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Distinct T Cell/Renal Tubular Epithelial Cell Interactions Define Differential Chemokine Production: Implications for Tubulointerstitial Injury in Chronic Glomerulonephritides

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Chemokines can promote interstitial fibrosis that is, in turn, a strong predictor of renal failure in chronic glomerulonephritides (GN). Resident renal cells, including renal tubular epithelial cells (RTEC), represent a prominent source of chemokine expression. Evaluating those factors responsible for sustained chemokine production by RTEC during GN is therefore crucial. The contribution of interstitial T cells to such expression, and in particular the precise nature of their interactions with RTEC, are poorly understood. Activated T cell/RTEC coculture induced production of high levels of monocyte chemoattractant protein-1 (MCP-1), RANTES, and IFN-inducible protein-10 from RTEC. Using double-chamber cultures and activated T cell plasma membrane preparations we demonstrated that both cell contact and soluble factors contributed to RTEC chemokine production. Importantly, different chemokines exhibited distinct activation requirements. Thus, for RANTES cell contact was essential, but not sufficient. In contrast, either soluble factors or cell contact induced MCP-1 and IFN-inducible protein-10 production, although both pathways were required for a maximal response.

Neutralization experiments identified critical roles in this process for proinflammatory cytokines such as TNF-α, IL-1β, and IFN-γ as well as membrane molecules such as LFA-1, CD40 ligand, and membrane bound TNF-α. Finally, chemotactic bioassays of T cell/RTEC coculture supernatants demonstrated 80% reduction of monocyte migration following MCP-1 neutralization, indicating a dominant role for this chemokine. In summary, activation of renal tubular cells by infiltrating T cells can amplify and perpetuate local inflammatory responses through chemokine production differentially mediated by soluble and cell contact-dependent factors. Recognition of this regulatory diversity has important implications in the choice of potential therapeutic targets in GN. The Journal of Immunology, 2000, 164: 3323–3329.

Most chronic human kidney diseases are characterized by progressive loss of renal function. This progression is thought to result from pathogenic processes that are independent of the original glomerular or interstitial injury and function as the final common pathway to end-stage renal failure (1–3). This pathway involves interstitial infiltration by inflammatory mononuclear cells consisting of both macrophages and predominantly T cells (4, 5).

Recruitment of circulating leukocytes to inflammatory sites is directed by a multistep cascade of molecular interactions coordinated by locally expressed chemoattractant and adhesion molecules (6). The 8- to 11-kDa chemokines, produced by a wide variety of stimulated cell types, including leukocytes and resident renal cells (7), play a central role in this process. They activate leukocyte-integrin molecules to facilitate firm endothelial adhesion (8) and provide a chemotactic gradient for trans- and subendothelial leukocyte migration (9). Recent studies have shown that chemokine functions extend to include activation of mononuclear cells (10, 11), modulation of collagen synthesis, and fibrosis (12, 13). Chemokines are subdivided into four major classes, based on cysteine motifs (14). Members of individual subgroups direct chemotaxis and activation of specific leukocyte subsets, such that differential expression at sites of inflammation dictates the profiles of tissue-infiltrating leukocytes (7, 15). The C-C chemokines, monocyte chemoattractant protein-1 (MCP-1),1 RANTES, and macrophage inflammatory protein-1α and -β (MIP-1α and MIP-1β), and the C-X-C chemokine IFN-inducible protein-10 (IP-10) are predominantly involved in the recruitment of macrophages and T cells (16).

Both in vitro and in vivo studies have demonstrated that RTEC represent a prominent source for the production of inflammatory mediators. Thus, upon stimulation by proinflammatory cytokines, cultured human RTEC produce MCP-1 and RANTES (17, 18). In a murine model of interstitial nephritis, up-regulation of interstitial expression of IP-10 correlates with the extent of mononuclear cell infiltration (19). Studies in humans demonstrated interstitial expression of MCP-1, RANTES, MIP-1α, and MIP-1β in a variety of glomerulonephritis (GN) (20).

