Identification of the Goodpasture Antigen as the $\alpha_3(IV)$ Chain of Collagen IV

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The organizational relationship between the recently identified $\alpha_3$ chain of basement membrane collagen (Butkowski, R. J., Langeveld, J. P. M., Wieslander, J., Hamilton, J., and Hudson, B. G. (1987) J. Biol. Chem. 262, 7874-7877) and collagen IV was determined. This was accomplished by the identification of subunits in hexamers of the NC1 domain of collagen IV that were immunoprecipitated with antibodies prepared against subunits M1, corresponding to $\alpha_1(IV)\text{NC1}$ and $\alpha_2(IV)\text{NC1}$, and M2*, corresponding to $\alpha_3\text{NC1}$, and by amino acid sequence analysis. The presence of at least two distinct types of hexamers was revealed, one enriched in M1 and the other enriched in M2*, but in both types, M1 and M2* coexist. Evidence was also obtained for the existence of heterodimers comprised of M1 and M2*. These results indicate that M2* is an integral component of the NC1 hexamer of collagen IV. The amino acid sequence of the NH$_2$-terminal region of M2* was found to be highly related to the collagenous-NC1 junctional region of the $\alpha_1$ chain of collagen IV. Therefore, M2* is designated $\alpha_3(IV)\text{NC1}$ and its parent chain $\alpha_3(IV)$.

These findings lead to a new concept about the structure of collagen IV: namely, 1) collagen IV is comprised of a third chain ($\alpha_3$) together with the two classical ones ($\alpha_1$ and $\alpha_2$), the $\alpha_3(IV)$ chain being within the same triple-helical molecule together with the $\alpha_1(IV)$ and $\alpha_2(IV)$ chains and/or within a separate triple-helical molecule, exclusive of $\alpha_1(IV)$ and $\alpha_2(IV)$ chains, but connected through the NC1 domains to the classical triple-helical molecule comprised of $\alpha_1(IV)$ and $\alpha_2(IV)$ chains. Additionally, a portion of those triple-helical molecules exclusive of $\alpha_1(IV)$ and $\alpha_2(IV)$ chains may be connected to each other through their NC1 domains; and 3) the epitope to which the major reactivity of autoantibodies are targeted in glomerular basement membrane in patients with Goodpasture syndrome is localized to the NC1 domain of the $\alpha_3(IV)$ chain.

Goodpasture syndrome is an autoimmune disorder in human beings which is characterized by rapidly progressive glomerulonephritis with or without lung hemorrhage. Classically, the autoantibodies distribute along the glomerular basement membrane in a linear manner (1), reflecting reactivity with specific GBM' antigens. The molecular origin and nature of the GP antigen has been the subject of many investigations in the past decade owing to the importance of this information in delineating the molecular basis of the syndrome.

Earlier studies revealed that the GP epitope is released from the insoluble GBM matrix upon degradation with bacterial collagenase and that the epitope is contained in a noncollagenous polypeptide fragment termed GP antigen, which exists in both monomer and dimer forms ($M_r = 26,000$ and 50,000, respectively) (2-11). More recently, we have obtained evidence that the major reactivity of GP antibodies is with a subunit, designated M2*, of the noncollagenous globular (NC1) domain of collagen IV, the major constituent of mammalian basement membranes, and that the epitope is sequestered under nondenaturing conditions (12, 13). This conclusion, however, raised the possibility of a structural heterogeneity in the collagen IV molecule, because three distinct subunits (M1, M2*, and M3) were described and only two were expected based on the current knowledge of the structure of collagen IV, which comprises two chains, $\alpha_1(IV)$ and $\alpha_2(IV)$.

