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Resident dendritic cells are the predominant TNF-secreting cell in early renal ischemia–reperfusion injury

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Renal ischemia–reperfusion injury (IRI) rapidly induces production of inflammatory mediators including, and in particular, tumor necrosis factor (TNF). Possible sources include resident parenchymal and bone marrow-derived cells as well as recruited leukocytes. Cell suspensions from kidneys subjected to IRI were examined by cell separation followed by *in vitro* culture and enzyme-linked immunosorbent assay (ELISA), immunoperoxidase and immunofluorescence microscopy, and multicolor flow cytometry to determine the contribution of dendritic cells (DCs) to early production of TNF and other inflammatory mediators. Secretion of TNF, interleukin (IL-6), monocyte chemoattractant protein-1 (MCP-1), and regulated on activation normal T cell expressed and secreted (RANTES) was increased in cell suspensions from IRI compared with control kidneys and was higher in DC-enriched preparations. Immunostaining identified TNF^{+ve} cells that coexpressed the DC marker CD11c. Flow cytometry of bone marrow-derived ($CD45^{+ve}$) cell populations at 24 h post-IRI demonstrated that $F4/80^{+ve}$/$CD11c^{+ve}$ DCs remained proportionately stable and exhibit higher levels of DC maturation markers, whereas the proportion of $F4/80^{-ve}$ DCs, monocytes, neutrophils, and T cells increased. Intracellular staining for TNF confirmed that $F4/80^{+ve}$ DCs were the predominant TNF^{+ve} cell and expressed higher levels than other TNF^{+ve} cells. *In vivo* depletion of DCs from the kidney substantially attenuated TNF secretion by total and $CD45^{+ve}$ cells following IRI. The results uncover a role for resident $F4/80^{+ve}$ DCs as the predominant secretors of TNF within 24 h of IRI.

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A critical role for inflammation in the pathophysiology of acute ischemia–reperfusion injury (IRI) of the kidney is currently well accepted on the basis of experimental and clinical studies carried out over the past two decades.[1–3] A number of proinflammatory cytokines and chemokines have been consistently implicated in the pathophysiology of acute renal failure among the most prominent of which is tumor necrosis factor (TNF). Upregulation of mRNA and protein levels of TNF occurs at a whole-organ level within minutes to hours of onset of IRI and other forms of acute renal parenchymal injury.[4–7] Less well appreciated at present is the degree to which individual cell populations contribute to the early production of TNF and other proinflammatory cytokines and chemokines within the renal interstitial compartment.[8] Although marginated and infiltrating cell populations, once recruited, likely secrete such products, the timing of the initial secretory burst in multiple models, and the requirement for secreted chemokines to initiate recruitment of these cells indicates a primary role for resident cell populations. Renal tubular epithelial cells are the predominant parenchymal cell type in the kidney and clearly can be induced to synthesize and secrete inflammatory mediators *in vitro* or *in vivo*.[9,10] The primary importance of epithelial cell-derived products, however, has not been established. Other resident cell types that may participate in the early inflammatory response to acute renal injury include endothelial cells, fibroblasts, mesangial cells, tissue macrophages, and dendritic cells (DCs).

Although least well studied among these cell types, DCs may be uniquely involved in the pathogenesis of acute renal injury for numerous reasons. DCs are known to be abundantly present throughout the renal interstitial space and directly interposed between the tubular epithelium and the peritubular capillaries in the healthy kidney.[11–13] Additionally, we and others have demonstrated that renal DCs (rDCs) undergo dynamic phenotypic alterations in the context of acute systemic or localized renal parenchymal injury.[14–19] Moreover, in keeping with the well-recognized role of DCs in initiating cognate immunity, rDC responses to such insults may include antigen uptake, migration to draining lymph nodes, and increased capacity for localized antigen-specific T-cell activation – functional activities which

occur within 2–7 days of an injurious event.[15,17] In this study, we have examined the contribution of rDCs to the early release of innate (proinflammatory) immune mediators within the acutely ischemic kidney in the mouse. Focusing predominantly on TNF production, the results identify rDCs, specifically F4/80-expressing rDCs, as potent 'first responders' in the innate response to renal IRI.

RESULTS

CD11c^{+ve} cells are potent early producers of proinflammatory mediators in the kidney following IRI

Unilateral renal artery clipping was employed to examine the contribution of intrarenal DCs to the production of proinflammatory cytokines and chemokines following IRI. Cell suspensions of clipped and unclipped kidneys, prepared 24 h after unilateral IRI, were divided into total, CD11c-enriched, and CD11c-depleted fractions using anti-CD11c microbeads and magnetic column separation (see Figure 1a) and were tested *in vitro* for secretion of TNF, interleukin (IL-6), monocyte chemoattractant protein-1 (MCP-1), and regulated on activation normal T cell expressed and secreted (RANTES) by enzyme-linked immunosorbent assay (ELISA) of culture supernatants. Secretion of these products by equal numbers of CD11c-enriched and CD11c-depleted cells was compared (Figure 1b). As shown, secretion of all four products was enhanced in culture supernatants from clipped compared to unclipped kidneys, and was markedly greater in the CD11c-enriched compared to CD11c-depleted fractions. The contribution of CD11c^{+ve} cells to overall secretion of the same products was examined by comparing concentrations in culture supernatants from unfractionated cells to those from equal numbers of CD11c-depleted cells (Figure 1c). For all products measured, depletion of CD11c^{+ve} cells from the ischemic kidney preparations was associated with reduced concentration. In this, and multiple similar experiments, the effect of CD11c depletion was most marked for TNF and RANTES compared with MCP-1 and IL-6. The increased secretion of proinflammatory products by CD11c-enriched fractions following IRI was reproduced in two other inbred mouse strains BALB/C and the lipopolysaccharide (LPS)-resistant strain C3H/HeJ, data not shown).

