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Immunopathological Role of Brush-Border (BB) Antigens in Animal Species and Man: from Nephritogenicity to Teratogenicity

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Summary. **Because the brush-border (BB) of the renal proximal tubule shares antigens with other epithelia including glomerular epithelial cells (GEC) and the visceral yolk sac (VYS) of the embryo, immune reactions directed to BB antigens can induce glomerulonephritis (GN), such as Heymann's nephritis, a rat model of membranous GN (MGN) and teratogenic effects. To identify the responsible antigens, we have raised monoclonal antibodies against the BB, selected those exhibiting cross-reactivity with the GEC and/or the VYS, and tested their pathogenicity *in vivo*. This led us to characterize the target antigen of Heymann's nephritis as a 330 kD glycoprotein closely associated with clathrin in the intermicrovillar areas of the BB and the coated pits of the GEC. Induction of the characteristic electron-dense deposits is a dynamic process including redistribution of the antigen on the surface of GEC triggered by cross-linking by polyclonal antibodies, followed by shedding of the immune complexes from the cell membrane, and increased antigen synthesis. Gp330 is not detected in human GEC and therefore is probably not implicated in the pathogenesis of MGN in man. Other antigenic candidates for the *in situ* formation of epimembranous deposlts in the glomerular capillary wall are dipeptidyl peptidase IV (gp90) and naeutral endopeptidase (gp85, CALLA), two enzymes shared by the BB and the GEC in numerous species including man. Using the same "monoclonal approach", we have identified another coated pit glycoprotein, gp280, as the main target antigen of teratogenic anti-kidney antibodies. Injection of any of the anti-gp280 monoclonal antibodies induces in a dose-dependent manner embryonic resorption and malformations in the surviving fetuses. Preliminary experiments suggest that the teratogenic effects are due to alterations of the endocytotic process that plays a key role in providing nutrients to the developing embryo. These observations indicate that binding of a monoclonal antibody to a single epitope can be pathogenic by blocking the function of the antigen. BB antlgens are thus implicated in various immune diseases affecting other structures than the renal tubule; full-blown expression of the disease may be observed with monoclonal antibodies or require polyclonal immune response, depending on the nature of the molecular mechanisms triggered by the deposition of the specific antibodies.**

Proximal tubule kidney cells are characterized by the presence at the cell apex of an unique organelle, the brush border. The latter faces the urine flow in the tubular lumen and owing to this strategic location, is implicated in resorption of solutes and water, degradation of small oligopeptides and reabsorption of proteins which reach the tubular lumen in glomerular diseases. On the opposite face of the proximal cell, the baso-lateral domain is also endowed with vectorialized transport activities supported by Na^+, K^+ ATPase pumps and, in addition, expresses specific adenylate cyclase-coupled hormone receptors which control activity of BB transporters. The BB which defines the apical domain of proximal cells is itself divided in two microdomains with distinct composition and function[1-3]: i) the microvillar domains containing BB hydrolases and ii) the "intermicrovillar" microdomains which bridge adjacent microvilli. The latter are characterized by a clathrin coat[2] and the presence of two high molecular weight (MW) proteins, gp330[4] and gp280[5] that are both implicated in adverse immune reactions affecting in the rat glomerulus and the development of the embryo. These two clathrin-associated proteins are concentrated in the intermicrovillar areas and virtually excluded from the microvilli. On the contrary, the enzyme maltase (MW = 300 kD) is localized on the

microvilli, and is absent from the coated membrane areas.[2] Whereas microvilli bear peptidase activities, the intermicrovillar domains seem to be preferentially involved in endocytosis, a major function of proximal cells.[3] Peptides and intact proteins are taken up via these specialized areas by a mechanism which resembles receptor-mediated endocytosis. These functional properties of the BB antigens as well as their precise distribution in the membrane microdomains must be considered when one analyzes their immunopathological role, especially the deleterious effects of specific antibodies.

Because the antigenic makeup of proximal tubule BB is partially shared with other epithelia including glomerulus, intestine, epididymis and yolk sac epithelia (reviewed in Ref. 6 and 7), pathologic processes affecting PCT epithelial cells may damage other structures than the renal tubule. This is illustrated by microvillus inclusion disease (MID), a familial lethal enteropathy[8] affecting intestinal and renal BB,[9] characterized by the presence of intracytoplasmic microvillus inclusions contrasting with the absence of a well-defined BB. Another example of interconnection between kidney and other organs is provided by the teratogenic effects of anti-kidney antibodies first established by Brent.[10] In this review article, we will focus on the molecular mechanisms at play in two diseases implicating BB antigens but affecting primarily the glomerulus and the embryo. The first is membranous glomerulonephritis (MGN), one of the most frequent histopathological types of glomerulonephritis with Berger's disease, which leads to end-stage renal failure in 20-30% of patients and may occur *de novo* after transplantation. It is characterized by the presence of subepithelial electron-dense deposits in the absence of glomerular cell proliferation. The latter become associated with excess of matrix material which demarcates the growing immune deposits and irregularly thickens the glomerular basement membrane, responsible for "spikes" in silver stained sections. The second part of this article will be devoted to the mechanisms involved in autoimmune malformations and abortions, with peculiar emphasis on the identification of antigenic targets of teratogenic anti-kidney antibodies. In both nephritogenicity and teratogenicity studies, we used a monoclonal approach which consisted in raising monoclonal antibodies (mAbs) against the BB from various species and testing their ability to induce glomerular deposits or teratogenic effects on the basis of their reactivity with the glomerulus or the embryo visceral yolk sac.

