

Chronic Antibody Mediated Rejection of Renal Allografts: Pathological, Serological and Immunologic Features in Nonhuman Primates

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The pathogenesis of late renal allograft loss is heterogeneous and difficult to diagnose. We have analyzed renal allografts in nonhuman primates to determine the relationship between alloantibodies and the graft pathology of late graft loss. Seventeen Cynomolgus monkeys were chosen from among those on several protocols for renal allotransplantation with mixed chimerism induction so that animals with and without alloantibodies were included. All animals received transient CD154 blockade and short-term cyclosporine treatment until day 28. Serial blood samples were tested for alloantibodies. Protocol biopsies and autopsy kidneys were scored for pathology and C4d deposition. Group 1, defined by complete lack of C4d deposition (24 tissue samples; 8 recipients), had no detectable alloantibodies (33 serum samples; 1–7 samples per recipient) and no evidence of chronic rejection. Three survived greater than 2 years with normal function and histology. Group 2, defined as having C4d deposition in peritubular capillaries, all made alloantibodies (100%), and most grafts later showed chronic allograft glomerulopathy (89%), and/or arteriopathy (89%). All grafts in Group 2 failed (3–27 months). Pathologic lesions of typical of chronic rejection in humans develop in monkeys, correlate with antecedent alloantibodies/C4d deposition and predict chronic rejection rather than durable accommodation.

Key words: Alloantibodies, chronic rejection, primate

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Introduction

Late graft loss remains a major obstacle to successful long-term kidney allograft transplantation. The factors con-

tributing to late graft loss include factors, both immunological (cellular and/or antibody mediated injuries) and nonimmunological (donor disease, recurrent disease, peritransplant ischemia, viral infection or drug toxicity). Considerable circumstantial evidence in humans implicates MHC alloantibodies as a cause of late graft loss (1). A more direct linkage between alloantibody and tissue injury is provided by deposition of C4d in renal peritubular capillaries (2). C4d, a split product of the classical pathway of complement activation, is present covalently bound on tissue near the sites of complement activation by alloantibody.

The presence of C4d staining of peritubular capillaries correlates well with the presence of serum alloantibodies and acute and chronic graft injury in humans (2–5). A substantial fraction of patients (60%, range 20–80%) with chronic allograft glomerulopathy or arteriopathy have C4d deposition in peritubular and/or glomerular capillaries, and 60–100% of these have alloantibodies (6–9). Furthermore, C4d deposition preceded the development of chronic allograft glomerulopathy (CAG) (9). Overall, among reports comprising 368 patients, 50% of those with CAG had C4d deposition vs. 15% of grafts without these lesions (6–9). However, alloantibodies (10,11) or C4d (12) in protocol grafts can be found in patients with normal function and without renal pathology. To account for these findings, we postulated that antibody mediated chronic rejection develops in stages, beginning with the production of alloantibody, leading to C4d deposition in the graft, followed by chronic pathological injury, and leading to graft dysfunction (13,14).

For the past decade, we have evaluated mixed chimerism protocols for induction of tolerance in Cynomolgus monkeys (*Macaca fascicularis*) by means of a combination of nonmyeloablative radiation, donor bone marrow, T-cell depletion, anti-CD40 ligand and short-term immunosuppression with cyclosporine (15–21). These protocols can lead to long-term graft survival without chronic allograft rejection. However, alloantibodies develop in some recipients, as well as glomerular and arterial lesions, similar to those in humans with chronic rejection (16).

In this report, we show evidence from protocol biopsies, autopsies and serological assays that these monkeys develop chronic antibody mediated rejection (glomerulopathy, arteriopathy and renal failure), which comes about

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after the development of alloantibodies and C4d deposition, but in the absence of confounding factors such as ongoing drug therapy, donor or recurrent disease.

Methods

Animals

Cynomolgus monkey recipients and donors weighed 3–8 kg (Charles River Primates, Wilmington, MA). Recipient and donor pairs were selected for compatible ABO blood types but mismatched for Cynomolgus leukocyte (CyLA) MHC antigens (15,16,20). CyLA-class I antigens were defined serologically and class II antigenic disparity was determined by positive mixed lymphocyte response *in vitro*. In addition, donor-recipient pairs were chosen to assure that at least one anti-class I monoclonal antibody could distinguish donor from recipient to allow post-transplant detection of chimerism (15,20). All surgical procedures and post-operative care of animals were carried out in accordance with National Institute of Health guidelines for the care and use of primates and were approved by the Massachusetts General Hospital Subcommittee on Animal Research.

