Virchows Arch A (1987) 411:245–252

Virchows Archiv A


# Basement membrane proteins and reticulin in a normal thymus and the thymus in myasthenia gravis

**Tuomo Karttunen**
Department of Pathology, University of Oulu, Kajaanintie 52D, SF-90220 Oulu, Finland

**Summary.** The distribution of basement membrane (BM) proteins, laminin and type IV collagen were studied immunohistochemically in a series of 12 normal thymuses representing different age groups (0–52 years) and in 10 cases of myasthenia gravis (age 7–53 years). The staining pattern was compared with that of conventional reticulin staining. BM proteins were present at the capsule-parenchyma interface and scantily distributed in the medullary stroma, where they were closely associated with reticulin fibres. The extrathymic perivascular space was effectively vizualized by the staining of the BM's marginal to it. The fiber network present in this space stained with reticulin stain and, less continuously, in BM stainings. Lymph node like tissue with germinal centers was occasionally present in the perivascular spaces in normal thymuses and commonly in the myasthenia gravis cases, where the perivascular spaces were often dilated. The BM's of the perivascular space were mostly continuous in normal cases, but discontinuities were observed in cases of myasthenia gravis, especially in the spaces which were widely dilated. Immunohistochemical detection of BM proteins seems to be useful in the study of thymic structure, particularly in the demonstration of the characteristic changes of the perivascular space in myasthenia gravis. It is suggested that the reticulin fibres present in the medulla and in the perivascular space contain laminin and type IV collagen.

**Key words:** Laminin – Type IV collagen – Thymus – Myasthenia gravis

## Introduction

The thymus is an exceptional organ in the lymphatic system. Being embryologically derived from the third pharyngeal pouch the primary sources of thymic tissues are entodermal and epidermal rather than mesenchymal in origin (Norris 1938; von Gaudecker 1986). Both developmentally and structurally, the thymus differs from the other components of lymphatic tissue. In contrast to lymph nodes, the thymus is characterized by an absence of the afferent lymphatic vessels and lymphatic sinuses. Epithelially derived cells make up the framework of the organ, being the counterpart of the mesenchymally derived reticulum cells of the lymph nodes and the spleen (Rosai and Levine 1976). The thymus contains structures unique to the organ, such as the epithelially derived, keratin-containing (Löning et al. 1981) Hassall's corpuscles, and a special organization of the perivascular spaces (Bearman et al. 1975).

The basement membrane (BM) proteins among the other components of the extracellular matrix are an important part of the microenvironment which plays a role in the traffic of lymphocytes across the interstitium (Fossum and Ford 1985). We have previously studied the distribution of BM proteins, laminin and type IV collagen, in normal spleen and lymph nodes and shown that they make up an important part of the reticular fibres (Apaja-Sarkkinen et al. 1986; Karttunen et al. 1986). We wished to study the distribution of these proteins in the thymus in order to find out the composition of the reticular network. The results were correlated with the physiological structural changes of aging and also the changes seen in myasthenia gravis, in which disease lymphoid hyperplasia characterized by germinal centers appears in the thymus (Castleman 1966).

## Material and methods

The material consisted of 12 thymuses from patients without myasthenia gravis (age 0–52 years; Table 1) and 10 thymuses

**Table 1.** Clinical and histological findings of the normal thymuses studied. In the cases 1–7 specimens were taken at autopsy, and the diagnosis refers to the terminal illness of the patient

| Case | Age/sex | Diagnosis | Histology of thymus | Germinal centers present |
|---|---|---|---|---|
| 1. | Abortion at 31 weeks/male | Sacral teratoma | Normal | None |
| 2. | 4 months/female | Microcefalia, pneumonia | Normal | None |
| 3. | 7 months/male | Pneumonia | Normal | None |
| 4. | 1,6 years/female | Septicemia | Normal | None |
| 5. | 2 years/female | Hydrocephalus | Normal | None |
| 6. | 3 years/male | Meningitis | Lymphocytic depletion | None |
| 7. | 6 years/male | Acute epiglottitis | Normal | None |
| 8. | 12 years/male | Cystic teratoma of thymus | Normal (in part) | A few |
| 9. | 14 years/male | Operation for suspected thyroid cancer | Normal | A few |
| 10. | 20 years/female | Dermoid cyst of thymus | Normal (in part) | A few |
| 11. | 22 years/female | Heterotopic thymus (lower part of neck) | Normal | A few |
| 12. | 52 years/female | Operation for suspected thyroid tumour | Normal | None |

