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Monoclonal Antibodies against a Human Juxtaglomerular Epithelioid Granular Cell Tumour

R. Andreessen[a], *W. Baier*[b], *P. Corvol*[c], *F. Pinet*[c], *M.R. Celio*[a,d]

[a]Institute of Anatomy, University of Kiel, FRG; [b]Institute of Anatomy, University of Zürich, Switzerland; [c]INSERM U 36, Paris, France; [d]Institute of Histology, University of Fribourg, Pérolles, Switzerland

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Abstract. With the exception of the renin-angiotensin system, the molecular composition of juxtaglomerular epithelioid granular cells (JEG cells) is unknown. We demonstrate the molecular peculiarities of these modified smooth muscle cells using monoclonal antibodies produced against a benign human JEG-cell tumour. Six out of 29 different clones produced antibodies that label JEG cells nearly exclusively. Antibodies from the other clones recognize JEG cells but also granularly or homogeneously distributed antigens of proximal tubule cells. This suggests antigenic similarity between granules of JEG cells and lysosomes of proximal tubule cells. Other clones produce antibodies which tag different cytoplasmic membranes or even mast cells. The antibodies directed exclusively against JEG cells promise to be useful tools to study their physiology and pathology.

Introduction

Juxtaglomerular epithelioid granular cells (JEG cells) of the kidney are modified smooth muscle cells with endocrine activity [1, 2]. They synthesize and deliver the enzyme renin (EC 4.3.99.19) to the blood circulation [3, 4]. Renin cleaves the circulating prohormone angiotensinogen to angiotensin I [5], which is further transformed to the bio-active peptide angiotensin II by the endothelially bound angiotensin-converting enzyme [6]. Angiotensin II, which may also be directly produced by JEG cells [7, 8], is a potent stimulator of aldosterone secretion [9, 10].

To study the peculiarities in the molecular structure of JEG cells, which differentiate them from smooth muscle cells, we produced monoclonal antibodies against a benign tumour [11, 12] of JEG cells and derived from them 29 different clones. In addition to JEG cells these antibodies also often recognize antigens of proximal tubule cells, of solitary cells and cell membranes.

Methods

Frozen pieces of tumour were ground with a mortar under liquid nitrogen. The crushed tissue was dissolved in complete Freund's adjuvant and injected into Balb/c mice at monthly intervals. After 4–8 months of immunisation the animals were sacrificed by cervical dislocation, and the spleen was used for cell fusion with AG 8-653 myeloma cells (kindly provided by Dr. Hämmerling, Heidelberg, FRG). The hybridoma technique was carried out according to Fazekas de St. Groth and Scheidegger [13]. Culture supernatants were screened by immunofluorescence on cryostat sections of rhesus monkey kidneys fixed with 4% paraformaldehyde in 0.1 *M* phosphate buffer, pH 7.3. Hybrids of interest were cloned twice by limiting dilution. For the production of larger amounts of antibodies, cells were injected intraperitoneally into Balb/c mice (5×10^7/mouse), and the ascitic fluid was collected. A solid-phase antibody assay utilizing subclass-specific rabbit antimouse antibodies (Nordic, Tilburg, Netherlands) was employed to determine the IgG subclasses.

The staining pattern of the monoclonal antibodies was further characterized on 12-µm cryostat sections of human and monkey tissue fixed with 4% paraformaldehyde in 0.1 *M* phosphate buffer, pH 7.3, or with Bouin's fluid [14], by using the indirect immunoperoxidase staining method with 3,3-diaminobenzidine tetrahydrochloride as substrate solution [15]. Supernatant fluid was tested undiluted or diluted up to

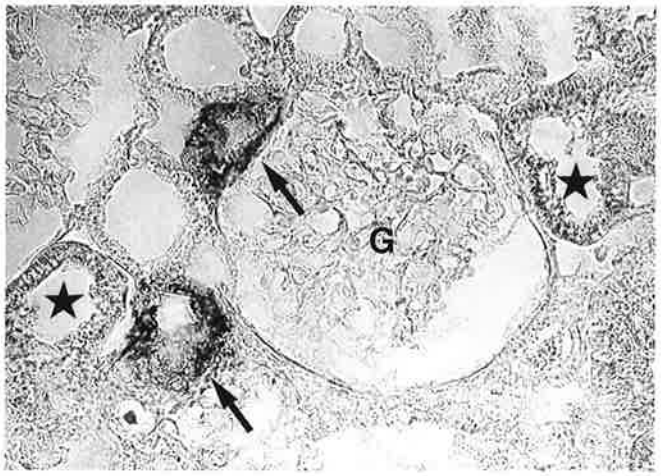

Fig. 1. Antibody 231, recognizing JEG cells nearly exclusively (arrows). Note faint granular immunolabelling in the cytoplasm of proximal tubule cells (stars). G = Glomerulus. ×250.

