Antigen presentation by dendritic cells in renal lymph nodes is linked to systemic and local injury to the kidney

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Antigen presentation by dendritic cells in renal lymph nodes is linked to systemic and local injury to the kidney.

Background. Dendritic cells (DCs) uniquely serve as conduits between innate and cognate arms of the immune system. The normal kidney contains an extensive population of interstitial DCs but their role in the pathogenesis of acute renal injury is not known.

Methods. Renal DCs were studied by flow cytometric analysis of collagenase-digested mouse kidneys, by immunohistochemistry, and by immunofluorescence microscopy. In vivo ingestion by DCs of intravenously administered fluorescein isothiocyanate (FITC)-dextran particles was examined. A model antigen system (presentation of ovalbumin-derived peptide to TCR transgenic CD4+ T-cells) was employed to examine the influence of systemic (lipopolysacchride injection) and localized (unilateral renal artery clipping) renal injury on DC-mediated T-cell activation in the renal lymph nodes (RLNs).

Results. Renal DCs were shown to constitute the predominant source of T-cell stimulatory capacity within the kidney, and to avidly ingest both filtered and nonfiltered particles. Lipopolysaccharide resulted in disappearance of DCs from the renal interstitium within 48 hours. This was accompanied by increased renal lymph node DCs, some of which contained intracellular Tamm-Horsfall Protein, indicating abnormal trafficking of kidney-specific antigens following renal injury. Lipopolysaccharide enhanced DC-mediated proliferation of ovalbumin-specific CD4[+ve] T-cells within the draining RLN. Unilateral renal ischemia augmented the capacity for DC-mediated T-cell activation in the lymph nodes draining both the ischemic and nonischemic kidney.

Conclusion. Renal DCs respond to systemic or localized acute renal injury by increasing the traffic of protein antigens from kidney to RLN, resulting in a concomitant increased potential for localized activation of antigen-specific CD4[+ve] T-cells.

Dendritic cells (DCs) are bone marrow–derived, antigen-presenting cells (APCs) with unique capacity to activate naïve T-cells [1, 2]. A wealth of research has established the importance of DCs for normal and abnormal immune function [1, 3, 4]. Central to DC biology is the phenotypic shift ("maturation") that regulates antigen-presenting potency [1, 3, 4]. In the immature state, DCs exhibit high phagocytic capacity, relatively low surface levels of class II major histocompatibility complex (MHC II) and costimulatory proteins, and weak ability to stimulate T-cell proliferation. Following maturation, DCs switch to low antigen-uptake capacity, high surface levels of MHC II and costimulatory ligands, and potent T-cell stimulatory ability [1, 3, 4]. The signals that drive DC maturation and, consequently, initiate antigen-specific T-cell responses, derive from products of tissue injury (microbial components, innate proinflammatory secretions) [1, 3–6]. In the absence of maturational stimuli, DCs play an ongoing, active role in maintaining peripheral immune tolerance through the induction of regulatory T-cells [7–9]. Thus, DCs serve as unique conduits for cross-talk between innate and cognate arms of the immune system and, through their varying interactions with T-cells, contribute actively to both tolerance and immunity.

The presence of DCs within the renal parenchyma has been established for both rodents and humans and, in the healthy kidney, these cells form a readily demonstrable population, expressing major histocompatibility complex (MHC) and costimulatory proteins [10–15]. The primary localization of renal DCs (rDCs) is within the peritubular interstitium, where they are in close contact with the basolateral surfaces of tubular epithelium and peritubular capillaries [13–15]. Renal DCs are thus positioned to sense and respond to substances diffusing from tubules to capillaries and are, therefore, likely to be directly influenced by alterations to the glomerular filtrate, as well as to other changes in the interstitial compartment. The recent expansion of knowledge regarding DC biology suggests that rDCs merit closer study as potentially important participants in forms of renal injury for which there is evidence of maladaptive communication between

innate and cognate arms of the immune system [16–21]. It is well established from animal and human studies that influx of inflammatory cells to the renal parenchyma characteristically occurs during acute renal failure associated with sepsis/endotoxinemia and in the wake of localized insults such as ischemia-reperfusion and ureteral obstruction [22, 23]. In elucidating the impact of intraparenchymal inflammation on the extent of experimental renal injury, much emphasis has been placed on the effector role of cell populations such as macrophages and neutrophils, which constitute components of the innate immune response. It is evident from experiments involving depletion of macrophages or inhibition of macrophage recruitment that this lineage contributes to the decline in renal function that follows a period of ischemia. More recently, however, T lymphocytes (T-cells) have also been identified as having a direct pathogenic role in the causation of acute renal failure in the context of generalized ischemia [24], as well as localized ischemia-reperfusion of the kidney [25]. Although the antigen specificity of T-cells involved in acute renal injuries remains to be determined, it is implicit from these observations that new or modified interactions between T-cells and antigen-presenting cells (APCs) occur following ischemia-reperfusion, and that these antigen presentation events are potentially injurious to the kidney. In further support of this concept, blockade of the CD80/CD86 costimulatory ligands attenuates ischemia-reperfusion injury [17, 19].

We hypothesized that the T-cell activation and subsequent T-cell–mediated injury associated with specific renal insults is induced by alterations to the kinetic and phenotypic characteristics of rDCs. Specifically, we postulated that whole body or localized proinflammatory events initiate early efflux of DCs from the renal parenchyma to the draining lymphoid tissue, leading to abnormal DC-mediated trafficking of antigens derived from the kidney and antigen-specific T-cell activation within renal lymph nodes (RLNs). In support of this thesis, and in a murine model system, we demonstrate (*1*) the predominant antigen uptake and presentation capacity of DCs within the kidney parenchyma, and (*2*) the potential for rDCs to mediate abnormal antigen transfer and antigen-specific T-cell activation within RLNs following renal injury.

METHODS

Experimental animals and reagents

C57BL/6, BALB/C (Jackson Laboratories, Bar Harbor, ME, USA) and DO11.10 mice were maintained in a specific pathogen-free facility. Tissue culture was carried out in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS) (Invitrogen Corp., Carlsbad, CA, USA). Mouse-specific detection reagents used were: fluo-rescein isothiocyanate (FITC)-, phycoerythrin (PE)-, and biotin-coupled anti-CD11c (clone HL3), anti-I-A^b-biotin (AF6-120.1), anti-H-2D^b-biotin (28–14-8), anti-H-2K^b-biotin (AF6-88.5), anti-CD86-PE (GL1), anti-CD80-PE (16-10A1), anti-CD40-PE (3/23), anti-ICAM-1-PE (3E2), anti-ICAM-2-biotin (3C4-mlC2/4), anti-CD8α-PE (53–6.7), anti-CD4-CyChrome (RM4.4), streptavidin-PerCP (BD Pharmingen, San Diego, CA, USA); sheep anti-Tamm-Horsfall Glycoprotein (THP) (BioTrend Chemikalien GmbH, Cologne, Germany); Alexa Fluor® 594-conjugated streptavidin, Alexa Fluor® 488-coupled donkey antisheep IgG (Molecular Probes, Eugene, OR, USA); biotin coupled anti-DO11.10 TCR (KJ1-26, kindly provided by Dr. Larry Pease, Dept. of Immunology, Mayo Clinic, Rochester, MN, USA).

Collagenase/DNase disruption of mouse organs

Kidneys and lymphoid organs were cut into 1 to 2 mm^3 pieces, placed in DMEM containing 1.6 mg/mL collagenase I (Sigma Aldrich, St. Louis, MO, USA), and 200 μg/mL DNase I (Roche Applied Science, Indianapolis, IN, USA) for 40 minutes at 37°C with intermittent agitation, washed, resuspended in DMEM/200 μg/mL DNase I at room temperature for 15 minutes, and washed twice in DMEM. Following erythrocyte lysis, cells were resuspended in DMEM/10% FCS or FACs buffer (PBS/0.2%BSA/0.02%NaN$_2$). Kidney cell suspensions were allowed to settle for 20 minutes, following which the upper two thirds (lower density cells) was removed for subsequent assays. For flow cytometry, aliquots of 0.25 to 4.0 × 10^5 cells were incubated in FACs buffer at 4°C, with fluorochrome-labeled and/or biotinylated antibodies followed by fluorochrome-labeled streptavidin. Samples were analyzed using a FACscan^{TM} flow cytometer and Cellquest^{TM} software (BD Pharmingen, San Diego, CA, USA).

