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Graft 2002; 5; 141

DOI: 10.1177/1522162802005003005

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Sequential Activation Patterns of Macrophages in Chronic Allograft Nephropathy

Erwei Song, Balazs Antus, Yousheng Yao, Jens Lutz, and Uwe Heemann

Infiltration of macrophages is associated with the development of chronic allograft nephropathy. Macrophages can be activated in a classical pathway characterized by TNF- α expression, and an alternative pathway characterized by the expression of alternative macrophage activation-associated CC-chemokine-1 (AMAC-1). The present study investigated the sequential activation patterns of macrophages in the course of chronic allograft nephropathy. Fisher (F344) rat kidneys were orthotopically transplanted into Lewis recipients and harvested at week 1, 2, 12, 16, 20, 24, or 28 after transplantation for histologic, immunohistologic, and molecular analysis. TNF- α mRNA was elevated at weeks 1 and 2 following transplantation, and could not be detected at late stages, whereas AMAC-1 mRNA began to be detectable at 12 weeks and increased steadily thereafter. Furthermore, AMAC-1 mRNA levels positively correlated with urinary protein excretion ($r = 0.685$, $P < 0.05$), and glomerulosclerosis ($r = 0.725$, $P < 0.05$) at the end of the follow-up. Hence, infiltrating macrophages are classically activated early and, alternatively, late after transplantation. Alternatively activated macrophages may be responsible for chronic allograft nephropathy.

ABBREVIATIONS

TNF- α	tumor necrosis factor alpha
AMAC-1	alternative macrophage activation-associated CC-chemokine-1
TGF- β_1	transforming growth factor-beta
ECM	extracellular matrix

Uwe Heemann, MD, PhD
AG Klin. Immunologie
und Transplantation
Dept. of Nephrology
Essen University Hospital
Hufelandstr 55
45122 Essen
Germany
Tel: +49.201.723.3395
Fax: +49.201.723.5633
email: uwe.heemann@uni-essen.de

Introduction

Macrophage infiltration and activation is associated with the development of chronic allograft nephropathy.¹⁻³ Recently, 2 distinct activation pathways of macrophages have been defined: classical and alternative activation. Whereas classical macrophage activation is characterized by the expression of tumor necrosis factor-alpha (TNF- α), alternative activation is associated with the expression of alternative macrophage activation-associated CC-chemokine-1 (AMAC-1).^{4,5} Classically activated macrophages synthesize and release constitutive amounts of proinflammatory factors and are responsible for tissue destruction during acute inflammation.⁶⁻⁷ By contrast, alternatively activated macrophages provide profibrogenic factors, enhance fibrogenesis of fibroblasts,⁸⁻⁹ and populate granulated healing wounds.¹⁰⁻¹²

The mode of activation of macrophages in chronic allograft nephropathy has not been studied.

Thus, we investigated the sequential activation patterns of macrophages in the course of chronic allograft nephropathy.

Materials and Methods

Renal allografts from male Fisher rats (F344, RT1v1) were transplanted orthotopically to male Lewis (LEW, RT1) recipients and treated briefly with low-dose cyclosporine (1.5 mg/kg per day for 10 days, intramuscularly) to reverse an initial acute rejection episode. Every 4 weeks, 24-h urine samples were collected using metabolic cages with a urine-cooling system, and quantitative urine protein was nephelometrically determined. Grafts were harvested at week 1, 2, 12, 16, 20, 24, or 28 after transplantation ($n = 8$ for each time point).

For histology, hematoxylin/eosin and periodic acid-Schiff (PAS) staining was performed to evaluate the percentage of sclerosed to total glomeruli. For immunohistology, monoclonal mouse anti-rat

CLASSICAL ACTIVATION

Characterized by TNF(α) and the release of proinflammatory cytokines.

ED1 antibody (Serotec Camon Labor-Service GmbH, Wiesbaden, Germany) was used as the primary antibody, followed by sequential incubation with rabbit anti-mouse IgG and the alkaline phosphatase antialkaline phosphatase (APAAP) complex (DAKO A/S, Hamburg, Germany). Cells staining positive were counted and expressed as cells per field of view (cells/fv).

