

# Immunohistochemical Detection of Small Lymphocytes in the White Matter of Jimpy Mice

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Using monoclonal antibodies against mouse T-cells (surface antigens: Thy-1,2 and Lyl-1,2), we tried to detect T-lymphocytes appearing in the white matter of Jimpy mice.

Immunohistochemistry was always performed on both the spleen and the brain of normal littermate and on those of a Jimpy mutant. Although the spleen in a Jimpy mouse was much smaller than that of the control, a moderate number of lymphocytes positive to anti T-cell antibodies were distributed in the periphery of splenic nodules and the splenic cords of Bilbroth of the Jimpy mice as well as those of the control ones.

While some immunopositive cells were always observed in the Jimpy brains, none of them were found in the brains of the controls. In the suckling Jimpy mice up to postnatal day 10, positive cells were scattered in the white matter in such regions as the cerebellar medulla and corpus callosum, in the periventricular area and in the perivascular space. With time after birth, however, it became increasingly difficult to demonstrate lymphocytes by immunohistochemistry, even though they could be seen in the white matter under an electron microscope. This change seemed to occur with the developing BBB. Most of cells immunopositive to anti T-cell antibodies were regarded as T-lymphocytes, but the larger ones were considered to be mast cells non-specifically labeled by the antibodies because of metachromatic granules in the cytoplasm.

**Key words :** immunohistochemistry, Jimpy mouse, T-lymphocyte, brain white matter, autoimmune disease.

## Introduction

It is well-known that the white matter of a Jimpy mouse, a recessive sex-linked mutant is myelin deficient through out the animal's life

(Privat et al., 1972; Kraus-Ruppert et al., 1973; Meier & Bischoff, 1974; 1975). It is clear that myelin deficiency in the Jimpy brain is caused by damage to the myelin forming cells rather than by secondary myelin breakdown. Previous authors reported that there were numerous lipid-

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containing glioblasts and oligodendrocytes in the Jimpy brain (Privat *et al.*, 1972; Meier & Bischoff, 1974). They considered that the lipid inclusions may reflect precursors of myelin which have failed to assemble into normal membrane and thus may have accumulated in the cytoplasm of oligodendrocytes. In our reports (Imamoto, 1986; Imamoto, *et al.*, 1988), however, we mentioned that most of the cells showing lipid inclusions types and the Jimpy specific cytoplasmic compartments (probably corresponding to membranous tubes described by Meier & Bischoff, 1974) were macrophages rather than oligodendrocytes. Furthermore, we emphasized the fact that hematogenous cells such as small lymphocytes and macrophages appeared in the brain parenchyma without any vascular injury and that these cells occasionally adhered to the surface of immature oligodendrocytes. This led us to postulate that degeneration of oligodendrocytes prior to their maturation might be induced by the contact of the surface with these hematogenous cells (Imamoto, 1986). In other words, the myelin deficiency owing to oligodendrocyte degeneration in Jimpy mice might be a kind of T-cell mediated autoimmune disease. Are there any similarities to the experimental allergic encephalomyelitis (EAE) studied in many species as a model of autoimmune disease (Raine, 1984; Sobel *et al.*, 1987)? The present study is only a preliminary one in which we have tried to find small lymphocytes by immunohistochemistry throughout the entire brain parenchyma of Jimpy mice.

## Materials and Methods

Jimpy-Tabby strain mice raised in our animal center were used at various ages from postnatal day 2 to day 25. Animals were anesthetized by somnopentyl. After washing out the blood using physiological saline, mice were perfused through the heart with a fixative made by PLP

