Induction of Passive Heymann Nephritis with Antibodies Specific for a Synthetic Peptide Derived from the Receptor-associated Protein

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Summary

Passive Heymann nephritis (pHN) is an experimental rat model for human membranous glomerulopathy. In pHN, the formation of subepithelial immune deposits (ID) involves as antigenic targets the membrane glycoprotein gp330/megalin and the 44-kD receptor-associated protein (RAP). A single binding site for ID-inducing antibodies (Abs) was previously mapped to the 86 NH_2-terminal amino acids of RAP (RAP_{1-86}). To further narrow this epitope, Abs eluted from the glomeruli were immunoblotted on membranes that were loaded with overlapping synthetic peptides representing the amino acid sequence of RAP (SPOTs system). Two adjacent Ab-binding domains with the sequences PVRLAE (amino acids 39–44) and HSDLKIQE (amino acids 46–53), which were separated by a single L residue at amino acid 45, were detected. Rabbit Abs raised against synthetic peptides containing these domains individually (P_{31-44} and P_{46-53}) failed to produce glomerular IDs. By contrast, Abs raised against a larger composite peptide (P_{31-53}) induced IDs within 3 d that were firmly cross-linked to the glomerular basement membrane. These data suggest that Ab binding in vivo depends on the conformation of the antigenic target sequence that is preserved in the synthetic peptide P_{31-53}, which covers the entire Ab-binding domain of RAP but not in its subdomains, P_{31-44} and P_{46-53}. Collectively, these results locate the sole ID-inducing epitope of RAP to amino acids 39–53.

The design of therapies for autoimmune diseases is primarily based on identification of antigenic targets and induction of specific immune tolerance. Considerable progress has been made recently in several areas, such as allergic encephalomyelitis, in which epitopes were identified and duplicated by synthetic peptides (1, 2). However, relatively little is known about antigens of glomerular immune complex diseases despite their tendency to cause chronic renal failure. To learn more about the molecular pathogenesis of one relatively frequent glomerular immune disease, membranous glomerulopathy (3), we have investigated in detail an established experimental model in rats, i.e., passive Heymann nephritis (pHN)[1] (4, 5). A hallmark of pHN is the presence of granular subepithelial immune deposits (IDs) that firmly adhere to the glomerular basement membrane (GBM) (6). The goal of our analyis of pHN is to eventually develop peptide-based therapies to specifically interfere with ID formation to provide a curative therapy for this model of membranous glomerulopathy.

In a series of investigations aimed at the identification of pHN antigens, we have used Abs eluted from glomeruli of diseased rats as the major tool, expecting that their specificities could guide us to the target antigen(s) and eventually to the pathogenic epitopes. This strategy led initially to identification of the >515-kD membrane glycoprotein megalin/gp330 (7, 8) and then to the 44-kD receptor-associated protein (RAP; 9, 10) formerly called C14 (11), which are associated together to form the Heymann nephritis antigenic complex (HNAC) (9). Search for pathogenic epitopes on HNAC has revealed one site that was originally thought to be localized on megalin (12), but was subsequently found to reside within the 86 NH_2-terminal amino acids of RAP (RAP_{1-86}) (12). Here, we further exploit the specificity of eluted glomerular Abs for the precise identification of the minimal antigenic target on RAP that is sufficient and competent for the induction of stable IDs.

[1] *Abbreviations used in this paper:* FxIA, crude renal cortex fraction; HN, Heymann nephritis; HNAC, HN antigenic complex; GBM, glomerular basement membrane; ID, immune deposit; pHN, passive Heymann nephritis; PLP, paraformaldehyde-lysine-periodate; RAP, receptor-associated protein; RAP_{1-86}, NH_2-terminal 86 amino acids of RAP.

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Materials and Methods

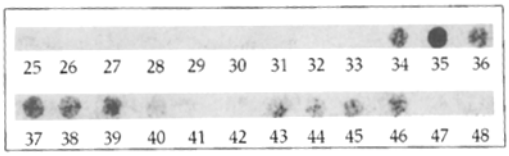
Antibodies. Anti-RAP Abs were prepared by intradermal immunization of rabbits with 30 μg rRAP from the cDNA clone C14, as described (11). IgG fractions were prepared on protein A–Sepharose 4B (Pharmacia, Uppsala, Sweden) and affinity purified further on rRAP fusion protein coupled to a cyanogen bromide-Sepharose 4B column (Pharmacia) (11). Some IgG fractions were also affinity purified using a recombinant RAP_{1-86} (12).

Induction of pHN and Elution of Glomerular IgG. PHN was induced by ~25 mg i.v. of anti-rRAP IgG into 200 g male Lewis rats (Charles River, Sulzfeld, Germany). Eluted IgG was prepared from the isolated glomeruli of 50 rat kidneys with 100 mM citric buffer, pH 2.8 (7), and was concentrated on protein A–Sepharose. The use of experimental animals was authorized by the Austrian Ministry of Science.

