Virchows Arch (2000) 437:167–172

ORIGINAL ARTICLE

Javier Pardo · Luisa Diaz · Pedro Errasti
Michel Idoate · Enrique de Alava · Iosu Sola
Lola Lozano · Angel Panizo

Mast cells in chronic rejection of human renal allografts

Received: 18 August 1999 / Accepted: 15 February 2000

Abstract An increased number of mast cells (MCs) is found in renal specimens of patients with diseases associated with persistent chronic inflammation. MCs proliferation is partly dependent on the presence of T lymphocytes. Both chronic inflammation and T-lymphocytes are essential in the development of chronic rejection (CR), and probably for the infiltration of MCs. MC-derived products such as heparin, histamine, and serine proteases may be responsible for endothelial proliferation and excess collagen production by fibroblasts. In this study, a quantitative evaluation of the MCs infiltration in kidney allografts with CR is performed. The extent of renal fibrosis was analysed in samples stained with Masson's trichrome. To evaluate the potential relationship between MCs and fibrosis in CR we analysed 30 kidneys with CR (25 from nephrectomies and 5 from autopsies). Ten transplanted kidneys obtained from patients died by causes not related with rejection were used as controls. CR was graded according to the Banff schema, which assesses the degree of vasculopathy, tubular atrophy, interstitial fibrosis and transplantation glomerulopathy. Giemsa-stained sections and immunohistochemistry using anti-MC tryptase and c-kit monoclonal antibodies were used to detect MCs. The mean number of MCs per 20 high-power fields (HPF) in the transplanted kidney with CR was 101.8±15.3 in the renal cortex and 46.60±6.52 in the medulla. MCs were significantly more numerous in CR with respect to normal kidneys, both in the cortex ($P<0.01$; Mann-Whitney U test) and in the medulla ($P<0.01$; Mann-Whitney U test). There was a positive correlation between the number of MCs and extent of fibrosis ($P<0.01$; Kruskal-Wallis one-way anova test) and tubular atrophy ($P<0.01$). These results suggest that MCs may play a role in the process of development of interstitial fibrosis in CR.

Key words Mast cell · Chronic rejection · Organ transplantation · Renal allograft · Interstitial fibrosis

J. Pardo · L. Diaz · M. Idoate · E. de Alava · I. Sola · L. Lozano
A. Panizo
Department of Pathology, Clínica Universitaria,
University of Navarra, 31080-Pamplona, Spain

P. Errasti
Department of Nephrology, Clínica Universitaria,
University of Navarra, 31080-Pamplona, Spain

J. Pardo (✉)
Dpto. de A. Patológica, Clínica Universitaria,
Universidad de Navarra, 31080-Pamplona, Spain
e-mail: jpardo@unav.es
Fax: +34-948-172294

Introduction

Renal transplantation is the treatment of choice for most patients with end-stage renal disease. Chronic rejection (CR) is the main cause of long-term graft failure and accounts for 50–80% of the patients who return to dialysis [19, 33]. CR of renal allografts is probably the result of a persistent immune response directed towards the endothelium [25] and mediated by cytotoxic T-lymphocytes [3]. Probably other, nonimmunological, mechanisms, such as the loss of organ mass, ischaemia–reperfusion damage, hypertension, hyperlipidaemia or the effect of cytomegalovirus on the renal allograft [8], are involved in the development of CR.

Characteristically, CR shows interstitial fibrosis and hyalinization of the artery wall with narrowing of the lumen. Renal architecture is altered by interstitial fibrosis, which causes marked tubular atrophy with basement membrane thickening and progressive replacement of tubules by fibrous tissue. Glomeruli show expansion of the mesangial matrix, hypercellularity, and basal membrane thickening [32]. Tubulo-interstitial fibrosis is a common pathway for progressive renal injury in most nephropathies. Indeed there is a close correlation between the level of tubulo-interstitial fibrosis and the degree of chronic renal dysfunction in most renal diseases [10, 30].