Regimens

All 17 recipients received nonlethal total body irradiation (TBI) (1.5 Gy) on day –6 and –5, local thymic irradiation (TI) (7 Gy) on day –1, *i.v.* ATG (ATGAM, Upjohn Pharmacia Co., Kalamazoo, MI.) (50 mg/kg/day) on day –2, –1 and 0, and *i.v.* donor bone marrow transplantation (DBMT) on day 0. Bone marrow was recovered from donor iliac bones by multiple percutaneous aspirations, and the number of mononuclear cells infused ranged from 0.4 to 4×10^9 /kg. As previously described monkeys under ketamine hydrochloride/diazepam anesthesia underwent heterotopic renal transplantation, nephrectomy of one kidney, and ureteral ligation of the second native kidney, which was nephrectomized about 1 month later at the time of a protocol biopsy (15). Anti-CD154 monoclonal antibody (5c8, Immerge Biotherapeutics) was given at 20 mg/kg, (2–6 doses) up to day 10. Cyclosporin (CyA, Novartis, Basel, Switzerland) was given *i.m.* beginning on day 1 and tapered from an initial dose of 15 mg/kg/day to maintain therapeutic serum levels (>300 ng/mL). CyA was discontinued on day 28 post-transplant after which the serum CyA levels became undetectable by day 60–70. Four animals also received a splenectomy at the time of transplant (1501, 4297, 6300, 3098) and four received anti-CD8 during the conditioning period (2800, 2702, 4102, 7801). These animals were intentionally chosen from among those on several protocols for renal allotransplantation with mixed chimerism induction, such that animals that did and did not develop DRA would be included. Our goal was to follow animals with and without alloantibody and not to evaluate a specific protocol.

Detection of anti-donor alloantibody

Anti-donor specific alloantibodies were assayed by flow cytometric analysis in 77 post-treatment and all 17 pre-treatment samples (16). Donor peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood by gradient centrifugation over freshly prepared 60% Percoll (Pharmacia Biotech, Uppsala, Sweden). Contaminating red blood cells were removed by standard water-shock treatment. PBMCs were incubated with recipient sera for 30 min at 4°C. After washing, FITC conjugated mouse anti-human IgG mAb was added and incubated for 30 min at 4°C, then washed twice. PBMC were further incubated with PE conjugated anti-CD20 mAb (Becton-Dickinson, Mountain View, CA) for 30 min at 4°C. After washing, PBMC were fixed with 2% paraformaldehyde. Cells were then acquired and analyzed with FACScan (Becton Dickinson, Mountain View, CA). A positive reaction was defined as a mean channel of fluorescence (MCF) of >10 for T cells and >50 for B cells. These thresholds are greater than two standard deviations above the mean channel level of donor cells stained with pre-treatment serum for these 17 animals (4.8 ± 1.7 and 23.3 ± 12.5 for T and B cells, respectively).

Pathology studies

Renal biopsies were obtained whenever a rise in creatinine occurred and protocol biopsies were obtained at 2–6-month intervals in animals with stable function. Overall, 59 renal allograft samples from 17 animals were studied, including 45 biopsies and 14 autopsies. Three animals were living with functional grafts at the time of this analysis. Tissue was processed for light microscopy and a portion frozen for immunofluorescence staining. Other organs obtained surgically (lymph nodes, native kidney and spleen) were similarly processed. After euthanasia, complete autopsies were performed and tissues were processed for study from the renal allograft, lymph node, heart, lung, liver, pancreas, thymus and skin.

Biopsy, nephrectomy and autopsy sections were stained with hematoxylin and eosin and periodic acid Schiff (PAS) stains and scored in coded samples, using Banff 2003 criteria without the knowledge of clinical or serological results (22). Banff scores were calculated using customized software (Colvin, unpublished; © The General Hospital Corporation). Chronic allograft glomerulopathy was defined as $>50\%$ glomerular capillaries with GBM duplication in the most involved glomerulus in PAS stains. Chronic allograft arteriopathy was scored only on autopsy and nephrectomy specimens, due to the paucity of arteries on the routine wedge biopsies. Polyoma virus was detected in formalin-fixed paraffin embedded tissue, using the avidin-biotin-peroxidase complex technique and a monoclonal antibody to the large T antigen of SV40 (Ab-2; Oncogene Science, Calbiochem, Cambridge, MA) as described by van Gorder et al. (23). Post-transplant lymphoproliferative disease (PTLD) was diagnosed by morphology, immunohistochemistry and *in situ* hybridization, as described by Schmidtke et al. (21). Lymphocytes were identified with polyclonal antibodies to CD3 (DAKO) or monoclonal antibodies to CD20 (L26, DAKO) in paraffin embedded sections. Tissue was frozen in OCT or fixed in Karnovsky's for subsequent evaluation by either direct immunofluorescence or electron microscopy.