The collagen chain origins of these three subunits were subsequently determined (14). M1 comprises two polypeptides which correspond to the noncollagenous segments of the $\alpha_1$ and $\alpha_2$ chains of collagen IV, designated $\alpha_1(IV)\text{NC1}$ and $\alpha_2(IV)\text{NC1}$, respectively. M2* and M3 have physicochemical properties remarkably similar to those of $\alpha_1(IV)\text{NC1}$ and $\alpha_2(IV)\text{NC1}$, but their amino acid sequences differ. Each has Gly-Y-Y triplets and hydroxyproline at their amino terminus, reflecting that each has a collagen chain origin, designated $\alpha_3$ and $\alpha_4$, respectively. These new chains may be integral components of the collagen IV molecule and therefore be classified as collagen IV, or they may comprise a new type of basement membrane collagen.

The purpose of the present study was to determine the relationship between the $\alpha_3$ and $\alpha_4$ chain and collagen IV. The results indicate that the $\alpha_3$ chain is an integral component of collagen IV and its NC1 domain has a sequence similarity highly related to that of $\alpha_1(IV)\text{NC1}$. Therefore, the chain is designated $\alpha_3(IV)$.\n
The abbreviations used are: GBM, glomerular basement membrane; GP, Goodpasture; LBM, anterior lens capsule basement membrane; HPLC, high pressure liquid chromatography; ELISA, enzyme-linked immunosorbent assay; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; mAb, monoclonal antibody.
The mixture was transferred to a conical centrifuge tube containing an excess of Protein A-agarose. After shaking for 20-30 min the purification of the NC1 domain in the presence of 2 M urea, using serum. Unless otherwise indicated, the general procedure was as described previously (13). To precipitate the immune complex formed at room temperature from a caproic acid, 10 mM CaCl2, and 0.05% NaN3 using a Brinkman mixture containing purified NC1 domain and the respective anti-antibodies, electrophoretic analysis, or Western blot studies.

Preparation of Polyvalent and Monoclonal Antibodies—Polyvalent antisera toward M1 (aM1), M2* (aM2*), and M3 (aM3) were raised in rabbits by three injections of the immunogen using standard procedures. The antisera were saturated with 50% (NH4)2SO4 and the soluble material removed after centrifugation. The pellets containing the immunoglobulins were suspended in 50 mM Tris-HCl, pH 7.5, 0.15 M NaCl, 0.05% NaN3 extensively dialyzed against the same buffer, with 0.2% bovine serum albumin. The bound antibodies were eluted by sequential washes with 3.5 M MgCl2 in incubation buffer (10 ml) and 6 M guanidine HCl in 50 mM glycine-HCl, pH 2.5 (10 ml). Immediately after elution, the material was neutralized with 1 M TRIS. The effluents (approximately 20 ml each) were pooled and dialyzed extensively against incubation buffer at 4 °C.

Alternatively, partially purified aM1 (140 µl) was diluted to 40 ml containing incubation buffer with 0.2% bovine serum albumin and passed through an affinity column (2 ml) bound with M1 or M2*, respectively. Columns were prepared using 0.5 mg of purified M1 or M2* and 2 ml of CNBr-activated Sepharose 4B. The column was equilibrated in phosphate-buffered saline, pH 7.4, 0.05% NaN3 (w/v), and 0.05% Tween 20 (incubation buffer) prior to injection onto a C18 reversed-phase HPLC column was from Vydac.

For some studies, aM1 or aM2* antibodies (125 µl) were passed through an affinity column (2 ml) bound with M1 or M2*, respectively. Columns were prepared using 0.5 mg of purified M1 or M2* and 2 ml of CNBr-activated Sepaphore 4B. The column was equilibrated in phosphate-buffered saline, pH 7.4, 0.05% NaN3 (w/v), and 0.05% Tween 20 (incubation buffer) prior to injection onto a C18 reversed-phase HPLC column was from Vydac.