Magnetic bead enrichment of CD11c^{+ve} cells and coculture with CFSE-labeled allogeneic T-cells

Cells prepared from collagenase/DNase-digested BALB/C kidneys and lymphoid organs were suspended in MACS buffer (PBS, 0.5% BSA, 2 mmol/L EDTA) at 90 μL/10^7 cells; incubated with anti-CD11c–coated microbeads (10 μL/10^7 cells; Miltenyi Biotech, Inc., Auburn, CA, USA) at 4°C for 20 minutes; washed and resuspended in 1 mL MACS buffer. Positive and negative fractions were separated using the miniMACS^{TM} separation system (Miltenyi Biotech), then washed and resuspended in DMEM/10% FCS. For mixed lymphocyte reactions, purified T-cells were prepared from spleens and lymph nodes of adult C57BL/6 mice by similar magnetic column separation following incubation with anti-Thy1.2 microbeads, then were labeled with CFSE. The CFSE-labeled T-cells were plated in 96-well round-bottom tissue

culture plates at 2×10^5 cells per well. CD11c-enriched and CD11c-depleted cell populations from BALB/C kidney and were added at 2×10^4 cells per well. As a positive control, CD11c-enriched cells from spleen were added at the same ratio to additional wells. All experimental conditions were carried out in triplicate. Following 72 hours of coculture, cells from individual wells were lifted, stained with anti-CD4-PE or anti-CD8-PE, and analyzed by 3-color flow cytometry. Proliferation of C57BL/6 T-cell subsets for each condition was expressed as mean $\pm$ SD percent divided on the basis of CFSE dilution of CD4^{+ve} or CD8^{+ve} cells.

Administration of FITC-dextran particles

FITC-Dextran of M_r 40,000, and 500,000 (Sigma Aldrich) were suspended in phosphate-buffered saline (PBS) and injected intravenously into groups of 3 C57BL/6 mice at 5 mg/animal. A control group received PBS. One hour later, the spleens and kidneys were digested with collagenase/DNase I, cell suspensions were stained with DC-specific antibody (anti-CD11c-PE), and analyzed by 2-color flow cytometry. Uptake of fluorescent particles by DCs and non-DCs cells within individual samples was expressed quantitatively as mean fluorescence intensity (MFI) on the FL-1 channel of CD11c^{+ve} and CD11c^{-ve} cell populations. Results for each experimental group were expressed as mean $\pm$ SD of the MFI.

Immunoperoxidase and immunofluorescence microscopy

C57BL/6 mice were injected intravenously with 50 μg lipopolysaccharide (LPS) in PBS or with PBS alone. At varying time-points later, kidneys, renal lymph nodes, spleens, and subcutaneous lymph nodes were removed, placed in embedding medium (OCT compound), and frozen in liquid nitrogen–cooled isopentene. For immunoperoxidase staining, 6 μm cryosections on silane-coated slides were fixed in acetone ($-20°C$ for 10 min), air-dried, and rinsed in PBS. Endogenous peroxidase activity was blocked with 0.3% H_2O_2 in PBS for 10 minutes (lymphoid sections) or 50 minutes (kidney sections), and slides were rinsed in PBS. Normal goat serum (5% in PBS) was applied for 1 hour, followed by blockade of endogenous biotin (Vector Laboratories, Burlingame, CA, USA) and sequential incubations with sheep anti-THP antibody followed by biotinylated antisheep antibody for 1 hour each in a humidified chamber. Slides were rinsed in PBS, incubated with ABC complex (Vector Laboratories) for 45 minutes, rinsed again, and DAB (brown) substrate solution was applied and allowed to develop to the desired color intensity. For 2-color immunoperoxidase staining, a second round of incubations with biotinylated anti-CD11c, ABC complex, and VIP

(purple) substrate (Vector Laboratories) was carried out. Finally, the slides were rinsed in tap water, counterstained with hematoxylin, rinsed again in tap water, dehydrated through 4 changes of alcohol (80%, 95%, 100%, and 100%), cleared in 3 changes of xylene and mounted with Cytoseal^{TM} 60 (Richard-Allan Scientific, Kalamazoo, MI, USA). For immunofluorescent staining, acetone-fixed sections were blocked for 1 hour with 5% normal donkey serum in PBS, followed by endogenous biotin blockade and incubation with sheep anti-THP and biotinylated anti-CD11c antibodies for 1 hour. After rinsing in PBS, slides were incubated with Alexa Fluor® 488-conjugated antisheep antibody and Alexa Fluor® 594-Streptavidin (1:500) for 45 minutes, then rinsed again, mounted using VECTASHIELD® mounting medium with DAPI (Vector Laboratories) and examined under confocal laser-scanning microscopy (LSM510; Carl Zeiss, Göttingen, Germany).

In vivo stimulation of adoptively transferred, CFSE-labeled DO11.10 cells

Erythrocyte-depleted cell suspensions were prepared from spleens and lymph nodes of adult DO11.10 as previously described [46], incubated with antimouse-CD4 microbeads (10 μL/10^7 cells, Miltenyi Biotech) at 4°C for 20 minutes, washed and resuspended in 1 mL MACS buffer, then separated into positive and negative fractions using the miniMACS^{TM} system. The CD4^{+ve} fraction was washed in PBS, labeled with CFSE, resuspended in PBS, then injected intravenously into gender-matched, adult BALB/C recipients at 2.5 to 3.5×10^6 cells per animal. Between 48 and 96 hours after adoptive transfer, recipient mice were anesthetized, a 1 cm incision was made in the left flank, the left kidney was briefly externalized, and 50 μL of ovalbumin solution (1 mg/mL in PBS) was injected beneath the capsule with a 1 mL syringe and 27-gauge needle. The flank incision was closed in a single layer using 5-O prolene suture, and animals were observed for 2 hours, after which groups were injected intraperitoneally with LPS (50 μg in PBS) or with PBS alone. Three days later, the groups of mice were sacrificed and analysis of DO11.10 T-cell division within spleens and lymph nodes was carried out by 3-color flow cytometric analysis of cell suspensions stained with anti-Thy1.2-PE and biotinylated KJ1-26 (anti-DO11.10 TCR) followed by streptavidin-PerCP. Expansion of DO11.10 T-cells was determined by comparing the mean $\pm$ SD for the proportion of KJ1-26^{+ve} cells within the total Thy1.2^{+ve} cells among individual groups of samples. Degree of DO11.10 T-cell division was determined by comparing the mean $\pm$ SD for the proportion of Thy1.2^{+ve}/KJ1-26^{+ve} cells that had passed through 5 or more cell divisions among individual groups of samples.

Unilateral renal artery clipping in mice

Unilateral renal ischemia was imposed in adult BALB/C mice under pentobarbitol anesthesia by total occlusion of the left renal artery as described [47]. The procedure was performed on a heated surface to ensure maintenance of normal body temperature. The kidneys were exposed via a midline abdominal excision; the left renal pedicle was carefully dissected, then clamped for 22.5 minutes using a nontraumatic sterile clamp (RS5426, Microaneuryam clip, straight 10 mm, 125-g pressure; Roboz Surgical Instrument, Rockville, MD, USA). For each experiment, a group of animals underwent a sham procedure including anesthesia and laparotomy but omitting clamping of the renal pedicle. Immediately after placement of the clip, ovalbumin (100 μL of a 2 mg/mL solution in PBS) was injected beneath the left renal capsule using a 27-gauge needle. The abdominal incision was closed in separate layers using 5–0 prolene sutures. Animals were observed for 2 hours after the procedure and sacrificed 24 hours later for preparation of cell suspension from lymphoid organs.

