Arch. Histol. Cytol., Vol. 65, No. 2 (2002)
p. 133-143

Basement Membrane Type IV Collagen Molecules in the Choroid Plexus, Pia Mater and Capillaries in the Mouse Brain*

Norio URABE[1,2], Ichiro NAITO[1,3], Kenji SAITO[1,4], Tomoko YONEZAWA[1], Yoshikazu SADO[5], Hidekatsu YOSHIOKA[1], Shozo KUSACHI[2,6], Takao TSUJI[2], Aiji OHTSUKA[7], Takehito TAGUCHI[8], Takuro MURAKAMI[7], and Yoshifumi NINOMIYA[1]**

Departments of Molecular Biology and Biochemistry[1], Medicine and Medical Science[2], and Human Morphology[7], Okayama University Graduate School of Medicine and Dentistry; Department of Nursing[4], Niimi College, Niimi; Departments of Medical Technology[6], and Radiological Technology[8], Faculty of Health Sciences, Okayama University Medical School, Okayama; and Divisions of Ultrastructural Biology[3], and Immunology[5], Shigei Medical Research Institute, Okayama, Japan

Received January 9, 2002

Summary. **We investigated the differential distribution of basement membrane type IV collagen α chains in the mouse brain by immunohistochemistry using α chain-specific monoclonal antibodies. Subendothelial basement membranes were found to contain α1 and α2 chains. Basement membranes surrounding smooth muscle cells on blood vascular walls were immunoreactive for α1 and α2 chains but not for α5 and α6 chains. Interestingly, the pia mater contained a thin basement membrane which was positive for α1, α2, α5, and α6 chains, suggesting that glia limitans superficialis coheres basement membranes containing $[\alpha1(IV)]_2\alpha2(IV)$ and $[\alpha5(IV)]_2\alpha6(IV)$ molecules. In contrast, capillaries always possessed thin basement membranes of $[\alpha1(IV)]_2\alpha2(IV)$ molecules.**

Cerebrospinal fluid is produced through filtration of blood at the choroid plexus, where two distinct basement membranes were detected by anti-α1 and anti-α2 antibodies. The subendothelial basement membrane appeared to consist of $[\alpha1(IV)]_2\alpha2(IV)$ molecules, whereas the subependymal basement membrane in the choroid plexus was strongly positive for α3, α4, and α5 chains, indicating that the filtering unit was composed of $\alpha3(IV)\alpha4(IV)\alpha5(IV)$ molecules. That the specific localizations of these molecules are shared by renal glomeruli and the choroid plexus leads us to hypothesize that the supramolecular network containing $\alpha3(IV)\alpha4(IV)\alpha5(IV)$ molecules may function as a permeability selective barrier.

Two circulatory systems, the blood vessels and cerebrospinal fluid routes, function to maintain and/or protect the central nervous system. Distribution of the vessels to introduce these systems into the brain is not the same as that in other tissues. Blood vessels penetrate the central nervous system through tunnels covered by pia mater, forming the so called perivascular space (WILLIAMS et al., 1995; GARTNER and HIATT, 2001). The pia mater disappears before the blood vessels are transformed into capillaries. There is a functional barrier, the blood-brain barrier, that prevents the passage of certain substances from the blood to the brain parenchyma. The blood-brain barrier results in a restricted reduced permeability that is a property of blood capillaries of the central nervous system (RISAU, 1991; RUBIN and STADDON, 1999). Tight junctions providing a continuous structure between the capillary endothelial cells represent the major component of the barrier. The other feature of the endothelial cells is that their cytoplasm does not have the fenestrations and pinocytotic vesicles that are often found in several other tissues. The most striking structural difference is that the capillaries are completely enveloped by the expansions of the neuroglial processes, glia limitans perivascularis, which have been suggested to be responsible for their selective permeability in the brain (JUNQUEIRA et al., 1992). In addition to these cellular components, an

*This study was supported in part by Grants-in-Aid (#11877280 to K. S. and #11694280 to Y. N.) from the Japan Society for the Promotion of Science.

**Present address: Department of Biochemistry, Oita Medical University, Oita 879-5593, Japan

extracellular structure, the basement membrane, is intercalated between the capillary endothelium and glial processes.

Cerebrospinal fluid, which is produced by the choroid plexus, fills the subarachnoid space between the subarachnoid and the pia mater. The pia mater and underlying basement membrane envelope the outermost layer of the brain, where the glial foot processes palisade to form the glia limitans superficialis. The choroid plexus consists of three structural components: blood capillaries, connective tissue derived from the pia mater, and a specialized ependymal epithelium, each of which is separated by a basement membrane (TAGUCHI et al., 1998).

One of the major functional components of the basement membrane is the type IV collagen subfamily, which influences the motility, proliferation, and differentiation of many cell types (ROHRBACH and TIMPL, 1993; TIMPL and BROWN, 1996). Six distinct genes have been identified as members of the type IV collagen gene family (ZHOU et al., 1993; OOHASHI et al., 1994). They are organized into three sets, COL4A1/COL4A2, COL4A3/COL4A4, and COL4A5/COL4A6, and are localized on three different chromosomes in humans, 13, 2, and X, respectively (HUDSON et al., 1993; SUGIMOTO et al., 1994). The genes are aligned head-to-head within each set and their expression is regulated by bidirectional promoters between the genes (PÖSCHL et al., 1988; SOININEN et al., 1988; BURBELO et al., 1988, KAYTES et al., 1988; SUGIMOTO et al., 1994; MOMOTA et al., 1998). While molecules composed of α1 and α2 chains are broadly distributed, the recently identified molecules comprising combinations of the other four chains, α3–α6, are localized at the specialized basement membranes (NINOMIYA et al., 1995; SADO et al., 1995, 1998). For instance, the major molecular component of the renal glomerulus is identified as the α3(IV)α4(IV)α5(IV) molecule, which can cause supramolecular structure associated with other molecules to filter blood fluid (SEKI et al., 1996; SAITO et al., 2000).

