INDIAN JOURNAL OF TRANSPLANTATION 8 (2014) S17–S20

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/ijt

Review Article

The emerging role of electron microscopy in renal allograft rejection

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ARTICLE INFO

Article history:
Received 9 December 2013
Accepted 6 January 2014
Available online 20 January 2014

Keywords:
Chronic rejection
Antibody-mediated rejection
Electron microscopy
Renal allograft
Transplant glomerulopathy

ABSTRACT

The current criteria for renal allograft rejection are primarily based on histology, and therefore the early treatable stage of rejection is often not detected. Ultrastructural examination of renal allograft biopsy is increasingly being recognized to be essential for detecting early changes of rejection, which may not be evident on histology. These early ultrastructural lesions usually involve the glomerular and peritubular capillaries. Additionally, the routine ultrastructural examination of renal graft biopsies in patients with chronic graft dysfunction can reduce the non-specific diagnosis of interstitial fibrosis and tubular atrophy by demonstration of changes indicative of rejection-associated injury. Ultrastructural evaluation along with histology and immunofluorescence of renal graft biopsies is also vital in proteinuria to differentiate transplant glomerulopathy from recurrent or de novo glomerular pathology. The need for specific specimen collection procedures, limited graft tissue, and cost are limiting factors for routine electron microscopy in allograft biopsies. However, electron microscopy is an excellent tool for the evaluation of early changes in graft biopsies not evident on routine histology.

1. Introduction

Traditionally, histological evaluation of renal transplant biopsy was considered sufficient to determine the etiology of renal allograft dysfunction. With the recent Banff classification of renal allograft dysfunction, immunofluorescence has become a part of the protocol due to the requirement for staining for C4d to diagnose antibody-mediated rejection. However, ultrastructural examination is still not considered mandatory for evaluation of renal transplant biopsies.

Recent evidence shows that ultrastructural examination of renal transplant biopsies is required for the detection of early rejection-associated changes in graft biopsies, early transplant glomerulopathy and in recurrent/de novo glomerular diseases. Ultrastructural examination is also often required in the confirmation of two or more coexistent pathologies that affects patient management and prognosis. Table 1 describes the conditions where ultrastructural examination of renal graft biopsies is helpful.[1]

While acute rejection can be diagnosed by the use of light microscopy and appropriate immunohistochemistry or immunofluorescence, the diagnosis of chronic rejection in certain cases requires the use of electron microscope (EM). Ultrastructural detection of multilamination of peritubular

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2212-0017/$ – see front matter
http://dx.doi.org/10.1016/j.ijt.2014.01.001

Table 1 – Role of electron microscope (EM) in renal graft biopsy.[1]

Conditions where EM is required
Essential
PTC multilamination in chronic ABMR
Early diagnosis of TG
Early diagnosis of de novo/recurrent FSGS
Other suspected de novo/recurrent glomerular diseases (as in native renal biopsies)
Hyperperfusion injury
Supportive
Thrombotic microangiopathy
Infections
Acute rejection

PTC – peritubular capillary; ABMR – antibody-mediated rejection; TG – transplant glomerulopathy; FSGS – focal segmental glomerulosclerosis.

capillary (PTC) basement membrane is one of the earliest manifestations of chronic antibody-mediated injury in renal allograft biopsies.[2] EM is also a more sensitive tool for the diagnosis of transplant glomerulopathy than light microscopy. With the increasing number of renal transplant recipients and the duration of allograft survival, the incidence of de novo and recurrent glomerular disease is rising; electron microscopy is often required for the definite diagnosis of these glomerular diseases similar to its role in native renal biopsies.

1.1. *Acute renal allograft rejection*

Ultrastructural examination of renal graft biopsies in acute rejection confirms the findings of histology and can help in excluding a coexistent pathology.

1.2. *Chronic renal allograft rejection*

The ultrastructural evaluation of renal capillaries is useful to diagnose chronic rejection as they show changes induced by repeated rejection episodes. Capillary endothelial cells express both HLA type I and II molecules and are therefore involved in the pathogenesis of rejection. The renal microvasculature is the main target of injury in rejection.

The basement membrane of PTC is a homogenous continuous structure with an average width of 88 nm[3] Repeated injury to endothelium of PTC and healing leads to formation of rings of basement membrane material with the new one deposited towards the luminal side. This multilamination of PTC basement membrane in renal allograft biopsies is useful in the diagnosis of rejection-induced tissue injury. The multilamination can be graded as mild (two to four layers), moderate (five or six layers) or severe (>seven layers).[4] Three or more PTC with five to six layers or even one PTC with seven or more circumferential multilamination are diagnostic of chronic rejection.[5] The multilamination can be regarded as circumferential if it is seen in at least three of the four portions of the capillary circumference.[5] One or two PTC with five to six layers can be found in other conditions like obstructive uropathy, chronic tubulo-interstitial nephritis, thrombotic microangiopathy, radiation nephritis and analgesic nephropathy.[5] However, many of these conditions can be excluded in the presence of adequate clinical information. PTC lamination also correlates with presence of multilamination in glomerular capillaries manifested as transplant glomerulopathy and with C4d deposition.[5,6]

Liapis et al extensively scored PTC multilamination on electron microscopy on the basis of the degree and extent of multilamination in three most affected capillaries.[2] They concluded that the presence of very severe PTC multilamination is helpful in the diagnosis of rejection-induced tissue injury without precisely predicting the Banff rejection category. Additionally, they also stated that the absence of very severe PTC multilamination is a strong indicator to exclude the possibility of chronic antibody-mediated rejection.[2]

The presence of PTC multilamination with unexplained interstitial fibrosis, suggests repeated episodes of rejection as the cause of fibrosis. Renal graft biopsies diagnosed as 'interstitial fibrosis tubular atrophy- not otherwise specified (IFTA-NOS) or as 'chronic allograft nephropathy' can be reduced to 17% and the diagnosis of chronic rejection increased from 26% to 68% by demonstrating PTC lamination on electron microscopy.[4,7] In severe cases, thickening of PTC basement membrane appreciated on histology correlates with PTC lamination found on ultrastructural examination. However, electron microscopy remains the gold standard for detection of PTC basement membrane lamination.[8]

1.3. *Transplant glomerulopathy*

Transplant glomerulopathy is characterized by reduplication of the glomerular basement membrane (GBM) and is a morphological evidence of repeated endothelial injury. On ultrastructural examination multilamination of GBM is seen around the entire circumference of the glomerular capillaries and even between the endothelium and mesangium where GBM is not normally present. It can be seen in graft rejection, thrombotic microangiopathy and in calcineurin inhibitor toxicity. Coexistent PTC lamination is found more commonly in rejection-associated transplant glomerulopathy as compared to non-rejection-associated transplant glomerulopathy.[2] Electron microscopy is also useful in differentiating transplant glomerulopathy from recurrent/de novo membranoproliferative glomerulonephritis (MPGN); the latter shows duplication of GBM along with presence of dense deposits. Ultrastructural examination of graft biopsies detects 40% more cases of transplant glomerulopathy as compared to light microscopy alone.[5]

The ultrastructural diagnostic criteria for transplant glomerulopathy, however, are not well established. Ivanyi et al have defined transplant glomerulopathy as the thickening of the capillary wall in at least three loops as a result of widening of the sub-endothelial space by abnormal basement membrane material and the formation of new layers of basal lamina.[4]

Longitudinal analysis of sequential protocol biopsies over a period of time has shown that endothelial and subendothelial ultrastructural abnormalities in glomerular and peritubular capillaries are sensitive early markers of transplant glomerulopathy.[9] One of the earliest events is