Preparation of Immobilized Overlapping Synthetic Peptides (SPOTs Assay). The SPOTs technique allows simultaneous solid phase synthesis of a large number of peptides that can be probed by immunoblotting. SPOTs membranes (Imperial Chemical Industries, Cambridge, UK) were obtained with 96 preformed spots in which a single amino acid was conjugated to the cellulose matrix as an anchoring site for peptides. NH_2-terminally acetylated peptides were synthesized manually by a miniaturized Fmoc-chemistry

A

SPOTs Assay with Eluted Glomerular Ab



B

SPOT#	Composition of Spot	Results
	RAP amino acid 30 40 50 55	
	N-TERMINUS«««««««WEKAKRLHLSPVRLAELHSDLKIQERDELN»C-TERM.	
29	WEKAKRLHLSP	0
30	EKAKRLHLSPV	0
31	KAKRLHLSPVR	0
32	AKRLHLSPVRL	0
33	KRLHLSPVRLA	0
34	RLHLSP**VRLAE**	++
35	LHLSP**VRLAE**L	+++
36	HLSP**VRLAE**LH	+++
37	LSP**VRLAE**LHS	+++
38	SP**VRLAE**LHSD	+++
39	P**VRLAE**LHSDL	++
40	**VRLAE**LHSDLK	+
41	RLAELHSDLKI	0
42	LAELHSDLKIQ	0
43	AEL**HSDLKIQE**	+
44	EL**HSDLKIQE**R	++
45	L**HSDLKIQE**RD	++
46	**HSDLKIQE**RDE	++
47	SDLKIQERDEL	0
48	DLKIQERDELN	0

C

RAP amino acid	33 40 45 50 55
Eluted Ab	KRLHLSP**VRLAEL HSDLKIQE**RDEL
Anti-RAP_{1-86}	KRLHLS**PVRLAEL HSDLKIQE**RDEL
Anti-rRAP	KRLHLS**PVRLAEL HSDLKIQE**RDEL

Figure 1. (*A*) Sections of a SPOTs membrane containing 11-mer peptides with one amino acid overlap representing part of the RAP amino acid sequence. The membrane was immunoblotted with Ab eluted from the glomeruli of rats in which pHN was induced by IgG specific for rRAP. This Ab labeled specifically two clusters of spots, 34–40 and 43–46, but it failed to bind to spots, 25–33, 41 and 42, and 47–48. (*B*) In this interpretative table of the results of *A*, intensity of labeling of individual spots was arbitrarily scored +, ++, and +++, and expressed by shading. The consensus amino acid sequence in each spot is marked by bold print. (*C*) Summary of results of SPOT experiments obtained with Abs specific for the glomerular eluate and for RAP1-86. Both Abs bind to amino acids 40–53 and apparently spare the L amino acid residue at 45.

Composition of Synthetic Peptides

Peptides Related to Binding Sites for ID Inducing Abs

RAP amino acid	30 40 45 50 53
Peptide P_{31-44}	WE**KAKRLHLSPVRLAE**LHSDLKIQER
Peptide P_{46-53}	WEKAKRLHLSPVRLAEL**HSDLKIQE**R
Peptide P_{31-53}	WE**KAKRLHLSPVRLAELHSDLKIQE**R

Control Peptides

Peptide P_{55-46}	**ERDELNWKKLK** (amino acids 55-64)
Peptide P_{64-73}	**KVEGLDGDGE** (amino acids 64-73)
Peptide $P_{116-126}$	**PRLEKLWHKAK** (amino acids 116-126)

Figure 2. Composition of synthetic peptides of various regions of RAP used for the generation of antibodies. The sequences of the peptides prepared are in bold print.

protocol, following the manufacturer's instructions. RAP-derived peptides were arranged as overlapping 11 mers with one amino acid offset, covering the entire sequence of RAP or RAP_{1-86}.

Epitope Mapping by Immunoblotting on SPOTs Membranes. SPOTs membranes were immunoblotted with affinity-purified Abs specific for RAP_{1-86} or with eluted glomerular IgG (11, 12). Membranes were developed and erased before reuse according to the manufacturer's instructions (Imperial Chemical Industries). The amino acid sequences of Ab-binding domains were deduced from the common amino acid sequence shared by all labeled spots (Fig. 1).