In this study, we define the extracellular barriers located at the perivascular areas, pia mater and choroid plexus and verify our hypothesis as to whether the specialized filtering unit of the choroid plexus contains the same molecular network as that in the renal glomerulus. Our data demonstrate that the basement membrane underneath ependymal cells at the choroid plexus contains α3(IV), α4(IV), and α5(IV) chains, indicating that they form a supramolecular network of α3(IV)α4(IV)α5(IV) molecules that are utilized for glomerular filtration.

MATERIALS AND METHODS

Animals and tissue preparation

Adult male 129Sv mice were obtained from the Animal Center for Medical Research, Okayama University Medical School. Under ether anesthesia, their brains were excised and embedded in OCT compound (Sakura, Tokyo, Japan) after snap-freezing in liquid nitrogen. The brain containing the olfactory bulb, cerebrum, cerebellum, and the upper cervical segments of spinal cord was frontally cut with a cryostat into 4 μm-thick serial sections, which were processed for immunohistochemistry. This frontal sectioning was done at intervals of 2 mm throughout the whole brain from rostral 6 mm to caudal 8 mm distant from the bregma, according to PAXINOS and FRANKLIN (2001).

In addition, paraffin sections of the mouse brains which had been perfusion-fixed with 2% paraformaldehyde and 0.5% glutaraldehyde in a 0.1 M phosphate buffer (pH 7.4), were prepared, and stained with hematoxylin and eosin.

Immunohistochemistry

Immunohistochemistry was performed as described previously (SADO et al., 1995; NINOMIYA et al., 1995) with slight modifications. In brief, the cryo-sections were denatured with 6 M urea in a glycine-HCl buffer (pH 3.5) for 20 min (in the case of B66, denaturation was performed for 10 min) at room temperature. This denaturation step, which presumably exposes the epitopes for several antibodies, was necessary to obtain better staining signals. An indirect immunofluorescence method using FITC-conjugated goat anti-rat IgG (Organon Teknika-Cappel, Durham, NC) was applied to detect the localization of primary antibodies (see below). After immunostaining, some of the sections were stained with 0.0005 % propidium iodide (Wako Pure Chemical Industries, Osaka) for 1 – 2 min. Some serial sections were directly immunostained with an anti-smooth muscle cell actin antibody (clone: 1A4, conjugated with Cy3, Sigma, St. Louis, MO) as described previously (SEKI et al., 1998). The samples were examined with a Axiophot microscope (Carl Zeiss) equipped with an DP-50 digital camera system (Olympus) in Shigei Medical Research Institute.

Antibodies

The monoclonal antibodies used for α chain analysis are listed in Table 1. We used H11, H22, H31, H43,

Table 1. α(IV) chain-specific monoclonal antibodies used for the study.

MAbs	specificity	Immunogen	Urea treatment	Reactivity
H11	α1(IV) chain	Peptide of human α1(IV)NC1	+	+++
H12	α1(IV) chain	Peptide of human α1(IV)NC1	+	+++
H22	α2(IV) chain	Peptide of human α2(IV)NC1	+	+++
M26	α2(IV) chain	Peptide of mouse α2(IV)helix	−	++
H31	α3(IV) chain	Peptide of human α3(IV)NC1	+	++
H43	α4(IV) chain	Peptide of human α4(IV)NC1	+	+++
RH42	α4(IV) chain	Recombinant α4(IV)NC1	−	+++
H53	α5(IV) chain	Peptide of human α5(IV)helix	+	+++
M54	α5(IV) chain	Peptide of mouse α5(IV)NC1	+	+
B66	α6(IV) chain	Bovine native α(IV)NC1s	− or +	++
M69	α6(IV) chain	Peptide of mouse α6(IV)NC1	+	+

Table 2. Distribution of α chains of type IV collagen in the mouse brain.

α(IV) chains	α1(IV)/α2(IV)	α3(IV)/α4(IV)	α5(IV)	α6(IV)
mAbs	H12, H22, M26	H31, H43	H53, M54	B66, M69
Blood vessels				
Capillary				
Subendothelial BM	++	−	−	−
BM surrounding pericyte	+	−	−	−
Artery and Vein				
Subendothelial BM	++	−	−	−
Smooth muscle cell BM	++	−	−	−
Pia matter surrounding vessels	+	−	+[*1)]	+[*1)]
Choroid plexus				
Subependymal BM	++	++[*2)]	++[*2)]	−
Subendothelial BM	+	−	−	−
Meninges				
Pia mater	+	−	+[*1)]	+[*1)]
Arachnoid	+	−	−	−
Artery in subarachnoid space				
Subendothelial BM	++	−	−	−
Smooth muscle cell BM	++	-	−,+[*3)]	−,+[*3)]
Venous sinus				
Subendothelial BM	++	−	−	−
Smooth muscle cell BM	+++	−	−	−

[*1)] Positive reactions for both α5 and α6 chains indicate the presence of $[\alpha5(IV)]_2\alpha6(IV)$ molecules.
[*2)] Positive reactions for both α3/α4 and α5 chains indicate the presence of $\alpha3(IV)\alpha4(IV)\alpha5(IV)$ molecules.
[*3)] There were two different arteries. Arteries positive for the α5 chain were positive for α6 chains.
BM: basement membrane.
Immunofluorescence intensity: − negative, + moderately positive, ++ strongly positive, +++ very strongly positive.