Direct evidence implicating chemokines in renal interstitial injury has come from animal studies. In a murine model of crescentic nephritis, neutralization of MCP-1 decreased both glomerular crescent formation and interstitial fibrosis, while neutralization of RANTES only decreased glomerular injury (12). In the same model a paucity of MCP-1 resulted in a dramatic reduction of tubular damage (21). Ectopic expression of RANTES in renal tubular epithelial cells in a murine model of lupus enhanced interstitial mononuclear cell infiltration (22).

As chemokine production by RTEC can be an important regulatory mechanism for interstitial infiltration by mononuclear cells,

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2 Abbreviations used in this paper: MCP-1, monocyte chemoattractant protein-1; MIP-1α, macrophage inflammatory protein-1α; GN, glomerulonephritis; RTEC, renal tubular epithelial cell; IP-10, IFN-inducible protein-10; REBM, renal epithelial basal medium; 24h-S, 24-h-stimulated; CD40L, CD40 ligand.
a major question is how this is regulated. Previous investigations have suggested a potential role of T cells in this process (17, 18, 23). T cells may stimulate RTEC either via soluble factors or by direct cell-to-cell contact. Cell-to-cell contact mediates important biologic effects of T cells that, in some cases, are distinct from those mediated by soluble factors (24–27). In this study we sought to identify the soluble factors and the cell surface molecules and their relative contributions to chemokine production by RTEC. We employed a cell culture system that uses a semipermeable membrane to physically separate T cells from RTEC (and thereby delineate the relative contribution of cell-to-cell contact) in combination with chemotaxis assays. Our data demonstrate that T cells stimulate RTEC to secrete chemokines through both soluble inflammatory cytokines and cell-to-cell contact-dependent pathways, and identify key molecules involved in this interaction.

Materials and Methods

Abs, cytokines, and CD40 ligand (CD40L) trimers

The following Abs, recombinant cytokines, and proteins were used in these experiments: monoclonal mouse anti-human TNF-α (R&D Systems, Minneapolis, MN), monoclonal mouse anti-human IL-1β (Genzyme, Cambridge, MA), monoclonal mouse anti-human IFN-γ (R&D Systems), monoclonal mouse anti-human CD40L (PharMingen), monoclonal mouse anti-human RANTES (R&D Systems), recombinant human TNF-α and IL-1β (R&D Systems), recombinant human IFN-γ (Genzyme), and trimERIC human CD40 ligand/leucine zipper fusion protein (a gift from Immunex, Seattle, WA).

T cell purification and culture

PBMC were isolated from normal donors by density gradient centrifugation. T cells were enriched by adherence on culture flask for 16 h at 37°C. T cell purity was assessed by FACS analysis using fluorescein-labeled mAb (Becton Dickinson, Mountain View, CA) with specificity for the following cell surface markers: CD14 (monocytes), CD3 (T cells), CD19 (B cells), and CD56 (NK cell). The T cell fraction routinely consisted of >80% CD3-expressing cells, <0.5% CD19-expressing cells, 3% CD41-expressing cells, and 10% CD56-expressing cells. In some experiments, CD4+ or CD8+ cells were positively selected from PBMC using anti-CD4 mAb- or anti-CD8 mAb-coupled magnetic beads (Dynal, Oslo, Norway) and then detached from beads by use of DETACHABEAD CD4/CD8 (Dynal), following the manufacturer’s manual. The purity of selected CD4+ or CD8+ cells was routinely >96%, as judged as CD4- or CD8-positive cells by FACS analysis. T cells were cultured in RPMI 1640 (Life Technologies, Gaithersburg, MD) supplemented with 10% heat-inactivated FBS (HyClone, Logan, UT), 2 mM t-glutamine, 100 IU/ml penicillin, and 100 μg/ml streptomycin (all from Life Technologies).

RTEC culture

Normal human RTEC were purchased from Clonetics (San Diego, CA). RTEC were cultured in renal epithelial basal medium (REBM), supplemented with 0.5% FBS, 10 ng/ml of recombinant human epidermal growth factor, 5 μg/ml of insulin, 0.5 μg/ml of hydrocortisone, 50 μg/ml of gentamicin, 50 ng/ml of amphotericin B, 0.5 μg/ml of epinephrine, 6.5 ng/ml triiodothyronine, and 10 μg/ml of transferrin (all from Clonetics). For these experiments we used RTEC passages 3–5.

