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Antigenic determinants of tubular basement membranes and Bowman's capsule in rats

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Antigenic determinants of tubular basement membranes and Bowman's capsule in rats. Kidney sections from several inbred rat strains were studied with an indirect immunofluorescence technique using five different, selected rat antisera which contained antibodies against the tubular basement membrane (TBM). The sera came from transplanted animals, an apparently normal animal, and animals treated with spleen cells in complete Freund's adjuvant. Upon panel analysis, at least two different antigenic determinants were recognized on the proximal TBM: One was present in nine and one was present in seven of the 15 strains tested; both determinants appear to be expressed in most antigen-positive strains. Using kidneys from major histocompatibility complex (MHC)-congenic strains, it was shown that the TBM antigens are encoded outside the MHC region. Three sera produced variable staining of Bowman's capsule in association with the proximal TBM; two sera produced clearly discordant staining of Bowman's capsule and proximal TBM. The capsular antigens were also coded outside the MHC region. These observations suggested the existence of at least two different antigenic determinants on proximal TBM. Bowman's capsule appeared to have antigenic determinants in common with the proximal TBM as well as distinct determinants.

Déterminants antigéniques des membranes basales tubulaires et de la capsule de Bowman du rat. Des sections de rein de plusieurs souches pures de rats étaient étudié par une technique d'immunofluorescence indirecte utilisant cinq anti-sérums de rats différents, sélectionnés, contenant des anticorps contre la membrane basale tubulaire (MBT). Les sérums provenaient d'animaux transplantés, d'un animal en apparence normal, et d'animaux traités avec des cellules spléniques dans de l'adjuvant complet de Freund. En faisant une analyse globale, au moins deux déterminants antigéniques différents étaient reconnus dans les MBT proximales. L'un était présent chez neuf, l'autre chez sept des quinze souches testées; les deux déterminants antigéniques semblaient exprimés chez la plupart des souches antigène positif. En employant des reins provenant de souches complexe de histocompatibilité majeur (CHM)-congéniques, nous avons montré que les antigènes de la MBT sont codés à l'extérieur de la région MHC. Trois sérums ont produit une coloration variable de la capsule de Bowman en plus des MBT proximales; deux sérums ont produit des colorations nettement discordantes de la capsule de Bowman et des MTB proximales. Les antigènes capsulaires ont également codés à l'extérieur de la région MHC. Ces observations suggèrent l'existence d'au moins deux déterminants antigéniques différents dans les MTB proximales. La capsule de Bowman sembla avoir des déterminants antigéniques communs avec les MTB proximales, mais également des déterminants différents.

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Occasionally, antibodies against the renal tubular basement membrane (TBM) are found in patients with renal diseases and also in renal allograft recipients [1–9]. The TBM antibodies in graft recipients appear to be directed against either autoantigens which are shared between donor and recipient [5] or alloantigens of the graft TBM which are absent in the recipient [6].

Anti-TBM antibodies and tubulointerstitial nephritis in the rat have been induced experimentally by injecting an allogeneic kidney suspension incorporated in complete Freund's adjuvant and a pertussis adjuvant [10], or by injecting bovine cortical TBM in (in)complete Freund's or pertussis adjuvant [11]. In vitro the antibodies reacted with the TBM and slightly with Bowman's capsule [10, 11], which suggests that Bowman's capsule has antigenic determinants in common with the TBM.

Most strains immunized with bovine cortical TBM develop anti-TBM antibodies that bind to autologous kidneys and thus are autoantibodies [11]. Panel analysis, however, revealed apparent allospecificity of the antibodies because no binding to kidneys from LEW, MAXX, and WF strain rats could be demonstrated [10, 11]. Allospecificity of TBM antigens was substantiated further by the results of transplantation experiments in which TBM-positive kidneys were implanted in TBM-negative recipients [12–15] and anti-TBM antibodies with an apparently similar strain specificity were formed [14, 15]. In this study we also have analyzed the antigenic characteristics of the TBM and Bowman's capsule of rat kidneys. Using panel analysis we found that the proximal TBM has at least two different antigenic determinants. In addition to the cross-reactive determinants between Bowman's capsule and proximal TBM, we also observed differences in staining which suggest the existence of different (allo) antigens on these two structures.

