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Critical Role for CD103+CD8+ Effectors in Promoting Tubular Injury following Allogeneic Renal Transplantation

Rongwen Yuan,* Riham El-Asady,† Kechang Liu,‡ Donghua Wang,* Cinthia B. Drachenberg,‡ and Gregg A. Hadley1,*‡

Immune destruction of the graft renal tubules is an important barrier to the long-term success of clinical renal allografts, and the underlying mechanisms remain obscure. CD103—an integrin conferring specificity for the epithelial cell-restricted ligand, E-cadherin—defines a subset of CD8 effectors that infiltrate the graft renal epithelium during clinical rejection episodes, predicting a causal role for CD103+CD8+ effectors in tubular injury. In the present study, we used rodent transplant models to directly test this hypothesis. Surprisingly, CD8 cells infiltrating renal allografts undergoing unmodulated acute rejection did not express significant levels of CD103. However, we demonstrate that a brief course of cyclosporine A to rat renal allograft recipients promotes progressive accumulation of CD103+CD8+ cells within the graft, concomitant with the development of tubular atrophy and interstitial fibrosis. As in the known clinical scenario, graft-associated CD103+CD8+ cells exhibited a T effector phenotype and were intimately associated with the renal tubular epithelium. Treatment with anti-CD103 mAb dramatically attenuated CD8 infiltration into the renal tubules and tubular injury. Mouse studies documented that CD103 expression is required for efficient destruction of the graft renal tubules by CD8 effectors directed to donor MHC I alloantigens. Taken together, these data document a causal role for CD103+CD8+ effectors in promoting tubular injury following allogeneic renal transplantation and identify novel targets for therapeutic intervention in this important clinical problem. The Journal of Immunology, 2005, 175: 2868–2879.

The infiltration of CD8+TCRαβ+ T effector populations (CD8 effectors) into the graft renal tubular epithelium has long been recognized as a cardinal feature of clinical renal allograft rejection (1, 2). Although the afferent phase of the anti-graft immune response is increasingly well-defined, the efferent pathways by which donor-specific CD8 effector populations access and ultimately destroy graft functional elements such as the renal tubules (rejection per se) remain poorly defined. This is an urgent problem in clinical renal transplantation because it is now clear that immune attack of the graft renal tubules, even that occurring subclinically, predisposes the graft toward development of tubular atrophy and interstitial fibrosis (3), which, with recent improvements in early graft survival, have emerged as the dominant lesions of late graft loss (4). In this regard, it is important to note that current immunosuppressive strategies effectively prevent acute rejection of clinical renal transplants but have proven ineffectual in preventing tubular injury and late graft loss (5).

We have reported previously that the integrin heterodimer αE/β7 (CD103)/β7, (herein referred to as CD103) defines a subset of CD8 effectors elicited in response to renal allografts (6, 7). Such expression is pertinent to mechanisms of allograft injury to the graft renal tubules because the principal ligand of CD103 is E-cadherin (8, 9), an epithelial cell-restricted marker highly and selectively expressed by cells comprising the renal tubular epithelium (10, 11). Moreover, gene knockout experiments document that CD103 expression is required for destruction of cellular epithelial allografts (pancreatic islets) by CD8+ T cells (12). Clinical observational studies indicate that CD8 effector populations expressing CD103 accumulate within the graft renal tubular epithelium at the time of graft dysfunction (13–15), leading to speculation that CD103+CD8+ effectors play a central role in promoting tubular injury following allogeneic renal transplantation. The goal of the present study was to directly test this hypothesis.

Progress in understanding immune mechanisms of tubular injury following allogeneic renal transplantation has long been hampered by the lack of models that reproduce this key aspect of renal allograft rejection. In the mouse, even fully MHC-disparate renal allografts are spontaneously accepted (16–19), and graft infiltrating CD8 T cells undergo an aberrant down-modulation of TCR-αβ expression (20). In the rat, renal allografts typically undergo rapid rejection, but the histopathology and cellular mechanisms of rejection are distinct from the clinical situation with an aberrant predominance of CD4+ T cells and Ab in the absence of cardinal features of clinical renal allograft rejection such as tubular inflammation and injury (21).

Therefore, in the present study, we initially developed rodent models of renal allograft rejection that reproduce the known clinical scenario, i.e., accumulation of CD103+CD8+ effectors within the graft concomitant with development of tubular inflammation and injury. We then used these models to assess the role of CD103+CD8+ effectors in promoting tubular injury. We use a rat model of renal allograft rejection to demonstrate that accumulation of CD103+CD8+ effectors in the graft renal tubules correlates with development of tubular atrophy and interstitial fibrosis and that anti-CD103 mAb significantly attenuates tubular inflammation and injury. We use parallel studies in the mouse with TCR transgenic and gene knockout strains to document that that CD103 expression is required for efficient destruction of the graft renal tubules by CD8 effector populations with specificity for donor MHC I alloantigens. Taken together, these data document a critical role for CD103+CD8+ effectors in promoting tubular injury following
allogeneic renal transplantation. The potential for CD103 blockade as a novel therapeutic concept to test late outcome of clinical renal allografts is discussed.

