Abstract. Progressive renal disease is frequently accompanied by renal interstitial inflammation and fibrosis in which the activity of resident fibroblasts may be of central importance. Because there are relatively few fibroblasts in the normal renal interstitium and there is no specific marker to permit their identification, these cells have proved difficult to characterize in vitro. In this study, these cells were isolated and established in culture, using CD90 as a positive selection marker. Antibodies to CD90 bound to tubular epithelial cells and fibroblasts, but not to glomerular cells in kidney sections. In culture, only fibroblasts were CD90-positive. These normal renal cortical fibroblasts (RCF) were α-smooth muscle actin- and vimentin-positive, but desmin-, cytokeratin-, and factor VIII-negative, identifying them as myofibroblasts. They expressed platelet-derived growth factor α and β receptors; CD44; and α2, β1, and β3 integrin chains: this combination of markers was also characteristic of fibroblasts in sections of normal cortex. These cells were positive for ICAM-1 but negative for VCAM-1. Similarly, proliferating or growth-arrested renal cortical fibroblasts (RCF) in culture expressed ICAM-1 but not VCAM-1. The expression of VCAM-1 was detected, however, and that of ICAM-1 was increased on fibroblasts associated with inflammatory infiltrates in sections from fibrotic kidneys, and ICAM-1 and VCAM-1 were up-regulated on RCF in culture after incubation with increasing doses of interleukin-1β or tumor necrosis factor α (maximum between 24 and 48 h). These adhesion molecules were functional, and neutrophils adhered to resting and cytokine-activated RCF. Binding was maximal between 24 and 48 h after cytokine treatment and was inhibited by anti-CD18 antibodies. ICAM-1 is the principal adhesion molecule controlling inflammatory cell infiltration of the interstitium. The study presented here suggests that cortical fibroblasts may be central to the control of this infiltration. (J Am Soc Nephrol 8: 604–615, 1997)
responses of normal cortical cells. Furthermore, the mechanisms that result in the stimulation of the normal fibroblast to adopt the fibrotic phenotype, described by Rodemann and Müller and other investigators (4–7), cannot be addressed by this approach.

To date, no specific surface marker has been described that will differentiate between fibroblasts and morphologically similar cells. In particular, with relevance to the kidney, although mesangial cells form hillocks in culture and fibroblasts do not, the two cell types otherwise have a very similar morphological phenotype. For this reason, the study presented here describes the detailed phenotypic characterization of the normal human renal cortical fibroblasts (RCF) in culture and compares this characterization with that of cells identified on tissue sections. This study also presents evidence that there is a specific marker (CD90) that will enable the routine isolation of the cells in the future.

Materials and Methods

Cell Culture

Primary human RCF cultures were established from 1-mm³ pieces of normal or fibrotic cortex of kidneys obtained at nephrectomy. The explants were cultured in Dulbecco’s modified Eagle medium (DMEM) supplemented with 2 mM l-glutamine, 0.02 M HEPES, 100 U/mL of penicillin/streptomycin (Basic Medium) and 10% fetal calf serum (FCS). All tissue-culture reagents were obtained from JRH Biosciences, Sera-Lab Ltd, Sussex, UK, except for the FCS, which was obtained from Biological Industries, Ward Park South, Cambridge, UK. Human lung fibroblasts (AG02262) were purchased from N.I.A Ageing Cell Repository, Corriel Institute, Camden, NJ, and were used for comparison in all experiments.

Magnetic Cell Sorting

To facilitate the positive selection of fibroblasts, mixed cultures of kidney cells (glomerular mesangial and tubular epithelial cells) were treated with trypsin/EDTA to remove the cells from their culture flasks, and were then resuspended in a solution containing 0.5% BSA, 0.2 mM EDTA in phosphate-buffered saline, pH 7.3 (PBS), together with a monoclonal antibody raised against human CD90 (a generous gift from Dr Julian Miller, Cymbus Bioscience Ltd, Southampton, UK). After 20 min of incubation at 4°C, the cells were pelleted by centrifugation and washed twice. Cells resuspended in buffer were mixed with magnetic beads (50 nm in diameter) coated with goat anti-mouse immunoglobulin (Ig) G and incubated for 30 min. The separation of bound cells was carried out using the Mini Macs system (Eurogenetics UK Ltd, Teddington, UK). Morphological and immuno histochemical analysis of the cells 24 h after seeding showed that selected cells were >95% viable (by trypan blue exclusion) after separation and had proliferative rates comparable with those of cells that had not been through the separation system.