Methods

Animals. Inbred rat strains [ACI($RT1^a$), Brown Norway (BN, $RT1^n$), Buffalo (BUF, $RT1^b$), Fisher (F344, $RT1^{lv1}$), Lewis (LEW, $RT1^l$), MAXX ($RT1^n$), Wistar Furth (WF, $RT1^u$)] and the hybrids (LEWxBN)F_1, (LEWxWF)F_1, and (LEWxWF)F_2 were purchased from M. A. Bioproducts, Walkersville, Maryland. Other sources were the Charles River Breeding Laboratory, Wilmington, Massachusetts [SHR($RT1^k$)], Dr. C. W. DeWitt, University of Utah College of Medicine, Salt Lake City, Utah [DA ($RT1^a$) and BN.B4 ($RT1^a$)], and Olac 1976 Ltd., Bicester, Oxon, United Kingdom [PVG.c ($RT1^c$), PVG.DA

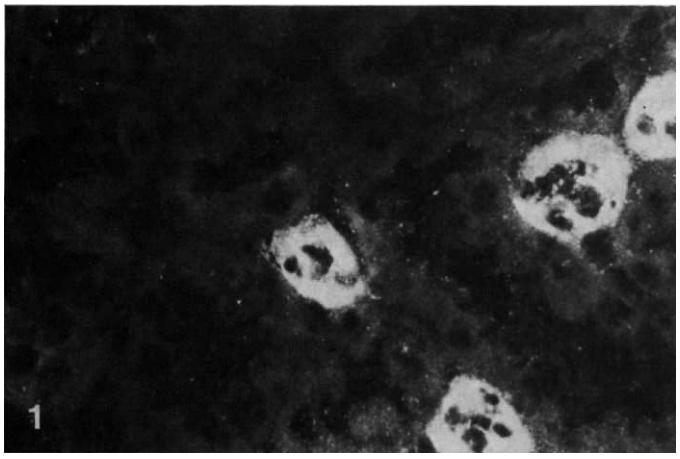


Fig. 1. *Immunofluorescence micrograph of a rat kidney section (LEW) incubated with rabbit anti-rat Tamm-Horsfall glycoprotein and stained with fluorescein-labeled goat anti-rabbit IgG.* The cytoplasm of the epithelium of the thick ascending limb of Henle's loops shows specific staining. (×500)

(RT1^a), PVG.AO (RT1^u) and PVG.1R [16]. RP (RT1^p) and NBR (RT1^l) strains are bred and maintained in a colony at the Animal Resources Center, Harvard Medical School, Boston, Massachusetts. Renal tissue from the following inbred strains was provided generously by Dr. H. W. Kunz, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania: PVG.c (RT1^c), KGH (RT1^g), BN (RT1^n), LEW (RT1^l), DA (RT1^a), OKA (RT1^k), WKA (RT1^k), BN.1L (N12F5, RT1^l), BN.WF (N12F7, RT1^u), BN.KGH (N12F6, RT1^g), BN.DA (N12F5, RT1^a), BN.WKA (N12F6, RT1^k), PVG.WKA (N12F7, RT1^k) and LEW.BN (N12F7, RT1^n).

Sera. The antisera that contained anti-TBM antibodies came from several different rat strains. Grafting of BN kidneys into bilaterally nephrectomized major histocompatibility complex (MHC)-identical MAXX recipients resulted in the formation of TBM antibodies 7 to 10 days after transplantation (manuscript in preparation); sera drawn on day 20 were used for this study. Other sources of anti-TBM antisera were unilaterally nephrectomized (LEWxBN)F_1 recipients of ACI kidneys (sera drawn 20 to 30 days after grafting) and bilaterally nephrectomized LEW recipients of (LEWxBN)F_1 kidneys. In the latter case permanent graft survival (> 100 days) was induced by administration of 0.5 ml LEW anti-BN hyperimmune sera at the time of transplantation (passive enhancement) [14]; sera were drawn 30 to 35 days after grafting. Furthermore, anti-TBM antibodies were found in the serum of an apparently normal male BN rat and in a pool of (WFxBN)F_1 anti-DA hyperimmune serum. The latter serum was obtained by injecting 10^6-10^7 pooled spleen and lymph node cells divided intraperitoneally in a hind footpad and subcutaneously at the back of the neck. Inoculation at the latter sites was combined with 0.2 ml complete Freund's adjuvant. Two and four weeks later, a boost injection of 10^7 cells was given intraperitoneally and in the back of the neck. Sera were drawn 10 days after the last injection [17]. Control sera included normal sera, sera from four animals treated with complete Freund's adjuvant, and hyperimmune sera produced by immunization with spleen and lymph node cells in complete Freund's adjuvant, as outlined above.

Analysis of antisera. The sera were studied using the indirect immunofluorescence technique and kidney sections [18]. Briefly, unfixed cryostat sections, 2 to 3 μ thick, were cut at −20° C and air-dried at room temperature for 15 min. Before and after each incubation, the sections were rinsed three times for 10 to 15 min in phosphate-buffered saline (PBS), pH 7.3. Incubation with serial dilutions of the sera and fluorescein-labeled antisera lasted 30 min and was carried out in a moist chamber. The sections were mounted in 10% v/v glycerin in PBS and examined the same day. The serum antibody titer was defined as the highest dilution of the serum in PBS that showed positive immunofluorescence. The variability in titers is within one dilution. Coded slides were read blindly. Two kidneys from two animals per strain were examined; in most instances, however, kidneys from three animals were studied. Biopsies were taken from the cortical and medullary portions of the kidney. A rabbit anti-rat Tamm-Horsfall glycoprotein was used to identify the epithelial cells of the ascending thick limb of Henle's loops (Fig. 1). In addition to binding to epithelial cells, binding to the luminal aspects of some distal convoluted tubular cells was observed [19]. This antiserum was a gift from Dr. Ramzi Cotran, Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts.

