

Noninvasive detection of renal allograft inflammation by measurements of mRNA for IP-10 and CXCR3 in urine

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Noninvasive detection of renal allograft inflammation by measurements of mRNA for IP-10 and CXCR3 in urine.

Background. We explored the hypothesis that measurements of mRNA encoding interferon-inducible protein-10 (IP-10) or the chemokine receptor CXCR3 in urinary cells offer a noninvasive means of elucidating cellular traffic causing acute rejection of human renal allografts.

Methods. We obtained 63 urine specimens from 58 renal allograft recipients who underwent 63 allograft biopsies to resolve the basis for graft dysfunction, and 27 additional urine samples from 24 other patients with stable allograft function. Twenty-seven of the 63 biopsies were classified as acute rejection, 20 as other, and 16 as chronic allograft nephropathy. We measured the levels of transcripts for IP-10 and CXCR3, and a constitutively expressed gene 18S rRNA in the urine specimens and correlated transcript levels with renal allograft diagnosis.

Results. mRNA levels of IP-10 ($P < 0.0001$) or CXCR3 ($P < 0.0001$) but not the levels of 18S rRNA ($P = 0.56$) predicted intra-graft cellular traffic causing acute rejection. Receiver-operating characteristic curve analysis demonstrated that acute rejection can be predicted with a sensitivity of 100% and a specificity of 78% using the (log-transformed) cutoff value of 9.11 copies of IP-10, and with a sensitivity of 63% and a specificity of 83% using the cutoff value of 11.59 copies of CXCR3. Immunohistologic analysis of allograft biopsies showed exuberant expression of IP-10 and CXCR3 during acute rejection whereas both were absent in grafts with stable function.

Conclusion. Our investigation demonstrates that intra-graft cellular events associated with acute rejection of human renal allografts can be noninvasively identified by measurements of mRNA for IP-10 and CXCR3 in urinary cells.

Chemokines constitute a family of structurally related small-molecular-weight cytokines which contribute to tis-

sue morphogenesis, hematopoiesis, cell proliferation and apoptosis, but their roles in leukocyte trafficking and recruitment during inflammatory responses have significance for renal medicine including renal transplantation [1, 2]. Studies in animal models have shown that all of the 50 known chemokines and their 20 receptors can be expressed at some stage during allograft rejection [3]. However, certain chemokine/chemokine receptor pathways have proven critical in the rejection of vascularized allografts in rodent models [4–7]. Comparative investigations have shown the particular importance of the chemokine receptor, CXCR3, in mediating host alloresponses and graft destruction [5]. Targeting of CXCR3 by homologous recombination [5], neutralizing monoclonal antibodies [5], or small molecule inhibitor [8], markedly prolonged cardiac allograft survival. Moreover, targeting of the CXCR3 chemokine ligand, interferon-inducible protein-10 (IP-10), by homologous recombination or monoclonal antibody in wild-type recipients, was also of therapeutic importance [9]. Both IP-10 and CXCR3 have also been implicated in human cardiac allograft rejection by their intra-graft expression during episodes of acute rejection [10–12], leading to preclinical trials of a neutralizing anti-human CXCR3 monoclonal antibody and a CXCR3 small molecule antagonist in nonhuman primate renal allograft recipients [13].

Acute rejection of a renal allograft is suggested by clinical features such as loin pain, fever, and oliguria and is currently established by histologic findings within a percutaneous renal biopsy [14]. While invasive and prone to sampling errors, renal transplant biopsies are reasonably safe and a standardized approach to their assessment has benefited clinical trials [15, 16], although surprising and significant interobserver variations in diagnosis have been noted [17]. The invasive nature of renal biopsy is particularly significant in the pediatric patient since the renal transplant is often placed in an intra-abdominal

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Table 1. Study cohorts for the measurement of mRNAs in urinary cells

| | Acute rejection ^a | Other findings ^a | Chronic allograft nephropathy ^a | Stable graft function ^a |
|---|------------------------------|-----------------------------|--|------------------------------------|
| Number of allograft recipients (<i>N</i> = 82) | 24 | 19 | 15 | 24 |
| Age (mean ± SD) years | 37 ± 11 | 49 ± 10 | 51 ± 12 | 47 ± 13 |
| Gender male/female | 10/14 | 14/5 | 8/7 | 19/5 |
| Graft donor source living/cadaver | 13/11 | 6/13 | 3/12 | 15/9 |
| Number of allograft biopsies (<i>N</i> = 63) | 27 | 20 | 16 | 0 |
| Number of urine samples (<i>N</i> = 90) | 27 | 20 | 16 | 27 |

^aThe diagnoses of acute rejection, other findings, and chronic allograft nephropathy were made by histologic evaluation of renal-allograft biopsy specimens. The clinical diagnosis of stable graft function was based on the finding that serum creatinine levels either had decreased or had not increased by more than 0.2 mg/dL during the 7 days before and the 7 days after the urine samples were collected.

