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Large Multinucleated Variant Endothelial Cells in Allograft Kidney Microvasculature: A Biopsy Series

Jonathan E. Zuckerman, John Brealey, Julie M. Yabu, and Anthony Chang

There are few published studies examining cytomorphologic alterations in endothelial cells in human tissue. One fascinating but largely unexplored endothelial morphologic variant is large multinucleated variant endothelial cells (MVECs). To our knowledge, there are no published reports of MVECs identified in the kidney. Here, we present a case series of 4 kidney biopsies from allograft kidneys whose microvasculature contained MVECs. Electron microscopy confirmed the endothelial identity in all cases. A broad immunohistochemical panel used in 1 case was also confirmatory of an endothelial cell origin. All cases occurred in the setting of chronic, active, antibody-mediated rejection, and alternative etiologies, such as viral infections, were excluded. Two patients were positive for concurrent donor-specific antibodies, and 3 of the 4 cases occurred in second kidney allografts. We speculate that MVECs are a rare or often overlooked finding often confused for megakaryocytes and may be associated with chronic endothelial cell injury in the setting of chronic antibody-mediated rejection.

Complete author and article information provided before references.

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INTRODUCTION

There are few published studies examining endothelial cell cytomorphologic alterations in humans. One largely unexplored endothelial cell morphologic variant is large multinucleated variant endothelial cells (MVECs). Multinucleated variant endothelial cells have been found in the human aorta, and their number increases with age and the severity of atherosclerosis; thus, these may represent a response to a chronic injury, age, or senescence.[1,2] To our knowledge, there are no published reports of MVECs identified in the kidney or other organ systems. We present a case series of allograft kidney biopsies whose microvasculature contained MVECs concurrent with chronic, active, antibody-mediated rejection. Data collection and waiver for informed consent were approved by the University of California Los Angeles institutional review board (protocol number IRB#20-001734).

CASE REPORTS

Case 1

A 50-year-old woman with a history of end-stage kidney disease of an uncertain etiology and a prior failed deceased-donor kidney transplant received a second deceased-donor kidney transplant, which was complicated by delayed graft function. The calculated panel-reactive antibody level was 100%. There was 1 current donor-specific antibody (DSA) to DPA1*01 MFI 10,939 and 1 historic DSA to A30 MFI 3084. She received antithymocyte globulin induction and high-dose intravenous immunoglobulin (IVIG), followed by tacrolimus, mycophenolate mofetil, and prednisone for maintenance immunosuppression. The DSA DPA1*01 MFI 6148 was detected 3 days after the transplant and was treated with rituximab and IVIG. One and a half years after the transplant, a kidney biopsy was performed because of an elevated fraction of donor cell-free DNA (Allosure) at 1.1% and persistent DSA DPA1*01 MFI 4181. She had a stable serum creatinine level (baseline level, 0.75 mg/dL) and no proteinuria. Polymerase chain reaction performed for cytomegalovirus and BK virus yielded negative results.

Light microscopy (Fig 1) demonstrated that the cortex contained 12 patent glomeruli, moderate glomerulitis ($G2^3$), diffuse severe peritubular capillaritis (ptc 3), and no interstitial fibrosis or tubular atrophy, arteriosclerosis, arteriolosclerosis, or endotheliitis. The glomeruli exhibited many enlarged endothelial cells containing syncytial aggregates of multiple nuclei, which stained positively for ETS-related gene and cluster of differentiation (CD)31. Staining for CD68, pan keratin AE1/E3, SRY-box transcription factor 10, CD45, and CD61 yielded negative results. No MVECs were present in other vessels. Immunofluorescence studies, including for C4d, yielded negative results. Electron microscopic studies demonstrated MVECs and early changes associated with chronic transplant glomerulopathy (cg1a).

The final diagnosis was that the patient had features suggestive of C4d-negative, chronic, active antibody-mediated rejection; was negative for acute T-cell-mediated rejection; and had multinucleated glomerular endothelial cells of uncertain clinical significance. She was treated with IVIG, with a resultant but persistent decrease in the DSA level.