T cells/RTEC coculture

T cells were cultured at 2 × 10^3/ml for 6 or 24 h in 10% FBS RPMI medium in the presence or the absence of 10 nM PMA (Sigma, St. Louis, MO) and 1 μM Monomycin (Calbiochem, La Jolla, CA). T cells were washed three times and resuspended to a final concentration of 1 × 10^6/ml in REBM. RTEC were harvested at 80% confluence and cultured in 24-well plates at 6 × 10^4 cell/0.6 ml/well in triplicate for 16 h. In preliminary experiments serial 2-fold increments (T cells/RTEC; 0.5:1 to 2:1) were examined. A dose response was observed, with higher ratios resulting in more stimulation. Although stimulation was detected at a ratio of 0.5:1, we elected to use the ratio of 1:1 because at this ratio an adequate amount of chemokine was induced to allow meaningful comparisons without using an excess of T cells. Subsequently, 6 × 10^5 of prepared T cells were added to 6 × 10^4 RTEC. In some experiments identical parallel cultures were established in which T cells were separated from RTEC by a 0.4-μm pore size semipermeable membrane (Biocomat, Falcon, Becton Dickinson Labware, Bedford, MA) while sharing the same medium. After 24-h incubation at 37°C supernatants were harvested and stored at −20°C for chemokine determination and chemotaxis assays.

Membrane preparations from T cells

Crude plasma membrane was prepared as described previously (25). Briefly, T cells were disrupted by sonication (five 5-s bursts of 90 W each) in PBS containing 0.68 M sucrose, 200 μM PMSF, and 5 mM EDTA. The lysate was centrifuged for 15 min at 4,000 × g to discard nuclei and unbroken cells. The supernatant was centrifuged for 45 min at 100,000 × g, and the pellet containing the membrane fraction was resuspended at the theoretical concentration of 10 × 10^6 cell equivalent/ml in supplemented REBM.

Inhibition of cytokine synthesis by neutralizing Abs

Abs against CD11a/LFA-1, CD40L, TNF-α, IL-1β, IFN-γ, or IgG1 of irrelevant specificity (Sigma) were incubated at 10 μg/ml with T cells or membrane preparations of T cells for 30 min at room temperature and were added without washing to RTEC as described above. Chemokine synthesis after 24 h was determined by ELISA.

Chemotaxis assay

Chemotaxis assay was performed as previously described (28). Supernatants of 24-h-stimulated (24h-S) T cells alone, RTEC alone, or coculture of 24h-S T cells/RTEC were centrifuged at 10,000 × g for 10 min to remove the contamination of cells and were diluted with supplemented REBM. Human PBMC (5 × 10^6) in 100 μl of supplemented REBM were added to the upper chamber of a 6.5-mm diameter, 5-μm pore size polycarbonate Transwell culture insert (Costar, Cambridge, MA) and incubated in duplicate with 500 μl of supplemented REBM or prepared supernatants in the lower chamber for 2 h at 37°C. For the neutralizing experiments, undiluted supernatants obtained from cocultures were preincubated with Abs against MCP-1, RANTES, or IgG of irrelevant specificity at 10 μg/ml for 30 min at room temperature. Cells that transmigrated into the lower chamber were vigorously suspended and counted with a FACSScan (Becton Dickinson, San Jose, CA) for 30 s at 60 μl/min, gating on the forward and side scatter of monocytes or lymphocytes. A 1/20 dilution of input PBMC was similarly counted, which was considered to be 100% migration.

Determination of chemokine production

MCP-1, RANTES, IP-10, MIP-1α, and MIP-1β were measured by ELISA (R&D Systems and Endogen, Woburn, MA).

Statistical analysis

All experiments were repeated at least three times. Results are presented as the mean ± SE from three separate experiments. Statistical significance was determined by Student’s t test. A value of p < 0.05 was considered to represent a statistically significant difference between group means.

