Practical Issues and Future Perspectives for Inflammation in Areas of Interstitial Fibrosis and Tubular Atrophy in Chronic Active T cell-Mediated Rejection: Three Case Reports with Commentary

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ABSTRACT

The 2017 Banff meeting provided specific criteria for the diagnosis of tubulointerstitial changes in chronic active T cell-mediated rejection (CATCR), with an emphasis on inflammation in areas of interstitial fibrosis and tubular atrophy, which was thought to reflect an ongoing T cell-mediated alloimmunity. CATCR is considered to occur as a consequence of persistent or recurrent acute T cell-mediated rejection. Acute T cell-mediated rejection is an acute cytotoxic T-cell reaction to HLA antigens on the donor kidneys and causes tubulitis, interstitial inflammation, and intimal arteries. However, unlike early T-cell transplant damage, CATCR can sometimes be difficult to diagnose because the subsequent chronic T-cell damage can become more complex from the accumulation of previous immune and nonimmune injuries. Furthermore, scoring inflammation in areas of interstitial fibrosis and tubular atrophy has potential problems because other diseases and not even native kidneys can have scattered inflammatory cells. Therefore, detailed insights on the pathogenesis of CATCR are indispensable for appropriate diagnosis and further treatment. In this study, the pathologic characteristics and possible factors involved in the interstitial lesions in both typical and complex cases of CATCR are discussed.

THE 2015 Banff classification for chronic active T cell-mediated rejection (CATCR) listed criteria only for chronic transplant vascular lesions, which were defined as arterial intimal fibrosis with mononuclear cell inflammation and formation of neointima (Banff cv score). Later, in the 2017 Banff meeting, specific criteria for tubulointerstitial changes in CATCR were provided and defined, with moderately high thresholds, as a combination of inflammation in areas of cortical interstitial fibrosis and tubular atrophy (Banff i-IFTA score), with inflammation in the total cortex (Banff ti score) plus tubulitis (Banff t score). i-IFTA was thought to reflect an ongoing T cell-mediated alloimmunity based on some studies, which demonstrated that i-IFTA often occurred as a consequence of acute T cell-mediated rejection (ATCR) under insufficient immunosuppression [1,2] and was associated with reduced graft survival [3]. CATCR in the tubulointerstitial compartment is pathologically classified into grades IA and IB. Both show extensive i-IFTA and ti, with the main difference being the severity of tubulitis, which is moderate (t2) in grade IA and severe (t3) in grade IB. Patients with tubulitis present only in severely atrophic tubules are excluded from the diagnosis of CATCR [4].

CATCR is thought to occur as a persistent or recurrent of ATCR. Because ATCR is an acute cytotoxic T-cell reaction to HLA antigens on the donor kidneys, T cells can affect the tubules, interstitium, and arteries, thereby leading to tubulitis (Banff t score), inflammation of the nonsclerotic cortex (Banff i score), and intimal arteritis (Banff v score). Injured tubules can stimulate surrounding fibroblasts through cross talks with the releasing cytokines, such as transforming growth factor-β, bone morphogenic protein, platelet-derived growth factor, and hepatocyte growth factor [5], which can cause sustained

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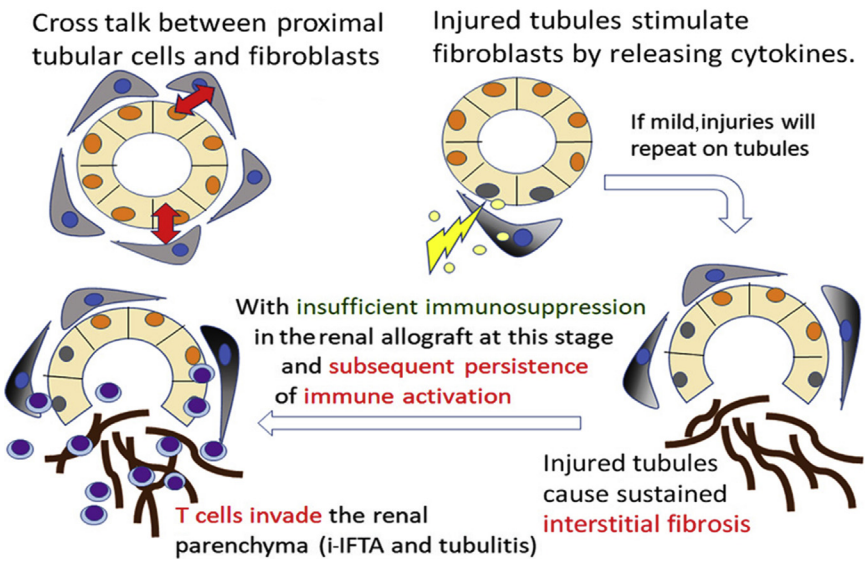