Detection of C4d

Preliminary results showed that the polyclonal rabbit anti-human C4d peptide (9) cross-reacted on Cynomolgus kidneys (in contrast to the negative results with two monoclonal antibodies to human C4d). Four-micron-thick sections on Superfrost Plus slides were baked at 60°C for 30 min and deparaffinized and rehydrated. For antigen retrieval, slides were heated in a pressure cooker for 3 min in Antigen Decloaker solution (Biocare Medical, Concord, CA), cooled and rinsed in PBS. Slides were blocked with normal goat serum 1:50 dilution for 20 min at room temperature. Two drops of avidin D (100 ug/mL in PBS) were added to the normal serum on each slide. Polyclonal anti-C4d (Biomedica, Vienna, Austria) was added at 1:50 in PBS and incubated at 4°C overnight. After PBS washing, Universal Link (Biocare Medical) was added and incubated for 20 min. After washing in PBS, Streptavidin-horse radish peroxidase (Biocare Medical) was added and incubated for 20 min. Slides were rinsed in distilled water and Romulin AEC Chromogen (Biocare Medical) was added, and the slides incubated for 2.5–5 min. The slides were rinsed in distilled water, counterstained, dehydrated in alcohol and xylene and cover slipped with permanent mounting media. Coded sections were scored for positive peritubular capillaries in 10 high power fields (40 \times , 412 μ diameter). A positive C4d result was defined as at least 6 of 10 randomly chosen high-power fields with circumferentially staining peritubular capillaries. Staining less than this was deemed focal and negative for statistical correlations. All native kidneys tested were negative for C4d in PTC and glomeruli; eight native kidneys, nephrectomized about 1 month after ureteral ligation (see surgical protocol above) with pathological changes of chronic obstruction were C4d negative.

Statistical analysis

Data were analyzed using Fisher's exact test (SPSS software, SPSS Inc., Chicago, IL).

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Results

Recipients chosen from several protocols (see Methods) were analyzed as two groups, based on whether or not they developed C4d deposition. Group 1 included eight recipients without C4d deposition in any graft sample (0/24)

(Figure 1). Their individual longitudinal data with creatinines are included in Figure 1. None of these animals developed alloantibodies or chronic rejection (no chronic allograft glomerulopathy or arteriopathy) and three survived with normal function and histology beyond 2 years. Graft loss in the remaining five was due to obstructive uropathy (2),

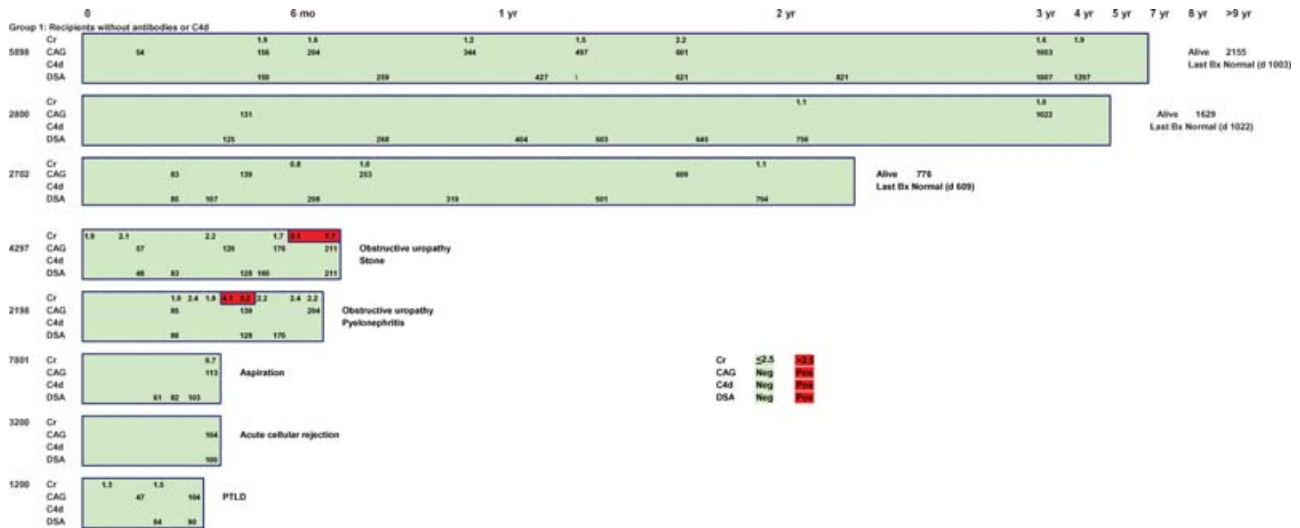


Figure 1: Longitudinal data for Group 1 monkeys without C4d deposition. Longitudinal creatinines are indicated with greater than 2.5 mg/dL considered abnormal in red. Numbers along the CAG row indicate the day post-transplantation on which a biopsy was performed for histological review. Numbers along the DSA row indicate the day on which serum was taken for DSA testing. Text at the end of the data column indicates status of animal or cause of death. Positive results are indicated by red bars (Cr > 2.5 mg/dL, CAG or positive DSA).