**Table 2.** Clinical and histological findings of the myasthenic thymuses studied

| Case | Age/sex | Diagnosis | Histology of thymus | Germinal centers present |
|---|---|---|---|---|
| 13. | 7 years/female | Myasthenia gravis | Normal | A few |
| 14. | 7 years/female | Myasthenia gravis | Normal | A few |
| 15. | 11 years/female | Myasthenia gravis | Normal | None |
| 16. | 18 years/female | Myasthenia gravis | Normal | A few |
| 17. | 28 years/female | Myasthenia gravis | Lymphoid hyperplasia | Many |
| 18. | 33 years/female | Myasthenia gravis | Lymphoid hyperplasia | Many |
| 19. | 34 years/female | Myasthenia gravis | Lymphoid hyperplasia | Many |
| 20. | 50 years/female | Myasthenia gravis | Atrophy | None |
| 21. | 50 years/female | Myasthenia gravis | Atrophy | Many |
| 22. | 53 years/male | Myasthenia gravis | Atrophy | None |

from patients with myasthenia gravis (age 7–53; Table 2) selected to represent the different ages in each group. The thymuses from the nonmyastenic cases were histologically normal, except in two cases with a focal abnormality (cystic teratoma and dermoid cyst) (Table 1). In seven cases the histological specimens were taken at autopsy, while five were surgical specimens. The thymuses in the myasthenic cases were all surgical specimens taken for therapeutic purposes (Table 2).

The specimens were fixed in 10% formalin and embedded in paraffin, and the 5 μm sections were stained with haematoxylin and eosin for routine histology and with Gomori's reticulin stain to evaluate the amount and distribution of reticulin. The antibodies against the human 7-S collagen domain of type IV collagen and the human fragment P1 of laminin were a kind gift from Dr. Leila Risteli and Dr. Juha Risteli, Collagen Research Unit, Department of Medical Biochemistry, University of Oulu. The P1 fragment of laminin was purified from human placenta (Risteli and Timpl 1981) and the 7-S domain of type IV collagen from human kidney (Risteli et al. 1980). The antisera were made in rabbits and purified by immunoabsorption (Karttunen et al. 1984). The sections for immunohistochemical staining were deparaffinized and treated with 0.4% pepsin (Sigma Chemical Co., St. Louis, MO) to enhance the availability of the antigenic determinants (Ekblom et al. 1982). The sections were exposed to a 0.1% solution of hydrogen peroxide in absolute methanol to inactivate the endogenous peroxidase and then stained with anti-laminin P1 or anti-7-S collagen using the peroxidase-antiperoxidase procedure (Sternberger 1979). Normal rabbit serum and phosphate-buffered saline (PBS) were used instead of the primary antibody for the control stainings.

## Results

In normal thymus the staining patterns of both laminin and type IV collagen were comparable in all cases. A continuous line of staining was present between the parenchyma and the capsule or capsular septae in both the cortex and the medulla. No BM material was present at the margin of the cortex and the medulla (Fig. 1). The reticulin staining pattern was identical with the BM stainings in these respects.

No fibres could be detected in the cortex in either reticulin or immunohistochemical stainings, with the exception of occasional fibres present close to the capsule in some cases. Some fibres were present in the medulla and their quantity did not seem to change with age. These fibres contained BM proteins, laminin and type IV collagen