Results

Activated T cells induce chemokine production by RTEC

MCP-1, RANTES, MIP-1α, MIP-1β, and IP-10 are major mononuclear cell-directed chemokines expressed in the interstitium of a variety of glomerulonephritides (19, 20). We first examined whether stimulated T cells induced the production of these chemokines by RTEC. After determining the optimal T cell/RTEC ratio and duration of coculture (see Materials and Methods), we stimulated T cells with PMA and ionomycin for both 6 and 24 h. This assures maximum expression of molecules such as TNF-α and CD40L, which are known to peak 6–8 h after stimulation and decrease 24 h later (29). T cells washed extensively were cocultured with RTEC at a ratio of 1:1 for 24 h. At the same time, identical parallel cultures were established in which T cells were separated from RTEC by a 0.4-μm pore size semipermeable membrane while sharing the same medium. In this coculture system separated T cells stimulate RTEC exclusively through soluble molecules, thus enabling determination of the contribution of cell-to-cell contact. As shown in Fig. 1, T cells induced up-regulation of MCP-1, IP-10, and RANTES production, but failed to induce up-regulation.
of MIP-1α and MIP-1β (data not shown). Because in the absence of RTEC stimulated T cells alone produced negligible amounts of MCP-1 and IP-10 and small amounts of RANTES (Fig. 1), we assumed that the major source of chemokine production in these cocultures was RTEC. This was corroborated by subsequent experiments using plasma membrane preparations from stimulated T cells (see below). Production of these chemokines was T cell contact dependent, because separation of T cells from RTEC significantly decreased their levels, although soluble factors alone induced significant amounts of MCP-1 and IP-10. Notably, RANTES synthesis was exclusively cell-to-cell contact dependent, as separation of T cells from RTEC decreased it to levels comparable to those obtained with T cells alone. In summary, activated T cells induce MCP-1, RANTES, and IP-10 by RTEC through both soluble factors and cell-to-cell contact-dependent mechanisms.

Both CD4+ and CD8+ cells induce chemokine production

In chronic renal diseases T cells infiltrating the renal interstitium consist of both CD4+ and CD8+ cells (4, 5). CD4+ or CD8+ cells were positively selected from PBMC using anti-CD4 mAb or anti-CD8 mAb-coupled magnetic beads and stimulated with PMA and ionomycin for 6 or 24 h. Stimulated cells were next cocultured both in contact with and separated from RTEC as described before. Both CD4+ and CD8+ cells induced comparable chemokine production at 24 h (Fig. 2). CD4+ and CD8+ T cells induce chemokine production by RTEC. CD4+ or CD8+ cells were positively selected from PBMC using anti-CD4 mAb or anti-CD8 mAb-coupled magnetic beads, then stimulated with PMA and ionomycin for 6 or 24 h. Subsequently, T cells were cocultured in contact with or separated from RTEC for 24 h, as described before. Chemokine production was determined by ELISA. Data are the mean ± SE from three separate experiments.

Role of soluble factors and cell surface molecules

We next sought to identify the cytokines and cell surface molecules involved in this interaction. TNF-α, IL-1β, and IFN-γ are major proinflammatory cytokines produced by activated T cells. Previous investigations have demonstrated that rTNF-α and rIL-1 induce MCP-1 by RTEC in vitro (17). CD40L, a costimulatory molecule expressed on activated T cells, and the LFA-1/ICAM-1 pathway have also been shown to be critical in many T cell-mediated inflammatory responses (30–32). Therefore, we blocked these molecules using neutralizing Abs by preincubating activated T cells with neutralizing Abs (or IgG of irrelevant specificity) for 30 min and adding them to RTEC. Twenty-four hours later, chemokine production was determined by ELISA. The percent inhibition relative to cocultures without Abs was calculated as described in Fig. 3.