Fig 1. Pathogenesis of chronic active T cell-mediated rejection as a consequence of persistent immune activation. There is cross talk between injured proximal tubular cells and fibroblasts. Injured tubules stimulate fibroblasts by releasing cytokines. Repeated injuries of proximal tubules can cause sustained interstitial fibrosis. With insufficient intake of immunosuppressive drugs in renal allograft at this stage and subsequent persistence of immune activation, T cells invade the renal parenchyma and can induce i-IFTA and tubulitis, which are the pathologic diagnostic components of CATCR in the tubulointerstitial compartments. CATCR, chronic active T cell-mediated rejection; i-IFTA, inflammation in areas of interstitial fibrosis and tubular atrophy.

interstitial fibrosis [6]. Therefore, with insufficient immunosuppression in the renal allograft at this stage and the subsequent persistence of immune activation, T cells will invade the renal fibrotic parenchyma and can induce i-IFTA and tubulitis, which are the pathologic diagnostic components of CATCR in the tubulointerstitial compartments (Fig 1). One microarray study on the use of renal tissue for the diagnosis of CATCR and ATCR demonstrated that both types shared almost all of the pathways, except for the OX40, which is known to be the evolving factor of CATCR due to T-cell proliferation [7,8]; these implied that CATCR can occur as an extension of ATCR.

i-IFTA is a key pathologic component for the diagnosis of CATCR. However, the i-IFTA scoring continues to have potential problems because tubulitis in the atrophic tubules may also be present in several diseases that are not related with rejection and even in native kidneys. Therefore, the 2017 Banff classification noted that the differential diagnoses for i-IFTA, such as BK virus (BKV) infection, pyelonephritis, antibody-mediated rejection, recurrent glomerulonephritis, and obstructive nephropathy, should be ruled out in order to diagnose CATCR [4] however, this can be sometimes difficult. This study aimed to discuss the pathologic characteristics and possible contributing factors to the formation of interstitial lesions in CATCR, based on 1 typical and 3 complex cases, in which the diagnosis of CATCR was not clear.

TYPICAL CASE OF CATCR

The recipient was a 50-year-old man with IgA nephropathy, and the donor was his 48-year-old wife. At 5 months after renal transplantation, renal biopsy was performed because of an increase in serum creatinine (sCr) from 1.5 mg/dL to 1.7 mg/dL. The renal tissue showed no tubulitis or interstitial inflammation, but there was an isolated v1 lesion with foam cell infiltration (Fig 2A). The patient was diagnosed with ATCR grade IIA (i0, t0, v1, cv0, i-IFTA1, ti1), for which steroid pulse therapy was administrated. The immunosuppressive drugs were reduced 3 months after the biopsy because his cytomegalovirus serology titer was elevated. At 4 months after the reduction of immunosuppressive drugs, a second biopsy was performed because the sCr increased from 2.1 mg/dL to 4.5 mg/dL. Compared with the prior biopsy, this biopsy showed more prominent interstitial fibrosis with scattered inflammatory cells and moderate tubulitis in the atrophic tubules (Fig 2B). Lesions of chronic transplant arteriopathy (Fig 2C) and intimal arteritis were also observed. The patient was diagnosed with CATCR grade IB (i1, t2, v1, cv1, i-IFTA3, ti3). However, the immunosuppressive drugs could not be

