Glomerular Basement Membrane Type IV Collagen Antigens in Goodpasture's and Alport's Syndromes

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Type IV collagen, the main constituent of the renal glomerular basement membrane, is involved in Goodpasture's syndrome, an autoimmune disease, and in Alport's syndrome, a genetic disease. There are 6 alpha chains, α1(IV) through α6(IV), in type IV collagen in mammals. Immunohistochemical studies, using α-chain-specific monoclonal antibodies on tissue specimens from healthy people and patients with Alport's syndrome, have shown that there are 3 forms of type IV collagen molecules in mammalian basement membranes, namely α1/α1/α2, α3/α4/α5, and α5/α5/α6. Antibody specificity analysis of sera from patients with Goodpasture's syndrome show that all sera have autoantibody, with the highest titer against α3(IV)NC1, although they also have titers against the other 5 α chains. This indicates that α3(IV)NC1 is the major target antigen of the disease, although the glomerular basement membrane contains the α1 through α5 chains. Experimental glomerulonephritis in rats, induced by the injection of 6 recombinant α(IV)NC1s with an adjuvant, has shown that α3(IV)NC1 and α4(IV)NC1 are nephritogenic. The lack of, or very poor, nephritogenicity of the other 4 α(IV)NC1s can be explained by the high immunologic tolerance against these chains, which are distributed widely in basement membranes of the whole body.


Key words: type IV collagen, Goodpasture's syndrome, Alport's syndrome, glomerular basement membrane, immunohistochemistry

The glomerular basement membrane is a 270- to 380-nm thick, flexible sheet between the endothelial and epithelial cells of the renal glomerulus. It works not only as an ultrafilter to produce a filtrate that is the origin of urine from the serum, but it also works as a supporting tissue against high blood pressure. The main components of the glomerular basement membrane are type IV collagen and other extracellular matrices, such as laminin, fibronectin, nidogen, heparan sulphate proteoglycan, and other glycoproteins. Recent progress in molecular biology has shown that there are 6 distinct isoforms (α1 to α6) of type IV collagen in basement membranes, and that the glomerular basement membrane contains both the 2 major ubiquitous chains, α1 and α2, and 3 newly recognized chains, α3 to α5 (Fig. 1).

The collagens are a family of highly characteristic fibrous proteins found in all multicellular organisms. A collagen molecule is composed of 3 α chains that contain the typical repeated structure of Gly-X-Y, and form a characteristic collagen triple helix. At present, 33 collagen α chains encoded by separate genes are known, and 19 collagen types have been identified. Type IV collagen was the fourth collagen type to be found. It is more flexible than fibrillar collagen. The triple helix of type IV collagen is interrupted more than 20 times, allowing multiple bends in an otherwise rigid molecule. This type of collagen is found only in basement membranes. The monomer of the type IV collagen network in basement membranes is a triple-helical molecule composed of 3 α chains, 2 α1 chains, and 1 α2 chain (α1/α1/α2). Like the fibril-associated collagens, they are not processed after secretion from cells, but retain the terminal regions that prevent them from forming fibrils by side-to-side lateralization. Instead, they interact through their unprocessed terminal ends to assemble into a sheet-like, multilayered meshwork (Fig. 1). Electron microscopic observations of type IV
collagen assemblies have suggested that these molecules associate, by means of their carboxyl termini, to form head-to-head dimers. These dimers then form an extended lattice of tetramers through amino-terminal interactions with 3 other type IV collagen molecules. The structure of the tetramers is further stabilized by numerous disulfide bridges and other covalent cross-links between the collagen molecules.

The mammalian type IV collagen genes have a unique arrangement, in that they are located pairwise in a head-to-head fashion on 3 different chromosomes, namely, 13, 2, and X (Fig. 1). This implies that the 6 genes evolved through duplication and inversion of an ancestral gene. The paired genes subsequently underwent 2 further rounds of duplication, resulting in the 3 closely opposed pairs.

Comparison of the genes and amino acid sequences of the 6 α chains of type IV collagen afford deduction of the evolutionary aspects of these molecules. The α1, α3, and α5 chains (odd-numbered chains) resemble one another. They are more alike than the α2, α4, and α6 chains (even-numbered chains), which also resemble one another. The α1 and α5 chains resemble one another more than they resemble the α3 chain, and the α2 and α6 chains resemble one another more than they resemble the α4 chain.

**PRODUCTION OF α-CHAIN–SPECIFIC MONOCLONAL ANTIBODIES**

To analyze type IV collagen molecules at the protein level, it was necessary to establish a group of monoclonal antibodies that recognize the α chains of type IV collagen. The α-chain–specific antibodies were obtained by the rat lymph node method, developed by us during a study of experimental anti-glomerular basement membrane nephritis. Immunization with emulsified antigen into the hind footpads of rats, a method usually used for the induction of active anti-glomerular basement membrane nephritis, causes enlargement of the medial iliac lymph nodes. These enlarged lymph nodes are used as the source of sensitized B lymphocytes for cell fusion. The efficiency of the method is about 10 times higher than that of the usual method, which uses mouse spleen. The immunogens used in nephritis induction were synthetic peptides, having a rather chain-specific sequence of human type IV collagen α chains. The clones thereby established were chain specific and epitope defined. They have been used for Western blotting, and for the immunohistochemistry of frozen and paraffin-embedded sections.