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Renal allograft rejection: the temporal relationship and predictive value of plasma TNF (alpha and beta), IFN–gamma and soluble ICAM-1

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Abstract Recently, close interactions have been described between the tumour necrosis factors alpha and beta (TNF-alpha and beta), interferon-gamma (INF-gamma) and intercellular adhesion molecule-1 (ICAM-1) in T-cell mediated immune activation. During the process of renal graft rejection, the properties of these cytokines to act as powerful stimulators of macrophages, to upregulate class II MHC expression and to stabilise cell-to-cell binding make them of great potential interest. The aim of the present study was to determine the plasma levels of each cytokine and soluble ICAM-1 in 16 renal allograft recipients. We examined plasmas of patients for the first 2 weeks after transplantation and correlated results with the clinical pattern of rejection. Our data suggest an immunopathologic involvement of TNF-alpha, TNF-beta and sICAM-1 in renal allograft rejection and showed that there was a significant elevation in plasma concentrations of these parameters 2 or 3 days prior to the diagnosis of clinical rejection. Rises in INF-gamma did not appear to be significant with regard to rejection as very high levels were found in patients showing no evidence of clinical rejection.

Key words Rejection, kidney, cytokines · Kidney, rejection, cytokines · Cytokines, rejection, kidney

Introduction

Activation of the immune system during renal allograft rejection is thought to be initiated by interaction between class II MHC molecules on donor cells and specific antigen receptors on recipient helper T lymphocytes. Indeed, soluble products of both activated T cells, such as interferon-gamma (IFN-gamma) and tumour necrosis factor-beta (TNF-beta), and activated macrophages, such as tumour necrosis factor-alpha (TNF-alpha), have been reported to be elevated in patients with acute graft rejection [7, 10, 18].

IFN-gamma is one of the first cytokines to be released during immune activation and may be considered pivotal to initiation of the rejection process [18]. TNF-alpha is also secreted early in the immune activation and can be detected in the circulation of patients showing renal graft rejection [7]. Preliminary clinical studies have suggested that these cytokines are associated with immune activation in graft rejection because they are both powerful stimulators of macrophages and also upregulate class II antigen expression [7, 18]. In addition, the interactions between MHC molecules on renal allograft epithelial cells and recipient helper T cells are stabilised by some adhesion molecules [4].

The antigen-independent adhesion of T cells to monocytes, B cells, target cells and vascular endothelium is mainly mediated by "accessory molecular pathways", i.e. lymphocyte function-associated antigen-1 (LFA-1; CD11a–CD18) binding to intercellular adhesion molecule-1 (ICAM-1; CD54), CD2 binding to LFA-3 (CD58) and very late antigen-4 (VLA-4) binding to vascular cell adhesion molecule-1 (VCAM-1) [1, 6, 11, 12, 17]. The most important of these paired adhe-

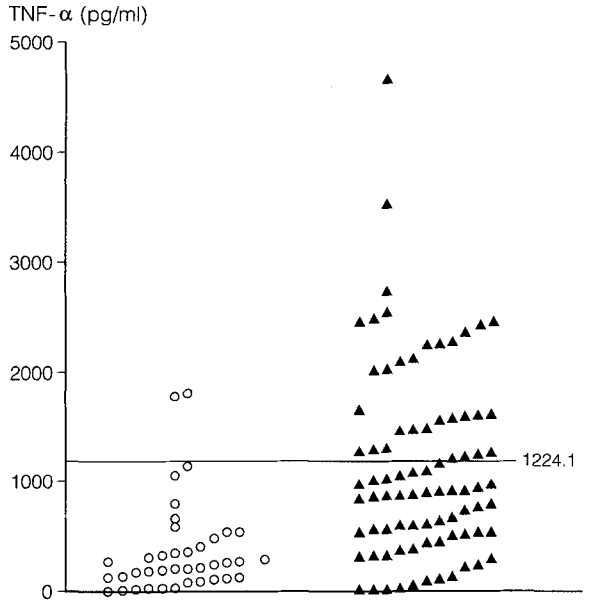

Fig. 1 TNF-alpha plasma concentrations of patients in the rejection (▲, $n = 83$; mean 1122.9 pg/ml) and no rejection (○, $n = 39$; mean 365.1 pg/ml) groups

sion molecule interactions is thought to occur between ICAM-1 and LFA-1 in reactions with renal epithelium [4]. Indeed, some studies have shown that therapy with monoclonal antibodies that block LFA-1 or ICAM-1 can prolong graft survival [2, 4].