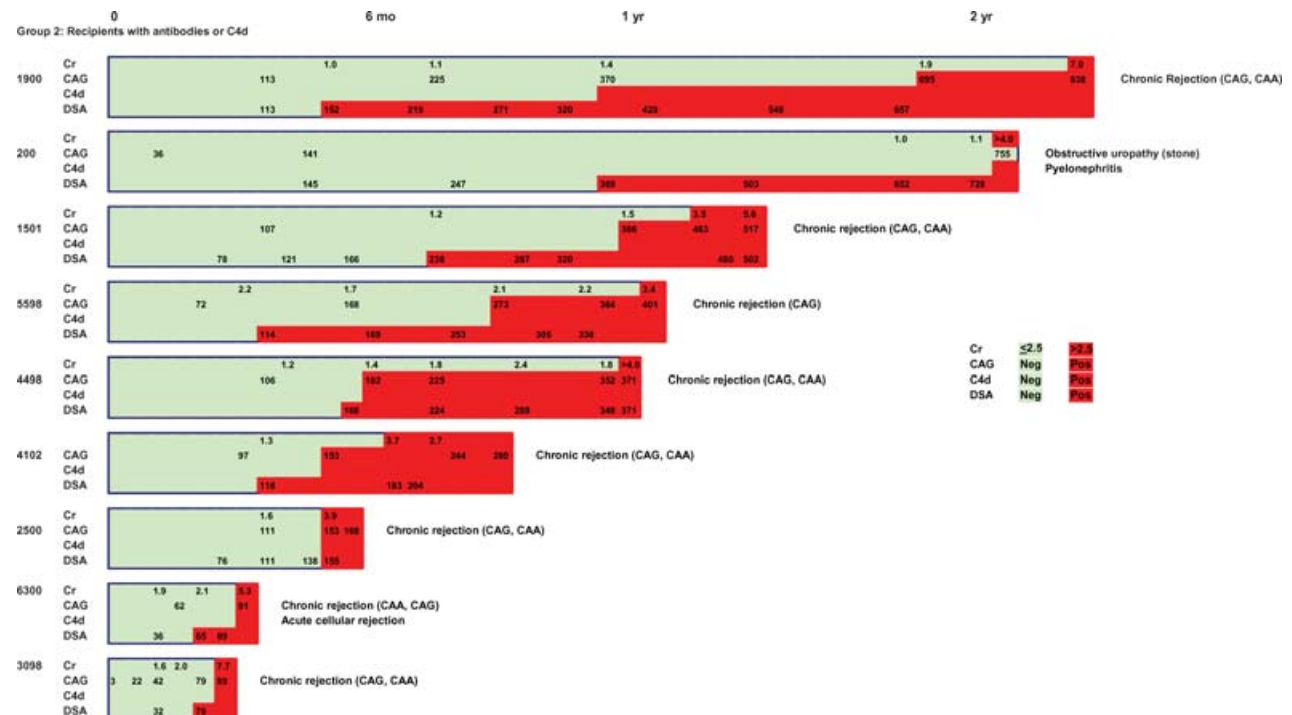


Figure 2: Longitudinal data for Group 2 monkeys with alloantibody and C4d deposition formatted as in Figure 1.

aspiration (1), acute cellular rejection (1) or post-transplant lymphoproliferative disease (1). Creatinines remained normal except for the two animals with obstruction.

Group 2 included all nine recipients that had C4d deposition in one or more allograft samples (Figure 2). Overall 20 of 35 samples showed widespread (>50% high-power fields), circumferential C4d deposition in peritubular capillaries. The staining was either in a broad linear or finely granular pattern along the peritubular capillaries in the cortex and medulla (Figure 3A). Glomerular C4d along the GBM was detected in seven recipients, Figure 3B. The first appearance of C4d among these animals ranged from 89–755 days after transplantation. C4d positive biopsies remained C4d positive after repeat biopsies. Alloantibodies were detected in all nine Group 2 recipients. The antibodies reacted with donor T and B cells (38%) or just B cells (62%). The antibodies were first detected 62–386 days after transplantation. Among all recipients in Groups 1 and 2, C4d deposition was highly correlated with the presence of

