The Presence and Prognostic Importance of Glomerular Macrophage Infiltration in Renal Allografts

RENAL ALLOGRAFTLARDA GLOMERÜLER MAKROFAJ İNFİLTRA S YON UNUN VARLIĞI VE PROGNOSTİK ÖNEMİ

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Summary

Aim: The purpose of this study was to analyze the role, degree, and frequency of intraglomerular macrophage infiltration in human renal allografts by examining biopsies from kidney grafts that were dysfunctional after transplantation.

Material and Method: Seventy-three transplant kidneys in states of chronic and acute rejection (33 and 40 grafts, respectively) were biopsied and specimens were examined for the presence of macrophage infiltration in the glomeruli. The infiltration of these cells was evaluated immunohistochemically using monoclonal antibody CD68, which labels macrophage cytoplasm.

Results: Of 73 biopsies, only 28 showed positive staining for CD68 in the glomeruli, indicating the presence of macrophages.The outcome for grafts that contained intraglomerular macrophages was significantly worse than the outcomes noted for other grafts during the 6 months after the biopsy was obtained. Also, patients with macrophage infiltration in the graft glomeruli did not respond well to steroid antirejection treatment.

Conclusion: We conclude that the presence of glomerular macrophages can be considered as a marker for rejection, and is a valuable additional criterion for rejection in the histological examination of renal allograft biopsies. The presence of intraglomerular macrophages indicates that the outcome of the graft is significantly worse than cases that do not show intraglomerular macrophage infiltration.

Key Words: Transplantation, Renal Allograft, Rejection, Macrophage, Banff Classification

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Özet

Amaç: Bu çalışmanın amacı transplantasyonu takiben fonksiyon bozukluğu olan böbrek graftlarının biopsilerini inceleyerek intraglomerüler makrofajların rolünü, derecesini ve sıklığını analiz etmektir.

Materyal ve method: Akut ve kronik rejeksion gösteren 73 transplant böbreğinden (33 kronik, 40 akut) biopsi yapıldı ve spesimenlerde glomerüler makrofaj infiltrasyonunun varlığı incelendi. Bu hücrelerin infiltrasyonu, makrofajların sitoplazmasını ayırt eden monoklonal antikor CD68 kullanılarak immünohistokimyasal olarak incelendi.

Bulgular: 73 biopsinin sadece 28'i glomerüllerde, makrofajların varlığına işaret eden, pozitif CD68 boyanması gösterdi. İntraglomerüler makrofaj içeren graftların gidişatı biopsiden sonraki 6 aylık dönemde diğer graftlara göre belirgin olarak daha kötü bulundu. Ayrıca graft glomerülleri içinde makrofaj infiltrasyonu gösteren hastalar steroid anti-rejeksiyon tedavisine iyi cevap vermedi.

Sonuç: Glomerüler makrofaj varlığı rejeksiyonun bir belirleyicisi olarak kullanılabilir ve renal allograft biopsilerinin histolojik incelemesinde rejeksiyon için değerli ek bir kriter olarak kullanılabilir. Bu hücrelerin varlığı, graft gidişinin makrofaj infiltrasyonu göstermeyen vakalara göre belirgin olarak daha kötü olduğunu gösterir.

Anahtar Kelimeler: Transplantasyon, Renal Allograft, Rejeksiyon, Makrofaj, Banff Klasifikasyonu

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The mononuclear phagocytic system functions via a cell line that originates from the bone marrow and eventually matures into macrophages in peripheral tissues. These cells have numerous immunologic and nonimmunologic functions.

Through their secretory and phagocytic properties, and their regulatory effect on the immune response, mononuclear phagocytes play a primary role in the body's defence against microorganisms and foreign materials (1). Macrophages interact with lymphocytes in both the afferent and the efferent limbs of the immune response, and are essential for the development of cellular and humoral immunocompetence (2,3). The relationship between the two is mutually exclusive, in that one cannot function without the other.

In recent years, there has been much interest in the role that macrophages play in glomerular disease (4,5). Investigations have clearly established that the monocyte-macrophage system is intimately involved in immune-mediated inflammatory reactions in certain types of glomerulonephritis (4-7). One study on experimental immunological glomerulonephritis suggested that macrophages were sometimes directly pathogenic to the structures in the renal parenchyma (6). Although many investigations have examined the role of glomerular macrophages in glomerulonephritis, few articles have focused on macrophages in renal allograft biopsies (8,9).