Proliferation Assays

The proliferation of RCF in culture was quantitated by direct counting of trypsinized cells and also by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay (17). For direct counting, cells were seeded at 10⁴ cells/well in 24-well plates. RCF were then grown in Basic Medium containing concentrations of FCS from 0.1 to 10% and for periods of up to 120 h. Incubations were ended by trypsinizing the cells and counting them in a hemocytometer.

In addition, the MTT assay was used to estimate RCF numbers and was performed on RCF cultured in the wells of 96-well plates for periods of up to 120 h. RCF were seeded at 2 × 10⁵ cells/well and grown in Basic Medium supplemented as above for periods of up to 120 h. The proliferative responses of the cells to different concentrations of FCS were established by seeding RCF at 2 × 10⁵ cells/well in 96-well plates, allowing them to establish in 10% FCS for 48 h, and then replacing the culture medium with Basic Medium containing concentrations of FCS from 0.1 to 10%. Cultures were monitored for periods of up to 120 h. At the end of incubations, 100 µL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (Sigma Chemical Co., Aldrich, Poole, UK) (1.25 mg/mL in Basic Medium) was added to the RCF monolayers. After 4 h at 37°C, the formazan precipitate was solubilized by overnight incubation with 10% sodium dodecyl sulfate-HCl. Absorbance was read at 600 nm. In preliminary experiments, the lower limit of sensitivity of the MTT assay was established as equivalent to 880 ± 79 cells, and there was a correlation coefficient of 0.95 between cell number and absorbance at 600 nm in the MTT assay.

Immunohistochemistry

Cortex obtained at nephrectomy from normal or fibrotic kidneys was snap-frozen, and 5 µm sections were fixed in cold 70% acetone: 30% methanol. Endogenous peroxidase activity was eliminated by incubation with 3% H₂O₂ in PBS for 5 min. Sections were blocked in 1% BSA in PBS before incubating with primary monoclonal antibody or polyclonal antiserum for 1 h at 37°C (see Table 1 for details of the antibodies used in this study). Normal mouse or normal rabbit antiserum at 1/500 dilution were used as controls. In addition, the antibodies to vimentin, cytokeratin, α-smooth muscle actin, CD90, and desmin were all mouse IgG, and therefore acted as internal controls. Horse-radish peroxidase conjugated anti-mouse or anti-rabbit secondary antibodies (Dako Ltd, High Wycombe, Bucks UK) were added, and the peroxidase was visualized with dianobenzidine (Sigma/Aldrich) after 1 h. Sections were counterstained with hematoxylin.

Characterization of Cultured Cells

The source and specificity of antibodies used in this study is shown in Table 1.

Immunohistochemistry was performed on cells grown in 8-well glass-chamber slides (Nunc, Gibco/BRL Life Technologies Ltd, Paisley, UK). For the identification of intracellular antigens, the cells were fixed in cold 70% acetone:30% methanol, and, for the identification of cell-surface antigens, cells were fixed in cold acetone alone. The slides were then blocked and treated with primary antibody or control sera as before. Primary antibody binding was detected as above or by using fluorescein isothiocyanate–conjugated secondary antibodies.

Staining for PDGFα and β receptors was performed on newly adhered fibroblasts that had been grown overnight afterpassaging. These conditions allow optimal receptor visualization (18). The fibroblasts were fixed in 100% methanol, followed by a 5-min wash in 1% Tween 20 (Sigma/Aldrich) in PBS, before being blocked with 1% BSA in PBS. The anti-PDGF receptor antibodies (Genzyme, West Malling, UK) were used at 50 µg/mL and visualized with anti-mouse IgG fluorescein isothiocyanate conjugate (Dako) (18).

For intercellular adhesion molecular analysis, cells were rested for 72 h in 0.1% FCS before being stimulated for periods of up to 48 h with recombinant human interleukin 1β (IL-1β) or tumor necrosis
factor alpha (rhTNFα) (kind gifts from Dr. G. R. Adolf, Ernst-Boehringer Institute, Vienna, Austria).