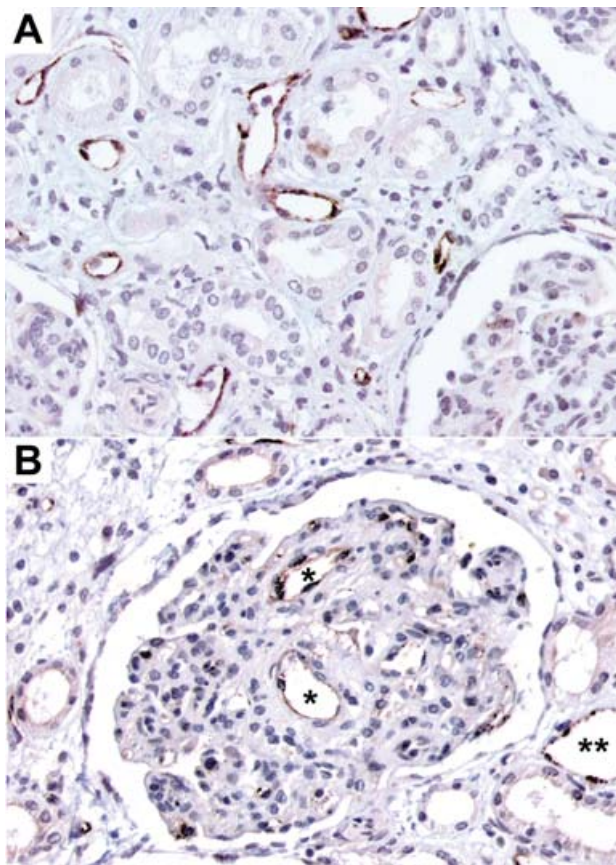


Figure 3: C4d staining. (A) Positive peritubular capillary immunoperoxidase staining for C4d. (B) Chronic allograft glomerulopathy with glomerular (*) and peritubular capillary (**) C4d staining. 400 \times , hematoxylin counterstain.

Table 1: C4d deposition correlates with donor-specific antibodies

	Alloantibodies*	
	Positive	Negative
C4d		
Positive	20	0
Negative	6	29
	p = 0.001	

*Alloantibody assays done within 1 month of the biopsy or before and after the biopsy with the same result. Fisher’s exact test.

alloantibodies in corresponding serum samples (p < 0.001, Table 1).

Pathology

Chronic allograft glomerulopathy (CAG), defined as GBM duplication affecting >50% of the capillaries of the most severely affected glomerulus on PAS stained sections, was found in eight of nine Group 2 recipients (89%) and in none of the samples from Group 1 animals (Figure 4A, B) (Table 2). Basement membrane duplication was confirmed by electron microscopy in selected samples (Figure 4C & D, white arrows). No immune complexes were detected by direct immunofluorescence or by electron microscopy. Of 20 positive C4d samples, 16 had CAG. In contrast, none of 29 C4d negative samples had CAG (p < 0.001). CAG was first detected in samples taken at 89–695 days. Most of the samples with CAG also had mesangial hypercellularity and/or mononuclear cell accumulation in >50% of glomeruli. Glomerular inflammation (glomerulitis), as measured by Banff g score was highly correlated with the C4d status of the same biopsy (Table 3; p < 0.00001). The typically observed sequential development of C4d deposition and glomerulopathy is illustrated in Figure 5.

Chronic allograft arteriopathy (CAA) was found in eight of nine recipients at autopsy in Group 2 animals and none of eight animals in Group 1 (p < 0.002, Table 2). Arteriopathy was only evaluated on autopsied allografts because too few arteries were present in biopsies. The intima showed proliferation of spindle-shaped cells with a variable mononuclear cell infiltration, similar to that observed in human renal allografts (data not shown).

Allograft biopsies in both groups showed interstitial mononuclear infiltrates at various times without graft dysfunction. The infiltrates in both groups were composed of mostly T cells, some B cells, and sometimes plasma cells (data not shown). The density of the infiltrate varied widely within each group, without obvious differences in numbers or cell type between groups. These infiltrates occasionally rose to Banff type 1 or type 2 acute cellular rejection (Table 2) on one biopsy during the life of the graft in about half of the recipients in both groups, but no statistically significant difference was detected between Group 1 or Group 2. The infiltrates of all individual biopsies graded

