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Molecular cloning of a cDNA encoding a major pathogenic domain of the Heymann nephritis antigen gp330

(membrane protein/autoimmune disease/renal pathology)

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ABSTRACT **Heymann nephritis is an experimental autoimmune disease in rats that is characterized by accumulation of immune deposits (IDs) in kidney glomeruli. The disease is initiated by the binding of circulating antibodies to a membrane glycoprotein, gp330, which is a resident protein of clathrin-coated pits on glomerular epithelial cells (podocytes). We have defined a domain representing about 10% of gp330 that appears to be responsible for the formation of stable glomerular IDs. A cDNA clone (clone 14) was isolated from a rat kidney cDNA expression library by screening with IgG eluted from glomeruli of rats in early stages (3 days) of passive Heymann nephritis. The clone 14 cDNA contains an open reading frame encoding the C-terminal 319 amino acids of gp330. The predicted amino acid sequence contains four internal repeats of 11 amino acids, which are also found in the putative ligand-binding region of carbohydrate-binding lectin-like receptors. An antibody raised against the clone 14 fusion protein recognized gp330 by immunoblotting and induced formation of subepithelial IDs typical of passive Heymann nephritis when injected into normal rats. When the clone 14 fusion protein was used to immunize rats, subepithelial IDs of active Heymann nephritis were found after 12 weeks. No IDs were formed by active or passive immunization of rats with fusion proteins derived from other regions of gp330. These results demonstrate that clone 14 encodes a region of gp330 responsible for antibody binding and ID formation *in vivo*.**

Most autoimmune diseases of the kidney are caused by the deposition of antigen–antibody complexes in glomerular capillaries, leading to functional damage of the glomerular filter (1–3). Knowledge of the pathogenic mechanisms involved is based largely on the analysis of animal models of which Heymann nephritis (HN), a model of human membranous glomerulonephritis, has been studied in greatest detail. It has been established that in HN formation of immune deposits (IDs) is initiated by the binding of circulating anti-gp330 antibodies to its antigen, gp330, a membrane glycoprotein (gp) located in coated pits at the base of the foot processes of glomerular epithelial cells (4, 5). Formation of IDs can be initiated either by immunization of rats with gp330 (active HN) or by transfer of serum from immunized animals to normal recipients (passive HN). The immune complexes thus formed are then shed and rapidly become crosslinked to the glomerular basement membrane (6).

Intriguingly, only certain antibodies to gp330 bind to the antigen *in vivo* and cause accumulation of granular IDs after intravenous injection into rats, whereas others (primarily monoclonal antibodies) fail to bind. Presumably, these antibodies recognize epitopes on gp330 that are not exposed to the circulation *in vivo*. Thus there appear to be certain "pathogenic epitopes" or domains on gp330 to which circulating antibodies bind and initiate the injury process (6). In this report we have identified a pathogenic domain of gp330 by using IgGs eluted from glomeruli of rats with passive HN to probe (*i*) CNBr peptides of gp330 and (*ii*) a rat kidney cDNA expression library and sequenced the corresponding cDNA clone, clone 14 (C14).‡ This approach may also prove to be applicable to analysis of antigens involved in other autoimmune diseases.

METHODS

Antibodies. Monospecific rabbit anti-gp330 IgG was prepared and affinity purified as described (5). A 2.5-mg sample of the latter in 0.5 ml of isotonic phosphate-buffered saline (PBS) was injected i.v. into ≈150-g rats (to induce passive HN). After 3 days, glomeruli were isolated and the bound IgG was eluted with 20 mM citrate buffer at pH 3.2 (4) and purified on protein A-Sepharose 4B (Pharmacia). Monoclonal anti-β-galactosidase (β-Gal) IgG was obtained from Thomas Mason (University of Massachusetts).

Peptide Mapping. gp330 was excised from 4–8% gradient SDS/polyacrylamide gels of rat kidney microvilli, prepared as described (4). The gel slices were adjusted to pH 5.0 and digested for 1 hr at 37°C with 5 M CNBr (Aldrich) in 70% (vol/vol) formic acid (7). The 1-hr digestion period was selected to yield peptides that could be resolved by SDS/PAGE. After neutralization with 1 M Tris·HCl (pH 8.0), the peptides were electroeluted from the gel slices (8), separated on 15% SDS/polyacrylamide gels, and transferred onto nitrocellulose membranes. Immunoblotting was carried out with affinity-purified rabbit anti-gp330 IgG or IgG eluted from glomeruli of rats injected with rabbit anti-gp330 IgG. Bound antibodies were detected using a goat anti-rabbit IgG conjugated to horseradish peroxidase (Biosys, Compiègne, France) and developed with 4-chloro-1-naphthol (Sigma).

