating level of TNFα. However, uri-
inary TNFα was not found consistently with infection 
periods26. Noroha et al extended their research to the expression of cytokines and their 
receptors in thirty-six renal allograft biopsies by 
means of an immunocytochemical assay. Numerous 
TNFα and TNFβ positive infiltrating mono-
nuclear cells were detected in all cases of acute 
rejection. No-rejection and chronic vascular rejec-
tion cases showed isolated TNFs-positive cells 
scattered throughout the interstitial. Both P75 
and P55 forms of TNF receptors (TNF-R) were 
expressed in infiltrating cells, as well as in glom-
erular and arterial endothelial cells in cases with 
acute rejection, and to a minor degree in no rejec-
tion and chronic rejection. The results showed 
that TNFs are produced in situ in acute cellular
rejection, reflecting marked immunologic activation. These findings support the hypothesis that TNFs play an important role in mediating allograft injury. TNFs expression in allograft biopsies is also the most reliable marker to differentiate acute cellular rejection from other abnormalities.49.

4. For the lung allotransplantation

In lung transplantation, bronchoalveolar cells (BAC) are thought to play an important role in the regulation of pulmonary immune response. The cellular and acellular components obtained from bronchoalveolar lavage fluid (BALF) reflect the immune responses in the lung more directly and precisely. Chang SC et al serially measured BALF levels of cytokines, including TNF, aiming at evaluating these biochemical markers in the early detection of canine lung allograft rejection in comparison with chest roentgenograms. The animals were divided into three groups. In Group 1, control group, (neither donor nor recipients were treated with cyclosporine) and Group 2, CsA-pretreated group, (only donors were treated with CsA orally at a single dose of 20 mg/kg/day for 3 days prior to transplantation), the BALF levels of TNF obtained from rejecting grafted lungs were significantly higher than those of normal and uncomplicated native lungs in both groups, and increased progressively with time. Pulmonary infection was found in the native lungs of two dogs in Group 2, TNF levels were also markedly increased. However, the titers were less than those obtained from samples harvested from rejected grafted lungs at the same time. BALF levels of TNF from the grafted lungs did not significantly differ from those obtained from normal, native lungs in dogs of Group 3 (only recipients were treated with CsA orally at a single dose of 20 mg/kg/day for 9 days after transplantation). After discontinuation of CsA therapy, the TNF levels from the graft began to increase. However, elevation of this marker could be measured 4-6 days ahead of the appearance of abnormal findings in grafted lungs detected by chest X-ray films. They were also highly correlated with the severity of the rejection response. Taken together, these studies strongly suggest that measurement of TNF on BALF level may serve as a useful means of monitoring the immunologic status of the canine lung allografts and predicting allograft rejection.5,6. Sato R et al confirmed through their experimental research that TNF is a major mediator of lung allograft rejection. First, the level of TNFα mRNA isolated from rejecting lung allografts showed a progressive increase with the development of rejection. Second, immunofluorescence studies with anti-TNF antibodies showed a marked increase in binding to mononuclear cells infiltrating rejecting allografts, but minimal immunofluorescence staining of syngeneic grafts or contralateral native lungs was seen. Finally, anti-TNFα and anti-TNFβ were synergistic in their ability to suppress necrosis, interalveolar hemorrhage and vasculitis of lung allografts.32.

5. For the pancreas allotransplantation

To determine whether TNF levels are predictive of rejection episodes in pancreas transplants, Grewal HP et al measured TNF levels and interleukin-6 (IL-6) on six pancreas transplant recipients intraoperatively and postoperatively. The peak TNF levels in 3 patients who experienced rejection episodes (mean 583 pg/ml, range 323 to 997 pg/ml) were significantly higher than those who did not (mean 22.3 pg/ml, range 0 to 41 pg/ml) (p<0.04). In addition, all rejection episodes were predicted by the elevated TNF levels prior to abnormal changes in other laboratory parameters, including a rise in serum creatinine, IL-6 and a reduction in urinary amylase. The results of this study suggest that the elevations of TNF intraoperatively may reflect early sensitization of the patient to the graft, and may be used as a reliable indicator of organ rejection at an earlier stage while currently used markers are relatively inaccurate. Also, elevated intraoperative TNF level may therefore suggest the use of more aggressive induction of immunosuppression.14.