Design and Production of Synthetic Peptides. Peptides were designed, two of which were extended by several amino acids towards the NH_2 terminus of RAP beyond the region, to which eluted glomerular IgG bound in the SPOTs analysis (see Fig. 2). Three peptides were produced: (*a*) P_{31-53} (KAKRLHLSPVRLAELHSDLKIQE, amino acids 31–53); (*b*) P_{31-44} (KAKRLHLSPVRLAE, amino acids 31–44); and (*c*) P_{46-53} (HSDLKIQE, amino acids 46–53). As controls, the irrelevant peptides P_{55-64}, P_{64-73}, and $P_{116-126}$ from outside the antigenic regions of RAP were synthesized. Peptides were produced in an automatic peptide synthesizer (model 430A; Applied Biosystems, Inc., Foster City, CA). Peptides were purified to >95% purity by reverse-phase HPLC, and their sequences were analyzed in an Applied Biosystems gas phase sequencer at the Institute of Applied Microbiology, University of Agriculture (Vienna, Austria).

Preparation of Peptide-specific Abs. Synthetic peptides were coupled to a multiple antigenic peptide-polylysine matrix (13) and rabbits were immunized with 2.5 mg i.d. of each synthetic peptide, followed by two boosts with 1 mg peptide, each at 3 and 6 wk. Sera were collected at weekly intervals from the second boost onwards. IgG fractions were purified on protein A–Sepharose 4B and further affinity purified on CNBr-Sepharose coupled to the corresponding peptides (5–10 mg peptide/ml) as follows: 4 ml serum/ml affinity adsorbent were circulated for 12 h at 4°C, washed with 200 column volumes of PBS, and eluted with 20 mM glycine-HCl buffer, pH 2.8, 150 mM NaCl, 0.01% gelatin at 4°C. Eluted IgG was neutralized with 1 M Tris-HCl buffer, pH 8.0, dialyzed against 4 liters PBS, and concentrated with Aquacide II (Calbiochem, San Diego, CA) to 1–5 mg IgG/ml. P_{46-53} prepared as a multiple antigenic peptide or conjugated to KLH or BSA was not immunogenic in rabbits. P_{46-53}-specific IgG was therefore prepared by affinity purification of anti-RAP IgG on a P_{46-53} CNBr column and used in lieu of Abs raised against the synthetic peptide.

Immunoblotting of Peptide-specific Antibodies. Rat kidney microvilli were prepared as described (7), and proteins were separated by SDS-PAGE and transferred onto nitrocellulose membranes. Lysates of isopropyl β-D-thiogalactoside (Sigma Chemical Co., St. Louis, MO)-induced *Escherichia coli* producing rRAP (11) were similarly transferred. Immunoblotting was performed with affinity-purified antipeptide Abs (~10 μg/ml) or with anti-

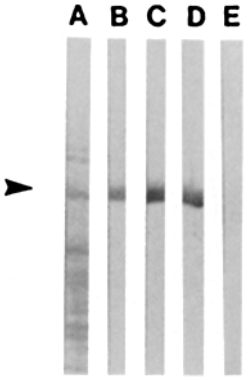


Figure 3. Antipeptide Abs specifically bind rRAP. Expression of a rRAP–GST fusion protein was induced in *E. coli*, the bacterial lysate was separated by SDS-PAGE (lane *A*) and transferred onto nitrocellulose membranes and immunoblotted with irrelevant rabbit IgG as negative control (lane *E*), affinity-purified antipeptide P_{31-53} IgG (lane *B*), affinity-purified antipeptide P_{31-44} IgG (lane *C*), and affinity-purified IgG raised against RAP_{1-86} (lane *D*). The arrow indicates RAP.

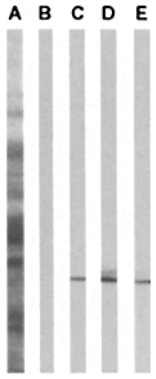


Figure 4. Antipeptide Abs specifically recognize RAP in microvillar fractions. Isolated rat tubular microvilli were separated by SDS-PAGE (lane *A*), transferred onto nitrocellulose membranes, and immunoblotted with affinity-purified IgGs specific for P_{31-53} IgG (lane *C*), P_{31-44} IgG (lane *D*) and RAP_{1-86} (lane *E*); and negative control blotted with irrelevant rabbit IgG (lane *B*).

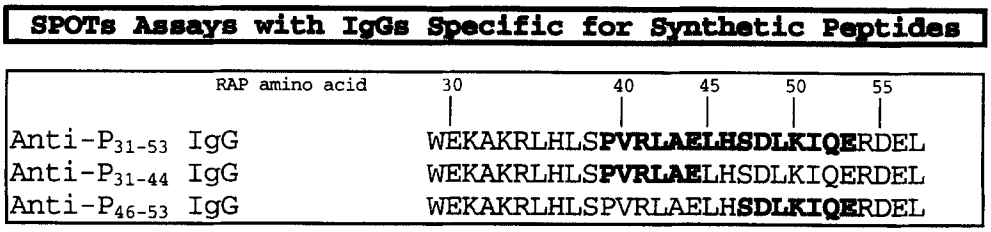


Figure 5. Results of SPOTs assays carried out with antipeptide IgGs. Note that Abs to P_{31-53} bind to amino acids 39–53, those raised to P_{31-44} bound only to amino acids 39–43, and those raised to P_{46-53} bound to amino acids 47–53.