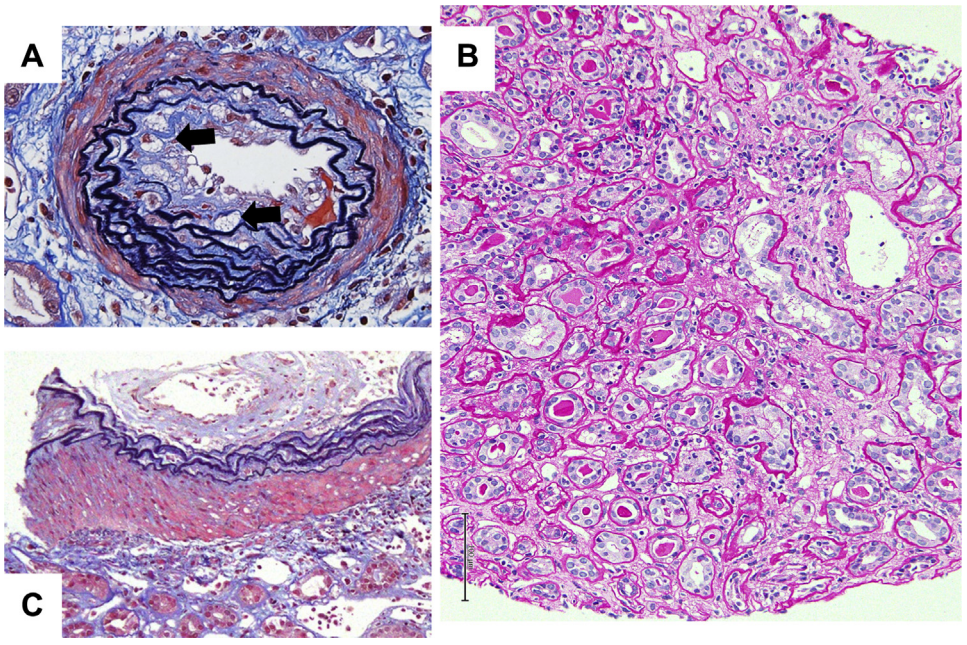

Fig 2. Histologic findings in a typical case of CATCR. **(A)** The first biopsy performed at 5 months after renal transplantation is diagnostic for acute T-cell rejection based on the presence of an isolated v1 lesion with foam cell infiltration. The second biopsy performed at 4 months after the reduction of immunosuppressive drugs is diagnostic for CATCR based on the presence of progressive interstitial fibrosis with scattered inflammatory cells [i-IFTA3] and moderate tubulitis in the atrophic tubules [t2] **(B)**, as well as a cv1 lesion in the renal tissue **(C)**. (**A** and **C**: Masson's trichrome stain, ×400; **B**: PAS stain, ×200). CATCR, chronic active T cell-mediated rejection; PAS, periodic acid-Schiff.

increased because the cytomegalovirus was not sufficiently controlled. A year later, hemodialysis (HD) was initiated.

The clinicopathologic characteristics of CATCR in this case were 1. a previous diagnosis of ATCR; 2. prior history of reduction of immunosuppressive drugs; and 3. a strong pathologic evidence for the diagnosis of CATCR based on the detection of cv lesions in addition to the presence of tubulointerstitial lesions that consisted of i-IFTA and tubulitis.

COMPLEX CASE 1: TUBULITIS IN THE ATROPHIC TUBULES IN THE SCARRED CORTEX: CATCR OR ISCHEMIC CHANGES?

The recipient was a 39-year-old man with reflux nephropathy, and the donor was his 66-year-old father. At 2 weeks after renal transplantation, a renal biopsy was performed because his sCr did not decrease to <2.0 mg/dL. The renal tissue showed transmitted severe arteriosclerosis and arteriolosclerosis with mild interstitial fibrosis. There was no evidence of rejection (i0,t0,v0,cv0,i-IFTA0,ci1,ct1,ah3) (Fig 3A).