**Preparation of Neutrophils**

Polymorphonuclear neutrophils (PMN) were prepared from citrated peripheral blood by dextran sedimentation and Ficoll-Paque (Pharmacia Biotech, Leicester, UK) density separation (19). Contaminating erythrocytes were removed by hypotonic lysis. More than 95% of the cells were PMN by morphology.

**Neutrophil Adhesion Assay**

Human RCF in 10% FCS were established in the wells of 96-well plates. After resting for 72 h in 0.1% FCS, RCF were exposed to various concentrations of recombinant cytokines for periods of up to 48 h. The monolayers were washed, and neutrophils (1 × 10⁶ cells/mL) in 100 μL DMEM were added to each well. The plates were incubated for 30 mm at 37°C and then washed twice to remove nonadherent neutrophils. The number of adherent neutrophils was calculated using a coborimetric assay based on intracellular myeloperoxidase activity. In brief, a citrate-phosphate buffer (pH 4) containing 0.5 mg/mL ABTS substrate (2,2'-Azino-bis(3-ethylbenzthioline-6-sulfonic acid; Sigma/Aldrich) and 0.01% H₂O₂ and 1% Triton-X100 (BDH Ltd., Poole, UK) was added to each well. This was also done to separate wells containing increasing numbers of neutrophils (as a standard curve). The released intracellular myeloperoxidase activity was estimated at an absorbance of 410 nm.

**Blocking of Adherence with Monoclonal Antibodies**

Neutrophils were incubated for 20 min at 37°C with a mononclonal antibody against CD18 (mAb 15/7; a kind gift from R. Rothlein, Boehringer Ingelheim, Ridgefield, NJ), which was diluted in DMEM to various concentrations up to 5 μg/mL. The neutrophils were added, in the presence of anti-CD18 antibody, to fibroblasts that had been stimulated with TNFα for 24 h, and the incubation continued for 30 min. Adherent neutrophils were quantified as discussed above.

**Analysis of ICAM-1 and VCAM-1 mRNA**

Time-dependent changes in mRNA expression for ICAM-1 and VCAM-1 were assayed using reverse transcription-polymerase chain reaction (RT-PCR). Total cellular RNA was isolated by lysing cells in 4 M guanidine isothiocyanate and centrifugation through 5.7 M cesium chloride in 0.1 M EDTA. One microgram of total fibroblast RNA was denatured at 95°C for 5 min, in the presence of 100 pmol random hexamers pd[N]₆ (Pharmacia Biosystems Ltd, Milton Keynes, UK), and cooled on ice for 2 min. The RNA was reverse-transcribed in a 20-μL final volume of 1× PCR buffer (10 mM Tris (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.001% gelatin), 6.25 μM of each dNTP, 20 U of RNasin (Gibco/BRL Life Technologies Ltd), 10 mM dithiothreitol, and 200 U of reverse transcriptase (Gibco/BRL Life Technologies Ltd). The reaction mixture was incubated at room temperature for 10 min, at 42°C for 40 min, and at 95°C for 5 min. Two microliters of cDNA product was PCR-amplified in a 50-μL final volume of 10 mM Tris (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 200 μM dNTP, 0.001% gelatin, 0.5 μM each primer, and 1.25 U of AmpliTaq DNA polymerase (Applied Biosystems Ltd, Warrington, UK) for 26 cycles in a Perkin Elmer 480 thermocycler (Applied Biosystems Ltd). Heating to 72°C for 10 min was followed by one cycle of 94°C for 3 min, 55°C for 1 min, 72°C for 1 min, 26 cycles (ICAM-1) or 34 cycles (VCAM-1) each of 94°C for 40 seconds, 55°C for 1 min, 72°C for 1 min, and finally 1 cycle of 94°C for 40 s and 60°C for 10 min on a thermocycler.