In addition to their effect in stabilising cell-to-cell binding, interactions between adhesion molecules may directly augment the recognition of specific MHC antigens by T lymphocytes [4]. It is also known that culture supernatants from activated T lymphocytes contain cytokines such as IFN-gamma and TNF-alpha, which augment the expression of ICAM-1 and LFA-3 [4, 13, 14]. Furthermore, a soluble product of ICAM-1 (sICAM-1) has been recently identified in the circulation of patients and in the supernatant of lymphocytes after in vitro culture [3, 5, 15, 16].

The aim of this study was to compare the time courses of production of TNF-alpha and beta, IFN-gamma and sICAM-1 beginning with the day of renal transplantation and to examine their relationship with the occurrence of clinically defined graft rejection.

Patients and methods

Patient groups

All patients ($n = 16$) in this study showed end-stage renal failure until they were transplanted in the Renal Transplantation Unit of the Royal Victoria Infirmary, Newcastle upon Tyne. The study group comprised 15 cadaveric renal transplants and a single living-related transplant. All of the patients received 500 mg methylprednisolone intravenously during the operation and also 24 h post-operatively. Immunosuppression consisted initially of cyclosporin monotherapy (10 mg/kg per day).

Table 1 Summary of the mean values of the cytokines measured in the patient group showing no rejection

	Mean	SD	Mean + 2 SD
TNF-alpha (pg/ml)	365.1	429.5	1224.1
TNF-beta (pg/ml)	33.4	21.2	75.8
Gamma-IFN (IU/ml)	0.72	1.71	4.14
sICAM-1 (ng/ml)	89.7	44.5	178.7

Over the course of the first 2 weeks after transplantation, renal biopsy confirmed that seven patients showed acute allograft rejection whilst four patients showed primary non-functioning (PNF) kidneys. Five patients showed no evidence of clinical rejection and therefore comprised the no rejection group (NRG).

In the rejection group (RG), three patients showed acute moderate cellular rejection episodes on day 6 and one patient on day 7 after transplantation. Three patients showed clinical rejection on day 4. Renal allograft rejection was diagnosed on the basis of clinical criteria and was confirmed by biopsy. Upon diagnosis of rejection, 500 mg methylprednisolone was given intravenously for 3 days. Over the course of the study no evidence of episodes of bacterial, viral or fungal infections were seen in any of the patients studied.

The study was approved by the ethics committee and the patients gave their informed consent prior to the study.

Plasma samples were obtained daily from each renal allograft recipient for 14 days after transplantation. To minimise changes in cytokine levels with storage of whole blood, cell-free plasma samples were stored at −20 °C within 30 min of collection by venipuncture.

TNF-alpha, TNF-beta and IFN-gamma assays

TNF-alpha (Medgenix, Belgium), TNF-beta (Amersham, UK) and IFN-gamma (Medgenix, Belgium) were measured quantitatively in test plasmas using enzyme-linked immunoassays (ELISA). Fourteen days of plasma samples were analysed for six patients and 8 days of plasma samples were analysed for ten patients (i.e. a total of 39 samples were analysed for the NRG, 83 samples for the RG and 37 samples for the PNF group).

sICAM-1 Assay

An ELISA was used for the quantitative determination of human sICAM-1. This assay involves the simultaneous reaction of sICAM-1 present in samples with two monoclonal antibodies directed against different epitopes on the sICAM-1 molecule. Six days of plasma sICAM-1 concentrations were analysed for 14 of the patients (a total of 24 for the NRG, 36 for the RG and 24 for the PNF group).

Statistical analysis

Statistical analysis of the data was performed with Student's t-test.