A sheep antiserum against a crude renal tubular epithelial fraction (Fx1A) was used to mark the brush border of the proximal tubular epithelial cells. This antiserum was a gift from Dr. Iekuni Ichikawa, Renal Division, Brigham and Women's Hospital, Boston, Massachusetts. Fluorescein-labeled rabbit IgG anti-rat IgG and rabbit IgG anti-sheep IgG as well as rhodamine-labeled rabbit IgG anti-rat IgG (heavy and light chains) were purchased from Chappel Laboratories, Inc., Cochranville, Pennsylvania.

Kidneys. Kidneys were harvested after exsanguination of the animals and perfusion with heparinized PBS. After the aorta was clamped and an incision was made in the inferior vena cava, 100 to 150 ml PBS were perfused at room temperature through both kidneys. A uniform blanching of the kidneys was used as an indication of effective perfusion.

Results

Characterization of the antisera-immunofluorescence patterns. Indirect immunofluorescence analysis of five anti-TBM antibody-containing rat sera with renal biopsies taken from inbred rat strains revealed three distinct staining patterns and combinations thereof. Two sera, LEW anti-(LEWxBN)F_1 and the serum from the BN animal with spontaneous anti-TBM antibodies, bound to the TBM of the proximal tubules (Fig. 2); there was also a weak staining of Bowman's capsule. Other renal structures, including the glomerular basement membrane, were not stained. The antibody titers in LEW anti-(LEWxBN)F_1 serum and in the BN serum were both 1:16. The intensity of staining with the two sera did not differ among the different strains. MAXX anti-BN antisera produced a similar staining of the proximal TBM and an additional clear-cut staining of Bowman's capsule of most glomeruli (titers 1:32 to 1:64 for both structures). The fluorescence of Bowman's capsule was continuous with that of the TBM. (LEWxBN)F_1 anti-ACI and (WFxBN)F_1 anti-DA gave a complex staining pattern dependent on the strains tested. On the basis of panel analysis and comparison with other antisera, at least three distinct

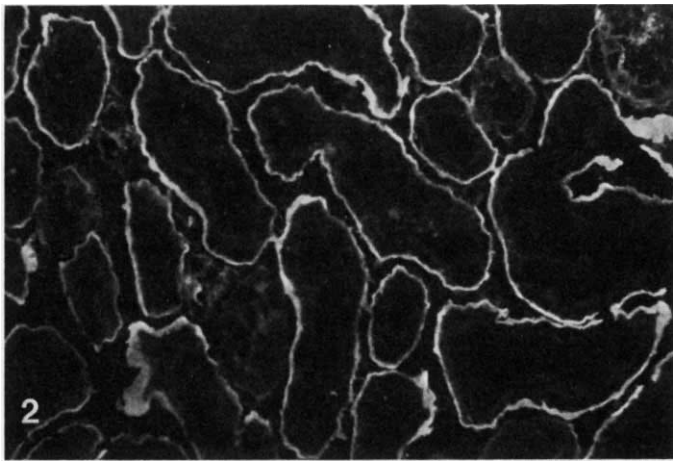


Fig. 2. *Immunofluorescence micrograph of a BN kidney section incubated with a BN serum containing anti-TBM antibodies and stained with fluorescein-labeled rabbit anti-rat IgG.* Staining shows proximal TBM. (×500)

specificities could be determined. In kidneys from RP and KGH rats (Table 1), there was antibody binding to the glomerular mesangium, Bowman's capsule, the TBM of approximately 10 to 15% of the cortical tubules, the basement membranes of peritubular capillaries and vasa recta, and the media of arteries and arterioles (titers 1:32) (Fig. 3). The antigen-positive tubules were never connected to glomeruli. To localize the TBM segment that bound the antibodies, double-labeling was performed using sera that marked either the proximal tubules or the ascending thick limb of Henle's loops (Fig. 1). When sections from KGH or RP kidneys were incubated with (LEWxBN)F_1 anti-ACI or (WFxBN)F_1 anti-DA and sheep anti-rat Fx1A and subsequently stained with rhodamine-labeled rabbit anti-rat IgG and fluorescein-labeled rabbit anti-sheep IgG, it was clear that no binding of rat antibodies to the TBM of Fx1A-positive tubular segments had occurred. Incubation of the sections with rat sera and rabbit anti-rat Tamm-Horsfall protein and staining with fluorescein-labeled goat anti-rabbit IgG followed by rhodamine-labeled rabbit anti-rat IgG demonstrated that the rhodamine-stained TBM did not come from the thick ascending limb of Henle's loops. Because clear staining of the basement membranes of the medullary collecting ducts was not observed, it appears that the anti-TBM antibodies in (LEWxBN)F_1 anti-ACI and in (WFxBN)F_1 anti-DA bind to the TBM of distal convoluted tubules in RP and KGH strain kidneys. Incubation of the two sera with kidneys from other strains produced either a negative reaction or a combination of the pattern of proximal TBM staining and the pattern obtained with RP and KGH kidneys (Fig. 4) (Table 1). With yet other strains (LEWxBN)F_1 anti-ACI resulted in the staining of only the proximal TBM (titer 1:32).