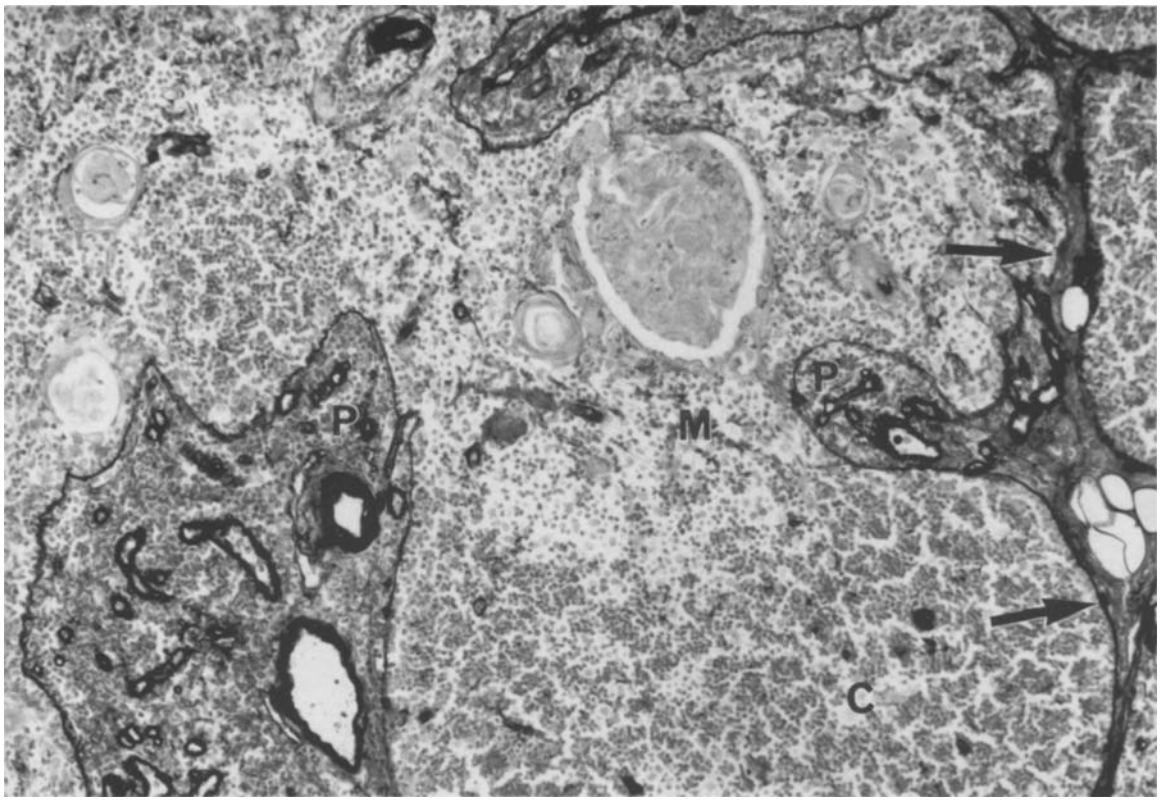


**Fig. 1.** Immunoperoxidase staining for laminin. Normal thymus of a 20-year-old female (case 10). A continuous line of staining is present between the fibrous septa and the parenchyma (*arrows*). This line extends to bound the perivascular space (*P*). No staining is present between the cortex (*C*) and the medulla (*M*). ×128

(Figs. 2, 3). Short strips of BM proteins were seen around Hassall's corpuscles, probably representing BM material of the adjacent reticulin fibres (Fig. 3).

The BM stainings effectively visualized the structure and course of the blood vessels. The staining pattern of the intrathymic blood the vessels did not differ from that of vessels in the surrounding tissues. However, a distinct space outlined by nearly continuous BM seen in both the BM and the reticulin stainings formed around a group of vessels arriving in the thymic parenchyma (Figs. 1, 2). This perivascular space contained a variable amount of lymphoid cells and fibres. Generally, the fibres made up a loose network which stained with reticulin stain and, less continuously, for BM (Fig. 2). The space was most prominent in older children and young adults. With increasing age it was gradually filled with fat cells with a concomitant disappearance of the reticular fibre pattern.

Lymphatic vessels with a few lymphocytes could be identified in the interlobular septae. They were mostly irregularly shaped, angulated or slit like. The vessels were characteristically outlined by a thin, discontinuous BM (Fig. 4). No clear-cut connections between the perivascular space and the lymphatic vessels could be demonstrated.

Germinal centers were present in four cases (Table 1). BM stainings showed that they were located in the perivascular space. The BM of the perivascular space was continuous in three out of four cases but showed discontinuities in one case (case 11) close to the germinal center. Discontinuities were also apparent in reticulin staining. The characteristic fibres of the perivascular space were not present on germinal centers.

The distributions of the BM components and reticulin in myasthenic thymuses were basically similar to those seen in the normal. The volume of the perivascular space was increased when compared with other patients of the same age. Germinal centers were present in all but three cases (Table 2) in which the staining patterns did not differ from those seen in normal thymuses. Germinal centers were scantily present in three cases, where they were located mostly in the perivascular space, assessed by BM stainings (Fig. 5). Germinal centers were present abundantly in four cases and were most commonly located in the medulla. The