Table 2 The relationship between the time of rise in TNF-alpha, TNF-beta, IFN-gamma and sICAM-1 plasma concentrations and the beginning of clinical rejection (*No* no plasma levels > mean + 2SD)

Patient no. Mean + 2 SD	TNF-alpha 1224.1 pg/ml	TNF-beta 75.8 pg/ml	IFN-gamma 4.14 IU/ml	sICAM-1 178.7 ng/ml
Rejection group[a]				
1	–3 (931.8)	+2 (1322.6)	–1 (12.15)	–3 (503.4)
2	–1 (1292.1)	not detectable	–2 (14.33)	–3 (371.5)
3	–3 (1620.0)	–3 (445.9)	No	–3 (825.4)
4	–3 (1278.0)	–2 (651.1)	No	–1 (806.5)
5	–6 (2270.0)	–5 (135.9)	No	–5 (549.4)
6	+1 (1270.0)	–3 (85.8)	No	–3 (893.4)
7	–5 (1232.0)	–5 (1236.8)	+5 (5.50)	–
Primary non-function group[b]				
1	No	Not detectable	8.99 (day 1)	320.6 (day 5)
2	No	1635.1 (day 1)	9.20 (day 1)	No
3	1690.4 (day 2)	Not detectable	6.29 (day 3)	426.2 (day 6)
4	2230.0 (day 1)	1861.6 (day 1)	No	405.7 (day 1)

[a] First day cytokine and sICAM-1 level exceeds mean + 2 SD value of no resection group with reference to the day of clinical rejection (day 0). [b] First day cy-tokine and sICAM-1 level exceeds mean + 2 SD value of no rejection group with reference to the day of transplantation (day 0)

Results

TNF-alpha

The time course of the mean values of TNF-alpha in the RG was very different from that in the NRG. There were significant differences on days 6, 7 and 8 after transplantation between the TNF-alpha plasma concentrations of these two groups. The mean values ± standard error of the mean (SEM) of the RG and the NRG on post-operative days 6, 7 and 8 were 1253.0 ± 123.2 and 266.0 ± 66.6, 972.4 ±118.2 and 186.6 ± 44.8, and 1074.6 ± 117.8 and 117.5 ± 18.8, respectively.

Figure 1 shows the distribution of TNF-alpha concentrations of all samples tested from both groups. The TNF-alpha concentrations of all plasmas tested in the NRG and RG were compared and found to be significantly different ($P < 0.05$). The mean value plus two standard deviations of TNF-alpha in the NRG was 1224.1 pg/ml (Table 1).

Whilst in patients showing no rejection only 2 of 39 (5.1 %) tests were above this level, in patients with clinical rejection 32 of 83 (38.6 %) tests exceeded it (Fig. 1).

In the RG the TNF-alpha plasma concentrations exceeded 1224.1 pg/ml in five of seven patients before the day of clinical rejection (Table 2). However, two of four patients in the PNF group had values higher than this level on days 1 and 2 with reference to the day of transplantation, day 0 (Table 2).

TNF-beta

There were significant differences ($P < 0.001$) in the TNF-beta concentrations over the first 8 days after transplantation between the RG and NRG (i.e. the mean ± SEM in RG and NRG was 265.0 ± 29.0 and 30.1 ± 5.3 for day 1 and 912.3 ± 36.0 and 56.7 ± 9.8 for day 7).

Figure 2 shows all measured TNF-beta concentrations in the RG and the NRG. The difference was significant ($P < 0.05$). None of the plasmas in the NRG had TNF-beta concentrations exceeding 75.8 pg/ml.

Five of the seven patients in the RG exceeded this level before the onset of clinical rejection (Table 2). In the PNF group, non-detectable levels were observed in two patients and the other two patients showed high levels at the beginning of the post-operative period (Table 2).

IFN-gamma

In both the NRG and the RG, the plasma concentrations of IFN-gamma showed peaks of increase and decrease within the 1st week. Over the period of the post-transplantation study there were no significant differences in IFN-gamma values between the two groups on the same days ($P > 0.05$).