In order to learn more about the level and frequency of intraglomerular macrophage infiltration, and to define the function of these cells in human renal allograft biopsies, we used immunohistochemical methods with monoclonal antibody CD68 to quantify these cells in dysfunctional allografts.

Materials and Methods

We obtained renal biopsy specimens from 73 kidney transplant recipients to investigate the cause of posttransplantation renal failure. The transplant was a first-time allograft in every case. Cyclosporine was the agent used to induce immunosuppression in the transplant recipients, and the target blood levels were 100 to 300 ng/ml. Rejection episodes were treated with a 1 g bolus dose of methylprednisolone daily for 3 days, and OKT3 was administered in steroid-resistant cases.

Renal biopsy was performed in suspected episodes of acute rejection (AR) where patients' creatinine levels were elevated, or in patients with posttransplantation oliguria and clinical signs of rejection. The specimens were obtained using a biopsy gun fitted an 18-gauge needle. At least two biopsy cores were collected for each case. A11 specimens were fixed in Formalin and embedded in paraffin. For light microscopy, 3-4 urn-thick sections were cut from the paraffin blocks and stained with hematoxylin and eosin, periodic acid Schiff (PAS), and Masson's trichrome stains. The light microscope sections were evaluated for evidence of rejection, and were graded according to the Banff classification (10). Only biopsies that contained a minimum of 7 glomeruli were included in the study. Of 73 biopsy specimens, 33 were classed as chronic rejection (CR) and 40 as acute rejection (AR). Of the 40 biopsies that exhibited AR, 10 were categorized as type IA, 8 were type IB, 17 were type IIA, and 5 were type IIB.

A11 the biopsies yielded adequate tissue for study. A 3 urn section from each specimen was stained for the presence of CD68 using a commercially available mouse monoclonal antibody (KPI clone, Dako, Denmark) that labels the cytoplasm of macrophages in a wide variety of nonlymphoid tissues. We used the avidin-biotin peroxidase staining procedure to be able to identify CD68. To derive the glomerular macrophage index (GMI) for each sample, we counted the total number of CD68-positive cells present in all glomeruli and then divided by the number of glomeruli in the section. Thus, the GMI is the number of CD68-positive cells per glomerulus. A11 slides were assessed by the examiners who were blinded to the clinical and pathological diagnoses. We evaluated the recipients' renal function with serial creatinine level measurements.

Statistical analysis was performed using the software SPSS for Windows. Differences between parameters were evaluated using the Kruskal Wallis test, and the chi-square test was used to analyze categorical values. The criterion for statistical significance was $p<0.05$.

Results

In the 73 patients who exhibited impaired kidney function after renal transplantation, the grafts exhibited chronic rejection (CR) in 33 cases and AR in 40 cases. Of the AR cases, 20 responded to steroid treatment and 20 were steroid-resistant, requiring OKT3 immunosuppressive therapy.

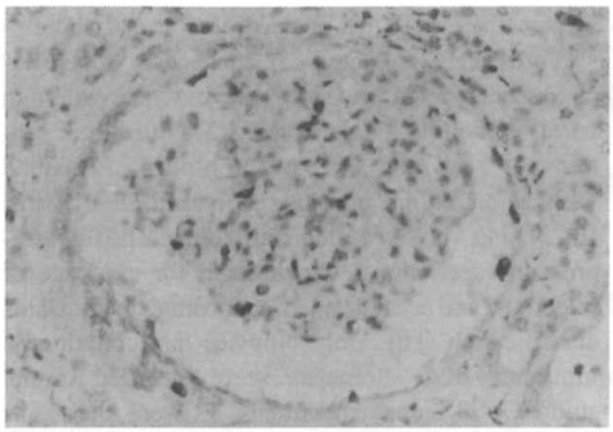

Figure 1. A biopsy section from a transplant kidney shows CD68-positive macrophages in the glomeruli.