Materials and Methods

Animals

Inbred male Brown Norway (BN) rats (200–250 g) (RT1a) and Lewis (LEW) (RT1b) rats (200–250 g) were supplied by Harlan Sprague Dawley. C57BL/6 (B6), BALB/c, and B6.CB17-Prkdcscid/SzJ (B6.scid) mice were purchased from The Jackson Laboratory. 2C transgenic mice (22) originally obtained from Dr. T. Hansen (Washington University School of Medicine, St. Louis, MO) were maintained by backcrossing heterozygous 2C males to C57BL/6 females and screening the offspring for expression of the clonotypic 2C TCR by FACS of peripheral blood using 1B2 mAb. 2C mice on a CD103−/− background were generated by crossing 2C (H-2b) mice with CD103−/− mice on the C57BL6 (H-2b) background (C57BL/6 129S2-F1 generation) to C57BL/6.129S2-C57BL/6 mice to generate offspring, which expressed the 2C TCR transgene as determined by FACS analyses using mAb 1B2 directed to the clonotypic 2C TCR, and were homozygous for the CD103−/− mutation as determined by PCR reactivity. All rats and mice were maintained under specific pathogen-free conditions in the animal facility at the University of Maryland. All rodent studies described herein were reviewed and approved by the Institutional Animal Care and Use Committee at the University of Maryland Baltimore.

Antibodies

For immunohistochemistry, mAbs to rat CD103 (OX62) (24), rat CD8, E-cadherin, and mouse CD8 were purchased from BD Pharmingen. For FACS, FITC-conjugated OX-62 was purchased from Serotec; biotin-conjugated mAbs to rat CD11a and mouse IgG1 were purchased from Caltag Laboratories; and conjugated mAbs to rat CD6a, rat αβTCR, mouse CD8, mouse CD103, and isotype-matched controls were purchased from BD Pharmingen. 1B2 mAb (anti-clonotypic 2C TCR; mouse IgG1) (25) was used as hybridoma culture supernatant in combination with anti-mouse IgG1-biotin and streptavidin-FITC (BD Pharmingen).

Rat renal and cardiac transplant models

Rat kidney and cardiac transplants from BN to LEW were performed under halothane (Halocarbon Products) inhalation anesthesia. For rat renal transplantation, the left donor kidney, ureter, and patch of the bladder were isolated and removed en bloc, then flushed with 100 U/ml heparin saline solution. After removal of recipient left kidney, the artery and vein of the renal allograft were anastomosed end to end with the same vessels of recipient. The bladder patch of allograft was anastomosed to the bladder of recipient. The right native kidney of the recipient was removed before closing the abdomen. After transplantation, serum creatinine was measured in blood samples from the tail. Where indicated, rat renal allograft recipients received cyclosporine A (CsA) (Novartis Pharmaceuticals) at 5 mg/kg/day i.m. for 10 days after transplantation. For Ab-blocking experiments, recipients were treated with the anti-rat CD103 mAb, OX-62, from postoperative days 12 through 28 (eight doses of 3.5 mg i.p. every other day). A control cohort (n = 5) received isotype-matched negative control mAb but otherwise was treated identically to the experimental group.

Intra-abdominal heterotopic cardiac transplantation was performed as described previously (26). Briefly, following infusion with cold lactated Ringer solution containing heparin (100 U/ml), the donor heart was harvested and implanted into the abdominal cavity with the aorta and pulmonary artery to the aorta and vena cava of the recipient in an end-to-side anastomosis.

Mouse adoptive transfer model

As recipients for adoptive transfer experiments, B6 scid hosts were transplanted with kidney allografts from BALB/c donors under halothane inhalation anesthesia as described by Zhang et al. (18). In brief, the graft was perfused in situ with 0.5 ml of cold, heparinized saline (100 U/ml). The left donor kidney attached to a segment of the aorta, and the renal vein along with ureter and patch of the bladder were isolated and removed en bloc. The donor aorta segment and renal vein was anastomosed end-to-side with the aorta and inferior vena cava of recipient. The bladder patch of the donor kidney was then anastomosed to the recipient bladder.

At day 7 posttransplantation, B6 scid hosts bearing vascularized BALB/c renal allografts were adoptively transferred with 15 × 106 splenocytes from 2C TCR transgenic mice crossed onto either a wild-type (WT) B6 or CD103−/− B6 backgrounds. Renal allografts were harvested after an additional 7 days for FACS analysis and histopathological evaluation.

