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Localization of thromboxane synthase in human tissues by monoclonal antibody Tü 300

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Summary. Using the monoclonal antibody Tü 300 we localized thromboxane synthase, a secondary enzyme of the arachidonic acid cascade, employing the alkaline phosphatase anti-alkaline phosphatase method and indirect double labelling immunofluorescence in frozen sections of human tissues. Aside from platelets, the source of the antigen, all cells of the mononuclear phagocytic system were positive, including epithelioid cells and associated giant cells, starry sky macrophages, dendritic cells of T-cell areas, Langerhans cells and Kupffer cells. In addition, some epithelial cells such as epithelia of tonsillar crypts, reticular epithelia of the thymic cortex and ductular epithelia in liver, pancreas, female breast and salivary glands showed occasional focal reactivity for thromboxane synthase. We suggest that the mAb Tü 300 is a key marker for the macrophage system and the thromboxane generating system in normal and pathological conditions. It may detect functional activities of as yet unknown significance in some specialized epithelial cells.

Key words: Thromboxane – Thromboxane synthase – Immunohistochemistry – Mononuclear phagocyte system – Epithelia

Thromboxane A2 (TXA2) is a labile product of the cyclo-oxygenase pathway and has powerful platelet aggregating and vascular smooth muscle contracting properties (Hamberg et al. 1975; Ellis et al. 1976). Its receptor-mediated action stimulates phospholipase C which hydrolyses membrane phosphoinositides, resulting in the release of two intracellular messengers, inositol-1,4,5-triphosphate and diacylglycerol. These, in turn, release intracellular calcium and stimulate protein kinase C (Brass et al. 1987). Because of the chemical instability of the oxane ring, TXA2 has a short half-life of 30 s and hydrolyses to the stable but biologically inactive derivative TXB2 (Hamberg et al. 1974). Thromboxane is thought to act as an autacoid affecting target cells only in the direct environment of the synthesizing cells. Platelets (Needleman et al. 1976) and macrophages (Murota et al. 1978) are known to contain thromboxane synthase (TX-synthase) by means of biochemical methods but only with antibodies against this cytochrome P450 enzyme can a clear localization be obtained. In view of the various pathophysiological effects of TXA2 (Morrison et al. 1985; Schützer et al. 1988; Lefer 1989; Smith 1989) a better knowledge of the producing cells in the various organs and tissues would be desirable.

Recently, we generated and characterized mAbs against TX-synthase (Nüsing et al. 1989) purified from human platelets (Haurand and Ullrich 1985). Now we have used the mAb Tü 300 to evaluate the localization of the enzyme in different human tissues.

Materials and methods

Tissues were collected from fresh surgical material or from autopsies and shock-frozen at −100° C. Frozen sections were mounted onto microscope slides, welded in plastic bags and stored at −70° C. The slides were thawed for 1–5 min at room temperature, fixed in acetone for 10 min at room temperature and incubated with purified anti-TX-synthase antibody (Tü 300, 1:800; Nüsing et al. 1989). The epitope Tü 300 was visualized by the alkaline/phosphatase anti-alkaline phosphatase method according to Mason (1988) using a rabbit anti-mouse antibody (Dako, Glostrup, Denmark, 1:30) as link and a commercial alkaline phosphate anti-alkaline phosphate complex (Dako A/S, Glostrup, Denmark, 1:80). Counter-staining with Mayer's haemalum solution and mounting in Aquamount completed the procedure. Evaluation was performed by conventional light microscopy.

To verify the mono-histiocytic nature of Tü 300-positive cells, immunofluorescence with double-staining antibodies to lysozyme (Dako, 1:40) and alpha-1-antitrypsin (Behring, Marburg, FRG; 1:800) detected by goat anti-rabbit fluorescein isothiocyanate (FITC) (Jackson Immunoresearch Laboratories, 1:80) was carried out.