Screening of a Rat Kidney cDNA Expression Library. Approximately 500,000 recombinant phages from a rat kidney cDNA library (Clontech) were screened (9) with anti-gp330 IgG (5 μg/ml in PBS), which was detected by alkaline phosphatase-conjugated goat anti-rabbit IgG (Promega) and Nitro blue tetrazolium/bromochloroindolyl phosphate (Kirkegaard and Perry Laboratories, Gaithersburg, MD) in 50 mM Tris·HCl, pH 9.5/100 mM NaCl/5 mM $MgCl_2$. The positive clones were pooled and rescreened with affinity-purified anti-gp330 (5 μg/ml in PBS).

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Abbreviations: HN, Heymann nephritis; ID, immune deposit; C14, clone 14; C15, clone 15; C14-fp, fusion protein of C14; C15-fp, fusion protein of C15; β-Gal, β-galactosidase; gp, glycoprotein.

‡The sequence reported in this paper has been deposited in the GenBank data base (accession no. M31051).

Preparation of β-Gal Fusion Proteins and Rabbit Antibodies. *Escherichia coli* lysogens were prepared from plaque-purified phage by infection of *E. coli* strain Y1089, and fusion proteins were produced (10). Bacterial lysates of induced clones were separated by SDS/PAGE and used for immunoblotting with (*i*) affinity-purified rabbit anti-gp330 IgG, (*ii*) IgG eluted from glomeruli of rats injected with anti-gp330 IgG, (*iii*) monoclonal anti-β-Gal IgG, or (*iv*) nonimmune rabbit IgG. Bound antibodies were detected with alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin. Fusion proteins were purified by preparative SDS/PAGE and electroelution (8). Rabbits were immunized with 50–75 μg of antigen emulsified in complete Freund's adjuvant and given two booster injections containing the same amount of antigen in incomplete Freund's adjuvant. IgG was prepared on protein A-Sepharose and affinity-purified on CNBr-activated Sepharose-immobilized lysates of *E. coli* infected with wild-type λgt11.

Immunofluorescence and Immunoelectron Microscopy. Cryostat sections of unfixed rat kidneys were incubated with fusion-protein-specific IgG (300 ng/ml) and then with fluorescein-conjugated goat anti-rabbit IgG (Cappel Laboratories). For immunoelectron microscopy, kidneys were perfused with modified Eagle's medium and periodate/lysine/paraformaldehyde fixative, and cryostat sections were incubated with the fusion-protein-specific IgG and then with goat anti-rabbit Fab-horseradish peroxidase conjugate (Biosys), as described (5).

Induction of IDs by Fusion Protein-Specific IgG. For production of passive HN, rats were injected i.v. with 2 ml of rabbit antiserum raised against clone 14 fusion protein (C14-fp), clone 15 fusion protein (C15-fp), or β-Gal. Animals were sacrificed at 1 hr, 3 hr, 3 days, or 8 days after injection; cryostat sections were prepared and used for detection of injected IgG by direct immunofluorescence or immunoperoxidase (5, 6). For production of active HN, rats were immunized with 0.5 mg of purified C14-fp, C15-fp, or β-Gal (Sigma) in complete Freund's adjuvant and given one booster injection after 6 weeks. Animals were sacrificed at 12 weeks, and their kidneys were processed as described for passive HN.

DNA Sequence Analysis. Phage DNA was prepared from large-scale liquid culture of *E. coli* Y1090 infected with plaque-purified phage (10). The cDNA insert was obtained by digestion of phage DNA with *Eco*RI (Promega), electrophoresis in a 1% agarose gel, and electroelution onto a DEAE membrane (Schleicher & Schuell) (11). The cDNA was ligated into *Eco*RI-digested M13mp19 and Bluescript plasmids (Stratagene). Both strands of overlapping exonuclease III deletion clones were sequenced with Sequenase (United States Biochemical) using the dideoxynucleotide chain-termination method (12). Sequences were analyzed using the software package from the University of Wisconsin Biotechnology Center Genetics Computer Group, as well as the FASTP (13) and FASTA programs (14).