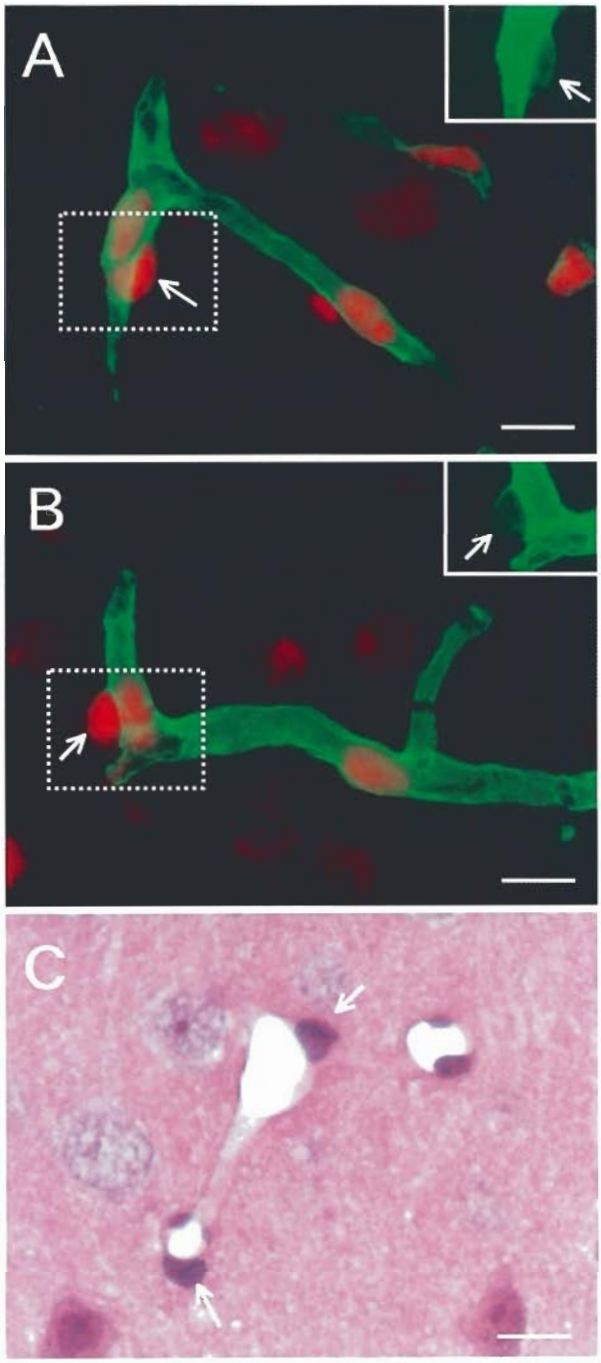

Fig. 1. Fluorescent immunohistochemical localization of α1 and α2 chains of type IV collagen in mouse brain capillary basement membranes. Cryosections of the cerebral cortex were stained with α1(IV)(**A**) and α2(IV)(**B**)-specific antibodies, H12 and H22, respectively. Cell nuclei are stained with propidium iodide emitting red fluorescence (**main figures** of **A** and **B**). The frames indicated by broken lined squares are taken without red fluorescence (**Insets** of **A** and **B**). Note that both α1 and α2 antibodies stain subendothelial basement membranes as well as basement membranes surrounding pericytes *(arrows)*. **C.** Hematoxylin-eosin stained sample. Scale bars = 10 μm

H53, and B66 antibodies for most of the experiments, the results of which were confirmed with the other antibodies H12, M26, RH42b, M54, and M69. We raised all these antibodies by the rat lymph node method (KISHIRO et al., 1995; SADO et al., 1995). Their specificity was established by immunofluorescence studies using human and mouse kidney sections, Western-blot analyses for human α(IV) NC1 fragments, and ELISA assays using recombinant α(IV) NC1s (NINOMIYA et al., 1995; KAGAWA et al., 1997). Most of the antigens were against the peptides within the NC1 domains. In this study we raised another antibody against the mouse sequence, NH2-(C)DTG VKRPIGGGQQVVVQPG-COOH, within the NC13 domain. This newly raised antibody, named M26, could demonstrate specific staining for α2(IV)-containing basement membranes such as capillary basement membranes in mouse kidney without urea treatment. The antibody H25, which had been raised against the peptide sequence, NH2-BCDTDVKRAV GGDRQEAIQPGC-COOH, in the NC13 domain of human α2 (IV), nicely reacted without urea treatment (KAGAWA et al., 1997). However, it did not cross-react with mouse tissues since the local peptide sequence used for antigen was quite different from the human sequence.

RESULTS

Generally, type IV collagen was detected only in three locations in the brain: 1) the blood vessels: subendothelial basement membranes of various sized arteries, veins and capillaries, and basement membranes surrounding smooth muscle cells in the tunica media; 2) the choroid plexus: subependymal basement membranes and subendothelial basement membranes; and 3) the pia mater: basement membranes beneath the pia mater. Wherever the basement membrane was present in the brain, type IV collagen was detected. The results of immunohistochemical staining are summarized in Table 2.

Basement membranes associated with brain blood vessels

There were many capillaries in brain parenchyma, where type IV collagen was detected along their basement membranes. As shown in Figure 1, α1(IV) and α2(IV) chains were clearly detected at subendothelial basement membranes, though α3-α6(IV) chains were hardly detectable there (Table 2). In the capillary walls, pericytes were often found outside of endothelial cells. Pericytes were encompassed by thin

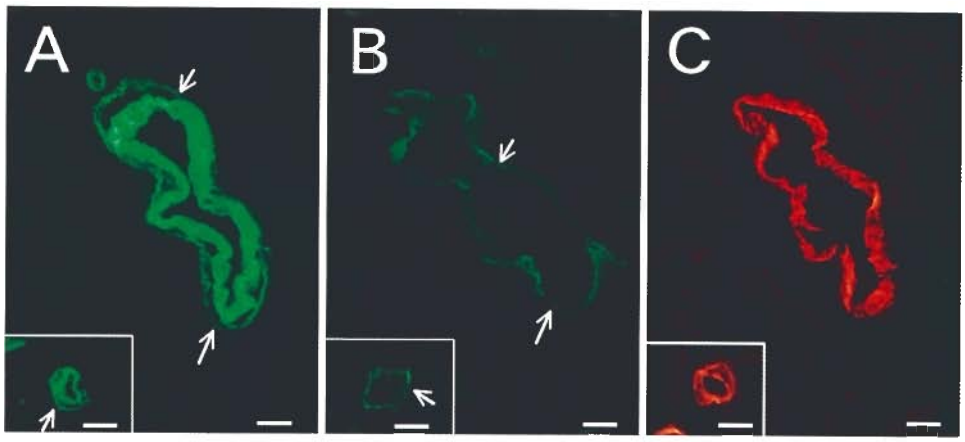

Fig. 2. Immunofluorescent staining of α2(IV) and α5(IV) chains in basement membranes of a medium size artery (**main figures**) and smaller arterioles (**insets**) containing smooth muscle cells. Sections were stained with antibodies against the α2 chain (H22, **A**), and α5 chain (H53, **B**), and anti-smooth muscle actin (1A4, **C**). Note that two basement membranes are recognized; the inner and thicker one surrounding smooth muscle cells is stained for the α2 chain, whereas the outer and thinner one *(arrows)* is positive for both α2 and α5 chains. **Insets** also show similar staining patterns in smaller arterioles. Scale bars = 10 μm

basement membranes, in which α1(IV) and α2(IV) chains were clearly detected (Fig. 1A, B). The nuclei of pericytes were indentified by the red fluorescence of propidium iodide (Fig. 1A, B) and the outside of the pericytes appeared to be covered by both α1(IV) and α2(IV) chains (insets of Fig. 1A, B).