Physical Methods—SDS-PAGE was performed as described by Laemmli (18) on 4–22% or 10–20% gels. Protein blotting to nitrocellulose paper was performed as described (19).}

RESULTS

The general experimental approach of this study was to identify the subsets of the NC1 hexamer which could be immunoprecipitated with polyclonal antibodies prepared against subunits M1, M2*, and M3 and with monoclonal antibodies to M1 and M2*. Coprecipitation of one of the subunits with antibodies prepared against another would suggest an integral relationship of the two in the parent hexamer. In addition, studies were conducted to identify a heterodimer comprised of a monomer of M1 covalently linked to a monomer of M2* and/or M3, which would provide additional independent evidence for such a relationship. An integral relationship would indicate that the α3 and α4 chains, from which the noncollagenous segments designated M2* and M3 are derived (14), coexist with the α1(IV) and/or α2(IV) chains of the collagen IV molecule.

Identification of NC1 Subunits—The identity of subunits, both monomer and dimer forms, of NC1 was previously established using SDS-PAGE in combination with Western blotting using polyclonal antibodies prepared against purified monomer subunits (M1, M2*, and M3) (12). These data are summarized in schematic form in Fig. 6 of Ref. 20. At least five different dimer-size polypeptides react with aM1 (D1a–e), five with M2* (D2a–e*), and three with aM3 (D3a, c, and d). In the monomer-size polypeptides, one band reacts with aM1, two with aM2* (M2a* and b*) and one with aM3. This information serves as the base-line data for the identification of subunits in the immunoprecipitates described below.

Immunoprecipitation with Polyclonal Antibodies—The NC1 hexamer was incubated separately with each of the three polyclonal antibodies (aM1, aM2*, and aM3). The resulting immune complexes (H1, H2, and H3) were precipitated with protein A-agarose and analyzed by SDS-PAGE. The subunits present in the immunoprecipitates were identified based on their mobility after SDS-PAGE and by Western blot analysis using the respective antibodies (Fig. 1). In the monomer-size region (Fig. 1), similarities and distinct differences were detected among H1, H2, and H3. H1 reacted with all three antibodies, showing the presence of M1, M2a*, and M2 b*, and M5. H2 and H3 both contain M2* and M3 subunits, but M1 was absent.

In the dimer region (Fig. 1) distinct differences were detected among H1, H2, and H3. When blotted with aM1, H1 yielded two main bands, D1b and D1d, and two minor ones, D1c and D1e. D1b was incompletely resolved from IgG-derived products, yet its presence was clearly evident. In con-
Precipitates, which suggests that they coexist in some hexamers.

The apparent heterogeneity in NC1 hexamer structure could be a result of other factors. 1) Urea (2 M) used in the hexamer preparation could cause successive dissociations and random reassociations, reflecting a subunit exchange between different hexamer populations. This possibility was eliminated when identical results were obtained from studies repeated without the use of urea (data not shown). 2) Genetic polymorphism could cause heterogeneity, because the NC1 hexamer was prepared from pooled bovine kidneys. However, identical results were obtained using hexamer prepared from a single kidney (data not shown). Therefore, these studies support the conclusion that structural heterogeneity is an inherent property of the hexamer.

**Immunoprecipitation with Monoclonal Antibodies**—Because of the potential existence of common epitopes among the dimers comprised of M1, M2*, or M3, monoclonal antibodies were used to investigate the coprecipitation of M1 and M2* in order to confirm whether M2* is an integral component of the NC1 hexamer. Two monoclonal antibodies were selected for study from clones, obtained from mice immunized with the NC1 hexamer, based on their reactivity to M1 and M2* in Western blot and ELISA analyses (data not shown). Their reactivity with the hexamer subunits was compared by Western blots to those of aM1 and aM2* (Fig. 2A). The staining patterns, for both the monomer and dimer regions of hexamers from LBM and GBM, using mAb-M1 and mAb-M2*, were identical to aM1 and aM2*, respectively, except the staining intensity with monoclonal antibodies was greater with the dimers than monomers. The specificity of mAb-M1 and mAb-M2* for subunits M1 and M2*, respectively, was further confirmed by Western blot analysis using purified monomers of M1 and M2* (data not shown).