Mast cells (MCs) are bone marrow-derived cells that show a wide distribution [18]. MCs are well known for

their role in anaphylactic hypersensitivity reactions, in which they release histamine, heparin, proteases and other mediators which induce multiple proinflammatory effects [14]. The role of MCs in delayed-type hypersensitivity reactions is still poorly understood. T-helper lymphocytes have been characterized by the production of gamma interferon, interleukin (IL)-2, tumour necrosis factor beta (lymphotoxin alpha), IL-4, IL-5, IL-6 and IL-10 and the ability to stimulate production of MCs, eosinophils and IgE [39–41]. In addition to their involvement in the pathogenesis of hypersensitivity reactions, MCs are implicated in many other biological responses, including wound repair [16], atherosclerosis [2], and reactions to neoplasms [7]. They are also believed to be involved in the pathogenesis of a variety of chronic inflammatory diseases, including scleroderma, rheumatoid arthritis, certain forms of pulmonary fibrosis, chronic graft-versus-host disease, and inflammatory bowel diseases [13]. Several reports have suggested that MCs may be involved in kidney fibrosis in certain primary glomerulonephritis (GN) [17], IgA nephritis [9], diabetic nephropathy [34] and acute cellular kidney rejection [24].

Both inflammation and T-lymphocytes are essential in acute rejection, which is among the most recognizable immunological risk factors for CR [21, 28]. To determine whether MCs are involved in tubulo-interstitial injury of CR, we evaluated MC infiltrate in patients who underwent nephrectomy after graft loss for CR and examined the relationship between the extent of MCs infiltration and tubulo-interstitial fibrosis.

Patients and methods

Patients

From October 1969 through December 1998, 525 renal transplantations were performed in the Clínica Universitaria de Navarra, University of Navarra, Spain. Nephrectomy was performed in 47 of the 525 patients (8.2%), 39 of whom had a primary diagnosis of CR. Nine of these were excluded: 5 had large infarcts, 2 had severe acute rejection, 1 had cytomegalovirus infection, and 1 had aspergillosis.

Medical records of the 30 remaining CR patients were available for review. The mean age of the renal allograft recipients at the time of transplantation was 36.17±2.73 (range, 12–63 years). The diseases leading to transplantation were diabetes mellitus (10 cases), chronic pyelonephritis (8 cases), IgA nephropathy (5 cases), other glomerulopathies (3 cases), and unknown causes in 4 cases. All grafts were from adult cadaver donors. Five patients who are included in the study with CR died of unrelated causes, such as myocardial infarction (2 patients), lymphoproliferative disease or an infectious disease (2 patients). The time that elapsed between the transplantation and the nephrectomy ranged from 11 to 139 months (median 50.41±7.12). Demographic data are presented in Table 1.

Table 1 Characteristics of 30 patients undergoing renal transplant nephrectomies

No. of cases	30
Age at nephrectomy (years)	36.17±2.73 (range, 12–63 years)
Donor age	51.5±5.3
Cold ischaemia time	21+5
Acute rejection episodes	0.6+1.1
Time (months) before nephrectomy	50.41±7.12
Male/female	21/9
Hypertension	4 (13.7%)

Biopsies

We fixed the 30 renal allografts removed in 10% formaldehyde. Six samples of each kidney were embedded in paraffin to obtain 4-µm-thick sections and were stained with haematoxylin-eosin, periodic acid–Schiff (PAS), Masson's trichrome, Giemsa and orcein techniques. Control sections were obtained from 10 transplanted kidneys (19–76 years old, mean age 51 years, mean time after transplantation 49 days) without any history of acute or chronic rejection, which were obtained from autopsies following death from causes not related to the transplant.