In the fissures and sulci or rugae, arteries were surrounded by pia mater to reach inside the brain. Subendothelial basement membranes in these relatively large arteries contained always α1 and α2 chains as did those in arteries of other organs (SEKI et al., 1998). The relatively larger arteries that contained smooth muscle cells in their tunica media showed two inner and outer basement membranes when stained with the α2 antibody (Fig. 2A). The inner, thicker basement membrane appeared to be a smooth muscle cell basement membrane because it was associated with a structure clearly labeled with the antibody against smooth muscle actin (Fig. 2C). The outer basement membrane was a thin and continuous structure immunolabeled for the α2 chain (Fig. 2A); its staining pattern for the α1 chain was the same as that for α2 (Table 2) (micrograph not shown), suggesting the presence of $[\alpha1(IV)]_2\alpha2(IV)$ molecules. This basement membrane was located between the pia mater and glia limitans, under which astrocytes sent foot processes aligned continuously from the outer surface of the brain. Intriguingly, only one of the two basement membranes was weakly positive for the α5(IV) antibody (Fig. 2B) but negative for α3(IV) and α4(IV) antibodies (Table 2) (micrograph not shown). These were positive for α6(IV)-specific antibodies as well (micrograph not shown), suggesting that the basement membrane of the glia limitans was composed of $[\alpha5(IV)]_2\alpha6(IV)$ molecules.

Larger arteries that were located close to the surface of the brain were analyzed with the same antibodies. Antibodies against α2(IV) and α5(IV) (Fig. 3) thorughly stained the outer thin basement membranes just as α1(IV) and α6(IV) antibodies (Table 2) (micrograph not shown). The inner basement membranes surrounding the smooth muscle cells were positive for α1 (micrograph not shown) and α2 (Fig. 3A).

Basement membranes at the pia mater

Basement membranes located between the pia mater and the brain parenchyma were immunopositive for α1, α2 (Fig. 2B), α5 (Fig. 3B) and α6 chains, indicating that they contained both $[\alpha1(IV)]_2\alpha2(IV)$ and $[\alpha5(IV)]_2\alpha6(IV)$ molecules. However, we could not detect any type IV collagen within the arachnoid or in the subarachnoid space except for arteries and veins, indicating a lack of any obvious basement membranes there, except in the vascular walls.

Basement membranes at the choroid plexus

In the choroid plexus, type IV collagen was detected

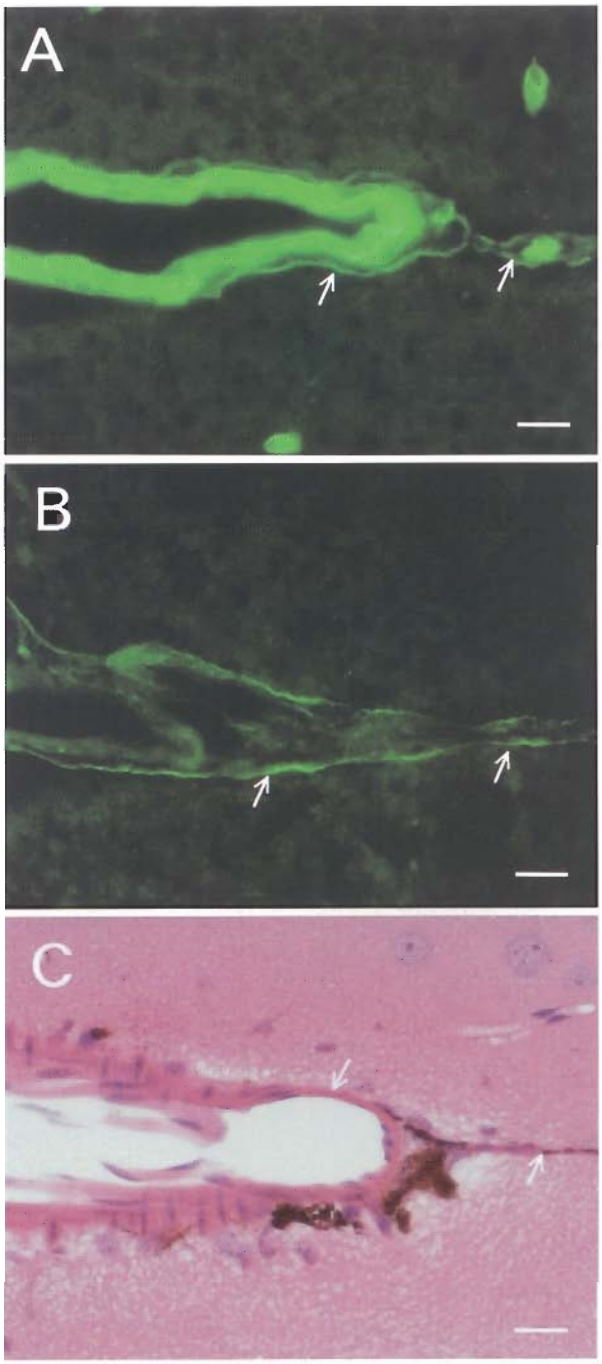

Fig. 3. The larger artery located close to the brain surface is stained with α2(IV) (H22, **A**) and α5(IV) (H53, **B**) antibodies, and with hematoxylin-eosin (**C**). The two basement membranes, the inner thicker and outer thinner *(arrows)* ones, are also stained for the α2 chain, and for α2 and α5 chains, respectively. Scale bars = 10 μm

in subendothelial basement membranes around the blood vascular endothelial cells and in subependymal basement membranes beneath the specialized ependymal cells (Fig. 4). However, we could not detect any type IV collagen chains beneath the ordinary ependymal cells at the surface of the ventricles (Figs. 4, 5). More interestingly, in the basement membranes under the choroid plexus ependymal cells, α3(IV), α4(IV) and α5(IV) chains were detected in addition to α1(IV) and α2(IV) chains (Fig. 5). In contrast, capillary basement membranes were only positive for α1(IV) and α2(IV) chains (Fig. 5A, B), and α3(IV), α4(IV) and α5(IV) chains were barely present at best.

