Immunoregulation in Urinary Tract Inflammation—A Role of Tamm-Horsfall Glycoprotein


The Tamm-Horsfall protein (THP), the most abundant protein in normal urine, was discovered five decades ago (1), or possibly even much earlier (2), but the role for the most abundant protein in normal urine had remained enigmatic for a long time. It is expressed in the thick ascending limb of Henle’s loop and the early distal convoluted tubule (3). Meanwhile, the important role of mutations of its gene in the genesis of an autosomal dominant form of progressive chronic renal failure with hyperuricemia with or without medullary cysts has been clearly established (4), consistent with an important biologic function of this protein.

THP seems to have some poorly defined role in tubular Na reabsorption (5,6). THP protects against oxalate crystal formation (7) and it has been postulated to play a role in the genesis of urolithiasis (8). Furthermore, THP promotes tubular cast formation (8) in conditions as diverse as acute renal failure or myeloma kidney.

THP is part of the antibacterial defenses of the urinary tract. It had been postulated that clearance of bacteria, specifically *Escherichia coli*, from the urinary tract was mediated by THP (6,9) and this hypothesis has since been proven in THP knockout mice (10).

Finally, it had been suspected for quite some time that THP was involved in inflammatory kidney diseases (3), not in the least because, in patients with interstitial renal disease and urinary tract infection, THP antibodies are found (11) and deposits of THP with THP antibodies are found in the renal interstitium. Such deposits may cause neutrophil stimulation and complement activation (12–14).

A new twist to understand the proinflammatory and immune mechanisms involved in urinary tract infection is provided by the study of Säemann *et al.* (15), which documents that THP uses a mechanism involved in pattern recognition of pathogenic molecules, *i.e.*, an isoreceptor of the Toll-like receptor (TLR) family (*e.g.*, TLR-4) to transform immature professional antigen-presenting cells into cells with a mature phenotype. After exposure to THP, these receptors express costimulatory molecules (CD 80 and CD 86) and HLA-DR molecules, as well as proinflammatory cytokines. Such immunostimulation is mediated via NFκB activation and involves rapid phosphorylation of a series of tyrosine kinases (ERK, p38, Akt). The crucial role of the Toll-like receptor TLR4 was definitively proven by comparing wild-type mice with TLR4 knockout mice. The latter failed to develop THP autoantibodies and proinflammatory cytokines when challenged with THP. It is curious to note that THP acts as both an antigen (causing autoantibody formation) and as an adjuvant (amplifying the immune response).

What is the relevance of this finding? It is obviously problematic and potentially dangerous for the kidney to produce such a potent proinflammatory and antigenic molecule, which can cause autoantibody formation. The authors argue convincingly that the kidney avoids unwanted activation and autoantibody formation by restricting the expression of the THP molecule to the luminal surface of the tubular epithelium and excreting the THP molecules in the urine. The aberrant deposition of THP in the tubulointerstitium, as seen in several inflammatory experimental (16) and human (17) kidney diseases, sets the stage for both an inflammatory and autoimmune response. This has been documented by experimental studies after immunization with THP (18) and after challenge with homologous urine or THP (18). It has been well known for a long time that THP antibodies are a
consequence of urinary tract infections and acute pyelonephritis (11,19), and the documentation by Säemann et al. of the Janus role of THP as both autoantigen and adjuvant will not fail to focus the attention of renal researchers once again on the notion that TPH may play an as yet underestimated role in the induction and progression of some renal diseases—not necessarily only bacterial infections but conditions as diverse as renal allograft nephropathy (20) or myeloma kidney.

References
Starvation in the Midst of Plenty? The Role of Hypoxia in Progression

Hypoxia in Renal Disease with Proteinuria and/or Glomerular Hypertension.


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The kidney, which receives 20% of the cardiac output, is at first glance an extremely unlikely candidate for a pathogenetic role of hypoxia, at least as far as the renal cortex is concerned. While the deleterious roles of hypertension and proteinuria in progression were suspected and accepted relatively early, suspicion of a pathogenetic role of hypoxia has been slow in coming.

With the introduction of a renal biopsy technique, it was noted early on that tubulointerstitial lesions were more closely related to loss of renal function than glomerular lesions (1–3). To explain this initially puzzling finding, Bohle proposed that interstitial lesions are associated with loss of peritubular capillaries, thus causing hypoxia (3,4). To put it mildly, this idea was not enthusiastically received because of the presumed rich oxygen supply of the renal cortex. However, the important functional implications of the loss of peritubular capillaries have recently been well documented (5,6).