Immunohistochemical analysis

Immunohistochemical analysis was done on 4-µm sections of formalin-fixed, paraffin-embedded tissue. Immunohistochemistry was performed on an automated immunostainer (TechMate 500; Dako, Copenhagen, Denmark) with the "EnVision+" system (Dako), in which the secondary antibody is coupled with a dextran polymer linked to peroxidase molecules. Endogenous peroxidase activity was quenched by treatment with 5% hydrogen peroxide in methanol for 30 min at room temperature. Antigen retrieval by microwave oven was performed for 15 min in an 800-W microwave. The primary antibody was an anti-MC tryptase monoclonal antibody (Chemicon no. MAB1222, Temecula, Calif.) diluted 1:1000, or a polyclonal CD117 antibody (the c-kit proto-oncogene product) diluted 1:400 (Santa Cruz Biotechnology, Santa Cruz, Calif.), both applied for 120 min at room temperature. The sections were then rinsed with washing buffer at room temperature. The next step was addition of EnVision+ system reagents and incubation for 30 min at room temperature. The slides were rinsed with washing buffer and treated with a solution containing 0.05% diaminobenzidine hydrochloride and 0.1% hydrogen peroxide in 0.05 mol/l TRIS-buffered saline, pH 7.4, at room temperature for 5 min. After rinsing in distilled water for 3 min, the slides were counterstained with modified Harris haematoxylin, dehydrated, and mounted. For negative controls, incubation with normal goat serum instead of primary antibody was used. Omission of the primary antibody likewise gave no background staining. Slides of a skin biopsy with urticaria pigmentosa were used as positive controls.

Immunosuppression

Patients were immunosuppressed with azathioprine, methylprednisolone and cyclosporine (CsA), which was kept at a blood concentration of 300 g/l (specific monoclonal radioimmunoassay, Sandimmun kit, Sandoz, Basel, Switzerland) immediately after transplantation, and between 50 and 100 g/l for 6 months after the transplant.

Assessment of CR

We diagnosed CR following the criteria of Burke et al. [4] and Solez et al. [37]. We graded CR according to the International Histologic Standards of Banff [37, 38]. We excluded subcapsular scars and cases with acute rejection.

We diagnosed CR in biopsies with concentric intimal proliferation and arterial fibrosis, and/or morphological signs of chronic ischaemia in the absence of other factors known to produce these

lesions [4]. All cases had one or several previous biopsies with normal vessels and absence of lesions of chronic ischaemia. Glomerular, tubular, interstitial and vascular lesions were semi-quantitatively evaluated in four categories: 0 absence of lesion, + slight, ++ moderate, +++ severe lesions.

Interstitial fibrosis was measured in Masson's trichrome-stained sections, using image analysis software (Olympus Micro-image, version 2.0), connected to an Olympus Provis microscope: the fibrosis was graded 1+ when it extended over 6–25% of the cortical area; 2+ when it involved 26–50% of the cortical area; and 3+ when it involved more than 50% of the cortical area.

Tubular atrophy was scored according to the following criteria: 1+ when the tubular atrophy involved less than 25% of the cortical area; 2+ when it involved 26–50%; and 3+ when the tubular atrophy involved more than 50%.

Vascular changes were characterized by fibrous intimal thickening, with or without breach of the internal elastic lamina or presence of foam cells or occasional mononuclear cells. These alterations were segmental in distribution. These changes were scored in the artery with most severe features. They were graded with the haematoxylin-eosin and orcein stains. A score of 1+ corresponded to up to 25% narrowing of the vascular luminal area of arteries; 2+, to a narrowing of the vascular luminal area by 26–50%; 3+ meant narrowing of the vascular luminal area by more than 50%.

Transplant glomerulopathy [27, 32] was diagnosed when a mesangial matrix expansion with associated basement membrane thickening, with or without double contours in capillary loops, was seen. These changes were graded using the following criteria: grade 1+ affecting up to 25% of nonsclerotic glomeruli; grade 2+ changes as above, but more pronounced, involving 26–50% of nonsclerotic glomeruli; and grade 3+ classic transplant glomerulopathy in more than 50% of nonsclerotic glomeruli.

