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ORIGINAL ARTICLE

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The localization of thromboxane synthase in normal and pathological human kidney tissue using a monoclonal antibody Tü 300

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Abstract Thromboxane, excreted in the urine in increased amounts in glomerular, vascular and tubulo-interstitial diseases, is considered to originate from the kidney. The localization of thromboxane synthase, a key enzyme of arachidonic acid metabolism, was studied in the human kidney by immunohistology using the monoclonal antibody Tü 300. In the interstitial tissue dendritic reticulum cells surrounding the tubules expressed high concentrations of the enzyme. In glomeruli the enzyme was weakly expressed in podocytes. This was confirmed by co-localization with an antiserum directed to podocalyxin, a marker of the visceral epithelial cells. In the study of various kidney diseases, massive accumulation of thromboxane synthase containing cells was observed in interstitial diseases, whereas in glomerular diseases there were no differences from normal kidney; in a case of thrombotic microangiopathy podocytes exhibited an increase in thromboxane-synthase. The thromboxane-synthase positive infiltrating interstitial cells were shown by conventional light microscopy to be mononuclear phagocytic cells. The physiological sources of renal thromboxane are dendritic reticular cells and podocytes. In interstitial renal disease infiltrating cells of the monocyte/macrophage system constitute the major site of thromboxane synthesis. In glomerular disease, a characteristic alteration of thromboxane-synthase was not found.

Key words Kidney · Thromboxane · Thromboxane synthase · Tü 300 monoclonal antibody

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Introduction

Thromboxane A_2 (TxA_2) is recognized as a potent derivative of arachidonic acid. This is first converted to prostaglandin endoperoxide by the enzyme cyclo-oxygenase and then isomerized to TxA_2 by thromboxane synthase (Hamberg et al. 1975). Since TxA_2 is highly active in platelet aggregation and vasoconstriction its role in cardiovascular disease is well-established (Needleman et al. 1977). Inhibitors of its biosynthesis or its receptor activation have been developed and are currently being tested for their therapeutic potential. It is well-known that blood platelets (Needleman et al. 1976) are the main sources of TxA_2 in these vascular diseases but also that monocytes/marcrophages (Murota et al. 1978) are rich in thromboxane synthase, which suggests a role in inflammatory disease. Immunostaining with the monoclonal antibody Tü 300 against thromboxane synthase (Haurand and Ullrich 1985; Nüsing et al. 1990c; Ullrich and Graf 1984) raised against the human enzyme (Nüsing et al. 1990a, b), has revealed its presence in histiocytes of all tissues. The synthesizing capacity of organs for TxA_2 is greatly increased by increasing numbers of monocytic cells (Murota et al. 1978) and by induced thromboxane synthase activity in these cells and in fibroblasts (Hopkins et al. 1978). TxA_2 produced under those conditions may be involved in smooth muscle contraction and the intercellular communication between cell types of the immune system and organ-specific cells. All responses elicited by TxA_2 are mediated by one or more TxA_2-receptors which are coupled to the so-called PI-response (Brass et al. 1987; Mené and Dunn 1986; Mené et al. 1988).

We wished to establish the localization of thromboxane synthase in cells of the kidney, since under various conditions increased thromboxane synthesis can be measured by following the release of thromboxane B_2 (Roberts et al. 1981) as a stable hydrolysis product of TxA_2 in the urine (Coffman et al. 1985; Lianos et al. 1983; Morrison et al. 1977; Patrono et al. 1985; Purkerson et al. 1985; Schwartz et al. 1984). In contrast to

