<原　著>

近畿福祉大学紀要
J. kinki welf
Vol. 1 (1) 20～25 (2000)

Basement Membrane beneath Serous Mesothelial Cells Contains α1(Ⅳ), α2(Ⅳ), α5(Ⅳ), and α6(Ⅳ) Chains of Type IV Collagen Demonstrated by Chain-specific Monoclonal Antibodies

Ichiro NAITO[1], Tatsuya MATSUBARA[3], Chieko TAKAHASHI[1], Hiroyuki OHMORI[3], Yoshikazu SADO[2], Yoshifumi NINOMIYA[4], and Tohru OKIGAKI[5]

Serous membrane (SM) covers inner surface of abdominal, thoracic and pericardial cavities, as well as outer surface of organs inside the cavities. It consists of surface mesothelial cells and loose connective tissue. Between them, a thin layer of basement membrane (BM) is located. Type IV collagen is a major constituent of BM, and consists of 6 different α(Ⅳ) chains, α1(Ⅳ) through α6(Ⅳ). Chain-specific functions are assumed by a chain-specific manner of localization. The α(Ⅳ) chain composition of skin, covering outer surface of the body, is demonstrated to have α1(Ⅳ), α2(Ⅳ), α5(Ⅳ), and α6(Ⅳ) chains, whereas that of SM, covering inner surface of the body, is yet to be analyzed.

Abdominal wall, small intestine, thoracic wall, lung, pericardium and epicardium of human materials were used in this study. Chain-specific monoclonal antibodies (mAbs) used were H11 (for α1), H22 (for α2), H31 (fo α3), H43 (for α4), H53 (for α5), and H63 (for α6). Fresh frozen sections were stained with indirect immunofluorescence staining using the mAbs.

Four out of six α(Ⅳ) chains, α1, α2, α5 and α6, were demonstrated in BM beneath the mesothelial cells of all types of SMs, whereas only capillary BM consisted of α1 and α2. Besides, epicardial SM expressed α3 and α4 moderately as extra components.

The α(Ⅳ) chain composition was same as those of epidermal skin BM. Therefore, these α(Ⅳ) chains are designated to be essential for BM covering inside and outside of the body.

Key words：Collagen type Ⅳ, basement membrane, serous membrane, peritoneum, CAPD
Ⅳ型コラーゲン，基底膜，漿膜，腹膜，腹膜透析

受付　平成13年１月９日，受理　平成13年１月17日

1　Division of Ultrastructural Biology, Shigei Medical Research Institute, Okayama, 701-0202 JAPAN.
2　Division of Immunology, Shigei Medical Research Institute, Okayama, 701-0202 JAPAN.
3　Division of Internal Medicine, Shigei Medical Research Hospital, Okayama, 701-0202 JAPAN.
4　Department of Molecular Biology and Biochemistry, Okayama University School of Medicine, 700-8558 JAPAN.
5　Department of Social Welfare, School of Social Welfare, Kinki Welfare University, Fukusaki, Hyogo, 679-2217 JAPAN.

Introduction

Serous membrane (SM), in peritoneum, pleura, and pericardium, is a thin layer of connective tissue delineating abdominal, thoracic and pericardial cavities, and some organs in these cavities (1). The cavities contain a small amount of liquid, serous exudate which moistens the surface of the SM. The SM permits the organs to slide freely within the cavities during peristalsis of digestive tracts, respiration of lung, and contraction of heart.

The SM consists of loose connective tissue covered by a single layer of mesothelial cells. Beneath the mesothelial cell layer, a thin basement membrane (BM) is localized (2, 3, 4). Generally, BM may act as a mechanical scaffold for supporting the surface epithelial cells (5, 6). It may also have functions in many biological processes, such as cell attachment, embryonic development, migration, tissue regeneration, and permeation through the BM (5, 6).

Continuous ambulatory peritoneal dialysis (CAPD) is known in nephrology to be a treatment for chronic renal dysfunction. Excess water and wastes can be excreted through peritoneum into dialysate, which is a hyper osmotic solution of glucose, inserted into the peritoneal cavity (7). The BM of the peritoneum is assumed to contribute to transportation during CAPD as well as supporting surface mesothelial cells.

