glomerular injury [5]. It could be supposed that tubulointerstitial inflammation yields cytokines, growth factors, vasoactive molecules, and free oxygen radicals that contribute to the development of arteriolar and glomerular lesions. Some authors go further and extend this hypothesis, assuming that subtle interstitial injury is not so much the manifestation of hypertensive kidney disease, but rather the underlying reason for hypertension [6]. Further studies are necessary to clarify this issue.

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Reply from the Authors

Our paper [1] focused primarily on the relationship of arteriolar disease and tubulointerstitial inflammation in renal progression. With renal mass reduction there is an increase in systemic blood pressure with glomerular hyperfiltration, glomerular hypertension, and proteinuria. Tubulointerstitial inflammation results and appears to mediate afferent arteriolar disease, which further impairs autoregulation, resulting in further increases in glomerular pressure and acceleration of glomerulosclerosis. Arteriolopathy in other conditions is also associated with glomerular hypertension, reduced plasma flow, ischemia, and fibrosis.

Drs. Tylicki and Rukowski ask about the relationship of arteriolopathy and tubulointerstitial inflammation in essential hypertension. This is an area of special interest to us because we have proposed that these changes are responsible for the development of salt-sensitive hypertension [2, 3]. As we have proposed, renal injury is initiated by vasoconstriction and glomerular hypertension induced by sympathetic nervous system overactivity, endothelial dysfunction, hyperuricemia, or activation of the renin-angiotensin system; this leads to tubulointerstitial inflammation, intrarenal oxidant and angiotensin II generation, and afferent arteriolar disease [4]. The arteriolopathy results in impaired autoregulation and worsens glomerular hypertension, and also decreases renal plasma flow, causing ischemia, thus perpetuating tubulointerstitial inflammation [2]. The observation by Drs. Tylicki and Rutkowski of urinary excretion of tubular markers in untreated hypertension [4] is consistent with our pathway. Thus, salt-sensitive hypertension may be considered a type of subtle acquired renal disease, which targeted therapies could possibly reverse and/or cure.

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GAPDH as housekeeping gene at renal level

To the Editor: In mRNA quantitative studies, choosing a reliable internal control is mandatory for monitoring intersample variations and comparing target RNA levels if assays are performed in different experimental sessions and come from different centers.

In microdissected kidney biopsies Schmid et al [1] studied the validity of three housekeeping genes commonly used in quantitative mRNA analysis: glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 18S rRNA, and cyclophilin A. The authors demonstrated that in the tubulointerstitium of 12 out of 165 microdissected specimens, GAPDH expression was significantly lower in comparison to 18S rRNA or cyclophilin A, thus warning investigators on the use of GAPDH in this type of setting. Nevertheless, our experience in renal biopsies and in in vitro studies suggests the possibility of using GAPDH as internal standard.

We had the opportunity to study by real-time reverse transcription-polymerase chain reaction (RT/PCR) the

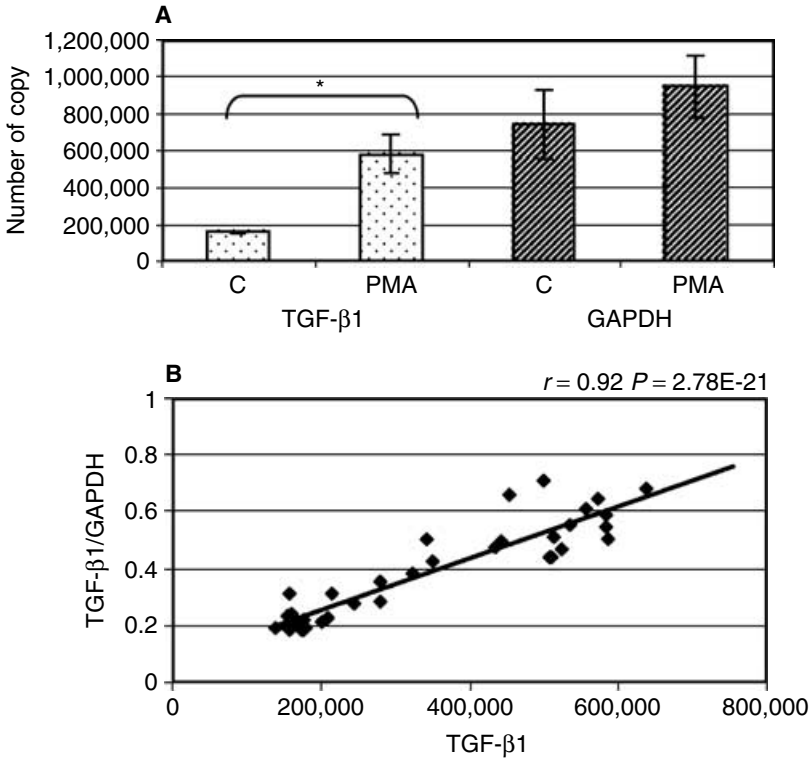

Fig. 1. Comparison between TGF-β1 and GAPDH gene expression in control (C) and in PMA-stimulated cells (* $P < 0.003$) (*A*). Relationship between absolute and relative amount of TGF-β1 mRNA (*B*).

expression of different genes in relation to GAPDH on mesangial cells in different culture conditions, including high glucose and phorbol 12-myristate 13-acetate (PMA) stimulation. In PMA-stimulated cells no significant modulation of GAPDH gene was observed, whereas transforming growth factor-β1 (TGF-β1) gene expression was increased by three times (Fig. 1A). Moreover, a linear correlation between absolute values of TGF-β1 and its relative amounts, calculated as a ratio between target and housekeeping gene copy number, was demonstrated (Fig. 1B). We wonder if the relative reduction of GAPDH gene expression levels observed in 12 biopsies by Schmid et al [1] is rather caused by the contemporaneous modulation of both 18S rRNA and cyclophilin A genes. We propose that, at least in microdissected glomeruli, GAPDH could be used as housekeeping gene in quantitative gene expression studies.

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Reply from the Authors

We appreciate the comments from Franca Anglani and the opportunity to clarify some open questions. To obtain reproducible results both in DNA array and real-time reverse transcription-polymerase chain reaction (RT-PCR) technology, adequate normalization of the mRNA expression data is crucial. Using a single reference gene [i.e., the "housekeeper" glyceraldehyde-3-phosphate dehydrogenase (GAPDH)], we could show differential regulation in a subset of human tubulointerstitial tissue.

This finding has been further supported by DNA array based expression profiling. Using this approach, we and others ([1] and unpublished observations) could show differential regulation of each housekeeper examined in an unpredictable subset of samples. However, simultaneous deregulation of two housekeepers were observed in only 7/156 comparisons, and parallel deregulation of all three housekeepers (GAPDH, 18S rRNA, and cyclophilin A) were not observed in any of the data sets evaluated.

If two housekeepers in RT-PCR studies show a stringent correlation in the samples analyzed, the probability of parallel differential regulation of these molecules is below 5%; using three housekeepers, it should be negligible. A note of caution should be used when extrapolating in vitro studies of single cell lines to in vivo conditions of complex tissue, as done by Anglani. Because only approximately one third of all glomerular cells are mesangial cells, in vitro studies on this cell line do not exclude differential regulation by the other glomerular