In vitro stimulation of CFSE-labeled DO11.10 T-cells

Stimulators. Cell suspensions were prepared by collagenase/DNase-digestion of pooled left RLNs, right RLNs, and SLNs from groups of experimental animals. A portion of each sample was retained for use as the "unsorted" stimulator fraction, and the remainder was labeled with anti-CD11c microbeads and subjected to magnetic column separation as described above. The resulting positive and negative fractions were retained as "CD11c-enriched" and "CD11c-depleted" stimulator fractions.

Responders. Purified CFSE-labeled DO11.10 T-cells were prepared as described above and plated in 96-well, round-bottom tissue culture plates at 1 or 2×10^5 cells per well. Stimulator cell populations were added at 5×10^4 (unsorted and CD11c-depleted) or 1×10^4 (CD11c-enriched) cells per well. Between 3 and 6 replicates of each experimental condition were initiated. Following 96 hours of coculture, cells from individual wells were lifted, stained with anti-CD4-PE and biotinylated KJ1-26 biotin followed by streptavidin-PerCP, and analyzed by 3-color flow cytometry. Proliferation of DO11.10 T-cells for each condition was expressed as mean ± SD percent divided on the basis of CFSE dilution of $CD4^{+ve}/KJ1-26^{+ve}$ cells.

Statistical analysis

For experiments comparing flow cytometric analysis of DCs from different organs, the mean fluorescence intensity (MFI) of DC-surface staining for a given organ was expressed as mean ± SD of the measured values for each animal. For assays of in vivo and in vitro lymphocyte expansion, each condition results were expressed as mean ± SD, and individual conditions were compared statistically using unpaired, two-sided Student *t* test with significance assigned to $P < 0.05$.

RESULTS

Dendritic cells are the predominant resident population of antigen-presenting cells in the healthy kidney

The phenotype and antigen presenting capacity of DCs from the healthy mouse kidney were initially characterized by employing a collagenase/DNase digestion protocol to prepare cell suspensions for flow cytometry and magnetic column purification, using CD11c as the primary distinguishing marker. Flow cytometric analysis of samples from multiple animals demonstrated that surface levels of MHC II (I-A) and MHC I (H-2D, H-2K), as well as of the costimulatory proteins CD80 (B7-1), CD86 (B7-2), CD40, and ICAM-1 were significantly lower on renal DCs (rDCs) than on DCs from subcutaneous lymph nodes, spleen, thymus, and renal lymph nodes (data not shown). In contrast to the lymphoid organs, there were no $CD8^{+ve}$ DCs within the kidney (data not shown). The results of this initial phenotypic characterization confirm the recent findings of Kruger [26].

To confirm that DCs constitute the major APC population in the healthy kidney, CD11c-enriched ($CD11c^{+ve}$) and -depleted ($CD11c^{-ve}$) cell fractions were prepared from collagenase/DNase-digested kidneys and were cocultured with purified allogeneic T-cells labeled with the fluorescent dye CFSE (Fig. 1). Counterstaining with appropriate PE-labeled antibodies was used to separately determine proliferation of MHC 1- ($CD8^{+ve}$) and MHC II-restricted ($CD4^{+ve}$) T-cells. Cocultures of T-cells with $CD11c^{+ve}$ and $CD11c^{-ve}$ populations from spleen were also carried out. As shown, the $CD11c^{+ve}$ cells from kidney induced proliferation of both $CD4^{+ve}$ and $CD8^{+ve}$ T-cells, while the $CD11c^{-ve}$ cells induced only minimal $CD8^{+ve}$ T-cell proliferation. Splenic $CD11c^{+ve}$ cells were more potent T-cell stimulators than those from the kidney. Thus, DCs from the kidney exhibit a relatively immature phenotype, are exclusively of the "myeloid" ($CD8^{-ve}$) subgroup, and constitute the predominant resident APC population in the healthy adult kidney.

Renal DCs phagocytoze filtered and unfiltered particles from the circulation

The ability of renal and splenic DCs to ingest material present in the circulation was examined by intravenous administration of FITC-dextran particles to healthy mice. To determine whether rDCs are preferentially exposed to materials that pass through the glomerular filtration barrier, uptake of FITC-dextran particles of approximate M_r 40,000 (filtered) or 500,000 (not filtered) were separately
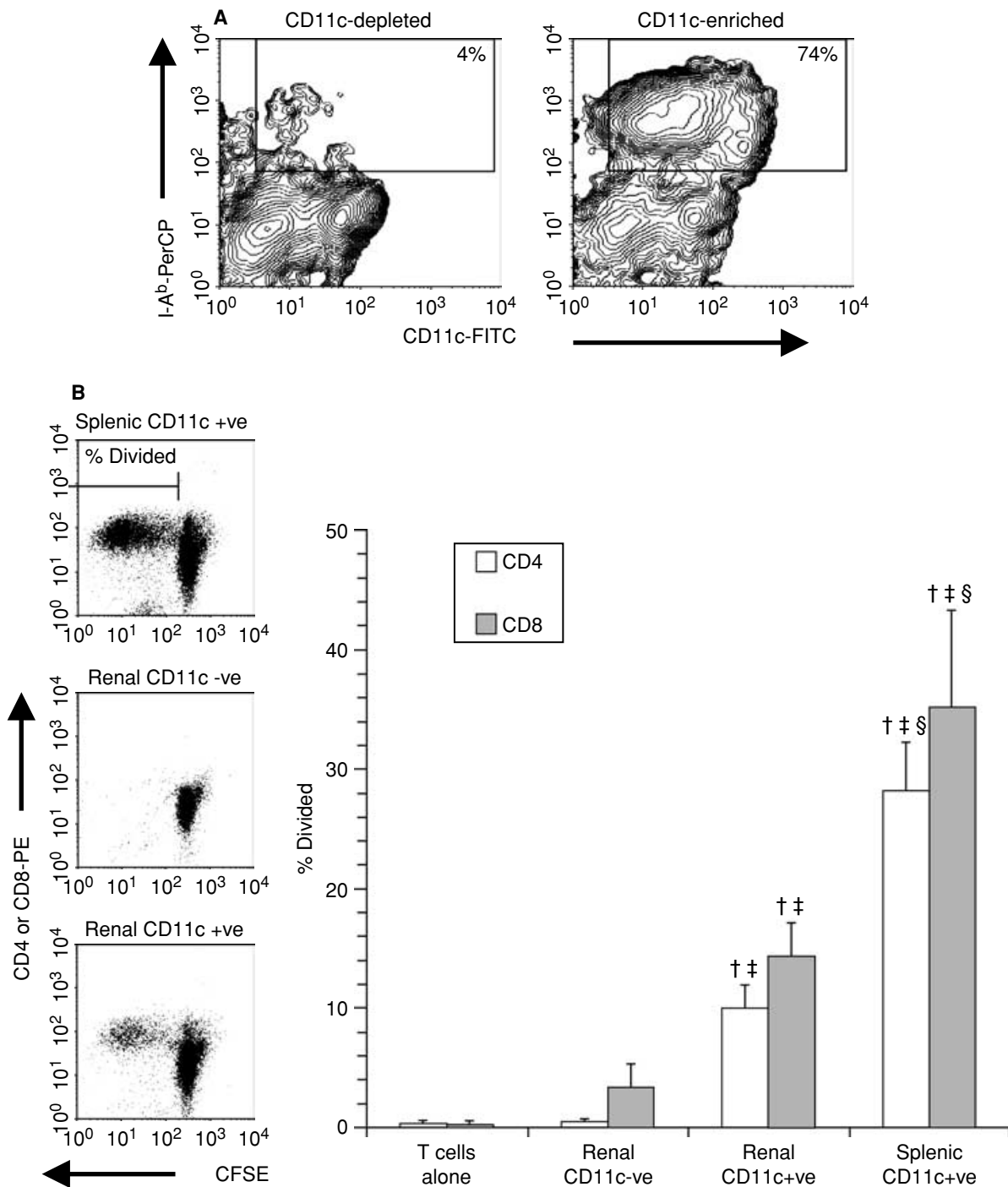
Fig. 1. Analysis of the T-cell stimulatory capacity of CD11c-depleted and CD11c-enriched cell populations from the kidney and comparison with splenic CD11c-enriched cells. Cell suspensions from kidney were separated into CD11c-depleted (CD11c^{-ve}) and CD11c-enriched (CD11c^{+ve}) fractions using anti-CD11c-coated microbeads and magnetic separation. (*A*) Restaining of the separated cells for MHC II (I-A^b) and CD11c demonstrated clear enrichment for double-positive cells in the CD11c^{+ve} (74%) compared with CD11c^{-ve} (4%) fractions. (*B*) Coculture of CD11c^{+ve} and CD11c^{-ve} kidney cells as well as CD11c^{+ve} splenic cells with CFSE-labeled fully allogeneic T-cells for 72 hours followed by flow cytometric analysis to separately detect cell division (reduced CFSE fluorescence) among the CD4^{+ve} and CD8^{+ve} T-cells (left panel). Cell division was quantified as the proportion of the total CD4^{+ve} or CD8^{+ve} cells that had undergone cell division (% divided) and was expressed graphically as mean ± SD for triplicate samples of each coculture condition (right panel). †$P < 0.05$ compared to T cells alone; ‡$P < 0.05$ compared to renal CD11c^{-ve}.