DISCUSSION

The brain is sort of a specialized organ where the extracellular matrix is extremely small, occurring only in meninges and along blood vessels. Instead, the brain is concealed by cranial bones, under which this most functionally active organ that sends out and allows in information to and from the other organs in the body in such sophisticated ways is protected. Although a number of papers have reported the presence of extracellular matrix components or collagen IV in the central nervous system, none of them systematically examined the localization of the six basement membrane α(IV) collagen chains throughout the mouse brain. Recently, we have raised α-chain specific antibodies (SADO et al., 1995) and it has become possible to distinguish the distribution of each α-chain (NINOMIYA et al., 1995). We know that the α2(IV) chain exists in all basement membranes with no exceptions and is always present together with the α1(IV) chain (SEKI et al, 1998; SAITO et al, 2000). After systematic screening studies of histochemical staining by use of the α2(IV)-specific antibody, we reached the conclusion that there were three major regions where type IV collagen was present in the brain: the blood vessels, choroid plexus, and pia mater.

Basement membranes of blood vessels and pia mater

The brain receives blood supplies through the internal carotid and vertebral arteries. When arterial branches enter the inside of the brain, they are accompanied for a short distance by the pia mater and even subarachnoid space containing cerebrospinal fluid. Such depths of the cerebral cortex in extensions of the subarachnoid space along blood vessels are called Virchow-Robin's space. At this level, we found

that the arteries contained smooth muscle cells in tunica media, which were stained for the smooth muscle cell actin antibody (Fig. 2C). When they enter further, the arteries become smaller and are called arterioles and finally capillaries, where the coverage disappears. Instead, a simple structure of endothelium surrounded by thin basement membranes persists here and pericytes enwrap them. Further, the outermost surface of the brain facing the pia mater is continuously lined by the foot-processes of astrocytes, which comprise what is called the glia limitans superficialis. Even if arteries become smaller, the structure of the pia mater and perivascular space continues, but when they become capillaries, it disappears. We confirmed here that the disappearance of the pia mater along with the perivascular space corresponded to the transition from arterioles to capillaries since smooth muscle cells disappeared at the corresponding level.

Interestingly, however, endothelial cells together with pericytes are completely surrounded by the processes of astrocytes, which is now called the glia limitans perivascularis. The glia limitans forms a special environment indispensable for the brain and protects the central nervous system. The former, the glia limitans superficialis, comprises the cerebrospinal fluid-brain barrier and the latter, the glia limitans perivascularis, functionally constitutes the blood-brain barrier. When arteries become capillaries in individual organs, they may be involved in tissue-specific functions. We previously reported that the basement membranes that ensheath such capillaries had different molecular compositions (SEKI et al, 1998). For instance, we demonstrated that basement membranes surrounding common capillaries were composed of $\alpha1/\alpha2$ molecules, whereas those wrapping capillaries in glomeruli in the kidney (NINOMIYA et al., 1995) and alveoli in the lung (NAKANO et al., 2001) contained supramolecular aggregates of $\alpha3/\alpha4/\alpha5$ molecules (BOUTAUD et al., 2000).

In the present study, we extensively analyzed vascular basement membranes in the mouse brain by immunohistochemistry and came to the conclusion that capillaries in the brain had basement membranes of $\alpha1/\alpha2$ molecules as commonly observed in other organs. On the other hand, basement membranes surrounding smooth muscle cells that appeared in arterioles and larger arteries contained only $\alpha1/\alpha2$ molecules, in contrast to those in other organs simultaneously revealing $\alpha1/\alpha2$ and $\alpha5/\alpha6$ molecules (SEKI et al., 1998, BORZA et al., 2001). This difference could be due to particular features of smooth muscle cells in the brain — for instance, the elasticity necessary for brain arterioles.

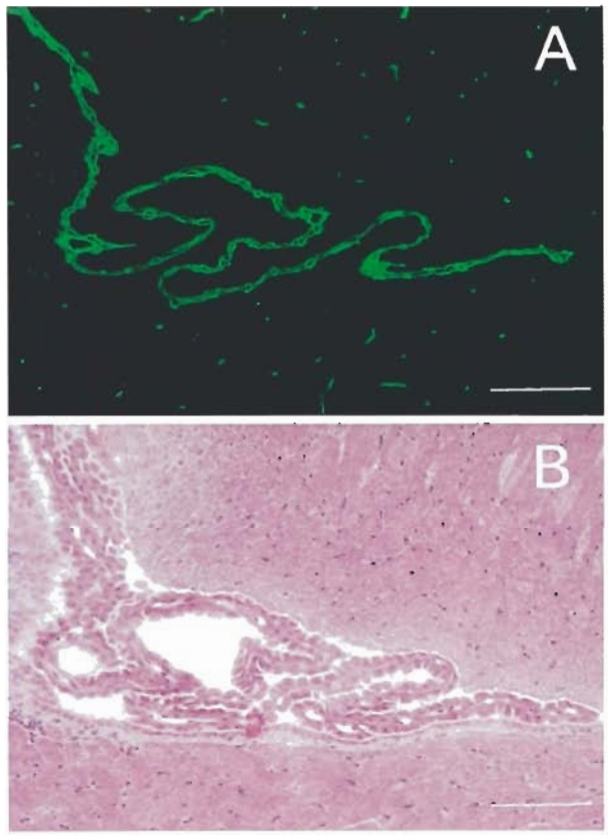

Fig. 4. Low magnification of the $\alpha1$(IV) chain staining of the basement membrane in the choroid plexus. Sections containing choroidal tissues in the lateral ventricle were stained with the anti-$\alpha1$ antibody, H12 (**A**) and hematoxylin-eosin (**B**). Linear staining of basement membranes under choroidal ependymal cells and small rings or spotty staining of capillary basement membranes are identified. Note that immunolabeling with the H12 antibody is not seen at all under the ordinary ependymal cell lining at the ventricular surface. Scale bars = 100 μm

Further, we have demonstrated in this study for the first time that the basement membrane in the pia mater contains type IV collagen molecules of $\alpha1/\alpha2$ and $\alpha5/\alpha6$. Although the basement membrane is very thin, it may play a key role in the barrier. Blood vessels penetrate, the central nervous system for a short distance, through a tunnel covered by the pia mater, where the glia limitans superficialis is present. At the capillary level astrocytes extend their foot processes and form the glia limitans perivascularis, which is fused with the basement membrane of endothelial cells. Such basement membranes were composed of only $\alpha1/\alpha2$ molecules in the mouse.