Primers for VCAM-1 and ICAM-1 were designed from the following published cDNA sequences and were obtained from Genosys, Cambridge, UK:

**ICAM-1**

<table>
<thead>
<tr>
<th>Primer DNA sequence</th>
<th>Product size</th>
</tr>
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<tr>
<td>5′-TCCAGAAGGTTGAAACTGGG-3′</td>
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</tr>
<tr>
<td>5′-GCAAGTCTTAGTGCACACCCTGGG-3′</td>
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</tr>
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**VCAM-1**

<table>
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<th>Primer DNA sequence</th>
<th>Product size</th>
</tr>
</thead>
<tbody>
<tr>
<td>5′-CATGACCTGTTCCAGGCGAGG-3′</td>
<td>523/247 bp</td>
</tr>
<tr>
<td>5′-GTAAGTGCTCCGGTGAGG-3′</td>
<td></td>
</tr>
</tbody>
</table>

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**Table 1. Details of antibodies used**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Clone*</th>
<th>Crossreactivityb</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD9O</td>
<td>Fl5-42-I</td>
<td>Specific</td>
<td>Cymbus</td>
</tr>
<tr>
<td>Vimentin</td>
<td>V9</td>
<td>Specific</td>
<td>Dako</td>
</tr>
<tr>
<td>α-smooth muscle actin</td>
<td>IA4</td>
<td>Specific</td>
<td>Dako</td>
</tr>
<tr>
<td>Desmin</td>
<td>D33</td>
<td>Specific across species</td>
<td>Dako</td>
</tr>
<tr>
<td>Cytokeratin</td>
<td>CY90</td>
<td>Specific</td>
<td>Sigma</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>F8/86</td>
<td>Specific</td>
<td>Dako</td>
</tr>
<tr>
<td>Myosin</td>
<td>Polyclonal</td>
<td>Specific across species</td>
<td>Sigma</td>
</tr>
<tr>
<td>β1 integrin chain</td>
<td>mAB 13</td>
<td>Specific</td>
<td>Becton Dickinson</td>
</tr>
<tr>
<td>β3 integrin chain</td>
<td>RUU-PL7F12</td>
<td>Specific</td>
<td>Becton Dickinson</td>
</tr>
<tr>
<td>α2 integrin chain</td>
<td>PIE6</td>
<td>Specific</td>
<td>Becton Dickinson</td>
</tr>
<tr>
<td>CD44</td>
<td>K176</td>
<td>Specific</td>
<td>Becton Dickinson</td>
</tr>
<tr>
<td>PDGFα receptor</td>
<td>1264-00</td>
<td>Specific to primates</td>
<td>Genzyme</td>
</tr>
<tr>
<td>PDGFβ receptor</td>
<td>1263-00</td>
<td>Specific to primates</td>
<td>Genzyme</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>LB2</td>
<td>Specific</td>
<td>Becton Dickinson</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>E1/6</td>
<td>Specific</td>
<td>Becton Dickinson</td>
</tr>
<tr>
<td>ELAM-1</td>
<td>H18/7</td>
<td>Specific</td>
<td>Becton Dickinson</td>
</tr>
</tbody>
</table>

* All antibodies were used at the manufacturer’s recommended dilutions.

b Crossreactivity: specific, specific for human antigen; specific across species, recognizes the same antigen in more than one species.
\[
\begin{align*}
\alpha-\text{actin} & \quad 3' \ G \ G \ G \ A \ G \ C \ A \ T \ G \ A \ T \ T \ T \ G \ A \ T \ C \ T \ T \ T \ 5' \\
& \quad 5' \ T \ C \ C \ T \ G \ A \ G \ G \ T \ A \ C \ G \ G \ G \ T \ C \ T \ T \ C \ C \ 3'
\end{align*}
\]

Product size: 204 bp

Laboratories Ltd, Hemel, Hemstead, UK), and the density of the bands were compared with those of the housekeeping gene (\(\alpha\)-actin). Results were expressed as ratios compared with \(\alpha\)-actin.

**Statistical Analysis**

The paired \(t\) test was used to assess significant differences between treatments.

Figure 1. Peroxidase immunohistochemistry of the normal human cortical interstitium. Tissue was stained with (A) anti-\(\alpha\)-smooth muscle actin, (B) anti-vimentin, (C) anti-CD90, and (D) anti-desmin. Interstitial cells were \(\alpha\)-smooth muscle actin-, vimentin-, and CD90-positive but desmin-negative. (Original magnification, \(\times310\).)
Results
Characterization of Interstitial Cells in Kidney Sections

Kidney sections from normal kidneys and from kidneys with interstitial lesions were stained with a range of monoclonal antibodies raised against cytoskeletal proteins and cell-surface antigens.