In addition to anti-basement membrane antibodies, both (LEWxBN)F_1 anti-ACI and (WFxBN)F_1 anti-DA contained alloantibodies against MHC-encoded antigens, which are expressed on the renal endothelium [17]. Consequently, both antisera stained the endothelium of $RT1^a$-positive kidneys. In $RT1^a$ positive kidneys that also bound TBM antibodies, the capillary endothelial staining was masked by TBM fluorescence, becoming evident only in high dilutions of the antisera.

To determine whether the antigens of the TBM and/or Bowman's capsule recognized by (LEWxBN)F_1 anti-ACI and (WFxBN)F_1 anti-DA are expressed on blood cells, both antisera were absorbed with mononuclear leukocytes or erythrocytes from ACI or PVG.DA rats (data not shown). This resulted in the disappearance of endothelial staining but not basement membrane staining. Furthermore, in more than 20 different alloantisera with high titers of lymphocytotoxic and hemagglutinating antibodies, including (LEWxBN)F_1 anti-ACI produced by immunization with lymphoid cells and complete Freund's adjuvant [17], no antibodies against TBM, Bowman's capsule, mesangium or basement membranes of capillaries and vasa recta were found.

Strain distribution and genetics of proximal TBM antigens (Table 1). Because the immunofluorescence patterns demonstrated a dissociation of proximal TBM and Bowman's capsule staining, we studied the strain distribution of both patterns separately. Four sera, LEW anti-(LEWxBN)F_1, MAXX anti-BN, (LEWxBN)F_1 anti-ACI and the BN serum with auto-anti-TBM antibodies, bound to the proximal TBM of most strains, except WF, MAXX, WKA, RP, KGH, and LEW. Furthermore, there was no binding to the kidneys from animals obtained by cross-breeding two TBM-negative strains (LEW and WF). The (WFxBN)F_1 anti-DA serum contained anti-proximal TBM antibodies with a more restricted strain distribution; in addition to the negative reactions already obtained with the four other anti-TBM antisera, negative reactions were also found with kidneys from BN and PVG.c strains. To examine whether or not the TBM antigens are encoded by genes within or closely linked to the MHC, kidneys from MHC-congenic lines[1] were studied. As shown in Table 1, the proximal TBM antigens are encoded by genes outside the MHC. BN serum with anti-TBM antibodies, MAXX anti-BN, LEW anti-(LEWxBN)F_1 and (LEWxBN)F_1 anti-ACI all reacted with BN and PVG.c kidneys but not with LEW, WF, KGH, WKA, and LEW.BN kidneys; because positive reactions were observed with BN.1L, BN.WF, BN.KGH, BN.WKA, PVG.AO, and PVG.WKA kidneys, the proximal TBM antigens are coded by gene(s) not closely linked to the MHC.

Genetically the proximal TBM antigens detected by (WFx BN)F_1 anti-DA also are not linked closely to the MHC because positive reactions were found with DA kidneys, whereas no staining was observed with kidneys from BN, PVG.c, BN.DA, PVG.DA, and PVG.1R rats.

Strain distribution and genetics of Bowman's capsule antigens (Table 2). Panel analysis of MAXX anti-BN revealed that the staining of Bowman's capsule was in complete concordance with the staining of proximal TBM, which suggests that common antigen(s) were detected on both structures. This concor-

[1] A MHC-congenic line is a line generated from two lines; one provides the genetic background (background strain) and the other donates the differential (MHC) locus (donor strain). With the use of several different mating systems, a line is developed which has the MHC loci (and most likely also a chromosomal segment of indeterminate length adjacent to these loci) of the donor strain whereas the rest of the genetic material is derived from the background strain. For example the BN.WF strain was derived from BN and WF; the MHC of this congenic line comes from WF whereas the rest of the genetic material comes from BN (background strain).