IFN-gamma concentrations for all of the plasmas in the NRG ($n = 39$) and the RG ($n = 83$) were compared statistically and no significant differences were seen. The mean value for IFN-gamma plus two standard deviations in the NRG was 4.14 IU/ml (Table 1). Most of the plasmas in both groups showed IFN-gamma concentrations that were lower than this level. Only the plasma of two of seven patients showing rejection exceeded the value of 4.14 IU/ml before clinical rejection was diagnosed (Table 2). In contrast, patients with PNF kid-

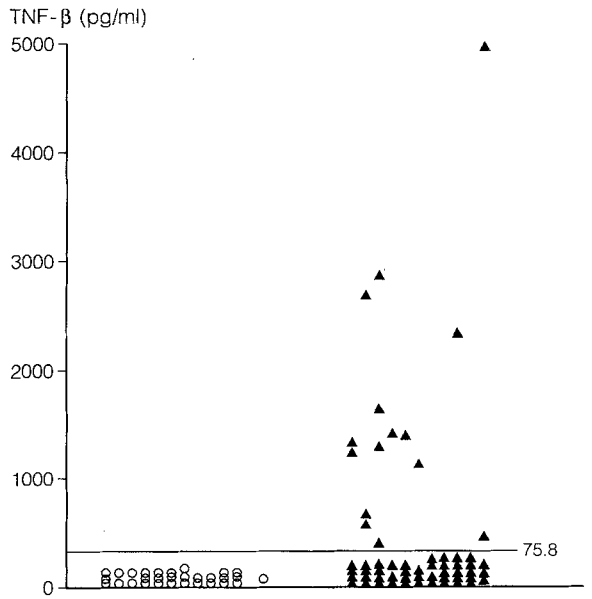

Fig. 2 TNF-beta plasma concentrations of patients in the rejection (▲, mean 328.4 pg/ml) and no rejection (○, mean 33.4 pg/ml) groups

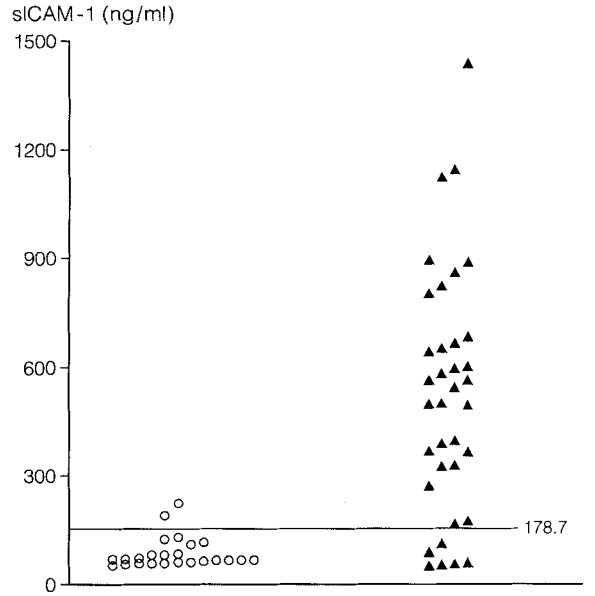

Fig. 4 sICAM-1 plasma concentrations of patients in the rejection (▲, $n = 36$; mean 524.6 ng/ml) and no rejection (○, $n = 24$; mean 89.7 ng/ml) groups

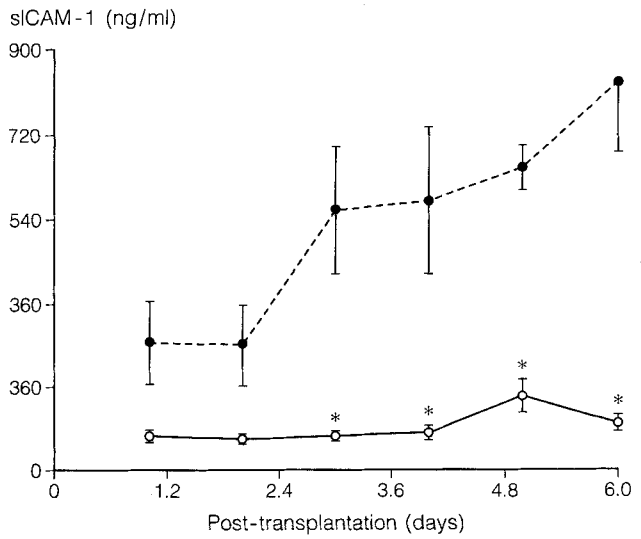

Fig. 3 Comparison between the mean daily plasma concentrations ± SEM of sICAM-1 in the rejection (---) and no rejection (——) groups. *$P < 0.05$

neys had high concentrations of IFN-gamma over the post-operative period (Table 2).

