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The Permeability of the Blood-Brain Barrier in Mice Suffering From Fatal Lymphocytic Choriomeningitis Virus Infection*

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Summary. The ultrastructure and the blood-brain-barrier (BBB) permeability were studied in mice suffering from lymphocytic choriomeningitis (LCM). Brains and meninges from mice suffering from LCM virus-induced lymphocytic choriomeningitis were studied by investigating the BBB function and by electron and light microscopy.

The cellular exudate in the leptomeninges was located in the subarachnoid space, in arachnoidea and pia, and it was dominated by proliferated pial cells and mononuclear cells, most of which were lymphocytes, while there were only a few neutrophil granulocytes. Many intravascular lymphocytes were seen adhering to as well as penetrating the vessel walls. Many of these lymphocytes were morphologically compatible with T cells. Lymphocytes and larger mononuclear cells were also accumulated in the choroid plexus, and lymphocytes were present in the ventricular system with a tendency to adhere to ependymal epithelial cells. Inspection of the ultrathin sections incubated for horseradish peroxidase (HRP)-activity revealed that the overwhelming part of the peroxidase activity was localized in the extracellular space of the meningeal vessel walls and especially in the abundant intercellular fluid which, like the inflammatory cells, was found in the subarachnoid space in arachnoidea and in pia. In the neuropil, only very small quantities of reaction product were seen intercellularly in the most superficial layers of the cortex. The tight junctions were always intact, but the possibility of a non-demonstrable opening is discussed. Evaluation of the BBB permeability for 2-amino[1-^{14}C]isobutyric acid (AIB) was made by quantitative autoradiography, and it was demonstrated convincingly that AIB concentrations in the subpial and perichoroidal tissues were markedly increased in diseased animals as compared to the controls.

Our results seem to contradict previous theories on the cause of death resulting from the LCM disease. The findings presented here do not speak in favor of a pronounced brain edema, just as results obtained by us and others do not speak for the possibility of the death being caused by convulsive seizures with subsequent brain anoxia. However, our observations are compatible with the hypothesis that cytotoxic T cells may interact in vivo with virus-infected targets, which are essential for the regulation of the composition of the cerebrospinal fluid. On the other hand, the dysfunction of the BBB demonstrated adds a new element to the pathologic mechanism in a model for the study of virus-induced meningitis.

Key words: Blood-brain barrier – Lymphocytic choriomeningitis – Mice

Introduction

Intracerebral inoculation of lymphocytic choriomeningitis virus (LCMV) into immunologically competent mice invariably leads to a characteristic clinical picture and to the death of the animals 8–9 days after the infection (Lehmann-Grube 1971). For several years it has been well established that the fatal outcome of this infection is not caused by the cytopathic effect of the infectious agent, but is the result of the host animals' virus-specific immune response (Rowe 1954).

While B cells seem to be of no importance in this acute lymphocytic choriomeningitis (LCM) disease (Johnson et al. 1978), substantial evidence has been presented establishing the T effector cells as a man-

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datory factor in the development of this condition (Cole et al. 1972).

From the comprehensive literature covering this field it may be noted that the development of clinical symptoms parallels the generation in the spleen of T cells which in vitro cause ^{51}Cr release from labeled, syngeneic cells expressing LCMV antigens on their surface (Marker and Volkert 1973), and that a substantial number of these cytotoxic T cells are found in the cerebrospinal fluid (CSF) during the late stages of the LCM disease (Zinkernagel and Doherty 1973).

The spread and proliferation of LCMV in the mouse brain have also been the subject of considerable interest (Marker et al. 1976; Thomsen et al. 1979), and it has been demonstrated that the cells of the ependyma and plexus choroideus are heavily infected with the virus, while infection is somewhat less pronounced in the leptomeninges, and that only trace amounts of viral antigen are found in the olfactory area and the diencephalon (Gilden et al. 1972).

It has been postulated that in vivo a population of T cells specific to LCMV antigen may act cytotoxically on epithelial cells of the plexus choroideus (Volkert et al. 1974) and thereby in some way reduce the integrity of the blood-brain barrier (BBB) which may cause an extravasation of serum protein, brain edema, and death during characteristic convulsive seizures (Doherty and Zinkernagel 1974).

In fact, observations have been made indicating that large molecules may pass the BBB during LCM disease (Doherty and Zinkernagel 1974), and very recently a morphological study has been published which lends some support to this hypothesis (Schwendemann et al. 1983).

It has been the purpose of our study to throw more light on the nature of the pathologic changes which may be observed during the LCM disease, and which may reflect pathophysiologic conditions leading to a fatal clinical course of a virus infection in the central nervous system (CNS). In the present study, the change in the BBB function in mice suffering from LCM disease was studied using various tracer techniques and light and electron microscopy (LM and EM).