Intragraft mRNA expression of TNF- α and AMAC-1 was determined by reverse transcriptase-polymerase chain reaction (RT-PCR). Briefly, cDNA was synthesized from isolated RNA by RT using the SuperScript preamplification system as described by the manufacturer (Gibco/BRL, Karlsruhe, Germany). PCR was performed on cDNA for the amplification of TNF- α , AMAC-1, and β -actin using the primer pairs designed according to published sequence.⁸ The conditions were optimized for each cytokine, allowing 28 cycles for β -actin, 30 for TNF- α , and 40 for AMAC-1. The amplified PCR product was identified by electrophoresis on 1.5% agarose gel stained with ethidium bromide. Semiquantitative comparison was performed by computerized densitometry of cytokine cDNA and β -actin from the same sample. The results are given as the ratio of intensity of cytokines to β -actin mRNA.

Results

Macrophage infiltration was noted early after transplantation (11 ± 3 cells/fv at week 1 and 19 ± 3 at week 2). From week 12 to week 28, macrophage infiltration constantly increased.

To evaluate the activation patterns of macrophages in the course of chronic allograft nephropathy, we examined the mRNA expression of TNF- α as marker for classical macrophage activation, and AMAC-1 for the alternative one. Early after transplantation, TNF- α mRNA expression was significantly higher than at later time points ($P < 0.001$; Fig 1a). On the other hand, intragraft AMAC-1 mRNA was not detectable in the first 2 posttransplantation weeks but was markedly enhanced after 12 weeks ($P < 0.001$, Fig 1b). In addition, AMAC-1 expression tended to increase steadily from week 12 to week 28 (Fig 1b).

As long-term graft dysfunction and deterioration manifested after week 24, we correlated intragraft

AMAC-1 mRNA expression to 24-h proteinuria and glomerular sclerosis at 28 weeks. AMAC-1 mRNA levels positively correlated not only with urinary protein excretion ($r = 0.685$, $P < 0.05$) but also with the percentage of sclerotic glomeruli ($r = 0.725$, $P < 0.05$).

Discussion

Macrophage infiltration and activation is actively involved in the pathogenesis of chronic allograft nephropathy.^{1,3} Monocyte/macrophage infiltration was identified in renal allografts during early episodes of reversible acute rejection.¹³ In the present study, we demonstrated that infiltrated macrophages were classically activated early after transplantation, whereas alternative activation pathways characterized the later posttransplantation stages.

In vitro studies have indicated that classically activated macrophages release constitutive amounts of proinflammatory cytokines, especially TNF- α , and matrix metalloproteinases,⁸ which may result in the destruction of parenchymal tissues and extracellular matrix. Hence, classically activated macrophages early after transplantation, along with their elaboration of inflammatory cytokines, may be responsible for initial insults to renal allografts.

Macrophage infiltration was also intense at later stages after transplantation (from week 12 to week 28). However, TNF- α mRNA expression was absent at these stages, suggesting that the infiltrating macrophages were not classically activated. By contrast, the macrophage subset present at late posttransplantation stages was primarily alternatively activated, as evidenced by an increased expression of AMAC-1. Furthermore, AMAC-1 mRNA level positively correlated with urinary protein excretion and glomerulosclerosis. This suggests that alternatively activated macrophages are associated with long-term allograft dysfunction and deterioration. Indeed, in vitro studies indicated that this macrophage subset may promote the proliferation and collagen production of fibroblasts by providing pro-fibrogenic growth factors, such as TGF- β , PDGF, and IGF.^{8,9} Additionally, lesions heavily populated by alternatively activated macrophages, such as granulomas and sites of chronic inflammation, are highly fibrotic.¹⁰⁻¹² Hence, alternative acti-

ALTERNATIVE ACTIVATION

Characterized by AMAC-1 and the release of profibrogenic factors.