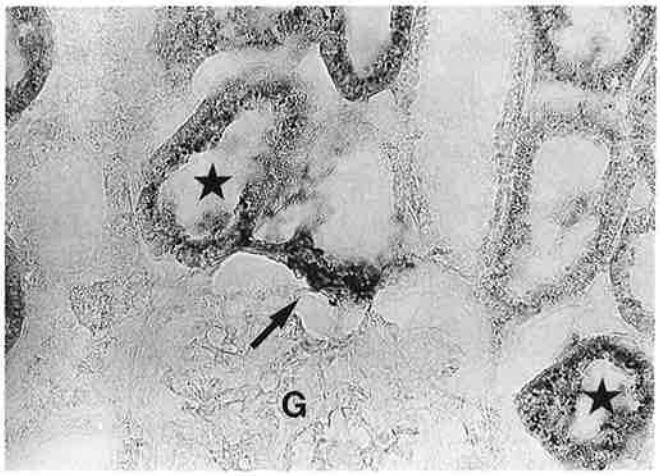

Fig. 2. Antibody 189, recognizing JEG cells (arrow) and granules in the cytoplasm of proximal tubule cells (stars). G = Glomerulus. ×250.

1:100 in 0.1 *M* Tris-buffered saline, pH 7.3. Ascitic fluid was diluted from 1:500 to 1:50,000. The second antibody – horseradish-peroxidase-conjugated antimouse IgG (H+L) prepared in rabbits (Bio-Makor, Rehovot, Israel) – was used in a 1:300 dilution. To identify mast cells we stained adjacent sections with Alcian blue at pH 1 [16].

Results

Using the antibodies produced by the 29 different clones we observe five different staining patterns (see also table 1).

Antibodies Recognizing JEG Cells Nearly Exclusively

The immunoreactive JEG cells are located exclusively in the media of the afferent arteriole in close proximity to the glomerulus and in the immediate neighbourhood of the parietal membrane of Bowman's capsule (fig. 1). The immunoreactive pattern is granulated, on a homogeneous background. Sparsely labelled structures are seen intermingled between mesangium cells of the glomerular tuft. The granules are 1–5 μm in diameter and occur at approximately 20/cell. Additionally the proximal tubule cells display faint granular immunolabelling.

Antibodies Recognizing JEG Cells and Granular Antigens in Proximal Tubule Cells

This second staining pattern of JEG cells does not differ from that described above. In addition, however, proximal tubule cells display strong granular immunoreaction in the cytoplasm (fig. 2). The granules are round, measuring 1–5 μm in diameter, and are located apically.

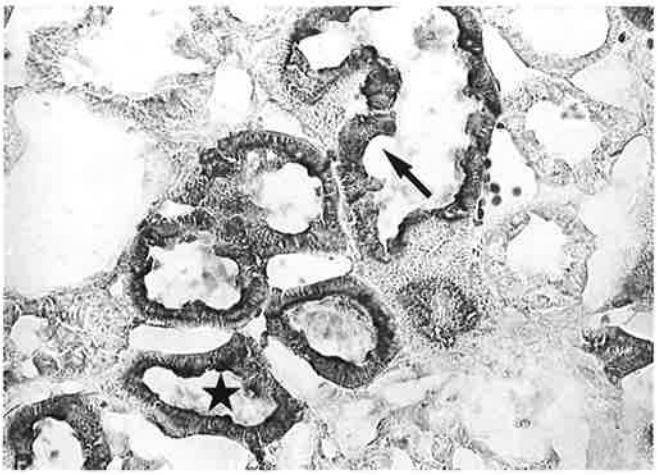

Fig. 3. Antibody 185: homogeneous cytoplasmic staining with intensive labelling of the apical tubule membrane (arrow). Star = proximal tubule. ×250.