Spindle-shaped fibroblastic cells within the interstitium of normal kidneys stained for α-smooth muscle actin and were vimentin-positive, CD90-positive, but desmin-negative (Figure 1). These cells also expressed the PDGFα and β receptors, ICAM-1, CD44, and α2, β1, and β3 integrin chains (Table 2). The smooth muscle of arterioles within the interstitium was positive for CD90, vimentin, α-smooth muscle actin, and desmin (not shown).

Although the epithelial cells of the tubules and of Bowman’s capsule were CD90-positive, structures within the glomerulus did not stain with anti-CD90 antibodies (Figure 2A), suggesting that the observed staining was specific for an epitope that was not expressed within the glomerulus. The widening of the interstitium that occurs during fibrosis was clearly visible in sections of fibrotic kidneys, as was the presence of increased numbers of CD90 (Figure 2B), vimentin-, and α-smooth muscle actin–positive interstitial cells. There was also an increase in CD90 staining that was associated with the interstitial matrix but not with the mesangial matrix or glomerular basement membrane. These interstitial cells were not of smooth muscle origin and remained desmin-negative. Cells within the glomerulus did not change their pattern of staining and remained CD90-negative (Figure 2B).

Immunomagnetic Separation

Approximately 5 to 10% of all nephrectomy specimens gave rise to fibroblast cultures that were free of contaminating cells. A further 5 to 10% of cultures were heterogeneous mixtures of fibroblasts, epithelial cells, and mesangial cells. The reasons for this low rate of success are not known. Digesting the tissue with either collagenase or trypsin before seeding the explants made no difference to the success of the primary outgrowth, and tubular epithelial cells overgrew fibroblastic cells in all situations of mixed co-culture. Morphological and histochemical analysis indicated that these cells were >95% fibroblasts, as were the cells isolated from mixed co-cultures by magnetic beads (Figure 3). These cells were vimentin-positive, cytokeratin-negative, and desmin-negative (Table 3), and stained positively for α-smooth muscle actin and CD90 (Figure 4). In all

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Normal Interstitial Cells</th>
<th>Fibrotic Interstitial Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD90</td>
<td>+++</td>
<td>++++</td>
</tr>
<tr>
<td>Vimentin</td>
<td>+++</td>
<td>++++</td>
</tr>
<tr>
<td>α-smooth muscle actin</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>Desmin</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cytokeratin</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>β1 integrin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>β3 integrin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>α2 integrin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD44</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>PDGFα receptor</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>PDGFβ receptor</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

* The intensity of staining was assessed independently by two observers and scored as − (none), + (very weak), ++ (weakly positive), +++ (positive), ++++ (strongly positive), or +++++ (very strong).
experiments, cells were used when they were between 80% and 90% confluent.

**Characterization of Cultured Interstitial Cells**

All CD90-positive fibroblasts cultured from explants of normal kidney cortex were examined for cytoskeletal protein expression. All exhibited an identical cytoskeletal profile and were positive for vimentin, α-smooth muscle actin, CD90, and myosin, but not for cytokeratin, factor VIII, or desmin (Table 3). The degree of staining of cells in culture was highly consistent between individual cultures. Only α-smooth muscle actin staining varied in intensity. However, although some cells stained weakly, the majority were strongly positive. In routine culture in our laboratory, glomerular mesangial and epithelial cells and proximal tubular epithelial cells were all CD90-negative (data not shown). No differences in phenotypic profile were observed between RCF from normal or fibrotic kidneys. In comparison, lung fibroblasts had the same profile, except that they did not express α-smooth muscle actin (Table 3).

In 10% FCS, normal RCF had a similar doubling time (approximately 3 days) to that of lung fibroblasts. Both grew more slowly, however, than RCF from fibrotic kidneys (Figure 5). RCF from normal or fibrotic kidneys had a similar lifespan.