About 6 months after the first biopsy, a second biopsy was performed because his sCr increased from 2.2 mg/dL to 2.7 mg/dL The renal tissue still showed severe arteriosclerosis, but no cv lesion was detected. Compared with the prior biopsy, the area of scarring in the cortex was more extensive (Fig 3B). The tubulitis in the atrophic tubules and inflammation were diffusely observed beneath the capsule (Fig 3C) and, to a lesser extent, in the other areas of the cortex (i3,t3,v0,cv0,i-IFTA3,ti3,ah2,aah1) (Fig 3D). The patient received steroid pulse therapy, and for the 7 months after biopsy, his sCr remained stable at 2.4 mg/dL.

COMPLEX CASE 2: i-IFTA IN A PATIENT WITH NEUROGENIC BLADDER: REFLUX NEPHROPATHY OR CATCR?

The recipient was a 36-year-old man with prune belly syndrome, and the donor was his 62-year-old mother. During transplantation, he underwent reconstructive surgery, using his left ureter for the urinary tract. Thereafter, he started to suffer from neurogenic bladder. At 3 months after transplantation, he developed ileus and could not take immunosuppressive drugs. Renal biopsy was performed because the sCr increased from 1.2 mg/dL to 1.9 mg/dL. The renal tissue showed moderate inflammation and severe tubulitis, predominantly in the medullary ray area, and the presence of Tamm–Horsfall protein (THP) in the glomerulus (Fig 4A). The patient was diagnosed with ATCR (i2, t3, v0, cv0, ct0, ci0, i-IFTA0).

After the renal biopsy, he was unable to take immunosuppressive drugs for about 2 weeks because he had surgery for bowel perforation. Subsequently, he developed anuria, with an increase of sCr to 5.0 mg/dL, and HD was initiated. Within 6 weeks of HD, there was a gradual restoration of urine output; when the sCr decreased to 2.0 mg/dL, a second biopsy was performed. The renal tissue showed several severe cv lesions that were almost occluded by arterial intimal fibrosis. Mononuclear cell inflammation in the area of fibrosis was not apparent, but there was severe narrowing of the branching arterioles (Fig 4B). Interstitial