Her subsequent course was complicated by a partial small bowel obstruction, sclerosing peritonitis, and an acute kidney injury. A second kidney biopsy demonstrated ongoing antibody-mediated rejection and glomerular MVECs, which were treated with plasma exchange and IVIG. Her kidney function recovered to a creatinine level of 0.9 mg/dL, with no DSA.

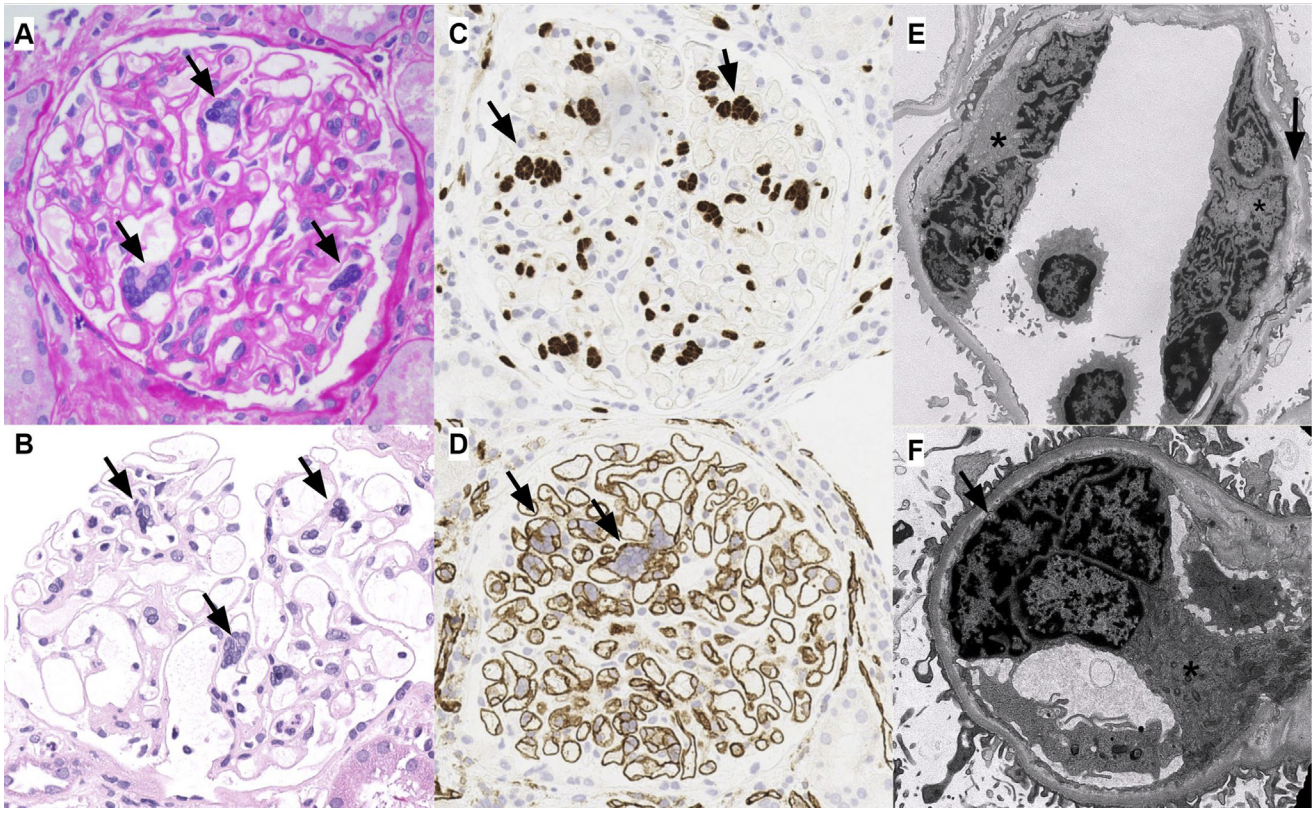

Figure 1. In the kidney biopsy findings of case 1, the glomeruli demonstrated segmental, large endothelial cells containing syncytia of multiple enlarged and mildly hyperchromatic nuclei, determined with light microscopy using (A) periodic acid–Schiff stain and (B) hematoxylin and eosin stain. Multinucleated endothelial cells were positive for endothelial cell immunohistochemical stains for (B) ETS-related gene and (C) cluster of differentiation 31. (E and F) (Original magnification, ×400 [A-D]; ×10,000 [E and F]). Electron microscopy further confirmed the presence of multinucleated endothelial cells lining the glomerular capillary loops. Some glomerular capillary loops also demonstrated early double contour formation (arrows). The asterisks indicate multinucleated endothelial cells.