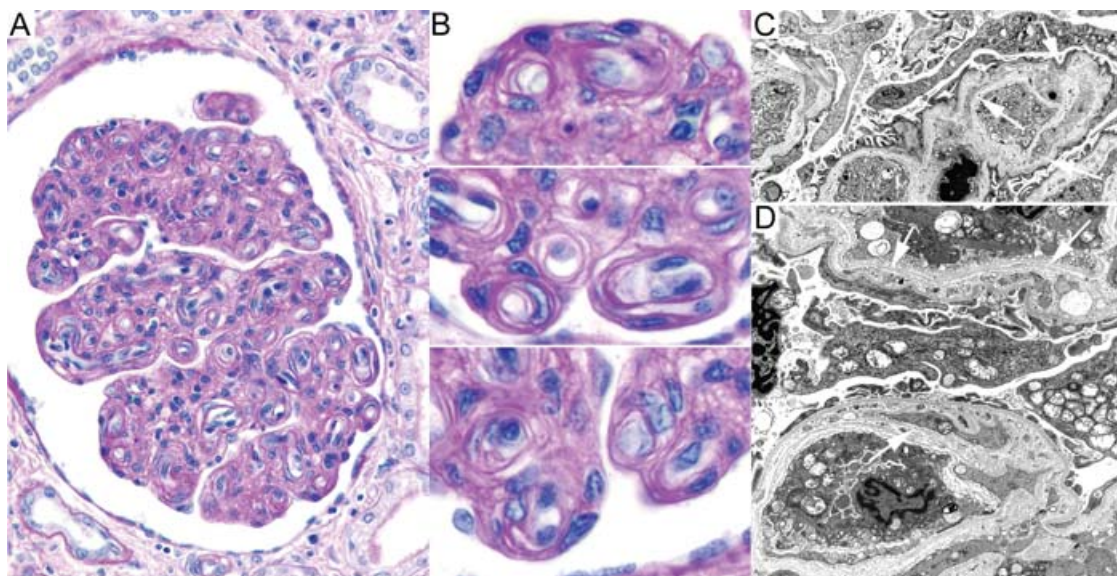


Figure 4: Glomerulopathy. (A) Glomerulus with severe glomerulopathy, and marked duplication of all glomerular basement membranes, 400× PAS. (B) Enlargement of selected glomerular segments in A showing multilamination of the GBM with luminal stenosis by enlarged endothelial cells. (C & D) Electron micrographs showing new multiple circumferential layers of glomerular basement membrane surrounding reactive endothelia, white arrows. (7100×–9100×, original magnification) Animal no. 4498, day 371, autopsy. See Figure 2 for clinical data. Representative of four animals examined.

Table 2: Frequency of pathologic lesions by group

Pathologic lesion	Group 1 [(N = 8; no C4d) (%)]	Group 2 [(N = 9; with C4d) (%)]	p value
Chronic allograft glomerulopathy	0	89	0.0004
Chronic allograft arteriopathy	0	78	0.002
Severe interstitial fibrosis (>50% cortex)	25	100	0.002
Acute cellular rejection, Type I	50	56	>0.05
Acute cellular rejection, Type II	25	33	>0.05

Table 3: Correlations of Banff scores with C4d deposition

	Banff scores*				p value
	0	1	2	3	
g	36	2	4	17	0.001
C4d positive (%)	11	50	75	71	
i	17	3	8	31	NS
C4d positive (%)	12	67	0	52	
t	11	28	15	5	NS
C4d positive (%)	9	46	33	20	
v	54	5	0	0	NS
C4d positive (%)	31	60	0	0	

For statistical analysis the 0–1 scores and the 2–3 scores were combined except for the v score.

*Number of biopsies with each score; percent of biopsies with C4d deposition given below.

according to Banff categories included for Group 1: none (10), suspicious (6), type 1A (5), type 1B (1), and type 2A (2), and for Group 2: none (7), suspicious (16), type 1A (6), type 1B (3) and type 2A (3). There was no statistical significant difference between the biopsies of Group 1 or Group 2 for any Banff category. Fibrosis involving >50% of the cortex was more frequent in Group 2 ($p < 0.002$; Table 2). Banff scores for glomerulitis correlated with C4d status (Table 3), but Banff scores for interstitial inflammation, tubulitis and vascular inflammation did not correlate with C4d status (Table 3). None of the allografts ever showed evidence of acute humoral rejection, as judged by neutrophil accumulation in peritubular capillaries or glomeruli, or fibrinoid necrosis of arteries or thrombi.

Progression of chronic humoral rejection

When the time of appearance of alloantibodies, C4d deposition, CAG and renal failure in individual animals was analyzed over time, a trend is evident, in which alloantibodies almost always were the first of these to appear. Five recipients (nos. 1900, 5598, 6300, 4102 and 3098) had alloantibodies at the time of a negative C4d biopsy. CAG was first detected at the same time as C4d positivity in seven recipients; one had C4d without CAG and one developed CAG 325 days after C4d positivity (no. 1900). Renal failure, defined as a serum creatinine >2.5 mg/dL, appeared after CAG in five recipients; three had an elevated creatinine at the time of first detection of CAG (Figure 2).