Table 1. Strain distribution of proximal TBM antigens recognized by five different anti-TBM antisera[a]

Rat strains	RT1 type	BN serum	MAXX anti-BN	LEW anti-(LEWxBN) F_1	(LEWxBN) F_1 anti-ACI	(WFxBN) F_1 anti-DA
WF	u	—	—	—	—	—
MAXX	n	—	—	—	—	—
WKA	k	—	—	—	—	—
RP	p	—	—	—	—	—
KGH	g	—	—	—	—	—
LEW[b]	l	—	—	—	—	—
(LEWxWF)F_1	l/u	—	—	—	—	—
(LEWxWF)F_2[c]	l/u, l/l, u/u	—	—	—	—	—
LEW.BN	n	—	—	—	—	—
(LEWxBN)F_1	l/n	+	+	+	+	—
BN[b,d]	n	+	+	+	+	—
BN.1L	l	+	+	+	+	—
BN.WF	u	+	+	+	+	—
BN.KGH	g	+	+	+	+	—
BN.DA	a	+	+	+	+	—
BN.WKA	k	+	+	+	+	—
BN.B4	a	+	+	+	+	+
PVG.c[b]	c	+	+	+	+	—
PVG.AO	u	+	+	+	+	—
PVG.DA	a	+	+	+	+	—
PVG.1R	a-c	+	+	+	+	—
PVG.WKA	k	+	+	+	+	—
DA[b]	a	+	+	+	+	+
ACI	a	+	+	+	+	+
OKA	k	+	+	+	+	+
SHR	k	+	+	+	+	+
BUF	b	+	+	+	+	+
F344	lv1	+	+	+	+	+
NBR	l	+	+	+	+	+

[a] Symbols used: —, no staining; +, staining of proximal TBM. Each individual serum gave approximately the same titer for the different strains.
[b] Animals came from two sources (see **Methods**).
[c] Three l/l, three u/u, and seven l/u animals were tested.
[d] The two BN strains differed at the RT2 (Ag-C)locus [24, 25].

dance also was observed for kidneys from the congenic line animals (Tables 1 and 2).

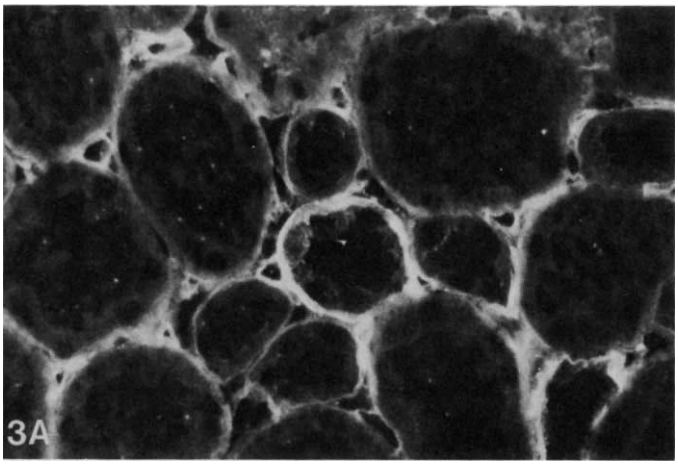
According to the strain distribution, (LEWxBN)F_1 anti-ACI and (WFxBN)F_1 anti-DA appeared to recognize the same antigen(s) on Bowman's capsule, distal TBM, vascular basement membrane, mesangium and the media of arterioles. Because positive reactions were found with Bowman's capsule from KGH and DA but not from BN, BN.KGH, BN.DA, PVG.c, PVG.DA, and PVG.1R rats, the genes that code the capsular antigens are not linked closely to the MHC. These capsular antigens seem to be distinct from the proximal TBM antigens because a dissociation of the staining was observed in several strains (Tables 1 and 2). For a number of strains (DA, ACI, OKA, SHR, BUF, F344, and NBR) concordant reactions of the proximal TBM and Bowman's capsule were observed.

Discussion

The renal tubular basement membrane (TBM) is a complex structure comprised of collagens as well as noncollagenous material [3, 20]. In this study we analyzed the serologic characteristics of rat TBM using an indirect immunofluorescence technique. For this purpose several different rat sera which contained antibodies against TBM were selected. Three anti-TBM antisera [MAXX anti-BN, LEW anti-(LEWxBN)F_1 and (LEWxBN)F_1 anti-ACI] were produced by renal allografting. (WFxBN)F_1 anti-DA hyperimmune serum, which was obtained by immunization with DA lymphoid cells and complete Freund's adjuvant, was studied because of the presence of anti-TBM antibodies in this serum. It is, however, not clear whether these anti-TBM antibodies developed as a result of immunization or spontaneously because anti-TBM antibodies occasionally are found in untreated, apparently healthy animals, as in a BN rat (serum from this animal was used in this study) and a MAXX rat (unpublished observation). Because no preimmunization serum sample was examined, this question remains unanswered.