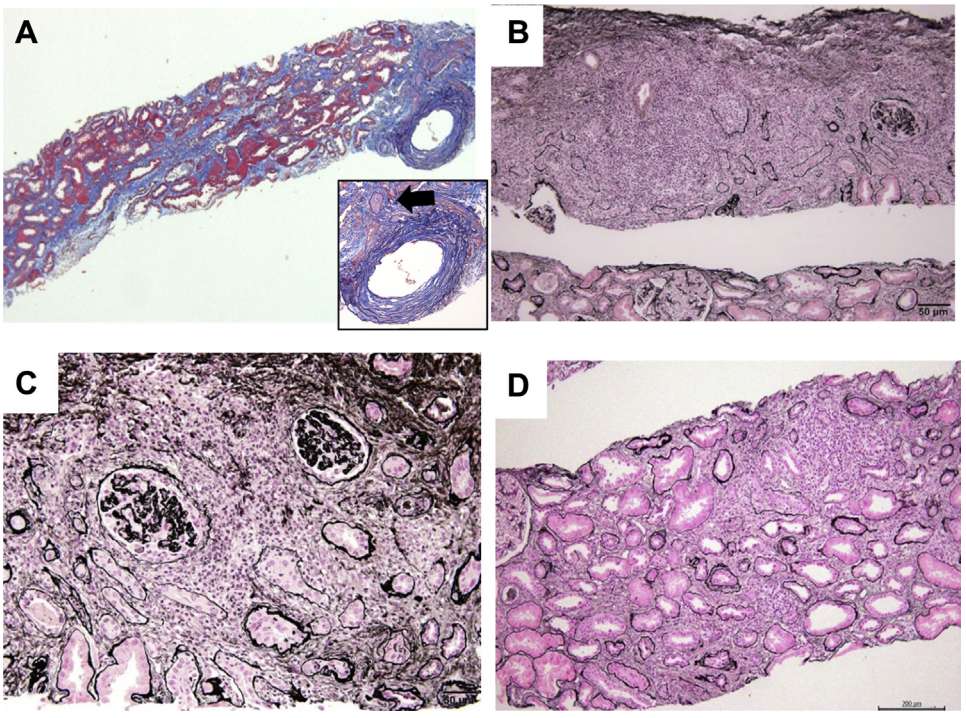

Fig 3. Histologic findings in case 1, which demonstrate difficulties in differentiating between CATCR and a cortical scar in severe arteriosclerosis. **(A)** The first biopsy performed at 2 weeks after renal transplantation shows severe transmitted arteriosclerosis (inset) and arteriolosclerosis (arrow) with mild tubulointerstitial fibrosis (Masson's trichrome stain, ×100). **(B-D)** A second biopsy is performed at 7 months after renal transplantation. Comparing the specimen obtained from the subcapsular area (upper specimen in **B** and **C**) and from the other areas of the cortex (lower specimen in **B** and **D**), the area beneath the capsule has more extensive scarring and inflammation; the tubulitis in the atrophic tubules is more prominent in the subcapsular area (**C**) and is less extensive in the other areas of the cortex (**D**) [i-IFTA3 and t3]. (**B-D**: PAM stain; **B** and **D** ×200, **C** ×400). CATCR, chronic active T cell-mediated rejection; PAM, periodic acid methenamine silver.

fibrosis with inflammation developed, and the tubules were either atrophic or dilated (Fig 4C). There was severe tubulitis in the atrophic tubules (i1, t3, v0, cv3, i-IFTA2, ti2) and THP in the glomerulus (Fig 4D). No pathologic evidence of chronic antibody-mediated rejection or serologicevidence of DSA was detected. The patient received steroid pulse therapy, and at 6 months after the biopsy, the sCr was stable at 1.7 mg/dL.

COMPLEX CASE 3: INTIMAL ARTERITIS WITH i-IFTA IN A PATIENT WITH LATE STAGE OF BK VIRUS NEPHROPATHY: ATCR OR CATCR?

The recipient was a 65-year-old man with tubulointerstitial nephritis of unknown etiology, and the donor was his 63-year-old wife. The patient was diagnosed with ATCR twice, at 6 and 18 months after transplantation, for which he received steroid pulse therapies. At 6 years after transplantation, a third biopsy was performed because his sCr increased from 1.5 mg/dL to 2.0 mg/dL. The renal tissue showed large and homogenous intranuclear inclusions in the tubular epithelium with several SV40-positive cells (Fig 5A) and accompanying moderate inflammation and severe tubulitis. He was diagnosed with BKV nephropathy stage B (i2, t3, v0, cv0, ct1, ci1, i-IFTA0), and immunosuppressive drugs were reduced.

A fourth biopsy was performed 3 months after the previous biopsy because the sCr increased from 2.0 mg/dL to 5.0 mg/dL. The number of SV40-positive cells significantly decreased, but the interstitial fibrosis and inflammation became severe (Fig 5B). There was a significant tubulitis in the atrophic tubules where the interstitial fibrosis progressed (Fig 5C). Moreover, the renal tissue showed a v1 lesion (i3, t3, v1, cv0, ct2, ci3, i-IFTA2, ti3) (Fig 5D). The patient received steroid pulse therapy, and at 6 months after the biopsy, sCr was stable at 1.7 mg/dL.

DISCUSSION

The 2017 Banff meeting established the specific criteria for CATCR in tubulointerstitial compartments with scoring for i-IFTA. However, CATCR can sometimes be difficult to diagnose, which may be due to the complex pathogenesis of lesion formation.

According to the 2017 Banff criteria, the first case could be categorized into CATCR grade IB (i-IFTA3 and t3). However, another diagnostic possibility was a cortical scar accompanying chronic inflammation, based on the findings of more prominent i-IFTA in the subcapsular area than in the other areas of cortex. Notably, grafts in elderly individuals tend to easily develop ischemic changes, including scars with chronic inflammation and tubulitis in the atrophic tubules, especially in severe arteriosclerosis. Moreover, the