Materials and Methods

Virus

LCMV of the viscerotropic Volkert strain was used throughout the study. This virus was originally obtained from Dr. Traub, Munich (FRG), and it has been used and propagated in our laboratory for more than 20 years (Volkert 1962). It appears to be non-identical to the Traub strain used in the laboratory of Dr. Traub and others, and it has consequently been designated the Volkert strain. The virus preparation used was tissue culture supernatant from monolayers of LCMV-infected L cells as described earlier (Marker and Volkert 1973).

Virus titrations were carried out by intracerebral inoculations into young adult Swiss albino mice. Titrations were calculated by the Kärber method (Kärber 1931) and expressed as mean lethal doses (LD_{50}) per 0.03 ml.

Mice

For the BBB studies adult (2–4-month-old) C3H/Sscl mice were used. This mouse strain was originally obtained from the State Serum Institute, Copenhagen (Denmark). The strain has been maintained in the Experimental Animal Department, The Medical Faculty, Copenhagen Unversity, by strict inbreeding.

Infection of Mice. Three, 5, and 7 days before the experiments were carried out and the mice killed, they received intracerebrally (i.c.) 10^2 LD_{50} of LCMV in 0.03 ml of phosphate-buffered saline (PBS) during a light ether anesthesia of short duration. When we used the above virus strain, mouse strain, dose and route for the virus inoculation 100% of the animals died of the LCM disease 9–12 days after infection. Control animals received 0.03 ml PBS i.c. in the same manner and under the same circumstances as the virus-infected mice, or were untreated.

Fixation and Incubation for Horseradish Peroxidase Activity, Light and Electron Microscopy

After 10 min of tracer circulation, the animals were anesthetized with Halothane, the thorax was opened, and a perfusion cannula was inserted into the ascending aorta. The animals were perfused with 2.0% paraformaldehyde and 2.5% glutaraldehyde in 0.08 M Na-cacodylate buffer, pH 7.3, for the first 5 min followed by 10 min of perfusion with 4.0% paraformaldehyde and 5.0% glutaraldehyde in 0.08 Na-cacodylate buffer, pH 7.3. Then the brain was carefully removed, and after 2-h immersion fixation cut into 1 mm thick frontal slices, which were kept overnight in the above mentioned dilute fixative at 4° C. The following day two slices were washed in 0.05 M tris buffer and incubated for peroxidase activity. They were placed into 40 ml 0.05 M tris buffer, pH 7.6, containing 0.3 ml glucose oxidase (Sigma, type V) at room temperature for 1 h and then for 3 h in a mixture of 40 ml of the solution described above enriched with 20 mg diaminobenzidine and 14.4 mg anhydrous dextrose. During the incubation the slices were constantly agitated on ice cubes.

The remaining slices were paraffin-embedded for light microscopy, and 7 µm sections were stained with HE and a.m. Klüver-Barrera.

Small pieces of parietal cortex from animals 7 days after infection were treated for 3 h with 2% osmium tetroxide in 0.2 M Na-cacodylate buffer, pH 7.35, at room temperature. This was followed by impregnation with 2% uranyl acetate in 0.68% maleate buffer for 1.5 h. The specimens were then dehydrated in graded ethanol and embedded in a low viscosity resin.

Survey sections were cut with glass knives, and from each block one unstained section and one stained with toluidine blue were examined in the light microscope. The mesas were cut to include the leptomeninges and upper cortical layers. Gray sections cut with a diamond knife were collected on Formvar-covered copper grids, stained with 5% uranyl acetate in 50% methanol for 30 min at 45° C followed by 2.66% lead citrate for 1 min at room temperature. These sections were examined in a Philips EM 201 electron microscope.

2-Amino[1-^{14}C] Isobutyric Acid Permeability

Three groups of animals were investigated: four animals injected i.c. with saline (control group), four untreated animals, and four animals

injected i.c. with an LCMV dose of 10^2 LD_{50}. Seven days later, the permeability of the BBB to 2-amino[1-^{14}C]isobutyric acid (AIB) was tested (Blasberg et al. 1983). Before tracer injection the animals were put into a heated cage to dilate their superficial veins. Then, with the animal in a restrain cage, 10 µCi AIB (CFA 203, 50 mCi/mmol, Amersham, England) in 0.5 ml Krebs' buffer was injected as a bolus into a tail vein.

After 10 min the animals were deeply anesthetized with chloroform, the thorax and abdomen were opened. and the heart, liver, and one kidney were removed and frozen in isopentane cooled with an acetone/solid CO_2 mixture. Finally, the brain was removed and frozen.

Cryostat sections, 20 µm thick, were cut from the brain as well as from the other organs mentioned above. The sections were collected on cover glasses and together with calibrated ^{14}C methyl metacrylate standards they exposed a Kodak MIN-R X-ray film for 24 days. After development, the tissue radioactivity was determined using a Leitz TAS plus computerized image analyzer (video-densitometry). Sections from all animals were digitized using 256 gray levels.