examined. Flow cytometric analysis of individual spleen and kidney cell suspensions was carried out 1 hour after injection of PBS alone, FITC-dextran 40,000, or FITC-dextran 500,000 with gating on CD11c^{+ve} cells (Fig. 2). Uptake of FITC-dextran by DCs and other cell populations could be readily detected by increased relative fluorescence (Fig. 2A). When expressed quantitatively (Fig. 2B), the results demonstrated ingestion of both filtered and unfiltered particles by rDCs. The fluorescence shift of rDCs was higher than that of splenic DCs for both FITC-dextran preparations, suggesting a relatively greater phagocytic capacity.

A systemic proinflammatory stimulus results in migration of DCs from the renal interstitium to draining lymph nodes and in DC-mediated transfer of a kidney-specific protein

Dendritic cells were visualized in situ by immunohistochemical staining of mouse kidneys and renal lymph nodes (RLNs) at different time points following intravenous administration of LPS (Fig. 3). Interstitial MHC II^{+ve} cells, morphologically consistent with DCs, were readily detected within the renal cortex at baseline and at 4 hours after LPS injection. At 24 hours, MHC II^{+ve} cells were substantially reduced in number. By 48 hours, the interstitium was essentially devoid of DCs. In RLNs at baseline and 4 hours, CD11c^{+ve} cells were present at moderate density in the medullary (T-cell) zone. At 24 hours and 48 hours, there was an increase in CD11c^{+ve} cells such that confluent staining of the RLN medulla was observed. Identical results were obtained from 5 animals.

The latter results suggested the potential for a systemic inflammatory stimulus to alter the trafficking of endogenous antigens from the kidney to the T-cell compartment of draining lymphoid tissue. In order to formally test this hypothesis, RLNs, removed 48 hours after treatment with PBS or LPS, were double-stained by immunoperoxidase for CD11c and for the kidney-restricted glycoprotein THP [28]. Rare cells with positive immunostaining for both CD11c and THP were detected within multiple renal lymph node sections from LPS-treated but not PBS-treated animals (Fig. 4A). In separate experiments, immunofluorescence staining for CD11c and THP was employed to definitively localize THP^{+ve} material to the cytoplasmic region of CD11c^{+ve} cells in the RLNs at 48 hours and 7 days post-LPS injection (Fig. 4B). Individual CD11c^{+ve} cells were detected at 48 hours containing discrete intracytoplasmic bodies that were strongly positive for THP. A total of 20 such cells were detected within 497 sections of RLNs from 9 LPS-treated animals. No CD11c^{+ve}/THP^{+ve} cells were detected in RLNs of PBS-treated animals or in nonrenal lymph nodes of LPS-treated animals. Seven days after LPS injection, THP-containing DCs could still be detected in RLNs, indicating an ongoing trafficking process.

Dendritic cell-mediated proliferation of antigen-specific T-cells in the renal lymph nodes is augmented by a systemic proinflammatory stimulus

An in vivo model system was derived to examine RLN T-cell activation in response to protein antigen localized to the kidney. Ovalbumin (OVA) solution was introduced beneath the left kidney capsule of mice containing CFSE-labeled, adoptively transferred TCR transgenic CD4^{+ve} T-cells with specificity for a MHC II-restricted OVA-derived peptide (DO11.10 T-cells). Shortly after OVA introduction, groups of mice received either PBS or LPS solution intravenously. Three days later, relative expansion and cell division profiles of DO11.10 T-cells were examined by flow cytometry in the left and right RLNs, mesenteric lymph nodes (MLNs), subcutaneous lymph nodes (SLNs), and spleens of the two groups (Fig. 5). The results demonstrated that, for both control and LPS-treated animals, a localized expansion of DO11.10 T-cells occurred in the left renal node. Analysis of cell division profiles (based on CFSE dilution of DO11.10 T-cells at each site) further demonstrated that LPS-treated animals exhibited accelerated progression of T-cell proliferation in the left RLN (as reflected in a significantly higher proportion of the cells that had undergone more than 5 cell divisions at the time of analysis). It was also evident that some DO11.10 T-cell proliferation occurred in the right RLN as well as at other lymphoid sites, likely due to low-level dissemination of OVA itself or of left kidney–derived DCs. LPS-associated acceleration of DO11.10 T-cell proliferation occurred within the right RLN and, to a lesser degree, within SLNs, but not within MLNs or spleen.