ANTIGENIC TARGETS IN MEMBRANOUS GLOMERULONEPHRITIS

Most of the efforts undertaken to unravel the pathogenic mechanisms have been centered on Heymann's nephritis, an experimental model of MGN described by Heymann et al in 1959.[11] Heymann's nephritis is induced by immunization of rats with the brush border (BB) of the proximal convoluted tubule. Within a few weeks of immunization, the animals develop heavy proteinuria leading to the nephrotic syndrome and glomerular lesions bearing close similarity with the human disease.[11,12] The pathogenetic mechanisms involved have been the object of intense controversy. For a long time, it was felt that the disease could only be explained by the deposition of immune complexes formed between BB antigens which had leaked in the circulation and the corresponding antibodies.[13] Subsequently a number of experiments performed by various groups showed that Heymann's nephritis could actually be induced by perfusing "ex vivo" or isolated kidneys with anti-BB antibodies.[14,15] Formation of circulating immune complexes was not possible since there was no circulating antigen in the system. The idea was therefore put forward that the deposits were in fact created by binding of free circulating antibody to a brush border antigen which would have to be also expressed in the glomerulus. In vitro demonstration of the glomerular expression of the nephritogenic antigen is difficult. Experiments performed by Neale and Wilson in 1982[16,17] showed that glomerular eluates from rats with active Heymann's nephritis bound to the proximal tubule BB but also to the glomerulus; they could induce immune deposits in an isolated perfused kidney system. The interpretation of the data was however difficult since, because of the polyclonal nature of the antibodies, one could not exclude that the eluates actually contained two populations of antibodies, one specific for the BB and one specific for the glomerulus.

The key to the problem was provided by isolation of the antigen involved in Heymann's nephritis. This was first achieved by Kerjaschki et al,[4,18] who isolated a 330 kD glycoprotein, referred to as gp330, by lentil lectin affinity chromatography and gel filtration of BB solubilized by sodium deoxycholate. Our approach to analyze this system was completely different and consisted in raising mAb against the BB and selecting those with glomerular reactivity. This strategy was rewarding since it provided us with mAb specific not only for a protein identical to gp330

(Fig. 1) but also for other yet unidentiffed phylogenetically conserved glomerulotubular antigens involved in the pathogenesis of MGN.[19-22]

Gp330: the pathogenic antigen of Heymann's nephritis

Tissue distribution. The distribution of gp330 within the body is limited since, apart from the kidney, it can only be detected on pneumocytes type II and on the epididymis and visceral yolk sac epithelial cells.[23] It is not found in other transport epithelia such as, for instance, the gut epithelium. When kidney sections are analyzed by indirect immunofluorescence with antibodies specific for gp330, the BB is very intensely stained (Fig. 1). In addition, there is a fine discrete staining of the glomerulus in a granular pattern which is quite typical of gp330. At the ultrastructural level, gp330 is exclusively located at the base of microvilli in the intermicrovillar domain, characterized on the cytoplasmic aspect by a clathrin coating.[4,24] Within the glomerulus, gp330 is actively synthesized by the glomerular epithelial cells and only expressed within clathrin coated pits.[18,24]

Demonstration of the pathogenic role of gp330. The pathogenic role of gp330 in the induction of Heymann's nephritis was established by two series of experiments.[25] To analyze the specificity of the Ig involved in Heymann's nephritis, we first compared by SDS-PAGE the apparent MW of the protein identified by our mAb with that of the antigen(s) immunoprecipitated from the BB either by glomer-