To demonstrate the presence of TX-synthase in epithelial cells, a double immunofluorescence method was employed. Acetone-fixed sections were first blocked for endogenous avidin/biotin using

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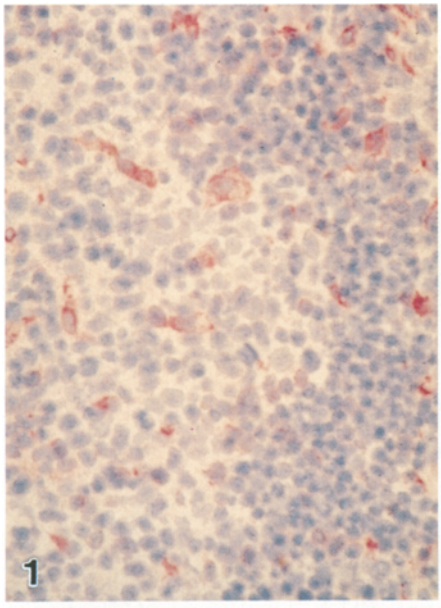
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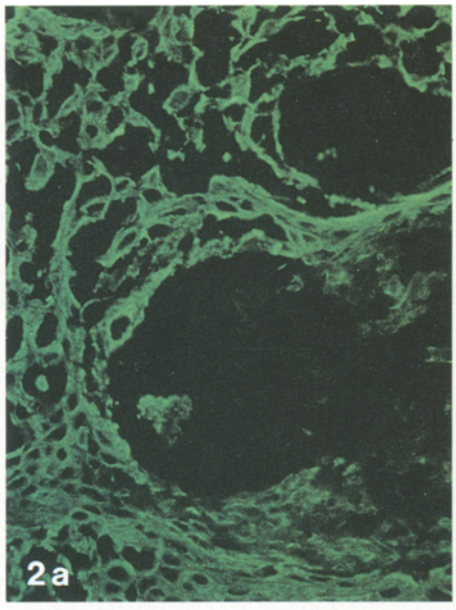
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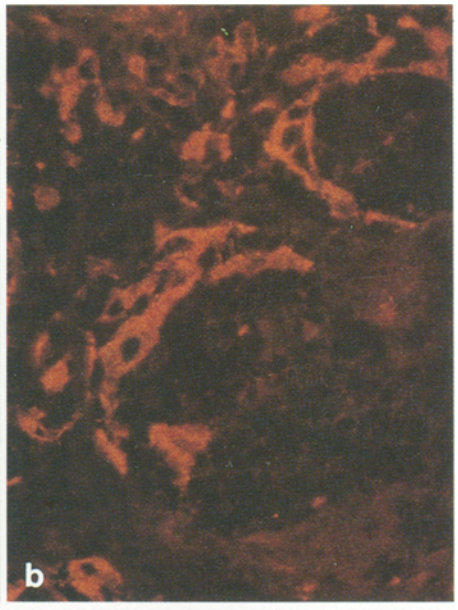
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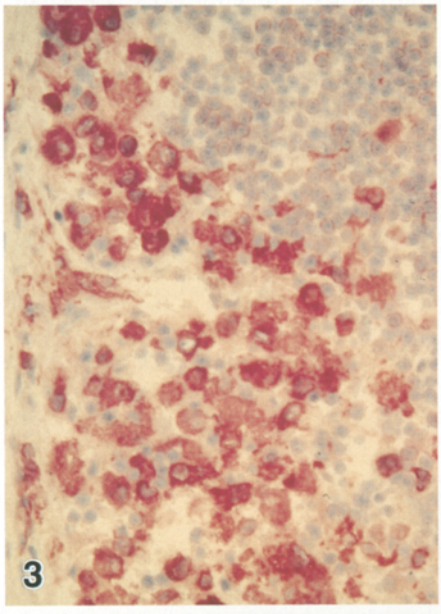
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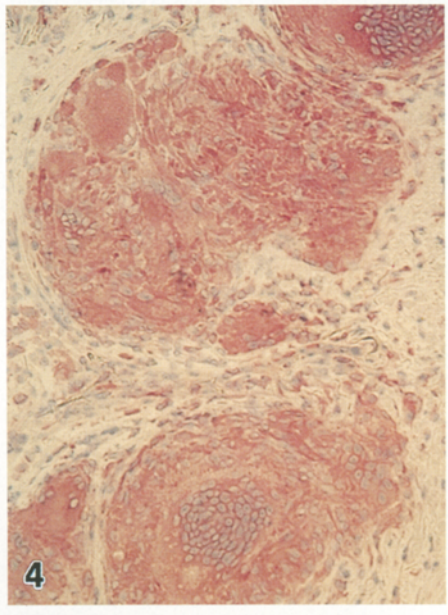
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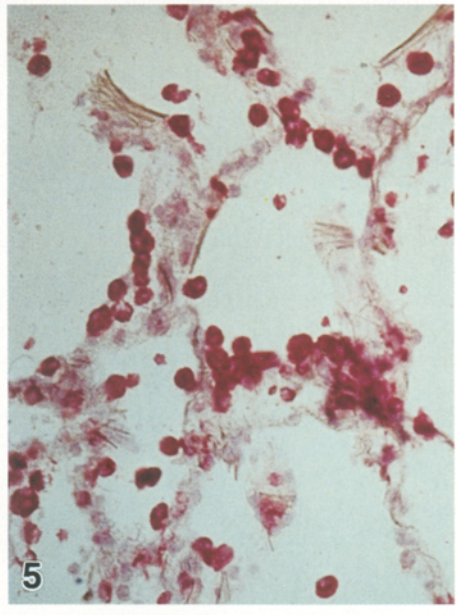
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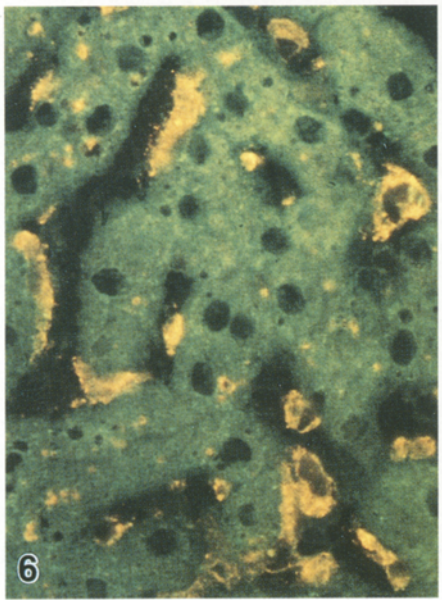
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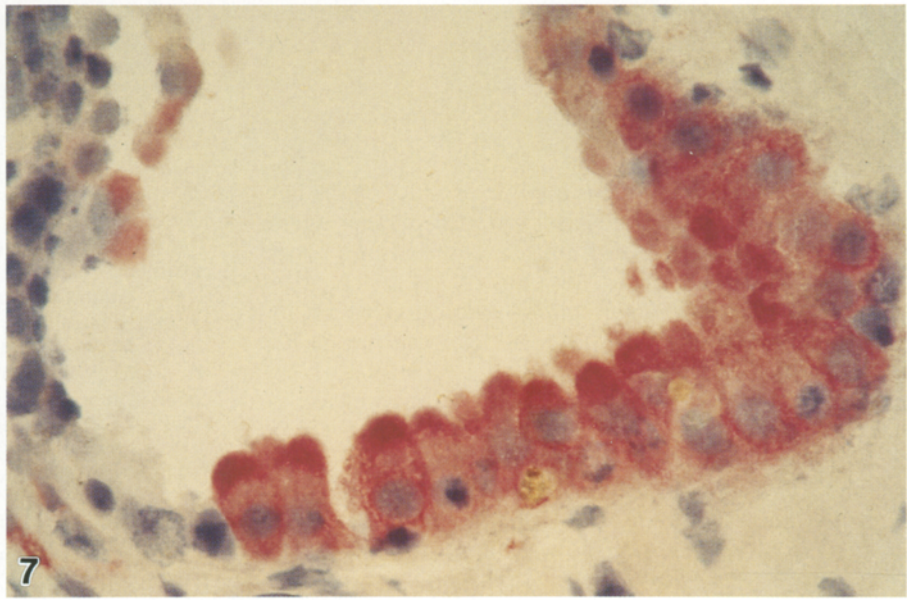
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the blocking kit of Zymed Laboratories (San Francisco, Calif., USA) according to the suggestions of the supplier. The sections were then incubated with mAb Tü 300 (IgG_{2a}), washed twice in phosphate-buffered saline (PBS) and stained with phycoerythrin-labelled goat anti-mouse IgG_{2a} (Southern Biotechnology Associates, 1:40). To block free binding sites, a second incubation with Tü 300 was applied followed by a sequence of biotinylated pan-epithelial mAb lu 5 (kindly provided by Roche Diagnostica, Basel, Switzerland), PBS (2 × 5 min), rabbit anti-biotin (Enzo Biochemistry of Ortho Diagnostic Systems 1:100), PBS (2 × 5 min.), FITC-labelled goat anti-rabbit immunoglobulin (Jackson Immunoresearch Laboratories; 1:80) and PBS (2 × 5 min). The sections were viewed in a Zeiss UV microscope with epi-illumination using band pass filter combinations for selective red or green fluorescence or broad range filters for simultaneous observation.