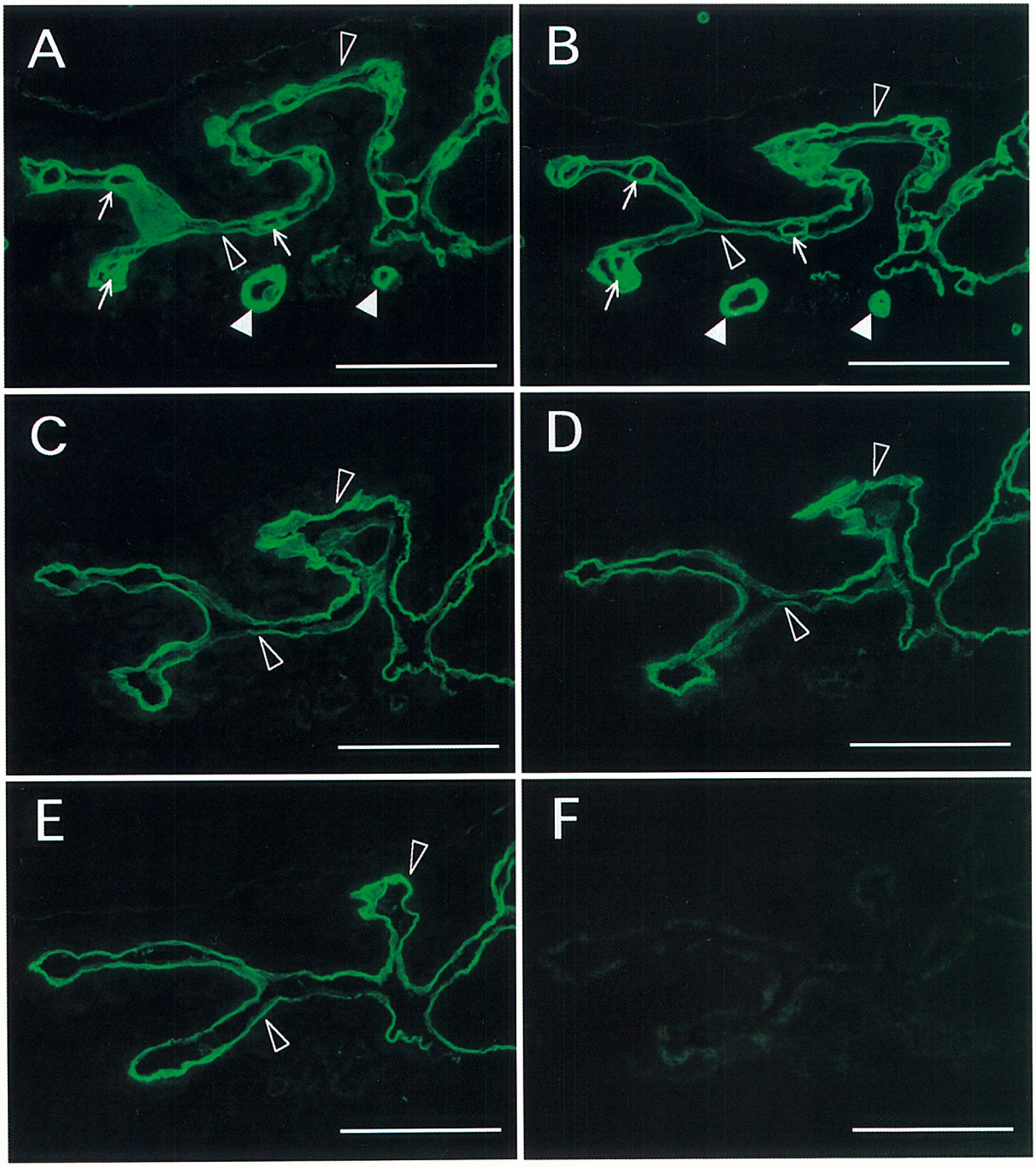

Fig. 5. Basement membranes of the choroid plexus, which are stained with antibodies against α1(IV) (H12, **A**), α2(IV) (H22, **B**), α3(IV) (H31, **C**), α4(IV) (H43, **D**), α5(IV) (H53, **E**), and α6(IV) (B66, **F**) chains. Note that basement membranes beneath endothelia and ependymal cells are stained for α1 and α2 chains, whereas the subependymal basement membranes *(open arrowheads)* are stained for α3, α4, and α5 chains. However, α3, α4, and α5 show no reactions in the subendothelial basement membrane. *Arrows* choroidal capillary subendothelial basement membrane, *white arrowheads* parenchymal capillary subendothelial basement membrane. Scale bars = 50 μm

Basement membranes in the choroid plexus

We reported immunohistochemically that the glomerular basement membrane contained α3(IV), α4(IV), and α5(IV) chains as major constituents, and that they were always present in the same amount (NINOMIYA et al., 1995; SADO et al., 1995). Recently we demonstrated by biochemical analysis that the three chains come from the same molecule, α3(IV)α4(IV)α5(IV) (BOUTAUD et al., 2000). The same method was applicd for vascular smooth muscle cell basement membranes and the presence of the $[\alpha5(IV)]_2$ α6(IV) was proved from among 56 different theoretical molecular species (BORZA et al., 2001).

