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The organ distribution of gp-330 (Heymann antigen) and gp-90 in the mouse and the rat

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Summary. The Heymann antigen (gp-330) and an antigen with lower molecular weight (gp-90) are major constituents of the brush border of the renal proximal tubules in the rat and the mouse. The Heymann antigen can also be found at discrete sites in the glomerular visceral epithelium of the rat, but not of the mouse. Gp-90 is present diffusely along the glomerular capillary wall of rat and mouse. The Heymann antigen is probably the target antigen for membranous glomerulonephritis in the rat, while in the mouse, where this form of glomerulonephritis can also be induced, gp-90 seems to be the antigen involved. We have separated the antibody populations against these two antigens by preparing eluates from kidneys of rats and livers of mice that had been injected with an antiserum against pronase-digested mouse renal tubular antigens. Using these purified antibodies we have examined by indirect immunofluorescence the distribution of the two antigens on normal mouse and rat tissues. The expression of the Heymann antigen is limited to the epithelia of several organs, while gp-90 has a more widespread distribution in many cells of different origin and function in both the mouse and the rat.

Key words: Organ distribution – Heymann antigen – gp-90 protein – Mouse – Rat

Introduction

Antisera directed against pronase-digested mouse renal tubular antigens contain antibodies against the Heymann antigen (gp-330) and against an antigen with a lower molecular weight (gp-90) (Assmann et al. 1984; 1985). Both antigens are present abundantly in the brush borders of the renal proximal tubules. The Heymann antigen can be found at discrete sites in the glomerular visceral epithelium of the rat but not of the mouse, while

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the gp-90 protein has a more homogeneous distribution in the cell membranes of the glomerular visceral epithelia and endothelia of both the mouse and the rat (Assmann et al. 1983; 1984; Kerjaschki and Farquhar 1983; Ronco et al. 1984a; 1984b). It is assumed that the Heymann antigen plays a key role in the formation of subepithelial deposits in the active and passive Heymann nephritis in rats. On the other hand, gp-90 is the target for antibodies that can induce a membranous glomerulonephritis in the mouse (Assmann et al. 1985).

Purified antibodies against gp-330 can be obtained by preparing eluates from kidneys of rats previously injected with antiserum against pronase digested mouse renal tubular antigens. Antibodies against gp-90 can be similarly purified using livers of mice previously injected with the antiserum (Assmann et al. 1984; 1985). In this report we describe in detail the localization of these two antigens in normal mouse and rat tissues as detected by immunofluorescence studies with the two eluates.

Materials and methods

Animals. The inbred mouse strain C57Bl/10 was originally obtained from the Jackson Laboratory, Bar Harbor, Maine, USA. Swiss mice and Wistar rats, all random bred, were bought from the Central Institute for the breeding of laboratory animals, TNO, Zeist, The Netherlands. New Zealand white rabbits were bought from a local breeder.

Preparation of antigens and antisera. Mouse F × 1A prepared from Swiss mouse kidneys, and the pronase-digested fraction thereof, designated M.TAPron, were prepared as previously described (Assmann et al. 1983). Rabbits were immunized with mouse F × 1A or TAPron, emulsified in complete Freund's adjuvant (Difco Laboratories, Detroit, MI, USA). The IgG fraction was obtained from a 50% ammonium sulphate precipitate, purified by affinity chromatography on a Sepharose-4B coupled protein-A column (Pharmacia, Uppsala, Sweden), and concentrated to approximately 10 mg/ml by ultrafiltration with a XM-50 Diaflow membrane (Amicon Corporation, Scientific System Division, Lexington, MA, USA) as described earlier (Assmann et al. 1983).