method at 4°C for 10 min (McLean & Nakane, 1974). Then the brain and spleen were removed and kept in the fresh fixative for 2 hs. Thereafter they were soaked in 15% sucrose phosphate buffer solution overnight and then embedded together in 10% gelatine. Sections of 30  $\mu$ m in thickness were cut by a vibratome and kept in 0.1 M phosphate buffered saline containing 0.03% Triton X (PBS). Just before the immunostaining, they were pretreated with 0.6% H<sub>2</sub>O<sub>2</sub> methanol solution for 30 min in order to block the endogenous peroxidase activity and rinsed with PBS. The immunohistochemistry was performed by an indirect method. The primary monoclonal antibodies against mouse T-cells were diluted in PBS (Thy-1,2: 1/2000; Lyt-1,2: 1/150, purchased from MIH, Japan), Sections were floated in the incubation medium for either 3 hs at room temperature or 18 hs at 4°C. After rinsing in PBS, the sections were reincubated in the medium containing peroxidase conjugated anti-mouse IgG (diluted into 1/2000 in PBS, purchased from DAKO, Denmark) for 2 hs at room temperature. The incubated sections were visualized with 0.01% diaminobenzidine solution containing 0.04M imidazol and 0.15% H<sub>2</sub>O<sub>2</sub> for 15 min. The counterstaining was done using either basic fuchsin or toluidine blue. Some of immunoreacted tissues were embedded in Epon after postfixation with 1% osmium tetroxide and dehydration in the routine manner. Ultrathin sections of immunoreacted and non-reacted tissues were lightly stained by uranyl acetate and lead citrate and examined under an H-700 electron microscope.

## Results

Small lymphocytes were frequently observed under an electron microscope in the non-myelinated white matter of Jimpy mice throughout this experiment, although they were never found in the controls (Fig. 1). They displayed

typical small round nuclei with dense chromatin patches. Such round nuclei were always encircled by thin electron-dense cytoplasm containing a few cell organelles. In the brain parenchyma, microvilli on the surface of the small lymphocytes were not prominent. However, lymphocytes were distinguishable from microglia which always have small dense nuclei of irregular shape and thin elongated cytoplasm often containing dense bodies and a few long cisterns of rough endoplasmic reticulum. Lymphocytes were occasionally located in the perivascular space, or within well-developed basement membrane (Fig. 2).

For the immunohistochemistry we tried to examine the entire brain parenchyma and the spleen in the Jimpy mice in parallel with those of

the control ones in order to confirm that the immunological reaction had occurred. We have noted no major difference between the immunoreactions of monoclonal antibodies against Thy-1, 2 and Lyt-1,2.

The Spleen: The spleens of Jimpy mice were usually much smaller than those of the control mice, and the splenic nodules were not as well-developed as those of the controls. Nevertheless, the cells positive against anti T-cell antibodies were scattered in the periphery of the nodules and splenic cords of Bilbroth as well as in the spleen of the control mice. Given the cell size and mononucleated figure, they were considered to be T-lymphocytes positive to the anti T-cell antibodies (Fig. 3). Under an electron microscope, the