The overall degree of CR was calculated by adding the scores for the other four parameters (interstitial fibrosis, tubular atrophy, vascular changes and transplant glomerulopathy). We recorded grade I when the total score was less than 4; grade II when the score ranged from 5 to 8; and grade III when the score was >8.

Cell count

The MCs were counted on the Giemsa-stained slides and immunohistochemistry using anti-MC tryptase or c-kit monoclonal antibodies on at least one set of randomly chosen 20 high-power fields (HPFs), in the medulla and in the cortex. Every high-power field corresponds to 0.55 mm^2 of surface area. Though the differences between the two techniques were not significant, the mean of the values was used. We did not consider occasional collections of metachromatic granules not associated with identifiable cells.

Statistics

Data were reported as mean ± standard error of the mean in 20 HPFs. Statistical analysis for comparison of means was performed by the Mann-Whitney U-test, the Wilcoxon signed-ranks test and the Kruskal-Wallis one-way anova test, using SPSS 7.5 for Windows. Correlation studies were also performed using the SPSS 7.5 and the Spearman test. A *P*-value less than 0.05 was considered statistically significant.

Results

According to the Banff schema, 3 cases of CR were classed as grade I, 10 cases as grade II, and 17 cases as grade III.

MCs could be identified in the cortex and medulla of all kidneys in slides stained with Giemsa stain and with immunohistochemistry with MC-tryptase or c-kit antibodies. The majority of the MCs were located diffusely throughout the interstitium without relation to inflammatory cell aggregates. MCs were more frequent in areas with a higher degree of fibrosis. The MCs were round or oval, with abundant metachromatic granules (Fig. 1). Some MCs had cytoplasmic processes or elongations (Fig. 2). No intratubular MCs were observed, but some intraglomerular MCs were identified in 1 case.

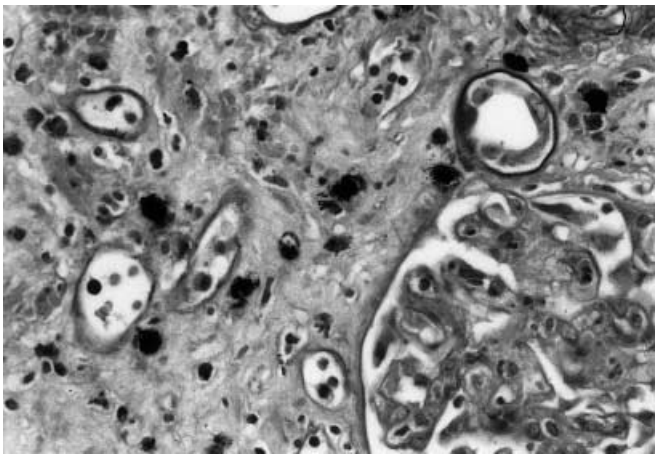

Fig. 1 Interstitial mast cells in a patient with grade III chronic renal rejection. Giemsa, original magnification ×450

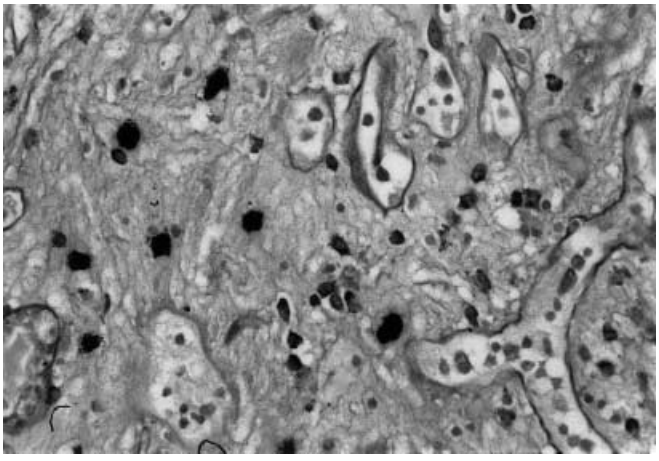

Fig. 2 Mast cells stained with anti-MC triptase in a renal allograft 61 months after transplantation. Original magnification ×400