Type Ⅳ collagen is known to be a major constituent of BM. It consists of 6 distinct α(Ⅳ) chains, α1 through α6 chains (6, 8, 9). Genes encoding these α(Ⅳ) chains are arranged in a head-to-head manner, and COL4A1 and COL4A2 (genes encoding α1(Ⅳ) and α2(Ⅳ), respectively) localize in chromosome 13, COL4A3 and COL4A4 (encoding α3(Ⅳ) and α4(Ⅳ), respectively) in chromosome 2, and COL4A5 and COL4A6 (encoding α5(Ⅳ) and α6(Ⅳ), respectively) in chromosome X (9).

Chain-specific monoclonal antibodies (mAbs) raised by us enabled to demonstrate localization of the α(Ⅳ) chains (10, 12, 13, 15). α1(Ⅳ) and α2(Ⅳ) are widely distributed in BM, while the others are restricted some parts of BM. Co-distribution of α(Ⅳ) chains and accompanied loss of α(Ⅳ) chains in Alport syndrome, that is genetic disorder of type Ⅳ collagen genes, indicate 3 different types of α(Ⅳ) chain assembly;α1/α1/α2, α3/α4/α5, and α5/α5/α6(15). For example, α3/α4/α5 form of assembly is found in glomerular BM (GBM) and lung alveolar BM, while α5/α5/α6 form is in skin epidermal BM, Bowman's capsular BM, and submucosal BM of digestive tracts (10, 12, 13, 15).

Functional differences among these assembly forms are not fully analyzed yet. α3/α4/α5 form in the GBM has important roles for preventing leakage of red blood cells and proteins through the BM which is demonstrated in patients with Alport syndrome (8). However, α5/α5/α6 form in the Bowman's capsular BM, epidermal BM and submucosal BM is essential for these BMs but its function is still unclear.

As previously described, the skin epidermal BM, covering outer surface of the body, and the submucosal BM, covering inner surface of the digestive tracts contain α5/α5/α6 form in addition to α1/α1/α2 form. On the other hand, subendothelial BM beneath capillary endothelial cells consists of only α1/α1/α2 form of assembly. Analysis of α(Ⅳ) chain composition of the SM, covering inner surface of the body cavities, is yet to be done. In this study, we have studied α(Ⅳ) chain composition of human SM using chain-specific mAbs raised by us, and discussed a possible role of type Ⅳ collagen in CAPD treatment.

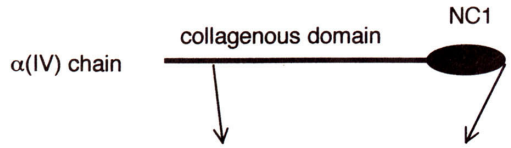
Materials and Methods

Tissue specimens

Human tissues used for analysis of type Ⅳ collagen chains were abdominal wall, small intestine, thoracic wall, lung, heart and pericardium. The specimens were obtained from 3 cases of autopsy : all males, 59, 69 and 78 years of age. The tissues were embedded in Tissue-Tek O.C.T. -compound (Sakura Finetechnical Co., Japan), and frozen with liquid nitrogen. (This experiment was performed in An Agreement of Informed Consent for Experimental Utilization of Human Materials, Shigei Medical Research Institute, 1995)

Monoclonal antibodies (mAbs)

Type Ⅳ collagen α chain-specific mAbs (10, 11) used in this study are listed in Figure 1. They were raised against synthesized peptides of human α(Ⅳ) chains.



α(IV) chains	mAbs
α1(IV)	H11
α2(IV)	H22
α3(IV)	H31
α4(IV)	H43
α5(IV)	H53
α6(IV)	H63

Figure 1. Positions of peptides used for antigens and a list of mAbs used for immunohistochemistry.

H11, H22, H31, H43, and H63 are raised against the peptides in C-terminal ends of human α1(IV), α2(IV), α3(IV), α4(IV), and α6(IV), respectively (10). H53 is against a peptide in imperfection of collagenous domain of α5(IV) (11). Enzyme-linked immuno-sorbent assay, immunostaining, and Western blotting confirm their specificity.

Immunohistochemistry

Cryostat sections of 4 μm were fixed with acetone for 10 min. They were incubated with 6 M urea in 0.1 M glycine-HCl buffer, pH 3.5, for 20 min., then washed 3 times with phosphate buffered saline (PBS) for 5 min. After10 min blocking with 5 % skimmed milk in PBS, the mAbs were applied to the sections for 60 min. Following to washing, the sections were incubated with FITC-labeled goat anti-rat IgG (CAPPEL, Organon Teknika Corp, PA), diluted to 1/60 with PBS. The sections were then studied under fluorescence microscopy (Zeiss Axiophot, Germany).