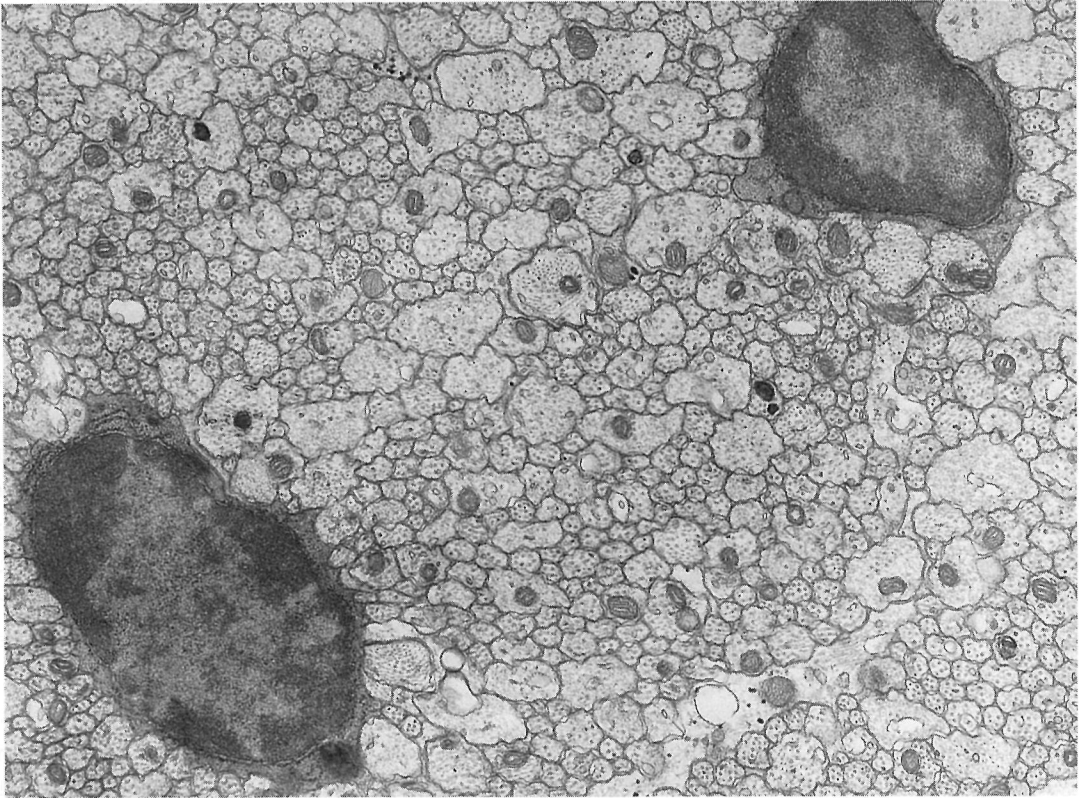


Fig. 1. Two lymphocytes observed among non-myelinated fibers in the corpus callosum of a Jimpy mouse on postnatal day 22. Note characteristic nuclei with dense chromatin patches and thin cytoplasm including a few organelles.