RESULTS

IgG Eluted from Glomeruli of HN Rats Binds to the 65-kDa CNBr Peptide of gp330. Cleavage of gp330 with CNBr under the conditions used produces a number of peptides, ranging from 10 to 120 kDa (Fig. 1, lane A). By immunoblotting, affinity-purified rabbit anti-gp330 IgG bound to ≈12 fragments, most prominently to those of 130, 120, 65, 54, 45, and 34 kDa (Fig. 1, lane B); however, IgG eluted from glomeruli of rats injected with rabbit anti-gp330 IgG specifically bound to the 65-kDa peptide and, to a lesser extent, to the 52-kDa peptide (Fig. 1, lane C). The fact that the eluted antibody (in contrast to the whole injected IgG) binds to a single peptide

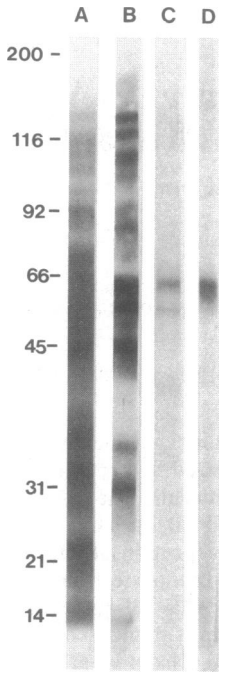

FIG. 1. Immunoblotting of CNBr peptides of gp330. Lane A: Protein stain (Ponceau S). Lane B: Affinity-purified anti-gp330 IgG. A number of peptides of gp330 are recognized. Lane C: IgG eluted from the glomeruli of rats 3 days after the i.v. injection of anti-gp330 IgG. A major ≈65-kDa peptide and a minor 52-kDa peptide are labeled. This indicates that the bound IgG in the IDs of early stages of passive HN is directed primarily against the 65-kDa peptide. Lane D: Anti-C14-fp IgG. This IgG binds exclusively to the 65-kDa peptide.

indicates that the bound IgG recognizes a specific domain of gp330 that is largely restricted to its 65-kDa CNBr fragment.

IgG Eluted from Glomeruli of HN Rats Recognizes the C14-fp. Initial screening with anti-gp330 IgG yielded 91 positive clones. These were pooled and rescreened with affinity-purified anti-gp330 IgG yielding 13 positive clones. When the fusion proteins of the latter were expressed and immunoblotted with IgG eluted from glomeruli of rats injected with anti-gp330 IgG, only the C14-fp (150 kDa, Fig. 2, lane A) was intensely labeled (Fig. 2, lane C). Two others, C15-fp (170 kDa) (Fig. 2, lanes E and G) and clone 21 fusion protein were not recognized at all, while the remaining clones bound the eluted IgG weakly (data not shown). Polyclonal anti-gp330 IgG (Fig. 2, lanes B and F) and monoclonal anti-β-Gal IgG (Fig. 2, lanes D and H) recognized the fusion proteins of all clones. The selective binding of eluted IgG to C14-fp pinpoints this clone as the one of interest, as it

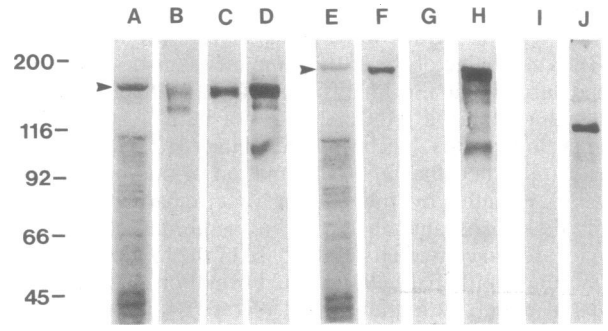

FIG. 2. Characterization of the clone 14 (C14) (lanes A–D) and clone 15 (C15) (lanes E–H) fusion proteins. Lanes A and E: Protein-stained *E. coli* lysates expressing C14 and C15, respectively. C14 and C15 encode ≈150- and ≈170-kDa fusion proteins (arrowheads), respectively. Lanes B and F: Immunoblots with affinity-purified rabbit anti-gp330 IgG. Both C14-fp and C15-fp are recognized. Lanes C and G: Immunoblots with IgG eluted from glomeruli 3 days after i.v. injection of anti-gp330. The eluted IgG strongly labels C14-fp but does not recognize C15-fp. Lanes D and H: Immunoblots with monoclonal anti-β-Gal IgG. Both C14-fp and C15-fp are labeled. Lanes I and J: Immunoblots of wild-type λgt11 *E. coli* lysate with eluted glomerular IgG and monoclonal anti-β-Gal IgG, respectively. The latter blots β-Gal (116 kDa).

encodes a region of gp330 that contains epitopes to which circulating antibodies bind *in vivo*.