Preparation of eluates from rat kidneys and mouse livers. As described earlier, antibodies against the Heymann antigen (gp-330) were purified by acid elution of a glomerular suspension of Wistar rats that had previously been injected with 2 ml of anti-mouse TAPron serum (Assmann et al. 1984). The glomeruli were isolated from a homogenate by a differential sieve technique and after a short sonication of the suspension bound antibodies were eluted with a 0.1M Glycine-HCl buffer, pH 2.8, for 1 h at room temperature. After concentration of the eluate by ultrafiltration with a XM-50 Diaflow membrane (Amicon Corp.), the protein content was adjusted to 5 mg/ml. Protein concentrations were measured with the method of Lowry et al. (1951). Antibodies to the gp-90 were obtained from livers of similarly injected C57Bl/10 mice killed 15–30 min after injection. We have previously shown that mouse liver expresses the gp-90, while lacking the gp-330. Bound antibodies were eluted with the same acid buffer from a homogenate as reported in an earlier study (submitted for publication). The Ig fraction from the eluate was obtained from a 50% ammonium sulphate precipitate. The protein content of the final preparation was 1.7 mg/ml.

In vitro incubations on normal mouse and rat organs. The distribution of the antigens by the eluates was examined in the indirect immunofluorescence technique on frozen sections of normal mouse and rat tissues. The fixation and staining techniques have been described earlier (Assmann et al. 1983). Bound rabbit antibodies were visualized by a fluorescein-labelled swine

anti-rabbit Ig (Dakopatt, Copenhagen, Denmark), absorbed with 500 mg/ml lyophilized non-immune mouse and rat serum. The staining intensities and the quantities of the immune reactants were recorded semiquantitatively (0+ = negative, 1+ = moderate, 2+ = relatively strong, 3+ = strong, 4+ = maximum intensity).

In vitro incubations of cell suspensions of mouse and rat thymus, spleen, and peripheral blood lymphocytes (PBLs). Single cell suspensions of thymocytes and spleen cells of C57Bl/10 mice and Wistar rats were obtained in petri dishes by teasing apart the organs with a needle. Mononuclear cells were isolated from 8 ml of citrated blood by Ficoll-Isopaque density gradient centrifugation. The cells were washed twice in PBS, pH 7.2, containing 5% bovine serum albumin and 0.01% Na azide (PBS/BSA/NaN_3). Subsequently $5–10 \times 10^6$ cells were incubated with 200 µl PBS/BSA/NaN_3 containing anti-mouse F × 1A (100 µg/ml), anti-mouse TAPron (100 µg/ml), the eluate from rat kidneys (50 µg/ml), or the eluate from mouse livers (50 µg/ml) for 30 min at 4° C. After washing, the cells were incubated with 100 µl of fluorescein-labelled swine anti-rabbit Ig (dil. 1:40) for 30 min at 4° C. The number of positive cells was expressed as the percentage of the total number of cells analysed. Normal rabbit Igs replacing the antisera and eluates in the first step of the incubations and the swine anti-rabbit Ig alone were included as controls.

Results

The two eluates have been used in previous studies and have been characterized by immunoprecipitation analysis on radiolabelled brush borders. The eluate from rat kidneys precipitated a single protein that comigrated with a protein bound by a monoclonal antibody to the gp-330. The mouse liver eluate predominantly bound to the gp-90 comigrating with a protein immunoprecipitated by a monoclonal antibody to the gp-90 protein (Assmann et al. 1985, and submitted for publication).

In vitro incubations of eluted antibodies from rat kidneys

The antigen detected by the rat eluate was present abundantly in the brush borders of the proximal tubular epithelia of both the mouse and the rat kidney and furthermore at discrete sites along the glomerular capillary wall of the rat, but not of the mouse (Fig. 1 A, E). Outside the kidney the antigen was present in several organs as summarized in Table 1. In both animals the type II alveolar cells in the corners of the alveolar septa of the lung, the apical parts of the epithelia of the epididymis, thyroid, ductules of the lacrimal gland, visceral yolk sac, and some ependymal cells of the ventricles of the brain contained the antigen in varying degrees (Fig. 1). Fine granules could also be observed in the endocrine cells of the parathyroid and especially in the basal epithelia of the ciliary body in the eye. Some organs, however showed a species specific localization. In the mouse the epithelia of the Harderian gland, a sebaceous-like gland situated behind the eye, and the outer cells in the lobules of the preputial and clitoral glands revealed the antigen. Only in the rat however, was the antigen present in the ductules of the parotid gland and, irregularly, in the coelomic epithelium of the ovary, on the oviduct, and the uterine epithelium. All other organs of both animals were negative, in particular the spleen, thymus, lymph nodes, heart, liver, small intestine, pancreas, and acinar cells of the lacrimal and salivary