RAP_{1-86} IgG (3–5 μg/ml; 12). As negative controls, the primary Ab was omitted or replaced by irrelevant rabbit IgG, or blotting was performed on lysates of uninduced *E. coli*. Immunoblotting was also performed on SPOTs membranes, as previously described.

Immunofluorescence. 3 d after injection of peptide-specific IgGs, direct immunofluorescence was performed on semithin 1.5-μm frozen sections of rat kidneys fixed with paraformaldehyde-lysine-periodate (PLP; 14) and cut on an Ultracut ultramicrotome equipped with an FC 4 cryoattachment (Reichert, Vienna, Austria). Sections were incubated with FITC-labeled goat anti–rabbit F(ab')2 fragments (DAKO, Copenhagen, Denmark), followed by FITC rabbit anti–goat F(ab')2 (DAKO) to enhance the fluorescence signal. Micrographs were taken on an Axiophot microscope equipped for epifluorescence (Zeiss, Oberkochen, Germany).

Immunohistochemistry on Isolated Basement Membranes. Isolated GBMs were prepared by incubation of 5-μm unfixed cryostat sections of rat kidneys injected with anti-P_{31-53} IgG with 1% Triton X-100 in PBS, followed by 4% deoxycholate in distilled water for 1–3 h at 20°C (15). These cell-free preparations were processed for direct immunofluorescence, as described above. Localization of IDs in similar preparations was performed by immunogold electron microscopy as described (6). Briefly, cell-free sections were quenched with 10% OVA in PBS and incubated with 1:100 diluted goat anti–rabbit IgG conjugated to 10-nm gold particles (Amersham International, Amersham, UK) for 1 h at 20°C. After five washes in PBS–10% OVA, samples were fixed with 2.5% glutaraldehyde in 100 mM cacodylate buffer, pH 7.2, followed by 1% osmium tetroxide in the same buffer and embedding in Epon 812.

Electron Microscopy. Samples of renal cortex from PLP-perfused kidneys of rats injected with anti-P_{31-53} Abs were postfixed in veronal acetate–buffered osmium, stained en bloc with uranyl acetate, and processed for routine electron microscopy (6).

BLAST Searches. Online BLAST searches were performed via the National Center for Biotechnology Information at the National Institutes of Health (Bethesda, MD). PredictProtein (European Molecular Biology Laboratory, Heidelberg, Germany) was used for structural protein analysis (16). We use the term "homology" as being identity plus conserved amino acid substitution.

Results

Anti-RAP Abs and Eluted Glomerular Abs Bind Selectively to Two Adjacent Domains of RAP. SPOTs membranes were prepared containing the primary sequence of RAP_{1-86} disassembled into 72 overlapping 11-meric synthetic peptides (Fig. 1, *A* and *B*). This permitted simultaneous screening of all peptides for Ab-binding sites by immunoblotting that was initially performed with IgG eluted from glomeruli of rats injected with rRAP-specific Abs. Eluted IgG specifically labeled two clusters of spots, one corresponding to VRLAE (amino acids 40–44) and the second to HSDLKIQE (amino acids 46–53), which were separated by an L residue at amino acid 45 (Fig. 1, *B* and *C*). IgGs specific for rRAP and RAP_{1-86} yielded similar results; however, both IgGs bound also to an additional P residue at amino acid 39 (Fig. 1 *C*). No labeling was observed in control experiments with irrelevant rabbit IgG or without primary Ab (data not shown).

These results indicate that the minimal binding sites on RAP of the most relevant Ab in this study, i.e., the eluted glomerular IgG, comprised a region of 14 amino acids with one intervening L residue. The accuracy of the NH_2-terminal border of the first Ab-binding region was limited by variation of ± 1 amino acid in repeated immunoblots on multiple SPOTs membranes.

Antipeptide Antibodies React Specifically with RAP. Two synthetic peptides were designated P_{31-44} and P_{46-53} according to their amino acid composition. P_{31-44} was extended beyond the binding region of eluted glomerular IgG by nine amino acids towards the NH_2 terminus of RAP to account for the variability of this border in the SPOTs assays. In addition, P_{31-53}, which covered both binding regions of eluted IgG, was synthesized (Fig. 2). Rabbit Abs specific for these peptides were prepared and affinity purified on CNBr peptide columns.