Both (WFxBN)F_1 anti-DA and (LEWxBN)F_1 anti-ACI contained antibodies against MHC antigens, which are expressed on the renal endothelium [17].

The anti-MHC antibodies did not, however, cause the staining of the renal basement membranes, Bowman's capsule, mesangium and the media of arteries because absorption of the sera with mononuclear leukocytes from the ACI or PVG.DA strains, which express the $RT1^a$ (MHC) antigens, did not influence significantly the antibody titers to the renal structures described. Furthermore, in some strains (NBR and RP, for example) antibody reactivity was found against antigens on



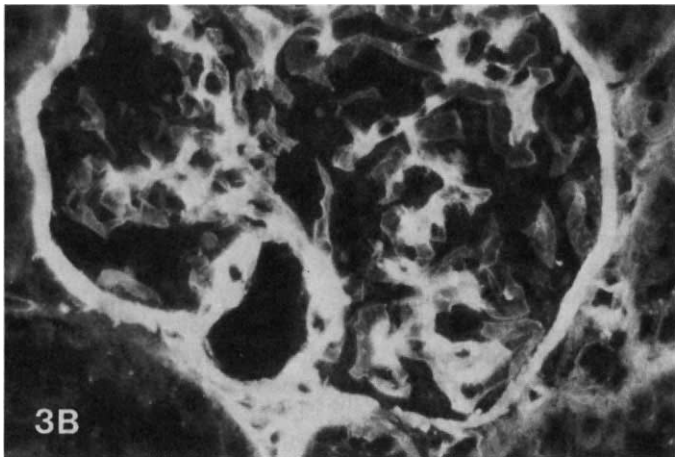
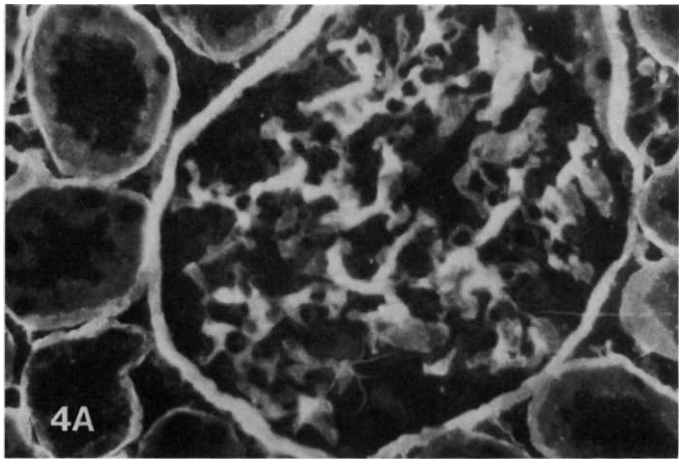


Fig. 3. *Immunofluorescence micrographs of a KGH kidney section incubated with (LEWxBN)F_1 anti-ACI and stained with fluorescein-labeled rabbit anti-rat IgG.* **A** *Staining of the distal TBM and the basement membranes of the peritubular capillaries.* (×500) **B** *Staining of Bowman's capsule, glomerular mesangium, media of an afferent arteriole entering the glomerulus and peritubular capillary basement membranes.* (×500)



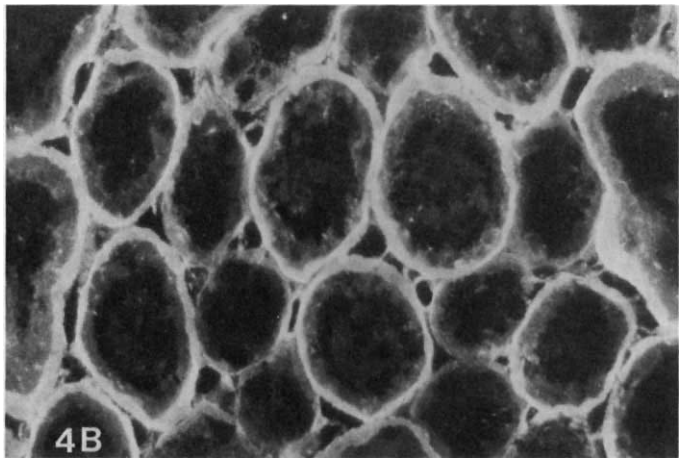


Fig. 4. *Immunofluorescence micrograph of a NBR kidney section incubated with (WFxBN)F_1 anti-DA and stained with fluorescein-labeled rabbit anti-rat IgG.* **A** *Staining of proximal TBM, Bowman's capsule and glomerular mesangium.* (×500) **B** *Staining of proximal TBM, distal TBM and basement membranes of peritubular capillaries.* (×500)

these renal structures but not on erythrocytes or lymphocytes (data not shown). Finally, alloantisera and monoclonal antibodies against MHC antigens do not bind to these structures [17].

