In summary, we report the first large case series of B7-1 staining in kidney biopsies with a podocytopathy. We failed to identify any cases with positive B7-1 podocyte staining after evaluating 60 biopsy specimens using 2 different antibodies. One other recent report also supports the uncommon nature of this B7-1 staining in FSGS.[7] We conclude that B7-1 immunostaining is unlikely to serve as a useful diagnostic stain supporting the use of abatacept therapy.

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Supplementary Material

Item S1: Detailed methods.

Note: The supplementary material accompanying this article (http://dx.doi.org/10.1053/j.ajkd.2014.07.023) is available at www.ajkd.org

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Viable Podocyturia in Healthy Individuals: Implications for Podocytopathies

To the Editor:

The podocyte is a terminally differentiated cell of the renal glomerulus that, by wrapping around the basement membrane through its foot processes, controls the selective permeability of the glomerular filtration barrier.[1,2] In diabetic nephropathy and other chronic glomerular diseases, a gradual decrease in intraglomerular podocytes, paralleled by increased urinary excretion of these same cells (up to 60,000 daily) has been demonstrated,[2-5] suggesting a direct role of podocyturia in disease progression.[2,5] Slow "physiologic" podocyturia (∼400 podocytes per day) recently has been found in healthy individuals.[5] Unexpectedly, in both health and disease, urinary podocytes show up as round viable cells able to proliferate once in culture,[5] possibly suggesting an underlying process of dedifferentiation.[6,7]

Whether urinary podocytes of healthy individuals consist of dedifferentiating cells and, if so, whether the dedifferentiation process precedes or follows detachment from the basement membrane presently is unknown. To clarify this, we collected sterile spot urine samples from 20 healthy individuals (mean age, 64.5 ± 4.1 [SD] years) and fragments of normal cortical tissue (derived from 15 adult kidneys removed for renal or urothelial carcinoma or from donor kidneys not suitable for transplantation) and searched for immature podocytes. Clinical characteristics of participants are provided in Table S1. The study was approved by the Ethics Committee of the San Raffaele Scientific Institute, and written informed consent was obtained from all participants.

Immature podocytes were defined as cells co-expressing the podocyte-specific antigens podocin, nephrin, and podocalyxin (Podxl)[1] and the embryonic transcription factors Nanog, Oct3/4, and Sox2 (with cytoplasmic localization) as markers of ongoing dedifferentiation.[8] The postmitotic podocyte marker synaptopodin also was evaluated.[9]

Analysis of RNA extracted from urine sediments showed expression of the podocyte and embryonic markers (Fig 1A). In experiments run in parallel, we identified (in cytospins of all 20 spot urine samples) viable podocytes, evident as round, mono- or binucleated, propidium iodide–negative, Podxl-positive cells (0.20 ± 0.02 viable podocytes per milligram of creatinine, equivalent to ∼300 podocytes per day; Fig 1B). We detected Nanog in 96% of viable podocytes, in line with their being immature cells (Fig 1B). These urinary podocytes also coexpressed nephrin and podocin with Nanog (Fig 1C), as well as Oct3/4 and Sox2 (not shown). Synaptopodin was undetectable. When we seeded urinary sediments into culture medium, clusters of immature podocytes coexpressing Nanog and Podxl could be detected within a few days (Fig 1D).

In immunofluorescence studies of glomerular sections, we found cell clusters coexpressing Nanog with podocin (Fig 2A) and Podxl (Fig 2B). We also observed colocalization of Nanog with Oct3/4 (Fig 2C) and Sox2 (not shown). Consistent with these cells being immature, we detected no colocalization of Nanog and synaptopodin (Fig 2D). Clusters of immature cells were detected in ∼60% of glomeruli considered, independent of donor age.

These study results show that viable podocyturia, as detectable in urine of healthy individuals, consists of dedifferentiating cells coexpressing mature podocyte antigens (with the exception of postmitotic synaptopodin) along with immaturity markers. The presence of immature podocytes inside the glomerulus alternatively could be interpreted as an ongoing differentiative process allowing a still unknown stem cell pool to replace dying or detaching podocytes. Although this hypothesis cannot be excluded, it nonetheless is not in line with the data in Fig 2, which show that no niches of undifferentiated stem cells expressing embryonic transcription factors

