http://www.kidney-international.org

Characterization of intra-graft B cells during renal allograft rejection

Valeriya Zarkhin[1,4], Neeraja Kambham[2,4], Li Li[1], Shirley Kwok[2], Szu-Chuan Hsieh[1], Oscar Salvatierra[1,3] and Minnie M. Sarwal[1,3]

[1]Department of Pediatrics, Stanford University Medical Center, Stanford, California, USA; [2]Department of Pathology, Stanford University Medical Center, Stanford, California, USA and [3]Department of Surgery, Stanford University Medical Center, Stanford, California, USA

Intra-graft CD20$^+$ B-cell clusters are found during acute rejection of renal allografts and correlate with graft recovery following rejection injury. Here using archived kidney tissue we conducted immunohistochemical studies to measure specific subsets of pathogenic B cells during graft rejection. Cluster-forming CD20$^+$ B cells in the rejected graft are likely derived from the recipient and are composed of mature B cells. These cells are activated (CD79a$^+$), and present MHC Class II antigen (HLADR$^+$) to CD4$^+$ T cells. Some of these clusters contained memory B cells (CD27$^+$) and they did not correlate with intra-graft C4d deposition or with detection of donor-specific antibody. Further, several non-cluster forming CD20$^-$ B-lineage CD38$^+$ plasmablasts and plasma cells were found to infiltrate the rejected grafts and these cells strongly correlated with circulating donor-specific antibody, and to a lesser extent with intra-graft C4d. Both CD20$^+$ B cells and CD38$^+$ cells correlated with poor response of the rejection to steroids. Reduced graft survival was associated with the presence of CD20 cells in the graft. In conclusion, a specific subset of early lineage B cells appears to be an antigen-presenting cell and which when present in the rejected graft may support a steroid-resistant T-cell-mediated cellular rejection. Late lineage interstitial plasmablasts and plasma cells may also support humoral rejection. These studies suggest that detailed analysis of interstitial cellular infiltrates may allow better use of B-cell lineage specific treatments to improve graft outcomes.

Kidney International (2008) **74,** 664–673; doi:10.1038/ki.2008.249; published online 11 June 2008

KEYWORDS: renal allograft rejection; intra-graft CD20$^+$ and CD38$^+$ infiltrates; B-lymphocytes; transplant biopsy; graft survival

Correspondence: *Minnie M. Sarwal, Department of Pediatrics, Stanford University Medical Center, G306, 300 Pasteur Drive, Stanford, California 94304, USA. E-mail: msarwal@stanford.edu*

[4]These authors contributed equally to this work.

Received 1 October 2007; revised 27 March 2008; accepted 1 April 2008; published online 11 June 2008

CD20$^+$ lymphocytic infiltrates have been demonstrated to be potentially pathogenic in transplant cellular acute rejection (AR), correlating with a phenotype of a rejection that is more recalcitrant to conventional treatment, an increased risk of graft loss, and independent of C4d peritubular deposition.[1–6] Other studies have not always shown this clinical correlation,[7–9] and it is unclear if these differences may, in part, be related to the patient group under study with underlying differences secondary to recipient age, the variability of specific transplant center induction and immunosuppression protocols, or because of differences in the specific lineage of CD20$^+$ B cells observed in different types of graft rejection.[1]

Detailed phenotypic analyses of CD20$^+$ B-cell infiltrates in graft rejection have not been performed to date. Given the number of immunohistochemistry stains required to comprehensively phenotype the lineage of infiltrating B cells and the limitation of human renal biopsy tissue available post-diagnostic evaluation, these studies are difficult to perform extensively on patient samples. With this in mind, tissue microarrays using transplant nephrectomies offer the best (if not ideal) alternative to biopsies for screening of possible immunohistochemical (IHC) predictors of graft outcome. Moreover, the transplant nephrectomy patients are often weaned off all immunosuppression prior to surgery, resulting in uninhibited full-blown immunological attack on the allograft. In our study, we have attempted to capture this phenotype of severe AR, utilizing tissue microarray tools, with a focus on the different lineages of infiltrating B cells in graft rejection.

RESULTS

Comprehensive immunostaining was performed initially on kidney tissue microarray (K-TMA) samples and selected, relevant immunostains were then performed in core biopsy samples with rejection, and the results were correlated with specific clinical graft outcomes.

Distinct B-cell lineages in rejecting grafts

Two predominant lineages of B cells were identified at the time of AR (Figure 1a): interstitial CD20$^+$ B-cell clusters and interstitial scattered CD38$^+$ plasmablasts and plasma cells. Fluorescence *in situ* hybridization (FISH) for the X and Y