The two monoclonals were each reacted with the NC1 hexamer from GBM. The immunoprecipitates were analyzed by Western blots for subunit content as shown in Fig. 2B. Lanes 1 and 4 represent GBM hexamers stained with aM1 and aM2*, respectively. The hexamers precipitated with mAb-M1 precipitated material.

**FIG. 1.** Western blot analysis of the NC1 hexamer bound to aM1, aM2*, or aM3 antibodies and precipitated by protein A-agarose. Incubation mixtures containing 800 μl of purified NC1 hexamer (A_{280 nm} = 0.744) and 10 mg of partially purified antibodies were diluted to 10 ml using incubation buffer (see “Methods”). After 4 h, 400 μl of protein A-agarose was added and allowed to react for 20 min. The material bound to the beads was collected by centrifugation and washed three times with 10 ml of incubation buffer. The agarose beads were then resuspended in 400 μl of sample buffer and heated at 100 °C for 5 min. Identical aliquots (30 μl) of material precipitated by aM1, aM2*, and aM3, lanes H1, H2, and H3, respectively, were analyzed by SDS-PAGE (10–20%), transferred to nitrocellulose, and blotted using aM1, aM2*, or aM3. The arrow on the right side of the figure shows the migration position of IgG-derived products in the antisera.

**FIG. 2.** Immunoprecipitation of NC1 hexamer with mAb-M1 and mAb-M2* antibodies. Western blot analysis was performed with NC1 hexamer precipitated by mAb-M1 and mAb-M2* with protein A-agarose. In A, approximately 4 μg of purified hexamer from LBM (L) or GBM (G) was analyzed by SDS-PAGE (4–22% gel), and the proteins were then transferred to nitrocellulose paper. The four sets of two lanes, LBM and GBM materials, were separated using the prestained protein markers as a guide. The four pieces of nitrocellulose were separately incubated with the indicated antibodies (at bottom of panel). In B, 10 μl of NC1 (GBM, A_{280 nm} = 0.744) and 250 μl of mAb-M1 or mAb-M2* (culture media) were diluted to 1 ml with incubation buffer. After 4 h or overnight at room temperature with shaking, four to six drops of a 1:5 (v/v) suspension of protein A-agarose in incubation buffer was added and allowed to react for 20 min. The material bound to the beads was collected by centrifugation and washed three times with 10 ml of incubation buffer. The beads were then suspended in 110 μl of sample buffer, heated at 100 °C for 5 min and used for SDS-PAGE analysis (4–22% gel) followed by Western blot analysis using aM1 or aM2. Lanes 1 and 4 were loaded with 4 μg of GBM hexamer, lanes 2 and 5 with 50 μl of mAb-M1-precipitated material, and lanes 3 and 6 with 50 μl of the mAb-M2*-precipitated material. D and M represent dimeric and monomeric region, respectively. mAb, monoclonal antibody.
are composed mainly of M1 dimers (lane 2) while those precipitated with mAb-M2* are composed mainly of M2* monomers and dimers (lane 6). However, as was observed with the polyclonals (see above), M1 is present in the mAb-M2* precipitate (lane 3) and M2* is present in the mAb-M1 precipitate (lane 5). Similar results were obtained when the Western blot analyses shown in Fig. 2B were performed with the respective monoclonals (data not shown).

In summary, these results confirm the conclusions reached from the polyclonal studies described above; namely, that 1) at least two different types of hexamers exist which differ in stoichiometry of subunits, one of which is composed mainly of M1 and the other of M2*, and 2) both M1 and M2* are present together in the immunoprecipitated hexamers.

Identification of a Heterodimer Comprised of M1 and M2*—Dimers were studied in search of a heterodimer comprised of M1 and M2* subunits to ascertain further the coexistence and integral nature of M2* in the NC1 hexamer. Such a dimer could exist based on the comigration of dimer bands which react with aM1 and aM2* (Fig. 6, Ref. 20) and with mAb-M1 and mAb-M2* (shown in Fig. 2A).