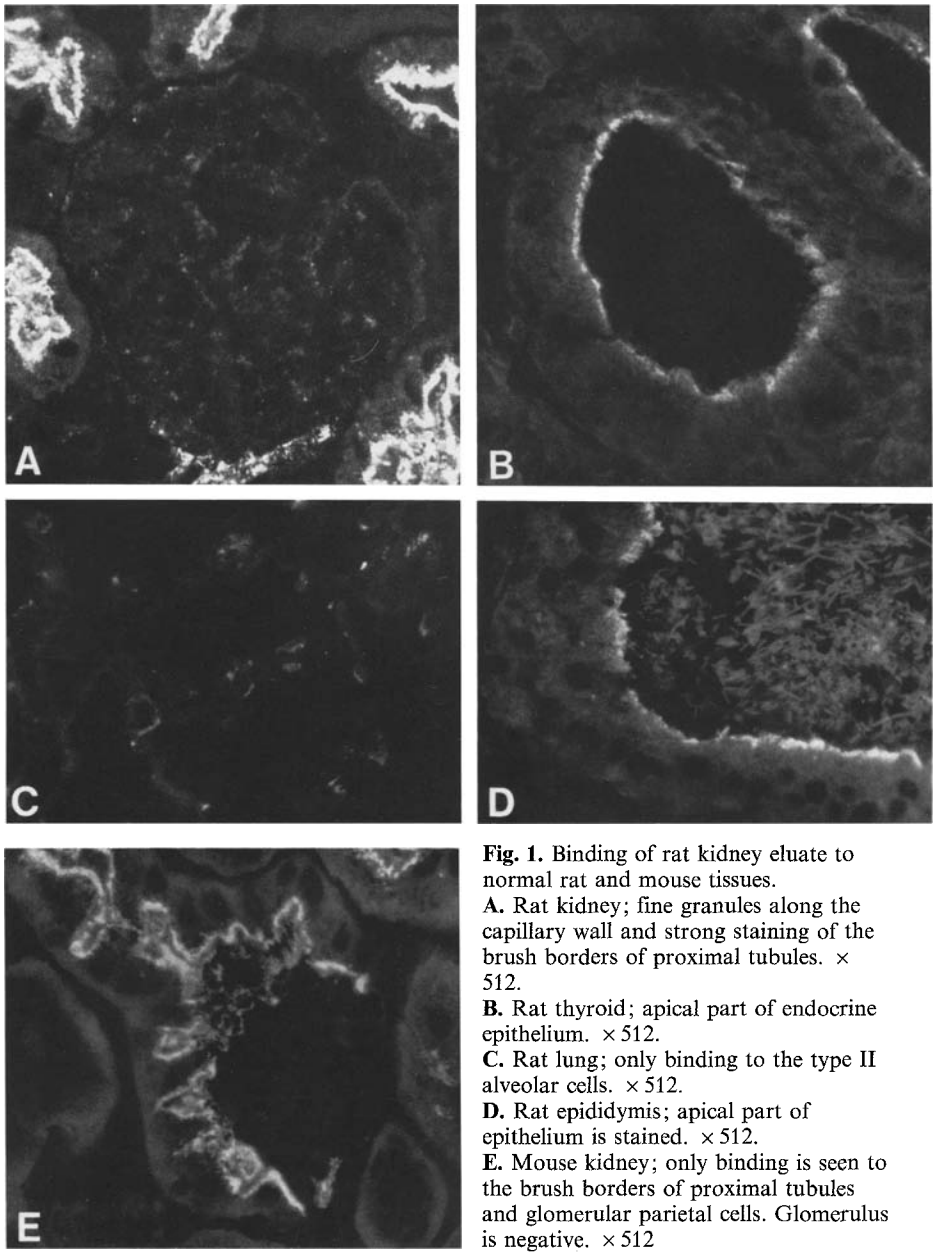

Fig. 1. Binding of rat kidney eluate to normal rat and mouse tissues.
A. Rat kidney; fine granules along the capillary wall and strong staining of the brush borders of proximal tubules. × 512.
B. Rat thyroid; apical part of endocrine epithelium. × 512.
C. Rat lung; only binding to the type II alveolar cells. × 512.
D. Rat epididymis; apical part of epithelium is stained. × 512.
E. Mouse kidney; only binding is seen to the brush borders of proximal tubules and glomerular parietal cells. Glomerulus is negative. × 512