Case 2

A 35-year-old man with a history of end-stage kidney disease secondary to posterior urethral valves and obstructive uropathy and a prior, failed, deceased-donor kidney transplant received a living, unrelated kidney transplant. The calculated panel-reactive antibody level was 98%, with no DSA. He received antithymocyte globulin induction and tacrolimus, mycophenolate mofetil, and prednisone for maintenance immunosuppression. The baseline creatinine level was 1.3 mg/dL. Eight years after the transplant, he was positive for class II de novo DSAs (DR53 MFI 1498 and DQA1*03 MFI 20,524) and had an elevated donor cell-free DNA fraction (Allosure) at 3.2%. A kidney biopsy was performed. The serum creatinine level was 1.4 mg/dL. Polymerase chain reaction performed for BK and cytomegalovirus yielded negative results.

A biopsy (Fig 2) demonstrated that the cortex contained 14 glomeruli (2 globally sclerotic), moderate glomerulitis (g2), focal moderate peritubular capillaritis (ptc 2), diffuse interstitial inflammation (i3), and focal mild tubulitis (ti). The focal glomeruli and peritubular capillaries exhibited MVECs. No MVECs were present in other vessels. The arterioles exhibited medial nodular hyalinosis. There was mild arteriosclerosis and no endotheliitis, interstitial fibrosis, or tubular atrophy. Simian virus 40 staining yielded a negative result. There was diffuse C4d staining of the peritubular capillaries. There was minimal granular mesangial staining for immunoglobulin A, immunoglobulin M, C1q, and kappa and lambda light chains. Electron microscopy demonstrated chronic transplant glomerulopathy, severe multilayering of the peritubular capillary basement membranes, and MVECs.

The final diagnosis was that the patient had C4d-positive, chronic, active antibody-mediated rejection; was borderline for acute T-cell-mediated rejection; had arteriolar medial hyalinosis, suggestive of chronic calcineurin inhibitor toxicity; and had focal multinucleated glomerular endothelial cells of uncertain clinical significance. He received IVIG and solumedrol, with a persistent DSA to DQA1*03 MFI 14,464 and a creatinine level of 1.4 mg/dL.

Case 3

A 35-year-old man presented 10 months after a second deceased-donor kidney transplant for end-stage kidney