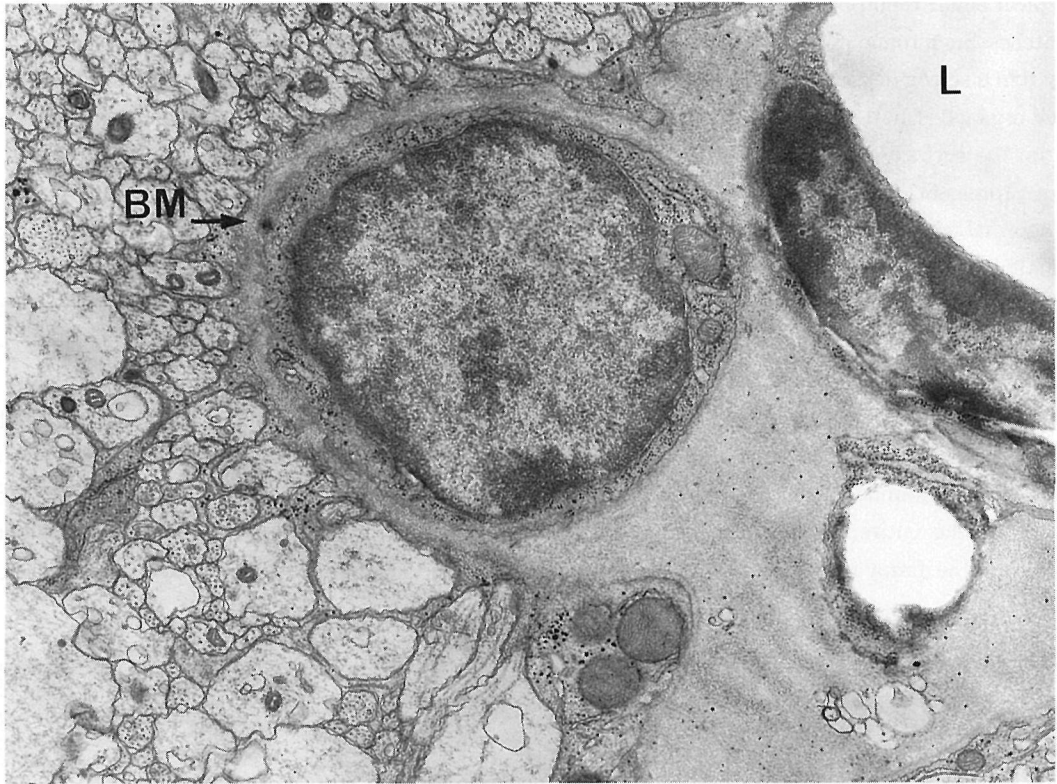


Fig. 2. Lymphocyte surrounded by the basement membrane (BM) in the perivascular space in the corpus callosum of a Jimpy mouse on postnatal day 22. L: lumen of the vessel  $\times 19,000$

immunoreaction products were seen on the microvilli of lymphocytes (Fig. 4). Some positive cells being larger than the lymphocytes and including metachromatic granules were regarded as mast cells in the splenic cords of Bilbroth. However, the immunospecificity of these cells to the anti T-cell antibodies was rather doubtful because they displayed a weak reaction even if the primary antibody reaction was omitted.

The white Matter: There were no cells immunopositive to anti T-cell antibodies in the brain parenchyma of the control mice. However, a moderate number of positive cells were found in the white matter such as the cerebellar medulla and corpus callosum, and in the periventricular and perivascular areas (Fig. 5). In the suckling Jimpy mice up to postnatal day 10, cells immuno-

positive to both anti Thy 1,2 antibodies were relatively numerous, but with time fewer and fewer cells were labeled by the antibodies in the brain parenchyma. The number of immunopositive cells was in fact much smaller than that normally found by electronmicroscopy. Under a light microscope, such positive cells with small dense nuclei were regarded as T-lymphocytes, but the finestructure of the latter has not been confirmed.

In addition, we noted larger immunopositive cells twice the size of a small lymphocyte in the brain parenchyma, the subependyma, the tela chorioidea, and on the leptomeninges (Fig. 6,7). They seemed to be the same as the mast cells observed in the spleen. Furthermore, a number of mast cells were observed showing metachromatic