glands. Although the majority of thymocytes, spleen cells, and PBLs showed positive staining with the unseparated antisera in single cell suspensions, the results of incubations with the rat eluate were completely negative (Table 3). All control incubations were negative.

Table 1. Organ distribution of the Heymann antigen[a]

Organ[b]	Mouse	Rat	Comments[c]
Kidney glomerulus	−	+	fine granules along capillary wall
brush border	+++	+++	basis of villi of BB
Epididymis	++	++	AP
Visceral yolk sac	+++	++	AP
Lung	++	++	fine granules in alveolar cells, type II
Eye	+	+	fine granules in basal cells of ciliary body
Harderian gland	+++	−	AP
Thyroid	++	++	AP
Parathyroid	+	+	fine granules in endocrine cells
Lacrimal gland	++	++	AP of ductules; acini negative
Parotid	−	++	AP of ductules; acini negative
Preputial gland	++	−	fine granules in outer cells of lobules
Clitoric gland	+	−	fine granules in outer cells of lobules
Ovary	−	+	AP; on some coelomic epithelia
Oviduct	−	++	AP, focal
Uterus	−	+	AP, focal
Brain	+	+	AP, on some ependym cells; choroid negative

[a] Detected in the indirect immunofluorescence technique with a kidney eluate of rats previously injected with rabbit anti-mouse TAPron serum
[b] All other organs examined are negative
[c] BB = brush border of the proximal tubules; AP = apical parts of the epithelium

In vitro incubations of eluted antibodies from mouse livers

As shown in Table 2 we found a widespread organ distribution of the antigen in both the mouse and the rat. Differences between the two animals were generally quantitative, the mouse showing a more prominent expression of the antigen in most tissues. The most common binding site was the endothelium of the capillary bed of almost all organs examined. Occasionally endothelial cells of arterioles also showed a faint staining. Differences in staining intensities in individual organs of the same species and interspecies variations were observed. In the mouse, the most intense staining was found in the endothelial cells of brain, liver (Fig. 2B), muscle, and thyroid, whereas in the rat the endothelial cells of liver, adrenal, thyroid, heart, spleen, muscle, and corpora cavernosa of the penis were highly positive. Several parts of the kidney contain the antigen as previously described (Assmann et al. 1985). A homogeneous staining along the glomerular capillary wall of the mouse and, to a lesser degree, of the rat, was seen while the brush borders of the proximal tubules were heavily stained in both animals (Fig. 2A). The loops of Henle and to a lesser extent the apical parts of the convoluted distal tubules showed the presence of the antigen, particularly in the rat. In the lung a more diffuse fluorescence was visible along the alveolar septae (Fig. 2D). A prominent staining was seen in the cells lining the cavities and in cells in the capsule of several organs (Fig. 2G). Furthermore, as is listed in Table 2, many epithelia in different organs con-

Table 2. Organ distribution of the gp-90 protein[a]