The number of MCs in the renal cortex of patients with CR was significantly higher than in control specimens (101.08±15.3 MCs/20 HPF vs 10.60±2.33 MCs/20 HPF; $P<0.01$). A similar difference was found between the medulla of CR and that of control kidneys (46.6±6.52 MCs/20 HPF vs 14.67±2.98 MCs/20 HPF, $P=0.008$). When we compared the number of MCs in the cortex and in the medulla of kidneys with CR we found the former significantly higher ($P<0.01$). The differences between the two areas were not significant in control kidneys.

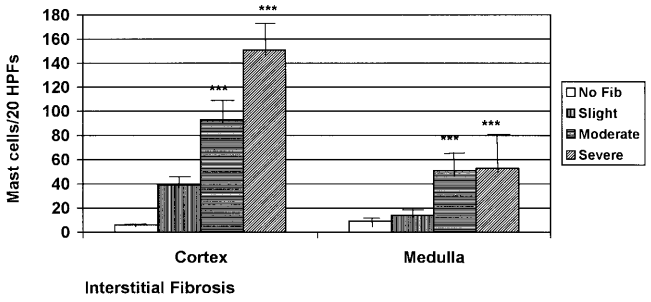

Fig. 3 The number of mast cells increases with the degree of interstitial fibrosis both in the cortex and in the medulla. The difference is statistically significant in moderate and severe degrees of interstitial fibrosis

Table 2 Number of mast cells (*MCs*) per 20 high-power fields (*HPF*) in control kidneys and in kidneys affected by chronic rejection (*CR*)

	n	MCs in the cortex (MCs/20 HPF)	MCs in the medulla (MCs/20 HPF)
Normal transplant kidneys	10	10.60±2.33	14.67±2.98
CR: all grades	30	101±17.01	53.34±8.66
CR grade I	3	20.00±11.15	10.67±5.04
CR grade II	10	69.50±14.30	34.20±8.04
CR grade III	17	132.29±22.35	60.24±9.08

The number of MCs increased with the degree of CR in the renal cortex and in the medulla, especially in moderate and severe CR (Table 2: r=0.755, P<0.01 in the cortical kidney; r=0.571, P<0.01 in the medulla). We did not find any correlation between the time that had elapsed since transplantation and the number of MCs.

We found a significant correlation between the number of MCs and the degree of interstitial fibrosis (P<0.01; Fig. 3) and tubular atrophy (P<0.01) both in the cortex and in the medulla. There was no correlation between the number of MCs and the presence of transplant glomerulopathy or the grade of transplant vasculopathy. The relationship between the number of MCs and the degrees of tubular atrophy and interstitial fibrosis was especially significant in severe CR (P<0.01).

We found no correlation between the number of MCs and those of other inflammatory cells, including eosinophils, which were present in 17 cases in percentages that varied between 1% and 6% of the inflammatory infiltrate.

Discussion

The anti-MC tryptase monoclonal antibody used in this study has been reported to be a good method for the detection of MCs in formalin-fixed paraffin-embedded renal tissues, as has been shown by others [22, 24]. The *c-kit* gene product (CD117) is known to be expressed in a variety of normal human tissue cell types, including breast epithelium, germ cells, melanocytes, immature myeloid cells, and mast cells [1]. The strong membrane reactivity for CD117 seen in mast cells may be useful in the diagnosis of mast cell disorders. We have not found statistically significant differences in the detection of MCs using Giemsa stain or immunohistochemistry with triptase or c-kit antibodies. Moreover, endogenous peroxidase activity is characteristic of MCs and is associated with the ability of the cells to synthesize PGD2. In this respect, MCs are well visualized with any immunohistochemical technique based on peroxidase activity [11, 12].