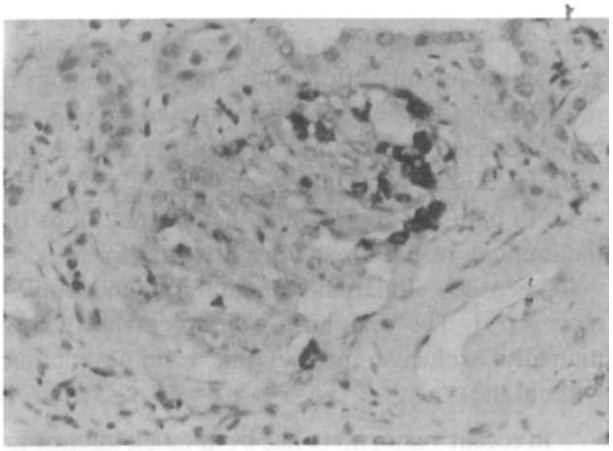

Figure 2. Dense macrophage infiltration in the glomeruli and in the periglomerular interstitium was noticed.

We regarded a biopsy as positive for macrophage infiltration if there were at least two CD68-positive cells present in one or more glomeruli (9). The number of CD68-positive macrophages observed in a single glomerulus varied between 2 and 15 (Figure 1). Only 28 (38.4%) biopsy sections stained positive for CD68 in the glomeruli. The glomeruli of the remaining 45 specimens contained no macrophages. Almost all biopsies that showed macrophages in the glomeruli also exhibited interstitial macrophage infiltration. Dividing the 28 cases according to pathology present, 7/33 CR and 21/40 AR patients were positive for CD68 in the glomeruli.

We observed intraglomerular macrophages in only 6 of the 20 AR cases that responded to steroid therapy (mean GMI, 0.3±0.1), and in 15 of the 20 steroid-resistant AR cases (mean GMI, 1.7±1.2) ($p<0.01$). In the AR biopsies that showed vascular injury in the graft tissue (types IIA and LIB), the intraglomerular macrophage density was 1.43±0.5, whereas the density in the AR biopsies with no vascular injury was 0.47±0.2 (types IA and IB) ($p<0.01$). The specimens from patients who exhibited vascular injury also showed macrophage infiltration on the walls of the arterioles. In the CD68-positive biopsies, we noted no statistical differences in GMI between patients with the various grades of AR and CR (Tables 1 and 2). Of the 33 CR biopsies, only 7 exhibited intraglomerular macrophages. All seven of these patients had previously experienced episodes of acute rejection.

Table 1. The relationship between GMI, severity of acute rejection, and response to treatment

	N	Responded to steroid	Steroid-resistant	GMI ± SE
AR Type IA	**10**	**8**	**2**	**0.31 ±0.1**
AR Type IB	**8**	**5**	**3**	**0.72 ±0.3**
AR Type IIA	**17**	**5**	**12**	**1.24 ±0.6**
AR Type IIB	**5**	**2**	**3**	**2.28 ± 0.8**
AR with vascular injury	**22**	**7**	**15**	**1.43 ±0.5**
AR No vascular injury	**18**	**13**	**5**	**0.47 ± 0.2**

GMI: Glomerular macrophage index
AR: Acute rejection

Table 2. The relationship between GMI and grade of chronic rejection.

	N	GMI ± SE
CR Grade 1	11	0.3 ± 0.1
CR Grade 2	11	0.9 ± 0.3
CR Grade 3	11	1.4 ± 0.9

GMI: Glomerular macrophage index
CR: Chronic Rejection

In the 6 months after the biopsies were examined, 20 of the 28 patients whose biopsies had shown intraglomerular macrophages rejected their graft completely or experienced deterioration of renal function. In two other cases, the grafts were irreversibly lost within a month of the time the biopsies were obtained. The remaining six patients

whose biopsies showed macrophages in the glomeruli had stable renal function during the 6 months following the biopsy. Of the 45 cases that showed no intraglomerular macrophages, 13 grafts went on to irreversible rejection or functional deterioration, 14 were stable, and 18 recipients experienced improved renal function during the 6 months postbiopsy. There was a statistically significant difference between graft outcome for patients with intraglomerular macrophages and graft outcome for patients whose biopsy showed no macrophages in the glomeruli ($p<0.01$).

Discussion

Several types of glomerular lesions occur in renal allografts. Transplant glomerulitis is one such abnormality, and is considered an important finding when evaluating renal biopsies according to the Banff classification (10). Renal graft glomerulitis may develop in the early posttransplantation period, or may appear later as a manifestation of CR. The cause of this glomerulitis and its relationship to classic AR is still not clear. Some authors have reported a significant correlation between the degree of glomerulitis and the grade of AR (11-13). In contrast, Axelsen et al. (14) found no correlation between the severity of rejection and glomerulitis, though they did find other evidence of cellular or vascular rejection in most of the biopsies that exhibited glomerulitis.