Organ[b]	Mouse	Rat	Comments
Urogenital system			
Kidney glomerulus	++++	++	homogeneous along capillary wall
proximal tubules	+++	+++	BB diffusely stained
distal tubules	+/−	+	AP of convoluted part
loops of Henle	+	+++	
Ureter	+/−	+/−	superficial epithelia
Bladder	−	−	E pos.
Prostate	+++	+/−	AP and BP
Seminal vesicles	+/−	+/−	AP and BP; faint staining of contents
Urethra	−	−	
Testis spermatozoa	−	+/−	
Sertoli and Leydig cells	−	−	
Epididymis	+++	++/+	AP and BP
Vas deferens	+	+	around epithelia
Penis	−	−	E of CC in rat strongly pos
Preputial gland	−	−	
Ovary follicles	++	+	theca cells of riping follicles
stroma	+	+	
Oviduct	+/−	+/−	AP
Uterus epithelium	++	−	AP
stroma	+	+	
Vagina	+	−	superficial layer of epithelia
Clitoral gland	−	−	
Gastrointestinal tract			
Tongue	−	−	
Esophagus	+	−	superficial layer of epithelia
Stomach	−	+/−	some glandular cells
Small intestine brush border	+++	+++	decreasing intensity toward distal end
crypts	+	+	AP
Large intestine	++	+	AP of lumen and crypts in mouse; in rat only crypts
Gall bladder	+	+	AP
Liver sinusoidal lining cells	++	++	
hepatocytes	+	+	only part of cell facing sinus
biliary epithelium	++++	++++	
Peritoneum	++++	++++	
Cardiovascular and pulmonary system			
Endothelium capillaries	++/+	++/+	in almost all organs; sometimes E small vessels
large vessels	−	−	
Heart myocardium	−	−	
peri-endocardium	++++	+	
Trachea surface epithelium	+	+	AP
gland	++	+	AP
Lung bronchial epithelium	+	+	AP
alveolar epithelium	++	++	diffuse along with E
pleura	++++	+++	

Table 2. (continued)

Organ[b]	Mouse	Rat	Comments
Endo- and exocrine glands			
Thyroid	+/++	−/±	AP
Parathyroid	±	−	fine staining endocrine cells
Adrenal cortex/medulla	−	−	
Pancreas acini	+	+	AP
ductal epithelium	++	+++	AP
islets of Langerhans	±	±	diffuse staining of some endocrine cells
Parotid acini	++	++	AP
ductal epithelium	+++	+++	AP
Submandibular gland	++	++	AP ductal epithelia; faint staining acini
Sublingual gland	+	+	AP ductal epithelia; faint staining acini
Nervous system			
Peripheral perineurium	+++	+++	
Central dura	+++	+++	
ependym	−	−	
neurons	−	−	in mice prominent staining E
Lymphatic tissue			
Thymus cortex	++	++	membrane bound staining of thymocytes
medulla	+	+	some cells with fine granular staining
Spleen follicles	+	±	membrane bound staining
red pulpa	±	+++	sinus endothelium; faint staining of stromal cells
Lymph nodes	+	+	membrane bound staining of follicle cells
Muscle smooth	±	−	media of some arteries (in mouse)
skeletal	−	−	
Placenta visceral yolk sac	++++	++	AP
Skin epidermis	++/−	+/−	outer cells
Fibroblast	+	+	in connective tissue of many organs; organ capsules
Eye cornea	++	+/−	outer squamous epitehlium; Descemet's membrane; F
ciliary body	−	−	
retina	−	−	
lens	−	−	
Harderian gland	++	−	AP irregularly

[a] detected by mouse liver eluate in the indirect immunofluorescence technique

[b] BB = brush border; AP = apical parts of epithelium; BP = basal parts of epithelium; CC = corpora cavernosa; E = endothelium of capillaries; pos. = positive staining; F = fibroblasts

Table 3. Incubations of suspensions of thymus, spleen, and PBLs of mouse and rat with antisera and eluates

Antiserum[b]	Percentage of positive cells[a]					
	Thymus		Spleen		PBLs[c]	
	Mouse	Rat	Mouse	Rat	Mouse	Rat
RaM.F × 1A	62[d]	80[d]	66	74	85	85
RaM.TAPron	80	80	86	84	91	87
R.eluate	–	–	–	–	–	–
M.eluate	70	85	83	39	90	72