C14-fp-Specific IgG Binds to the 65-kDa CNBr Peptide of gp330. When an antibody was raised to the C14-fp, it recognized C14-fp but not C15-fp by immunoblotting. It also recognized gp330 by immunoblotting and immunoprecipitation (data not shown). This antibody also selectively bound the 65-kDa CNBr peptide of gp330 (Fig. 1, lane D). Anti-C15-fp IgG recognized gp330 but not the 65-kDa peptide.

C14-fp-Specific IgG Labels the Coated Pits of Proximal Tubule Brush Borders. Antibodies to C14-fp, as well as those to all other gp330-derived fusion proteins, labeled exclusively the brush border region of proximal tubules by indirect immunofluorescence (Fig. 3*a*). By indirect immunoperoxidase with anti-C14-fp IgG, reaction product was found in clathrin-coated pits (Fig. 3*b*) located at the base of the microvilli.

IgG Specific for C14-fp Forms IDs. When anti-C14-fp IgG was injected i.v. into rats, it bound to glomeruli where it accumulated in a fine granular pattern as early as 1 hr after injection. The deposits progressively increased in size up to 8 days (Fig. 4*a*). No IDs were formed after injection of antibodies to C15-fp or β-Gal. A similar pattern of glomerular IDs was seen when rats were immunized against the C14-fp, but no deposits were seen after immunization with C15-fp or purified β-Gal (data not shown).

By immunoperoxidase the IDs formed by injection of anti-C14-fp IgG or by immunization with C14-fp were found either in clathrin-coated pits at the base of the epithelial foot processes or under the slit diaphragms (Fig. 4 *b* and *c*). Their location was identical to IDs found in active and passive HN (4, 6), but their size was consistently smaller. This demonstrates directly that C14 encodes a region of gp330 responsible for antibody binding and subsequent ID formation *in vivo*.

C14 Encodes the C-Terminal End of gp330. The complete 1260-base-pair sequence of the cDNA of C14 (Fig. 5) contains an open reading frame of 957 base pairs and would be expected to encode an ≈35-kDa peptide, which agrees with the predicted size of C14-fp (Fig. 2, lane A). The termination codon at position 957 is followed by a putative 3′ untranslated region containing multiple termination codons in all frames. Also present is the sequence TATAAA (16), which is a variant of the predominant AATAAA sequence encoding the polyadenylylation signal (17), and two additional polyadenylylation sequences, CATTG and CACTG (18). This indicates that C14 encodes the C-terminal end of gp330. Partial sequence analysis of C15 cDNA shows no homology with C14.

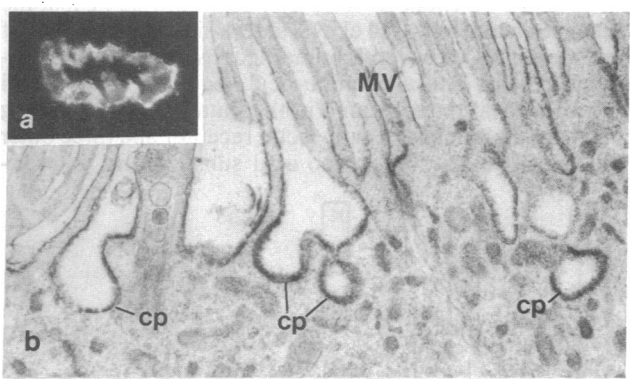

FIG. 3. Antibodies to C14-fp stain the brush border of the proximal tubule by indirect immunofluorescence (*a*) and immunoelectron microscopy (*b*). The localization in coated pits (cp) at the base of the microvilli (mv) is identical to that of gp330 (15). Reaction product is found largely on the outer surface of the intermicrovillar microdomain of the apical membrane. (*a*, ×405; *b*, ×18,000.)