MCs are seen along with lymphocytes and monocytes in GN [17], and they contribute to the process of renal deterioration in GN through tubulo-interstitial injury. The number of MCs is higher in chronic GN, such as focal segmental GN or diabetic nephropathy, than in acute GN, such as in rapidly progressive GN [9, 17, 34]. The number of MCs is slightly higher in CR than in chronic GN, including diabetic nephropathy. Comparison of MCs in GN and in CR with similar degrees of fibrosis would probably reveal whether MCs have a special pathogenetic role in CR.

According to our results, MCs have an important role in the development of interstitial fibrosis in CR of kidney allografts. MCs are activated by C5a and C3a and some cytokines (Il-5, Il-8), participate in type I hypersensitivity anaphylactic reaction and in both acute and persistent inflammatory reactions, and secrete different primary mediators such as biogenic amines, chemotactic mediators, enzymes and proteoglycans, and secondary mediators such as leukotrienes, prostaglandin D2, platelet-activating factor and cytokines [5]. The presence of MCs in connective tissues has been linked to the development of fibrosis through the production of cytokines and growth factors, such as histamine, heparin, tryptase, basic fibroblastic growth factor, tumour necrosis factor-α and transforming growth factor-β, which stimulate the proliferation of myofibroblasts and fibrosis [15, 16, 31, 35]. Lajoie et al. [24] suggest that the presence of a large number of MCs in renal allografts could indicate an unfavourable prognosis of the allograft. Early measurement of interstitial fibrosis has been demonstrated to predict long-term renal function and graft survival [30, 34].

Some authors [23] think that the MCs' secretion of interleukin 5, a eosinophil chemoattractant, induces an eosinophilic infiltrate in acute and CR. Thus, eosinophils and MCs would increase together in CR. Lajoie et al. [24] suggest that the presence of a large number of eosinophils in renal allografts could indicate an unfavourable prognosis. This hypothesis has not been proved so far, but to our mind provides an explanation for the presence of eosinophils in cellular rejection, which were thought to be an adverse histological feature for the outcome in renal allografts [23]. The absence of correlation among the number of MCs, lymphocytes and eosinophils may be due to the long evolution of the CR, since the re-

lationship among these three cell types has always been described in acute phases [3, 23, 39–41].

Immunological rejection and ischaemia are probably the mechanisms of induction of MC infiltration in renal allografts [6, 17, 20, 27, 36]. The relation between MCs and rejection has been described in the heart [26], lung [42], and intestine [40], and in graft-versus-host disease [29] and acute cellular rejection of the human kidney [24].

In conclusion, MCs play a part in the development of fibrosis in CR, and should be included in any protocol of evaluation of renal allograft biopsies.