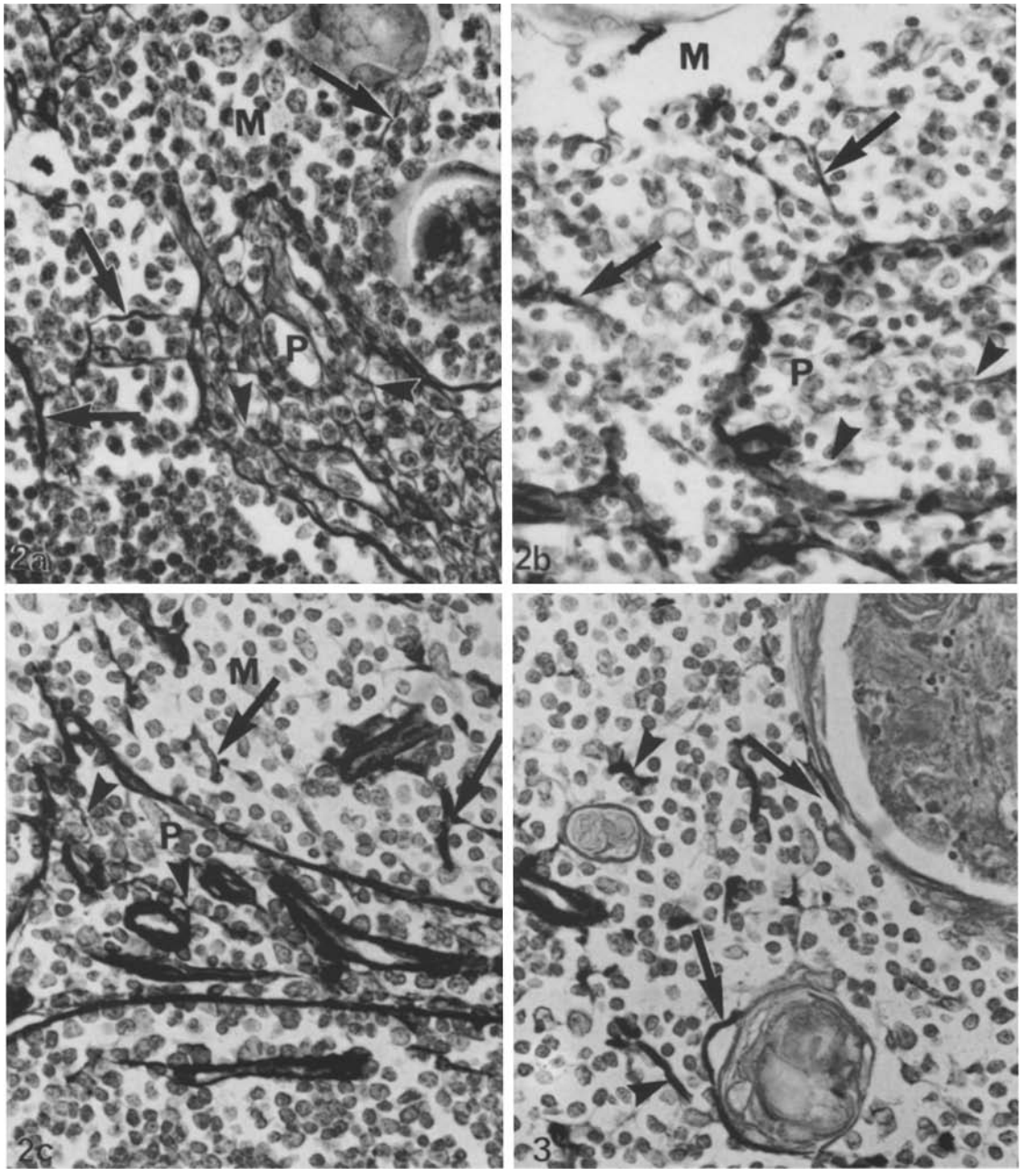


**Fig. 2a, b, c.** Gomori's reticulin stain (**a**) and immunoperoxidase staining for type IV collagen (**b**) and laminin (**c**). Normal thymus of a 20-year-old female (case 10). BM between the perivascular space (*P*) and the medulla (*M*) is continuous and is also stained with reticulin stain. Reticulin fibres containing BM proteins (*arrows*) are present in the medulla. In the perivascular space the fibres are thinner and only a minor component contain BM proteins (*arrow heads*). × 512

**Fig. 3.** Immunoperoxidase staining for laminin. Normal thymus of a 20-year-old female (case 10). Short fibre like strips of staining partly surround two Hassall's corpuscles (*arrows*) and similar fibres are scantily present in the medullary parenchyma (*arrow heads*). × 512

epithelial BM of the perivascular space showed discontinuities in both the reticulin and BM stainings. Discontinuities were clearly more apparent in cases with abundant germinal centers or close to the germinal centers located in the medulla (Figs. 6, 7). The general architecture of the thymus in cases with abundant germinal centers was profoundly distorted (Fig. 7), and it was difficult to observe the relationship between the tissue components. Hassall's corpuscles were closer to each other than