The predominant role of DCs in processing and presenting OVA-derived peptides in this model system was confirmed by examining the ability of unsorted, CD11c-depleted, and CD11c-enriched cell populations to induce in vitro proliferation of resting DO11.10 T-cells 24 hours after introduction of OVA beneath the left kidney capsule (Fig. 6). Cell populations from left RLNs, right RLNs, and SLNs were cocultured with CFSE-labeled DO11.10 T-cells for 72 hours, after which cell division was examined by flow cytometry. As shown, unsorted and CD11c-enriched cells from the left RLN of PBS-treated mice stimulated DO11.10 T-cell proliferation, while the CD11c-depleted fraction did not (Fig. 6A). The relative potency of the CD11c-enriched fraction is highlighted by the fact that the number of stimulator cells used for this fraction was reduced to 20% of that used for unsorted and CD11c-depleted populations. For PBS-treated animals, T-cell stimulatory capacity was absent in all cell fractions from right RLN and SLNs. In a group of animals
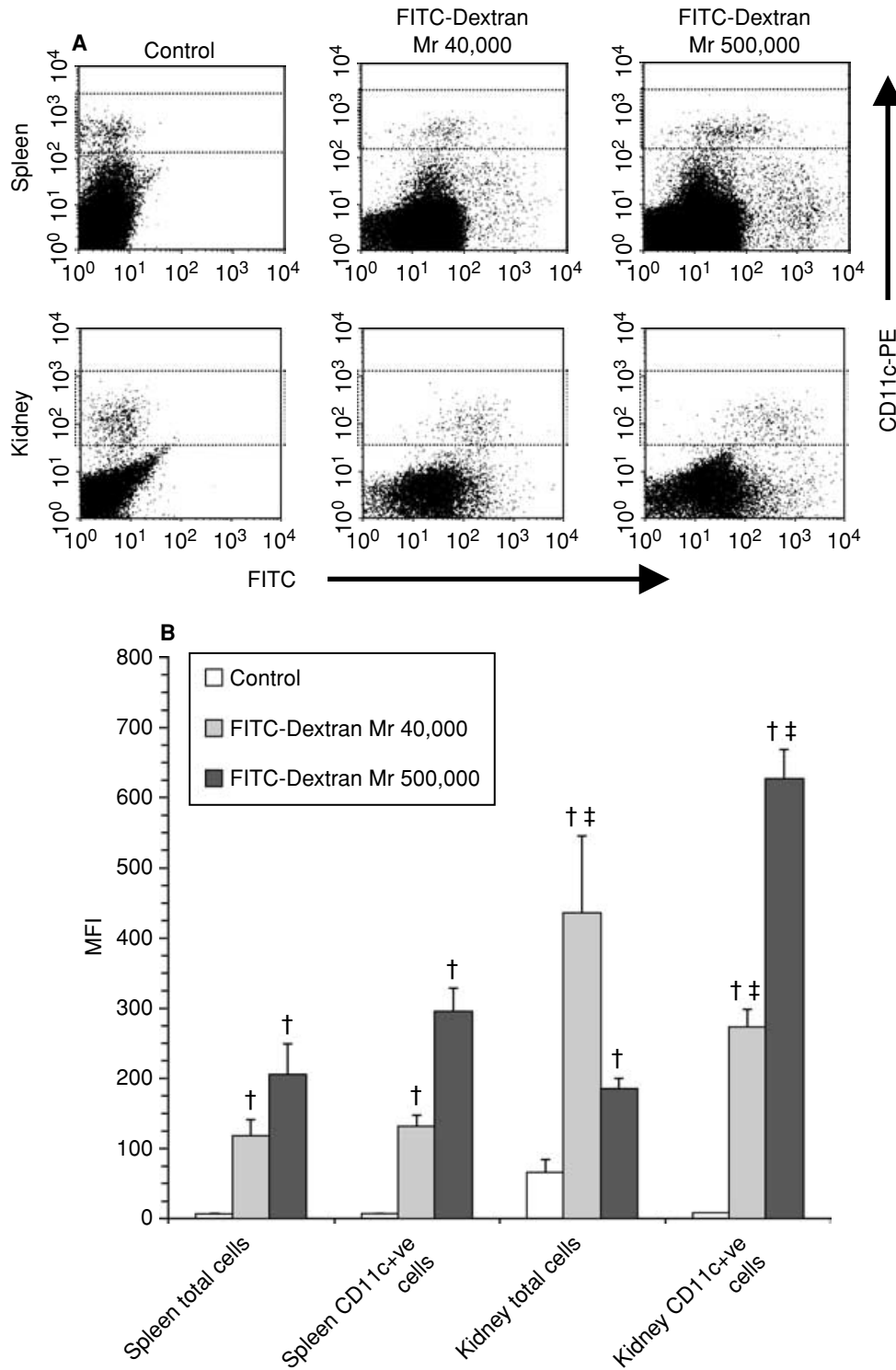
Fig. 2. Uptake of FITC-dextran particles by renal and splenic DCs. Cell suspensions were prepared from kidneys and spleens of groups of 3 healthy adult mice 1 hour after administration of PBS ("Control"), FITC-dextran particles that pass through the glomerular filtration barrier and into the urinary space (FITC-dextran Mr 40,000), and FITC-dextran particles that are precluded from the urinary space (FITC-dextran Mr 500,000). Following surface staining with anti-CD11c-PE, flow cytometry was carried out to examine uptake of FITC by DCs (CD11c+ve cells) and all other cells (total cells). Examples of dot-plots for spleen and kidney from each group are shown (upper panel, DCs are indicated by boxes). Graphic representation of the results is also shown (lower panel). The presence of fluorescent material within total and CD11c^{+ve} cell populations was expressed quantitatively for each cell population as mean $\pm$ SD of the mean fluorescence intensity (MFI) on the FL-1 channel. $\dagger P < 0.05$ compared with control group, $\ddagger P < 0.05$ for kidney compared with spleen cells.
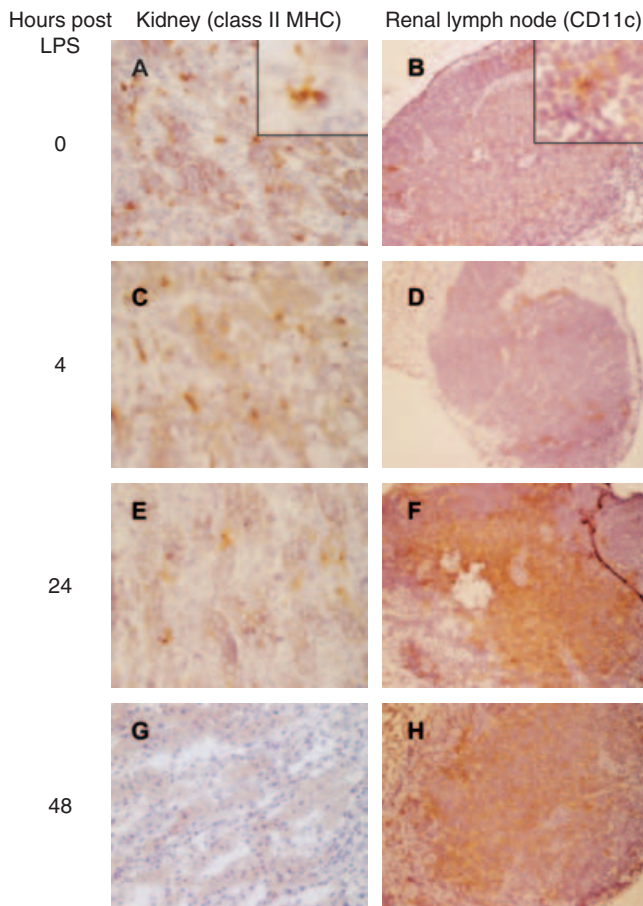
Fig. 3. Immunoperoxidase staining for DCs in kidney and renal lymph nodes of mice at various time-points following intravenous injection of LPS. Examples are shown of immunoperoxidase staining for DCs in the kidney (anti-MHC II) and renal lymph nodes (anti-CD11c) of mice before ("0," panels *A* and *B*), and 4, 24, or 48 hours after intravenous injection of LPS (panels *C* to *H*). For both organs, DCs are identified at 0 and 4 hours by strong staining of individual cells (high-power views of single cells shown in inserts to A and B). At 24 and 48 hours, a large increase of DCs numbers within renal lymph nodes is indicated by virtually confluent staining of the central (medullary) zone (panels F and H). At these same time-points, MHC II–positive cells in the kidney interstitium are greatly reduced in frequency (24 hours, panel E) or absent (48 hours, panel G).

treated with LPS shortly after OVA introduction (Fig. 6B), DO11.10 T-cell proliferation was induced by CD11c-enriched fractions from right RLNs and SLNs also, again suggesting an effect of the systemic proinflammatory stimulus to cause dissemination of antigen or antigen-containing DCs beyond the site of local antigen uptake.