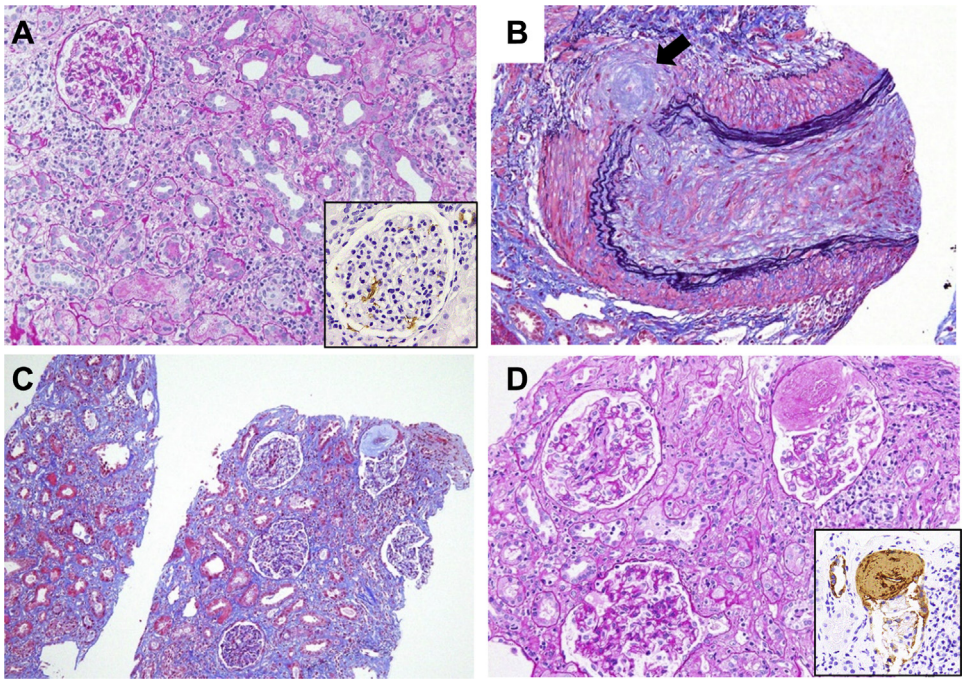

Fig 4. Histologic findings in case 2, which demonstrate difficulties in differentiating between CATCR and reflux nephropathy. **(A)** The first biopsy performed at 3 months after renal transplantation is diagnostic for acute T-cell rejection based on the presence of moderate inflammation in the medullary ray area and severe tubulitis [i2 and t3]; the THP is positive in the glomerulus (inset). **(B-D)** The second biopsy performed at 5 months after renal transplantation demonstrates arterial occlusion from arterial intimal fibrosis [cv3] and severe narrowing of the branching arterioles (**B**, arrow). The interstitial fibrosis with inflammation [i-IFTA2] and the severe tubulitis in the atrophic tubules are prominent [t3] **(C, D)**, with positive THP in the glomerulus (**D**, inset). (**A** and **D**: PAS stain, ×200; inset ×400; **B** and **D**: Masson's trichrome stain; **B** ×400; **C** ×100). CATCR, chronic active T cell-mediated rejection; PAS, periodic acid-Schiff; THP, Tamm–Horsfall protein.

recipient had no history of reduced immunosuppressive drugs. This case suggested that nonimmune factors, such as ischemia, could lead to the formation of i-IFTA lesions.

The second case was diagnosed with CATCR grade II based on the presence of cv3 lesions. The tubulointerstitial lesions in this case also met the criteria for CATCR grade IB (i-IFTA2 and t3), but a clear pathologic conclusion could not be made because the etiology of the lesions was difficult to differentiate between CATCR, reflux nephropathy, or both. Clinically, the recipient had a history of insufficient intake of immunosuppressive drugs and suffered from neurogenic bladder, with pathologic evidence of THP deposition in the glomerulus. This case suggested the difficulty in differentiating between reflux nephropathy and CATCR in the tubulointerstitial compartments, especially when the i-IFTA in the background was aggravated by other reasons, such as ischemia. In this case, the severe cv lesions were thought to have hastened i-IFTA formation.