Results

Basement membrane in the serous membrane

A thin layer of BM was found beneath the surface mesothelial cells (Figure 2).

Type IV Collagen α Chains in Peritoneum

Immunostaining of type IV collagen α(IV) chains in parietal peritoneum of the abdominal wall was shown in Figure 2. H11 (anti- α1), H22 (anti- α2), H53 (anti- α5), and H63 (anti- α6) were moderately positive, but H31 (anti- α3), and H43 (anti- α4) were absolutely negative at all, whereas subendothelial BM of capillaries

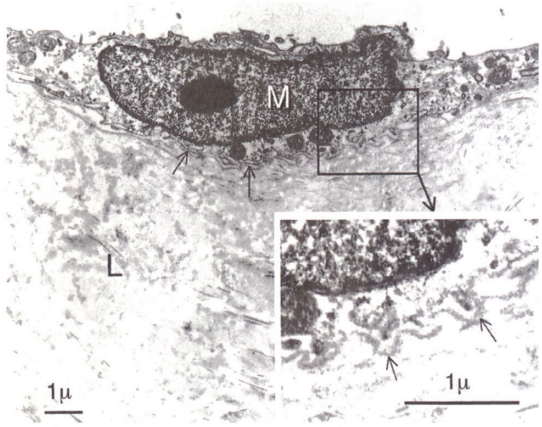


Figure 2. Electron Micrograph of the parietal peritoneal serous membrane.

Thin BM (arrows) is demonstrated beneath the surface mesothelial cell (M). Loose connective tissue (L) is rich in fibrous components. An inset shows enlargement of the area enclosed with a square.

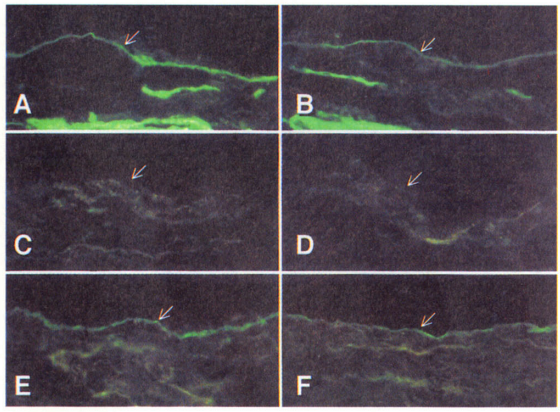


Figure 3. Collagen type IVαchains in parietal peritoneal membranes.

A, stained with mAb H11 specific for α1(IV) ; B, H22 specific for α2(IV) ; C, H31 specific for α3(IV) ; D, H43 specific for α4(IV) ; E, H53 specific for α5(IV) ; F, H63 specific for α6(IV). Serous BM (arrows) is stained continuously with mAbs specific for α1(IV), α2(IV), α5(IV), and α6(IV) chains.

consisted of only α1(Ⅳ) and α2(Ⅳ). The staining pattern was continuously linear, and detected along the possible position of the BM.

The visceral peritoneum in the intestine revealed similar staining pattern as those of the abdominal wall (Figure 4A-D). These findings indicated the peritoneum, both parietal and visceral, contained α(Ⅳ) chains same as those of epidermal BM of the skin.

Type Ⅳ Collagen α Chains in Pleura

Type Ⅳ collagen α(Ⅳ) chains of pleura were studied in parietal SM of thoracic wall and visceral SM of lung surface. The results were shown in Figure 4E-L. The α(Ⅳ) chain composition of pleura was the same as that of the peritoneum.

Type Ⅳ Collagen α Chains in Pericardium

Pericardium, outer SM of the heart, contained small amount of α3(Ⅳ) and α4(Ⅳ) chain in addition to α1(Ⅳ), α2(Ⅳ), α5(Ⅳ), and α6(Ⅳ) chains (Figure 4Q-T). However, epicardium, corresponding to the parietal pericardium consisted of α1(Ⅳ), α2(Ⅳ), α5(Ⅳ), and α6(Ⅳ) chain (Figure 4M-P).