Antibodies Recognizing JEG Cells and Homogeneously Distributed Antigens in Proximal Tubule Cells and Some Parts of the Apical Tubule Membranes

In contrast to the antibodies described for the second staining pattern, some antibodies show a homogeneous staining pattern, not only of JEG cells but also of proximal

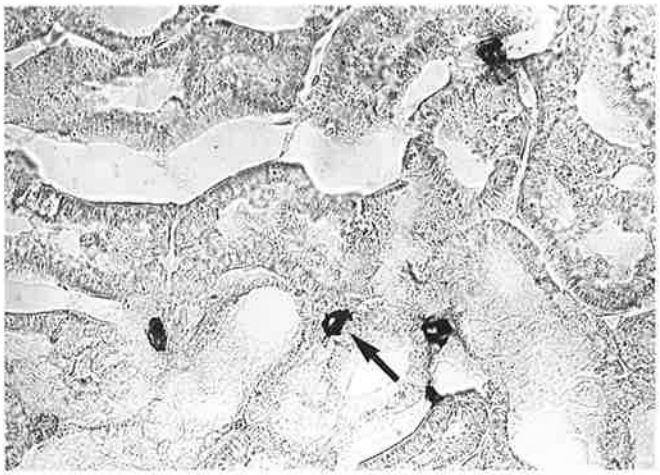

Fig. 4. Antibody 233: labelling of solitary cells in the renal cortex. This labelling is intensive, granular and spares the nucleus (arrow). ×250.

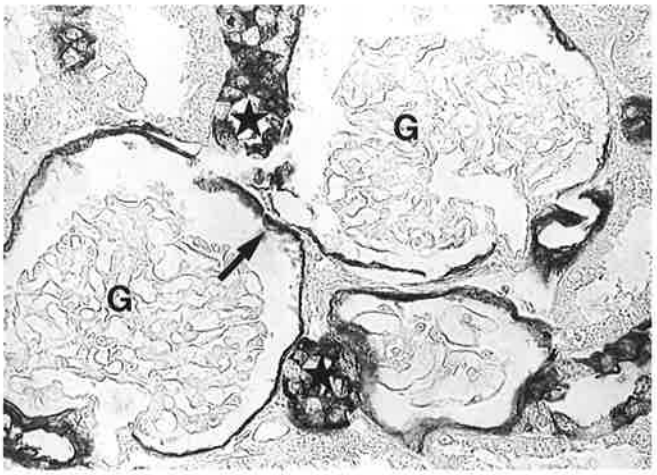

Fig. 6. Antibody 227 (tissue fixed with Bouin's fluid): intensive labelling of the parietal membrane of Bowman's capsule and parts of the distal tubules (arrow). G = Glomerulus; stars = proximal tubules. ×250.

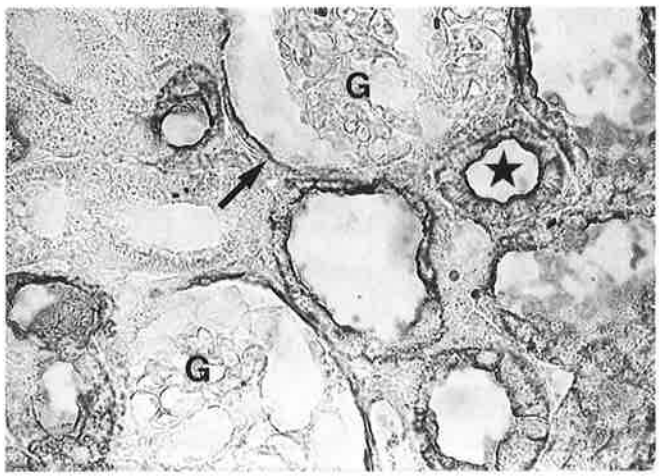

Fig. 5. Antibody 227 (tissue fixed with 4% paraformaldehyde): intensive labelling of the apical tubule membrane (star) and the parietal membrane of Bowman's capsule (arrow). G = Glomerulus. ×250.

tubule cells and, additionally, an intensive labelling of parts of the apical tubule membranes (fig. 3).

Antibodies Recognizing Solitary Cells

A few antibodies label solitary cells in the interstitium of the renal cortex (fig. 4). These cells are more or less round, with a diameter varying between 8 and 25 μm. They are inhomogeneously distributed, with a concentration of up to 50 cells/mm^2.