**Table 3. Immunophenotyping of interstitial fibroblasts in culture**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Lung Fibroblasts</th>
<th>Normal RCF†</th>
<th>Fibrotic RCF†</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD90</td>
<td>++ +</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Vimentin</td>
<td>+++ +</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>α-smooth muscle actin</td>
<td>+   +</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Desmin</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cytokeratin</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Myosin</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>β1 integrin</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
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<td>β3 integrin</td>
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<td>++</td>
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<tr>
<td>PDGFβ receptor</td>
<td>++</td>
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</tbody>
</table>

*The intensity of staining was assessed independently by two observers and scored as – (none), – + (very weak), + (weakly positive), ++ (positive), +++ (strongly positive), or ++++ (very strong).
† RCF, renal cortical fibroblasts.
in culture and maintained their morphology for between eight and 11 passages. Thereafter, large senescent cells appeared. All experiments were carried out using cells between their second and eighth passage. Fibroblasts from both normal and fibrotic kidneys became quiescent after 72 h in medium with serum concentrations below 1% (Figure 6). These conditions were routinely adopted to maintain cells in a nonproliferative state for subsequent adhesion molecule analysis.

**Intercellular Adhesion Molecule Expression**

We examined the expression of the intercellular adhesion molecules ICAM-1 and VCAM-1 on interstitial fibroblastic cells and compared this to their expression on lung fibroblasts.

Immunofluorescence demonstrated the presence of ICAM-1 at low levels on cultured cortical fibroblasts and lung fibroblasts in 10% FCS and also on cells made quiescent by incubation in medium containing 0.1% FCS. There was a time- and concentration-dependent increase in ICAM-1 expression, however, in response to IL-1β or TNFα, which became maximal at 24 to 48 h. IL-1β and TNFα were equally potent inducers of adhesion molecules. For clarity, only experiments with TNFα are shown (Figure 7, A and B).

In contrast, quiescent cells stained poorly for VCAM-1, and there was a variable and heterogeneous increase in VCAM-1 expression induced by cytokine treatment (Figure 7, C and D). ELAM-1 was not expressed under any growth conditions or as a result of cytokine treatment and therefore served as a control for the expression of other adhesion molecules. Using reverse transcription and PCR, we detected ICAM-1 and very low levels of VCAM-1 mRNA in both quiescent and cytokine-stimulated cells. There was an increase in message over 4 h for both ICAM-1 and VCAM-1 in TNFα-treated cells (Figure 8).

**Leukocyte Binding to RCF**

Neutrophil binding to TNFα-activated RCF, during a 30-min incubation, was increased above resting levels, and reached a maximum at 24 to 48 h after RCF exposure to TNFα. At 48 h of TNFα treatment, this binding was twofold that of resting fibroblasts (Figure 9).

Monoclonal antibodies directed against CD18 inhibited this binding in a dose-dependent manner, whereas there was no effect of an irrelevant IgG1 isotype-matched control. At 5 μg/mL antibody, there was maximal inhibition of 58.2 ± 9.2% (mean ± SE; N = 5, P < 0.05).

**Discussion**

The isolation and characterization of the human cortical interstitial fibroblast from normal kidney tissue by using the CD90 cell-surface marker, is presented in this report. The use of this marker allowed the specific sorting and characterization of fibroblastic cells, even in the presence of morphologically
similiar mesangial cells. CD90 was identified in sections of normal kidney on interstitial cells, proximal tubular epithelium, and on the uroepithelial cells of Bowman’s capsule. In agreement with previous studies (20–22), the location of this antigen was specific, and no intraglomerular staining was observed. CD90, otherwise known as Thy-1, is an antigen that is present on a variety of tissues but shows considerable variation in distribution between species. It is a GPI-anchored protein of approximately 21 kD that is present on the cell membranes of brain cells (23,24). In renal tissue, it has been described principally as a marker of rodent mesangial cells. Recently the localization of human CD90 to proximal tubular epithelium and to Bowman’s capsule has been presented (20–22), but whereas CD90 is thought to play a role in the transduction of proliferation signals (25,26) and as a form of adhesion protein (27,28), a definite function for the molecule has not been described. Interestingly, we observed that anti-CD90 antibodies recognized epitopes associated with interstitial extracellular matrix and that this staining was increased in fibrotic kidneys. There was no concomitant staining, however, of the glomerular matrix. Whether this suggests the release of this glycoprotein from the cell surface and its association with specific components of the interstitial matrix is not known.