Table 2. Sequence and location of oligonucleotide primers and fluorogenic probes^a

| Gene | Accession number | Sequence |
|----------|------------------|--|
| IP-10 | NT 022760 | Sense 5'-ATTTTGTCCACGTGTTGAGATCA-3' Antisense 5'-TGGCCTTCGATTCTGGATTC-3' |
| CXCR3 | NM 00154 | Probe 5'-FAM-GACATCTCTTTCTCACCCCTCTTTTTCATGTAGCA-TAMRA-3' Sense 5'-ACCCAGCAGCCAGAGCAC-3' Antisense 5'-CAACCTCGGCGTCATTTAGC-3' |
| 18S rRNA | K 03432 | Probe 5'-FAM-CTTGGTGGTCACTCACCTCAAGGACCAT-TAMRA-3' Sense 5'-GCCCCAAGCGTTTACTTTGA-3' Antisense 5'-TCCATTATTCTAGCTGCGGTATC-3' Probe 5'-FAM-AAAGCAGGCCCGACGGCGG-TAMRA-3' |

^aThe probes for interferon-inducible protein-10 (IP-10) mRNA, CXCR3 mRNA, and 18S rRNA were labeled with 6-carboxy-fluorescein (FAM) at the 5' end and with 6-carboxy-tetramethylrodamine (TAMRA) at the 3' end. FAM functioned as the reporter dye and TAMRA as the quencher dye.

location making the biopsy procedure challenging. mRNA profiling of allograft biopsy specimens, including the use of DNA microarray profiling for analysis of human renal biopsies, may refine the mechanistic information gleaned from biopsy specimens [18–21], but such analysis requires invasive tissue sampling as well.

An important goal for the transplant community is the development of noninvasive probes for the assessment of renal allografts. Radermacher et al [22] recently showed that use of Doppler ultrasonography to prospectively measure the renal segmental arterial resistance in more than 600 renal transplant recipients allowed prediction of long-term allograft function even more accurately than did measurements of glomerular filtration rates (GFR). With respect to noninvasive diagnosis of acute rejection, the most serious complication of organ transplantation, Vasconcellos et al [23] showed that heightened expression of perforin, granzyme B, and fas ligand mRNA in peripheral blood leukocytes is a correlate of acute renal allograft rejection, particularly when two or more of these genes are up-regulated [23]. We reported that measurement of mRNAs for cytotoxic proteins perforin and granzyme B in urinary cells offers a noninvasive means of diagnosing acute rejection of renal allografts [24].

In light of preclinical data that IP-10 and CXCR3 participate in the immigration of alloreactive cells in to the allograft as well as in the development of acute rejection, we designed and developed kinetic quantitative polymerase chain reaction (PCR) assay for the measurements of transcripts for IP-10 and CXCR3 in urinary cells and investigated the hypothesis that measurements of mRNAs

encoding IP-10 or CXCR3 in urinary cells noninvasively identify critical cellular traffic causing acute rejection of human renal allografts.

METHODS

Collection of urine samples and kidney biopsies

We obtained 90 urine specimens from 82 kidney transplant recipients (Table 1). Fifty-eight of the 82 patients underwent 63 allograft biopsies to resolve the basis for graft dysfunction and 63 urine specimens were collected within 24 hours of the core needle biopsy of the allograft. All biopsy specimens were evaluated by a single pathologist who was unaware of the results of molecular studies. Using the Banff 97 classification, 27 of the 63 biopsy specimens were classified as acute rejection, 20 specimens as other histologic findings (other), and 16 biopsies as chronic allograft nephropathy. The remaining 27 urine samples were from 24 patients who were classified as having stable allograft function after transplantation. In these patients, the serum creatinine levels either had decreased or had not increased by more than 0.2 mg/dL during the 7 days before and 7 days after urine collection. Immunosuppression consisted of either a cyclosporine-based or tacrolimus-based regimen, and the acute rejection episodes were treated with a course of intravenous methylprednisolone [24]. Antilymphocyte antibodies (CD3 monoclonal antibodies or antithymocyte globulin) were given to patients who had an episode of glucocorticoid-resistant acute rejection.