systemically produced thromboxane B_2 which is further metabolized to 2,3-dinor-TxB_2 and 11-dehydro-TxB_2 (Roberts et al. 1981), the urinary TxB_2 seems to originate largely from the kidney (Benigni et al. 1989; Frohlich et al. 1975; Zoja et al. 1989). Increased thromboxane synthesis has been reported in experimental glomerulonephritis (Lianos et al. 1983), ureteral obstruction (Morrison et al. 1977), renal allograph rejection (Coffman et al. 1985), renal venous occlusion (Schwartz et al. 1984), renal ablation (Purkerson et al. 1985) and lupus nephritis in humans (Patrono et al. 1985). The rapid beneficial effects of inhibition of thromboxane synthesis or receptor blockade on impaired haemodynamics in the majority of these conditions point to a functional role for enhanced local synthesis. In lupus nephritis, treatment with sulatroban, a recently developed TxA_2-receptor blocker, improved renal function (Pierucci et al. 1989). Renal ischaemia also leads to a selective increase in TxB_2-accompanied by extensive acute tubular necrosis. Pretreatment with OKY 046, a selective thromboxane synthase inhibitor, blocked ischaemia-induced TxB_2-synthesis and tubular damage (Lelcuk et al. 1985). In the early phase of acute renal failure induced by glycerol injection in rats significant increase of TxB_2 was observed, while creatinine clearance decreased and the administration of OKY 046 prevented the decrease in creatinine clearance (Chatziantoniou and Papanikolaou 1989). Thromboxane synthase inhibitors reduced proteinuria in animal models of nephrotoxic serum nephritis (Lianos et al. 1983), adriamycin-induced nephrosis (Remuzzi et al. 1984) and immunocomplex-glomerulonephritis (Saito et al. 1984). Foegh et al. (1981) reported that the urinary thromboxane excretion in renal transplant recipients increased during episodes of acute rejection. In animal models of graft rejection administration of thromboxane synthase inhibitors was reported to improve renal function (Coffman et al. 1989; Mangino et al. 1989). In spontaneously hypertensive rats TxA_2 synthesis increased during development of hypertention and this was delayed by cotreatment with a specific TxA_2 synthase inhibitor (CV 4151) (Shibouta et al. 1981, 1985).

These many findings suggest that TxA_2 plays an important role in renal disease. Our study was designed to identify the sources if TxA_2 in normal and diseased kidney tissue.

Materials and methods

Twenty-five kidney biopsies (2 normal kidneys from tumour nephrectomies, taken from the opposite pole, 12 biopsies with glomerular diseases, 10 with interstitial diseases and 1 case of thrombotic micro-angiopathy) were shock-frozen at −100° C. Cryostat sections were mounted on microscope slides, welded in plastic bags and stored at −70° C. The slides were thawed for 15 min at room temperature and incubated with anti-thromboxane synthase antibody Tü 300 (1:800). This was visualized by the alkaline phosphatase anti-alkaline phosphatase method as described in detail in elsewhere (Mason 1988). As a negative control, the first antibody was omitted and replaced by buffer. A positive control was provided by intravascular monocytes which always gave a strong reaction. The evaluation of the renal biopsies was done blind by two of us (P.M.F. and F.G.).

To analyse the localization of thromboxane synthase in the glomerulus, immunofluorescence double staining with Tü 300 (1:100) and rabbit anti-podocalyxin antiserum (Kerjaschki et al. 1986) (1:80), kindly provided by Dr. D. Kerjaschki, Vienna, was carried out. Monoclonal antibody Tü 300 was visualized by goat anti-mouse IgG_{2A} phyco-erythrin (1:80) and the polyclonal antiserum was detected by the use of goat anti-rabbit fluorescein isothiocyanate (FITC) (1:80).

To localize prostacyclin synthase in the glomerulus we used the monoclonal antibody isn-1 (DeWitt and Smith 1983), kindly provided by Dr. W.L. Smith, visualized with goat anti-mouse FITC (Grub, 1:30).

Results

The monoclonal antibody Tü 300 showed a granular staining of the cytoplasm without affecting cell membranes or nuclei. In glomerular capillaries and vessels circulating platelets and monocytes were strongly stained, endothelial and smooth muscle cells were negative. A distinct but weak reactivity with podocytes was observed, whereas mesangial cells were negative (Fig. 1A). The tubules and collecting ducts were always negative. In the interstitium dendritic reticulum cells gave a strongly positive reaction (Fig. 1B). The staining of podocytes by Tü 300 was confirmed by double incubation of Tü 300 with an antiserum generated against podocalyxin. The visceral glomerular epithelial cells are endowed with a highly polyanionic glycocalyx where podocalyxin is the major sialoprotein. Both antibodies reacted with the same cell type in the glomerulus (Fig. 2A). Prostacyclin synthase, in contrast, could be localized only in endothelial cells of the glomerulus, no reactivity was observed in podocytes (Fig. 2B).