Mesangial and epithelial cells isolated from the glomerulus and placed in culture did not express the CD90 antigen, and proximal tubular cells in primary culture lost their positive staining. Only fibroblastic cells retained the expression of CD90 in culture. This antigen was used, therefore, as a marker for the cells in culture. A functional role for CD90, however, was not investigated in this study. The populations of fibroblastic CD90-positive cells isolated using this marker allowed us to perform extensive characterization of other cell-surface antigens expressed by these cells in culture.

Previously it has been difficult to isolate fibroblasts from the normal renal interstitium because of their relatively low numbers, compared with other cell types, and the lack of specific markers to ensure reliable characterization. Fibroblasts from fibrotic kidneys have been characterized more successfully (4–7). More recently, viral transformation of isolated cell lines has allowed comparisons to be made with fibroblasts isolated from other tissues, such as skin (15,16). These studies have begun the preliminary analysis of renal fibroblast functions and have demonstrated that the transformed cells synthesize and respond to cytokines that initiate a large number of morphological and functional changes. Many of the responses of the cells, however, are likely to be affected by the process of transformation. For example, the transformed cells from both normal and fibrotic kidneys lost their sensitivity to the proliferative effects of FCS and continued to proliferate in serum-free conditions. In contrast, normal (nontransformed) skin fibroblast growth was absolutely dependent on serum in the culture medium. We now demonstrate that RCF isolated from normal kidney cortex become quiescent in serum-free conditions and need a concentration of greater than 1% FCS to stimulate proliferation in vitro. Thus it is essential to select the appropriate conditions for individual studies. The report presented here will facilitate the routine and reproducible isolation and culture by other investigators of normal renal fibroblasts.

This will allow the study of nontransformed cells expressing a phenotype identical to that of interstitial fibroblasts described by others (4–7,10,15,16) and which we have now demonstrated is very similar to that expressed in vivo (14,21–23).
Fibroblasts within the cortical interstitium have a well-developed endoplasmic reticulum, indicating a high capacity for protein synthesis (29). They synthesize many of the components of the interstitial matrix, such as fibronectin, laminin, glycosaminoglycans, and collagens. Fibroblasts in other tissues contribute to the composition of the surrounding matrix through the synthesis of proteolytic enzymes and their inhibitors (11,30). Fibroblasts respond to numerous cytokines produced by cells of the immune system and also synthesize and secrete a variety of growth factors with pleiotropic effects (31–34). There is a degree of diversity, however, between the phenotype of fibroblasts analyzed in vivo and in vitro. In culture they become flattened and polarized and develop actin-stress fibers and intercellular gap junctions. Phenotypic changes have also been characterized in vivo in the cells found in the granulation tissue of healing wounds. These myofibroblast cells have both fibroelastic and smooth muscle-like properties and provide the force for wound contraction. Although all fibroblasts express the interstitial filament protein vimentin, the contractility characteristic of myofibroblasts is associated with the expression of α-smooth muscle actin. Cortical interstitial myofibroblasts have been described previously in sections of normal human kidneys (14), and it is possible that they have a contractile role in maintaining the structure of the renal cortex. Adhesive interactions between myofibroblasts and collagen fibrils that result in the contraction of the collagen matrix are mediated by the α2β1 integrin (35,36). Because cultured RCF in the study presented here expressed both β1 and β3 integrin chains, they would be expected to interact with a variety of matrix molecules. The expression of the α2 integrin chain in combination with the β1 chain suggests that RCF are able to bind to and remodel collagenous extracellular matrices (37). No detailed analysis of other associated α-chains has yet been performed to allow specific interactions to be identified.