As shown in Fig. 3A, blockade of TNF-α, IL-1β, or IFN-γ alone significantly reduced RTEC MCP-1 synthesis in cocultures of 6h-S T cells, but had no effect in cocultures of 24h-S T cells. In contrast, simultaneous blockade of TNF-α, IL-1β, and IFN-γ decreased RTEC MCP-1 production in cocultures of both 6h-S and 24h-S T cells (Fig. 3A), suggesting that different mechanisms operate through sequential T cell activation. Similarly, blockade of TNF-α, IL-1β, or IFN-γ separately decreased RANTES synthesis in cocultures of 6h-S T cells, while only simultaneous blockade significantly reduced it in cocultures of 24h-S T cells. Interestingly, blockade of LFA-1 or CD40L resulted in dramatic reduction of RANTES (Fig. 3B), but not MCP-1 or IP-10 (data not shown), synthesis induced by 6h-S T cells. T cell induction of IP-10 by RTEC was IFN-γ dependent. Blockade of IFN-γ alone achieved a >60% decrease in IP-10 synthesis by RTEC in cocultures of both 6h-S and 24h-S T cells. Thus, chemokines manifest differential requirements for cell and soluble effector pathways that we sought to better define in subsequent experiments.
against TNF-α). These results suggest that in addition synthesis by 50% (Fig. 4), but not RANTES (data not shown). Simultaneous blockade of the theoretical ratio of 10:1 (T cells:RTEC). Membrane preparations at the theoretical ratio of 10:1 and cultured for 24 h (B). Chemokine synthesis was determined by ELISA. Data are the mean ± SE from three separate experiments. Note that the concentrations shown in the two y-axes are different.

**FIGURE 3.** Inhibition of chemokine synthesis by RTEC by neutralizing Abs. Peripheral blood-derived T cells, stimulated with PMA and ionomycin for 6 or 24 h, were preincubated with 10 μg/ml of neutralizing Abs against TNF-α (anti-TNF-α), IL-1β (anti-IL-1β), IFN-γ (anti-IFN-γ). Mixtures of them (anti-TNF-α/IL-1β/IFN-γ), LFA-1 (anti-LFA-1), CD40L (anti-CD40L), or IgG of irrelevant specificity (cont IgG) for 30 min. Subsequently, T cells were added to RTEC at a ratio of 1:1 as described before. Twenty-four hours later, chemokine production was determined by ELISA. Data are the mean ± SE from three separate experiments. The percent inhibition was calculated as follows: 100 – (chemokine synthesis with neutralizing Ab/chemokine synthesis without neutralizing Ab).

**Role of membrane-bound cytokines**

Several studies have suggested a significant role for T cell membrane-bound TNF-α and IFN-γ in inflammatory responses (25, 33). To address the role of membrane-bound TNF-α and IFN-γ in the interactions between RTEC and activated T cells, we next examined whether plasma membrane preparations from stimulated T cells could induce chemokine production by RTEC. Crude plasma membrane fractions from T cells were cocultured with RTEC at the theoretical ratio of 10:1 (T cells:RTEC). Membrane preparation from 6h-S or 24h-S T cells induced MCP-1 and IP-10 (Fig. 4A), but not RANTES (data not shown). Simultaneous blockade of TNF-α and IFN-γ by specific neutralizing Abs decreased MCP-1 synthesis by 50% (Fig. 4B). These results suggest that in addition to the soluble form, the membrane form of TNF-α and IFN-γ may be involved in T cell induction of chemokines by RTEC.

**Production of chemokines by RTEC in response to inflammatory cytokines and CD40L**

Since their cooperative effects on production of a panel of chemokines by RTEC have not previously defined, we next characterized in more details the role of cytokines and CD40L. In preliminary experiments we determined the optimal concentrations of cytokines to induce maximum response by RTEC. RTEC were cultured for 24 h in the presence or the absence of rTNF-α (10 ng/ml), rIL-1β (2 ng/ml), rIFN-γ (500 IU/ml), or combinations of these cytokines and soluble CD40L, and chemokine synthesis was determined by ELISA. As shown in Fig. 5A, IL-1β induced a higher level of MCP-1 compared with TNF-α. IFN-γ in combination with TNF-α or IL-1β, but not alone, enhanced MCP-1 synthesis. The production of RANTES required multiple cytokines, as neither TNF-α, IL-1β, nor IFN-γ alone induced significant RANTES synthesis; it required the combination of all these cytokines. Of interest, although IP-10 synthesis was IFN-γ dependent, IFN-γ alone could not induce significant IP-10 synthesis. IFN-γ in combination with TNF-α or IL-1β (but not combination of TNF-α with IL-1β) induced a high level of IP-10. These data demonstrate that IFN-γ is necessary, but not sufficient, to induce IP-10 synthesis.