The staining results with Tü 300 in glomerular and interstitial diseases are summarized in Table 1. In the case of glomerular diseases the staining pattern with Tü 300 was similar to normal kidneys. A case of thrombotic microangiopathy (Fig. 3A) exhibited a stronger antigenicity of the podocytes when compared with normal tissue. In the different interstitial diseases, the reaction in the glomeruli did not differ from normal kidneys, however, numerous mononuclear cells, monocytes, histiocytes, macrophages, stained strongly positive in the tubular interstitial space (Fig. 3B).

Discussion

The normal kidney contains interstitial cells with a strong positive reaction against the Tü 300 monoclonal antibody (Nüsing et al. 1992). In addition, however, we were able to identify a weaker but distinct activity in podocytes identified by the cell-specific antibody against podocalyxin. In contrast, mesangial cells were negative by immunohistology in contrast with our preliminary studies (Nüsing et al. 1990c). This is in agree-

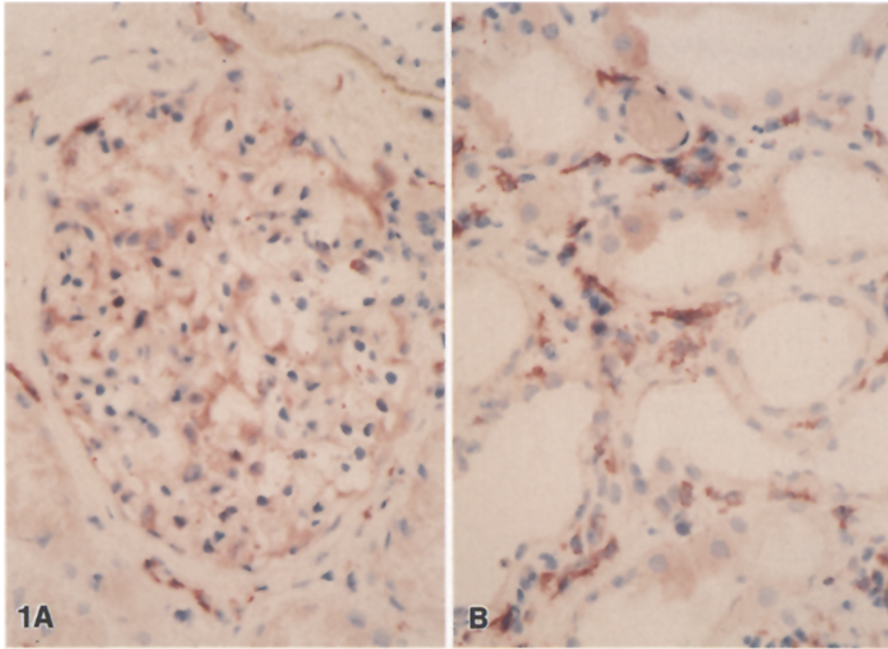

Fig. 1A, B Normal kidney (cryostat sections). **A** Glomerulus of normal kidney stained with anti-thromboxane synthase monoclonal antibody Tü 300. The cytoplasm of podocytes shows a positive reaction, whereas cells of the mesangial stalk do not (×450). **B** Section of interstitium of normal kidney stained with Tü 300. Strong staining reaction of dendritic reticulum cells in the surrounding of tubules (× 450).