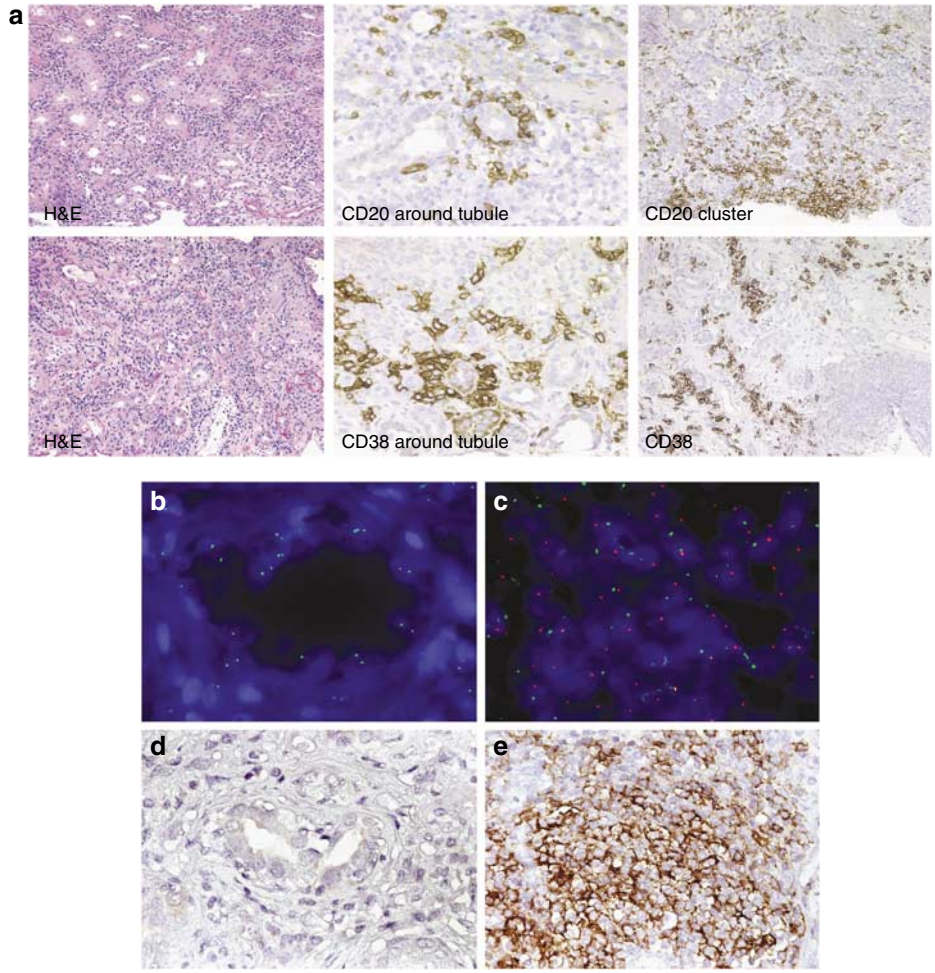

Figure 1 | Different B-cell subsets populate rejecting kidney allografts (a) and these B cells are recipient derived (b–e). (**a**) Two different B-cell subsets are identified in AR: representative renal allograft core biopsies were stained for H&E, CD20, and CD38 markers. Two major nonoverlapping populations of B cells are seen and these include $CD20^+$ but $CD38^-$ cells mostly in clusters (upper panel) and $CD20^-$ but $CD38^+$ interstitial plasmablasts and plasma cells (lower panel, original magnification $\times 200$). The $CD20^+$ and $CD38^+$ cells can be seen in the interstitium, surrounding the tubules, but not causing tubulitis (original magnification $\times 300$). (**b–e**) $CD20^+$ B-cell clusters in rejecting kidney allografts are recipient derived. FISH for X (green signal) and Y (red signal) chromosome probes was performed on all K-TMA cores and a representative core with AR (T-11) from a male recipient of a female donor kidney is shown here (original magnification $\times 400$). CD20 stain and FISH were performed on consecutive sections to be able to locate the B-cell clusters on FISH sections accurately. As shown, the tubule is from a female donor kidney (XX, **b**) and the B-cell clusters seen in the interstitium are likely recipient derived (XY, **c**), although few Y-chromosome-positive cells within the B-cell cluster may also be colocalizing T cells or macrophages. The serial section stained with CD20 is also shown, corresponding to the area of the tubule (**d**) and B-cell cluster (**e**).

chromosomes in sex-mismatched donor and recipient pairs suggests that the B cells in AR were recipient derived (Figure 1b–e).

$CD20^+$ clusters. As elucidated by the K-TMA immunostains, the $CD20^+$ B-cell clusters were of early lineage, with an activated phenotype, with positive staining for CD79a. Few, but not all cluster cells positive for CD20 and negative for CD3, expressed the CD27 marker, suggesting that B-cell clusters contain some memory B cells (Figure 2a; Figure S3). B-cell clusters also stained positive for MHC Class II (HLADR; Figure 2b), and were surrounded by $CD4^+$ lymphocytes, suggesting a putative role of $CD20^+$ cells in antigen presentation.[10] Occasional $CD8^+$ cytotoxic T cells and $CD68^+$ macrophages were also seen in the B-cell aggregates. Dense interstitial $CD20^+$ B-cell aggregates[1,2,5] were seen in 53% (17/32) of core biopsy samples with graft rejection. The follicular dendritic cell network as highlighted by CD21 was seen in the $CD20^+$ clusters in 11% of core biopsies (Figure 3a).

$CD38^+$ plasmablasts and plasma cells. Another predominant population of B cells in graft rejection consists of $CD38^+$ plasmablasts and plasma cells. Many of these cells co-segregate with plasma cells, which are positive for CD138 and negative for early B-lineage markers (Figures 1a and 2c). $CD38^+$-rich cellular infiltrates were seen in 59% (19/32) of core biopsies. Many AR biopsies contained both $CD20^+$ clusters and $CD38^+$ B-lineage cells (in 44% (14/32) of core biopsies).