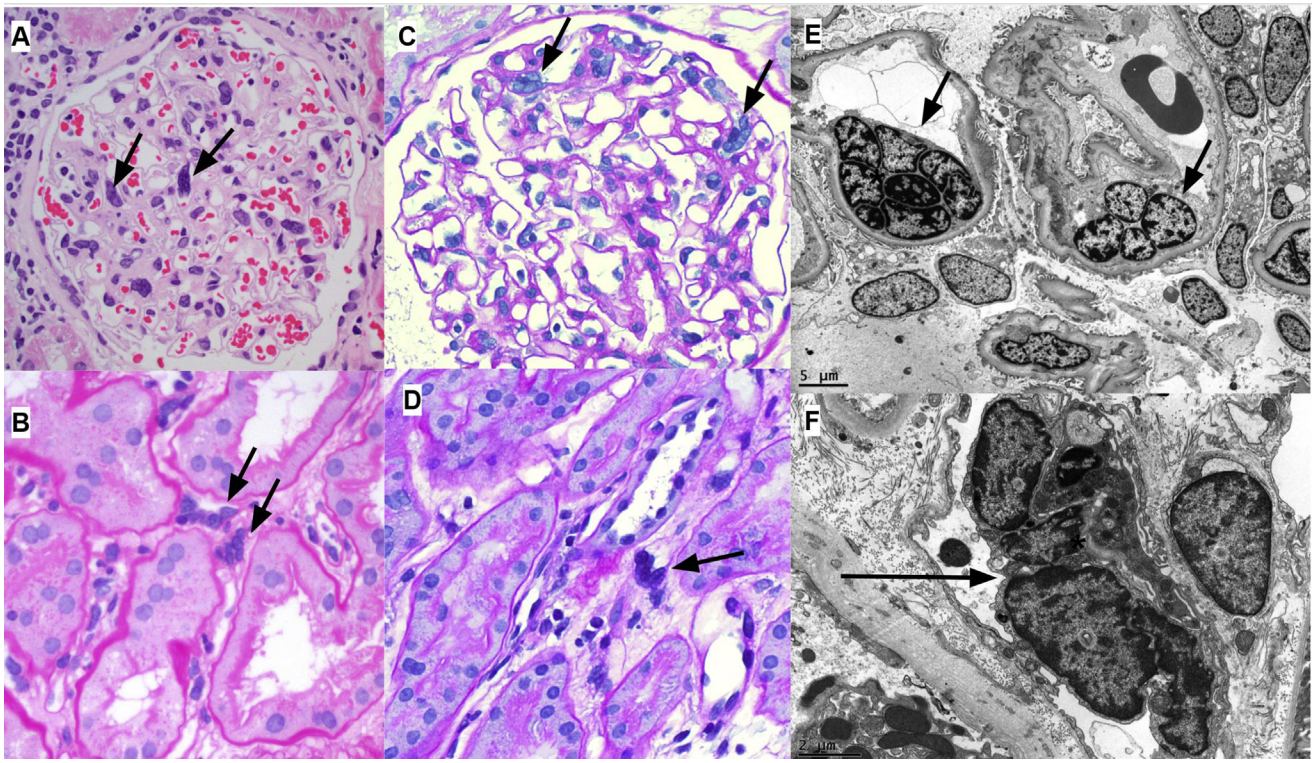

Figure 2. The microscopic findings of cases 2 and 3 showed that both (A and B) case 2 and (C and D) case 3 demonstrated glomeruli with multiple enlarged multinucleated endothelial cells. (B, D, and F) Occasional peritubular capillaries were also lined with enlarged multinucleated endothelial cells. The images show staining with periodic acid–Schiff stain (Original magnification, ×400). The arrows point toward multinucleated endothelial cells. (E and F) Electron micrographs from case 4 demonstrated multinucleated endothelial cells in the glomerular capillary loops and peritubular capillaries. The arrows point toward multinucleated endothelial cells. Double contours of the glomerular capillary loops were also present.

disease attributed to hypertension. He had been treated for cytomegalovirus infection with the development of massive ascites of an unknown etiology without hepatosplenomegaly. The serum creatinine level increased from 1.3 to 1.6 mg/dL.

A kidney biopsy (Fig 2) demonstrated that the cortex had 8 patent glomeruli, which contained many MVECs within the glomerular capillary loops and peritubular capillaries. There was mild congestion of a few peritubular capillaries because of leukocytes and patchy, mild interstitial edema, without significant interstitial fibrosis, tubular atrophy, or interstitial inflammation. Immunostaining for Epstein-Barr virus, herpes simplex virus 1/2, human herpesvirus 8, varicella, and measles yielded negative results. Forty-fifty percent of the peritubular capillaries demonstrated weak C4d deposition. Electron microscopy demonstrated MVECs within the glomerular capillary loops. No viral particles were present.

A subsequent biopsy 12 months after the transplant demonstrated similar findings, with many MVECs and with features of chronic, active, antibody-mediated rejection. Biopsies at 13.5 and 15 months after the transplant demonstrated a decreased number of MVECs, persistent features of chronic, active antibody-mediated rejection, and progressive cortical scarring. An allograft nephrectomy was performed 18 months after the transplant, which showed features of chronic antibody-mediated rejection and focal cryptococcal granulomatous interstitial nephritis.

Case 4

A 53-year-old woman had received simultaneous pancreas and kidney transplants for type 1 diabetes 6 years before presentation. Her immunosuppressive medications included tacrolimus, prednisolone, and azathioprine. Donor-specific antibodies were absent. A biopsy was performed because of an elevated serum creatinine level (1.3 mg/dL).