Isolation of RNA and design of primers and probes

Urine was centrifuged at 10,000g for 30 minutes at 4°C. RNA was isolated from the cell pellet using an RNeasy minikit (Qiagen, Chatsworth, CA, USA), quantified and reverse-transcribed to cDNA as described [24,25]. We designed and synthesized oligonucleotide primers and fluorogenic probes for the measurement of IP-10 and CXCR3 with the use of kinetic quantitative PCR assay (Table 2). We also designed and developed primers and probes for the quantification of 18S ribosomal RNA (rRNA).

Kinetic quantitative PCR assay

mRNA levels of IP-10 and CXCR3 and the levels of 18S rRNA were measured with the use of ABI Prism 7700 sequence detection system (PE Biosystems, Foster City, CA, USA). PCR reaction for each sample was set up in duplicate as a 25 µL reaction volume using 12.5 µL TaqMan Universal PCR Master Mix, 2.5 µL of 1:10 diluted (1:1000 for 18S rRNA measurement) template cDNA, 300 nmol/L of primers, and 200 nmol/L of probe. PCR amplification included an initial incubation at 50°C for 2 minutes and denaturation at 95°C for 10 minutes. This was followed by heating at 95°C for 15 seconds and 60°C for 60 seconds repeated for 40 cycles. The PCR amplicon for 18S rRNA was prepared, quantified, and used for developing standard curves. The standard curves were based on the principle that a plot of the log of the initial target copy number of a standard versus threshold cycle results in a straight line. mRNA levels in the samples were expressed as number of copies per microgram of total RNA isolated from the urinary cells.

Immunohistology

We localized IP-10 and CXCR3 within renal biopsies in those patients with either stable function posttransplant or acute rejection. Cryostat sections were labeled using monoclonal antibodies to IP-10, CXCR3, or isotype-matched controls (Pharmingen, San Diego, CA, USA) and an EnVision immunoperoxidase kit (Dako, Carpinteria, CA, USA), as described [26].

Statistical analysis

We used GraphPad Prism 3.03 Statistical Software for Windows (GraphPad Software, San Diego CA, USA). The levels of mRNA for IP-10 and CXCR3 and 18S rRNA levels deviated significantly from a normal distribution ($P < 0.0001$), which was substantially reduced by use of a log transformation. The natural log mRNA levels were used as the dependent variable in a one-way analysis of variance (ANOVA) to test for any differences among the various diagnostic groups. Dunnett's test for multiple comparisons was then used to control the risk of a type I error while comparing the mRNA levels in the