Previous studies showed that the monomers M1, M2*, and M3 could be separated by reversed-phase HPLC based on their different hydrophobicity (12, 14). Likewise, their corresponding dimers also appear to resolve with this technique (12). Therefore, the hexamer subunits were fractionated by HPLC in order to isolate a potential heterodimer. Pools I–IV were rechromatographed and analyzed by SDS-PAGE (Fig. 3A).

Pool I contained a single monomer band with M1 mobility. Pool II contained four bands in the dimer positions of b, c, d, and e, and one band in the M1 monomer position. Pool III contained three dimer bands in position a, b, and d, and one band in the M3 monomer position. Pool IV contained five dimers in positions a–e and two monomer bands in positions M2a and M2b. The identity of subunits in each fraction was determined by Western blotting with aM1, aM2*, and aM3. The main composition of the four pools was M1, D1, M3-D3, and M2*-D2* and D3, respectively (Fig. 3B, and data not shown).

As indicated, the M1 subunit in monomer and dimer form exists primarily in pools I and II, respectively. However, pool IV displayed dimers which react with both aM1 and aM2*, primarily in positions a, b, and d (compare lanes 5 and 6, Fig. 3B), suggesting that a portion of the bands are heterodimers. To confirm this conclusion, aM1 was affinity-purified against monomer M2* (aM1*) and shown to have no detectable reactivity in direct ELISA against coated M2*, aM1* was subsequently used for Western blot analysis of pool IV. As shown in Fig. 3C, dimers in positions a, b, and d react with aM1*, aM2*, and aM1. Only dimer in position c was more evident with the aM2*. Additionally, the M2* monomer did not react in aM1* and aM1 blots indicating the specificity of both M1 antibody preparations. Equivalent results were obtained when mAb-M1 and mAb-M2* or aM1 and aM2 affinity-purified using their respective monomers were used as reacting antibodies in Western blots (data not shown).

These results suggest that dimers in positions a, b, and d of pool IV contain heterodimers comprised of monomers M1 and M2* because 1) these dimers react with antibodies to M1 and M2*, and 2) homodimers (D1) of M1 elute much earlier than M2* monomers and dimers (18 min for pool II versus 28 min for pool IV, respectively). Therefore, M2* is an integral component of the NC1 domain of collagen IV because M1 constitutes the NC1 regions of the α1 and α2 chains of collagen IV.

Amino Acid Sequence Alignment of Bovine M2* with Homologous Regions of α1(IV) Chain—In our recent report (14), the partial amino-terminal amino acid sequence for bovine M2* was compared to the homologous regions in α1(IV) and α2(IV) from murine and human beings, respectively. It was concluded that M2* was neither the α1(IV)NC1 nor the α2(IV)NC1 but was derived from a novel α3 chain. An additional 10 residues of sequence for M2* has been obtained, as shown in Fig. 4.

This sequence was aligned for maximal matching with those homologous regions in the α1(IV) chain from mouse, human, and bovine species (21–28), α1(IV) chain from Drosophila (29, 30), and α2(IV) chain from mouse and human (28, 31–37). The residues which are identical with those of M2* are shown in Fig. 4 in the boxed areas. A 50% identity was found between M2* and α1(IV) chain from the mouse, human, and bovine species (which are 100% identical in this region), while the identity was 43 and 36% when compared with Drosophila α1(IV) chain and mouse α2(IV) chain, respectively. It is particularly noteworthy that the hydroxyproline residues in positions 6 and 12 are conserved in the three α chains of the mammalian species. Hence, M2* is highly related to the NC1 domain of the α chain of collagen IV, and its sequence
properties of their respective noncollagenous domains, and composition of the triple-helical molecule. One, denoted as A, ever, distinct from each other and from those of al(1V)NCl chain origins (14). Hence, many of the properties of M2* and all exist in monomer- and dimer-size polypeptides, denaturation-renaturation curves for the individual subunits are similar, and all reassociate to form a hexamer even after extensive unfolding in 6 M guanidine-HCl (13). Moreover, the reactivity of M2* with Goodpasture antibody is concomitant with dissociation of hexamer, and the reactivity is reversibly lost upon reassociation of subunits (13). Both M2* and M3 have Gly-X-Y triplets at the amino terminus, indicating their collagen chain origins (14). Hence, many of the properties of M2* and M3 are highly related to those of al(1V)NC1 and a2(IV)NC1 and all exist in a hexamer form under non-denaturing conditions.