References

1. Arber DA, Tamayo R, Weiss LM (1998) Paraffin section detection of the c-kit gene product (CD117) in human tissues: value in the diagnosis of mast cell disorders. Hum Pathol 29: 498–504
2. Atkinson JB, Harlan CW, Harlan GC, Virmani R (1994) The association of mast cells and atherosclerosis: a morphologic study of early atherosclerotic lesions in young people. Hum Pathol 25:154–159
3. Barth C, Von Menges A, Zanker B, Lammerding P, Stachowski J, Baldamus CA (1996) Restricted T cell V beta repertoire in renal allografts during acute and chronic rejection. Kidney Int 50: 2020–2026
4. Burke BA, Chavers BM, Gillingham KJ, Kashtan CE, Manivel JC, Mauer SM, Nevins TE, Matas AJ (1995) Chronic renal allograft rejection in the first 6 months posttransplant. Transplantation 60:1413–1417
5. Cotran R, Kumar V, Collins T (1999) Diseases of immunity. In: Robbins' Pathologic basis of disease. Saunders, Philadelphia, pp 196–199
6. De Heer E, Davidoff A, van der Wal A, van Geest M, Paul LC (1994) Chronic renal allograft rejection in the rat. Transplantation-induced antibodies against basement membrane antigens. Lab Invest 70:494–502
7. Dimitriadou V, Koutsilieris M (1997) Mast cell-tumor cell interactions: for or against tumour growth and metastasis? Anticancer Res 17:1541–1549
8. Dodd SM (1999) Chronic allograft nephropathy: the inevitable outcome of renal transplantation? Curr Top Pathol 92:37–60
9. Ehara T, Shigematsu H (1998) Contribution of mast cells to the tubulointerstitial lesions in IgA nephritis. Kidney Int 54: 1675–1683
10. Eknoyan G, McDonald MA, Appel D, Truong LD (1990) Chronic tubulo-interstial nephritis: correlation between structural and functional findings. Kidney Int 38:736–743
11. Escribano LM, Gabriel LC, Villa E, Navarro JL (1987) Endogenous peroxidase activity in human cutaneous and adenoidal mast cells. J Histochem Cytochem 35: 213–220
12. Fritz P, Muller J, Reiser H, Saal JG, Hadam M, Tuczek HV, Wegner G, Laschner W (1986) Avidin–peroxidase. A new mast cell staining method. Acta Histochem [Suppl] 32:235–239
13. Galli SJ (1993) New concepts about the mast cell. N Engl J Med 328:257–265
14. Galli SJ, Wershil BK (1996) The two faces of the mast cell. Nature 381:21–22
15. Gruber BL, Kew RR, Jelaska A, Marchese MJ, Garlick J, Ren S, Schwartz LB, Korn JH (1997) Human mast cells activate fibroblasts: tryptase is a fibrogenic factor stimulating collagen messenger ribonucleic acid synthesis and fibroblast chemotaxis. J Immunol 158:2310–2317
16. Hebda PA, Collins MA, Tharp-MD (1993) Mast cell and myofibroblast in wound healing. Dermatol Clin 11:685–696
17. Hiromura K, Kurosawa M, Yano S, Naruse T (1998) Tubulointerstitial mast cell infiltration in glomerulonephritis. Am J Kidney Dis 32:593–599
18. Horny HP, Ruck P, Xiao JC, Kaiserling E (1993) Immunoreactivity of normal and neoplastic human tissue mast cells with macrophage-associated antibodies, with special reference to the recently developed monoclonal antibody PG-M1. Hum Pathol 24:355–358
19. Hostetter TH (1994) Chronic transplant rejection. Kidney Int 46:266–279
20. Huang C, Sali A, Stevens RL (1998) Regulation and function of mast cell proteases in inflammation. J Clin Immunol 18:169–183
21. Humar A, Kerr S, Hassoun A, Granger D, Suhr B, Matas A (1999) The association between acute rejection and chronic rejection in kidney transplantation. Transplant Proc 31:1302–1303
22. Irani AM, Bradford TR, Kepley CL, Schechter NM, Schwartz LB (1989) Detection of MCT and MCTC types of human mast cells by immunohistochemistry using new monoclonal anti-tryptase and anti-chymase antibodies. J Histochem Cytochem 37:1509–1515
23. Kormendi F, Amend WJ Jr (1988) The importance of eosinophil cells in kidney allograft rejection. Transplantation 45: 537–539
24. Lajoie G, Madasdy T, Laszik Z, Blick KE, Silva FG (1996) Mast cells in acute cellular rejection of human renal allografts. Mod Pathol 9:1118–1125
25. Lemstrom K, Koskinen P, Hayry P (1995) Molecular mechanisms of chronic renal allograft rejection. Kidney Int (Suppl) 52:S2–S10
26. Ly QY, Raza Ahmad A, MacAulay MA, Lalonde LD, Rowden G, Trethewey E, Dean S (1992) The relationship of mast cells and their secreted products to the volume of fibrosis in post-transplant hearts. Transplantation 53:1047–1051
27. Maryniak RK, First MR, Weiss MA (1985) Transplant glomerulopathy: evolution of morphologically distinct changes. Kidney Int 27:799–806
28. Monaco AP, Burke JF Jr, Ferguson RM, Halloran PF, Kahan BD, Light JA, Matas AJ, Solez K (1999) Current thinking on chronic renal allograft rejection: issues, concerns, and recommendations from a 1997 roundtable discussion. Am J Kidney Dis 33:150–160
29. Nagler A, Segal V, Slavin S, Levi-Schaffer F (1995) Ketotifen therapy in chronic graft-versus-host disease (cGVHD): effect on mast cells and fibroblasts. Clin Exp Immunol 100: 529–535
30. Nicholson ML, McCulloch TA, Harper SJ, Wheatley TJ, Edwards CM, Feehally J, Furness PN (1996) Early measurement of interstitial fibrosis predicts long-term renal function and graft survival in renal transplantation. Br J Surg 83: 1082–1085
31. Nilsson G, Metcalfe DD (1996) Contemporary issues in mast cell biology. Allergy Asthma Proc 17:59–63
32. Pardo-Mindan FJ, Salinas-Madrigal L, Idoate M, Garola R, Sola I, French M (1992) Pathology of renal transplantation. Semin Diagn Pathol 9:185–199
33. Paul LC, Häyry P, Foegh M, Dennis MJ, Miharsch MJ, Larsson E, Fellström B (1993) Diagnostic criteria for chronic rejection/accelerated graft atherosclerosis in heart and Kidney transplants. Transplant Proc 25:2022–2023
34. Ruger BM, Hasan Q, Greenhill NS, Davis PF, Dunbar PR, Neale TJ (1996) Mast cells and type VIII collagen in human diabetic nephropathy. Diabetologia 39:1215–1222
35. Ruoss SJ, Hartaman T, Caughey GH (1991) Mast cell tryptase is a mitogen for cultured fibroblasts. J Clin Invest 88: 493–499
36. Shoskes DA, Parfrey NA, Halloran PF (1990) Increased major histocompatibility complex antigen expression in unilateral ischemic acute tubular necrosis in the mouse. Transplantation 49:201–207

