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Review

The Role of Tumor Necrosis Factor in Allograft Rejection

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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[37]. 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 predicted[18]. Sankary H's

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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 parameters including total leukocyte count, total bilirubin, 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[31]. Another clinical study suggested that an intraoperative elevation of TNFα could be of predictive value in early rejection reaction. Preoperative TNFα values were not different in patients developing rejection (n=8) compared with those without rejection (n=20). After re-establishment of circulation in the graft, the median TNFα 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 rejection 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 rejection could be predicted in 23/28 (82%), an error in 5 of 28 (18%)[12].

2. For the cardiac allotransplantation

Lowey and Blais first demonstrated the presence 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 recombinant product itself, to measure quantitatively serum TNFα activity of rejecting rat cardiac allografts and culture supernatant of graft infiltrating cells respectively. The results strongly verified that TNFα is released by cells of monocyte/macrophage lineage infiltrating in rejecting grafts[24]. 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 presently mainly based on endomyocardial biopsy (EMB). However, this procedure is invasive, and early findings in the rejection process are still difficult. Chollet-Martin S et al recently reported a marked elevation of plasma TNF level during episodes of cardiac allograft rejection in human beings. 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, increased TNF plasma levels were observed during all 8 episodes of rejection in 6 heart transplant recipients. The mean of the peak TNF levels during 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 antirejection therapy[8]. 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 transplant rejection.

3. For the renal allotransplantation

In a cadaveric renal transplantation study, Maury and Teppo, using a sensitive double-antibody 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 levels 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 level (880 pg/ml) was associated with an irreversible rejection[25]. Similarly, McLaughlin et al evaluated the sequential changes of plasma and urinary 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, $\bar{x}$=135 pg/ml, range 80-5000 pg/ml), and high urinary TNFα was also presented in almost half of these cases (18/37, 49%; $\bar{x}$=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%, $\bar{x}$=62 pg/ml, range <80-625 pg/ml; and $\bar{x}$=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, urinary TNFα was not found consistently with infection periods[26]. 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 mononuclear cells were detected in all cases of acute rejection. No-rejection and chronic vascular rejection 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 glomerular and arterial endothelial cells in cases with acute rejection, and to a minor degree in no rejection 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[29].

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 dogs 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 rejecting 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 correlatd 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 esisodes were predicted by the elevated TNF levels prior to abnormal changes in other laboratorian 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 synergetic 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 TNFs has been shown to prolong significantly allograft survival not only in prophylactic treatment, but also as therapeutic administration. Moreover, the significant synergetic 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[9,10,13]. 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 administrated 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 antibody therapy, 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 most 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[16,17,34].

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[19,23,33,35,36,38]. 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 showd 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 expectd, 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[19,35] 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 immunohistochemistry, 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[33].

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)[3]. 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 effects[38].

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 infiltrate[2,20,27,28,30,40]. 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.

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