According to the Banff criteria, the third case was diagnosed with BKV nephropathy and ATCR based on the presence of a v1 lesion. However, a diagnosis of CATCR was likewise possible based on the progression of interstitial fibrosis and inflammation with severe tubulitis, as well as the decrease in the number of SV40-positve cells after the reduction of immunosuppressive drugs. These findings implied that i-IFTA progression may have been caused by the persistent T cell-mediated rejection and not by the BKV infection. According to the 2017 Banff classification, CATCR should be diagnosed after ruling out the other differential diagnoses known to be associated with i-IFTA. In this case, i-IFTA formation may have been brought about by nonimmune factors, such as continued inflammatory response in the late stage of BKV nephropathy. This case suggested the difficultly in making a diagnosis of CATCR when other diseases coexist because the tubulointerstitial lesions of CATCR could be easily missed, unless v or cv lesions are detected in the specimens.

Based on the 3 complex cases presented, multiple factors were thought to be involved in the formation of the tubulointerstitial lesions of CATCR (Fig 6). In ATCR, the recipient T cells become activated when they recognize donor-specific antigens and migrate into the graft tubules, interstitium, and vessels. Early treatment of rejection can prevent graft dysfunction and i-IFTA progression. However, insufficient intake of immunosuppressive drugs for a certain period may lead to recurrent subclinical T-cell rejection and the development of CATCR, which histologically consists of cv and i-IFTA lesions. Unlike an i-IFTA lesion, a cv lesion is

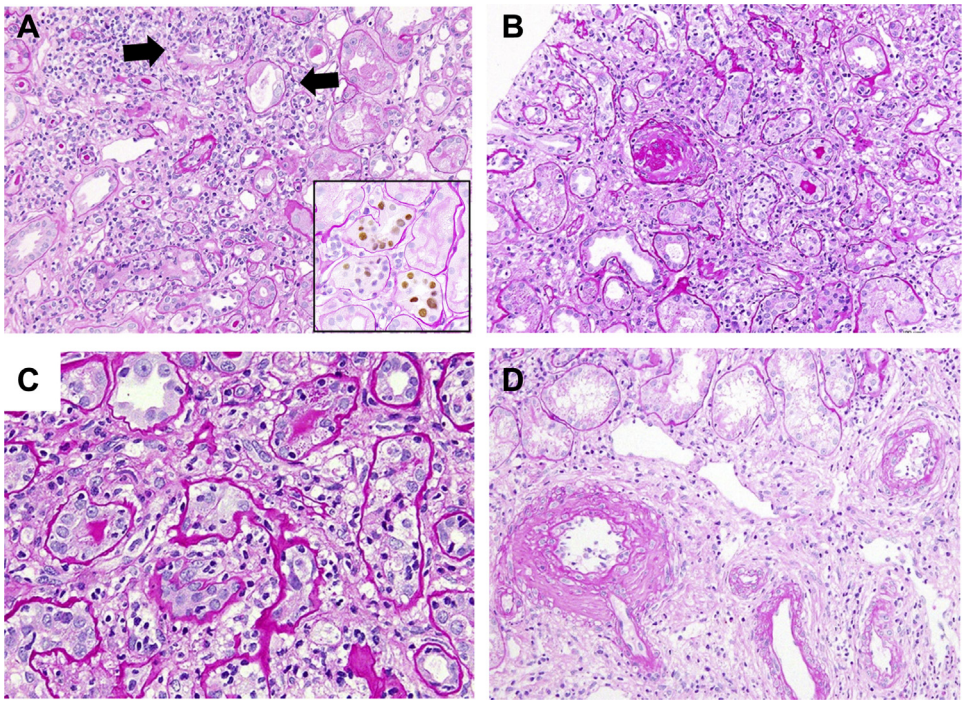

Fig 5. Histologic findings in case 3, which demonstrate difficulties in differentiating between acute T-cell rejection and CATCR in the late stage of BKV nephropathy. **(A)** The third biopsy performed at 6 years after renal transplantation is diagnostic for stage B BKV nephropathy based on the presence of large and homogenous intranuclear inclusions in the tubular epithelium (arrow), with several SV40-positive cells (inset) and accompanying moderate inflammation and severe tubulitis [i2 and t3]. **(B-D)** The fourth biopsy performed at 3 months after the third biopsy demonstrates progressive interstitial fibrosis and inflammation [i-IFTA3 and t3] **(B)**; the presence of tubulitis in the atrophic tubules where the interstitial fibrosis is prominent **(C)**; and a v1 lesion **(D)**. (**A-D**: PAS stain; **A** and **B**: ×200; **C** and **D**: ×400). BKV, BK virus; CATCR, chronic active T cell-mediated rejection; PAS, periodic acid-Schiff.