It is worth reminding that the renal community had to accept the equally surprising finding that the sensor detecting oxygen deficiency and stimulating synthesis of erythropoietin was, of all places, located in the peritubular space of the renal cortex, which had been thought to have an abundant supply of oxygen.

Pursuing the proposal of Bohle, Leon Fine put forward the hypothesis that hypoxia is the consequence of the depletion of peritubular capillaries and plays an important role as progression promoter (7,8). This hypothesis found support by *in vitro* work (8,9), which documented that a low pO$_2$ stimulated collagen production by interstitial fibroblasts. Although low pO$_2$ values were indeed measured in the renal interstitium (10), methodological problems remained and the reported results were conflicting (11). The smoking gun, *i.e.*, evidence of very low interstitial pO$_2$ values in renal damage models and documentation of a topographical relationship between regions of hypoxia and of fibrogenesis, were lacking.

The work of Tanaka *et al.* has now achieved a breakthrough and put hypoxia squarely back on the map. The investigators reasoned that the chemical tools used hitherto for this purpose, *e.g.*, nitroimidazoles, lacked sensitivity and resolution with respect to the time course. Therefore they developed a novel transgenic rat model, taking advantage of the hypoxia-responsive element (HRE) of the rat vascular endothelial growth factor (VEGF) gene to create a HRE-driven tagged luciferase construct. Hypoxia sensing by this construct was validated and quantitated by *in vitro* studies, and induction of the transgene by hypoxia was proven using cobalt chloride as a surrogate stimulus of hypoxia. Not unexpectedly, even under normoxic conditions the construct indicated the presence of hypoxia in the renal medulla. Cobalt increased the hypoxia signal, however, in tubular cells, glomerular epithelial cells, and interstitial cells.

The authors then went one step further and examined whether the construct indicated hypoxia in rats with chronic progressive renal damage. To exclude model-specific artifacts, they wisely studied two different models: The puromycin aminonucleoside model of nephrotic syndrome and the remnant kidney model. The transgene construct was upregulated, although to a different degree, in both models. The degree of impairment of renal function (*e.g.*, blood urea nitrogen levels), as well as the extent of tubulointerstitial injury, were clearly correlated to the intensity of hypoxia indicated by the transgene construct. An impressive regional correlation was noted between areas of hypoxia and areas of macrophage accumulation, apoptosis, and cell proliferation, indicated by proliferating cell nuclear antigen (PCNA). The peritubular capillaries showed signs of damage recognized from the previous experiments of Kang *et al.* (5), suggesting that impaired tubulointerstitial blood flow, presumably caused by a deficit of VEGF...
and nitric oxide (NO) (6), had created the regional foci of hypoxia postulated by Bohle (3). The hypothesis that hypoxia is at least one of the factors triggering and perpetuating progression is further supported by previous findings that hypoxia even precedes evidence of renal damage (12,13). Oxygen-sensing in the kidney is obviously mediated by hypoxia-inducible factor(s)-1 and -2 (HIF-1 and HIF-2) as recently documented by Rosenberger et al. (14,15). HIF is involved not only in the control of the synthesis of erythropoietin (EPO), but more generally triggers metabolic and structural responses to hypoxia such as stimulation of glycolysis, angiogenesis, etc. in different organs (16).

What are the implications of the findings of Tanaka et al.? Because hypoxia sensing is such a central mechanism, the implications of the findings are presumably far-reaching in situations of progressive renal damage. Renal hypoxia is obviously of moderate intensity and fails to induce hypoxic necroses, but nevertheless it may become a target for therapeutic intervention in the future. So far, tantalizing observations from the Irbesartan Diabetic Nephropathy Trial (IDNT) (unpublished) and the Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan (RENAAL) trial (17) indicate that a low hemoglobin value is a predictor of more rapid progression of renal failure. Whether this is due to hypoxia induced by anemia or a result of EPO deficiency is currently unknown. We shall soon have the results of ongoing trials, however, to clarify at least whether correction of anemia by EPO attenuates the rate of progression. Possibly the beneficial effects of other interventions as well are mediated by modulating hypoxia, at least in part (18).

At any rate it is safe to predict that the communication of Tanaka et al. will spawn further experimental and clinical studies to clarify the exact role of hypoxia as a progression promoter.