In the rat model of anti-TBM tubulointerstitial nephritis induced with homologous kidney homogenate or bovine cortical TBM, it was first noted that anti-TBM antibodies exhibit strain specificity [10, 11]. Anti-proximal TBM antibodies with an apparently similar strain specificity were induced by renal allografting [13–15] or immunization with kidney homogenates [15]. The strain distribution of the previously recognized proximal TBM antigens is summarized in Table 3. As shown in this table, all antisera seem to recognize proximal TBM antigen(s) in most strains except LEW, AS, MAXX, and WF. The possibility that LEW rats have no TBM alloantigen(s) or that they are expressed only in low densities is supported by the observation that no anti-TBM antibodies are formed in the BN or DA strains upon stimulation with (LEWxBN)F_1 or LEW kidney tissue [13, 15]. This observation, however, does not imply that the proximal TBM of TBM-negative strains is devoid of xenoantigenic determinants, because positive staining of LEW TBM has been found using a heterologous anti-GBM/TBM serum that contained several anti-basement membrane antibody reactivities [21].

In this study we corroborate earlier studies concerning the strain distribution of proximal TBM antigen(s) (Tables 1 and 3). Using different rat sera which contained anti-TBM antibodies, we confirmed that the LEW, WF, and MAXX strains do not express allogeneic TBM antigens. We further showed that the proximal TBM of WKA, RP, and KGH rats probably also does not express allogeneic antigens. We cannot, however, exclude the possibility that such antigenic determinants might be expressed below the level of detection of the immunofluorescence technique.

With (WFxBN)F_1 anti-DA serum the same strains were found to be negative as were the other four anti-TBM antisera. Furthermore, additional negative reactions were found with kidneys from BN and PVG.c rats and lines derived thereof. Because the reaction of (WFxBN)F_1 anti-DA serum to proximal TBM antigenic determinants obviously appeared in fewer strains than that of the other four anti-TBM antisera, this

Table 2. Strain distribution of Bowman's capsule antigens recognized by three different antisera[a]

Rat strains	RT1 type	MAXX anti-BN[b]	(LEWxBN) F_1anti-ACI[c]	(WFxBN)F_1anti-DA[c]
WF	u	—	—	—
MAXX	n	—	—	—
WKA	k	—	—	—
RP	p	—	+	+
KGH	g	—	+	+
LEW[d]	l	—	—	—
(LEWxWF)F_1	l/u	—	—	—
(LEWxWF)F_2[e]	l/l, l/u, u/u	—	—	—
LEW.BN	n	—	—	—
(LEWxBN)F_1	l/n	+	—	—
BN[d,f]	n	+	—	—
BN.1L	l	+	—	—
BN.WF	u	+	—	—
BN.KGH	g	+	—	—
BN.DA	a	+	—	—
BN.WKA	k	+	—	—
BN.B4	a	+	+	+
PVG.c[d]	c	+	—	—
PVG.AO	u	+	—	—
PVG.DA	a	+	—	—
PVG.1R	a-c	+	—	—
PVG.WKA	k	+	—	—
DA[d]	a	+	+	+
ACI	a	+	+	+
OKA	k	+	+	+
SHR	k	+	+	+
BUF	b	+	+	+
F344	lv1	+	+	+
NBR	l	+	+	+

[a] Symbols used: —, no staining; +, staining of Bowman's capsule. Each individual serum gave approximately the same antibody titers for the different strains.

[b] MAXX anti-BN serum produced clear-cut staining of Bowman's capsule of most glomeruli; the capsular staining was similar to the staining of proximal TBM (Table 1).

[c] (LEWxBN)F_1 anti-ACI and (WFxBN)F_1 anti-DA produced concordant staining of Bowman's capsule, distal TBM, basement membranes of capillaries and vasa recta, arteriolar media, and glomerular mesangium.

[d] Animals came from two sources (see **Methods**).

[e] Three l/l, three u/u, and seven l/u animals were tested.

[f] The two BN strains differed at the RT2 (Ag-C)locus [24, 25].

Table 3. Summary of the strain distribution of TBM antigens recognized by several xeno- and alloantisera

Rat strains	RT1 type	BN and (LEWxBN)F_1 anti-SD [10]	Rat anti-bovine TBM [11]	LEW anti-(LEWxBN)F_1 [14]	LEW anti-DA [15]
LEW[a]	l	—	—	—	—
AS	l				—
MAXX	n	—			
WF	u	—			
AGUS	l				+
F344	lvl	+	+	+	+
(LEWxBN)F_1	l/n	+	+	+	
ACI	a	+	+		
DA	a	+			+
COP	a			+	
BUF	b		+	+	
SD[b]	(b)	+		+	
August	c	+	+	+	+
PVG.c	c				+
WAG	u				+
BN	n	+	+		+
WF (fz)	u		+		

[a] LEW animals obtained from various sources.