The present study on the choroid plexus successfully demonstrated the presence of the two basement membranes in this location. One was the capillary subendothelial basement membrane which was composed of $\alpha1/\alpha2$ molecules (Figs. 4, 5) as observed in other tissues. The other was the subependymal basement membrane which was positive for $\alpha1$ to $\alpha5$ but not for $\alpha6$, indicating that it is composed of $[\alpha1(IV)]_2$ α2(IV) and α3(IV)α4(IV)α5(IV) molecules together. This was the fourth tissue that was shown to contain α3(IV)α4(IV)α5(IV) molecules after their demonstration in the renal glomeruli, pulmonary alveoli, and testicular seminiferous tubules. Possible common features for these organs may be a barrier and their selective permeability. A filter function similar to that observed in these tissues could be present in the choroid plexus, because blood should be filtered so that cerebrospinal fluid may be produced there. However, it is intriguing that the basement membranes detected in this tissue were two different membranes instead of one such fused basement membrane as seen in other organs. This difference could be related to the function of this particular basement membrane. Cuboidal ependymal cells lining the surface of the choroid plexus are active in transporting various ions, glucose, and other substances (JUNQUEIRA et al, 1992). Similar active transport systems are found in some parts of renal tubules and the cochlear duct, where α3(IV)α4(IV)α5(IV) molecules exist (SADO et al., 1995, HARVEY et al., 2001). Therefore, such a filter function or an active transport system could be related to the supramolecular aggregates of α3(IV)α4(IV)α5(IV) molecules in basement membranes. It is interesting to note that basement membranes of the choroid plexus are not lined with glia limitans and do contain α3(IV)α4(IV)α5(IV) molecules. In contrast, basement membranes lined with glia limitans do not contain such molecules. In view of different barrier functions, some other key molecules can be postulated to be present in basement membranes lined with glia limitans. As a matter of fact, SIXT et al. (2001) defined the two different laminin barriers encountered by extravasating T cells through the capillary basement membranes in the mouse brain.

In fact, in Alport syndrome cases which result from mutations in coding regions of α5(IV) gene, COL4A5, abnormal α5 protein causes the protein suicide of normal α3 and α4 chains. Since the three α chains always need the other two chains to form a α3(IV)α4(IV)α5(IV) molecule within cells, when an abnormal α chain forms, the other two normal chains are always degraded inside the cells (TRYGGVASON, 1996). Such an event takes place in the renal glomeruli and inner ear in Alport cases, resulting in progressive kidney dysfunction and hearing loss (HARVEY et al., 2001). Autosomal dominant Alport cases caused by mutations in COL4A3 or COL4A4 genes in humans (TRYGGVASON, 1996) and α3-knock out mice (COSGROVE et al., 1996) showed similar symptoms. These directly prove the importance of the function of the α3(IV)α4(IV)α5(IV) molecules in the organs concerned.

In conclusion, the choroid plexus basement membrane was analyzed by monoclonal antibodies specific for 6 α(IV) chains and found to contain a supramolecular network containing α3(IV)α4(IV)α5(IV) molecules. Such a network was previously detected in organs where a filtering function is needed — such as the renal glomeruli, suggesting that it may function as a permeability barrier.

Acknowledgements. The authors would like to thank all members of the Department of Molecular Biology and Biochemistry, Okayama University Graduate School of Medicine and Dentistry, for their continuous support and stimulating discussions.

REFERENCES

BORZA, D. B., O. BONDAR, Y. NINOMIYA, Y. SADO, I. NAITO, P. TODD and B. G. HUDSON: The NC1 domain of collagen IV encodes a novel network composed of the α1, α2, α5, and α6 chains in smooth muscle basement membranes. J. Biol. Chem. 276: 28532-28540 (2001).

BOUTAUD, A., D. B. BORZA, O. BONDAR, S. GUNWAR, K. O. NETZER, N. SINGH, Y. NINOMIYA, Y. SADO, M. E. NOELKEN and B. G. HUDSON: Type IV collagen of the glomerular basement membrane. Evidence that the chain specificity of network assembly is encoded by the noncollagenous NC1 domains. J. Biol. Chem. 275: 30716-30724 (2000).

BURBELO, P. D., G. R. MARTIN and Y. YAMADA: α1(IV) and α2(IV) collagen genes are regulated by a bidirectional promoter and a shared enhancer. Proc. Nat. Acad. Sci. USA 85: 9679-9682 (1988).

COSGROVE D, D. T. MEEHAN, J. A. GRUNKEMEYER, J. M. KORNAK, R. SAYERS, W. J. HUNTER and G. C. SAMUELSON: Collagen COL4A3 knockout: a mouse model for autosomal Alport syndrome. Genes Devel. 10: 2981-2992 (1996).

GARTNER, L. P. and J. L. HIATT: Color textbook of histology, 2nd ed., W. B. Saunders Co., Philadelphia. PA, 2001.

HARVEY, S. J., R. MOUNT, Y. SADO, I. NAITO, Y. NINOMIYA, R. HARRISON, B. JEFFERSON, R. JACOBS and P. S. THORNER: The inner ear of dogs with X-linked nephritis provides clues to the pathogenesis of hearing loss in X-linked Alport syndrome. Amer. J. Pathol. 159: 1097-1104 (2001).

HUDSON, B. G., S. T. REEDERS, and K. TRYGGVASON: Type IV collagen: structure, gene organization, and role in human diseases. Molecular basis of Goodpasture and Alport syndromes and diffuse Leiomyomatosis. J. Biol. Chem. 268: 26033-26036 (1993).

JUNQUEIRA, L. C., J. CARNEIRO and R. O. KELLEY: Basic histology. Appleton & Lange, Englewood Cliffs, NJ, 1992.

KAGAWA, M., Y. KISHIRO, I. NAITO, T. NEMOTO, H. NAKANISHI, Y. NINOMIYA and Y. SADO: Epitope-defined monoclonal antibodies against type-IV collagen for diagnosis of Alport's syndrome. Nephrol. Dial. Transplant. 12: 1238-1241 (1997).

KAYTES, P., L. WOOD, N. THERIAULT, M. KURKINEN and G. VOGELI: Head-to-head arrangement of mouse type IV collagen genes. J. Biol. Chem. 263: 19274-19277 (1988).

KISHIRO, Y., M. KAGAWA, I. NAITO and Y. SADO: A novel method of preparing rat-monoclonal antibody-producing hybridomas by using rat medial iliac lymph node cells. Cell Struct. Funct. 20: 151-156 (1995).