References


**Microinflammation in Renal Failure—Is Periodontal Disease One of the Culprits?**


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There is consensus that microinflammation is common in patients on dialysis (1,2). The evidence for microinflammation consists of increased concentrations of acute phase reactants (3) such as high-sensitivity C reactive protein (hsCRP) (4,5); of cytokines (6), particularly IL-6 (7,8); and of oxidative stress indicators (9,10). Novel markers include fetuin (11) and adiponectin (12,13). Many factors have been suspected or documented to be responsible for microinflammation. They are related to renal replacement therapy (e.g., exposure to proinflammatory stimuli such as bacterial toxins or bacterial DNA in the dialysate, bioincompatibility of dialysis membranes and tubing, etc.) or terminal renal failure (e.g., uremic toxins, advanced glycosylation end (AGE) products, hypervolemia with intestinal leak of endotoxin [14] etc.). Nevertheless it is of note that increased markers of microinflammation and endothelial cell dysfunction can be found even in nondialysed patients with renal failure early in the course of chronic kidney disease (CKD) (15–17). In that stage of CKD one also finds accelerated atherogenesis (18), possibly triggered by increased oxidative stress (14), providing a link between microinflammation and cardiovascular risk.

It is unlikely, however, that one single cause accounts for all microinflammation. In this context it is of interest that a high prevalence of periodontal disease is found in chronic hemodialysis patients (19), particularly in diabetic patients (20). Unpublished preliminary data even suggest that periodontal disease is predictive of survival (21). Is there a causal link between periodontitis and microinflammation?

Following the observations of Ridker and colleagues (22,23) that CRP concentrations predict cardiovascular events, it is of interest to examine whether periodontal disease contributes to the inflammatory burden. Indeed, inflammation markers are found in patients with periodontal disease: Increased hsCRP concentrations, together with reduced flow-mediated vasodilatation of the brachial artery, were noted by Amar *et al.* (24), although not confirmed by others (25), and higher IL-1β, TNFα, and IL-2 levels are also common (26). After periodontal treatment, white
blood cell, neutrophil, and platelet counts decrease significantly (27). Antibodies against and T cells reactive with heat shock protein of Porphyromonas gingivalis were found in the circulation and in atherosclerotic plaques of patients with periodontitis (28).

How about cardiovascular risk? A correlation, though modest, was found between periodontal disease and coronary artery calcification as a surrogate marker (29). In the Third National Health and Nutrition Examination Survey (NHANES III), the odds ratio for a heart attack increased with increasing clinical severity of periodontitis (29), and such a relationship was confirmed in the Atherosclerosis Risk in Communities (ARIC) study (30). Sceptics will be impressed by the observation that radical elimination of periodontitis by extraction of all teeth failed to lower cardiovascular risk, however (31). So criticisms had been definitively justified that the available evidence was soft and that more sensitive and comprehensive measures of periodontal disease were required (32).

It is the sophisticated methodology used by Desvarieux in the Oral Infection and Vascular Disease Epidemiology Study (INVEST) that now provided more scientifically convincing evidence. Six hundred fifty-seven patients without history of cardiovascular events and with teeth were studied. Subgingival plaque samples were collected and quantitatively assessed by DNA–DNA checkerboard hybridization targeting 11 bacterial species known to be involved in periodontal disease. In addition, the carotid artery intima-media thickness (IMT) was measured and white blood cell counts as well as CRP values were obtained. The main result is that increasing tertiles of overall periodontal bacterial burden, but more importantly increasing tertiles of the burden of causative bacteria (i.e., bacteria involved in plaque formation) were correlated to the carotid IMT. The adjusted IMT values across tertiles of etiologic bacterial burden were 0.84, 0.85, and 0.88—a modest, but statistically significant, increase ($P < 0.02$). White blood cell counts, but not CRP values, were similarly correlated.

The strength of the study is that rigorous molecular bacteriological techniques were used. The weakness is that a surrogate marker (i.e., IMT) but not hard endpoints (i.e., events) were assessed. Nevertheless, the study identifies periodontitis as a definite candidate for, although not yet a convicted culprit in, the genesis of microinflammation and cardiovascular risk.

Against the background of the high prevalence of malnutrition, inflammation, and atherosclerosis (MIA syndrome) (1) and of periodontal disease (19,21) in dialysis patients, it is a reasonable hypothesis to propose a link between periodontal disease and microinflammation in these patients. Rigorous studies are currently underway to test this hypothesis.

What are the practical consequences? When examining uremic patients it is not medical malpractice but a most sensible procedure to check whether the patient has periodontal disease. And the patient is not hurt by the advice to adopt careful oral hygiene.

References
7. Kalantar-Zadeh K, Kopple JD, Humphreys MH, Block G: Comparing outcome predictabil-