Antibodies Recognizing Membranes and Different Parts of the Nephron

Some antibodies label membranes, especially the apical tubule membrane and the parietal membrane of Bowman's capsule (fig. 5). Additionally to this labelling some parts of the nephron are homogeneously stained. The intensity of this immunoreaction, however, differs from cell to cell (fig. 6).

All 29 different clones have been tested unsuccessfully on kidneys of rats and mice.

Discussion

The monoclonal antibodies described in this paper recognize antigens in the JEG cells, proximal tubule cells, mast cells and cytoplasmic membranes of primate kidneys. A striking feature is the absence of any staining of smooth muscle cells, from which JEG cells derive. On the other hand, the staining pattern indicates an antigenic relationship between the granules of JEG cells and lysosomes of proximal tubule cells.

The secretory granules of JEG cells may be evolutionary homologues to lysosomes, and similarities between the matrix components of these organelles have been suggested by de Duve and Wattiaux as early as 1966 [17]. In support of this contention, Taugner et al. [18] report that intravenously injected peroxidase not only becomes apparent in lysosomal compartments, but also in renin-containing secretory granules. Furthermore, acid phos-

Table 1. Staining patterns

Clone	IgG subclass	JEG cells	Proximal tubule (granulated)	Proximal tubule (homogeneous)	Proximal tubule (apical membrane)	Bowman's capsule	Solitary cells	Figure No.
175	IgG 1	++	+					
176	IgG 1	++	+					
177/178	IgG 2a	(+)	+++					
179/180	IgG 1	++	+++					
183/184	IgG 1	++	+					
185/186	IgG 1	(+)		+++	++			3
187/188	IgG 1	+++	+					
189/190	IgG 1	++	+++					2
191/192	IgG 1	++	+++					
193/194	IgG 2b	+	++					
195/196	IgG 1	+	++					
197/198	IgG 1	+	+++					
199/200	IgG 1	++	++					
201	IgG 1	+++	++					
202/203	IgG 3	(+)		+++	++			
204/205	IgG 3	+	(+)					
206/207	IgG 1	+	+++					
208/209	IgG 1	+	++					
210/211	–	+	(+)					
216/217	–	+	(+)					
218/219	–						++	
220/221	–						++	
222/223	–	+	(+)					
224	–						++	
225/226	–	+	(+)					
227/228	–			+a, +++b	+++a, b	+a, +++b		5, 6
229/230	–						+++	
231/232	–	+	(+)					1
233/234	–						+++	4

– = IgG subclass determination not performed; a = tissue fixed with 4% paraformaldehyde; b = tissue fixed with Bouin's fluid; +++ = very strong labelling; ++ = strong labelling; + = average labelling; (+) = weak labelling.

phatase, a lysosomal marker enzyme, is present in both types of organelles [18], which also react similarly to the application of lysosomotropic substances [18–21]. Not only are the two intracytoplasmic organelles analogous in their structure, but also their secretory products, renin (granules of JEG cells) and cathepsin D (lysosomes), share 33% of their amino acid residues [22] and both belong to the group of aspartyl proteases [23]. Substances biochemically related to aspartyl proteases or originating from a common evolutionary ancestor could represent the antigen immunolabelled in both proximal tubule cells and JEG cells.

The JEG-cell tumour was not reported as containing tubular epithelial cells [24]; however, the possibility that surrounding normal tissue was excised and homogenized with the tumour has to be kept in mind. Therefore, the labelling of tubular membranes could arise from the contamination of the immunogen with normal kidney tissue. Similarly, the family of antibodies recognizing mast cells may have been raised against mast cells infiltrating the original JEG-cell tumour.

In conclusion, one third of the antibodies raised against the tumour recognize JEG cells nearly exclusively, in the normal human and in the monkey kidney. A large number of antibodies also detect lysosomes of proximal tubule cells, pointing to the presence of a strong antigenic component common to both organelles. The antibodies directed against mast cells and membranes could have derived from contamination of the tumour with normal tissue constituents.

Those antibodies labelling JEG cells selectively may help to understand the physiology of JEG cells and could be used in clinical pathology.