[a] Expressed as the percentage of 300–400 cells analysed.
[b] RaM.F × 1A = rabbit anti-mouse F × 1A; RaM.TAPron = rabbit anti-mouse TAPron; R.eluate and M.eluate = eluates from kidneys of rats and livers of mice previously injected with RaM.TAPron
[c] PBLs = peripheral blood lymphocytes
[d] Intensity of staining of RaM.F × 1A was weak on thymocytes

tained the antigen in varying densities where it was located mostly in the apical part of the cells (Fig. 2A, B, C, E, F, I). In connective tissues, for instance the stroma of ovary, oviduct, and uterus, there was a faint to moderate staining of fibroblasts and some other unidentified cells. Thymocytes in the cortex of the thymus (Fig. 2H) and lymphocytes in the follicles of the spleen and lymph nodes demonstrated a cell membrane bound fluorescence. Examination of suspensions of the thymus, spleen, and PBLs of the mouse and the rat revealed that the majority of cells was positive as listed in Table 3. All control incubations were negative.

Discussion

The microvillous fraction of the brush borders of the renal proximal tubules, the starting material for the preparation of our antisera, contains numerous proteins, many of which are cell membrane-bound (Kenny and Maroux 1982). Two proteins, gp-330 and gp-90 are reported to play a crucial role in evoking a membranous glomerulonephritis in the rat (Heymann et al. 1959; Barabas et al. 1970; Feenstra et al. 1975; Kerjaschki and Farquhar 1982; 1983), and in the mouse respectively (Assmann et al. 1983; 1985). By preparing eluates from kidneys of rats and livers of mice injected with anti-mouse TAPron antiserum we have demonstrated that this antiserum actually contains antibodies against both specificities (Assmann et al. 1984; 1985, and submitted for publication). With these eluates, previously characterized by immunoprecipitation analysis and used for studying the distribution of the antigens in the kidney, we could now examine the extrarenal distribution by indirect immunofluorescence in both the mouse and the rat.

Earlier studies on the extrarenal distribution of brush border antigens in rats by means of indirect immunofluorescence were done with less well-