Renal ischemia is associated with augmented antigen-specific T-cell activation that is localized to the renal lymph nodes and mediated by DCs

The influence of renal ischemia on DC-mediated antigen-specific T-cell activation within RLNs was next determined. Groups of mice were subjected to unilateral (left) renal ischemia-reperfusion (IR) or to sham
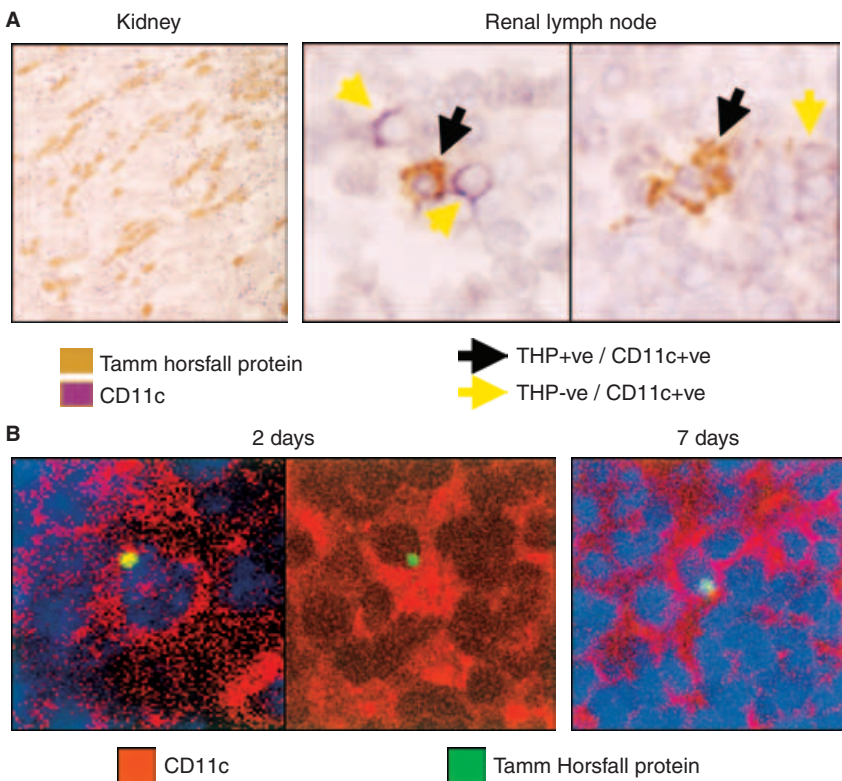

Fig. 4. Detection of DCs containing Tamm-Horsfall protein (THP) in renal lymph nodes following intravenous injection of LPS. (*A*) Left panel: Immunoperoxidase staining of normal adult mouse kidney for THP demonstrated the expected restriction of staining to distal tubular epithelium. Right panel: Examples of 2-color immunohistochemical staining of sections of renal lymph nodes from adult mice that had received LPS 48 hours previously. Sections were double-stained for CD11c (mauve) and THP (brown). Individual cells that clearly stained positive for both CD11c and THP are shown. (*B*) Examples of immunofluorescent staining for CD11c (red) and THP (green) of renal lymph node sections from adult mice that had received LPS 48 hours (left two panels) or 7 days (right panel) previously. Nuclei are indicated by DAPI (blue) staining. Individual CD11c[+ve] cells contained THP-positive intracytoplasmic bodies are shown.
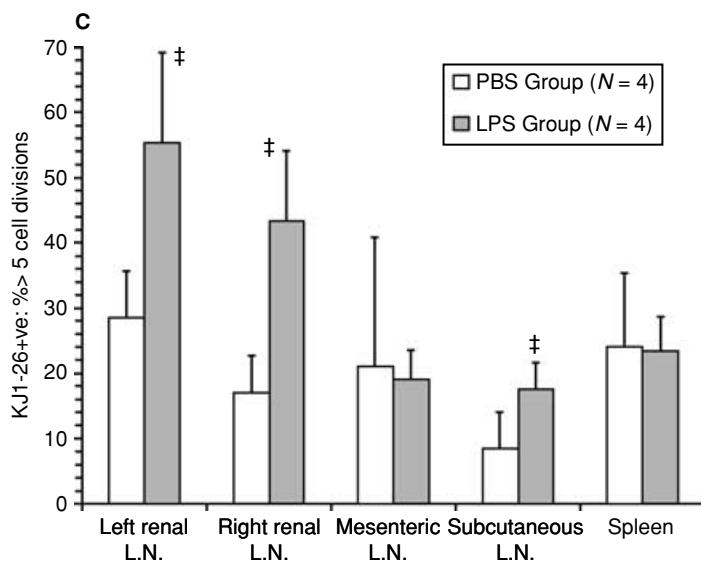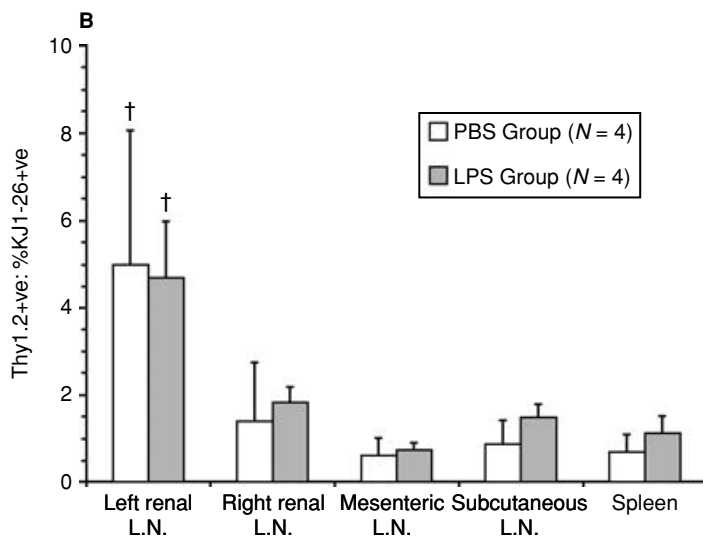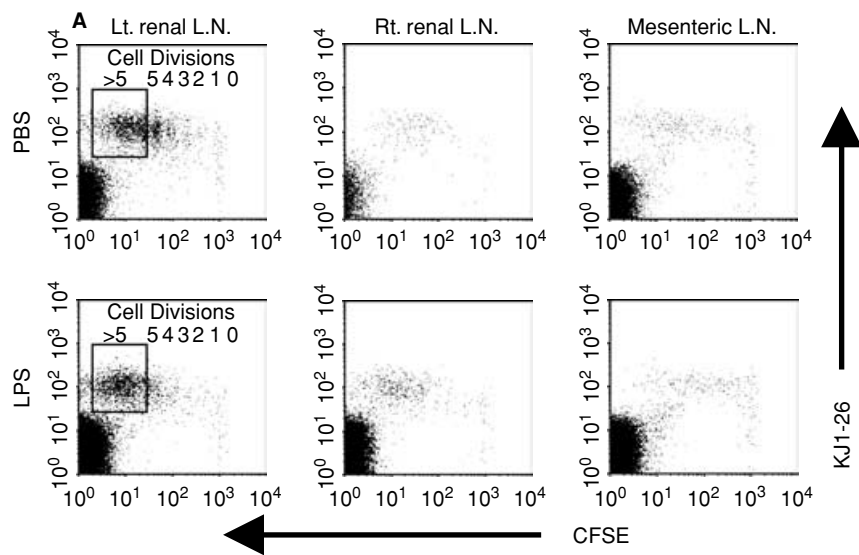
Fig. 5. Proliferation of antigen-specific T-cells in the renal lymph nodes following subcapsular inoculation of ovalbumin and augmentation by systemic administration of LPS. (*A*) Examples of flow cytometric analysis of adoptively transferred, CFSE-labeled DO11.10 T-cells from left and right renal lymph nodes (L.N.) and mesenteric L.N. of mice at 72 hours' post-inoculation of ovalbumin beneath the left renal capsule followed by subsequent intraperitoneal injection of PBS (upper dot plots) or LPS (lower dot plots). Dot plots are gated on Thy1.2^{+ve} cells and demonstrate CFSE dilution of DO11.10 (KJ1-26^{+ve}) T-cells. Adoptively transferred T-cells that had undergone 6 or more cell divisions are indicated in boxes (left renal L.N. samples only). (*B*) Relative expansion of adoptively transferred DO11.10 T-cells within individual left and right renal, mesenteric, and subcutaneous L.N.s and spleens of PBS and LPS-treated mice at 72 hours' post-inoculation of ovalbumin beneath the left renal capsule. Results are expressed as mean ± SD of the proportion of total T-cells (Thy1.2^{+ve}) that were also positive for the DO11.10 TCR (KJ1-26^{+ve}). (*C*) The proportion of adoptively transferred DO11.10 (Thy1.2^{+ve}/KJ1-26^{+ve}) T-cells that had undergone more than 5 cell divisions is shown graphically for each lymphoid organ from PBS- and LPS-treated mice. †$P < 0.05$ for left RLN compared with right RLN, MLN, SLN, and spleen; ‡$P < 0.05$ for LPS group compared with PBS group.
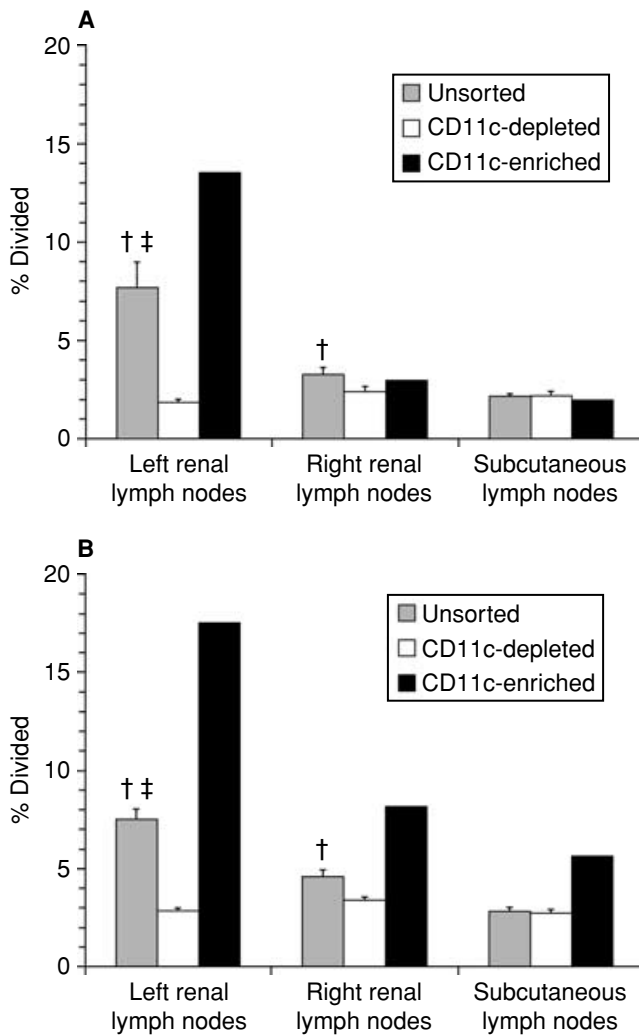
Fig. 6. Presentation of ovalbumin-derived peptide by renal lymph node cells following subcapsular inoculation of ovalbumin is restricted to the CD11c^{+ve} (DC) fraction. In vitro proliferation of CFSE-labeled DO11.10 T-cells is shown graphically following 96 hour coculture with unsorted, CD11c-depleted, and CD11c-enriched stimulator populations derived from the pooled left and right renal and subcutaneous lymph nodes of groups of mice inoculated, 24 hours previously, with ovalbumin beneath the left renal capsule. Results are shown for groups additionally inoculated intraperitoneally with PBS (*A*) or LPS (*B*). For each stimulator population, proliferation was expressed as mean ± SD of the proportion of DO11.10 T-cells that had undergone cell division ("% divided"—derived by flow cytometric analysis of replicate samples for CFSE dilution of CD4^{+ve}/KJ1-26^{+ve} cells). †$P < 0.05$ compared to CD11c-depleted; ‡$P < 0.05$ compared to right RLN and SLNs.
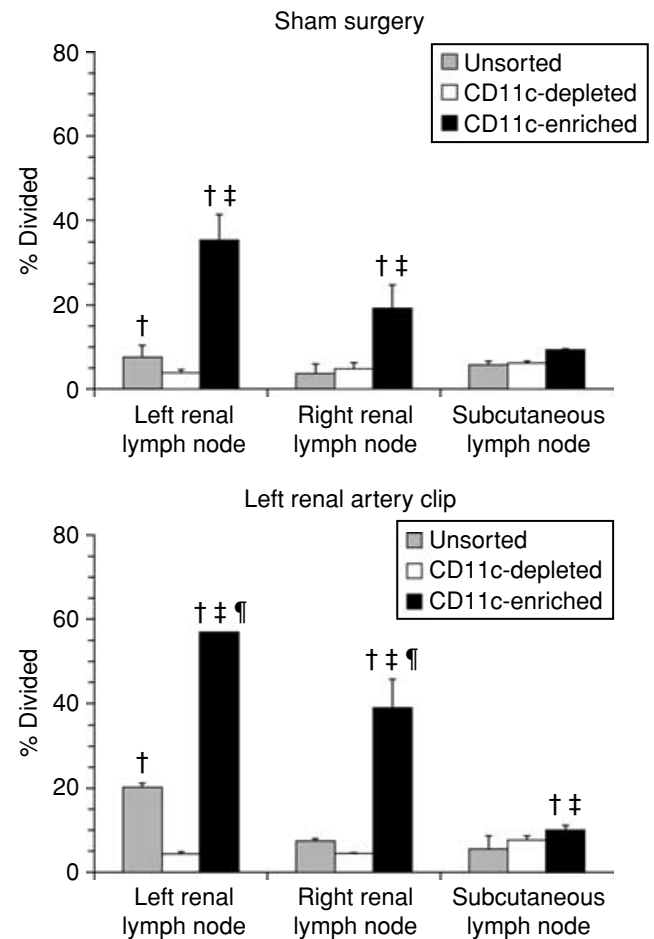