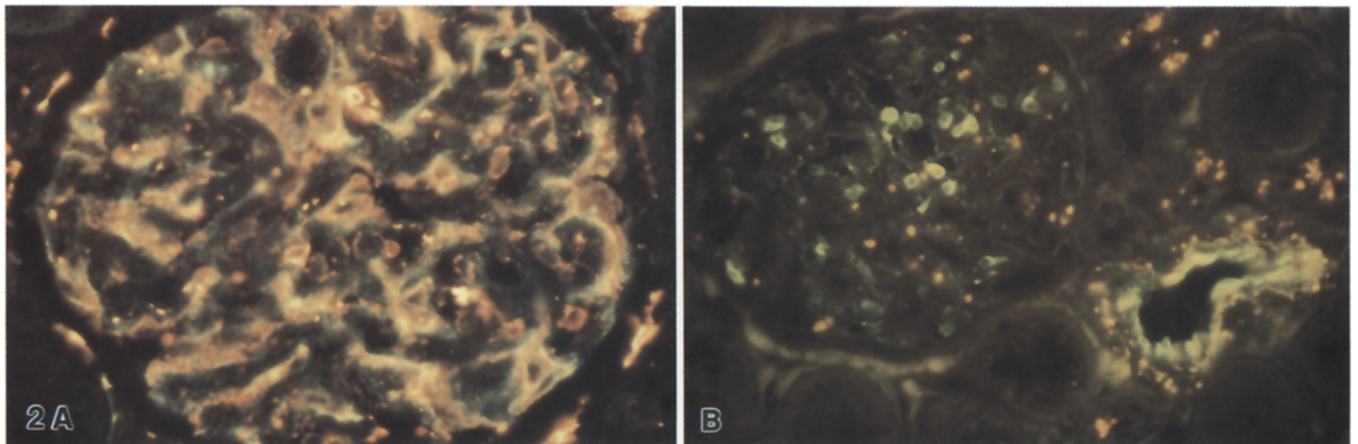

Fig. 2A, B Normal kidney (cryostat sections). **A** Fluorescence photomicrograph of glomerulus of normal kidney double-stained with Tü 300 (red) and anti-podocalyxin antiserum (green). Co-localization of anti-podocalyxin antiserum and Tü 300 confirm the presence of thromboxane synthase in podocytes (×450). **B** Fluorescence photomicrograph of glomerulus of normal kidney stained with anti-prostacyclin synthase monoclonal antibody. In contrast to A only endothelial cells give a positive reaction (×450).

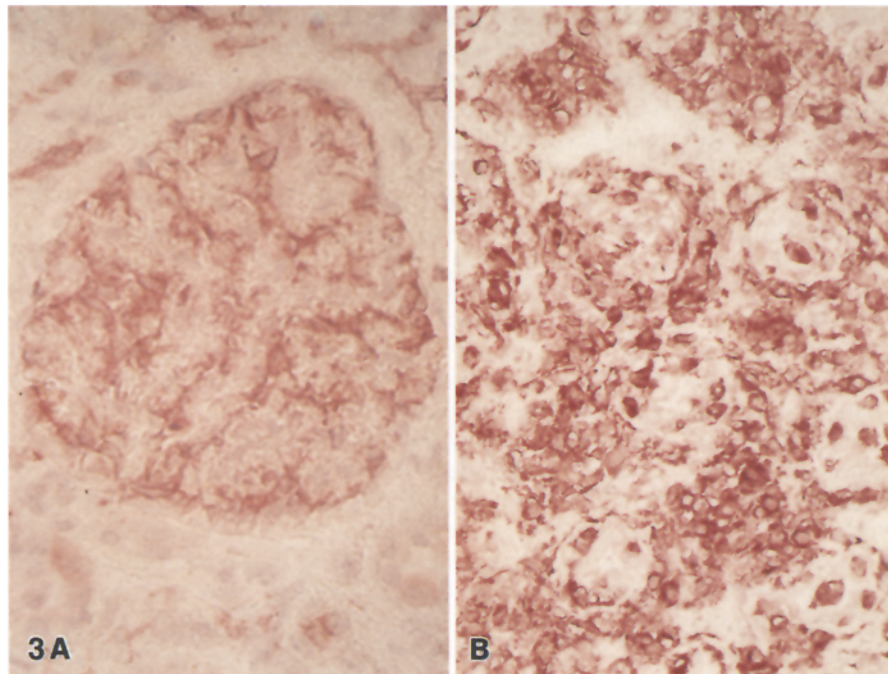

Fig. 3A, B Tü 300 reactivity in diseased kidneys (cryostat sections). **A** Glomerulus of kidney with thrombotic microangiopathy stained with Tü 300. Note stronger staining of podocytes in compartison with Fig. 1A (× 450). **B** Interstitium of kidney with pyelonephritis stained with Tü 300. Note massive positive reaction in comparison with Fig. 1B due to the accumulation of cells of the monocyte/macrophage system (×450)