[b] Unknown whether or not inbred SD animals were used.

finding implies the existence of at least two different proximal TBM antigenic determinants. The reaction pattern of human anti-TBM antisera on a panel of human kidney biopsies is in agreement with this possibility [8]. We do not know, however, whether the remaining positive reactions were attributable to one or more anti-proximal TBM antibodies. At present it is also uncertain whether the different antigenic determinants are present on the same molecule or on physically separate molecules. Although no obvious differences in proximal TBM staining were noted, the resolution of the immunofluorescence technique is too low to discover different sites of antibody binding within the structure that, according to immunofluorescence criteria, is considered to be the TBM.

Because four anti-TBM antisera [BN serum with spontaneous anti-TBM antibodies, MAXX anti-BN, LEW anti-(LEWx BN)F_1, and (LEWxBN)F_1 anti-ACI] all reacted with the BN, PVG.c, BN.1L, BN.WF, BN.KGH, BN.WKA, PVG.AO, and PVG.WKA kidneys but not with the LEW, WF, KGH, WKA, and LEW.BN kidneys (Table 1), we conclude that the gene(s) coding the proximal TBM antigens are not linked closely to the MHC. Preliminary data, obtained in a study of 32 animals in the second generation of LEW and BN, indicate that the TBM antigen(s) as recognized by MAXX anti-BN, appear to segregate independently from the MHC gene complex, which is mapped in the ninth linkage group [22], or the albinism trait and peripheral thymocyte Ag-F genes [23], which are in the first linkage group [22]. This data seem to be in accordance with data from Hart and Fabre [15] who demonstrated in a back-cross analysis of LEWx(LEWxDA)F_1 that the TBM antigen(s) detected by LEW anti-DA segregate independently from the MHC.

The proximal TBM antigen(s) recognized by (WFxBN)F_1 anti-DA also is (are) not linked closely to the MHC because this serum did not react with TBM from PVG.DA, PVG.1R, and BN.DA kidneys (Table 1). We do not know whether or not these TBM antigens are linked genetically to the antigens detected by the other anti-TBM antisera. The positive reactions with BN.B4, which has the MHC of DA ($RT1^a$) on a BN background, may be explained by the presence of DA genes in the background. Another possibility is that inbred BN strains are heterogeneous for TBM gene(s), an observation made of the RT2 (AgC) system [24, 25].

In addition to proximal TBM staining, two antisera [(LEWxBN)F_1 anti-ACI and (WFxBN)F_1 anti-DA] produced additional staining of endothelium, glomerular mesangium, Bowman's capsule, distal TBM, vascular basement membranes, and the media of arteries. The anti-endothelial activity was caused by antibodies against MHC determinants [17]. The antigens detected on the other structures are not well defined. Similar staining patterns have been observed in human kidney sections with xenoantibodies against different collagens [20]. In this study we demonstrated the allospecificity of these different structures, although the chemical nature of the antigen(s) is not known, nor is it known whether this staining pattern was caused by one antibody or a mixture of antibodies. In all instances, however, the reactions of the glomerular mesangium, Bowman's capsule, distal TBM, vascular basement membranes, and media of arteries were concordant.

Indirect immunofluorescence studies of anti-TBM antisera have shown variable staining of Bowman's capsule, either obvious staining [11], faint staining [10], or an occasional partial staining [15] has been reported. The BN serum and LEW anti-(LEWxBN)F_1 produced no clear staining of Bowman's capsule. MAXX anti-BN, (LEWxBN)F_1 anti-ACI and (WFxBN)F_1 anti-DA, however, yielded clear-cut staining of Bowman's capsule. It is conceivable that the first two sera did not give clear staining of Bowman's capsule because of the relatively low antibody titers.

Panel analysis of the three sera that stained Bowman's capsule (Table 2) demonstrated that MAXX anti-BN detected capsular antigens with the same strain distribution as the proximal TBM antigens (Table 1). Furthermore, preliminary data have shown that this serum gives concordant staining of Bowman's capsule and proximal TBM in a F_2 population of LEW and BN parental strains. This suggests that the same antigens were detected on Bowman's capsule and the proximal TBM. (LEWxBN)F_1 anti-ACI and (WFxBN)F_1 anti-DA, however, revealed the existence of allospecific capsular antigens which are not expressed on proximal TBM (Tables 1 and 2). The reaction pattern with kidneys from BN, PVG.c, BN.KGH, BN.DA, PVG.DA, PVG.1R, KGH, and DA rats indicates that these antigens are coded by genes outside the MHC.

We conclude that the proximal TBM of rat kidneys expresses at least two serologically distinct antigenic determinants that are encoded outside the MHC region. Some of these determinants are shared by Bowman's capsule. Furthermore, Bowman's capsule also has determinants that are not present on proximal TBM.

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