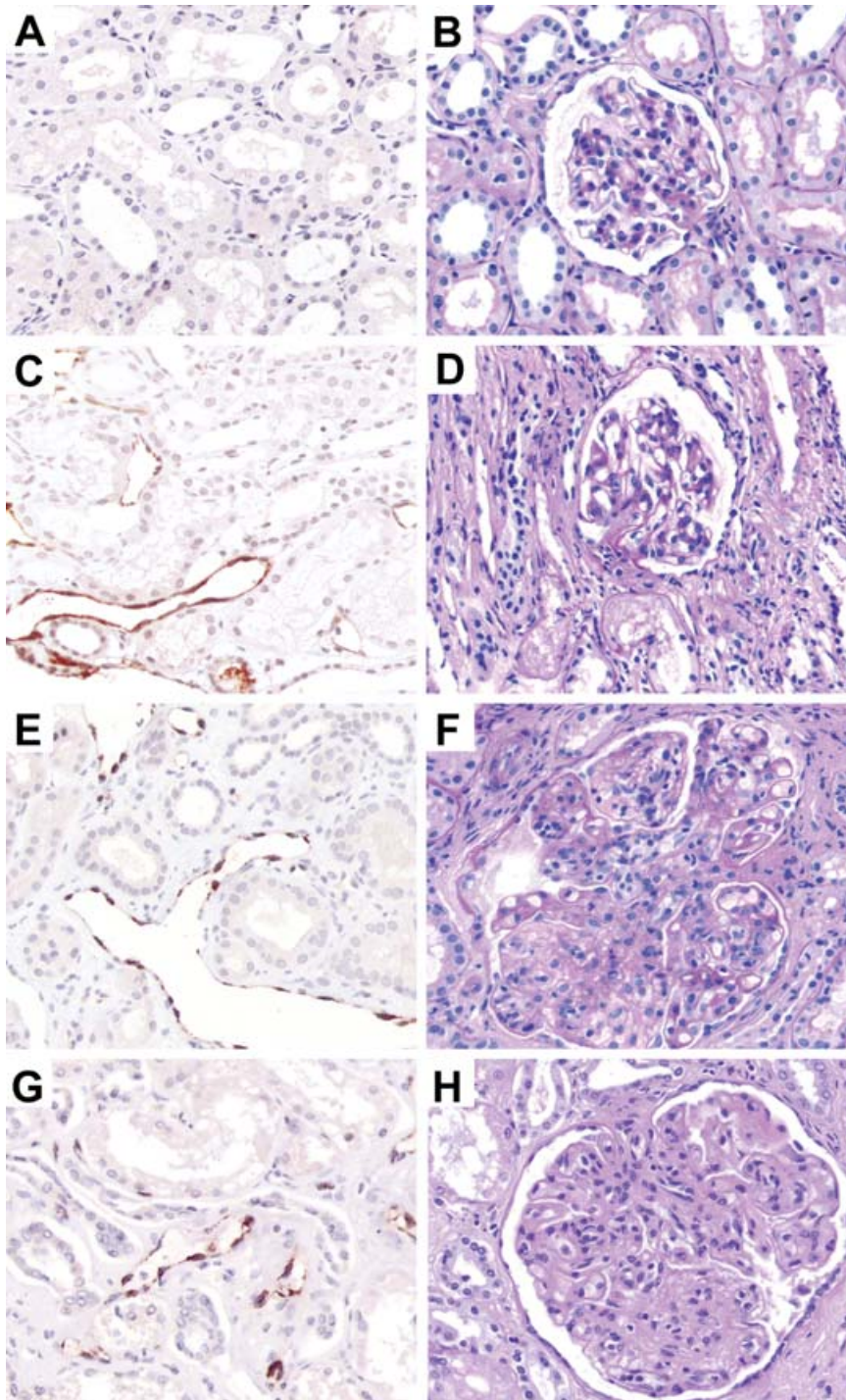


Figure 5: Progression of glomerulopathy in animal 1900. (A, C, E, & G) C4d immunoperoxidase stains, 400 \times , hematoxylin counterstain. (B, D, F, & H) PAS stains, 400 \times . Day 225 (A & B): The biopsy is normal looking and C4d is negative. Alloantibodies were present beginning on day 152 and persisted to autopsy. Day 370 (C & D): The biopsy appears normal looking, but C4d is positive in the peritubular capillaries. Day 695 (E & F): Glomerulopathy and glomerulitis are now present with persistent C4d staining; renal function remained normal. Day 838 (G & H): At autopsy the glomerulopathy is worse, C4d staining persists, and renal failure has developed (Cr = 7.0 mg/dL).

Discussion

This study demonstrates that a nonhuman primate develops chronic renal allograft rejection with pathological patterns similar to that found in humans, confirming studies in Rhesus monkeys on other protocols (24,25). The features considered characteristic of chronic, immunolog-

ically mediated graft injury (chronic rejection) are duplication of the glomerular basement membrane (chronic allograft glomerulopathy, CAG) and proliferative, fibrous intimal thickening with scattered mononuclear cells (chronic allograft arteriopathy, CAA) (26). These findings were seen in the Group 2 allografts in the absence of confounding causes often present in humans, such as calcineurin

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inhibitor toxicity, recurrent disease, ischemia and arteriosclerotic donor disease. The advantages of this model include the lack of long-term immunosuppression and the opportunity to analyze protocol biopsies, thereby, providing a unique opportunity to follow the natural history of late alloimmune mediated graft rejection.