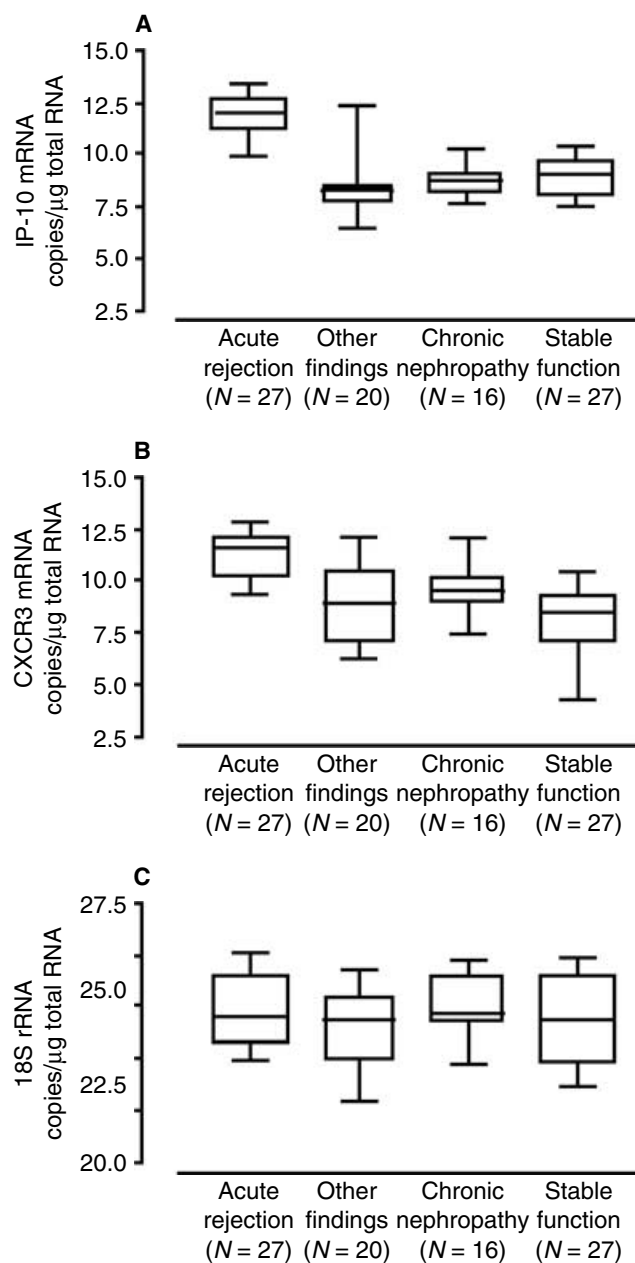


Fig. 1. Levels of mRNA and rRNA in urinary cells. Box and whisker plots show the 10th, 25th, 50th (median), 75th, and 90th percentile values (log) for interferon-inducible protein-10 (IP-10) mRNA (A), CXCR3 mRNA (B), and 18S rRNA (C) in urine samples from patients classified as having an episode of acute rejection, other findings on allograft biopsy, chronic allograft biopsy, or stable course after renal transplantation. The levels of IP-10 mRNA ($P < 0.0001$) and CXCR3 mRNA ($P < 0.0001$), but not those of 18S rRNA ($P = 0.56$), were significantly higher in patients with an episode of acute rejection than in the patients in the other diagnostic groups [P value by one-way analysis of variance (ANOVA)]. Values in parenthesis are the numbers of urine samples.

acute rejection group with those in the “other,” chronic allograft nephropathy, and stable posttransplant groups. The relationship between the levels of mRNA for IP-10 and CXCR3 was estimated with Pearson's correlation. Receiver-operating characteristic curve analysis of

Table 3. Levels of mRNA (copies/ μ g of total RNA) in urinary cells of patients postrenal transplantation^a

| Type of mRNA | Acute rejection (N = 27) ^b | Other findings (N = 20) | Chronic allograft nephropathy (N = 16) | Stable graft function (N = 27) | P value ^c |
|--------------|--|----------------------------|---|-----------------------------------|----------------------|
| IP-10 | 11.9 \pm 0.3 | 9.0 \pm 0.6 | 8.7 \pm 0.3 | 8.9 \pm 0.3 | <0.0001 |
| CXCR3 | 11.7 \pm 0.3 | 8.3 \pm 0.9 | 9.9 \pm 0.5 | 7.5 \pm 0.7 | <0.0001 |
| 18S rRNA | 24.6 \pm 0.3 | 24.0 \pm 0.4 | 24.3 \pm 0.4 | 24.3 \pm 0.3 | 0.56 |

^aCopies of mRNA and rRNA were measured with the use of gene-specific primers and probes by real-time polymerase chain reaction (PCR) assay. Plus-minus values are mean (\pm SE) of the natural logarithm of mRNA and rRNA copy number.

^bValues in parentheses are the numbers of urine samples.

^cP values were calculated with the use of log-transformed mRNA copy numbers as the dependent variable in one-way analysis of variance (ANOVA). Dunnett's test was then used to compare the mRNA copy numbers in samples showing acute rejection with the mRNA copy numbers in each of the three other groups of samples. It showed that copy number of interferon-inducible protein-10 (IP-10) mRNA in urinary cells obtained during an episode of acute rejection was significantly higher than those in specimens with other findings ($P < 0.01$), chronic allograft nephropathy ($P < 0.01$), or stable function ($P < 0.01$). Dunnett's procedure showed that the copy number of CXCR3 mRNA in urinary cells obtained during an episode of acute rejection was significantly higher than those in specimens with other findings ($P < 0.01$) or stable function ($P < 0.01$) but not in specimens with chronic allograft nephropathy ($P > 0.05$). None of the pair-wise comparisons copy numbers of 18S rRNA in urinary cells were significant.

mRNA levels was used to determine cutoff values that maximized the combined sensitivity and specificity for distinguishing patients with acute rejection from those without acute rejection. The area under the curve was calculated, and sensitivity and specificity at the selected cutoffs were determined.