Flow cytometry

Graft-infiltrating lymphocytes (GIL) were isolated as described previously (7). Briefly, after removal of the renal capsule, the graft cortex was minced into ~1-mm3 pieces, rinsed to remove contaminating peripheral blood, then incubated for 30 min in DMEM:F12 (50:50) containing 0.1% collagenase (type IV; Worthington Biochemical), 0.1% soybean trypsin inhibitor (Sigma-Aldrich), and 0.01% DNase 1 (Boehringer Mannheim). Following vigorous agitation, the resulting cell suspension was centrifuged on Lympholyte-Rat or Lympholyte-Mouse (Cedarlane Laboratories) to isolate lymphocytes. Lymphocytes from lymph nodes of recipients were isolated by gentle mincing with forceps. Freshly isolated lymphocytes were stained for three- or four-color (rat and mouse studies, respectively) FACS analyses using anti-CD103 and anti-CD8 in conjunction with mAbs to markers of interest. Separate sets of mAbs were used for the rat and mouse systems; appropriate species- and isotype-matched negative control mAbs were included in all experiments. IB2 mAb (anti-clonotypic 2C TCR; mouse IgG1) (25) was used as hybridoma culture supernatant (1:2) followed by anti-mouse IgG1-biotin in combination with streptavidin-fluorochrome (BD Biosciences).

After staining, cells were fixed with 0.5% paraformaldehyde, and 50,000–100,000 events were analyzed using a FACScalibur (BD Biosciences). Lymphocyte populations were gated by forward scatter/side scatter analysis to exclude dead cells and nonlymphocytes. WinMDI version 2.8 developed by Dr. J. Trotter (The Scripps Institute, San Diego, CA) was used for analysis and graphical display of flow cytometry data. Percent-positive cells for a given marker and quadrant settings were based on cutoff points chosen to exclude >99% of the negative control population.

Real-time PCR

Total RNA was isolated from renal cortex of rat allografts using an RNeasy Mini kit and a RNase-Free DNase set (Qiagen). Four hundred nanograms of RNA were reverse transcribed using the Ominiscrypt RT kit (Qiagen). One microliter of cDNA was used as the template to quantitate mRNA content of mRNA by iCycler IQ Real-Time PCR Detection System (Bio-Rad) using respective primers and SYBR Green. The following primers were designed by Primer Express (version 1.5; Applied Biosystems) on the basis of the published sequence of rat TGF-β1 (GenBank accession no. X52498): forward primer, 5′-GCTAATGGTGGACCGCAACAAC-3′; and reverse primer, 5′-ACTGCTTCCTCAGATGTCGAC-3′. PCR were performed with the IQ SYBR Green Supermix (Bio-Rad) on an iCycler IQ (version 3.0; Bio-Rad).

Graft histology and immunohistochemistry

For graft histology, sections were incubated with primary mAb or isotype-matched control mAbs. Separate sets of mAbs were used for the rat and mouse systems; appropriate species- and isotype-matched negative control mAbs were included in all experiments. IB2 mAb (anti-clonotypic 2C TCR; mouse IgG1) (25) was used as hybridoma culture supernatant (1:2) followed by anti-mouse IgG1-biotin in combination with streptavidin-fluorochrome (BD Biosciences). Lymphocyte populations were gated by forward scatter/side scatter analysis to exclude dead cells and nonlymphocytes. WinMDI version 2.8 developed by Dr. J. Trotter (The Scripps Institute, San Diego, CA) was used for analysis and graphical display of flow cytometry data. Percent-positive cells for a given marker and quadrant settings were based on cutoff points chosen to exclude >99% of the negative control population.

2 Abbreviations used in this paper: BN, Brown Norway; LEW, Lewis; GIL, graft-infiltrating lymphocyte; TBM, tubular basement membrane; IEL, intestinal intraepithelial lymphocyte; WT, wild type.
HRP (Vectastain Elite ABC kit; Vector Laboratories) and development with diaminobenzidine. Subsequently, sections were incubated with 3% horse serum, anti-human E-cadherin (10 μg/ml, 4°C overnight), biotinylated horse anti-mouse IgG (H+L) (rat adsorbed, 5 μg/ml; Vector Laboratories), then avidin DH:biotinylated alkaline phosphatase (Vectastain ABC-AP kit; Vector Laboratories), followed by development with Vector Red containing levamisole to inhibit endogenous alkaline phosphatase activity.

To quantitate the degree of tubular atrophy present in renal allografts, slides were stained with Jones-methenamine silver (Sigma-Aldrich). Ten random fields in the upper pole, middle, and lower poles of the cortex were viewed at 2600× to 2000× resolution under 200 magnification using a Zeiss Axioskop microscope fitted with AxioCam digital camera (Carl Zeiss Optical). AxioVision 4.2 software (Carl Zeiss Optical) with an interactive measurement package was used to quantitate the density of tubules with intact TBM.

Statistics

Data are expressed as mean ± SEM. Student’s t test and linear regression analyses were performed using SigmaPlot 2000 software (SPSS). Values of p < 0.05 were considered statistically significant.