In addition to extracellular matrix adhesion molecules, the constitutive expression of ICAM-1 was demonstrated on quiescent (serum-deprived) cells. This reflected the reported expression of ICAM-1 on the interstitial cells of normal kidneys (reviewed in reference 38). ICAM-1 expression on cultured RCF was augmented by incubation with inflammatory cyto-

Figure 7. Immunofluorescence staining of cultured normal RCF stained for ICAM-1 (A, B) and VCAM-1 (C, D). Cells were cultured in 0.1% FCS for 72 h, followed by a further 24 h with either medium alone (A, C) or medium containing 10^{-10} M TNFα (B, D). (Original magnification, ×310.)
Figure 8. Time-dependent increase in ICAM-1 or VCAM-1 mRNA. Growth-arrested normal RCF were stimulated with $10^{-10}$ M TNFα for the times indicated and after RT-PCR. ICAM-1 (A) and VCAM-1 (B) were separated from the α-actin PCR product by flat-bed electrophoresis. The results express the densitometric ratio of ICAM-1 product or VCAM-1 product to α-actin at each time point. One representative experiment is shown.

=kines. Furthermore, this correlated with a functional increase in the ability of the cells to bind leukocytes, which was inhibited by monoclonal antibody to CD18, the β chain of the ICAM-1 counter receptors on neutrophils. The confirmation of functional ICAM-1 on RCF is important because in other systems, the expression of the ICAM-1 molecule has not always correlated with its functional capacity (39). Under resting conditions, there was a very low expression of VCAM-1, which was only moderately induced by cytokine treatment. Interestingly, the alternatively spliced VCAM-1 RNA was also detected and amplified by the primers used in this study, because the primers recognize sequences in domains 3 and 4 of the VCAM sequence, into which the extra 276-base pair of the splice variant is inserted (40). These results reflected the reported distribution of these molecules in the normal renal interstitium and their increase in disease (41). Recent studies have described the localization of leukocytes adherent to interstitial fibroblast cells expressing ICAM-1 and demonstrated (42) a clear relationship between the increased expression of ICAM-1, the infiltration of leukocytes, and the degree of tissue damage in the interstitium of rats with experimental glomerulonephritis.

Figure 9. Increasing neutrophil adherence to normal RCF with time of TNFα pretreatment. Growth-arrested normal RCF monolayers were incubated for the times indicated with $10^{-10}$ M TNFα. The neutrophil adhesion assay was then performed (see the Methods section) and numbers of adherent neutrophils calculated from standard curves run in parallel. The results were corrected for the unstimulated control values at each time point and are expressed as mean ± SE for five experiments, each performed on RCF cultured from different nephrectomy specimens. * $P < 0.05$; ** $P < 0.01$. 
study is a report of the comprehensive analysis of the phenotypic character of fibroblastic cells isolated from the normal human cortical interstitium. Although Rodemann and Müller (4–7) previously established renal fibroblasts with a similar phenotype in culture, they did not separate cortical tissue from tissue from the medulla, and, thus, their cells were not isolated specifically from the renal cortex. Indeed, in subsequent reports by Lonnemann et al (15,16), their methods of isolation and culture are referenced for the isolation of medullary fibroblasts. Future extensions of our study may confirm findings in animal models (43,44) and identify important phenotypic and functional differences between fibroblasts in the cortical and medullary compartments.

Fibroblasts grown from normal or fibrotic kidney biopsy material by clonal selection have been described as having several premiotic and postmotic phenotypes (4–7). In addition, the heterogeneous nature of fibroblasts isolated from the gingiva, lung, and dermis is well recognized (8,9,11,15,16,45). Collagen and C1q receptors have been used to identify some of the differences between subpopulations of fibroblasts from the same source (45). The expression of CD90 antigen has also been used previously to identify subpopulations of fibroblasts (41). We have described a remarkably homogeneous population of CD90-positive RCF in our studies. In all kidney sections examined, interstitial cells stained CD90-positive. However, given the heterogeneity of CD90 expression on fibroblasts from other tissues, we cannot exclude the possibility that there are subpopulations of CD90-negative fibroblasts that were not identified in this study. Further studies may reveal other antigens that could be used to identify other fibroblast populations, such as those recently identified in subpopulations of cells within breast tumors (46,47), which showed differential expression of CD10, CD13, and CD26. In addition, because dendritic cells also occupy the interstitial space, the identification of markers differentiating between fibroblasts and dendritic cells will also be important (48).

Normal RCF have been characterized and shown to express a contractile, myofibroblastic phenotype. Given their potential for expression of a wide range of matrix and functional cell adhesion molecules, the experimental manipulation of these cells in culture will provide important information on the role of RCF in the normal kidney and in a variety of disease states.

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