The amino-terminal sequences of M2* and M3 are, however, different from each other and from those of al(1V)NC1 and a2(IV)NC1, indicating that they are derived from two new collagen chains, α3 and α4, respectively (14). Moreover, the amounts of these chains are minor relative to al(1V) and a2(IV) chains, and the amounts are tissue-specific (38). Thus, the existence of four distinct α chains, the remarkably similar properties of their respective noncollagenous domains, and the occurrence of these domains in hexamer form indicate that several distinct but related triple-helical molecules exist in basement membrane. Distinctions occur in the chain composition of the monomer forms of the triple-helical molecule and in the association of these forming dimers (38).

Several distinct monomers are possible which differ in chain composition of the triple-helical molecule. One, denoted as A, is the classical collagen IV molecule with a chain composition of (α1)2α2. In a second molecule, the α3 and α4 chains could comprise exclusively a separate triple-helical molecule [(α3)2α4 or α3(α4)2], denoted as B, and a third in which the α3 and α4 chains could coexist with either the al(1V) or a2(IV) chain in a separate molecule [α1,α2,α3, or α1,α2,α4], denoted as C. Other molecules are possible in which the α3 and α4 chains occur separately, or the chain stoichiometries vary.

Additional distinct molecules are possible because the monomer form of the triple-helical molecule is connected end-to-end at the carboxyl-terminal NC1 domain, forming dimers in the membrane matrix. The various monomer molecules cited above could theoretically connect to yield six different dimers (homodimers AA, BB, and CC and heterodimers AB, AC, and BC). Additional dimers are possible considering that the α3 and α4 chains may comprise separate monomers. Upon collagenase digestion, each of these molecules would release their respective NC1 domains in the form of hexamers, yielding several distinct hexamers which differ in subunit composition, depending on the chain composition of the two parent triple-helical molecules. The existence of any hexamer containing NC1 subunits derived from the al(1V) and a2(IV) chains, as M1 in this study, and derived from the α3 chain (M2*), such as AB, AC, BC, and CC would indicate that the M2* subunit is an integral part of the collagen IV molecule.

In the present study of bovine GBM, this relationship was examined by using antibodies to immunoprecipitate hexamers with the subsequent identification of subunits in the immunoprecipitates. Studies with polyclonal and monoclonal antibodies to M1 revealed the presence of hexamers comprised mainly of M1 with small amounts of M2*. Analogue studies with antibodies to M2* revealed the presence of hexamers comprised mainly of M2* with small amounts of M1. These findings show the existence of at least two distinct types of hexamers, one enriched in M1 subunits and the other enriched in M2* subunits, but in both types M1 and M2* coexist, Hence, both immunoprecipitates likely contain some combination of hexamers AB, AC, BC, and CC described above, possibly along with AA and BB.