II. Efficacy of anti-TNFs therapy and synergistic response in combination with immunosuppressive agents in allograft rejection

Animal and human studies have demonstrated a high level of circulating TNFs in serum of transplanted recipients experiencing rejection episodes. The important role of TNFs in the pathogenesis and regulation of the rejection process has led investigators to attempt to inhibit TNFs and their actions as one of the effective immunosuppressive managements. Antibody therapy against TNFα has been shown to prolong significantly allograft survival not only in prophylactic treatment, but also as therapeutic administration. Moreover, the significant synergistic response of anti-TNFs and other immunosuppressants combination therapy have improved prognosis and assisted in the reduction of immunosuppressive toxicity.

1. Effect of prophylactic anti-TNFs administration

Recently, experimental studies have demonstrated that TNF would be irremissibly released following both moderate-severe hepatic ischemia
and subsequent reperfusion, and is then involved in the rapid development of the grafted liver failure as well as pulmonary insufficiency. The injured liver has a unique capacity for significant TNF production, because TNF is produced primarily by the monocyte/macrophage lineage cells and Kupffer cell mass in the liver is the largest fixed macrophage population in the body. Animals treated with anti-TNF antiserum prior to the induction of hepatic ischemia had a significantly improved liver functions and reduced pulmonary edema, intra-alveolar hemorrhage as well as neutrophil sequestration, compared to controls without pretreated TNF-blocking administration. These findings may provide a prophylactic strategy to prevent hepatopulmonary damage by blocking TNF actions with anti-TNF antibodies or TNF inhibitors in clinical orthotopic liver transplantation. In heterotopic cardiac transplant, Imagawa DK and Seu P found that control animals which received no immunotherapy had a mean survival of 11+/−0.4 days. Experimental animals received anti-TNFs either intraperitoneally or intravenously from days 1 to 10. The i.p administered anti-TNF prolonged graft survival to 16+/−2.7 days (p<0.05 vs. controls); the i.v administration prolonged survival to 15+/−1.4 days (p<0.004). Animals treated with i.p anti-TNFβ survived 17+/−1.7 days (p<0.002), and combination therapy with anti-TNFα and anti-TNFβ increased the living period to 21+/−2.2 days (p<0.001). In order to determine whether circulating levels of TNFs were affected by antibodystory, serum cytotoxic activity was determined by bioassay in vitro. The control animal which received no immunosuppression had a maximum of 40 cytotoxic units/ml 3 days prior to rejection. In contrast, treatment with either anti-TNFα alone or anti-TNFα plus anti-TNFβ markedly decreased cytotoxic activity between 5–10 units/ml, the cytotoxic activity provoked a rise once the antibody was discontinued. In addition, histologic examination of the cardiac graft from control animals on postoperative day 7 showed classical evidence of severe rejection, characterized by mononuclear cells infiltrate. The transplant grafts showed no evidence of hemorrhagic or coagulative necrosis in animals treated with anti-TNFs; only a small amount cellular infiltration was present, indicating no acute rejection.

2. Effect of Therapeutic anti-TNFs administration

Further evidence for TNFs’ role in rejection is also demonstrated by successful anti-TNFs strategies resulting in prolonging allograft survival. Heterotopic cardiac transplants were performed using Buffalo rats as donors and Lewis rats as recipients. A total of 9 groups were studied. Transplant grafts from untreated animals revealed significant rejection at day 4 with an average survival of 10.8+/−0.4 days, histologic examination showed the transplanted heart to be significantly edematous with an intense infiltration of mononuclear cells, consistent with a diagnosis of moderate to severe acute rejection. In order to assess the efficacy of therapy to reverse acute rejection, animals in the experimental groups received anti-TNFs therapies from postoperative days 4–13. The results showed that administration of polyclonal anti-TNFα in combination with polyclonal anti-TNFβ increased graft survival 14.6+/−0.4 days (p<0.001 vs controls), microscopic analysis of cardiac graft on postoperative day 9 showed only a minimum amount of cellular infiltrate. Use of a monoclonal anti-TNFα antibody was even more effective, with graft survival of 17.4+/−0.7 days (p<0.001 vs controls). These animals also showed histologic reversal rejection. Addition of polyclonal anti-TNFβ to monoclonal anti-TNFα regimen further increased survival to 25.7+/−1.0 days (p<0.001 vs monoclonal anti-TNFα alone). The most effective treatment in this series was the combination immunotherapy with monoclonal anti-TNFα and low-dose oral cyclosporine A, prolonging graft survival to 37+/−1.3 days (p<0.001 vs controls). As expected, administration of recombinant TNFα accelerated the time of graft failure to 7.4 days (p<0.001 vs controls). These cardiac graft showed a pronounced mononuclear cell infiltration histologically. To further determine whether anti-TNF antibodies could prolong cardiac allograft survival, the hearts from Brown rats were transplanted to the necks of Lewis rats. Complete graft rejection was defined by cessation of contraction. In untreated rats, the hearts were rejected 6.8+/−0.6 days (n=10) after transplantation. The mononuclear cell infiltrate in grafts stained intensively for TNF by immunohistochimistry, indicating that TNF was present within the inflammatory cells in the rejection process. In rats receiving a single injection of anti-TNF antibody at the time of transplantation (n=6), graft survival was nearly doubled (12.7+/−1.4 days, p<0.001 vs controls). Prolonged cardiac graft survival was also evident if the anti-TNF therapy was delayed until 1 day (n=5, 16.2+/−2.4 days; p<0.001 vs controls) or even 3 days after transplantation (n=5, 11.4+/−2.3 days; p<0.005 vs controls). The data indicate that a single bolus of anti-TNF antibodies can delay heart transplant rejection. In order to assess the efficacy of anti-TNF therapy, a micro ELISA was developed to quantify total circulating levels of TNFs. Animals receiving either cyclosporine A or anti-TNF antibodies alone showed only minimal levels of circulating TNF (1.9+/−1.9, 13.6+/−5.2 pg/ml
respectively). In contrast, animals not receiving immunotherapy had TNF levels of 121.4±32 pg/ml. This demonstrates that administration of anti-TNF antibodies can successfully suppress circulating levels of TNF and that TNF levels rise again once immunotherapy is terminated