Fig. 7. Renal ischemia-reperfusion augments the capacity for DC-mediated stimulation of antigen-specific T-cells within draining and contralateral renal lymph nodes. In vitro proliferation of CFSE-labeled DO11.10 T-cells is shown graphically following 96 hour coculture with unsorted, CD11c-depleted, and CD11c-enriched stimulator populations derived from the pooled left and right renal and subcutaneous lymph nodes of groups of mice ($N = 5$) subjected, 24 hours previously, to sham surgical procedure (*A*) or to left renal artery clipping for 22.5 minutes (*B*). For each stimulator population, proliferation is expressed as mean ± SD of the proportion of DO11.10 T-cells that had undergone cell division ("% divided"—derived by flow cytometric analysis of replicate samples for CFSE dilution of CD4^{+ve}/KJ1-26^{+ve} cells). †$P < 0.05$ compared to CD11c-depleted; ‡$P < 0.05$ compared to unsorted; ¶$P < 0.05$ compared to corresponding sample from sham surgery group.

surgery (control). Ovalbumin was introduced beneath the left renal capsule in both groups at the time of surgery. Twenty-four hours later, the capacity of unsorted, CD11c-enriched, and CD11c-depleted cell populations from left RLNs, right RLNs, and SLNs to induce in vitro proliferation of resting CFSE-labeled DO11.10 T-cells was examined (Fig. 7). As for previous experiments, stimulator cell numbers for CD11c-enriched fractions were reduced to 20% of those for unsorted and CD11c-depleted fractions. For both IR and control groups, un-

sorted and CD11c-enriched cells from the left RLNs as well as CD11c-enriched cells from the right RLNs stimulated DO11.10 T-cell proliferation. In both groups, the CD11c-enriched fraction induced significantly greater T-cell proliferation compared to unsorted cells, while the CD11c-depleted fractions were devoid of T-cell stimulatory capacity (Fig. 7A and B). Comparison of results for IR and control groups demonstrated that the CD11c-enriched fraction from left RLNs of mice subjected to IR induced higher levels of DO11.10 T-cell proliferation than the corresponding fraction from the control group (Fig. 7A and B). The CD11c-enriched fraction from the right RLNs of the IR group was also associated with

increased T-cell proliferation compared to the corresponding control group fraction. Finally, for SLNs, a modest increase in T-cell proliferation occurred following coculture with CD11c-enriched cells compared with unsorted and CD11c-depleted fractions from the IR but not the control group. Comparable findings were observed in multiple similar experiments. The results were interpreted as indicating that unilateral renal ischemia-reperfusion is associated with: (*1*) increased capacity for DC-mediated activation of antigen-specific T-cells within lymph nodes draining the ischemic kidney; and (*2*) a concomitant increase in DC-mediated antigen presentation within the contralateral, nonischemic kidney and, to a lesser extent, within distant lymphoid tissue.