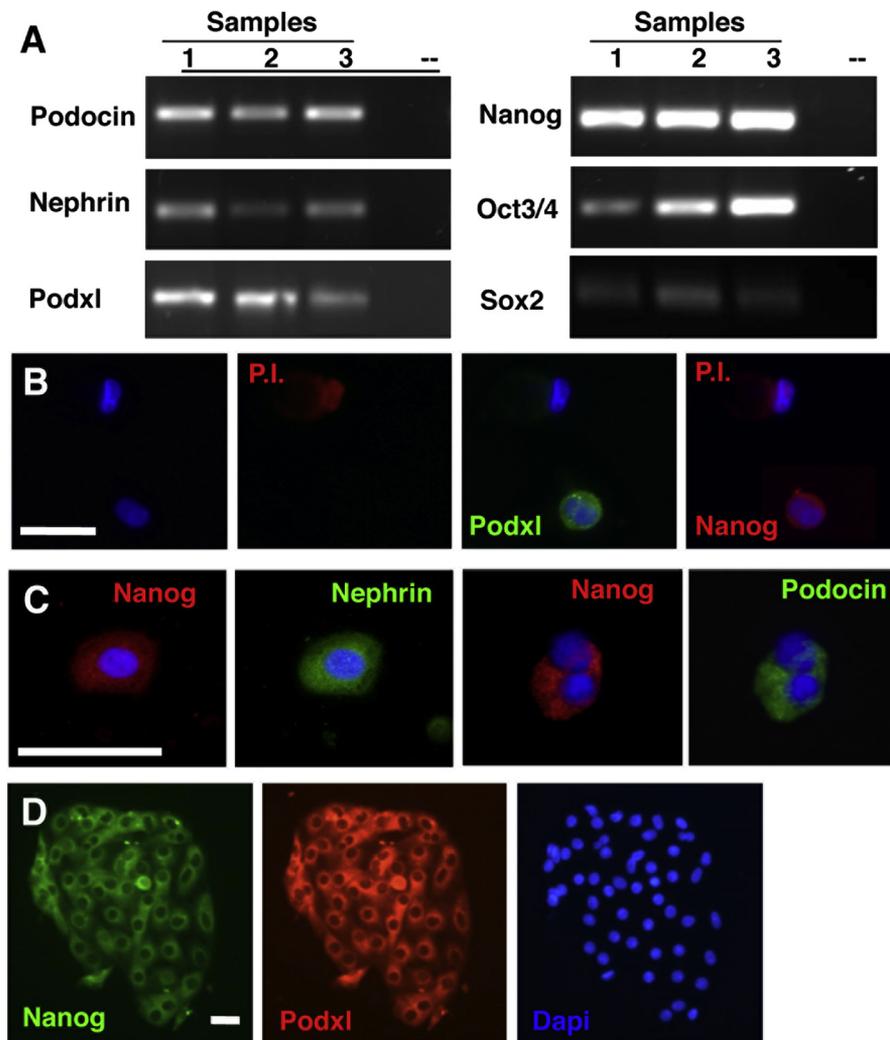**Figure 1.** Analysis of podocyturia in healthy individuals. (A) Reverse transcription–polymerase chain reaction analysis shows the expression of podocyte markers podocin, nephrin, and Podxl and embryonic transcription factors Nanog, Oct3/4, and Sox2. RNA was extracted from 3 independent urinary collections. (B) Cytospin analysis of urine. Image 1: 2 cells (at top, a single cell, and below, a binucleated cell) are visible on the cytospin slide (nuclei are stained with DAPI; scale bar, 20 μm). Image 2: the top cell stains with propidium iodide (P.I.), suggesting lack of viability; the lower cell is viable. Image 3: Podxl staining confirms the viable cell is a podocyte. Image 4: cytoplasmically localized Nanog is visible in the viable cell, consistent with it being an immature cell. (C) Cytospin analysis confirms the presence in urine of immature podocytes co-expressing Nanog along with nephrin and podocin; scale bar, 20 μm. (D) Proliferating colonies of immature podocytes arising from culturing the urinary pellet in Dulbecco modified eagle medium and 10% fetal bovine serum; scale bar, 20 μm.

in absence of podocyte markers could be detected inside the glomerulus. Our study also demonstrates that the process of podocyte dedifferentiation precedes detachment from the basement membrane because clusters of immature podocytes were detected inside normal glomerular sections. Of course, this evidence does not exclude the possibility that some podocytes may detach from the basement membrane when still differentiated. To confirm the relationship between intraglomerular immature podocytes and urinary immature podocytes, lineage tracking experiments in an appropriate mouse model will be required.