Discussion

The present study demonstrated presence of type Ⅳ collagen beneath the surface mesothelial cells by electron microscopy as well as immunohistochemistry using α(Ⅳ) chain-specific mAbs. SM consists of 3 different layers of mesothelial cells, BM, and loose connective tissue (2, 3, 4). The existence of collagen type

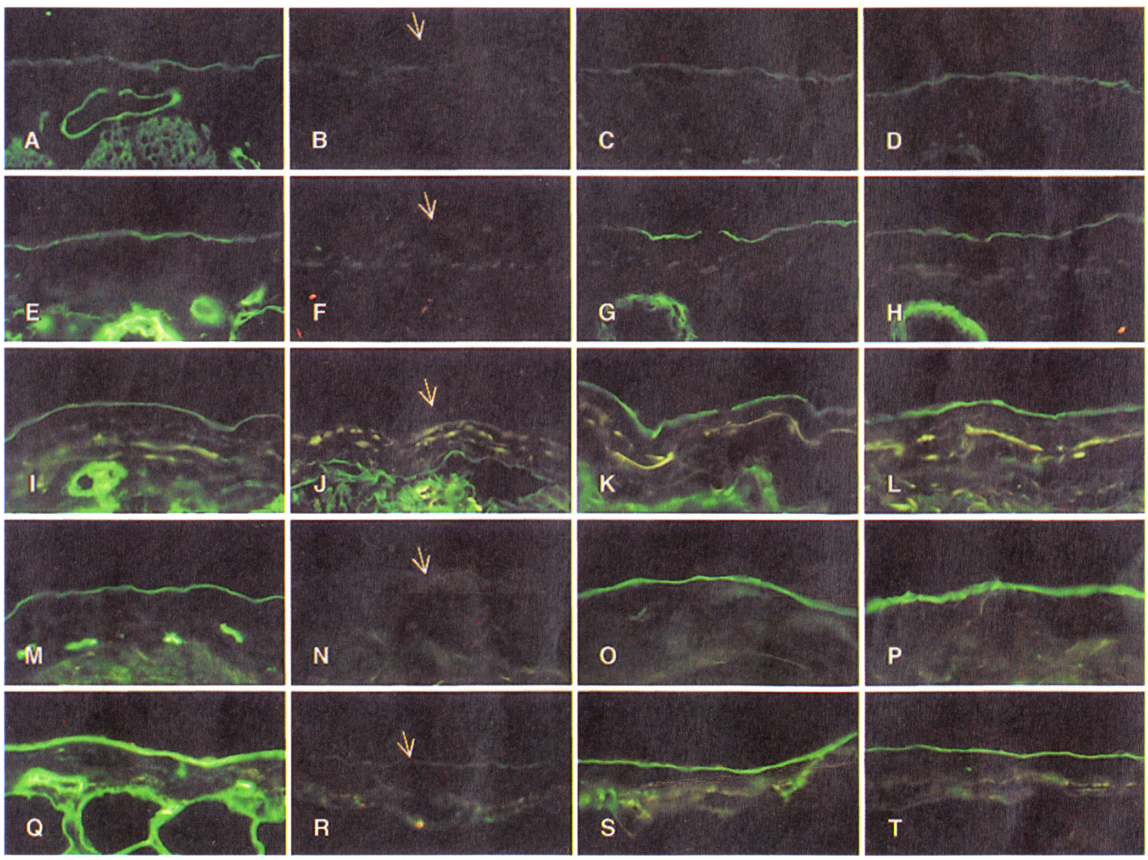


Figure 4. Collagen type Ⅳα chains in human SMs.

A-D, SM of small intestine ; E-H, pleura ; I-L, lung ; M-P, pericardium ; Q-T, epicardium. A, E, I, M and Q, α1(Ⅳ) or α2(Ⅳ) ; B, F, J, N, and R, α3(Ⅳ) or α4(Ⅳ) ; C, G, K, O and S, α5(Ⅳ) ; D, H, L, P and T, α6(Ⅳ). α1(Ⅳ) and α2(Ⅳ) show identical staining patterns. α3(Ⅳ) and α4(Ⅳ) also show identical patterns. Note that the α3(Ⅳ) and α4(Ⅳ) chains do not appear in the SM except of epicardium.

Ⅳ in the SM has been shown by immunohistochemistry (16), although in vitro experiment of cultured mesothelial cells have not demonstrate the production of the collagen type Ⅳ (17, 18).