RESULTS

mRNA levels in urinary cells

The level of IP-10 mRNA was higher in urinary cells from patients with acute rejection as compared with those without acute rejection. Figure 1A and Table 3 show IP-10 mRNA levels in urinary cells from the patients with an episode of acute rejection, patients with other findings on allograft biopsy, patients with biopsy evidence of chronic allograft nephropathy, and patients with stable graft function after renal transplantation. The natural log transformed IP-10 mRNA levels were used as the dependent variable in a one-way ANOVA to test for any differences among the four diagnostic groups. IP-10 mRNA levels, measured during an episode of acute rejection, were significantly higher compared with the three other three diagnostic groups ($P < 0.0001$). Dunnett's test for multiple comparisons demonstrated that IP-10 mRNA levels in urinary cells obtained during acute rejection were significantly higher than in those classified as "other" ($P < 0.01$), chronic allograft nephropathy ($P < 0.01$), or stable posttransplant group ($P < 0.01$).

The level of CXCR3 mRNA was also higher in urinary cells from patients with acute rejection as compared with those without acute rejection (Fig. 1B) (Table 3). CXCR3 mRNA levels, measured during an episode of acute rejection, were significantly higher compared with the three other three diagnostic groups ($P < 0.0001$, one-way ANOVA). Dunnett's test showed that CXCR3 mRNA levels in urinary cells obtained during an episode of acute rejection were significantly higher than those classified as "other" ($P < 0.01$) or stable posttransplant ($P < 0.01$) but not chronic allograft nephropathy ($P > 0.05$).

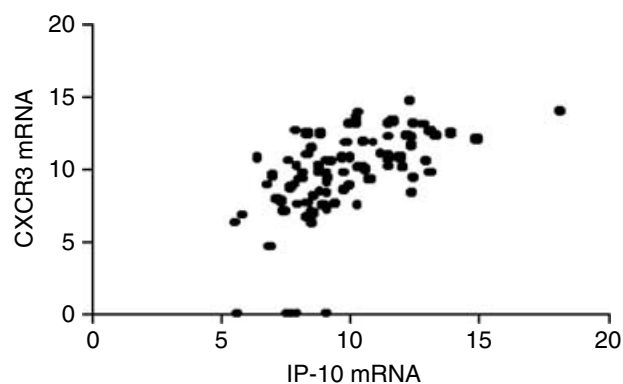


Fig. 2. Coordinated expression of mRNAs for interferon-inducible protein-10 (IP-10) and CXCR3 in urinary cells. The relationship between the levels of IP-10 mRNA and CXCR3 mRNA in urinary cells was estimated with the Pearson correlation, and was significant at $P < 0.0001$.

Figure 1C and Table 3 show that, in contrast to the levels of mRNA for CXCR3 or IP-10, the levels of constitutively expressed 18S rRNA do not vary significantly among the four diagnostic categories ($P = 0.56$).

We used Pearson's correlation to estimate the relationship between the levels of mRNA for IP-10 and CXCR3. Our evaluation revealed a significant and positive correlation between the levels of expression of IP-10 mRNA and CXCR3 mRNA in urinary cells (Fig. 2) ($r = 0.57$, $P < 0.0001$).

Receiver-operating characteristic curve analysis of mRNA levels

The receiver-operating characteristic curves (Fig. 3) show the true positive fractions (sensitivity) and false positive fractions (1-specificity) for various cut points for mRNA levels of IP-10 (Fig. 3A), CXCR3 (Fig. 3B), and 18S rRNA (Fig. 3C). The natural log-transformed cut point (threshold) that maximized the combined sensitivity and specificity for IP-10 mRNA was 9.11 copies/ μ g total RNA (Fig. 3A). At this threshold, the sensitivity was 100% and the specificity was 78% ($P < 0.0001$). The