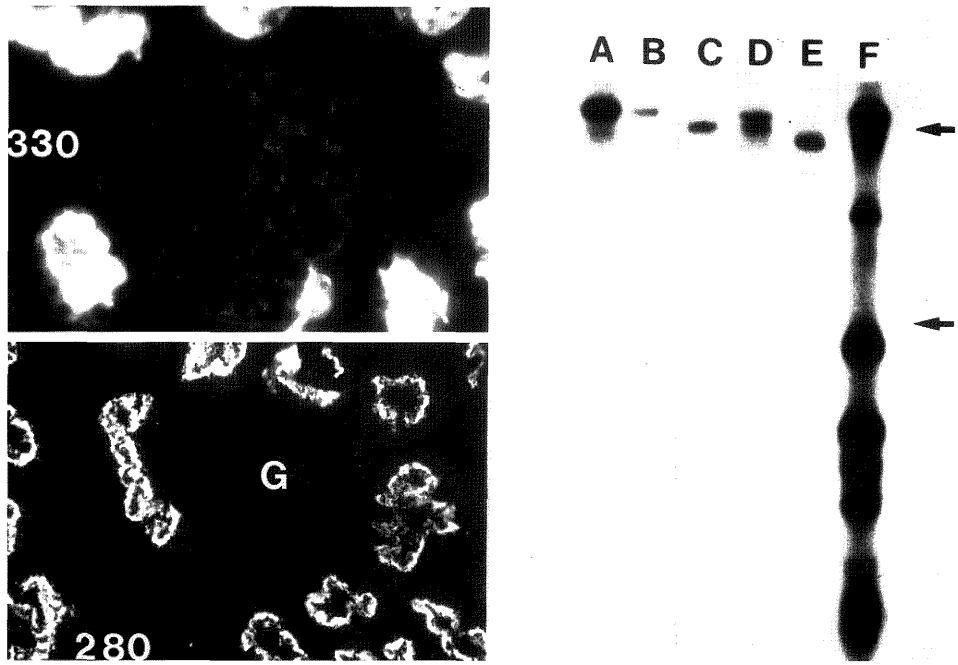

Fig. 1. *Left panel:* Indirect immunofluorescence (IF) of normal rat kidney using monoclonal antibodies (mAb) specific for the 330-kD Heymann antigen (*upper picture*) and the 280-kD teratogenic antigen (*lower picture*). Both antigens are expressed on the brush-border of proximal tubule cells, but only gp330 is detectable in the glomerulus with a fine punctuated distribution. Note lack of staining of the glomerulus (G) by anti-gp280 mAb. *Right panel:* Comparative immunoprecipitation analysis of the 330-kD Heymann antigen and the 280-kD teratogenic antigen. BB vesicles were ^{125}I radiolabeled using lactoperoxidase, solubilized with NP40 and incubated, after ultracentrifugation, with the following antibodies: A Ig eluted from glomeruli of rats with active Heymann nephritis induced using FX1A (a crude BB membrane preparation); *B* mAb anti-gp330; C mAb anti-maltase; *D* rabbit anti-RTEα5 (the nephritogenic antigen prepapared by Edgington et al.); *E* mAb anti-gp280; *F* polyclonal rabbit antibody raised against FX1A. Markers are 330-kD fibrinogen (*top arrow*) and unreduced 150-kD IgG (*lower arrow*). In lane *A*, active Heymann nephritis eluate binds massively the 330-kD antigen; trace amounts of the 280-kD antigen are also bound. In lane *D*, anti-RTEα5 identifies two distinct proteins which comigrate with the Mab-defined 330- and 300-kD antigens.

ular eluates from rats with active HN or by antibodies specific for RTE α5, the antigen initially described as nephritogenic by Edgington et al.[13] The results (Fig. 1) show that the main antigen recognized by the antibodies eluted from nephritic glomeruli and by anti-RTE α5 comigrates with gp330, thus providing a link between classical studies performed by Edgington who isolated RTE α5 but could not determine its MW precisely, and more recent experiments that resulted in the identification of gp330. Second, to demonstrate that gp330 could itself induce Heymann's nephritis, rats were immunized with gp330 previously affinity purified using Sepharose beads covalently linked to three of our anti gp330 mAb. All rats developed heavy proteinuria as well as abundant epimembranous deposits. Immunoperoxidase electron microscopy confirmed that the subepithelial electron-dense deposits contained both rat IgG and gp330. This further demonstrates that gp330 is the predominant -if not the sole- antigen implicated in the pathogenesis of Heymann's nephritis.

Induction of electron dense deposits is a dynamic process. Characterization of the nephritogenic antigen made it possible to study the events which take place from the initial antigen-antibody interaction on the epithelial cell membrane to the formation of the typical extracellular electron-dense deposits. We compared the ability of polyclonal and monoclonal antibodies (defining six distinct epitopes on gp330) to induce electron dense deposits.[26] We found that although immune deposits could be induced by both monoclonal and polyclonal anti-gp330, electron-dense deposits were only observed in rats given polyclonal antibodies. These observations are to some extent akin to those reported by Barba et al.[27] and Matsuo et al.[28] in the lung and the oocyte after injection of antibodies to the angiotensin converting enzyme. We believe that polyclonal antibodies bind to gp330 within the coated pits on the base of the foot processes facing the basement membrane, where they induce redistribution of gp330 and formation of large immune complexes which are shed from the cell membrane to give rise to the classical electron dense immune deposits. In this process, the antigen is removed from the cell membrane thus triggering increased synthesis of gp330.[26] When the antibody is no longer present in the circulation, the antigen becomes re-expressed. Further evidence in favour of this hypothesis was provided by Kerjaschki et al.[29] who showed that immune deposits remained in contact with coated pits up to 8 days after injection. Monoclonal antibodies also bind to the antigen but cannot induce cross-linked antigen-antibody complexes and do not give rise to electron dense deposits.