All antipeptide IgGs specifically and strongly reacted with RAP by immunoblotting on transfers of rRAP (Fig. 3) or on isolated microvillar fractions (Fig. 4). On SPOTs membranes, anti-P_{31-44} IgG specifically labeled PVRLAE (amino acids 39–44), and anti-P_{46-53} IgG bound to SDLKIQE (amino acids 47–53). IgG raised against P_{31-53} bound to PVRLAELHSDLKIQE (amino acids 39–53) but not to amino acids 31–38 (Fig. 5). In control experiments, Abs raised to peptides distant from the NH_2 terminus of RAP bound only to their respective antigenic peptides (data not shown). These data provide evidence that Abs raised against synthetic peptides selectively bound to their respective sequences on RAP and mirrored the binding sites of IgG eluted from glomeruli.

Antibodies Specific for Peptide P_{31-53} Induce IDs That Firmly Attach to the GBM. Abs produced against peptides P_{31-44} and P_{46-53} failed to induce IDs at 3 and 6 d after injection into rats (data not shown). By contrast, fine granular IDs were observed in the peripheral capillary walls of glomeruli after injection of anti-P_{31-53} IgG (Fig. 6), in a pattern typical for HN. Some IgG was also detected within the mesangium. The glomerular pattern of IDs was confirmed by

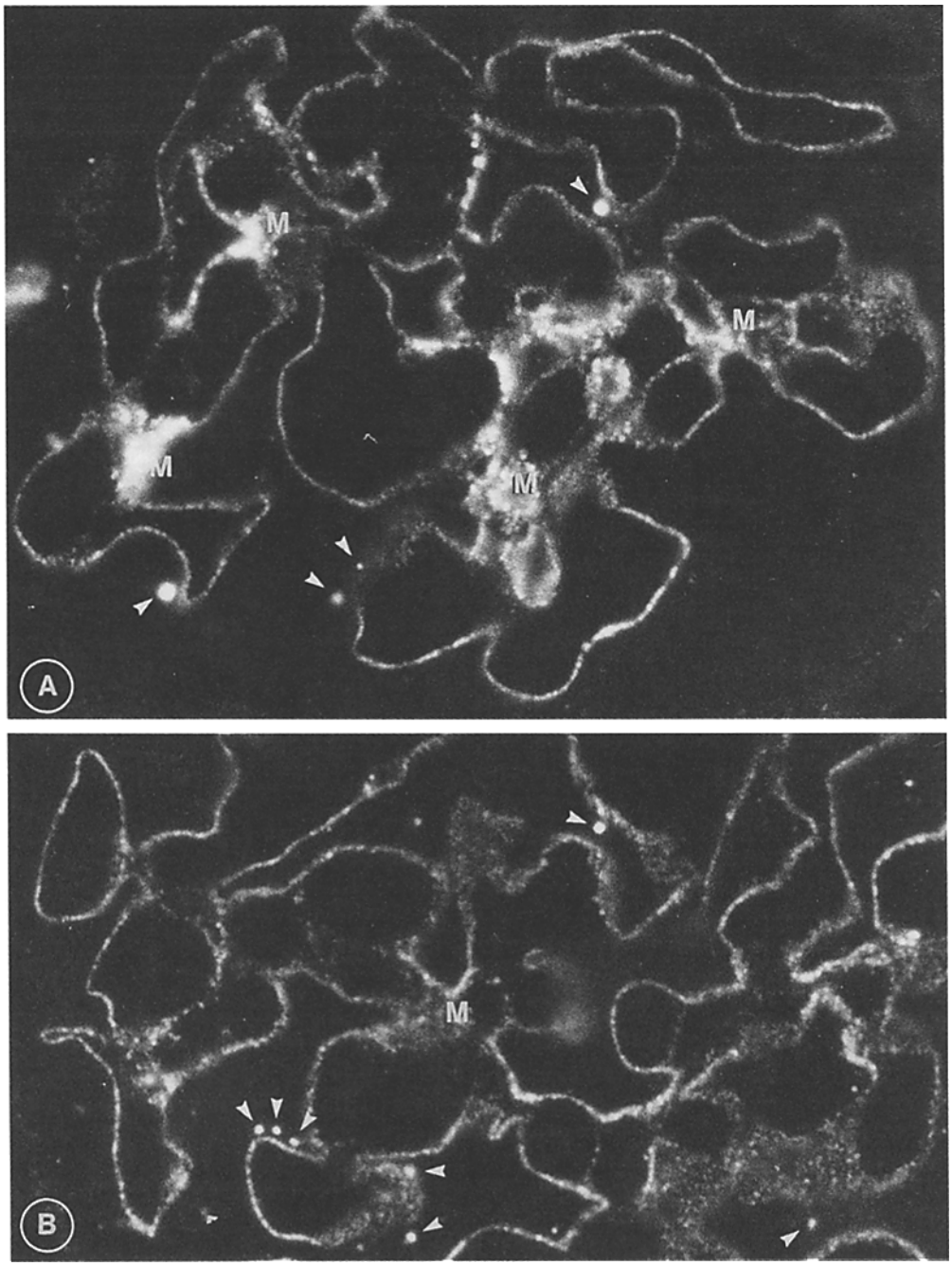


Figure 6. Affinity-purified IgG specific for peptide P_{31-53} produces IDs 3 d after intravenous injection. IDs are visualized by direct immunofluorescence on semithin 1.5-μm frozen sections. There are numerous fine granular IDs in the peripheral glomerular capillary walls. The mesangium is also labeled in a granular pattern (*M*). Several large intracellular vacuoles of glomerular epithelial cells (*arrowheads*) contain rabbit IgG. ×1200.

electron microscopy, which revealed small deposits primarily within clathrin-coated pits at the base of the foot processes of podocytes (Fig. 7).