Results

CD8 cells infiltrating acutely rejecting renal allografts do not express significant levels of CD103

We initially sought to determine whether CD103 is significantly expressed by CD8 effector populations that infiltrate renal allografts undergoing unmodified acute rejection. In these experiments, fully MHC-disparate kidneys from BN rat donors were transplanted into LEW rat hosts. As shown in Fig. 1A, renal allografts in this strain combination are rapidly rejected in the absence of immunosuppression as evidenced by an abrupt rise in serum creatinine. However, note that rejection in this model is accompanied by severe arteritis and transmural inflammatory infiltration, fibrinoid change, and interstitial hemorrhage (Fig. 1B), graded as severe vascular rejection (type IIIv3), according to the Banff97 classification system (1). Also note the sparse mononuclear cell infiltrate and conspicuous absence of mononuclear cells within the renal tubular epithelium (tubulitis), the sine qua non of cellular renal allograft rejection (Fig. 1B). Moreover, as shown in Fig. 2A, CD8 cells that infiltrated allografts undergoing unmodified acute rejection showed negligible expression of (<5%) of CD103.

Progressive accumulation of CD103+CD8+ effectors in renal allografts undergoing CsA-delayed rejection

Clinical observational studies indicate that CD103+CD8+ effectors are most abundant in renal allografts undergoing late rejection episodes (27). To model this rejection scenario, we administered a brief course of CsA (10 days at 5 mg/kg/day) to LEW recipients of BN allografts. As shown in Fig. 1C, CsA-treated recipients exhibited a brief elevation in serum creatinine levels, which returned to normal and remained stable for >80 days (data not shown). However, note that grafts in CsA-treated recipients undergo significant

FIGURE 1. Characterization of renal allograft rejection models. Kidneys from BN rats were transplanted into fully MHC-mismatched LEW recipients in the absence of immunosuppression (A and B) or with a brief course of CsA at 5 mg/kg/day for 10 days (C–F). A, Time course of serum creatinine levels during unmodified acute rejection; the different symbol/line combinations represent individual rat recipients (n = 4). B, Graft histology of unmodified acute rejection at day 7 posttransplantation (H&E, ×200). Note the severe tubular injury/necrosis with sparse lymphocytic infiltrates and fibrinoid necrosis of arteriole (arrow). C, Time course of serum creatinine levels in the CsA-delayed rejection model; the different symbol/line combinations represent individual rat recipients (n = 14). D, Graft histology in the CsA-delayed rejection model at day 40 (H&E, ×200). Note the prominent tubulointerstitial infiltration of mononuclear cells. E, Graft histology in the CsA-delayed rejection model at day 35 (H&E, ×100). Note focal nature of tubulointerstitial inflammation. F, Graft histology in CsA-delayed rejection model at day 84 (H&E, ×200). Note the tubular dropout and the interstitial fibrosis.
subclinical rejection as evidenced by massive infiltration of mononuclear cells into the graft interstitium accompanied by severe tubulitis (Fig. 1D). Tubulointerstitial inflammation (grade type IB by Banff97 criteria) gradually progressed from focal (Fig. 1E) to diffuse (data not shown), ultimately leading to massive tubular loss and interstitial fibrosis by 12 wk posttransplantation (Fig. 1F).

FACS analyses of lymphocytes infiltrating renal allografts under undergoing CsA-delayed rejection revealed a progressive increase in the frequency of CD103+ CD8+ cells within the graft from 3 through 8 wk posttransplantation (Fig. 2B). Note that ~40% of graft infiltrating CD8+ T cells expressed significant levels of CD103 × 8 wk posttransplantation. As shown in Fig. 3A, left panel, CD103 expression by GIL was strongly biased toward the CD8 subset, a distribution highly similar to that previously described for rejecting clinical renal allografts (27). Thus, this model recapitulates the known clinical scenario in which subclinical tubulointerstitial inflammation in renal allografts is accompanied by accumulation of CD103+ CD8+ cells at the graft site concomitant with development of tubular atrophy and interstitial fibrosis.

Characterization of graft-associated CD103+ CD8+ cells
Renal isografts (LEW-to-LEW) transplanted into hosts receiving a brief course of CsA were virtually devoid of CD103+ CD8+ cells at all time points examined (Figs. 2C and 4A), demonstrating that accumulation of CD103+ CD8+ cells at the site of vascularized renal transplants is alloantigen dependent. CD8 cells isolated from heart allografts transplanted into CsA-treated hosts (BN to LEW) were similarly devoid of CD103 expression (Fig. 2D), suggesting that this phenomenon may be unique to allografts containing epithelial elements.

As shown in Fig. 3A, right panel, CD8 cells in the peripheral lymph nodes of renal allograft recipients did not express significant levels of CD103, suggesting that CD103-expressing cells remain sequestered at the graft site. Fig. 3B shows that graft-associated CD103+ CD8+ cells were TCR-αβ+, CD11ahigh, and CD62Llow and thus exhibited a classic CD8 T effector phenotype.