References

1 Ruyter JHC: Über einen merkwürdigen Abschnitt der Vasa afferentia in der Mäuseniere. Z Zellforsch Mikrosk Anat 1925;2: 242–248.
2 Barajas L, Müller J: Structure of the juxtaglomerular apparatus; in Johnson JA, Anderson RR (eds): The Renin-Angiotensin System. Adv Exp Med Biol 130. New York, Plenum Press, 1980, pp 85–103.
3 Tigerstedt R, Bergman PG: Niere und Kreislauf. Skand Arch Physiol 1898;8:223–271.
4 Davis JO, Freeman RH: Mechanisms regulating renin release. Physiol Rev 1976;56:1–56.
5 Page IH, Helmer OM: A crystalline pressor substance (angiotonin) resulting from the reaction between renin and renin activator. J Exp Med 1940;71:29–42.
6 Skeggs LT, Kahn JR, Shumway NP: The preparation and function of the hypertensin-converting enzyme. J Exp Med 1956;103: 295–299.
7 Celio MR, Inagami T: AII immunoreactivity coexists with renin in the juxtaglomerular epithelioid granular cells of the rat kidney. Proc Natl Acad Sci USA 1981;8:3897–3900.
8 Taugner R, Hackenthal E: Angiotensin II in epithelioid (renin containing) cells of rat kidney. Histochemistry 1981;72:499–509.
9 Page IH, Bumpus FM: Angiotensin. Physiol Rev 1961;41:331–390.
10 McCaa RE, Guyton AC, Young DB, McCaa CS: Role of aldosterone biosynthesis; in Johnson JA, Anderson RR (eds): The Renin-Angiotensin System. Adv Exp Med Biol 130. New York, Plenum Press, 1980, pp 227–252.
11 Galen FX, Corvol MT, Devaux P, Gubler MC, Mounier F, Camilleri JP, Houot AM, Menard J, Corvol P: Renin biosynthesis by human tumoral juxtaglomerular cells. Evidences for a renin precursor. J Clin Invest 1984;73:1144–1155.
12 Pinet F, Corvol MT, Dench F, Bourguignon J, Feunteun J, Menard J, Corvol P: Isolation of renin producing human cells by transfection with three simian virus 40 mutants. Proc Natl Acad Sci USA 1985;82:8503–8507.
13 Fazekas de St. Groth SF, Scheidegger D: Production of monoclonal antibodies: Strategy and tactics. J Immunol Methods 1980;35:1–31.
14 Romeis B: 305. Pikrin-Formol-Eisessig; in Romeis B (ed): Mikroskopische Technik. München, Leibniz, 1948, pp 69–70.
15 Graham RC, Karnovsky MJ: The early stages of absorption of injected horseradish peroxidase in the proximal tubules of the mouse kidney: Ultrastructural cytochemistry by a new technique. J Histochem Cytochem 1966;14:291–302.
16 Lev R, Spicer SS: Specific staining of sulphate groups with Alcian blue at low pH. J Histochem Cytochem 1964;12:309.
17 De Duve C, Wattiaux R: Functions of lysosomes. Annu Rev Physiol 1966;28:435–492.
18 Taugner R, Whalley A, Angermüller S, Bührle CP, Hackenthal E: Are the renin-containing granules of juxtaglomerular epithelioid cells modified lysosomes? Cell Tissue Res 1985;239:575–587.
19 Lüllmann-Rauch R: Drug-induced lysosomal storage disorders; in Dingle JT, Jacques PJ, Shaw IH (eds): Lysosomes in Applied Biology and Therapeutics. Amsterdam, North-Holland Publishing Company, 1979, vol 6, pp 49–130.
20 De Duve C: Lysosomes revisited. Eur J Biochem 1983;137: 391–397.
21 Hruban Z, Slesers A, Hopkin E: Drug-induced and naturally occurring myeloid bodies. Lab Invest 1972;27:62–70.
22 Shewale JG, Tang J: Amino acid sequence of porcine spleen cathepsin D. Proc Natl Acad Sci USA 1984;81:3703–3707.
23 Cordes EH: Structure and function of carboxyl proteases; in Doyle AE, Bearn AG (eds): Hypertension and Angiotensin System. New York, Raven Press, 1983, pp 77–91.
24 Pinet F: Etablissement de lignées cellulaires humaines rénales et extra-rénales productrices de rénine; thèse de doctorat de l'Université de Paris-VI, 1987.

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Dr Marco R. Celio
Institute of Histology and General Embryology
University of Fribourg
Rue A.-Goeckel
CH–1700 Fribourg (Switzerland)