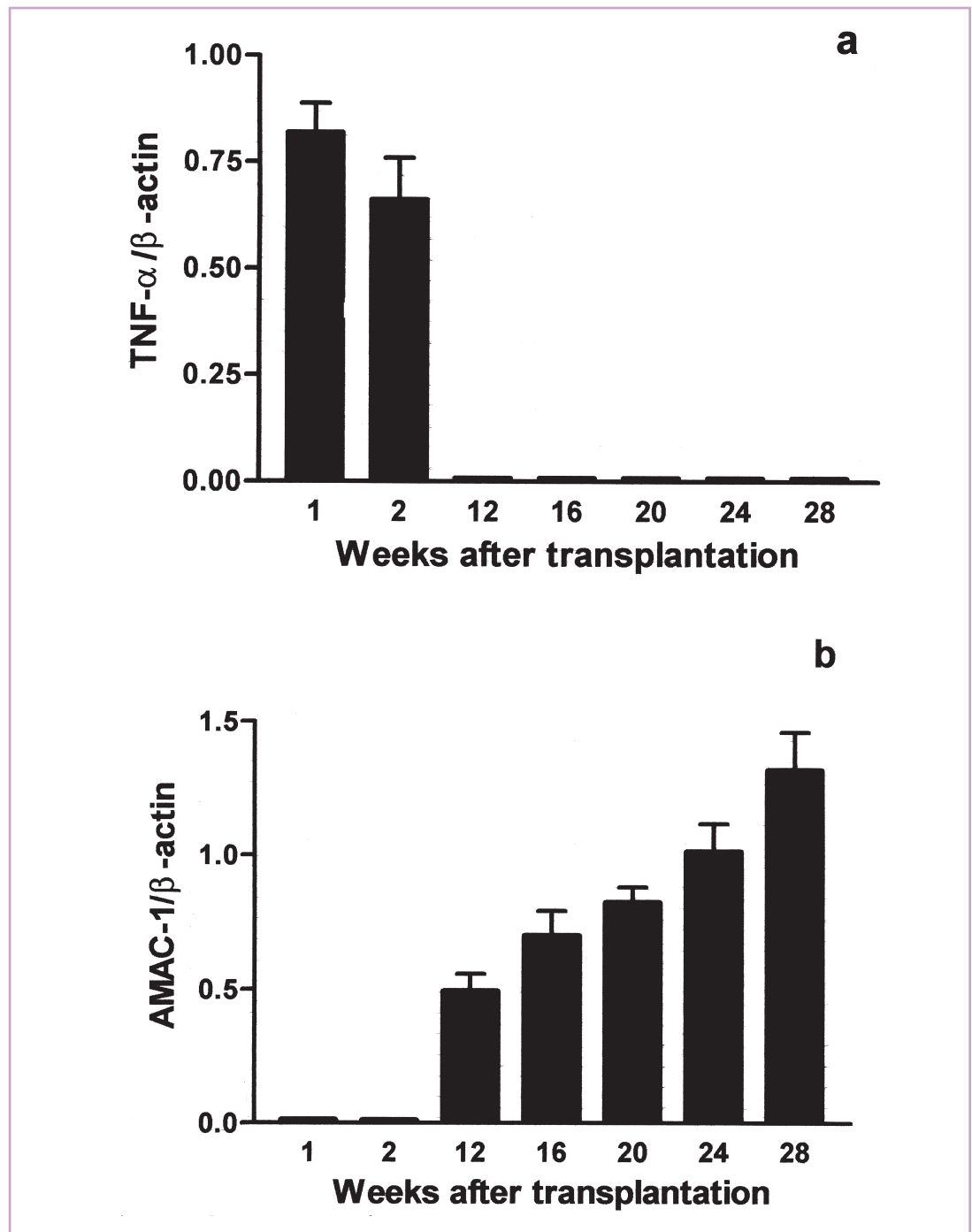


Figure 1. Intra-graft mRNA expression of TNF- α (a) and AMAC-1 (b) in the course of chronic allograft nephropathy ($n = 8$ /time point).

PRO-INFLAMMATORY CYTOKINES

Cytokines that induce and uphold an inflammation.

vation of macrophages at late posttransplantation stages may be responsible for allograft fibrogenesis that leads to chronic allograft nephropathy.

In summary, we conclude that differential macrophage activation patterns occur at different stages after kidney transplantation. Classically activated macrophages that release proinflammatory factors may be related to initial insults to the grafts, whereas alternatively activated macrophages may be responsible for late allograft fibrogenesis.

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