To determine the localization of CD103+ CD8+ effectors within the graft, double staining for CD103 and its ligand E-cadherin was performed. As shown in Fig. 4A, the number of CD103+ CD8+ effectors in the graft progressively increased from the time of CsA withdrawal through 9 wk, then declined sharply by 10–12 wk posttransplantation. As shown in Fig. 4B, the abundance of CD103+ CD8+ effectors in the graft during weeks 3–9 correlated inversely with the
density of undamaged tubules, i.e., those with intact TBM. In contrast, the total number of CD103^+CD8^+ effectors bore no discernible relationship to progression of tubular injury (data not shown).

We previously documented a key role for TGF-β in regulating CD103 expression by donor-specific CD8^+ effectors that infiltrate mouse renal allografts (7). That these data are relevant to the rat model used in the present study is supported by data showing that TGF-β activity in the graft increases in parallel with accumulation of CD103^+CD8^+ effectors at the site (Fig. 4C).

The above observations were consistent with a causal role for CD103^+CD8^+ effectors in tubular injury. To test this hypothesis, we assessed the capacity of the anti-rat CD103 mAb, OX-62 (mIgG1), to prevent tubular inflammation and destruction of the graft renal tubules. The epitope recognized by OX-62 and its capacity to block the functional activity of CD103 remain uncharacterized (24, 28). However, as shown in Fig. 5, injection of normal rats with OX-62 readily coats CD103^+ intestinal intraepithelial lymphocytes (iIEL)—CD103^+CD8^+ cells of unknown function that reside within the intestinal epithelium of normal vertebrae—as evidenced by its capacity to mask subsequent binding of OX-62 to CD103^+iIEL (Fig. 5A) and by the presence of mIgG1 on the iIEL cell surface in treated but not untreated rats (Fig. 5B). However, note that bound OX-62 has no discernible effect on total numbers of lymphocytes in the iIEL compartment (data not shown) or on iIEL expression of TCR-αβ (Fig. 5C) or the activation markers CD44 (Fig. 5D) and CD11a (Fig. 5E). Similar results were obtained in vitro using CD103^+CD8^+ effectors generated from splenic CD8^+ T cell (data not shown). Thus, it is unlikely that OX-62 has the capacity to deplete or activate CD103^+CD8^+ effectors in vivo.

As shown in Fig. 6A, prophylactic treatment of recipients with OX-62 at days 10–26 dramatically reduced accumulation of CD8^+ T cells in the graft renal tubules as compared with isotype control. Furthermore, OX-62 treatment sharply attenuated tubular injury as
FIGURE 4. The abundance of CD103⁺CD8⁻ effectors in the graft correlates with development of tubular injury. BN renal allografts were transplanted into LEW hosts receiving a brief course of CsA (5 mg/kg/day for 10 days) then harvested at the indicated time points. A, Total number of graft-associated CD103⁺CD8⁻ effectors as a function of time posttransplantation. B, Density of undamaged tubules (defined as tubules with intact basement membranes) as a function of the total number of CD103⁺CD8⁻ effectors in the graft. Data shown are for weeks 3–9 posttransplantation. Numbers shown in the box indicate the sample size (N), correlation coefficient (R), and the statistical error (P) for linear regression analyses. C, Real-time PCR analysis of TGF-β1 mRNA expression in allografts with time posttransplantation. Data shown are the ratios of TGF-β1 mRNA to 18S rRNA. Asterisks show statistically significant (p < 0.01) increases compared with control (nontransplanted LEW kidney).

FIGURE 5. OX-62 is a nondepleting, nonactivating Ab. Normal LEW hosts were untreated (gray peaks) or treated (solid line) with OX-62 mAb (three doses of 3.5 mg i.p. every other day). Two days following the final injection, iIEL were harvested and subjected to two-color FACS analyses using mAb to CD8 in combination with OX-62 (A) or mAbs to mouse IgG1 (B), TCR-αβ (C), CD44 (D), or CD11a (E). Data shown are expression of the indicated markers by gated CD8⁺ lymphocytes. Data are representative of two independent experiments.
indicated by a markedly higher density of undamaged tubules (tubules with intact TBM) in OX-62-treated recipients as compared with control cohorts (Fig. 6).

Quantitative image analyses revealed that the impact of OX-62 on CD8 infiltration into the renal tubules (Fig. 6E) and development of tubular injury (Fig. 6F) was highly significant as compared with an isotype-matched (IgG1) negative control mAb (p < 0.01). Importantly, the total number and phenotype of CD8 cells infiltrating grafts in OX-62-treated recipients were not significantly different from control cohorts (data not shown), indicating that OX-62 does not prevent tubular injury by generally depleting CD8 cells from the graft. Rather, CD8 effectors in OX-62-treated recipients successfully extravasated into the general graft site but were confined to the graft interstitium (Fig. 6B), suggesting that OX-62 blocks local migration and/or retention of CD103+CD8+ effectors in the graft renal tubular epithelium.