To more specifically visualize TNF^{+ve} cells within kidney cell suspensions, and to colocalize TNF and CD11c expression, cell preparations from clipped and control kidneys were examined by immunoperoxidase staining with anti-TNF and by three-color immunofluorescent staining with anti-TNF, anti-CD11c, and 4′,6-diamidino-2-phenylindole (DAPI) (Figure 2). Compared to control kidneys, cell preparations from IRI kidneys contained numerically greater numbers of TNF^{+ve} cells that were predominantly small and rounded (Figure 2a and b). Immunofluorescence microscopy confirmed the presence of TNF and CD11c double-positive cells within cell preparations from IRI kidneys (Figure 2c). This series of experiments was interpreted, as indicating that CD11c^{+ve} cells, presumably DCs, constitute potent inducible

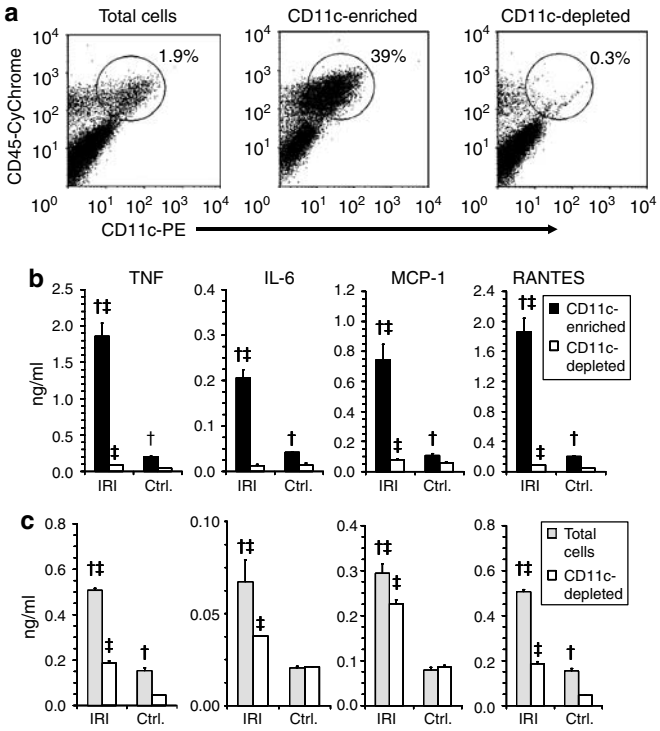

Figure 1 | CD11c^{+ve} cells are a potent source of TNF, IL-6, MCP-1, and RANTES in kidney cell suspensions following IRI. (**a**) Examples of flow cytometric analysis of total kidney cell suspensions and of CD11c-enriched and CD11c-depleted fractions prepared by anti-CD11c magnetic column separation. Proportions of each cell population stained positively for CD45 and CD11c are indicated. (**b**) Cell suspensions from clipped (IRI) kidneys and unclipped (Ctrl.) kidneys of a group of four mice were prepared 24 h after being subjected to 30 min of unilateral renal artery clipping. The cells were separated into CD11c-enriched and CD11c-depleted fractions, and were cultured overnight in equal numbers. Results are shown for ELISAs of culture supernatants from the cell fractions expressed as mean ± s.d. concentration in ng/ml. Concentrations of all four products were higher in supernatants from CD11c-enriched fractions, and were increased in CD11c-enriched cells from IRI compared to Ctrl kidneys. (**c**) Unsorted and CD11c-depleted cell fractions from the same organs were cultured overnight in equal numbers. Results are shown for ELISAs of culture supernatants from the cell fractions expressed as mean ± s.d. concentration in ng/ml. For IRI kidneys, concentrations of all four products were higher in supernatants from unsorted compared with CD11c-depleted fractions. For control kidneys, only TNF and RANTES were higher in unsorted fractions. Levels of all products were higher in all fractions from IRI compared with the equivalent fraction of Ctrl kidneys. † = $P < 0.05$ for CD11c-enriched vs CD11c-depleted (**b**) or total cells vs CD11c-depleted (**c**). ‡ = $P < 0.05$ for IRI vs Ctrl kidneys.

secretors of TNF and other proinflammatory mediators within 24 h of IRI.

CD11c^{+ve} DCs are resident intrarenal cells that are distinct from infiltrating monocytes, neutrophils, and T cells and undergo maturation *in situ* following IRI

To more definitively distinguish rDCs from other intrarenal cell populations and to further characterize TNF^{+ve} DCs during IRI, flow cytometric analysis was undertaken. Characterization of CD45^{+ve}/CD11c^{+ve} cells from healthy mouse kidneys revealed that, consistent with DCs, they were