Function of gp330. The function of gp330 is at present unknown. Its predominant localization in coated pits and its association with clathrin suggest that it plays a role in receptor-mediated endocytosis. However, its restricted distribution to a selected population of epithelial cells involved in bulk endocytosis, tends to relate it more to a "specialized" receptor-mediated endocytosis system in which gp330 might function as a receptor for a yet undefined ligand than to the "clathrin system" itself. The receptor hypothesis is supported by partial molecular cloning of gp330 cDNA which shows homology with the low density lipoprotein receptor[30] and with the putative ligand-binding region of carbohydrate-binding lectin-like receptors such as hepatic asialoglycoprotein receptors and lung surfactant.[31]

Pathogenic role in other species. This important question was addressed using rabbit polyclonal antibodies and rat monoclonal antibodies. gp330 is detected in the BB of all the species studied including mouse, rabbit, pig and human species[32,33]; however, its expression in the glomerulus can be demonstrated only in the rat. This finding is in keeping with the fact that it seems impossible to induce Heymann nephritis in other species than the rat. It further suggests that gp330 is not implicated in human MGN due to in situ formation of immune complexes.

Gp90, an enzymatic antigen endowed with peptidase activity

Tissue distribution. In the process of producing antibodies reactive with rat BB and glomeruli, we have selected two additional mAb specific for a 90-kD protein[20,22] (Fig. 2). The latter is intensively expressed not only in the BB but also along the glomerular capillary wall[24] (Fig. 2). At variance with gp330, gp90 i) is not restricted to the intermicrolillar region of the BB but is detected over the entire surface of microvilli; ii) is evenly distributed on the membrane of GEC; iii) is further expressed on endothelial cells lining the glomerular capillary wall; iv) assumes a wide extrarenal distribution[20,23] including transport epithelia such as gut and biliary pole of hepatocytes, but also capillary endothelia and the vast majority of normal lymphocytes.[34]

Identification of gp90 as dipeptidyl peptidase IV (DPPIV). This distribution suggestive of the enzymatic nature of the antigen, led us to identify gp90 as dipeptidyl peptidase IV (DPPIV).[35] Gp90 is therefore probably identical to a 108 kD glycoprotein also identified as DPPIV by Natori et al in 1987.[3] It is of