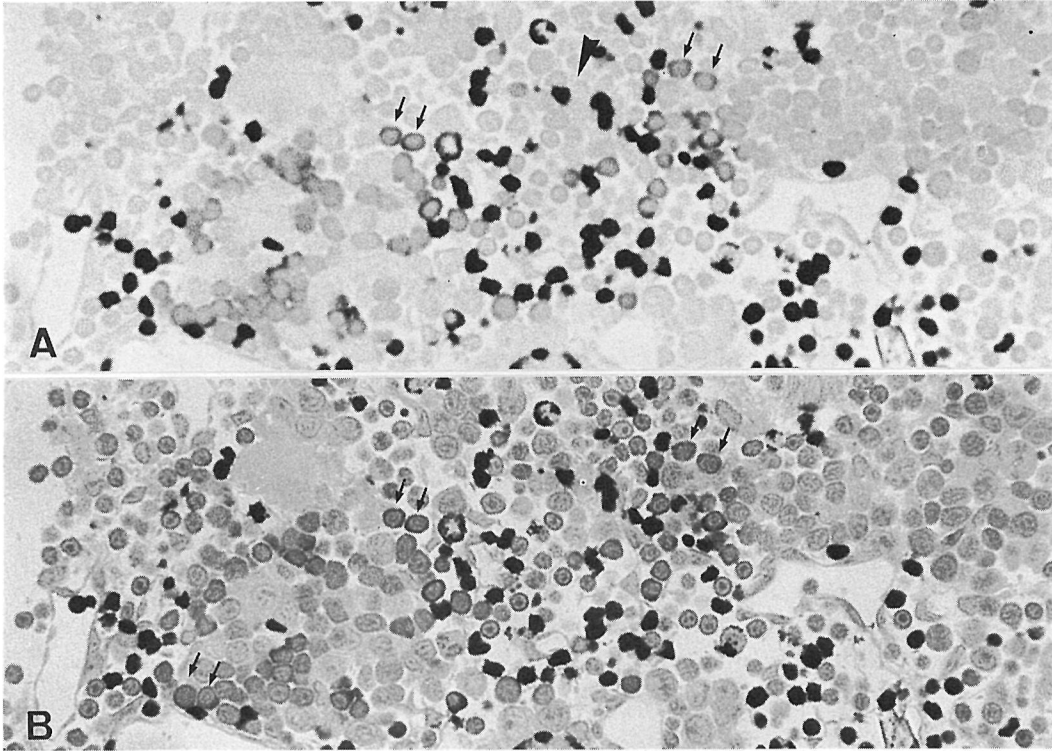


Fig. 3. Semithin Epon section of the spleen of a control mouse on postnatal day 13, after reaction with anti T-cell antibodies. A: before the counterstain, B: after the counterstain with basic fuchsin. Some of the positive lymphocytes in the splenic cords are marked by arrows.  $\times 550$

granules in the leptomeninges and the tela chorioidea during the newborn period, but they generally decreased in number with time. In Jimpy mice, however, mast cells in such regions remained till later in the animal's life compared with the control and moreover they tended to be distributed also in the brain parenchyma, particularly in the white matter and the subependymal area. By electronmicroscopy, such mast cells were often noted to display dense immunoreactive products not only on the cell membrane but also on the peripheral part of the cytoplasmic matrix and granules located in the periphery.

## Discussion

In this preliminary experiment, we focused on T-lymphocytes appearing in the white matter of Jimpy mice, although it is important to estimate both cell-mediated and humoral immune responses in order to establish the existence of the autoimmune disease in Jimpy mice. For the detection of T-cells, we employed monoclonal antibodies against T-cell surface antigens, Thy-1,2 and Lyt-1,2. It seemed that the antibodies against lyt-1,2 specifically label the T-cell line and that those against Thy-1,2 react not only with T-cells but also with some cells in the brain and the skin (Raff, 1971, Fields, 1979, Sinclair et al., 1987). During this experiment, however, immuno-

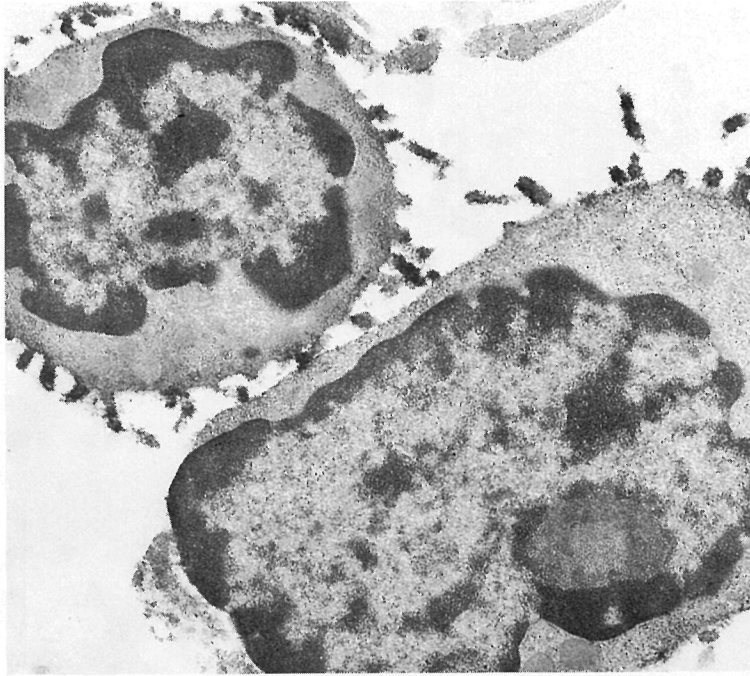


Fig. 4. Electron micrograph of immunopositive lymphocytes observed in the splenic cords of a control mouse on postnatal day 13. Immunoreaction products were located on the membrane of microvilli. Lightly stained with uranyl acetate and lead citrate.  $\times 16,000$

reactions by these two types of antibodies displayed no major difference in the immunoreactivity of T-cells. We also noted that none of the neurons and glial elements reacted with antibodies against Thy-1,2.