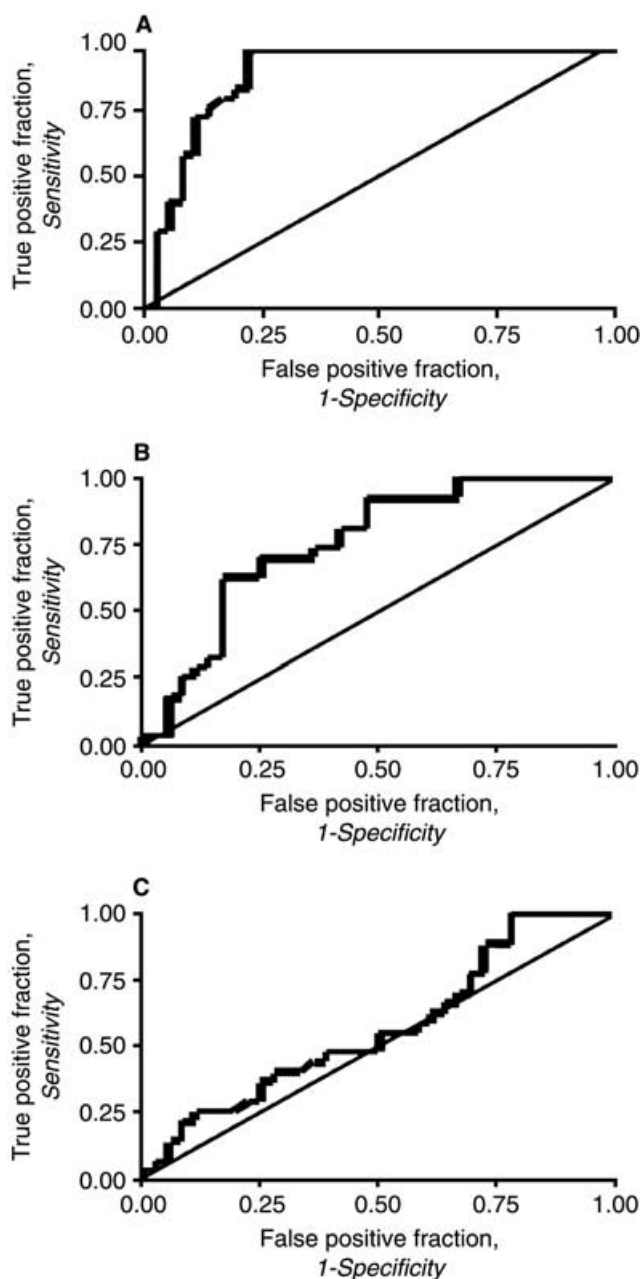


Fig. 3. Receiver-operating characteristic curve of mRNA and rRNA levels. The fraction of true positive results (sensitivity) and false positive results (1-specificity) for interferon-inducible protein-10 (IP-10) mRNA levels (A), CXCR3 mRNA levels (B), and 18S rRNA levels (C), as markers of acute rejection are shown. The calculated area under the curve was 0.903 for IP-10 mRNA levels, 0.762 for CXCR3 mRNA levels, and 0.574 for 18S rRNA levels. A value of 0.5 is no better than expected by chance, and a value of 1.0 reflects a perfect indicator.

calculated area under the curve was 0.903 (95% CI 0.824 to 0.982). The cut point that maximized the combined sensitivity and specificity for CXCR3 mRNA was 11.59 copies/ μ g total RNA (Fig. 3B). At this threshold, the sensitivity was 63% and the specificity was 83% ($P < 0.0001$). The calculated area under the curve was 0.762 (95% CI 0.6444 to 0.881).

Figure 3C shows the receiver-operator characteristic curve for 18S rRNA with respect to presence or absence of acute rejection. 18S rRNA levels did not discriminate acute rejection from other renal diagnoses and the calculated area under the curve was 0.574 (95% CI 0.429 to 0.715).

Localization of IP-10 protein and CXCR3 protein within renal allograft biopsies

We performed immunohistochemical studies of renal allograft biopsy specimens from patients with stable renal function and patients with acute rejection to investigate whether IP-10 and CXCR3 are expressed in a differential fashion in the allograft biopsy specimens. In accordance with mRNA data, only minor staining for IP-10, localized to sparsely distributed interstitial cells, and no CXCR3 expression was observed in the allograft biopsy specimen classified as no acute rejection (Fig. 4C and E); however, dense staining of tubular cells and infiltrating mononuclear cells for IP-10 was observed in the biopsy specimen obtained during an episode of acute rejection (Fig. 4D), and CXCR3 was localized to host mononuclear cells, including leukocytes in areas of tubulitis (Fig. 4F).

DISCUSSION

The new observation that has emerged from our investigation is that cellular traffic into human renal allografts causing acute rejection, a remediable risk factor for allograft failure, can be identified noninvasively by measurements of mRNA for the chemokine IP-10 and the chemokine receptor CXCR3 in urinary cells.