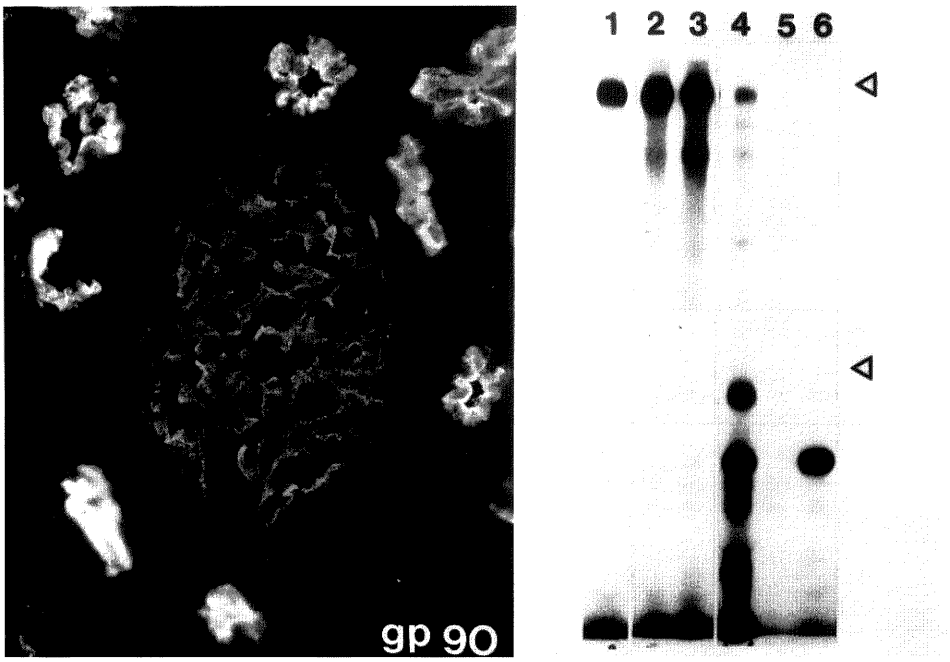

Fig. 2. *Left panel:* Indirect IF of normal rat kidney using a mAb specific for the 90-kD antigen, identified as dipeptidyl peptidase IV (DPPIV). Note intense staining of the brush-border and diffuse florid expression of DPPIV along the glomerular capillary wall. *Right panel:* Comparative immunoprecipitation analysis of the 330-kD Heymann antigen and the 90-kD DPPIV. Radiolabeled BB vesicles processed as in Fig. 1 were incubated with the following antibodies: *1* mAb anti-gp330, *2* glomerular eluate of passive Heymann nephritis, *3* polyclonal rabbit anti-gp330, *6* mAb anti-gp90 (DPPIV). Untreated BB was applied to lane 4. Lane 5 is control immunoprecipitate made with normal mouse serum. Markers are 330-kD fibrinogen (*top arrowhead*) and 150-kD IgG (*lower arrowhead*).

note that DPPIV, one of the major hydrolases of gut and kidney BB, is unable to cleave native proteins. In the gut, it may be involved in the final breakdown of peptides resulting from porotein digestion. A more likely role for DPPIV in other sites including the kidney is the hydrolysis of physiologically active peptides.

Pathogenic role of gp90-DPPIV in the rat. The kinetics of the immune deposits that appear in the glomerulus after intravenous injection of anti-gp330 monoclonal antibody contrast sharply with those of deposits appearing after injection of anti-gp330 monoclonal antibody.[20,23] Glomerular binding is maximal 4 h after injection but decreases very rapidly from then on. During the same period, the anti-gp90 monoclonal antibody can be found on the brush border and on the basolateral aspect of proximal tubule cells. Similar kinetics are observed after injection of polyclonal antibodies to gp90 and suggest that the transient character of glomerular immune deposits is not due to the antibody itself but is more likely attributable to as-yet-undefined properties of the antigenic target. The reasons whereby some immune deposits are removed rapidly from the glomeruli where as others remain stable for prolonged periods of time are not entirely defined. Part of the explanation at least has been provided by Kerjaschki et al.[29] who showed that gp330-antigp330 immune complexes had the property to adhere very strongly to the glomerular basement membrane. The chemical nature of the link is unknown. However, because binding resists incubation with detergents, covalent linkage may be considered.

The role played by gp90 in renal immunopathology in the rat is poorly defined. Immunoglobulins eluted from glomeruli of rats with active Heymann nephritis do not precipitate significant amounts of gp90.[25] This finding does not rule out the possibility that the short-lived immune complexes involving DPPIV formed on endothelial and/or epithelial cells are responsible for an increase in glomerular permeability that may enhance binding of antibodies to gp330. The gp90-gp330 cooperation hypothesis is supported by the fact that rats injected with certain anti-DPPIV antibodies develop transient proteinuria which conin-

cides with the presence of DPPIV immune complexes in the glomerular basement membrane.[37,38] Along this line, a role for endothelial immune complexes formed in situ was recently suggested by Jeraj et al.[39] in passive Heymann nephritis and by Matsuo et al.[40] in a model of MGN induced in the rabbit by polyclonal antibodies to angiotensin-converting enzyme.

Role in a mouse model of MGN. In the mouse, the role of gp90 is easier to establish because gp330 is not expressed in the glomerulus of this species. Assmann and colleagues studied a model of MGN induced by passive immunization with rabbit antibodies raised against mouse brush border digested by pronase (anti-TAPRON).[41,42] The kinetics of the heterologous phase bear close similarity to those described in the rat given anti-gp90 antibodies, but the amount of antibody remaining in the glomeruli is sufficient to allow the development of an autologous phase.[41] Immunoprecipitation of radiolabeled rat and mouse brush border demonstrated that anti-TAPRON antisera contain antibodies to the 330 and 90 kD brush-border proteins described: whereas the anti-gp330 antibodies induce MGN after injection of anti-TAPRON in the rat, only the anti-gp90 are nephritogenic in the mouse.[42-43]

Role in a rabbit model of MGN. Although these observations clearly showed that antibodies to gp90 could induce subepithelial deposits and be nephritogenic, we could not be certain that the pathogenic process involved essentially formation of immune complexes on the surface of epithelial cells because gp90 was also expressed by endothelial cells. This difficulty led us to study monoclonal antibodies raised against rabbit brush border and specific for antigens shared by brush-border and glomerular epithelia.[44] The first one we identified has a molecular weight of 90 kD and is DPPIV. In contrast with the rat, the rabbit lacks expression of this enzyme on glomerular endothelial cells. At the ultrastructural level, the antigen is evenly distributed on the surface of brush-border microvilli and glomerular epithelial cells.[44] The kinetics of deposits induced by injection of monoclonal antibody to DPPIV in rabbits is reminiscent of that observed in the rat; goat polyclonal antibodies raised against rabbit DPPIV purified on a column of sepharose 4B linked to our monoclonal antibody induced more persistent deposits and the development of an autologous phase (Fig. 3). This observation further demonstrates that antigens expressed by glomerular epithelial cells, but distinct