Unfixed kidney sections of rats injected with anti-P_{31-53} IgG were extracted with detergents to remove cellular material. By direct immunofluorescence, granular IDs containing rabbit IgG were observed to adhere to the cell-free GBMs of glomerular capillaries (Fig. 8) similar in size and distribution to IDs in unextracted sections. By immunoelectron microscopy of isolated GBMs, IDs were observed in the lamina rara externa of the GBM that were specifically labeled by anti–rabbit IgG–gold conjugate (Fig. 9).

These results indicate that IgGs specific for P_{31-53} form small IDs in peripheral glomerular capillary loops and that these IDs are firmly attached to the GBMs. Thus, this peptide-specific Ab is able to generate small IDs similar to those observed in pHN induced by IgGs specific for rRAP or RAP_{1-86} (11, 12).

Predictions of Homologies and Structure of the P_{31-53}. P_{31-53} was found to contain twice a putative RT-1B[1] MHC class II–binding motif, S x x x x x E (Fig. 10), thought to be important for generation of Abs in Lewis rats (17). Search for sequence similarities revealed no other significant resemblance of P_{31-53} to other rat proteins. Predictions of second-

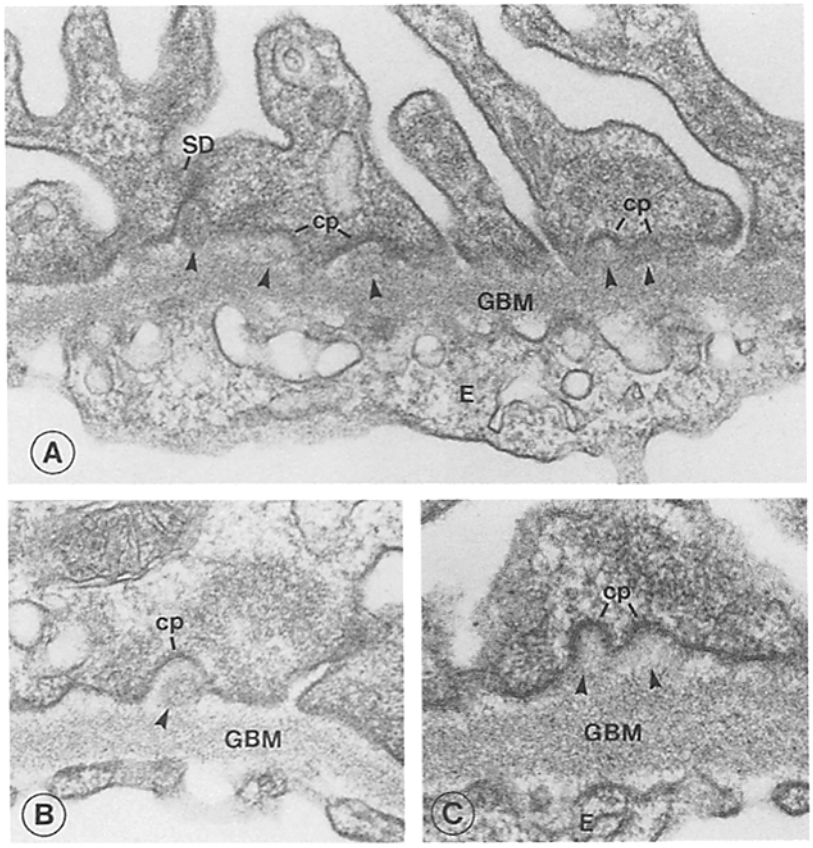


Figure 7. Gallery of electron micrographs of IDs induced by anti-P_{31-53}-specific IgG. The IDs are localized within clathrin-coated pits (*cp*) and under the slit diaphragms (*sd*). ×85,000.

ary structure with 70% certainty (16) indicated that P_{31-53} contains two helical regions at amino acids 31–35 and 41–53 connected by a short loop ranging from amino acids 36 to 40 (Fig. 11).