Results

In all human tissues tested, interstitial histiocytes and macrophages reacted strongly with the mAb Tü 300, particularly in areas of non-specific inflammation whenever this was present in the test tissues. As expected from earlier data (Nüsing and Ullrich 1990), platelets and monocytes in the lumen of vessels exhibited strong antigenicity. The identity of monocytic cells was verified by double labelling with antisera directed against lysozyme and alpha-1-antitrypsin. Endothelial and muscle cells were always negative.

In Table 1 we have indicated the extension of the histiocyte distribution as well as the staining intensity of other cell types reacting with the mAb.

Special features observed in individual organs were as follows. In central nervous tissue only the microglia was stained. Peripheral neurons were negative and only in the endoneurium of nerve bundles was a relatively sparse population of histiocytic cells positive. The endocrine cells proper of parathyroid, thyroid, adrenal glands and islets of the pancreas did not stain. All tissues of the lymphatic system were particularly rich in cells of the macrophage lineage. Starry sky macrophages of the germinal centres and interdigitating cells of the interfollicular areas were positive (Fig. 1). In lymph nodes, sinusoidal macrophages (Fig. 3) and in case of granulomatous inflammation (sarcoidosis, cat scratch disease) epithelioid cells and admixed multinucleated giant cells also showed intensive staining (Fig. 4). In the tonsils, the epithelia of the crypts reacted focally but strongly with mAb Tü 300 (Fig. 2a, b). In human lung, the alveolar macrophages (Fig. 5) represent the main cell type containing TX-synthase; epithelial cells were negative. No epithelial cells in bronchi were seen to be positive in the present series. In human skin the epidermis was nearly free of TX-synthase-containing cells and in the dermis only a sparse population of histiocytes was observed. Additionally, a few Langerhans' cells, primarily in the stratum spinosum, reacted with the mAb as seen in all stratified epithelia. Among the tissues of the digestive system, positively reacting histiocytes were widely represented, except for the tongue, where only a few monocytic cells in the interstitial tissue were present. Some epithelia of serous acini of small salivary glands were stained. All other glandular epithelia as seen in the submandibular gland, the parotid gland and pancreas exhibited no antigenicity. In tissue sections of the oesophagus we observed a moderate number of histiocytic cells, but intra-epithelial Langerhans' cells were stained too. As a rule, the gastroinestinal tract was rich in labelled histiocytic cells, particularly within the mucosa. In the stomach, the surface epithelium was negative for TX-synthase but on the apical site of the mucosa histiocytes were observed, distributed in an umbrella-like fashion. Towards the muscularis mucosae the accumulation of histiocytes decreased. Mucous neck cells and parietal cells were negative. In the liver the Kupffer cells showed very strong antigenicity (Fig. 6), whereas the endothelial cells were negative. In the connective tissue of portal tracts, many histiocytes could be observed. Epithelia of the bile ducts frequently showed weak reactivity. Likewise, in the pancreas, in addition to the widely distributed histiocytes, occasional epithelia of intercalated ducts were labelled. In the kidney, a few histiocyte cells in the interstitium of medulla and cortex were positively stained. A weak but distinct reaction of the monoclonal antibody with podocytes was found in the glomeruli. All other structures of the urinary system as well as of the female and male reproductive system were unreactive with mAb Tü 300 aside from the obligatorily positive histiocytic cells. A focal epithelial staining was observed in ductular epithelia of the mammary gland (Fig. 7).