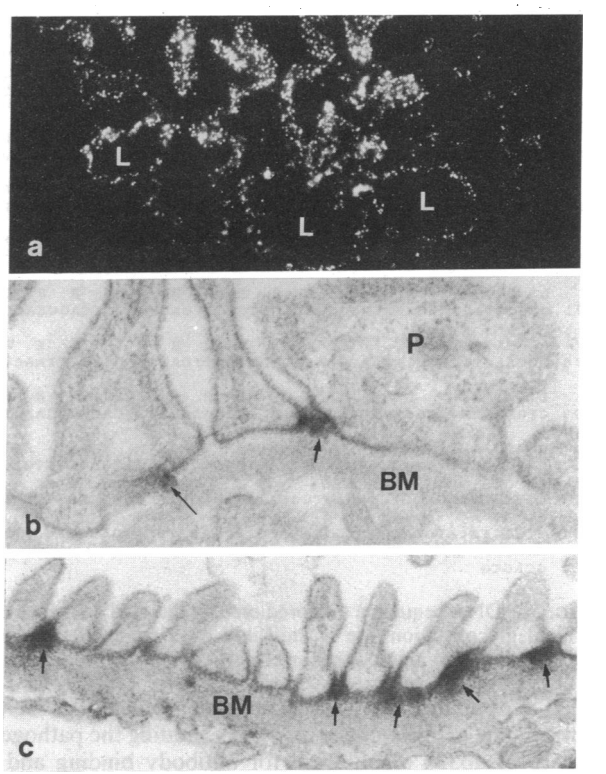

FIG. 4. Formation of IDs after injection of rabbit anti-C14-fp IgG into rats (*a* and *b*) or by immunization of a rat with purified C14-fp (*c*). (*a*) Granular IDs are found in the capillary loops by direct immunofluorescence 3 days after i.v. injection. (*b*) By immunoperoxidase, IDs are located between the epithelial foot processes (short arrow) or between the foot process membrane and the glomerular basement membrane (long arrow). (*c*) Numerous subepithelial IDs (arrows) containing endogenous rat IgG are present 12 weeks after immunization with C14-fp (active HN). L, capillary lumen; BM, glomerular basement membrane; P, foot process. (*a*, ×405; *b*, ×37,800; *c*, ×25,200.)

C14 Contains Internal Sequence Repeats Resembling Those Found in Carbohydrate-Binding Receptors. A dot matrix analysis of the predicted amino acid sequence of C14 with itself revealed four internal repeats of 11 amino acids, each containing a tryptophan residue (Fig. 5). Interestingly, the third repeat contains a 5-amino acid sequence, Trp-His-Lys-Ala-Lys, which is identical to that found in the putative ligand-binding domain of the chicken hepatic lectin receptor (Fig. 6) and is similar to the corresponding regions of several other carbohydrate-binding proteins, such as rat hepatic lectin, rat liver mannose-binding proteins, apoprotein of dog pulmonary surfactant, and invertebrate soluble lectin (19). No significant homologies with any known proteins were found in the sequences outside the repeats.

DISCUSSION

In this investigation we have devised a strategy that involves the utilization of antibodies bound to glomeruli of nephritic rats for the identification of a pathogenic domain of gp330 responsible for antibody binding and initiation of immune deposit formation in HN. Initially the pathogenic domain was localized to a 65-kDa CNBr peptide of gp330 by immunoblotting with the eluted IgG. By using anti-gp330 and the eluted antibody to screen a rat kidney cDNA expression library, we identified a clone, C14, that encodes the pathogenic domain of gp330. The fact that an antibody raised against the C14-fp recognized gp330 and selectively bound to only the 65-kDa CNBr peptide corroborated that C14 en-

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    R  S  A  E  K  N  E  P  E  M  A  A  K  R  E  S  G  E  E  F  R  M  E  K  L  N  Q  L  W  E  K  A  K  R  L  H  L  S
    CGCTCGGCGGAGAAGAATGAGCCCGAGATGGCCGCCAAGCGTGAGTCCGGGGAGGAGTTCCGCATGGAGAAGCTGAACCAGCTATGGGAGAAGGCCAAGCGGCTGCATCTGTCT

    P  V  R  L  A  E  L  H  S  D  L  K  I  Q  E  R  D  E  L  N  W  K  K  L  K  V  E  G  L  D  G  D  G  E  K  E  A  K
115 CCTGTGAGGCTGGCCGAGCTGCATTCTGACCTGAAGATACAAGAGAGGGATGAACTCAACTGGAAGAAGCTGAAGGTGGAAGGCCTAGACGGGGATGGGGAGAAAGAAGCAAAA