Little is known about why macrophages appear in transplant glomerulitis. As outlined, our aim was to investigate the presence of macrophages in graft glomerulitis, and to try to assess the importance of these macrophages in rejection episodes. Earlier investigations of transplant kidney pathology examined macrophage accumulation in the glomeruli of allograft biopsies using a nonspecific esterase reaction (9,15). In the present study, we evaluated the presence of intraglomerular macrophages using a monoclonal antibody CD68 (KP1 clone), which labels macrophage cytoplasm. This method is easier and cheaper than the previous method.

Recognizing the moderate (G2) and severe (G3) degrees of transplant glomerulitis according to the definitions of the Banff classification is easier than identifying mild (G1) glomerulitis in renal allograft biopsies. Mild transplant glomerulitis is characterized by scattered intraglomerular T cells and monocytes. Glomerular monocytes can be prominent in rejection (16,17). Brentjens and colleagues noted a strong relationship between intraglomerular macrophage infiltration and rejection (18). Atkins et al. (19) reported that the more severe the rejection process, as judged by light microscopic study, the greater the number of macrophages present in the glomeruli; however, they did not comment on graft outcome. To date, Harry and coworkers have been the only authors to publish a description of graft outcome for cases of renal transplant rejection in which intraglomerular macrophages were noted. They found that grafts containing glomerular macrophages had a significantly poorer prognosis than those without intraglomerular macrophage infiltration (9).

Similarly, in our study, most patients whose grafts contained intraglomerular macrophages were in the state of AR. Within this group of patients, the highest numbers of intraglomerular macrophages were found in cases of severe AR involving vascular injury. This finding concurs with the results reported by other authors (16,17). It is noteworthy that most of our AR patients did not respond to steroid treatment. We also found intraglomerular macrophages in patients with chronic allograft rejection, but all of these individuals had experienced at least one episode of histologically confirmed AR prior to our study. Our results show that there are associations between macrophage infiltration in graft glomeruli and severe allograft rejection, and between this type of cellular infiltration and poor outcome.

The presence of intraglomerular macrophages in AR suggests that they may function as effector cells in the rejection process, and shows the direct damage that macrophages cause in the renal parenchyma. Monocytes and lymphocytes are the first recipient cells to infiltrate the renal graft. Monocytes initiate the immune response by presenting antigen, activating T cells via interleukin (IL)-1, and releasing additional cytokines, such as interferon-* and tumor necrosis factor (20). Several

authors have shown that AR is accompanied by increased monocyte and macrophage infiltration in both renal and cardiac allografts (16,21,22).

The goal of immunosuppression treatment is to inhibit this monocyte and lymphocyte interaction, and thus prevent rejection. Cyclosporine, the immunosuppressive agent that was used routinely in the patients we studied, is known to inhibit IL-2 production by acting directly on macrophages (23). In our study, we found that patients with the highest proportions of intraglomerular macrophages tended to be those experiencing severe acute graft rejection, even though they were taking cyclosporine. This finding is in line with the results of Mcintosh and Thompson (24), who found that the phagocytic activity of macrophages is unaffected by cyclosporine. Macrophages are thought to react as effector cells in the AR state under cyclosporine treatment.

In conclusion, the results of our study suggest that all cases of macrophage infiltration in graft glomeruli are associated with renal transplant rejection. Furthermore, the presence of these cells indicates that the prognosis for the graft is significantly worse than cases that do not show intraglomerular macrophage infiltration. Of the cases we observed, none of the grafts with macrophages in the glomeruli showed any improvement in function, and the majority were rejected within 6 months of the biopsy. Also, none of these patients responded to steroid treatment, and only some responded to OKT3 as the next line of therapy. We also noticed intraglomerular macrophages in biopsies from individuals who were in chronic graft rejection, but each of these patients had exhibited clinical and histopathological AR at some point prior to our study. Our findings indicate that renal transplant recipients who have macrophages in the graft glomeruli require aggressive immunosuppressive therapy. Finally, these data underline the importance of intraglomerular macrophages in the rejection process.

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