Our findings suggest in vivo podocyte dedifferentiation as the origin of viable podocyturia in healthy individuals. Whether podocyturia in healthy individuals may represent a "side effect" of physiologic podocyte turnover (based on a dedifferentiation-redifferentiation process[10]) and, if so, by what means chronic glomerular diseases may unbalance this scenario (leading, in some podocytopathies, to huge numbers of podocytes being lost in urine), remains to be clarified.

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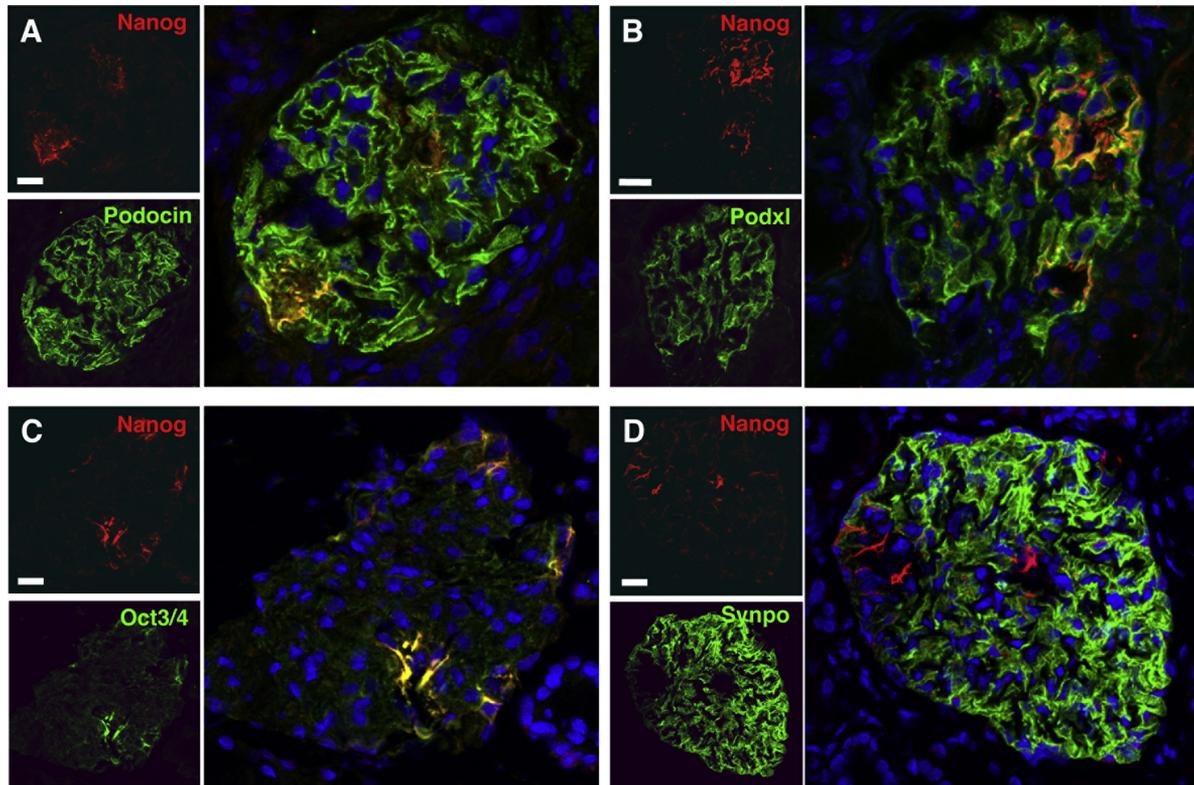Corresponding author: g.zerbini@hsr.it

Figure 2. Identification of immature podocytes within normal glomeruli. In each panel, upper left image shows intraglomerular Nanog staining (scale bar, 20 µm). Lower left images show the podocyte markers (A) podocin and (B) Podxl, (C) the embryonic marker Oct3/4, or (D) the mature podocyte marker synaptopodin. For each panel, the larger image at right is a merged view of staining for Nanog and the relevant marker. Nanog colocalizes with podocin, Podx1, and Oct3/4, but not with synaptopodin.

content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved. GZ takes responsibility that this study has been reported honestly, accurately, and transparently; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

Supplementary Material

Table S1: Clinical characteristics of the participants.

Note: The supplementary material accompanying this article (http://dx.doi.org/10.1053/j.ajkd.2014.08.016) is available at www.ajkd.org

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