MOMOTA, R., M. SUGIMOTO, T. OOHASHI, K. KIGASAWA, H. YOSHIOKA and Y. NINOMIYA: Two genes, COL4A3 and COL4A4 coding for the human α3(IV) and α4(IV) collagen chains are arranged head-to-head on chromosome 2q36. FEBS Lett. 424: 11-16 (1998).

NAKANO, K. Y., K. I. IYAMA, T. MORI, M. YOSHIOKA, T. HIRAOKA, Y. SADO and Y. NINOMIYA: Loss of alveolar basement membrane type IV collagen α3, α4, and α5 chains in bronchioloalveolar carcinoma of the lung. J. Pathol. 194: 420-427 (2001).

NINOMIYA, Y., M. KAGAWA, K.-I. IYAMA, I. NAITO, Y. KISHIRO, J. M. SEYER, M. SUGIMOTO, T. OOHASHI and Y. SADO: Differential expression of two basement membrane collagen genes, COL4A6 and COL4A5, demonstrated by immunofluorescence staining using peptide-specific monoclonal antibodies. J. Cell Biol. 130: 1219-1229 (1995).

OOHASHI, T., M. SUGIMOTO, M.-G. MATTEI and Y. NINOMIYA: Identification of a new collagen IV chain, α6(IV), by cDNA isolation and assignment of the gene to chromosome Xq22, which is the same locus for COL4A5. J. Biol. Chem. 269: 7520-7526 (1994).

PAXINOS, G. and K. B. J. FRANKLIN: The mouse brain in stereotaxic coordinates. Academic Press, San Diego, 2001.

PÖSCHL, E., R. POLLNER and K. KÜHN: The gene for the α1(IV) and α2(IV) chains of human basement membrane collagen type IV are arranged head-to-head and separated by a bidirectional promoter of unique structure. EMBO J. 7: 2687-2695 (1988).

RISAU, W.: Induction of blood-brain barrier endothelial cell differentiation. Ann. New York Acad. Sci. 633: 405-419 (1991).

ROHRBACH, D. H. and R. TIMPL: Molecular and cellular aspects of basement membranes. Academic Press, London, 1993.

RUBIN, L. L. and J. M. STADDON: The cell biology of the blood-brain barrier. Annu. Rev. Neurosci. 22: 11-28 (1999).

SADO, Y., M. KAGAWA, Y. KISHIRO, K. SUGIHARA, I. NAITO, J. M. SEYER, M. SUGIMOTO, T. OOHASHI and Y. NINOMIYA: Establishment by the rat lymph node method of epitope-defined monoclonal antibodies recognizing the six different α chains of human type IV collagen. Histochem. Cell Biol. 104: 267-275 (1995).

SADO, Y., M. KAGAWA, I. NAITO, Y. UEKI, T. SEKI, R. MOMOTA, T. OOHASHI and Y. NINOMIYA: Organization and expression of basement membrane collagen IV genes and their roles in human disorders. J. Biochem. (Tokyo) 123: 767-776 (1998).

SAITO, K., I. NAITO, T. SEKI, T. OOHASHI, E. KIMURA, R. MOMOTA, Y. KISHIRO, Y. SADO, H. YOSHIOKA and Y. NINOMIYA: Differential expression of mouse α5(IV) and α6(IV) collagen genes in epithelial basement membranes. J. Biochem. (Tokyo) 128: 427-434 (2000).

SEKI, T., I. NAITO, T. OOHASHI, Y. SADO and Y. NINOMIYA: Differential expression of type IV collagen isoforms, α5(IV) and α6(IV) chains, in basement membranes surrounding smooth muscle cells. Histochem. Cell Biol. 110: 359-66 (1998).

SOININEN, R., M. HOUTARI, S. L HOSTIKKA, D. J. PROCKOP and K. TRYGGVASON: The structural genes for α1 and α2 chains of human type IV collagen are divergently encoded on opposite DNA strands and have an overlapping promoter region. J. Biol. Chem. 263: 17217-17220 (1988).

SIXT, M., B. ENGELHARDT, F. PAUSCH, R. HALLMANN, O. WENDLER, and L.M. SOROKIN Endothelial cell laminin isoforms, laminins 8 and 10, play decisive roles in T cell recruitment across the blood-brain barrier in experimental autoimmune encephalomyelitis. J Cell Biol. 153: 933-946 (2001).

SUGIMOTO, M., T. OOHASHI and Y. NINOMIYA: The genes COL4A5 and COL4A6, coding for basement membrane collagen chains α5(IV) and α6(IV), are located head-to-head in close proximity on human chromosome Xq22 and COL4A6 is transcribed from two alternative promoters. Proc. Nat. Acad. Sci. USA 91: 11679-11683 (1994).

Taguchi, T., A. Ohtsuka and T. Murakami: Light and electron microscopic detection of anionic sites in the rat choroid plexus. Arch. Histol. Cytol. 61: 243-252 (1998).

Timpl, R. and J. C. Brown: Supramolecular assembly of basement membranes. Bioassays 18: 123-132 (1996).

Tryggvason, K.: Mutations in type IV collagen genes and Alport phenotypes. Contrib. Nephrol. 117:154-171 (1996).

Williams, P. L., L. H. Bannister, M. M. Berry, P. Collins, M. Dyson, J. E. Dussek and M. W. J. Ferguson: Gray's Anatomy. 38th ed. Churchill Livingstone, New York, 1995.

Zhou, J., T. Mochizuki, H. Smeets, C. Antignac, P. Laurila, A. Paepe, K. Tryggvason and S. T. Reeders: Deletion of the paired α5(IV) and α6(IV) collagen genes in inherited smooth muscle tumors. Science 261: 1167-1169 (1993).

Prof. Yoshifumi Ninomiya, MD, Ph D.
Department of Molecular Biology and Biochemistry
Okayama University Graduate School of Medicine and Dentistry,
2-5-1, Shikata-cho, Okayama
700-8558 Japan

Phone: +81-86-235-7127
Fax: +81-86-222-7768
E-mail: yoshinin@cc.okayama-u.ac.jp

二宮 善文
700-8558 岡山市鹿田町 2-5-1
岡山大学大学院医歯学総合研究科
分子医化学