DISCUSSION

To the best of our knowledge, studies to date have not explored the functional significance of DCs derived from the kidney following localized ischemic insults or systemic inflammation induced by LPS. As such, our present analysis breaks new ground, providing novel insights into the interplay between the immune system and acute renal injury in general, and the involvement of DCs in such interplay in particular. By focusing on DCs from the kidney and its draining lymph nodes, we demonstrate that this population of cells has a broad capacity to ingest material in the renal vascular and interstitial compartments, and is primarily responsible for presenting peptides derived from renal proteins to antigen-specific CD4^{+ve} T-cells within RLNs. Furthermore, in vivo model systems confirm that, in the context of systemic and localized proinflammatory states, DCs specifically mediate abnormal transfer of renal proteins to the RLNs and enhancement of T-cell proliferation.

Dendritic cells within the kidney were first characterized in the course of ultrastructural studies of the peritubular interstitium rather than by their immunologic properties. Renal DCs were defined on the basis of MHC II expression as well as the presence of multiple finger-like processes abutting the tubular basement membrane [13–15], and were shown to be abundant within the cortex and outer medulla [11–15]. Based on this anatomic location it might be speculated that rDCs primarily acquire antigenic material from reabsorbed glomerular filtrate or derived directly from tubular epithelial cells and interstitial fibroblasts. Nevertheless, in the normal kidney, we demonstrated equally efficient uptake by rDCs of filtered and nonfiltered dextran particles. Thus, DCs within the interstitial compartment of the kidney possesses the capacity for uptake of endogenous as well as exogenous antigens from all compartments of the normal kidney, and for delivery of high loads of antigenic peptides to lymphocytes within draining lymphoid tissue. Counterbalancing this high capacity for antigen presentation, DCs in the healthy kidney are of relatively low maturity [26] and, compared with DCs from lymphoid organs [27], undergo low, steady state turnover (<20% over 2 weeks by in vivo bromodeoxyuridine labeling—Matthew D. Griffin, unpublished observation). The capacity of a systemic proinflammatory stimulus to substantially accelerate the rate of transit of DCs from the kidney to regional lymph nodes was highlighted by a large increase in DC density within the T-cell compartment of RLNs within 48 hours of LPS injection, accompanied by virtual disappearance of DCs from the renal parenchyma [12]. Taken together, these observations confirm the potential for rDCs to broadly and continuously sample the microenvironment of the renal interstitium and to be directly influenced by proinflammatory stimuli.

Recent studies have revealed that DC-mediated trafficking of endogenous antigens from individual organs to draining lymph nodes represents an important mechanism for maintenance of immune tolerance to peripheral antigens [7, 29–33]. In these studies, DCs containing tissue-specific proteins have been directly visualized within afferent lymph or draining lymph nodes [29, 30]. Interestingly, Scheinecker et al have recently shown that the frequency within gastric lymph nodes of DCs containing a gastric epithelial antigen was greatly increased during autoimmune gastritis [34]. Thus, alterations in cycling of self-antigens to lymph tissue may represent a key event in organ-specific autoimmunity. In this context, the detection of DCs containing THP in RLNs shortly after an inoculum of LPS indicates that altered trafficking of kidney-specific proteins occurs as an early consequence of renal injury. The fact that THP-positive DCs were not detected in RLNs of control animals may indicate that, in contrast to mucosal organs, trafficking of endogenous proteins from the kidney does not occur during health. Alternatively, it is possible that low-level trafficking from the kidney occurs and is involved in maintenance of tolerance to kidney-restricted antigens. The latter possibility is supported by the results of Kurts et al in transgenic mice expressing ovalbumin in pancreatic islets and renal proximal tubular epithelium [35, 36]. In this report, adoptive transfer of ovalbumin-specific T-cells was associated with proliferation of the T-cells specifically within pancreatic and renal lymph nodes, indicating that antigen from the kidney had been processed and presented by an APC population (most likely DCs). Using a model system in which ovalbumin was introduced beneath the kidney capsule, we show here definitively, that productive, DC-mediated presentation of MHC II/peptide complex to CD4^{+ve} T-cells occurs in localized fashion in the draining RLN. Because the experimental system necessitated manipulation of the kidney, it cannot be concluded that the degree of localized T-cell expansion observed in non-LPS–treated animals represents the product of steady state DC kinetics. Nonetheless, the observed

effect of LPS to increase the rate of ovalbumin-specific T-cell division, together with the previously demonstrated increase in DC trafficking and antigen transfer, indicate that DC-induced activation of CD4^{+ve} T-cells in RLNs is augmented in the context of acute sepsis/endotoxinemia. Additional studies with transgenic model antigen systems may further elucidate the role of rDC-mediated transfer of endogenous renal antigens in the pathogenesis of acute tubular injury.

T-cells mediate renal parenchymal injury in a variety of disorders for which "foreign" antigenic stimuli (infectious organisms, medications, toxins) are absent. Examples include ischemia-reperfusion (IR), partial nephrectomy, and hereditary nephritis [16, 18, 20, 21, 23–25, 37–43]. Ischemia-reperfusion represents the most common cause of acute renal failure and is associated with high mortality in hospitalized patients. Many recent studies have demonstrated the important role of mononuclear cells in the pathogenesis of acute renal failure due to ischemic injury. In experimental models of IR, monocyte/macrophages and T-cells accumulate at the outer strip of medulla, in the absence of a simultaneous increase in polymorphonuclear leukocytes, within 24 hours of injury [17, 22, 23]. A specific pathogenic role for CD4^{+ve} T-cells has been demonstrated by several experimental approaches. The use of mice genetically lacking CD4^{+ve} T-cell populations, depletion of CD4^{+ve} T-cells before induction of ischemia, and administration of immunosuppressive agents or costimulatory blockade are all associated with attenuation of renal injury, even though innate immune mechanisms remained intact [16, 21, 23–25, 37–41]. The extension of our experiments to a unilateral renal artery clip model in the mouse provides clear evidence that acute ischemia of the kidney is also associated with abnormal DC-mediated presentation of antigen to CD4^{+ve} T-cells in the RLNs. The finding that CD11c-depleted cells from the RLN draining the ischemic kidney remained incapable of initiating antigen-specific CD4^{+ve} T-cell proliferation suggests that other MHC II–expressing APC populations (macrophages, B-cells) do not participate in early T-cell activation following IR. This increase in DC antigen-presenting capacity occurred within 24 hours of the ischemic episode and, while clearly most evident in the lymph node draining the ischemic kidney, was also observed within the contralateral RLN. The latter observation is consistent with an immunologic component to the contralateral renal dysfunction reported by several groups following experimental unilateral renal ischemia [44, 45].

CONCLUSION

We suggest that our present findings are significant from several perspectives. First, they provide, as best we can tell from the present literature, the first evidence that

speaks directly to the involvement of DCs in relaying information from the kidney injured by localized (renal ischemia) or systemic (LPS) insults to the immune system. Second, they provide the first evidence incriminating DCs as critical mediators of T-cell activation, which is known to occur following renal ischemia. Third, they provide a hitherto unrecognized mechanism (namely a DC-dependent pathway) whereby a localized insult to the kidney, such as that induced by regional ischemia, initiates a systemic inflammatory process with adverse effects on such vital organs as the heart and lungs. Fourth, they provide a mechanism whereby injury inflicted on one kidney evokes inflammatory responses in the contralateral kidney. The results suggest that interventions targeting DC antigen uptake, DC trafficking, or DC/T-cell interactions merit further evaluation in the prevention or attenuation of acute renal failure of diverse causes.

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