A large number of chemokines are generated at sites of inflammation, although differences in the tempo, cellular source, and pattern of expression occur, partly relating to the type of tissue and partly as to whether acute or chronic injury is involved. On occasion, "spill-over" into the circulation can be detected and may be of diagnostic or prognostic significance. Thus, serum IP-10 levels are a good indicator of disease activity in patients with various autoimmune diseases, including systemic lupus erythematosus [27], new diagnosed type 1 diabetes [28], recent-onset Grave's disease [29], autoimmune liver diseases [30], and relapsing remitting or secondary progressive multiple sclerosis [31, 32]. Although absent in normal kidneys, IP-10 hyperexpression within the kidneys of patients with proliferative glomerulonephritis [33], and IP-10 production by both human glomerular mesangial cells [33, 34] and renal proximal tubular cells [35], following their activation with interferon gamma ($\text{IFN-}\gamma$) and tumor necrosis factor- α (TNF- α), has been communicated. Experimental data are consistent with these reports, with IP-10 expression detected in vivo during glomerulonephritis [34, 36, 37] and tubulointerstitial nephritis [38, 39], and production of IP-10 by mesangial [40–42], tubular [41,

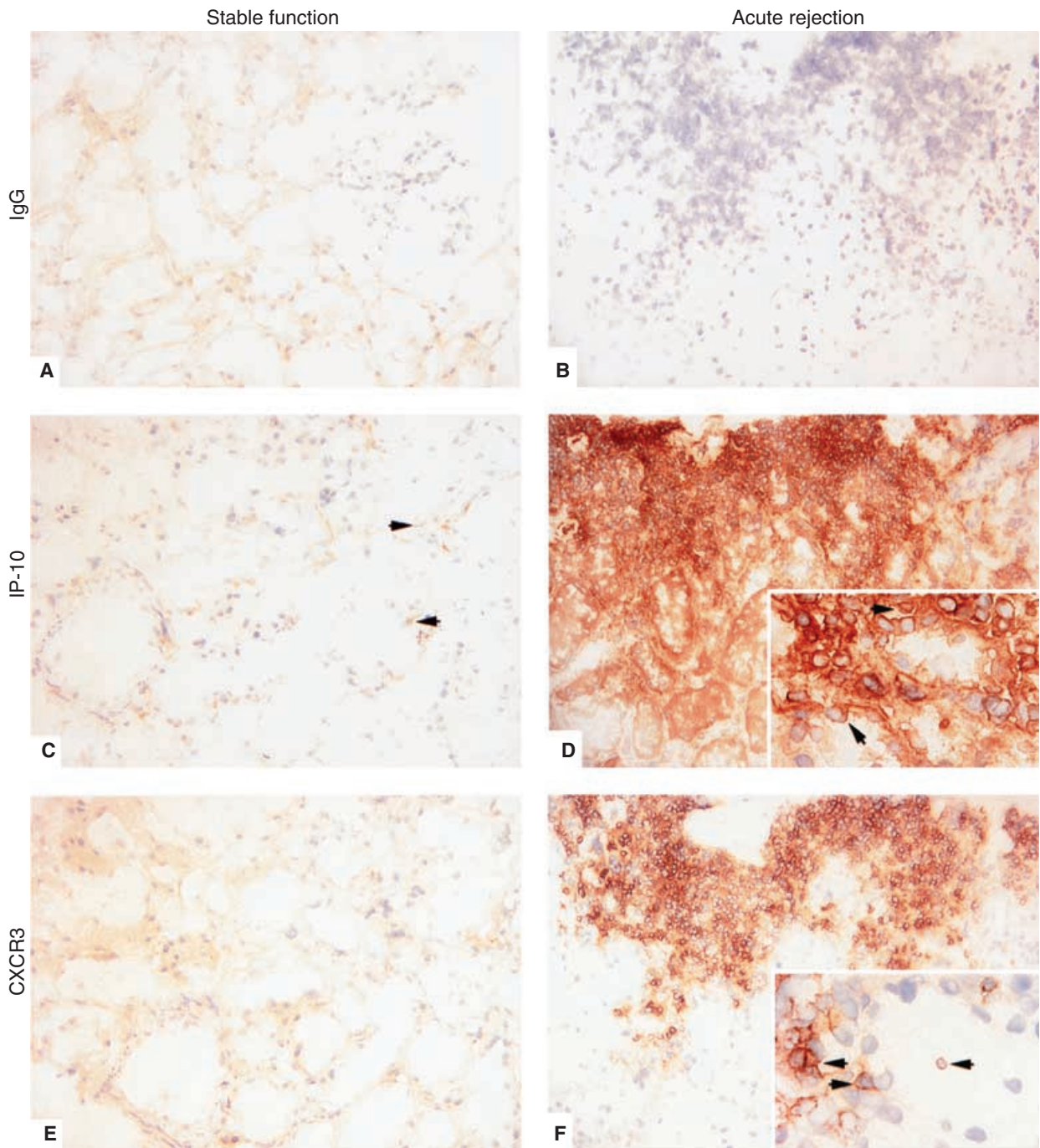


Fig. 4. Immunoperoxidase localization of interferon-inducible protein-10 (IP-10) and CXCR3 within representative renal biopsies of patients with stable graft function or acute rejection. Sections were unstained with control monoclonal antibodies (A and B). IP-10 was expressed by occasional interstitial cells (arrows) during stable renal function (C), but was strongly up-regulated within tubules and infiltrating mononuclear cells during acute rejection (D); inset shows IP-10+ leukocytes crossing tubules. CXCR3 expression was absent during stable renal function (E) but was localized to infiltrating mononuclear cells during acute rejection (F); inset shows several CXCR3+ leukocytes in varying stages of crossing a proximal tubule (cryostat sections, hematoxylin counter-stain, original magnifications $\times 200$ except insets $\times 750$).

42], and interstitial fibroblast [42, 43] cells upon exposure to cytokines in vitro.

Our study was stimulated in part by prior mechanistic data as to the key roles of IP-10 [9] and CXCR3 [5] in cardiac allograft rejection in mice, and on the evidence of

expression of this pathway during episodes of human cardiac [10–12], lung [44], and liver [45] allograft rejection. However, little is known as to IP-10 or CXCR3 expression during an episode of acute rejection of human renal allografts. In the one report dealing with IP-10, Segerer

et al [46] reported IP-10 mRNA expression within several failed renal allografts. Similarly, in the only report regarding CXCR3 expression during renal allograft rejection, intragraft CXCR3+ leukocytes were detected during development of transplant glomerulopathy in patients with chronic allograft nephropathy [26].