Fig. 1. Tonsil: demonstration of macrophages in a germinal centre, few within the mantle zone and several within the bordering interfollicular area. APAAP method, × 310

Fig. 2. Tonsil: simultaneous demonstration of keratin (**a**: mAb lu5, green) and thromboxane (TX)-synthase (**b**: mAb Tü 300, red) in many epithelial cells of a crypt. Double labelling fluorescence, × 200

Fig. 3. Lymph node: accumulation of sinusoidal histiocytes strongly positive with Tü 300. APAAP method, × 310

Fig. 4. Lymph node: epithelioid granulomas in sarcoidosis exhibiting a strong positivity for TX-synthase in all epithelioid and giant cells as well as in surrounding histiocytes contained in the non-specific granulation tissue. APAAP method, × 160

Fig. 5. Lung: alveolae with heavily labelled alveolar macrophages. APAAP method, × 158

Fig. 6. Liver: simultaneous demonstration of keratin- (lu 5-) positive liver cells (green) and TX-synthase (Tü 300) in Kupffer cells (orange). Double labelling fluorescence, × 310

Fig. 7. Mammary gland: portion of a duct with focal appearance of TX-synthase in ductular epithelia. APAAP method, × 400

Discussion

As outlined in the introduction, TXA2 is a powerful, short-range and short-acting mediator involved in inflammation and thrombosis. Its promoting role in tumour biology has been pointed out recently (Nigam and Zakrzewicz 1990). The ubiquitous occurrence of the synthesizing enzyme in sessile and circulating, highly specialized cells underlines the key role of this mediator system.

Table 1. Localization of thromboxane synthase in different human tissues by monoclonal antibody Tü 300

	Interstitial histiocytes Frequency	Special cell types Staining intensity
Nervous tissues		
Cerebellum	+	Glial cells + +
Cerebrum	+	Glial cells + +
Peripheral nerve	+	
Endocrine system		
Pituitary	+ +	
Thyroid	+ +	
Parathyroid	+ + +	
Adrenals	+ + +	
Lymphatic system		
Lymph node	+ + +	Interdigitating reticular cells + + + Sinus macrophages + + +
Spleen	+ + +	Interdigitating reticular cells + + +
Thymus	+ + +	Thymic macrophages (cortex) + + + Interdigitating cells (cortex) + + + Thymic dendritic cells (medulla) + + +
Tonsil	+ +	Interdigitating cells + + + Epithelium of crypts + + + (focal)
Respiratory system		
Lung	+	Alveolar macrophages + + +
Bronchus	+ +	
Digestive system		
Tongue	+	Epithelium of serous acini + (focal)
Submandibular gland	+ +	
Parotid gland	+ +	
Oesophagus	+ +	Langerhans cells + +
Stomach	+ + +	
Duodenum	+ + +	
Jejunum	+ + +	
Ileum	+ + +	
Colon	+ + +	
Appendix	+ + +	
Liver	+ +	Kupffer cells + + + Epithelium of bile duct + (focal)
Pancreas	+ + +	Epithelium of intercalated ducts + (focal)
Urinary system		
Kidney	+	Podocytes +
Ureter	+	
Urinary bladder	+ +	
Reproductive system		
Ovary	+ +	
Uterus	+ +	
Vagina	+ +	
Cervix	+ +	Langerhans cells + +
Breast	+ +	Langerhans cells + +
Testis	+ +	
Prostate	+ +	
Seminal vesicles	+	
Muscular tissue		
Skeletal muscle	+	
Cardiac muscle	+	
Skin	+	Langerhans cells + +

Frequency/intensity: + few/weak; + +, several/medium; + + +, many/strong

The present immunohistochemical study on the localization of TX-synthase in frozen, unfixed human tissues confirms our previous report (Nüsing and Ullrich 1990) that TX-synthase is not restricted to thrombocytes, from which the enzyme was extracted for immunization. It is also an obligatory constituent of cells of the mononuclear phagocytic system (MPS; van Furth et al. 1972). Therefore, the mAb Tü 300 may be used to evaluate the distribution of histiocytes and macrophages in human tissues. As a supplement to many restricted antibodies against differentiation-associated membrane markers, this antibody may be considered as a "pan-MPS" marker recognizing an intracellular key enzyme of all phagocytosing and secretory members of this cell family. As an example for the latter group epithelioid cells in granulomas may be cited which are heavily labelled with this antibody.