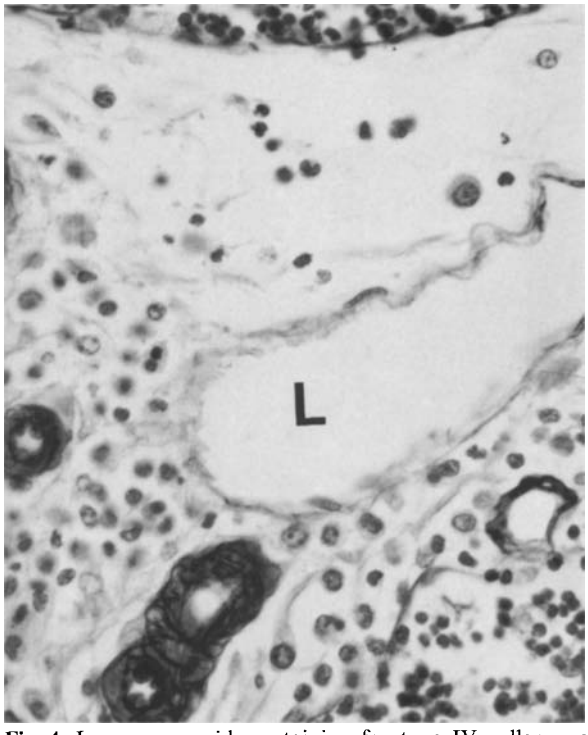


**Fig. 4.** Immunoperoxidase staining for type IV collagen of a normal thymus of a 7-month-old male (case 3). A lymphatic vessel (*L*) with a thin and discontinuous BM is present in the perivascular space. × 280

normally. The reticulin fibre network in the medulla was denser than normal, and the fibres again contained BM proteins.

## Discussion

The present study demonstrates the distribution of two well characterized components of BM's, laminin and type IV collagen, in normal and myasthenic thymuses. BM stainings effectively illustrates the basic structure of the thymus, particularly the course of the blood vessels and the unique perivasvular space. The alterations associated with the disease and aging in these structures could be studied easily.

The distribution of BM proteins, as described in this study, is generally in accord with the distribution of electron microscopic "basal lamina" in human thymic tissue (Kameye and Watanabe 1965; Pinkel 1968; Bockman et al. 1972; Bearman et al. 1975, 1978). In normal thymuses, BM proteins are present in the BM separating the thymic parenchymal tissue from the connective tissue capsule. These structures are also stained in the reticulin staining. In the cortex, BM proteins are only present in the BM's of blood vessels and there are no extracellular fibres demonstrable in either the reticulin staining or the BM stainings. In the medulla, the distribution of reticulin fibres is scant and the fibres contain BM proteins laminin and type IV collagen.

Previous light microscopic studies have suggested that reticulin fibres are also absent from the medulla (Henry 1978; Steinmann 1986). Similarly, Bofill et al. (1985) and Berrih et al. (1985) only found laminin or type IV collagen in blood vessels and at the capsule-parenchyma interface, but not in the fibres of medulla and perivascular space. The reason for this disagreement is not apparent. The antibodies against murine laminin (used by Bofill et al. and Berrih et al.) have been shown to be less sensitive than the antibodies against human laminin (used in the present study) in immunohistochemistry (Salonen et al. 1984). However, it is suggested that the immunoreactivities of both laminin and type IV collagen are slightly weakened in formalin fixed, paraffin embedded tissues when compared with frozen sections (Barsky et al. 1984). Only one electron microscopic study comments on the presence of reticular fibres in the thymic parenchyma in association with epithelial cells (Pinkel 1968). Tamaoki et al. (1971) observed BM between epithelial cells and collagen fibres around Hassall's corpuscle. Scantiness though not total absence of the reticular fibres in the thymic parenchyma is also demonstrated in this study, and the amount of reticular meshwork is definitely much less than in the spleen and the lymph nodes (Apaja-Sarkkinen et al. 1986; Karttunen et al. 1986).

The perivascular space between the endothelial and epithelial BM's is a unique structure present only in the thymus and is thought to make up a blood-thymus barrier, so that only the structures outside this space are intrathymic (Raviola and Karnowsky 1972; Bearman et al. 1975). It is outlined by endothelial BM of the blood vessel on one side and BM of the thymic epithelium on the other. Electron microscopically this space is narrow around small vessels (Bearman et al. 1975), and it could generally not be demonstrated around capillaries in immunohistochemical stainings. However, the perivascular space around larger vessels arriving in the medulla was effectively demonstrable in BM stainings. This space was most prominent in young adults as has been reported previously (Steinmann 1986). The network of reticulin fibres is constantly present in the perivascular space (Steinmann 1986) and the present study shows that these fibres contain BM proteins in places. Germinal centers can be found in normal