T-cells in the splenic cords of Bilbroth were more easily identified than those in the brain parenchyma. As mentioned before, it was rather difficult to demonstrate the presence of lymphocytes in the Jimpy brain by immunohistochemistry, especially from postnatal day 10 onwards. Although we were still able to find some lymphocytes in the brain during this later period by electronmicroscopy, they were only weakly stained by immunohistochemistry. We assumed that this change might depend on the alteration of membrane antigenicity. Probably it is due to

either a loss of microvilli on the lymphocyte surface in the brain parenchyma or the immunomasking effect by the well-developed surrounding tissue. The lymphocyte penetrated before the establishment of BBB may maintain the original membrane property. Morphological changes in lymphocytes and other cells after invasion into the brain parenchyma probably imply the alteration of the membrane property of such cells.

It is possible that the presence of the intact BBB might prevent larger protein molecules and hematogeneous cells from penetrating into the brain parenchyma and also immunological effects from extending into the central nervous system (CNS). However, there have been a number of reports concerning the EAE caused by inoculation of extracts from the CNS (Weigle, 1981). It is



Fig. 5. Immunopositive cells in the cerebellar medulla on postnatal day 4, reacted with anti T-cell (Lyt-1,2) antibodies, counterstained with basic fuchsin.  $\times 320$

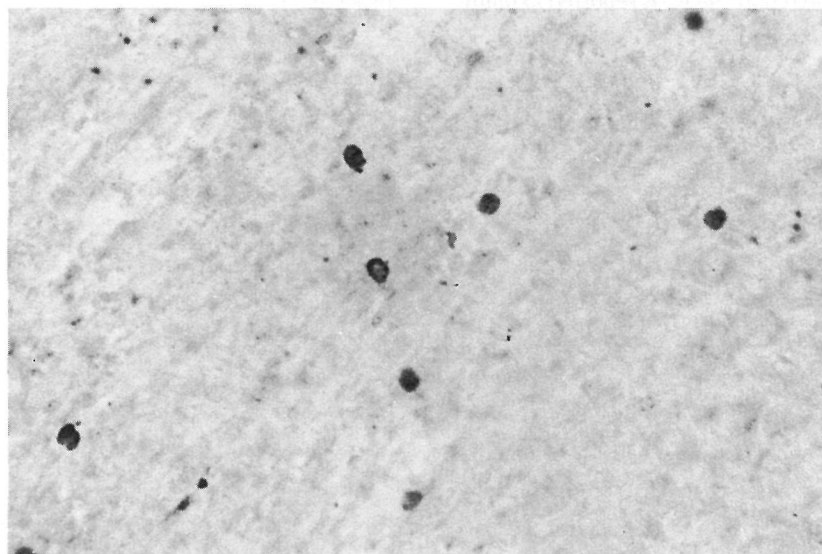


Fig. 6. Immunopositive mononuclear cells in the cerebral peduncle of a Jimpy mouse on postnatal day 18. The immuno-specificity of these cells are uncertain but they were regarded as mast cells because of cell size and the metachromatic granules in the cytoplasm.  $\times 320$