    L  V  H  N  L  N  V  I  L  A  R  Y  G  L  D  G  R  K  D  T  Q  T  V  H  S  N  A  L  N  E  D  T  Q  D  E  L  G  D
229 CTGGTCCACAATCTCAATGTCATCCTGGCCAGGTACGGACTGGATGGGAGGAAGGACACCCAGACAGTGCACAGCAACGCCCTCAATGAAGACACCCAGGATGAGCTGGGGGAC

    P  R  L  E  K  L  W  H  K  A  K  T  S  G  I  S  V  R  L  T  S  C  A  R  V  L  H  Y  K  E  K  I  H  E  Y  N  V  L
343 CCCAGGTTGGAAAAGCTGTGGCACAAGGCAAAGACATCAGGAATCTCAGTGAGACTGACAAGCTGTGCGAGAGTTCTGCACTACAAAGAGAAGATCCACGAGTACAATGTACTG

    L  D  T  L  S  R  A  E  E  G  Y  E  N  L  L  S  P  S  D  M  T  H  I  K  S  D  T  L  A  S  K  H  S  E  L  K  D  R
457 CTAGATACACTGAGCAGAGCTGAAGAAGGTTATGAGAACCTTCTCAGCCCCTCTGACATGACCCACATCAAGAGTGACACCCTGGCCAGCAAGCACAGTGAACTGAAGGACAGA

    L  R  S  I  N  Q  G  L  D  R  L  R  K  V  S  H  Q  L  R  P  A  T  E  F  E  E  P  R  V  I  D  L  W  D  L  A  Q  S
571 CTGCGTAGTATCAACCAGGGCCTAGACCGCCTGAGGAAGGTCAGCCACCAGCTACGCCCCGCCACTGAGTTTGAAGAGCCCCGAGTGATAGATCTGTGGGACCTGGCTCAGTCT

    A  N  F  T  E  K  E  L  E  S  F  R  E  E  L  K  H  F  E  A  K  I  E  K  H  N  H  Y  Q  K  Q  L  E  I  S  H  Q  K
684 GCCAACTTCACCGAGAAGGAACTGGAGTCGTTCAGGGAGGAGCTTAAGCACTTCGAGGCCAAAATCGAAAAGCACAACCACTACCAGAAGCAGCTGGAGATTTCCCACCAGAAG

    L  K  H  V  E  S  I  G  D  P  E  H  I  S  R  N  K  E  K  Y  V  L  L  E  E  K  T  K  E  L  G  Y  K  V  K  K  H  L
799 CTGAAGCACGTGGAGAGCATCGGTGACCCCGAGCACATCAGCCGCAACAAGGAGAAGTACGTCCTGCTGGAGGAGAAGACAAAGGAGCTGGGCTACAAGGTGAAGAAGCATCTG

    Q  D  L  S  S  R  V  S  R  A  R  H  N  E  L
912 CAGGACCTGTCCAGCAGGGTCTCAAGGGCTCGGCACAATGAGCTCTGAGACCAGAAGCCACTGGCAGTAGCCTAGAGAGGCTCTTGAAGACACTGGGAACTGTCAACTTGTCAT

1027 ACTGGTGTGGCTGTCCACAGTGGCAAGGAGAAATACCGATCTGATCCTGCTGCGGCTGGCAAGGACTAATTTTCTTTCAAGCAAGTGTAGCTGTCACCGCCCTGGTTGAGGGCT

1141 TTGGGTACATCTACCAATGAGATATGACCGGTCTCCATGCTGCAGCAAAAGTTTACATTGAAATTATATAAATCTGTCACTGGAAATGTTATGTACAGAGTCCTTAAATACATG

1255 GCAGCG
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FIG. 5. DNA sequence and predicted amino acid sequence of C14. Four tryptophan-containing repeats (see Fig. 6) are boxed. A potential N-linked glycosylation site is indicated by a triangle. Polyadenylylation signals (TATAAA, CATTG, and CACTG) are underlined. The single-letter amino acid code is used.

codes a domain of the molecule that coincides with this peptide. The identity of the clone as encoding the pathogenic domain of gp330 responsible for antibody binding and ID formation was corroborated by the demonstration that glomerular IDs could be generated in rats either by injection of C14-fp-specific IgG (passive HN) or by immunization with C14-fp (active HN).