**CD103 expression is required for destruction of the graft renal tubules by donor-specific CD8 effectors**

In addition to CD8+ T cells, CD103 is potentially expressed by a variety of non-T cell populations, including dendritic cells (29), macrophages (30), and mast cells (31), which could theoretically account for the impact of OX-62 on tubular injury. To directly assess the role of donor-specific CD103+CD8+ effectors in promoting tubular injury, we developed a mouse adoptive transfer model. Consistent with previous reports (19), BALB/c allografts transplanted into naive B6 hosts are spontaneously accepted and do not exhibit tubular inflammation/injury characteristic of clinical renal allograft rejection (data not shown). To circumvent this limitation, spleen cells from 2C TCR transgenic mice (H-2Ld-specific TCR on 99% of peripheral CD8 cells) crossed onto either WT (2C-WT) or CD103−/− backgrounds (2C-CD103−/−) were adoptively transferred into syngeneic B6 scid hosts bearing vascularized renal allografts from BALB/c (H-2Ld-positive) donors. mAb 1B2 directed to the clonotypic 2C TCR was used to track donor-specific CD8 cells in vivo.

As shown in Fig. 7, donor-specific CD8 cells (CD8+ T cells) carrying either the WT (Fig. 7A) or CD103−/− (Fig. 7B) alleles (here referred to as 2C-WT and 2C-CD103−/− effectors, respectively) acquired an activated/effector phenotype (CD44high) and migrated into H-2Ld-expressing renal allografts. As previously reported (7), a major subset of 2C-WT effectors up-regulates CD103

![Image](https://www.jimmunol.org/Downloadedfrom)
expression following migration into the renal allografts (Fig. 7A), and as expected, 2C-CD103−/− effectors that infiltrated the graft were devoid of CD103 expression (Fig. 7B). The total number of 2C-CD103−/− cells that infiltrated renal allografts was not significantly different from that of WT cohorts (5.4 + 0.5 × 10⁶ for 2C-KO vs 6.0 + 0.5 × 10⁶ for 2C-WT, n = 5), thus arguing against a significant role for CD103 in driving homeostatic expansion of 2C cells in SCID recipients.

As shown in Fig. 8, migration of 2C-WT effectors into L4-expressing renal allografts was accompanied by massive tubular injury as evidenced by a dramatic decrease in tubule density (Fig. 8, A and E), an aberrant vacuolization of the remaining tubular epithelial cells (Fig. 8C), and a marked decrease in cortical thickness of allografts compared with mock-transferred controls (Fig. 8F). In contrast, renal tubules in grafts infiltrated with 2C-CD103−/− effectors remained pristine (Fig. 8D) with minimal loss in total number of tubules (Fig. 8, B and E), and there was no significant loss of cortical depth compared with controls (Fig. 8F). Thus, these data document a requirement for CD103 expression in promoting tubular injury mediated by donor-specific CD8 effector populations and provide compelling evidence that donor-specific CD103+CD8+ effectors can be an important mechanism of tubular damage following allogeneic renal transplantation.

Importantly, BALB/c renal allografts transplanted into WT B6 mice previously primed to donor alloantigens (at day −10) exhibited extensive mononuclear infiltrates and tubular inflammation/injury (Fig. 9A) as compared with B6-CD103−/− cohorts by day 7 posttransplantation (Fig. 9B). Similar to experiments using 2C-WT donors, a subset (12−15%) of primed CD8 cells from WT donors infiltrating rejecting allografts up-regulate CD103 expression concomitant with infiltration into the graft site (Fig. 9C). Note that CD103+CD8+ cells in the graft were uniformly CD44high (Fig. 9D). Thus, these data confirm that tubular injury mediated by CD103+CD8+ effector populations is relevant to the normal (polyclonal) anti-graft response.

Discussion
Immune attack of the graft renal tubules by CD8 T effector populations has long been recognized as a cardinal feature of clinical renal allograft rejection (32), and with recent success in preventing acute graft loss, tubular atrophy and interstitial fibrosis have emerged as key barriers to long-term renal allograft survival (4). Although nonimmunologic insult clearly contributes to these lesions, recent clinical data implicate prior immune assault of the graft renal tubules as the major predisposing factor (3). The present study provides mechanistic insight into this clinicopathological phenomenon by experimentally documenting that CD103+CD8+ effector populations play a critical role in promoting tubular injury following allogeneic renal transplantation.

A key accomplishment of the present study was the development of a novel rat model that reproduces a clinically relevant pattern of tubular injury following allogeneic renal transplantation. We demonstrate that a brief course of CsA administered to rat renal allograft recipients diverts the rejection response from one of predominantly vascular involvement to a clinically relevant tubulo-interstitial pattern of rejection in which CD103+CD8+ effectors infiltrate the graft renal tubules concomitant with progressive tubular atrophy and interstitial fibrosis. This model recapitulates the known clinical scenario in which subclinical tubulointerstitial inflammation accompanied by accumulation of CD103+CD8+ cells at the graft site (13, 27) precedes development of tubular atrophy and interstitial fibrosis (3). Importantly, the intragraft localization and extended phenotype of CD103+CD8+ cells (Fig. 3) that accumulate in rat renal allografts in our model are virtually identical to those associated with rejecting clinical renal allografts (13, 27). The clinical relevance of the CsA-delayed rejection model is further supported by the observation that CD103+CD8+ effectors are undetectable in clinical renal allografts undergoing early rejection episodes (33) but are abundant in grafts undergoing delayed rejection episodes (13, 27).