Our analyses of urinary IP-10 and CXCR3 mRNA levels indicate the utility of these genes as biomarkers of acute renal allograft rejection. Measurement of IP-10 mRNA proved to be a particularly sensitive marker of acute rejection, with 100% sensitivity. Measurement of CXCR3 mRNA had a lower sensitivity (63%) for acute rejection but a higher specificity (83% vs. 78%) than assay of IP-10. This difference in sensitivity may reflect a delay between the timing of chemokine production and shedding of cells, including potentially tubular as well as host inflammatory cells, and the subsequent recruitment and transmigration of CXCR3+ mononuclear cells through the graft and across the tubular cells (Fig. 4F, inset). Further studies are required to assess the value of longitudinal monitoring postrenal transplant, how mRNA levels for these genes are modulated by antirejection therapy, levels in patients experiencing vascular or mixed vascular and cellular rejection, and whether measurements of mRNAs for IP-10 and CXCR3 provide diagnostic and/or prognostic information capable of further refining noninvasive diagnosis of acute rejection made by measurements of mRNA for cytotoxic proteins granzyme B and perforin [24]. Because IP-10 is expressed by tubular cells as well as mononuclear cells and is an "invitational signal" for CXCR3 expressing CD4 T cells and because granzyme B and perforin are prominently expressed by CD8 T cells, measurements of urinary cell levels of IP-10 and CXCR3, as well as levels of granzyme B and perforin may heighten the diagnostic accuracy.

In biopsies from patients without histologic or functional evidence of acute rejection, as well as in several donor nephrectomy samples, we detected minor IP-10 expression in association with small numbers of renal interstitial cells, and no CXCR3 expression was detected. By contrast, acute cellular rejection was accompanied by dense staining for IP-10 of renal tubules and adjacent interstitial cells, plus infiltrating host mononuclear cells. In addition, CXCR3 was localized to a subset of host mononuclear cells, including in areas of tubulitis, which is one of the diagnostic hallmarks of acute cellular rejection. This tubular staining for IP-10, and the accompanying lymphocyte expression of the sole chemokine receptor for IP-10 and CXCR3 during episodes of acute rejection, provide a compelling explanation for the high degree of accuracy in diagnosing acute rejection by measurements of mRNA for IP-10 and CXCR3 in urinary cells.

The first several months postrenal transplantation remain a critical period for development of acute rejection.

The development of noninvasive techniques for detection of acute rejection and its differentiation from other causes of graft dysfunction, including drug toxicity, ureteral complications, infections, and disease recurrence, are major goals of the transplantation community, especially since the appropriate diagnosis and therapy may be of critical import [47–49]. Multiple or ongoing but unrecognized instances of low-level rejection (e.g., subclinical rejection) are thought to determine long-term survival of an allograft. The monitoring of chemokine and chemokine receptor genes such as described in this communication may be an important new and noninvasive approach for the detection of critical cellular traffic into the allografts and also provide practical laboratory tools useful for the tailoring of immunosuppressive protocols.

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