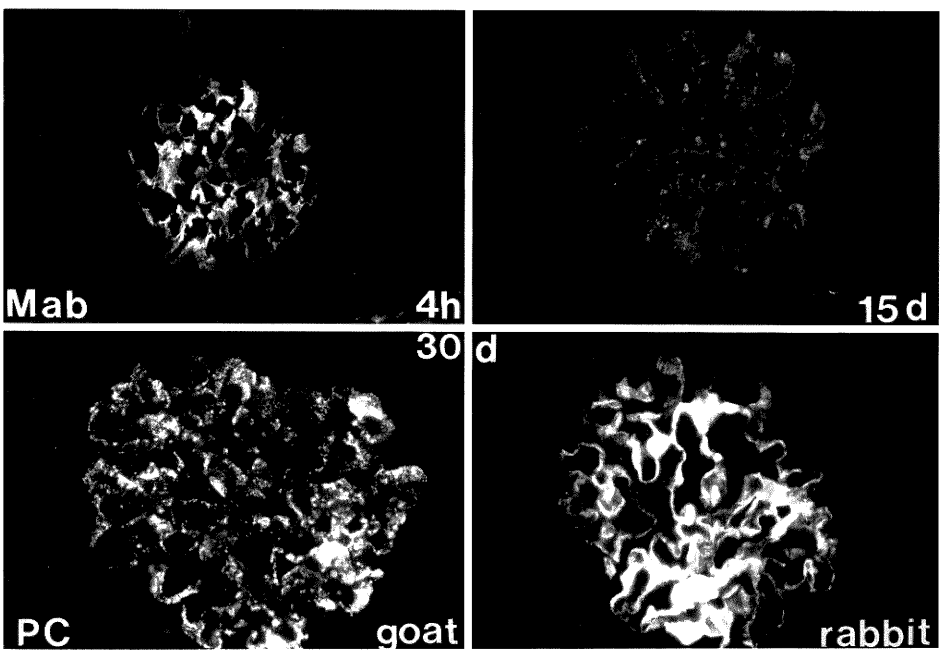

Fig. 3. Direct IF of renal cortex from rabbits previously injected with either monoclonal mAb 130 (*upper panel*) or polyclonal (*lower panel*) anti-DPPIV antibodies. Note bright deposits of mAb 130 four hours after injection, which decrease very slowly and remain detectable at day 15 Rabbits injected with polyclonal goat anti-DPPIV antibodies develop persistent granular goat Ig deposits (*left*) and an autologous phase characterized by pseudo-linear rabbit Ig deposits (*right*), shown here 30 days after injection.

from gp330, can be implicated in the formation of long-lasting immune deposits.

Gp85, another enzymatic antigen identical with the common acute lymphobalstic leukemia antigen (CALLA)

The second brush-border protein shared by glomerular epithelial cells in the rabbit is enkephalinase also referred to as neutral endopeptidase (molecular weight, 85 kD); it exhibits the same ultrastructural distribution as DPPIV.[44] Glomerular deposits observed after injection in vivo of monoclonal antibody to enkephalinase are particularly transient; their almost complete disappearance within 24 h coincides with the appearance of the antibody on the brush border of some proximal convoluted tubules, as already noted in the rat with anti-DPPIV.

Some of the mAb that we have produced against the rabbit enzyme are cross-reactive with the human antigen which has the same distribution on BB microvilli and the surface of GEC (Fig. 4). Using these antibodies, we have shown in collaboration with the group of M. Letarte and V. Jongeneel[45] that neutral endopeptidase is identical with CALLA, a marker of acute lymphoblastic leukemias also expressed on neutrophils, melanoma and kidney adenocarcinoma cells. This makes CALLA a potential antigenic candidate for malignancy-associated MGN.

Antigenic targets in humans

Because of the close similarities between Heymann nephritis and human MGN, substantial efforts have been made to identify antigenic targets expressed by human GEC. However, circulating Ig and Ig eluted from glomeruli of patients with MGN do not usually display reactivity with normal glomeruli and brush border.[46] This does not exclude a pathogenic role for GEC antigens such as gp90 and gp85 because reactivity studies i) are performed lately in the course of MGN, when content of deposits is probably markedly different from their initial composition, and ii) often use unsensitive methods. By western blotting, we have recently detected in the serum (kindly provided by Pr. Niaudet, Paris) of a child with MGN and various auto-immune disorders, antibodies reactive with a 58 kD antigen expressed in the glomerulus and on the brush border that might be the renal target of auto-immunization.[47,48] Although this finding may permit to follow an interesting track, one has to keep in mind that MGN is an heterogeneous disease that