The basis for the dramatic shift in mechanisms of graft injury elicited by a transient course of CsA is not clear. However, recent studies indicate that CD8 responses to allogeneic transplants are resistant to immunosuppressive strategies that effectively blunt the anti-graft CD4 response (34, 35). Therefore, it is possible that CsA blocks vascular rejection by selectively inhibiting the anti-graft Th response, thereby leading to the observed dominance of CD8 effector mechanisms. By blocking acute graft loss, CsA may allow

**FIGURE 7.** Mouse adoptive transfer model. Spleen cells (15 × 10⁶) from 2C TCR transgenic mice carrying WT (A) or CD103−/− alleles (B) were adoptively transferred into B6 scid hosts bearing BALB/c renal allografts. GIL were isolated at day 7 and processed for four-color FACS analyses. Data shown are for electronically gated total lymphocytes (left) or gated 1B2+CD8+ lymphocytes (right). Numbers shown above histograms indicate percentage of gated cells expressing CD103 at levels above isotype control staining (gray peaks).
ROLE FOR CD103+ CD8+ EFFECTORS IN PROMOTING TUBULAR INJURY

CD103 expression is required for efficient destruction of the graft renal tubules by donor-specific CD8 effectors. Spleen cells (15 x 10⁶) from 2C TCR transgenic mice carrying WT (A, C, and E) or CD103−/− alleles (B, D, and F) were adoptively transferred into B6.scid hosts bearing BALB/c renal allografts. At day 7, grafts were harvested and processed for paraffin sections. A and B, Jones-methamine silver staining of sections to highlight disruptions in the TBM (×200). C and D, Standard H&E sections (×200). E, Quantitation of tubular injury. F, Quantitation of cortical thickness. Data shown for E and F are mean values ± SE for 2C-WT (n = 5), 2C-CD103−/− (n = 5), and mock (n = 3) transfers.

For the elaboration of local TGF-β activity, a factor which induces CD103 expression by CD8+ T cells responding to renal allografts (7). Indeed, chronically rejecting renal allografts are associated with increased levels of TGF-β at the graft site (36). Although CsA is known to promote intrarenal TGF-β production (37, 38), two lines of evidence argue against an essential role for CsA in promoting the generation of CD103+CD8+ effector populations at the graft site. For one, CD103+CD8+ effectors accumulate within rat renal allografts transplanted into partially MHC-matched recipients in the complete absence of immunosuppressive drug treatment (data not shown). In this scenario, rejection is delayed (mean survival, 24 wk) by improved histocompatibility rather than CsA immunosuppression. In further support of this point, CD103+CD8+ effectors accumulate within mouse renal allografts in the absence of immunosuppressive drugs (Fig. 6A). However, we cannot exclude the possibility that CsA treatment enhances such accumulation. Given that other commonly used immunosuppressive agents such as sirolimus and tacrolimus (39, 40) also have been reported to promote intrarenal TGF-β production, it will be important to determine how the different immunosuppressive protocols in current clinical usage impact on the accumulation of CD103+CD8+ effectors within renal allografts.

That CD103+CD8+ effectors are causally related to tubular injury was established by two complementary approaches: by anti-CD103 mAb blockade in a rat model of renal allograft rejection and by targeted disruption of the CD103 gene in mouse adoptive transfer studies. We initially demonstrated that prophylactic treatment with anti-CD103 mAb dramatically reduces tubular inflammation and disruption of the TBM in a rat model of tubular injury (Fig. 5A). In contrast, anti-CD103 mAb did not prevent migration of CD8 cells into the graft interstitium (Fig. 5A). These data are consistent with our previous findings in a mouse islet transplant model that CD103 expression is not required for extravasation of CD8 effectors into the general graft site but is absolutely required for efficient migration/retention into graft epithelial compartments (12).

The above rat studies clearly documented that a CD103-expressing cell population is critically involved in promoting tubular injury. However, these data failed to conclusively document a role for CD103+CD8+ effectors in tubular injury because CD103 is expressed by non-CD8 populations (29–31), which could conceivably contribute to tubular injury. It was also possible that anti-CD103 mAb nonspecifically prevented tubular injury by generally depleting CD8 effectors from the graft rather than by specifically inhibiting CD103+CD8+ effectors responding to donor alloantigens. To circumvent these inherent limitations of the rat system, we used a mouse adoptive transfer model to document that CD8+ T cells responding to a defined donor MHC class I alloantigen require CD103 expression to efficiently mediate tubular injury (Fig. 7). Together with the above mAb-blocking data, these data provide compelling evidence that donor-specific CD103+CD8+ effector populations are a dominant mechanism of tubular injury following allogeneic renal transplantation. Conversely, our data...
suggest that CD103+CD8+ effector populations present in rejecting allografts (Fig. 3A) may account for extratubular lesions associated with the rejection process such as vascular injury and/or interstitial inflammation.