sICAM-1

In the NRG, the levels of sICAM-1 did not change significantly during the 1st week after transplantation. A level of 72.5 ± 14.8 ng/ml was found on the 1st post-operative day and a level of 96.3 ± 19.6 ng/ml was observed 5 days later ($P > 0.05$). In the RG the plasma concentration of sICAM-1 was 274.6 ± 45.7 ng/ml on day 1 and increased rapidly, reaching 823.3 ± 138.0 ng/ml on day 6 ($P < 0.05$).

The time course of mean plasma concentrations of sICAM-1 showed a different pattern between the RG and the NRG (Fig. 3). There were significant differences on days 3–6 after transplantation in the plasma sICAM-1 concentrations of these groups ($P < 0.05$; Fig. 3).

Figure 4 shows the distribution of all sICAM-1 plasma concentrations assayed in the patient groups. At almost all time points the sICAM-1 plasma concentrations in both the NRG and RG were statistically different ($P < 0.05$; Fig. 4).

The mean plasma level plus two standard deviations of sICAM-1 in the NRG was 178.7 ng/ml (Table 1). In the NRG only 8.3 % (2/24) were higher than this level while 83.3 % (30/36) exceeded it in the RG (Fig. 4).

In the RG, the plasma sICAM-1 concentrations of all patients exceeded 178.7 ng/ml before the day of rejection (Table 2). In the PNF group, three patients had values higher than this within the 1st week after transplantation (Table 2).

Discussion

TNF-alpha is a macrophage-derived polypeptide that plays a prominent role as a cytokine in numerous inflam-

matory and infectious diseases [8, 10]. It has been reported that in acute renal allograft rejection, TNF-alpha is released with interleukin-1 by cells of the monocyte-macrophage lineage and by other cells infiltrating the rejected allograft [8]. However, the exact mechanisms of how TNF-alpha and TNF-beta induce or produce graft damage are not yet fully understood. They may be directly cytotoxic to vascular endothelium or may render endothelial cells more susceptible to T-cell attack by increasing the expression of class I and II MHC antigens and cell surface adhesion molecules. Noronha et al. [9] and McLauglin et al. [7] have reported raised serum levels of TNF-alpha during episodes of human renal allograft rejection. Meulders et al. [8] have indicated that important amounts of TNF-alpha are released by peripheral blood mononuclear cells during such crises.

In this study, we have examined the plasma TNF-alpha and beta concentrations over a 14-day post-transplantation time course in our clinical groups. We have also attempted to determine if there was a discriminating plasma level that was indicative of rejection. TNF-alpha and beta showed consistently higher levels in the RG than in the NRG. Elevation of plasma levels was seen before the day of clinical rejection. Although there was a significant decrease in TNF-alpha levels at the end of the 1st week in the NRG, we did not observe a decrease in TNF-alpha or beta levels in the RG. The release of these cytokines by mononuclear cells and by T lymphocytes had increased, possibly due to the presence of a rejecting allograft.

Statistical analysis of TNF-alpha and TNF-beta values showed a significant difference between the NRG and the RG not only on the same days but also in all of the samples from these groups. The mean value plus two standard deviations in the NRG was designated as a "discriminating level". In the RG, TNF-alpha and beta values for 71.4 % of cases (5/7) exceeded the discriminative level a few days before the onset of clinical rejection. Comparison of the timing of the rises in TNF values with reference to the day of rejection showed elevation before rejection was diagnosed. Indeed, acute renal allograft rejection may be predicted 2 or 3 days before by detecting elevated TNF-alpha and TNF-beta plasma concentrations, provided the patients do not have any infections or any other inflammatory events since these are inflammatory cytokines and potent mediators of the inflammatory response. The discriminatory value of high TNF levels may be reduced in the presence of infections.