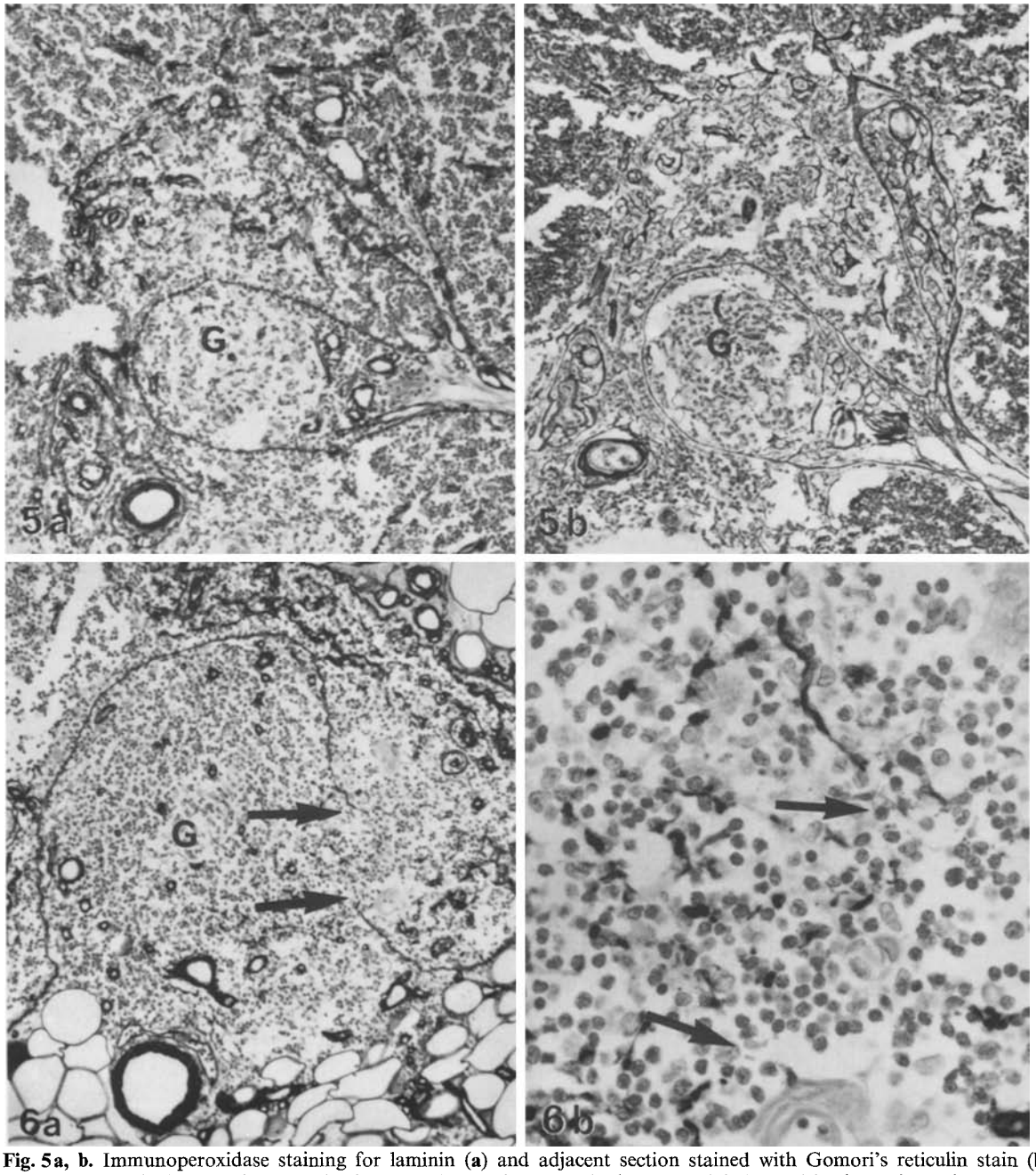


**Fig. 5a, b.** Immunoperoxidase staining for laminin (**a**) and adjacent section stained with Gomori's reticulin stain (**b**). Thymus from a 7-year-old female with myasthenia gravis (case 13). A germinal center (*G*) is located in the perivascular space surrounded by a continuous BM. ×128

**Fig. 6a, b.** Immunoperoxidase staining for type IV collagen. Thymus from a 50-year-old female with myasthenia gravis (case 21). A germinal center (*G*) is present in a wide perivascular space. The BM of the perivascular space is discontinuous (*arrows*). **a** ×128; **b** ×512

thymuses (Middleton 1967). The present study also shows that, as a rule, the germinal centers are located in the perivascular space, as suggested by Levine and Rosai (1978).

BM staining proved to be beneficial in the demonstration of lymphatic vessels in the thymic septae. These vessels characteristically had thin and discontinuous staining of BM, which is in agreement with the electron microscopic findings of the lymphatic capillaries in other tissues (Leak 1976) and also in agreement with our previous immunohistochemical study on the lymphatic capillaries