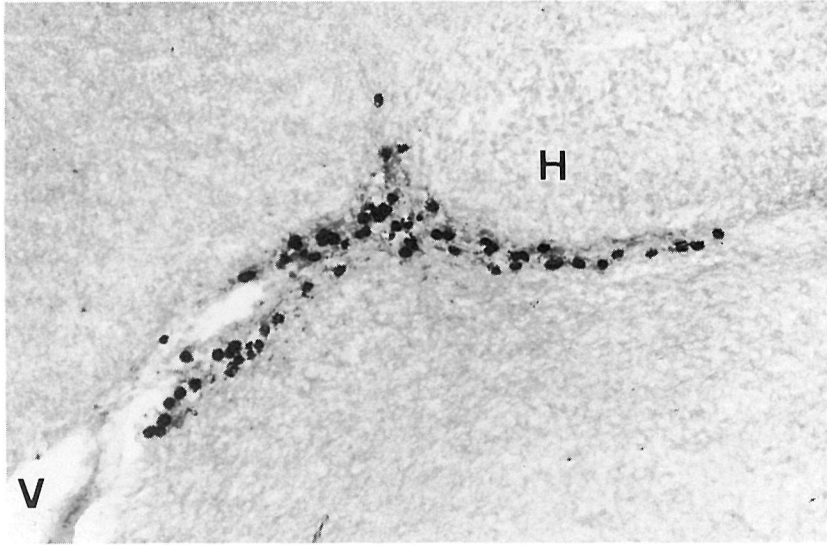


Fig. 7. Immunopositive cells in the subependyma and the tela chorioidea of the ventriculus tertius of a Jimpy mouse on postnatal day 7, after reaction with anti T-cell antibody. They are considered to be mast cells. Sagittal section. H: hippocampus; V: entriculus tertius.  $\times 160$

currently accepted that the EAE reported in many species is an autoimmune disease mediated by T-cells (Zamvil *et al.*, 1985). Moreover, not only activated T-cells but also antisera from EAE animals seems to cross the BBB and to transfer EAE in the recipients (Bernard and Mackay, 1983; Holda and Swanbog, 1982), suggesting an increased permeability of the BBB (Raine and Dolich, 1986).

Even though antigenicity of oligodendrocytes and myelin components are revealed by several approaches, the initiation of demyelination in EAE is still obscure. However, it is said that the Fc receptors on oligodendrocytes may be of particular importance in the multiple sclerosis showing demyelination, because the complex of IgG and the surface membrane of these cells may create favorable conditions for the initiation of immunodestruction by macrophages (Ma *et al.*, 1981). Similarly, we might be able to give an explanation for the degeneration of oligoden-

drocytes in Jimpy mice.

In the brain parenchyma of Jimpy mice, we found a moderate number of mast cells. This fact may suggest that the allergic reaction had occurred in the brain, although the immunological specificity of mast cells against anti T-cell antibodies is doubtful. Mast cells were common in the leptomeninges but rare or absent in the normal CNS of human beings and other primates (Dropp, 1972). With the autoimmune disease, Prineas & Wright (1978) reported occasional typical mast cells in the perivascular space. The binding of monoclonal antibodies to mast cells might be nonspecific and is probably mediated by Fc receptors on the cell membranes.

Further experiments are required in order to confirm whether the myelin deficiency in a Jimpy mouse is due to the autoimmune disease.



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## Jimpy マウスの脳内白質部における小リンパ球の検索

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Jimpy マウスの脳内白質部に出現する T リンパ球を広範囲に検索するために, T 細胞の表面抗原 (Thy-1,2 と Lyt-1,2) に対するモノクローナル抗体を用いて免疫組織化学的に観察した。

Jimpy マウスの脾臓は健常マウスのものに比べ著しく小さいが, 抗 T 細胞抗体陽性のリンパ球は正常のものと同様脾小節の辺縁部及び脾索内に十分に検出できた。

脳内白質部では, これら抗体に対して陽性となる細胞は正常のものでは認められないが, Jimpy マウスでは少数ながら常に観察された。生後10日までの

授乳期の Jimpy マウスでは, 抗 T 細胞抗体陽性のリンパ球様細胞が, 小脳髄質や脳梁をはじめとする白質部, 脳室周囲層及び灰白質の血管周囲に散在するのが観察された。

生後の日数が経つと, 電顕的にはリンパ球の存在を認めても, 免疫組織化学的には次第に検出が困難となった。この変化は BBB の発達に伴い生じるように思われた。抗 T 細胞抗体に対して陽性な小型細胞は T リンパ球と考えられるが, 大型の陽性細胞はメタクロマジアを示す顆粒を持つため非特異的反応を示した肥満細胞と思われる。