IFN-gamma can produce two important effects in renal allograft rejection. First, it induces increased expression of class I and II MHC and adhesion molecules on graft tissue, which potentially makes the graft more vulnerable to immune effector mechanisms [10]. Second, it activates monocytes to mediate a destructive delayed hypersensitivity response against the graft [10]. Elevations of IFN-gamma concentrations in serum have been reported to occur during rejection and infection [18]. However, during rejection episodes IFN-gamma was also frequently measured within the normal range [18]. We therefore addressed the question as to whether increases in plasma IFN-gamma could also be observed in our patient groups.

The IFN-gamma values in the RG did not significantly differ from those in the NRG ($P > 0.05$). The rises observed before or during rejection episodes were not significantly different from the same day values in the NRG. In the RG, only 28.5 % of patients (2/7) exceeded the discriminating level before clinical rejection and one patient had a higher level 5 days after the rejection was diagnosed. As a result of this comparison between the NRG and the RG, the plasma IFN-gamma level was not found to be a predictive parameter for acute renal allograft rejection. Our data suggest that highly elevated levels of IFN-gamma may occur in almost all renal allograft recipients despite the graft rejection status during the 1st week following transplantation. We conclude that the operation of transplantation itself might be a major stimulus for IFN-gamma release. The induction and subsequent upregulation of class II MHC antigens in the graft ist, however, almost certainly due to IFN-gamma. Yet, the elevated graft levels produced in the kidney milieu were not reflected in plasma concentrations.

ICAM-1 is a surface glycoprotein induced on endothelial cells, fibroblasts, macrophages, epithelial cells and activated lymphocytes by various inflammatory mediators, such as IL-1, TNF and IFN-gamma [2, 13]. A soluble form of ICAM-1 (sICAM-1) has been detected in human serum using ELISA techniques [3]. It has been suggested that inhibition of LFA-1/ICAM-1 interaction in vivo might suppress T-cell mediated immunologic reactions such as renal allograft rejection [2]. Indeed, Cosimi et al. [2] prolonged the survival of renal allografts in non-human primates by using monoclonal antibody against ICAM-1. By using monoclonal antibodies against sICAM-1, we also examined the plasma concentration of human sICAM-1 in renal allograft recipients.

We compared the post-transplantation time courses in the RG and the NRG and found that they were significantly different ($P < 0.05$). Although sICAM-1 levels did not change during the 1st week in the NRG, there was an increase in the RG. The mean value plus two standard deviations in the NRG was 178.7 ng/ml and 100 % of patients in the RG exceeded this discriminative level 3 days (on average) before clinical rejection was detected. Our data allow us to conclude that sICAM-1 rises rapidly 3 days before the clinical diagnosis of rejection and, therefore, its plasma concentration may be used as a predictive parameter for acute renal rejection.

We also determined the plasma concentrations of TNF-alpha, TNF-beta, IFN-gamma and sICAM-1 in four patients with primary non-functioning kidneys. In this group, at least two of these four parameters exceeded the discriminating levels already defined within the 1st week after transplantation. In two cases (the third and fourth), three parameters showed the pattern we obtained in the RG. Histologic examination of graft tissues of these cases was not performed and the patients were treated with high-dose methylprednisolone for 3 days during the 1st week. Clearly, it is difficult to determine the efficacy of monitoring these inflammatory cytokines and sICAM-1 in the PNF group, due to uncertainty about the underlying pathology. However, the recovery of the third and fourth patients after methylprednisolone therapy suggests that the process of renal rejection may well have occurred.

In conclusion our data suggest an immunopathologic involvement of TNF-alpha, TNF-beta and sICAM-1 in renal allograft rejection and demonstrate that there was a significant elevation in plasma concentrations of these parameters 2 or 3 days before the onset of clinical rejection. However, the clinical value of plasma TNF monitoring may be limited by the association of systemic infections with concomitant high TNF release. The high degree of discrimination afforded by the assessment of sICAM-1 in rejection may well be due to its role as an effector molecule involved synergistically with many other amplifying cytokines. On the other hand, IFN-gamma rises do not necessarily indicate allograft rejection because of the occurrence of significant levels in patients without rejection.

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