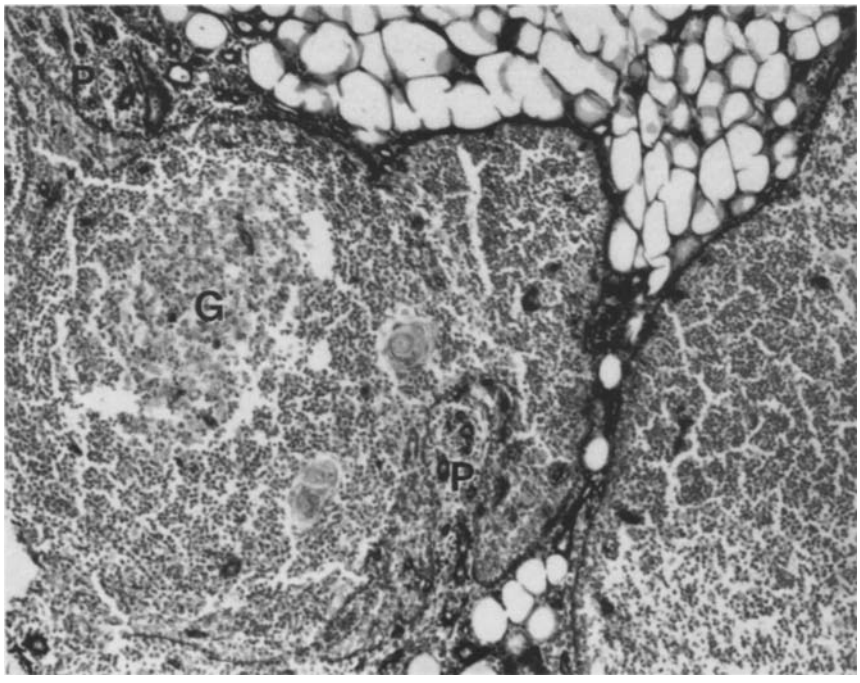


**Fig. 7.** Immunoperoxidase staining for laminin. Thymus from a 34-year-old female with myasthenia gravis (case 19). Profound alteration of the basic structure becomes evident from the BM stained section. A germinal center (*G*) is present in the medulla. The perivascular spaces (*P*) appear fairly small. ×128

of human skin (Autio-Harmainen et al. 1986). The perivascular space is continuous with efferent lymphatics in guinea pigs (Kotani et al. 1966). No terminal structures such as marginal sinuses of lymph nodes could be demonstrated and lymphatic vessels seemed to end blindly close to the perivascular space.

The most prominent finding in the thymuses of patients with myasthenia gravis is, according to this study, dilatation of the perivascular space in the medulla. This agrees with the results of Wekerle and Müller-Hermelink (1986). Germinal centres are located both in the perivascular space and, when abundantly present, in the medulla. With an increased number of germinal centres there appeared to be discontinuities in the BM of the perivascular space. So far germinal centers have been thought to be located in the medulla of the thymus (Castleman 1966) but recently both electron microscopic (Wekerle and Müller-Hermelink 1986), histochemical (Wekerle and Müller-Hermelink 1986) and immunohistochemical studies (Bofill et al. 1985; Berrih et al. 1985) have suggested that they are extrathymic and separated from the thymic tissue by a BM, which may be discontinuous. The present study confirms this notion and further suggests that the occurrence of discontinuities in the BM of the perivascular space is associated with the degree of expansion of the space. The disruption of the blood-thymus barrier may not be an initial event in the pathogenesis of the autoimmune phenomena in myasthenia gravis, but rather one expression of the expansion of lymph node like tissue in the perivascular space.

*Acknowledgements.* I gratefully acknowledge critical reading of the manuscript and valuable comments by Dr. Helena Autio-Harmainen. Dr. Leila Risteli and Dr. Juha Risteli are acknowledged for donating antibodies against laminin and type IV collagen. I thank Ms Mirja Vahera, Ms Marja Tolppanen and Mr Tapio Leinonen for expert technical assistance. This study was in part supported by Emil Aaltonen Foundation and Cancer Foundation of Northern Finland.

## References

Apaja-Sarkkinen, Alavaikko M, Karttunen T, Autio-Harmainen H (1986) Basement membrane proteins in the spleen: immunohistochmical demonstration and relation to reticulin. Histopathol 10:295–302

Autio-Harmainen H, Karttunen T, Apaja-Sarkkinen M, Dammert K (1986) Basement membrane distribution in different histologic stages of Kaposi's sarcoma not associated with AIDS. Abstracts, XVI International congress of the International Academy of Pathology and 7th Congress of Academic and Environmental Pathology, Wien, 121 (abstr)

Barsky SH, Rao NC, Restrepo C, Liotta LA (1984) Immunocytochemical enhancement of basement membrane antigens by pepsin: Applications in diagnostic pathology. Am J Clin Pathol 82:191–194

Bearman RM, Bensch KG, Levine GD (1975) The normal human thymic vasculature: an ultrastructural study. Anat Rec 183:485–498