How CD103+CD8+ effector populations promote tubular injury remains to be determined. Consistent with the intratubular localization of CD103+CD8+ effectors (Fig. 3C), the known ligand of CD103 (E-cadherin) is selectively expressed by cells within the renal tubular epithelium (11, 41). CD103 is known to promote T cell adhesion to E-cadherin-expressing epithelial cells in vitro (8, 9, 42), and we have recently documented that CD103 expression promotes accumulation of CD103+CD8− effectors in the host intestinal epithelium during graft-vs-host disease (43). These data raise the possibility that enhanced adhesion/retention of CD8 effectors within the graft renal tubular epithelium promotes tubular injury via classic CTL mechanisms (i.e., via perforin/granzyme or Fas/FasL pathways) (44). However, it is important to note that CD8 effectors possess a diverse armamentarium for destruction of allogeneic targets, any or all of which could conceivably underlie tubular injury mediated by donor-specific CD8 effector populations. An intriguing possibility is suggested by recent studies documenting that CD8 effectors can promote destruction of allogeneic tissues indirectly by producing cytokines and/or chemokines that promote site-specific recruitment of inflammatory leukocyte populations into the graft (45–48). Consistent with this possibility, renal tubules in mouse allografts adoptively transferred with CD103-deficient CD8 cells (Fig. 7D) remain strikingly free of mononuclear cell infiltration, despite the fact that CD8 T cells displayed an activated/effector phenotype and successfully extravasated into the general graft site (Figs. 3 and 6). This is an appealing hypothesis because leukocytic infiltration into the graft renal tubules (tubulitis) is the sine qua non of clinical renal allograft rejection (32). However, clearly, additional studies are required to discriminate between these nonmutually exclusive hypotheses.

The present findings have implications for the field of immune monitoring. Our data indicate that CD103+CD8+ effectors are present at the site of renal allografts but not in the recipient spleen (data not shown) or lymph nodes (Fig. 3A). The clinical relevance of this observation is corroborated by our previous demonstration that CD103+CD8+ effectors are present at high levels in rejecting renal allografts but are not detectable in the peripheral blood or tonsils (14). These data indicate that CD103+CD8+ cells either fail to recirculate from the graft to peripheral lymphoid compartments or rapidly down-regulate CD103 expression subsequent to exiting the graft site. The present data do not discriminate between these possibilities. Regardless, these data document that an important component of the anti-graft response is not detectable by conventional immune monitoring approaches, which typically rely on sampling of peripheral blood. An alternative approach for detection of CD103+CD8+ effectors is suggested by Ding et al. (49), who have shown that CD103-expressing cells are detectable in the urine of renal transplant patients and that such levels correlate with rejection episodes. It now will be important to determine whether such assays possess the sensitivity and specificity to reliably predict tubular injury.

The present findings point to novel therapeutic strategies to preserve long-term function of renal allografts. Current immunosuppressive strategies protect the graft from immune assault at early posttransplant intervals but are ineffectual in preventing tubular atrophy and late graft loss (5). The present study provides proof-of-principle that anti-CD103 mAb can be used as a toll to combat progression of tubular injury in renal transplants. These data raise the possibility that blockade of CD103+CD8+ effectors as an adjunct to conventional immunosuppressive strategies provides a
plausible means of preserving long-term function of clinical renal transplants. It will now be important to identify the most effective strategies for therapeutic blockade of the CD103 pathway. That CD103 is selectively expressed by CD8 cells that infiltrate renal allografts (see above) suggests that blockade of graft-associated CD103+CD8+ effectors can be accomplished without global suppression of the immune system. To our knowledge, blockade of the CD103 ligand, E-cadherin, as a means to inhibit the function of CD103+CD8+ effectors is not practical due to its ubiquitous expression and its critical roles in embryogenesis and maintenance of epithelial integrity (50).

In summary, the present data document a causal role for CD103+CD8+ effector populations in promoting tubular injury following allogeneic renal transplantation. These data provide novel insight into mechanisms of late graft loss in clinical renal transplant recipients and identify a novel set of targets for therapeutic intervention in this important clinical problem.

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Disclosures

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References


Corrections


An affiliation for the second author was omitted in the article. The corrected author line and affiliations are shown below.

Rongwen Yuan,* Riham El-Asady,†,‡ Kechang Liu,† Donghua Wang,* Cinthia B. Drachenberg,§ and Gregg A. Hadley*†

*Department of Surgery, University of Maryland Medical School, Baltimore, MD 21201; †Department of Microbiology and Immunology, University of Maryland Medical School, Baltimore, MD 21201; §Ain Shams University, Cairo 11566, Egypt; and ‡Department of Pathology, University of Maryland Medical School, Baltimore, MD 21201

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