The present study is the first animal study to correlate C4d deposition with alloantibodies and is entirely consistent with human studies (3,6,27). In monkeys 89% with C4d developed CAG during the time of observation, compared with none of the recipients that remained C4d negative. Alloantibodies preceded C4d in five of nine cases. Although C4d and DSA were strongly correlated, a small fraction of discordant cases exist with C4d but without DSA or the reverse on any given biopsy (R. N. Smith, unpublished data). This occasional discordance has been observed in humans (3,28–31). Positive C4d staining without serum DSA (which occurs in about 10% of human biopsies) might be due to non-DSA mediated C4d deposition, non-HLA antibodies (32–34) or adsorption of antibodies by the graft. Other investigators have shown that elutes of renal allograft nephrectomies moved for chronic rejection contain DSA in 71% of cases but only 31% of these patients had detectable DSA in the circulation (35). Cross reactive *de novo* alloantibodies may be a more sensitive indicator of a humoral response to the graft because they may not be absorbed as efficiently by the graft (1).

Overall, 100% of the animals that developed CAG had concurrent or prior biopsies that were C4d positive and had positive alloantibodies. A similar sequence was found in humans (9) although the frequency of subsequent CAG after a positive C4d biopsy was lower at 46%, perhaps due to the mitigating effects of ongoing immunosuppression. In this study no C4d positive cases became C4d negative on subsequent biopsies, in contrast with humans in which about 38% become negative, also probably a consequence of therapy (9). CAG showed no relationship to prior episodes of subclinical infiltrates, tubulitis or endothelialitis, arguing that CAG is not an invariant consequence of cellular rejection *per se*. However, these studies do not exclude a role for some population of mononuclear cells in either tolerance induction or chronic graft injury.

C4d and alloantibodies also predicted CAA. Overall, 89% of the monkeys with alloantibodies and C4d positive staining developed arteriopathy. In this study no correlation was found between CAA and either type 1 or 2 acute cellular rejection, although the series is small. The correlation of C4d or alloantibodies with CAA in humans is present in some but not all series (6,9,36–38). In mice, alloantibodies may participate in the development of allograft arteriopathy (39,40). Arteriopathy can also develop in the absence of alloantibodies, possibly mediated by T or NK cells (41,42).

C4d and alloantibodies correlated with interstitial fibrosis but not with acute cellular rejection type 1 or 2. In humans interstitial fibrosis (without CAG or CAA) may be related to episodes of late acute rejection and humoral presensitization (8). Our data support an immunologic component of fibrosis, perhaps related to the stimulation of growth factors and receptors by interaction of antibody and complement with peritubular capillary endothelium.

These data support the hypothesis that alloantibodies lead to shortened renal allograft survival (1,10,11). In this small series we were unable to show that alloantibody, C4d deposition or CAG predicts shortened median allograft survival. However, in a review of a larger group of 102 animals, each individually predicted with statistical significance shortened allograft survival (R. N. Smith et al., unpublished data).

How alloantibody and complement activation promote glomerulopathy, arteriopathy and fibrosis is unclear. Antibodies to class I MHC antigens can stimulate endothelial and smooth muscle proliferation and expression of FGF receptors (43). Soluble terminal complement components (C5b-9) trigger the production of FGF and PDGF by endothelial cells (44). Thus antibodies and activated complement might induce gene products that promote endothelial activation and injury with consequent basement membrane duplication and arterial smooth muscle proliferation (45,46).

Five of nine animals in Group 2 had an interval of normal graft function (from 148 to 547 days) with persistent alloantibodies, thereby meeting the definition of accommodation (47). Three of nine animals in Group 2 had persistent C4d deposition with normal graft function (91–325 days) meeting another suggested definition of accommodation (48). However, in this model without immunosuppression after post-transplant day 28, the antibodies and C4d deposition persisted and were associated inexorably with graft pathology and renal failure. Thus, accommodation in this model is either unstable or incomplete. In humans C4d deposition also can occur without simultaneous graft dysfunction (12), most commonly in ABO incompatible grafts (49). Further studies are required to determine the long-term outcome of this apparent stable state of graft accommodation.

An open question is the relationship between acute and chronic humoral rejection, which have different histological features. In both, alloantibodies target the endothelium of glomeruli, peritubular capillaries and arteries. Acute inflammation, necrosis and thrombosis were absent in all these cases of chronic humoral rejection; no grafts suffered an episode of acute antibody mediated rejection. We propose that chronic humoral rejection may represent attenuated acute humoral rejection mitigated by sufficient resistance (accommodation) within the graft to prevent acute inflammation but with insufficient resistance

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(accommodation) to prevent activation pathways that lead to chronic rejection.

This model is particularly relevant to clinical protocols that include reduction or elimination of immunosuppression. Alloantibody production and chronic rejection are significant problems in other tolerance protocols (25,50,51). Why B-cell responses are refractory to the techniques that are successful in preventing acute cellular rejection is unclear. Presumably, this is related to lack of tolerance or regulation in the indirect pathway of T-cell activation and/or in the generation of alloantigen reactive B cells. Further work is needed to clarify these putative mechanisms and devise interventions that will permit B-cell tolerance to develop.

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