Bearman RM, Levine GD, Bensch KG (1978) The ultrastructure of the normal human thymus: A study of 36 cases. Anat Rec 190:755–782

Berrih S, Savino W, Cohen S (1985) Extracellular matrix of the human thymus: Immunofluorescense studies on frozen sections and cultured epithelial cells. J Histochem Cytochem 33:655–664

Bockman DE, Lawton AR, Cooper MD (1972) Fine structure of thymus after bone marrow transplantation in an infant with severe combined immunodeficiency. Lab Invest 26:227–239

Bofill M, Janossy G, Willcox N, Chilosi M, Trejdosiewicz LK, Newsom-Davis J (1985) Microenvironments in the normal thymus and the thymus in myasthenia gravis. Am J Pathol 119:462–473

Castleman B (1966) The pathology of thymus in myasthenia gravis. Ann NY Acad Sci 135:496–504

Ekblom P, Miettinen M, Rapola J, Foidart JM (1982) Demonstration of laminin, a basement membrane glycoprotein, in routinely processed formalin fixed human tissues. Histochem 73:301–307

Fossum S, Ford WL (1985) The organization of cell populations within lymph nodes, their origin, life history and functional relationship. Histopathol 9:469–499

von Gaudecker B (1986) Development of the human thymus microenvironment. In Current topics in pathology, Vol 75, The human thymus, Ed by Müller-Hermelink HK. Springer, Berlin, Heidelberg, New York, pp 1–41

Henry K (1978) The thymus gland. In Systemic pathology, Volume 2. second edition, ed by W St C Symmers. Churchill, Edinburgh, pp 894–924

Kameya T, Watanabe Y (1965) Electron microscopic observations on human thymus and thymoma. Acta Pathol Jap 15:223–246

Karttunen T, Autio-Harmainen H, Räsänen O, Risteli J, Risteli L (1984) Immunohistochemical localization of basement membrane laminin and type IV collagen in bullous lesions of dermatitis herpetiformis. Br J Dermatol 111:389–394

Karttunen T, Alavaikko M, Apaja-Sarkkinen M, Autio-Harmainen H (1986) Distribution of basement membrane laminin and type IV collagen in human reactive lymph nodes. Histopathol 10:841–850

Kotani M, Seiki K, Yamashita A, Horii I (1966) Lymphatic drainage of thymocytes to the circulation in guinea pig. Blood 27:511–520

Leak LV (1976) The structure of lymphatic capillaries in lymph formation. Fed Proc 35:1863–1871

Levine GD, Rosai J (1978) Thymic hyperplasia and neoplasia: a review of current concepts. Hum Pathol 9:495–515

Löning T, Caselitz J, Otto HF (1981) The epithelial framework of the thymus in normal and pathological conditions. Virchows Arch A (Pathol Anat) 392:7–20

Middleton G (1967) The incidence of follicular structures in the human thymus at autopsy. Aust J Exp Biol Med Sci 45:189–199

Norris EH (1938) The morphogenesis and histogenesis of the thymus gland in man. Contrib Embryol 27:191–221

Pinkel D (1968) Ultrastructure of human fetal thymus. Am J Dis Child 115:222–238

Raviola E, Karnovsky MJ (1972) Evidence for a blood thymus barrier using electron-opaque tracers. J Exp Med 136: 466–478

Risteli L, Timpl R (1981) Isolation and characterization of pepsin fragments of laminin from human placental and renal basement membranes. Biochem J 193:749–755

Risteli J, Bächinger HP, Engel J, Furthmayr H, Timpl R (1980) 7-S collagen: characterization of an unusual basement membrane structure. Eur J Biochem 108:239–250

Rosai J, Levine GD (1976) Tumors of the thymus. In: Atlas of tumor pathology. Second series, Fascicle 13. Washington 1976

Salonen J, Pelliniemi LJ, Foidart JM, Risteli L, Santti R (1984) Immunohistochemical characterization of the basement membranes of the human oral mucosa. Archs Oral Biol 29:363–368

Steinmann GG (1986) Changes in the human thymus during aging. In: Müller-Hermelink HK (ed). Current topics in pathology. Vol 75. The human thymus. Springer, Berlin, Heidelberg, New York, pp 43–88

Sternberger LA (1979) Immunocytochemistry. 2nd ed. Wiley, New York, pp 104–129

Tamaoki N, Habu S, Kameya T (1971) Thymic lymphoid follicles in autoimmune disease. II. Histological, histochemical and electron microscopic studies. Keio J Med 20:57–68

Wekerle H, Müller-Hermelink HK (1986) The thymus in myasthenia gravis. In: Müller-Hermelink HK (ed), Current topics in pathology. Vol 75. The human thymus. Springer, Berlin, Heidelberg, New York, pp 179–206

Accepted March 13, 1987