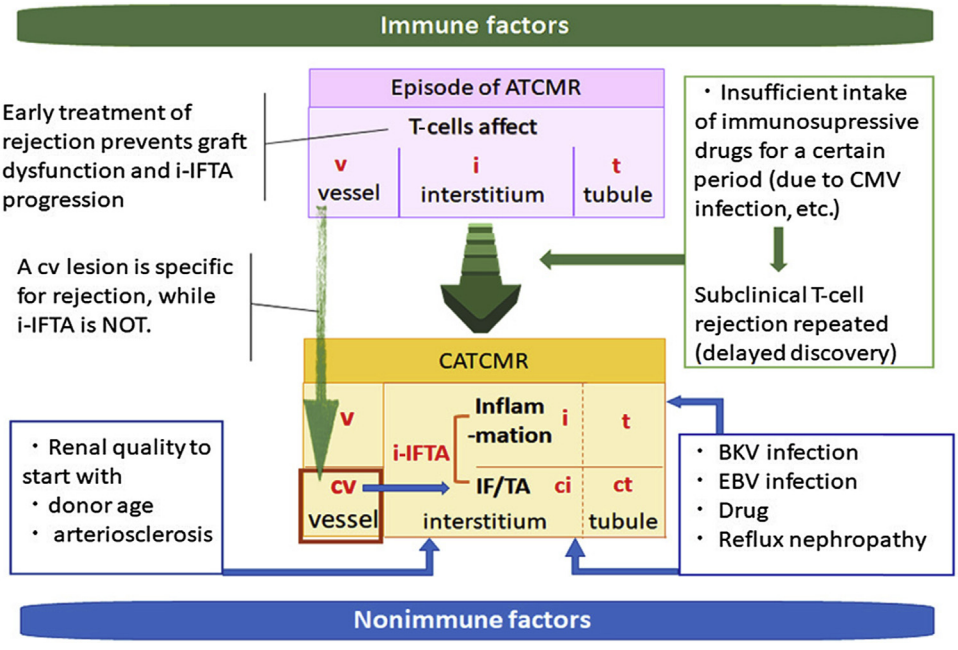

Fig 6. The proposed pathogenesis of CATCR in the tubulointerstitial compartments based on the 3 complex cases presented. ATCMR, acute T cell-mediated rejection; BKV, BK virus; CATCMR, chronic active T cell mediated rejection; CATCR, chronic active T cell-mediated rejection; CMV, cytomegalovirus; EBV, Epstein-Barr virus; i-IFTA, inflammation in areas of interstitial fibrosis and tubular atrophy; IF/TA, interstitial fibrosis and tubular atrophy.

specific for rejection. Unlike early T-cell transplant damage, subsequent chronic T-cell damage can become more complex because of accumulated immune and nonimmune injuries. As presented in case 2, the cv lesions accelerated i-IFTA formation. The other factors that can contribute to the injury process of CATCR include the baseline renal quality, such as donor age and the severity of arteriosclerosis (case 1) and the presence of graft infections (case 3), drug toxicity, and reflux nephropathy (case 2).

In summary, CATCR can be pathologically characterized by the presence of vascular and tubulointerstitial components. Unlike cv lesions, i-IFTA lesions are not specific findings of rejection because multiple factors can contribute to the formation of i-IFTA. However, "pure" CATCR, as a consequence of persistent or recurrent ATCR, exists among cases with i-IFTA with tubulitis. The 2017 Banff criteria shed light on the use of i-IFTA for the diagnosis of CATCR, which requires prompt treatment. Detailed insights on the pathogenesis of CATCR are indispensable for appropriate diagnosis and further treatment.

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