Direct Immunization with Pathologic Urine Samples: A New Approach to Monoclonal Antibody-Based Urinary Assays of Renal Disorders

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Dear Sir,

Diagnosis of kidney disorders so far mainly relies on proteinuria measurements, changes in serum creatinine levels, urine output and release of various serum proteins or renal enzymes into the urine following renal injury. However, these routine tests are rather unspecific and often renal biopsy is required to verify diagnosis. Urinary shedding of renal tissue constituents, specific for distinct renal structures, could be a reliable indicator of kidney damage. Establishing a highly specific urinary assay using monoclonal antibodies (Moabs) detecting renal antigens would constitute a simple noninvasive system for diagnosing renal tissue injury. The development of a Moab-based sandwich enzyme immunoassay which reveals a 120-kd glycoprotein, the adenosine desaminase binding protein, mostly associated with proximal tubular epithelial cells, has been reported [1]. So far, Moabs

Fig. 1. Indirect immunoperoxidase staining with three selected Moabs on cryostat sections of normal human kidney tissue $\times 300$. TN14 detects an antigen on the luminal site of distal tubules (a), whereas TN16 recognizes proximal tubules (b). In contrast to these tubular markers, TN17 stains glomerular mesangial cells and in addition smooth muscle cells of vessels (c).

raised against renal antigens in order to establish an assay for urinary diagnosis were generated by a conventional immunization with kidney tissue or cell lines of isolated human kidney cells. Although these Moabs can detect epitopes of renal cells it seems to be uncertain whether antigens recognized by the selected antibodies are shed into the urine.

In order to overcome this problem we have taken a quite different approach in our efforts to detect renal antigen shedding by urinary Moab-based enzyme immunoassays. Moabs were produced by fusing mouse myeloma cells with spleen cells of mice directly immunized with pathologic urine samples using a rapid single-shot intrasplenic immunization [2]. Supernatants of hybrids were screened for Moabs recognizing renal tissue antigens shed into the urine by use of immunoperoxidase staining of frozen sections of a normal kidney. Antibodies reactive with structures of the normal kidney should therefore indicate the original anatomical site of the antigen released into the urine by the injured kidney. With this new approach a panel of Moabs was selected which is specific for distinct parts of the human nephron and allows its immunohistologic dissection (fig. 1a-c). The reactivity pattern of at least four Moabs (TN14-TN17) with normal adult renal tissue as well as their immunoglobulin subclasses are given in table I.

Preliminary data point to the usefulness of this Moab set in urinary enzyme immunoassays. Further urinary analyses of different renal diseases are currently being performed to establish a highly sensitive sandwich immunoassay. Such a multiple Moab assay system will be useful to detect the anatomical region of a renal disorder, the course of a pathologic process, its response to therapy and probably may give an early hint to renal graft rejection.

Table I. Characteristics of Moabs TN14-TN17 raised against pathologic urine samples

Moab Ig subclass Reactivity pattern with normal adult renal tissue

TN14	IgM	distal tubules
TN15	IgM	proximal tubules, thick limb of Henle's loop
TN16	IgM	proximal tubules
TN17	IgM	mesangial cells, smooth muscle

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