Discussion

Binding of antibodies in situ to specific epitopes on HNAC, a complex of RAP and megalin/gp330 (9, 18), triggers the formation of persistent IDs in the GBM (6). We and others have observed that Abs raised against rRAP form IDs after injection into rats (11, 19). We had previously identified a single ID-forming epitope at the NH_2 terminus of RAP (RAP_{1-86}) (12) by the expression of a series of RAP cDNA deletion clones, raising of specific Abs, and probing of their ability to induce glomerular IDs. Here, we precisely determine the minimal amino acid sequence on RAP_{1-86} that is required for the binding of

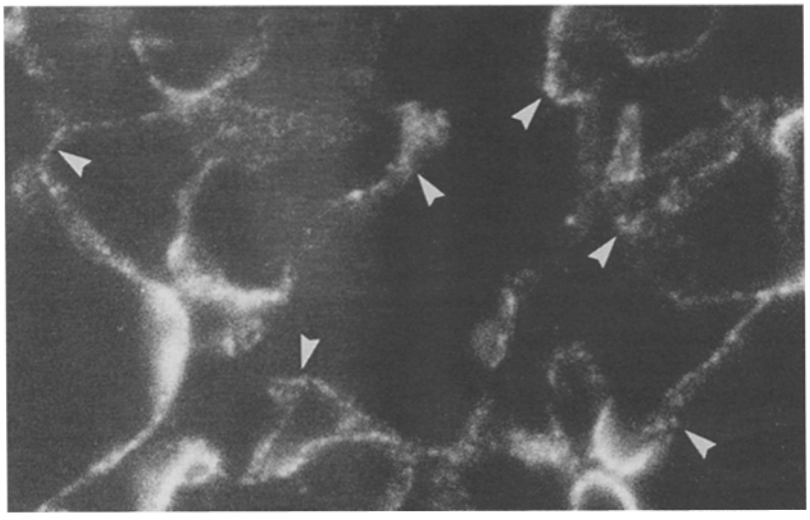


Figure 8. Immune deposits induced by anti-P_{31-53} IgG firmly adhere to isolated GBMs. Cryostat sections of unfixed kidneys of rats injected with anti-P_{31-53} IgG were extracted with detergents to remove the cellular material and to leave behind only cell-free basement membranes. IDs were visualized by direct immunofluorescence in glomerular capillary loops as fine granular deposits that are particularly well resolved in sections grazing through capillary loops (*arrowheads*). ×1,200.

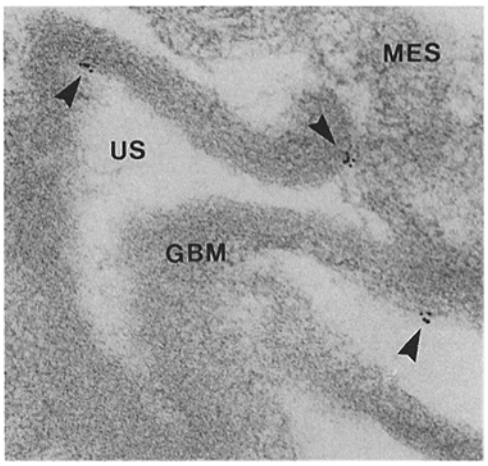


Figure 9. Localization of IDs in a preparation of isolated GBMs by direct immunogold immunoelectron microscopy. Small clusters of gold particles (*arrowheads*) are located on the external side of the isolated GBM of a rat that was injected 3 d before death with anti-P_{31-53} IgG. *M*, mesangial matrix; *US*, urinary space. ×65,000.

eluted glomerular IgG by mapping of IgG-binding domain(s) on overlapping synthetic peptides. In a second step, synthetic peptides that duplicated the Ab-binding domains were produced, and specific Abs were raised and probed for their capacity to induce stable glomerular IDs.

Mapping of binding sites of eluted Abs on a large number of RAP_{1-86}-derived synthetic peptides was performed by SPOTs assays. The results provided evidence for two adjacent Ab-binding domains within the sequences VRLAELH (amino acids 40–44) and HSDLKIQE (amino acids 46–53), with an apparent inaccuracy of ± 1 amino acid at the NH_2-terminal border of the first binding site. These findings raised the possibility that RAP amino acids 40–53 were involved in ID formation in vivo and that appropriate peptide-specific Abs could induce IDs. At first, peptides P_{31-44} and P_{46-53} were synthesized, and specific rabbit Abs were prepared which, however, failed to form IDs when injected into rats. Therefore, a large peptide spanning both P_{31-44} and P_{46-53} was designed and designated P_{31-53}. Intravenous injection of Abs raised against this peptide promptly induced small IDs in the peripheral capillary walls of glomeruli. Moreover, these IDs strongly adhered to the GBM in cell-free preparations of GBMs. Thus, in most respects, IDs produced by injection of anti-P_{31-53} IgG were similar to those induced by conventional Abs (6).