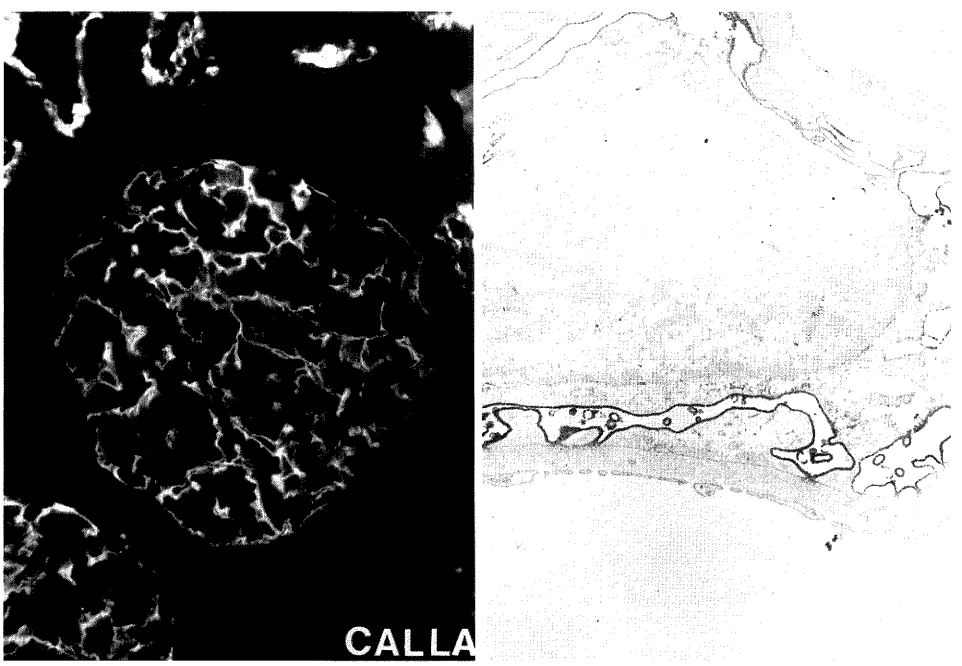

Fig. 4. Immunohistological analysis of normal human kidney using anti-neutral endopeptidase (CALLA) monoclonal antibody (FAH99). Indirect IF shows intense staining of brush borders and glomerulus (*left*). By immunoperoxidase electronmicroscopy (*right*), neutral endopeptidase is diffusely expressed on the entire surface of glomerular epithelial cells. Note that glomerular endothelial cells are not stained.

may involve a variety of target antigens either synthetized by GEC or "planted" onto the outer aspect of the glomerular basement membrane. Further studies are required to analyze reactivity of "early" glomerular eluates from human biopsies. They could be complemented by a careful analysis of the antigens contained in subepithelial immune deposits using monoclonal antibodies of defined specificity.

ANTIGENIC TARGETS OF TERATOGENIC ANTI-KIDNEY ANTIBODIES

Identification and tissue distribution of a new coated pit-associated 280-kD glycoprotein (gp280)

In the wake of studies performed on Heymann's nephritis, we produced three anti-BB mAb which identified a 280-kD protein selectively expressed on the proximal tubule and yolk sac brush borders[5] (Fig. 1 and 5). In contrast with gp330, gp280 is not detected in GEC coated pits (Fig. 1). At the ultrastructural level, the protein is predominantly expressed in the intermicrovillar domain of the renal BB but is occasionally more diffuse in some tubular segments. In the yolk sac of the embryo, both gp330 and gp280 are associated with the clathrin-coated vesicular system, but gp280 has a more diffuse distribution on the cell surface, involving both microvilli and intermicrovillar areas.

Teratogenicity of monoclonal anti-gp280 antibodies

The induction of birth defects by anti-kidney antibodies was first described by Brent almost 30 years ago[10] and confirmed by David et al.[49] and Leung.[50] The first biochemical informations were provided by Leung[50] who isolated a 340 kD BB antigen, reported as diffuse on BB microvilli, which elicited teratogenic antibodies reactive with epithelial cells of the visceral yolk sac. However, the antigen isolated from