As blockade of CD40L effectively decreased RANTES production induced by 6h-S T cells (Fig. 3), we also examined the effect of rCD40L. Thus, we next cultured RTEC in the presence or the absence of rCD40L (3 μg/ml), rIFN-γ (500 IU/ml), or their combination. CD40L in combination with IFN-γ, but not alone, induced dramatic up-regulation of MCP-1 and RANTES (Fig. 5B).

**MCP-1 induces significant monocyte migration**

Direct evidence from animal models of GN and circumstantial evidence from analysis of tissues obtained from patients with a variety of GN have suggested an important role of monocyte migration in mediating tubulointerstitial injury. Because several chemokines are produced during T cell/RTEC interaction, it was necessary to examine their relative contributions to monocyte...
migration in chemotaxis assays. Freshly isolated human PBMC in supplemented REBM were added to the upper chamber of a 5-μm pore size polycarbonate Transwell filter. Supernatants of T cells alone, RTEC alone, or cocultures of 24h-S T cells/RTEC were added to the lower chamber, either directly or after dilution with supplemented REBM. After a 2-h incubation, monocytes or lymphocytes that transmigrated into the lower chamber were counted by FACScan gating on the forward and side scatters. As shown in Fig. 6A, coculture supernatants induced significant monocyte migration in a dose-dependent fashion, while supernatants of RTEC or T cells alone did not. Interestingly, monocyte migration was only blocked by neutralization of MCP-1, not that of RANTES (Fig. 6B). Moreover, although high levels of IP-10 and RANTES were present in the coculture supernatants, lymphocytes did not significantly migrate in response to them (data not shown). This may be due to the low level of chemokine receptor expression on rested lymphocytes (34, 35). We did not use activated T cells in these chemotaxis experiments, because T cells produce chemokines upon activation, and this may confound the results.

Discussion

Accumulation of T cells in the renal interstitium is a prominent feature of a variety of progressive renal diseases, although the mechanisms by which T cells promote interstitial injury remain ill defined. Chemokines released by damaged or activated renal resident cells probably direct such T cell infiltration (36). We now demonstrate that T cells once recruited can, in turn, promote further chemokine production through combined cognate and soluble factor-mediated interactions with resident tubular epithelial cells. These interactions are discrete for different chemokines, vary within T cell subsets, and depend upon the duration of prior T cell activation.

Previous in vitro studies have shown that proinflammatory cytokines, such as TNF-α, IL-1β, and IFN-γ, or ligation of CD40 on the surface of RTEC induce MCP-1 and RANTES production by RTEC (17, 18, 37). These studies, however, did not address the relative importance of these inflammatory mediators in the context of adjacent activated T cells as would be expected in vivo. We have now systematically examined such T cell/RTEC interactions. Our observations indicate that MCP-1, IL-10, and RANTES are synthesized in distinct regulatory cascades that differentially use soluble and cell contact-dependent pathways. Functional assays indicated that MCP-1 accounted for ~80% of the chemotactic activity toward monocytes arising from T cell/RTEC interactions. Monocyte migration in the interstitium is important for subsequent tubular injury (12). Moreover, animal models of GN define an important role of MCP-1 in interstitial infiltration and tubular injury. Of interest, MCP-1 exhibited the least stringent activation requirements in our assay. Thus, although soluble factors and cell contact-dependent pathways. Functional assays indicated that MCP-1 accounted for ~80% of the chemotactic activity toward monocytes arising from T cell/RTEC interactions.
Neutralizing experiments implicated TNF-α of cells significantly decreased RANTES production, and membrane presence of both soluble factors and cell contact. Separation of MCP-1 and IP-10, synthesis of RANTES required the simultaneous expression through the pathways defined above. Resting lymphocytes express very low levels of chemokine receptors (34, 35), and it is unlikely to be involved in the migration of mononuclear cells, thus amplifying and perpetuating inflammatory responses. The end results of these processes is atrophy of tubular cells, interstitial fibrosis, and loss of renal function.

