Dear Editor:

Mesangial cell (MC) proliferation and extracellular matrix production play a central role in the progression of chronic renal disease, and MC culture using rodent and human cells has been extensively employed as a model for the study of glomerular disease in vitro. Fibroblasts share numerous characteristics with MC, including similarities in cytoskeletal protein composition, extracellular matrix production, Thy 1.1 expression, cytokine elaboration, and contraction in response to vasoactive peptides (1,13). Unlike other resident glomerular cell types, however, MC lack a specific marker. Verification of the purity of MC cultures thus requires the use of both exclusion and confirmation criteria.

Fibroblast exclusion is a particular problem in the primary culture of cells of mesenchymal origin. Fibroblasts are widely distributed as stromal cells, and fibroblasts grow readily in vitro. Consequently, fibroblast contamination is difficult to eliminate in primary cultures of cells derived from explant outgrowth or enzymatic cell dissociation techniques. Numerous techniques have been developed to select against fibroblasts and enhance the cellular homogeneity of primary cultures. These techniques include differential adherence, detachment and digestion, clonial selection, fluorescence-activated cell sorting, and the use of selective media (4). Many investigators studying rodent and human MC have used selective media in which the L-isomer of valine is substituted with the D-isomer to select against renal fibroblasts, which are thought to lack D-amino oxidase (2,5). Although D-amino oxidase is highly expressed in kidney in comparison with other tissues, this technique has not met with uniform success in the exclusion of fibroblasts (6,12). We have also found similar growth in primary cultures of canine fibroblasts maintained in selective or nonselective media.

Mesangial cells contain desmin, an intermediate filament protein characteristic of cells of myogenic differentiation (1,9). In contrast, desmin is generally considered to be absent from both cultured fibroblasts and most fibroblasts in vitro (1,10). Desmin has been described in a few nonmyogenic cell types, including hepatic perisinusoidal (Ito) cells (11), human intestinal pericryptal cells (11), and a subpopulation of myofibroblasts found in hypertrophic scars and fibromatoses (10). Therefore, we have chosen to confirm fibroblast exclusion from primary cultures of canine MC using expression of desmin as a marker of MC differentiation.

Glomeruli were obtained from fresh kidneys aseptically collected from nephrectomized or euthanized adult dogs of either sex (n = 3). The glomeruli were isolated using a differential sieving technique (7) and suspended in RPMI 1640 medium containing 20% fetal calf serum (FCS) and antibiotics (100 U penicillin/ml, 100 μg streptomycin/ml, and 25 μg amphotericin B/ml). Glomeruli were plated at a density of 400 glomeruli/cm² tissue culture flask surface area, and were maintained in a humidified atmosphere of 5% CO₂ in air. Cells derived from outgrowth of glomerular explants were subcultured and split 1:3 using standard techniques. Immunohistochemistry was used to characterize third-passage cells as MC by the expression of actin, smooth muscle myosin, and desmin, and the absence of cytokeratin, factor VIII-related antigen, and CD45 (leukocyte common antigen). Third through eighth passage cells were used in this study.

Canine renal fibroblasts were obtained using explant outgrowth techniques from minced sections of renal capsule (n = 8). Fibroblasts were maintained in RPMI 1640 medium containing 20% FCS and antibiotics. Second and third passage renal capsule fibroblasts were used for characterization and proliferation assays. Mouse embry AKR2B fibroblasts (a generous gift from Dr. J. Halper, Department of Veterinary Pathology, University of Georgia College of Veterinary Medicine) were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% FCS and antibiotics. To evaluate the efficacy of D-valine-substituted medium as a selective medium against renal fibroblasts, third passage renal capsule fibroblasts were grown in DMEM that had been modified to contain either the D- or the L-isomer of valine. FCS was dialyzed against DMEM containing D- or L-valine using 1000 MW cutoff dialysis tubing (SpectraPor/7, Fisher Scientific, Pittsburgh, PA). Test media containing 20% FCS and either D- or L-valine were prepared using FCS that had been dialyzed against the respective substituted medium.

Renal capsule fibroblasts were either grown for a single passage in D-valine-substituted selective medium prior to cell proliferation assays (n = 3), or subcultured directly from nonselective maintenance medium into test wells (n = 4). All cells were plated in triplicate in D-valine-substituted selective medium with 20% FCS at a density of 20,000 cells/well in 24-well plates (Costar, Cambridge, MA) for attachment overnight. After attachment, the cells were washed twice with serum-free D-valine-substituted MEM, and test media were added to triplicate wells. Cells were trypsinized and counted using a hemocytometer at 24-h intervals. No significant differences were seen between renal capsule fibroblasts that were grown in media that was deficient or replete with D-valine. The growth of renal capsule fibroblasts that were maintained for an entire passage in D-valine-substituted medium with dialyzed serum prior to proliferation assay (Fig. 1) was comparable to the growth of renal capsule fibroblasts that were subcultured directly from maintenance medium to test medium (Fig. 2).

Desmin expression in MC and renal capsule fibroblasts was evaluated using immunocytochemical and immunoblotting techniques. For characterization studies, mesangial cells and renal capsule fibroblasts (both n = 3) were grown in eight-chambered Lab Tek slides (Nunc, Cambridge, MA) until subconfluent. Cells were fixed with 4% paraformaldehyde in 0.25 M phosphate buffer and permeabilized with 0.01% triton X-100 and 4% normal goat serum in phosphate-buffered saline (PBS) prior to a 12-h incubation with the primary antibodies at 4°C. The slides were washed three times with PBS and...
FIBROBLAST EXCLUSION FROM CULTURED MC

Fig. 1. Renal capsule fibroblast proliferation in selective medium over time. Third passage renal capsule fibroblasts were grown in Dulbecco's modified Eagle's medium (DMEM) that was modified to contain either the D- or the L-isomer of valine with 20% fetal calf serum (FCS) that had been dialyzed against the respective medium. Cells were grown for an entire passage in D-valine-substituted medium prior to proliferation assay, and subsequently plated in 24-well plates at a density of 20,000 cells/well in medium that contained D- or L-valine. Solid and open symbols represent mesangial cells (MC) grown in D-valine- and L-valine-substituted medium, respectively. Cell proliferation did not differ significantly in medium that was deficient or replete with D-valine.

Fig. 2. Growth curve of renal capsule fibroblasts that were plated directly from L-valine-replete maintenance medium into test media. Proliferation of cells did not differ between cells grown in medium that contained D- or L-valine (open and closed symbols, respectively).

Fig. 3. Mesangial cells expressed desmin when evaluated using immunochemistry. Diffuse staining was observed in mesangial cells (MC) reacted with rabbit polyclonal anti-desmin antibodies. Staining was absent in renal capsule fibroblasts. × 400.

Fig. 4. Immunoblot of third passage mesangial cells (MC) and renal capsule fibroblasts. Cells were normalized for protein content and run on a 10% SDS-PAGE gel against chicken gizzard extract (lane 1) and AKR2B cells (lane 2) as positive and negative controls, respectively. Chicken gizzard desmin was expressed as a triplet, whereas MC (lane 4) expressed desmin as a single 53 kDa band. Renal capsule fibroblasts (lane 3) and AKR2B mouse embryo fibroblasts did not contain desmin. Experiment was repeated three times with similar results.