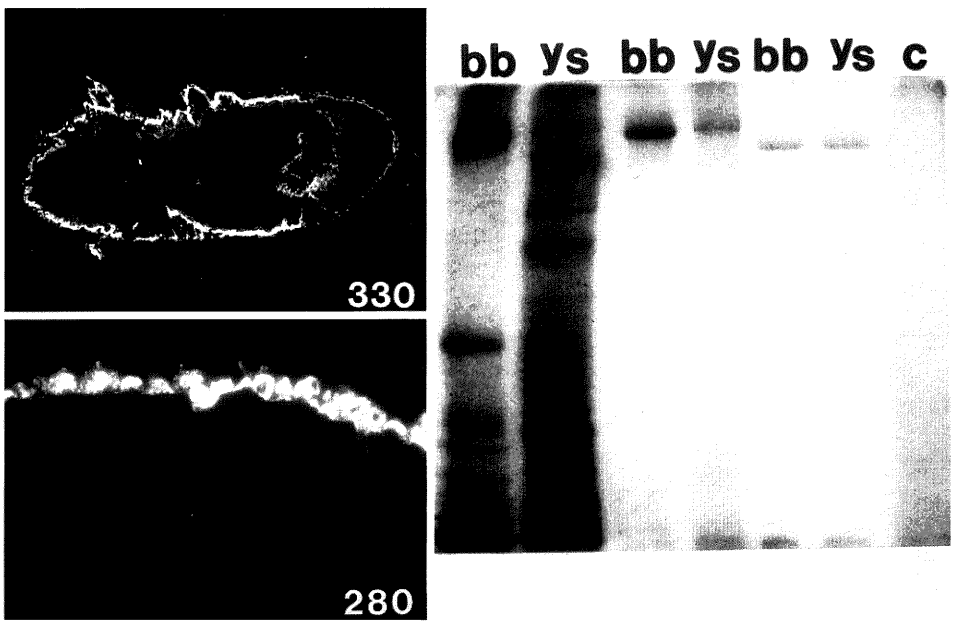

Fig. 5. *Left panel:* Indirect IF of rat embryo and visceral yolk sac using mAb specific for the 330-kD Heymann antigen (*upper picture*) and the 280-kD teratogenic antigen (*lower picture*). Note intense staining of visceral yolk sac by both antibodies. In addition, anti-gp330 binds to embryonic structures (*arrow*). *Right panel:* Immunoaffinity chromatography of solubilized BB membrane vesicles (bb) and yolk sac (ys): proteins separated by SDS-polyacrylamide gel electrophoresis were stained by Coomassie blue. Lanes 1 and 2: solubilized BB membrane vesicles (bb) and yolk sac (ys); lanes 3 and 4: material purified from bb and ys by mAb Anti-gp330; lanes 5 and 6: material purified from bb and ys by mAb anti-gp280; lane 7: control immunoadsorption using S4B-LPC1, a monoclonal antibody devoid of reactivity with bb and ys. Note identical apparent molecular weight of the protein purified by the same mAb from bb and ys.

the yolk sac consisted of two proteins of lower MW (30 and 60 kD).[51] This led us to test the teratogenic effects of monoclonal and polyclonal antibodies specific for gp280 and gp330 which are in the same range of MW as the putative 340 kD teratogenic antigen described by Leung.

Injection of any of the anti-gp280 mAb induced in a dose-dependent manner a high frequency of embryonic resorption and fetal malformations in the surviving fetuses.[5] The most frequent malformations concerned the eyes and the central nervous system. By contrast, monoclonal antibodies specific for gp330 had no effect. Twenty-four hours after injection, anti-gp330 were localized on visceral yolk sac as well as in the mother's glomeruli and in embryonic structures, whereas anti-gp280 were only detectable on the visceral yolk sac, thus suggesting that anti-gp280-induced embryonic abnormalities are not related to immunological disease of the mother or to antibody binding to embryonic structures.

Function of gp280

Clues to the function of gp280 are provided by teratogenicity experiments and ultrastructural localization of the protein. In rodents, the visceral yolk sac which envelopes the developing embryo plays a major role in providing nutriments to the embryo, essentially via catabolism of proteins taken up by fluid-phase and/or receptor-mediated endocytosis.[52] Alteration of these processes by chemical agents including trypan blue, leupeptine or chloroquine, results in malformations reminiscent of those induced by anti-gp280 antibodies. Close association of gp280 with clathrin containing structures together with teratogenic effects of anti-gp280 antibodies suggest that gp280 plays a specific role in the formation or the subsequent handling of endocytotic vesicles.

What gp280 can teach us about deleterious effects of antibodies

Our observations show that binding of a monoclonal antibody to an antigen probably endowed with an important functional role in endocytosis can induce dramatic effects although the antibody does not fix complement and cannot trigger antigenic redistribution. It is likely that binding of antibody to gp280 is sufficient to alter or annihilate its function. Similar "immunological blockade" may be at play in various auto-immune conditions. In contrast, the nephritogenic potential of anti-gp330 antibodies is only achieved by polyclonal antibodies which are required to induce redistribution of the antigen and shedding of the complexes on the cell membrane as well as complement activation.

Very interestingly, like gp330, gp280 is conserved in human proximal tubule BB and can also be detected in the placenta and the visceral yolk sac. Preliminary data show auto-immune reactivity with gp280 and gp330 in sera from patients with lupus disease. They lead to investigate the role of anti-gp280 antibodies in auto-immune repetitive abortions.

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