37. Solez K, Axelsen RA, Benediktsson H, Burdick JF, Cohen AH, Colvin RB, Croker BP, Droz D, Dunnill MS, Halloran PF, Häyry P, Jennette JC, Keown PA, Marcussen N, Mihatsch MJ, Morozuni K, Myers BD, Nast CC, Olsen S, Racusen LC, Ramos EL, Rosen S, Sachs DH, Salomon DR, Sanfilippo F, Verani R, von Villebrand E, Yamaguchi Y (1993) International standardization of criteria for the histologic diagnosis of renal allograft rejection: the Banff working classification of kidney transplant pathology. Kidney Int 44:411–422
38. Solez K, Benediktsson H, Cavallo T, Croker B, Demetris AJ, et al (1996) Report of the Third Banff Conference on Allograft Pathology (20–24 July 1995) on classification and lesion scoring in renal allograft pathology. Transplant Proc 28:441–444
39. Suga M, Yamasaki H, Nakagawa K, Kohrogi H, Ando M (1997) Mechanisms accounting for granulomatous responses in hypersensitivity pneumonitis. Sarcoidosis Vasc Diffuse Lung Dis 14:131–138
40. Walgenbach KJ, Heeckt PF, Stanson JD, Whiteside TL, Bauer AJ (1996) Increased presence of mast cells and interleukin-4 during chronic rejection of rat intestinal allografts. Transplant Proc 28:2454
41. Wood PR, Seow HF (1996) T cell cytokines and disease prevention. Vet Immunol Immunopathol 54:33–44
42. Yousem SA (1997) The potential role of mast cells in lung allograft rejection. Hum Pathol 28:179–182