The coexistence of M1 and M2* in these hexamers was further substantiated with independent evidence which suggests the existence of disulfide-cross-linked heterodimers comprised of M1 and M2*. Hence, we conclude that M2* is an integral component of the NC1 hexamer of collagen IV because M1 constitutes the NC1 regions of the α1 and α2 chains of collagen IV (14). M3 is also likely an integral
component because it appears to coprecipitate with M1 and M2*, but its distribution remains unknown because of the unavailability of monoclonal antibodies to it at the present time. Of particular significance is the finding, reported herein, that the amino acid sequence of the NH2-terminal region (residues 1–28) of M2* is highly related to that of a region of the a1 and a2 chains of collagen IV (Fig. 4). This region includes the terminal sequence of the collagenous domain and the beginning sequence of the NC1 domain of the a1(IV) chains (Fig. 4). The sequence similarity between M2* and this region of the a1(IV) chain is 50% while that between M2* and a2(IV) chain is 36%. For comparison, the sequence similarity of the same region between a1(IV) and a2(IV) is 50%, and between the a1(IV) and Drosophila a1(IV) is 43%; the sequence similarity between the entire NC1 domain of a1(IV) and a2(IV) chains is 63–65% as reported by others (28, 32, 35–37) and that between a1(IV)NC1 and Drosophila a1(IV)NC1 is 59% (29). Moreover, both the a1(IV) and a2(IV) chains and M2* each contain hydroxyproline in positions 6 and 12, reflecting a further similarity in their structures. Hence, M2* is highly related in primary structure to the collagenous NC1 junctional region of the a1 chains of collagen IV, and its sequence similarity is more closely related to that of the a1(IV) chain than to the a2(IV) chain.

The present study together with previous ones (11–13) lead us to designate M2* as a3(IV)NC1 and its parent chain as a3(IV). The findings include: 1) the sequence similarity between M2* and the a1 and a2 chains of collagen IV; 2) the integral nature of M2* as a component of the NC1 hexamer of collagen IV; and 3) the multiple similarities in the physical-chemical properties of M2* in comparison to those of the NC1 domains of the a1(IV) and a2(IV) chains, as summarized above. Furthermore, this identity of M2*, which contains the major reactivity of Goodpasture antibodies, localizes the Goodpasture epitope to the NC1 domain of the a3(IV) chain.

The overall findings of these studies lead to a new concept about the structure of collagen IV: namely, 1) collagen IV is comprised of a third chain (a3) together with the two classical ones (a1 and a2) and possibly a fourth chain (a4) from which M3 is derived (14). 2) The a3(IV) chain exists within the same triple-helical molecule together with the a1(IV) and a2(IV) chains and/or within a separate triple-helical molecule, exclusive of a1(IV) and a2(IV) chains, but connected through the NC1 domains to the classical triple-helical molecule comprised of a1(IV) and a2(IV) chains. Additionally, a portion of those triple-helical molecules exclusive of a1(IV) and a2(IV) chains may be connected to each other through their NC1 domains. 3) Finally, the epitope, to which the major reactivity of autoantibodies are targeted in GBM in patients with Goodpasture syndrome, is localized to the NC1 domain of the a3(IV) chain.

The existence of the a3(IV) chain as a subunit of the collagen IV molecule adds additional levels of structural heterogeneity to this molecule. Previous work has shown that collagen IV is heterogeneous with respect to disulfide and non-disulfide cross-linking, isoforms of the NC1 subunits, and proportion of a chains, depending on tissue origin (38). Such variations may confer important functional properties to the membranes.

Finally, the identification of the a3(IV) chain provides the conceptual framework for the interpretation of the results of a recent study on the molecular defect in GBM of patients with Alport Familial Nephritis (39). This study revealed that the human GP antigen, 28-kDa monomers generated by catabolism of GBM, is absent in these patients with this disease. Since the GP antigen is indicative of the a3(IV) chain, it follows that collagen IV molecules containing this chain are absent in these patients. It is particularly noteworthy that the ultrastructure of the defective GBM is characterized by diffuse splitting and multilaminations of the lamina densa (40–43). This leads to hematuria in childhood with progression to end-stage renal disease. Conceivably, molecules containing the a3(IV) chains play a key role in the fusion of epithelial and endothelial basement membranes during embryonic development of GBM (44, 45). In this regard, it is of interest that the amount of a3(IV) in basement membranes is tissue-specific, with GBM containing the highest quantity of a3(IV) of the basement membranes examined thus far (38).

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