The BM acts as a basal scaffold for supporting the surface epithelial cells (6, 8, 9), therefore, in the same way the surface mesothelial cells may be supported by the BM beneath the cells. It is well known that BM has some biological functions in the body other than the supporting epithelial cells (6, 8, 9). α(Ⅳ)-chain composition may correlate with function of the BM, such as selective permeability of α3(Ⅳ)/α4(Ⅳ)/α5(Ⅳ) chains in the GBM (5, 6). In the present study the BM beneath the mesothelium contained α5(Ⅳ) and α6(Ⅳ) chains in addition to ubiquitous α1(Ⅳ) and α2(Ⅳ) chains. This composition of α(Ⅳ) chains are the same as those of skin epidermal BM, renal Bowman's capsule, submucosal membrane of digestive tract, and some other subepithelial BMs (14). On the other hand, alveolar BM, GBM, BM of testis and choroid plexus consist with α3(Ⅳ)/α4(Ⅳ)/α5(Ⅳ) (12, 14). These tissue-specific manners of α(Ⅳ) chain localization indicate characteristic functions of the BM determined by the α(Ⅳ) chain composition. However, the function of the BM containing α5(Ⅳ) and α6(Ⅳ) is not fully understood, but they are characteristic to the BM covering outer surface, and inside tube structure in the body. Thus, the α5(Ⅳ)/α5(Ⅳ)/α6(Ⅳ)-assembly form of collagen type Ⅳ may be important for the BM delineating outside the body and inside of the tube and the body cavities.

The SM is delineating 3 different body cavities; peritoneal, pleural and pericardial cavities. Furthermore, they are divided into two parts; parietal and visceral SMs. The α(Ⅳ) chain-composition in the SMs was the same except the pericardium of small amount of α3(Ⅳ) and α4(Ⅳ) chains in addition to α1(Ⅳ), α2(Ⅳ), α5(Ⅳ), and α6(Ⅳ) chains.

CAPD is a treatment for chronic renal dysfunction performed in the peritoneal cavity, and excess water and wastes can be excreted through the peritoneum, both parietal and visceral. Therefore intrinsic structure of the peritoneum must be important for maintaining CAPD treatment. Detachment of surface mesothelial cells from the mesothelium is a major irregularity found in CAPD patients, and defined as a proceeding abnormality in CAPD treatment (7). It may influence the CAPD efficiency, and initiate the thickening of the peritoneum. BM beneath the mesothelium may act as a scaffold for the mesothelium, so that the type Ⅳ collagen, one of the major components of BM, should be focused in order to clarify possible mechanisms of the detachment of the mesothelial cells.

An exceptional expression of α3(Ⅳ) and α4(Ⅳ) chains were found in the epicardium of heart SM. As previously described, they are expressed in some specific BM such as GBM and lung alveolar BM, certainly correlating with selective permeability in urinary excretion and gas exchange (7). Although, possible functions of the α3(Ⅳ) and α4(Ⅳ) are not clarified in the epicardium.

In this study, we described specific localization of α1(Ⅳ), α2(Ⅳ), α5(Ⅳ), and α6(Ⅳ) chains in the SM, and the α(Ⅳ) chain-composition was identical to those of skin epidermal BM and submucosal BM of digestive tract. Those findings indicate that these chains are physiologically necessary for maintaining active functions of the BMs covering the outer body surface as well as inside the body.

Acknowledgement

This study has generously been supported financially by Baxter Research Funds.

References

1. Bloom, W., Fawcett, D. W. : Connective tissue proper. in "A Textbook of Histology" 10th. ed, W. B. Saunders Co., Philaderphia, pp186-188, 1976
2. Wang, N.-S.: The regional difference of pleural mesothelial cells in rabbits. American Review of Respiratory Disease. 110 (5), 623-633, 1974
3. Michailova, K. N. : The serous membranes in the cat. Electron microscopic observations. Anatomischer Anzeiger. 178 (5), 413-424, 1996
4. Allen, D. J., DiDio, L. J., Zacharias, A., Fentie, I., McGrath, A. J., Puig, L. B., Pomerantzeff, P. N., Zerbini, E. J. : Microscopic study of normal parietal pericardium and unimplanted Puig-Zerbini pericardial valvular heterografts. Journal of Thoracic Cardiovascular Surgery. 87 (6), 845-855, 1984
5. Ninomiya, Y. : Type Ⅳ collagen:gene expression, supramolecular aggregates, and role in human diseases. Seikagaku. 67 (11), 1301-1307, 1995 (Japanese)
6. Miner, J.H.:Renal basement membrane components.