Activated T cells may also themselves secrete chemokines, such as RANTES, MCP-1, and IP-10. Chemokines produced by these cells promote further infiltration of the renal interstitium by T cells and macrophages. In addition to serving as chemoattractants for T cells and macrophages, these chemokines may activate mononuclear cells, thus amplifying and perpetuating inflammatory responses. The end results of these processes is atrophy of tubular cells, interstitial fibrosis, and loss of renal function.

FIGURE 7. Cellular interactions between T cells and RTEC in the pathogenesis of interstitial injury in GN. T cells infiltrate the kidney in response to chemokines released by injured resident renal cells. T cells, activated either systemically or locally in response to Ags or cytokines/chemokines (bystander activation), induce MCP-1, RANTES, and IP-10 production by RTEC through soluble proinflammatory factors, such as TNF-α, IL-1β, and IFN-γ, and cell-to-cell contact-dependent pathways, such as CD40L/CD40, LFA-1/ICAM-1, and membrane-bound cytokines. Activated T cells may also themselves secrete chemokines, such as RANTES, MIP-1α, and MIP-1β. Chemokines produced by these cells promote further infiltration of the renal interstitium by T cells and macrophages. Resting lymphocytes express very low levels of chemokine receptors (34, 35), and this may account for their lack of migration in our assay.

A pathogenic role for RANTES has been proposed for glomerular injury, interstitial mononuclear cell infiltration, and interstitial rejection of renal allografts (12, 22, 37). Our chemotaxis assay, however, suggested that IP-10 is unlikely to be involved in the migration of monocytes, consistent with previous findings that monocytes do not possess CXCR3, the only receptor reported to date for IP-10 (34). In contrast, it is probable that IP-10 may be involved in the migration of activated CXCR3+ T cells into inflamed interstitium. Such T cell recruitment will probably further enhance IP-10 and other chemokine expression through the pathways defined above. Resting lymphocytes express very low levels of chemokine receptors (34, 35), and this may account for their lack of migration in our assay.

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Although in many glomerulonephritides tubulointerstitial disease is thought to represent a secondary event resulting from downstream events that follow glomerular injury, once the latter is established, T cells and macrophages may assume a more important role in its perpetuation. T cells infiltrating the interstitium probably activate interstitial macrophages that are abundant sources of TNF-α and IL-1β. T cell- and macrophage-mediated activation of RTEC could promote a variety of other biologic effects in RTEC including 1) increased expression of accessory molecules and enhancement of their ability to present Ags and activate local T cells, 2) trans-differentiation of tubular epithelial cells to fibroblast-like cells that may promote fibrosis, 3) production of vasoactive mediators such as endothelin-1 and nitric oxide that exacerbate ischemia, and 4) induction of RTEC apoptosis resulting in tubular atrophy. The net effect of such processes will be atrophy of tubular cells, interstitial fibrosis, and loss of renal function (44).
The present study, although focussed on chemokine and sustained leukocyte recruitment, demonstrates the biological plausibility of these possibilities.

The availability of specific inhibitors of cytokines, such as TNF-α and IL-1, and Abs recognizing CD40L and ICAM-1 for clinical use in humans provides unique opportunities to examine the contributions of these molecules in human GN (45–48). Our data suggest that in addition to their well-described effects on mesangial cells (49), these membrane-bound and secreted molecules may also affect the biology and behavior of human RTEC, and hence pathology, in the renal interstitium (Fig. 7). Our studies have thus defined clear therapeutic targets, but demonstrate significant diversity and potential redundancy, recognition of which will be essential to logical therapeutic selection.

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