Table 1 Reactivity of Tü 300 in normal and diseased kidneys. GN, Glomerulonephritis

	No of cases	Reactivity in	
		Glomerulus	Interstitium
		Intensity in podocytes	Interstitial cells (1–4)[a]
Normal kidney	2	Normal	1
Glomerular diseases			
Mesangio-proliferative GN	4	Normal	2
IgA nephritis	2	Normal	1
Epimembranous GN	1	Normal	1
Membranoproliferative GN	1	Normal	1
Anti-glomerular basement membrane GN	1	Normal	1
Segmental focal glomerulosclerosis	1	Normal	1
Glomerular minimal change with nephrotic syndrome	2	Normal	1
Vascular diseases			
Thrombotic microangiopathy	1	Increased	2
Interstitial diseases			
Transplant rejection	6	Normal	3
Interstitial nephritis	1	Normal	3
Acute renal failure	1	Decreased	2
Severe subacute pyelonephritis	2	Normal	4

[a] Semi-quantitive score of the amount of positive cells (1 = minimal, 4 = many), which always gave a strong reaction

ment with published reports about thromboxane formation in glomeruli (Folkert and Schlöndorff 1979; Hassid et al. 1979; Sraer et al. 1989) but is at variance with others reporting that mesangial cells also contribute to thromboxane formation (Kreisberg et al. 1982; Petrulis et al. 1981; Sraer et al. 1979). Studies of cultured human mesangial cells have not verified their ability to form TxB_2 (Ardaillou et al. 1983; Floege et al. 1990). Podocytes however, have been reported to generate 10-fold higher amounts of cyclo-oxygenase metabolites than mesangial cells (Kreisberg et al. 1982).

We assume that the release of TxA_2 by podocytes affects the contractility of the mesangial cell, modified smooth muscle cell in the glomerulus involved in the control of blood flow, filtration surface area and, finally, the ultrafiltration coefficient (Kreisberg et al. 1985; Schlöndorff 1987). Vasodilatory arachidonic acid metabolites, such as prostacyclin, are a product of glomerular endothelial cells which counteract locally the action of TxA_2 on mesangial cells. In cultured mesangial cells prostacyclin receptors and, to a lesser extent PGE_2 receptors, are present coupled to adenylate cyclase and stimulate a rapid rise of intracellular cAMP (Mené and Dunn 1988). Experiments with cultured human mesangial cells showed significantly enhanced proliferation after this stimulation (Mené et al. 1990). The role of thromboxane in the control of mesangial cells and the signals that trigger arachidonate release should be studied further as this is this pre-requisite of thromboxane formation in all thromboxane synthase-positive cells.

In the pathologically modified kidney we found that, in glomerular disease, the thromboxane synthase intensity in podocytes appeared normal. With the exception of the four cases of mesangio-proliferative glomerulonephritis, where the interstitial cells were approximately doubled in number, no change in the numbers of these cells or their thromboxane synthase content could be established. In contrast, in vascular disease such as the one case of thrombotic microangiopathy, there was a clearly increased staining in the podocytes and an increased number of interstitial cells. In all cases of interstitial disease the interstitial cells were greatly increased whereas the staining intensities in podocytes were normal or even slightly decreased.

Thus our results indicate a new, interesting physiological role for thromboxane in podocyte mesangial cell interaction. There is possibly a pathophysiological effect of increased thromboxane levels produced by invading interstitial cells acting on the contractility of the mesangial cells with presumed consequent effects on glomerular function. It should be borne in mind, however, that the relative amount of thromboxane synthase in glomeruli is low when compared with that in circulating and infiltrating cells of the monocyte/macrophage system. Thus, interstitial infiltrating cells may contribute significantly to the elevation of TxA_2. Its metabolites in tissue extracts and urine are mainly contributed by these cells rather than podocytes.

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