Kidney International. 56 (6), 2016-2024, 1999
7. Burkart, J. M., Nolph, K. D.: Peritoneal Dialysis. in "The Kidney" 5th ed., ed. by Brenner, B. M., PP. 2507-2575, W. B. Saunders, Philadelphia, 1996
8. Tryggvason, K.: Mutations in type Ⅳ collagen genes and Alport phenotypes. Contributions to Nephrology. 117, 154-171, 1996
9. Sado, Y., Naito, I., Ninomiya, Y.: Expression of collagen Ⅳ genes and Goodpasture antigens. in "Extracellular Matrix-Cell Interaction ; Molecules to Diseases" ed. by Ninomiya, Y., Olsen, B. R., and Ooyama, T., Japan Scientific Societies Press, Tokyo/S. Karger AG, Basel, pp 235-260, 1998
10. Sado, Y., Kagawa, M., Kishiro, Y., Sugihara, K., Naito, I., Seyer, J. M., Sugimoto, M., Oohashi, T., Ninomiya, Y.: Establishment by the rat lymph node method of epitope-defined monoclonal antibodies recognizing the six different α chains of human type Ⅳ collagen. Histochemistry and Cell Biology. 104 (4), 267-275, 1995
11. Kagawa, M., Kishiro, Y., Naito, I., Nemoto, T., Nakanishi, H., Ninomiya, Y., Sado, Y.: Epitope-defined monoclonal antibodies against type-Ⅳ collagen for diagnosis of Alport's syndrome. Nephrology Dialysis and Transplantation. 12 (6), 1238-1241, 1997
12. Ninomiya, Y., Kagawa, M., Iyama, K., Naito, I., Kishiro, Y., Seyer, J. M., Sugimoto, M., Oohashi, T., Sado, Y.: Differential expression of two basement membrane collagen genes, COL4A6 and COL4A5, demonstrated by immunofluorescence staining using peptide-specific monoclonal antibodies. Journal of Cell Biology. 130 (5), 1219-1229, 1995
13. Seki, T., Naito, I., Oohashi, T., Sado, Y., Ninomiya, Y.: Differential expression of type Ⅳ collagen isoforms, α5(Ⅳ) and α6(Ⅳ) chains, in basement membrane surrounding smooth muscle cells. Histochemistry and Cell Biology. 110 (4), 359-366, 1998
14. Saito, K., Naito, I., Seki, T., Oohashi, T., Kimura, E., Momota, R., Kishiro, Y., Sado, Y., Yoshioka, H., Ninomiya, Y.: Differential expression of mouse α5(Ⅳ) and α6(Ⅳ) collagen genes in epithelial basement membrane. Journal of Biochemistry. 128 (3), 427-434, 2000.
15. Naito, I., Kawai, S., Nomura, S., Sado, Y., Osawa, G.: Relationsip between COL4A5 gene mutation and distribution of type Ⅳ collagen in male X-linked Alport syndrome. Kidney International. 50 (1), 304-311, 1996
16. Davila, R. M., Crouch, E. C.: Role of mesothelial stromal cells in matrix remodeling following pleural injury. American Journal of Pathology. 142 (2), 547-555, 1993
17. Stylianou, E., Jenner, LA., Davies, M., Coles, G. A., Williams, J. D.: Isolation, characterization of human peritoneal mesothelial cells. Kidney International. 37 (6), 1563-1570,1990
18. Harvey, W., Amlot, P. L.: Collagen production by human mesothelial cells in vitro. Journal of Pathology. 139 (3), 337-347, 1983

漿膜中皮細胞下の基底膜Ⅳ型コラーゲンは α 1鎖，α 2鎖，α 5鎖，α 6鎖で構成される

内藤一郎[1]・松原龍也[3]・高橋千恵子[1]
大森浩之[3]・佐渡義一[2]・二宮善文[4]
沖垣達[5]

漿膜は腹腔や胸腔，心嚢腔の内側と内部臓器の表面を被う疎性結合組織の膜で，その表面は薄い一層の内皮細胞に被われ，内皮細胞下には一層の基底膜が存在する。Ⅳ型コラーゲンは基底膜の主要成分で，6種のα鎖により構成される。各α鎖の局在は特有であることから基底膜の機能と構成α鎖に関連性があるものと考えられている。腹膜は腹膜透析療法を行う場であり，それを長期間維持する上でも構成成分の解析が重要である。今回，特異的モノクローナル抗体を用い，腹壁を含め複数の漿膜基底膜の構成α(Ⅳ) 鎖を解析した。その結果，漿膜基底膜にはα 1，α 2，α 5，α 6の4種のα(Ⅳ) 鎖が存在し，例外的に心外膜には少量のα 3とα 4を認めた。またこれらのα(Ⅳ) 鎖は体表を被う皮膚基底膜と共通する成分であった。