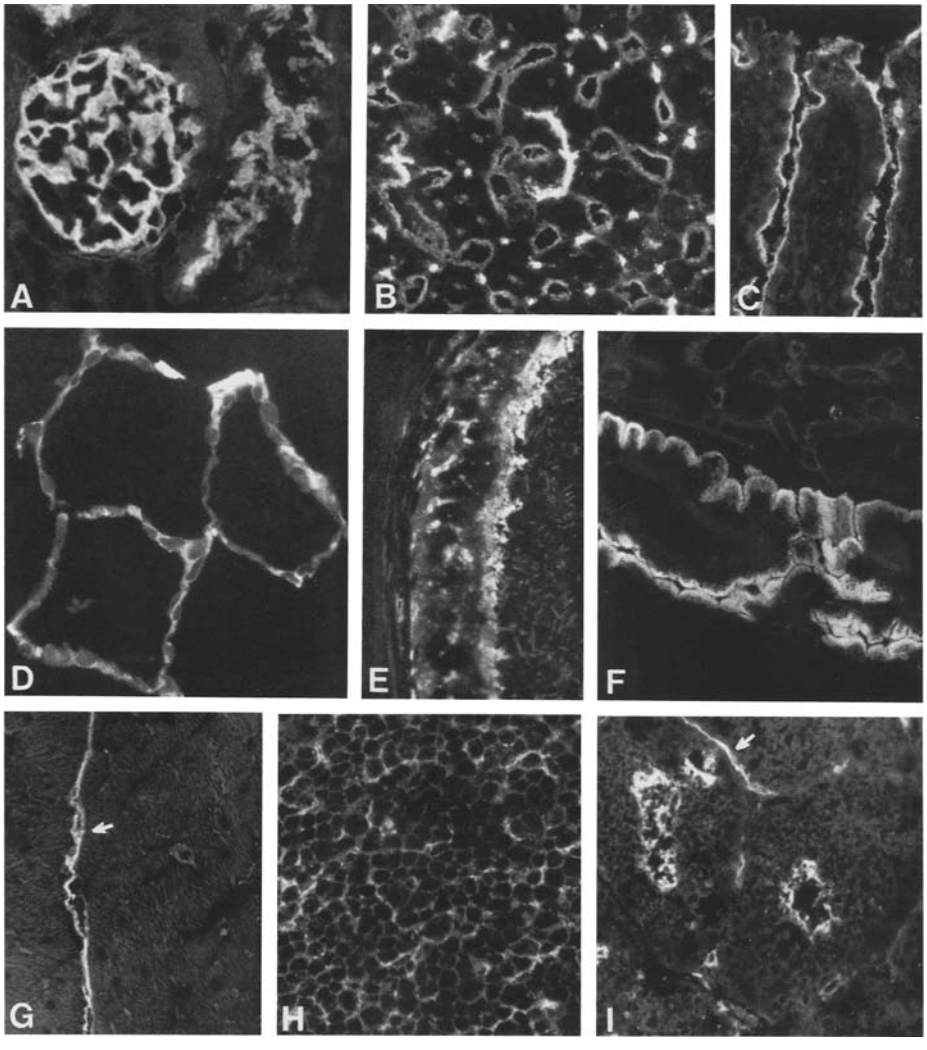

Fig. 2. Indirect immunofluorescence on frozen sections of normal mouse tissues incubated with mouse liver eluate. **A.** Glomerulus and brush borders of proximal tubules are stained. × 512. **B.** Liver with positive canaliculi and sinuses. × 512. **C.** Brush border of the small intestine. × 256. **D.** Homogeneous staining of the alveolar septa of the lung. × 512. **E.** Heavy staining of the apical part of the epididymal epithelium; also granules in the basal parts of the cell. × 512. **F.** Yolk sac epithelium with strong apical staining. × 256. **G.** Heart; Endocardium (*arrow*) and capillary endothelium. × 256. **H.** Cell membrane staining of cortical thymocytes. × 512. **I.** Harderian gland; apical part of the epithelium and capillary endothelium (*arrow*) are positive. × 512

defined antisera against the crude F × 1A fraction (Linder 1969; Miettinen and Linder 1976; Chant and Silverman 1978; Bakker et al. 1979; Naruse 1981; Makker and Singh 1983). In later studies with eluates from kidneys of rats with active or passive Heymann nephritis only a restricted number of organs were examined and the results were conflicting (Chant et al. 1980;

Miettinen et al. 1984). In recent studies in which the extrarenal localization of the Heymann antigen was examined by monoclonal antibodies directed to the gp-330 protein, only four organs were found to contain the antigen. It was concentrated in coated pits in the epithelium of the epididymis, visceral yolk sac, hepatocytes, and brush border of the small intestine (Doxsey et al. 1983; Kerjaschki and Farquhar 1985). Ronco et al. (1984b) who also used monoclonal antibodies described additional localization in cells of the alveolar septae of the lung. The results suggest that the Heymann antigen is not very widely expressed outside the kidney. On the basis of our study with the rat eluate we can now add some ogans of both mouse and rat in which the Heymann antigen is present. Besides the kidney, epididymis, lung and visceral yolk sac, we could locate the antigen in several glandular epithelia and some cells with known reabsorptive capacities. Although the antigen showed in general a similar localization in both species, a few disparities were observed as indicated in Table 1.

Studies of Bakker et al. (1979) have suggested that the Heymann antigen is also present on thymocytes and other lymphoid cells. Our studies with the rat eluate did not confirm this. However, since we found positive reactions with both the unseparated sera and the mouse liver eluate, it seems most likely that the positive findings of the abovementioned authors were caused by the presence of anti gp-90 antibodies in their sera.

Contamination with gp-90 antibodies is probably also the explanation for the apparently widespread distribution of the Heymann antigen recently described by Singh and Makker (1985) who used a "monospecific" antiserum against gp-600. Actually these authors also identified antibodies against gp-90 in their serum, but they considered this protein to be a breakdown product of gp-600. Our results suggest that this conclusion is not justified and that gp-90 is an antigen completely different from the Heymann antigen. Our inability to detect the antigen in the liver and the small intestine is more difficult to explain in the light of the positive findings with monoclonal antibodies. Comparison of the eluate with monoclonal antibodies under otherwise similar test conditions will be necessary to resolve these discrepancies.