3. Synergistic efficacy of combining anti-TNFs with immunosuppressant therapy

It is well known that some immunosuppressants can induce adverse side effects. For instance, the application of cyclosporine A (CsA) is in many cases temporarily discontinued by the occurrence of serious nephrotoxicity. While decreasing the dosage of CsA may reduce its toxicity. However, this also lowers its immunosuppressive effectiveness. Further evidence for TNF's role in rejection is successfully demonstrated by combination therapy of anti-TNFs with low-dose immunosuppressive agents resulting in extended transplant allograft survival. Bolling SF et al investigated the synergistic effect of combination anti-TNF with low-dose CsA in rat heterotopic cardiac allotransplant model. Heart graft rejection was significantly delayed by administration of CsA (14.8±2.4 days vs 6.8±0.6 days in controls). Treatment with anti-TNF antibodies significantly extended graft survival to 12.7 days (p<0.001 vs control) and 11.8 days (p<0.005 vs control), when given on the day of transplant or 3 days after operation. Anti-TNF antibody administration with low-dose CsA (1.5 mg/kg/day, 1/10 the dose of CsA required to permanently prevent rejection in this model) was significantly more effective than either one drug used alone, as evidenced by the graft survival time which was three-fold that of controls (22.1±3.1, 23.8±2.9 and 21.0±1.5 days on the day 0, 3, 5 following transplant, respectively; all p<0.001 vs control)35. In another study, hepatic allotransplantation model was performed by utilizing male DA rat as donors and male BN rat as recipients. Mean graft survival time of control animals receiving no immunosuppression was 15.1±1.5 days. Recipients treated with a 10-day course of anti-TNFβ (5000 U/day iv) prolonged survival to 29.8±2.1 days. Animals treated with the combination of anti-TNFβ and CsA survived over 60 days, being statistically significant when compared with control (p=0.0001). In conclusion, anti-TNF administration was synergistic with low-dose CsA. This finding suggests that clinical use of anti-TNF treatment may permit a reduction in the dose of CsA, hence decreasing the incidence of these immunosuppressant-related side effects36.

TNFs participation in the induction and course of rejection remains to be investigated further. Nevertheless, rejection is typically a local reaction triggered by alloantigen presentation to immunocompetent cells of host origin. Macrophages/monocytes, lymphocytes and Kupffer cells play a very prominent role in the antigen presentation as well as being greatly involved in the rejecting process, since the more severe and necrotic the rejection, the more these immune cells are present in the graft infiltrate2,20,27,28,30,40. One possible explanation for their potentially deleterious effect in situ is their synthesis and secretion of TNFs secondary to their activation. On the other hand, TNFs immunologic monitoring that provides relevant and early information on allograft rejection would certainly facilitate clinical decisions because of their relative sensitivity and reliability. It is believed that knowledge and technique of both TNFs and anti-TNFs will progress with the passage of time on organ transplantation, although the clinical utilization is now still in its infancy.

ACKNOWLEDGEMENT

We thank Dr. Hiroshi WATANABE very much for his helpful advice and responsible correction on this review.

(Received August 24, 1993)
(Accepted October 26, 1993)

REFERENCE


in rat cardiac transplants during allograft rejection. Transplantation 56: 399-404.