Preliminary experiments indicate that it was crucial to use IgG derived from rats early in the course of passive HN for screening, because IgG eluted at later times (>3 days) proved to recognize additional CNBr fragments of gp330. A likely explanation for this phenomenon is that with time an increasing number of antibody binding sites are exposed by processing of gp330 within the IDs and, as a consequence, circulating IgG specific for additional sites can also bind to gp330 present in the IDs. Indirect indication that the number of pathogenic sites on gp330 might be limited came from the finding (6) that IgG eluted from glomeruli 3 days after injection consisted mainly of acidic isoelectric variants (pI 5.0–6.1), whereas the polyclonal anti-gp330 IgG injected had a broader pI range (5.0–8.5).

The 319-amino acid sequence encoded by C14 is contained within the 65-kDa CNBr fragment of gp330. The resultant ≈35-kDa peptide represents a relatively small region (≈10%) of the gp330 molecule that must be located at the extreme C terminus of gp330 based on the presence of a termination codon and multiple polyadenylylation signals. By definition, the C terminus must be exposed on the ectodomain of the molecule since it binds antibody *in vivo*. This result differs from that obtained in a previous study (20) in which the C terminus was assigned to the cytoplasmic domain based on the presence of a single termination codon. The explanation for these discrepant results should become apparent when the complete amino acid sequence of gp330 has been obtained.

The number of antibody binding sites present in the ≈35-kDa peptide contained in the C14-fp is not known, but our data suggest that most, if not all, of the pathogenic epitopes required to initiate HN reside within this C-terminal region of gp330. Additional work is needed to further narrow the specific sequences involved. For experimental allergic encephalitis in mice, the autopathogenic domain has been precisely defined as a nonapeptide located at the N terminus of myelin basic protein (21). The identification of such sites in gp330 might allow the development of strategies to interfere with the initial epitope-dependent immune complex formation between gp330 and anti-gp330 antibodies.

We have postulated (3) that gp330 might function as a receptor because, like a number of other receptors (e.g., the low density lipoprotein, asialoglycoprotein, and transferrin receptors) (22), it is located in clathrin-coated pits (23). It differs from the other receptors mentioned in that it is found in a selected population of epithelial cells—i.e., those of the proximal tubule, yolk sac, and epididymis—that are heavily involved in bulk endocytosis (24, 25). It is intriguing that the sequence encoded by C14 cDNA shows similarities to that of the C terminus of several animal lectins. It contains tryptophan repeats and a 5-amino acid sequence in the third repeat identical to that found in the putative ligand-binding domain of the chicken hepatic lectin receptor and three other repeats with conserved amino acid substitutions. Interest-

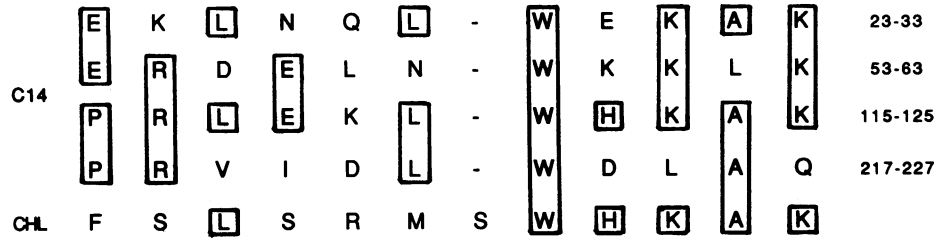

FIG. 6. Alignment of the four tryptophan-containing repeats in C14 with a conserved region in the chicken hepatic lectin receptor (CHL) (19). Boxed amino acids indicate identical residues between the sequences. The internal repeats of C14 each contain a conserved tryptophan residue as well as conserved substitutions at other positions. The sequence Trp-His-Lys-Ala-Lys (WHKAK) in the third internal repeat of C14 is identical to a sequence in the putative ligand-binding domain of the chicken hepatic lectin receptor. The single-letter amino acid code is used.

ingly, yet another repeating sequence motif was reported in gp330 (20) that contains cysteine-rich domains found in the low density lipoprotein receptor and the 500-kDa low density lipoprotein receptor-related protein (26). The function of gp330 and several other antigenically related brush border membrane glycoproteins [maltase (15) and a 280-kDa protein (27, 28)] remain to be established.

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