Apart from the known effector functions in inflammation, such as smooth muscle contraction and platelet aggregation, our findings indicate that the TXA2 generating system is not only involved in the effector phase of the immune response, for example in graft rejection, but also in the afferent limb during antigen presentation. Dendritic cells in T-lymphocyte areas of lymphoid organs, Langerhans cells of the dermis and interstitial reticular cells of all organs were found to harbour TX-synthase. Dendritic cells are the principal accessory cells of the vertebrate system (Gordon et al. 1981; Steinman and Inaba 1989) and known to present antigen effectively and to stimulate T-cells for primary immune responses. In a recent in vitro study it has been shown that TXA2 probably enhances lymphocyte proliferation in response to mitogens (Gordon et al. 1981). The Langerhans cell in the epidermis is critically needed for the initiation of the cutaneous immune response and plays an important role in induction of contact hypersensitivity and graft rejection (Wolff and Stingl 1978; Rowden 1981). A possible involvement of thromboxane in graft versus host reactions has been suggested in different reports (Coffman et al. 1985; Mangino et al. 1989) and TXB2 has been mentioned as an early indicator of allograft rejection (Foegh et al. 1981). With all these functional interactions it is worth noting that the dendritic processes extend three-dimensionally and extensively thus establishing physical contact with many more than immediately adjacent cells. Therefore a short-lived signal such as TXA2 would be adequate for such a cellular microenvironment.

The role of thromboxane in B-lymphocyte stimulation is less clear since the antigen-presenting dendritic reticular cells of lymphoid follicles were not labelled. Instead, the starry sky macrophages were rather weakly stained but their role in B-cell regulation is ill-defined.

Kupffer cells of the liver are considered part of the MPS and thus it is not surprising that they are apparently the major site of TXA2 generation in the liver. It remains to be established whether this is also involved in their function as antigen presenting cells.

The present study has also shed new light on the role of epithelial cells in the generation of TXA2. Although skin, especially the epidermis layer, possesses a high arachidonic acid-transforming capacity (Ruzicka and Printz 1984), the cellular source of the products has not yet been identified. Our results clearly show that dermal generation of TXA2 comes from cells of the MPS and not from the epithelium. Within the epithelial layer Langerhans cells are the only immunohistochemical identifiable source of thromboxane, whereas in the underlying connective tissue histiocytes and reticular cells constitute the main source.

However, in other locations we were able to document a focal presence of TX-synthase in certain epithelial cells. This was consistently seen in groups of crypt, but not superficial epithelial cells, of the tonsil and less frequently in ductular epithelial cells at diverse sites. At present, the functional meaning of this expression is unknown. It is tempting to speculate, however, that this might also be associated with antigen presentation by epthelial cells in inflammatory processes. In this context the expression of TX-synthase in epithelium-derived reticular cells of the thymic cortex is of particular interest since these cells are considered to be involved in cell to cell interaction with cortical thymocytes.

Another interesting aspect of the potential expression of TX-synthase is its appearance in carcinomas derived from such epithelial tissues. By double labelling immunofluorescence we have shown that 30% of breast and 78% of colon carcinoma tumour cells expressed TX-synthase focally (Sauter et al. 1992) but none of 24 bronchial carcinomas did. These positive tumors did not reveal significant differences in their overall behaviour, but it remains an open question whether TXA2-producing tumour cells differ in their ability to spread locally and/or to metastasize.

The podocytes of glomeruli contain TX-synthase at low levels. An obvious short-range responder cell might be the contractile mesangial cell (Nüsing et al., in prep.).

In conclusion, a monoclonal antibody (Tü 300) is described which, on frozen tissue sections, is able to detect TXA2 synthesizing cells as part of the systemic MPS and in certain subpopulations of epithelial cells. The role of such cells in inflammation, graft rejection and tumour biology can be studied with the aid of this antibody.

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