The eluate from the mouse liver revealed a much more widespread distribution of the corresponding antigen than the gp-330 protein. One of the most common and prominent sites was the endothelium of the capillaries, although quantitative differences between the two species and between various organs within the same animal were observed. Apart from the reported occurrence of the antigen in normal kidneys, many epithelia in glands, liver canaliculi, brush borders of the small intestine, and crypts of the small and large intestine, cells covering or lining the organs and cavities, as well as thymocytes, lymphocytes, and stroma cells were positive. The expression corresponds with the localization of the gp-90 protein as detected with a monoclonal antibody for some organs by Ronco et al. (1984a; 1984b). At this moment the nature and function of this antigen is unknown. The widespread distribution of the antigen in the cell membranes of many cells with apparently different functions closely resembles that of several hydrolytic

enzymes, e.g. aminopeptidase A or angiotensinase A, and dipeptidyl peptidase IV. These enzymes form a major group of cell membrane bound proteins in the renal tubular brush borders (Gossrau 1979; Lojda and Gossrau 1980; Fukasawa et al. 1981). Future studies will hopefully reveal whether this protein is a cell membrane bound proteinase or a molecule with completely different function.

The differences in kinetics of the anti gp-90 and anti gp-330 specificities after administration of the anti-mouse TAPron antibodies, can be explained to a large extent by the different localization of the respective target proteins (Assmann et al. 1983; 1985). The widespread occurrence and the easy accessibility of the gp-90, notably on the endothelial cells of the capillary bed, account for the prompt binding of the specific antibodies and its rapid disappearance from the circulation after injection into the mouse. We have actually observed a rapid fixation to the capillary endothelium of particularly the liver and the lung with a quick disappearance of the antibodies from the blood stream. In rats the same was seen by Ronco et al. (1984a) after intravenous administration of monoclonal antibodies directed against gp-90. Paired label studies showed that most of the injected antibodies fixed to the lung and the liver, and only small amounts to the kidney. On the other hand, a slow and gradual binding takes place to gp-330 in the rat glomerulus during several days after injection of anti-mouse TAPron antibodies, while circulating antibodies to gp-330 remain detectable for almost a week (unpublished observation). These results are in agreement with the more elaborate studies of Salant et al. (1980). The specific localization of gp-330 along with factors determining the permeability of the glomerular capillary wall may account for the characteristic kinetics in the PHN of rats.

In spite of the fact that many organs contain the Heymann antigen, active Heymann nephritis in rats can only be induced with rat kidney preparations. Therefore it is assumed that only this organ possesses the nephritogenic antigen (Naruse 1980; Miettinen et al. 1984; Heymann et al. 1971; 1959; Fleuren et al. 1980; Klassen et al. 1971; Sugisaki et al. 1973). A report by Naruse (1981) of the successful induction of active Heymann nephritis with liver and lung tissues has not been confirmed by others (Miettinen et al. 1984; Fleuren et al. 1980; Sugisaki et al. 1973). Immunization of rats with suspensions of epididymis containing the Heymann antigen, was also not successful (Miettinen et al. 1984). It is yet unknown why renal tissue is such a superior material for the induction of active Heymann nephritis. The antigen concentration and epitope densities of the gp-330 might be important factors.

The induction of a passive Heymann nephritis with heterologous antisera against non-renal tissues has been examined far less extensively. Antisera directed to liver and small intestine gave negative results (Miettinen et al. 1984). Bakker et al. (1979, 1980) found that that antisera against rat thymocytes, fixed homogeneously to the glomerular wall after injection in rats. However, as discussed above their binding is probably related to the presence of gp-90, both on thymocytes and in rat glomeruli, and not to the Heymann antigen. Also the homogeneous glomerular pattern which they

described fits in better with the diffuse distribution of gp-90 than with the discrete pattern that is always found for gp-330.

In conclusion, the antigen that we have identified and that is involved in the membranous glomerulonephritis of the mouse is different from the Heymann antigen and has a much more widespread tissue distribution. Like the Heymann antigen, its normal physiologic function is unknown. It is also not clear whether its involvement in membranous glomerulonephritis is limited to the mouse or extends to the rat or even other species.

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