These data indicate that anti-P_{31-53} IgG binds in vitro and in vivo to the same amino acid sequence of RAP as Abs eluted from glomeruli of rats with pHN. Intriguingly, Abs raised against P_{31-44} and P_{46-53} failed to produce IDs when injected into rats. It is possible that P_{31-53} acts as a composite peptide that mimics a natural structural epitope by simultaneously presenting two linked antigenic peptides to the immune system. Thus, while Abs raised against the composite peptide bind in vivo to the native protein, Abs raised against individual smaller peptides bind to their respective amino acid sequences only in vitro in unfolded proteins. A similar situation was recently observed for the proliferating cell nuclear antigen in lupus erythematosus (20). Abs specific for individual synthetic peptides modeled after immunogenic domains failed to bind in vivo. Abs raised against a dimer of these peptides, however, readily bound to their nuclear antigen in vivo, presumably because this composite peptide antigen resembled the corresponding conformation-dependent natural epitope. It remains to be seen to which extent the structural predictions of a helix-loop-helix motif in the region of the P_{31-53} peptide are related to the topography of this epitope on RAP.

HN is characterized by relatively large IDs and by proteinuria. Although large amounts of Ab specific for P_{31-53} were injected into rats, the IDs obtained were considerably smaller than those induced by conventional Abs, such as antimegalin/gp330 IgG (6, 21). It is possible that anti-P_{31-53} IgG serves only as nidus for ID formation, and that additional Abs directed against epitopes on megalin bind subsequently. We have provided evidence that antimegalin Abs without reactivity to RAP induce IDs (21), and one epitope on megalin was recently identified on the fifth cystine-rich repeat of the second LDL receptor-like domain of megalin (22). Thus, while the RAP epitope identified in this study provides a single nidus for the formation of an initial ID, it is clear that additional epitopes on megalin must be operative to yield IDs of the large sizes typically found in passive and active HN. Lack of proteinuria after injection of anti-P_{31-53} IgG is presumably caused by the absence of C5b-9–activating Abs specific for glycolipids that were recently identified as components of anti-Fx1A IgG (23).

In a next step, it will be of interest to attempt to produce active HN by immunization of Lewis rats with synthetic peptides, which may be facilitated by two putative MHC class II peptide–binding motifs specific for Lewis rats (17) within P_{31-53}. Further on, attempts to interfere with the immunologic basis of the disease by use of synthetic peptides will be possible. One example indicating the feasibility of this approach is derived from studies on experimental murine interstitial nephritis (24), which was ameliorated by specific synthetic peptides.

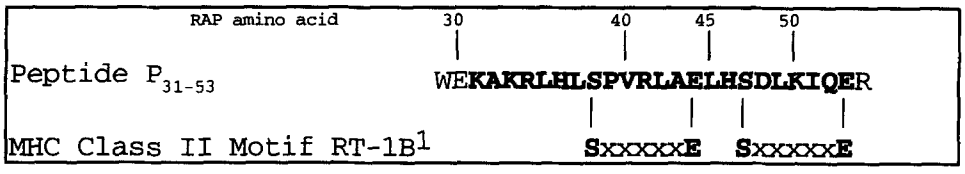


Figure 10. Homolgy searches with online BLAST revealed that the Lewis rat MHC class II–binding motif S x x x x x E is contained twice within P_{31-53}.

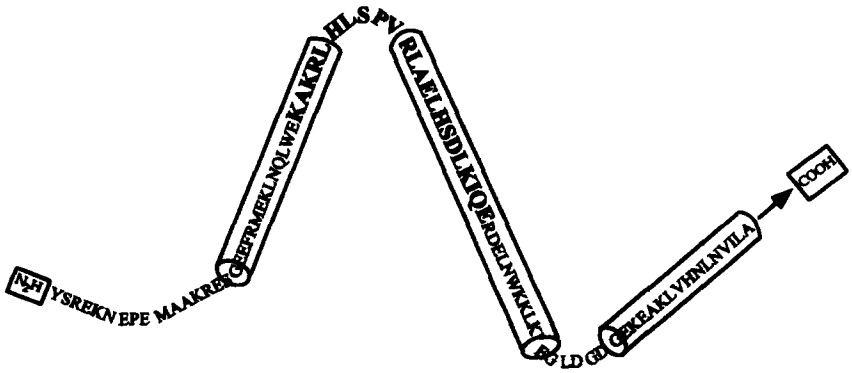


Figure 11. Predicted secondary structure by the ProteinPredict program (70% accuracy) of the P_{31-53} region by the EMBL-PredictProtein program. As indicated by cylinders, this region comprises two helical structures connected by a short loop. A third helix distant to the Ab-binding region and extending towards the COOH terminus of RAP is depicted.

Collectively, the results of this study provide evidence for a presumably structural epitope on RAP that is responsible for the formation of subepithelial IDs in pHN and spans 14 amino acids. These findings are also the first example of induction of glomerular IDs with Abs specific for synthetic peptides modeled after natural Ab-binding sites.

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