The biopsy demonstrated that the cortex and medulla contained 8 glomeruli (6 globally sclerotic), mild glomerulitis (g1), severe peritubular capillaritis (ptc 3), and MVECs. There was mild interstitial fibrosis and tubular atrophy associated with chronic inflammation. Immunofluorescence studies, including for C4d, yielded negative results. Electron microscopy (Fig 2) demonstrated many MVECs in the glomerular capillary loops and peritubular capillaries. The glomerular capillary loops showed increased luminal leukocytes, possible chronic transplant glomerulopathy, and segmental podocyte foot process

effacement. There were no tubuloreticular inclusions or viral particles.

The final diagnosis was peritubular capillaritis and glomerulitis, suggestive of antibody-mediated activity; possible transplant glomerulopathy, determined using electron microscopy; and alterations in the endothelial cells, which were difficult to categorize.

DISCUSSION

There are no prior literature reports documenting the presence of MVECs in kidney microvasculature. Multinucleated variant endothelial cells may be overlooked or mistakenly interpreted as circulating megakaryocytes. In all our cases, multinucleated endothelial cells were clearly visualized using electron microscopy. In case 1, immunohistochemical characterization confirmed the multinucleated cells to be of an endothelial cell origin (positive for ETS-related gene and CD31) and not of a megakaryocyte lineage (negative for CD61).

The etiology of MVECs is uncertain. Chronic endothelial cell injury is a hallmark of chronic, active antibody-mediated rejection.[3] All the MVEC cases demonstrated ultrastructural features of chronic antibody-mediated rejection. We speculate that MVECs represent a manifestation of chronic endothelial cell injury and cellular senescence possibly related to chronic antibody-mediated rejection.

Follow-up biopsies were performed in 2 cases. In 1 case (case 1), a similar number of MVECs was present at the time of a 1-year follow-up biopsy. Surprisingly, the number of MVECs in the second case (case 3) decreased, with progressive, chronic, active antibody-mediated rejection and allograft failure. The reason for this decrease is uncertain; the possible explanations include that the MVECs underwent apoptosis or other cell death and were replaced, MVECs divided or lost their nuclei, or MVECs are a focal phenomenon not always sampled.

All the biopsies were performed because of an indication. In cases 1 and 2, the biopsies were performed because of elevated serum cell-free DNA levels and DSA, although the patients had stable kidney function. In cases 3 and 4, the biopsies were performed because of a modest elevation in the serum creatinine levels. It should be noted that not all centers might perform biopsies for such indications.

Multinucleated variant endothelial cells have rarely been reported in human tissues. The presence of MVECs in the human aorta is correlated with age and the severity of atherosclerosis and, thus, may represent a response to a chronic injury, age, or senescence.[1,2] Multinucleated variant endothelial cells have been demonstrated in the cerebral microvasculature in patients with possible mycoplasma infection associated with intraluminal, mycoplasma-like particles.[4] In the lung, multinucleate endothelial cells have been reported in patients with Nipah virus infection.[5,6] No viral particles or mycoplasma-like particles were present in our cases, as determined using electron microscopy. An extensive, negative, viral workup was performed in case 3. A more extensive viral workup was not performed in the other cases because there was no clinical concern for a systemic viral infection.

The details regarding the formation and function of MVECs outside the setting of viral infections are few. Bovine aortic endothelial cells develop a giant, multinucleated phenotype at a high passage number in a senescent monolayer culture.[7] Multinucleated variant endothelial cells in culture do not incorporate ^{3}H-thymidine, suggesting that they are a product of cellular fusion rather than of intracytoplasmic nuclear division.[2] Multinucleated variant endothelial cells also express p53 and B-cell lymphoma 2, potentially to prevent apoptosis in the setting of an injury.[8] Simulated diabetes can lead to the formation of MVECs with altered mitochondrial function in cultured human coronary artery endothelium.[9] Multinucleated variant endothelial cell functional alterations, including augmented low-density lipoprotein uptake, have been observed, hinting at a possible role for MVECs in cardiovascular disease.[1]

Additional studies evaluating larger numbers of biopsies are needed to further understand this phenomenon. It is unknown whether this phenomenon occurs without rejection, in native kidneys, or in other tissue vascular beds.

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