Since no arterial catheter was inserted, only the final arterial AIB concentration was known. Instead of calculation of the transfer factor K_{in} for AIB ($K_{in} = C^*_{br}/\int C_a T(dt)$ permeability was expressed as the distribution volume: C^*_{br}/C^*_a (10 min). For 10-min circulation periods, the relationship between this and the plasma AIB-integral is close to 0.056 (Benveniste et al. 1984).

Results

Light Microscopy

Uninfected Mice. The brains from six sham-injected and four non-injected animals showed all normal histology, especially with regard to the choroid plexus, ependyma, leptomeninges, and the vessels. There were no changes in the neuropil and no demyelination. Apart from this normal picture, the sham-injected animals showed a small cannula track in the cortex and basal ganglia. In and around the cannula track mononuclear cells and phagocytosing macrophages were seen. Otherwise, these animals displayed no changes. As to vascular permeability, the survey sections from control animals showed only slight amounts of horseradish peroxidase (HRP)-reaction product in a few capillaries. There was no leakage into the perivascular space and the parenchyma of HRP-reaction product. In the leptomeninges there were no signs of HRP extravasation either. The few remaining erythrocytes showed endogenous peroxidase reaction. The 1 µm thick plastic-embedded section confirmed these observations.

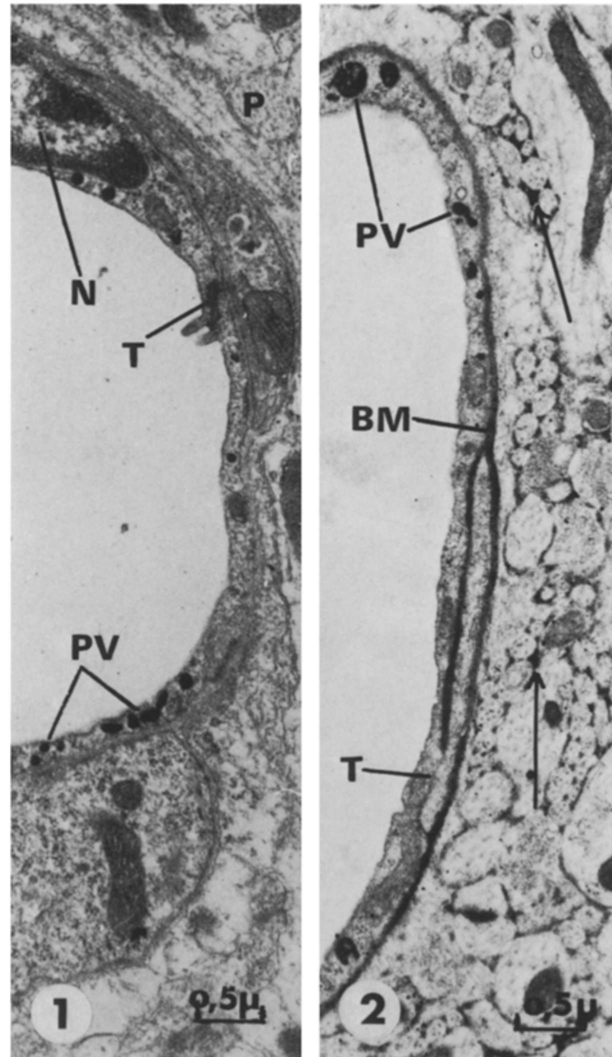


Fig. 1. Part of cerebral capillary from noninfected mouse. *T* denotes tight junction of the endothelial cell, *N* the nucleus, and *PV* peroxidase positive cytoplasmic vesicles. Pericyte cytoplasm is marked *P*. ×18,100

Fig. 2. Part of cerebral capillary from LCMV-infected mouse. *T* denotes tight junction of the endothelial cell and *PV* peroxidase positice cytoplasmic vesicles. The peroxidase reaction product is confined to the space normally occupied by the capillary basement membrane (*BM*) and to the extracellular fluid of the neuropil (*arrows*). ×18,100

Three Days After LCMV Infection. In the two animals examined, small bilateral hemorrhages and necrotic cell nuclei were seen in the central forebrain, in the vicinity of the anterior part of the subependymal region. No cell infiltration of the choroid plexus, ependyma or leptomeninges was seen, and all vessels were free from luminal and perivascular lymphocytes. No increased permeability to HRP could be demonstrated in the animals tested for BBB function.

Five Days After LCMV Infection. Two mice were examined. Small hemorrhages, in combination with infiltration of mononuclear cells, were seen centrally in the olfactory bulb or, more caudally, in the subependymal region.

At this time, lymphocytes were seen in the leptomeninges, attached to the luminal endothelial membrane and around the leptomeningeal vessels. HRP-injected animals now presented a high degree of

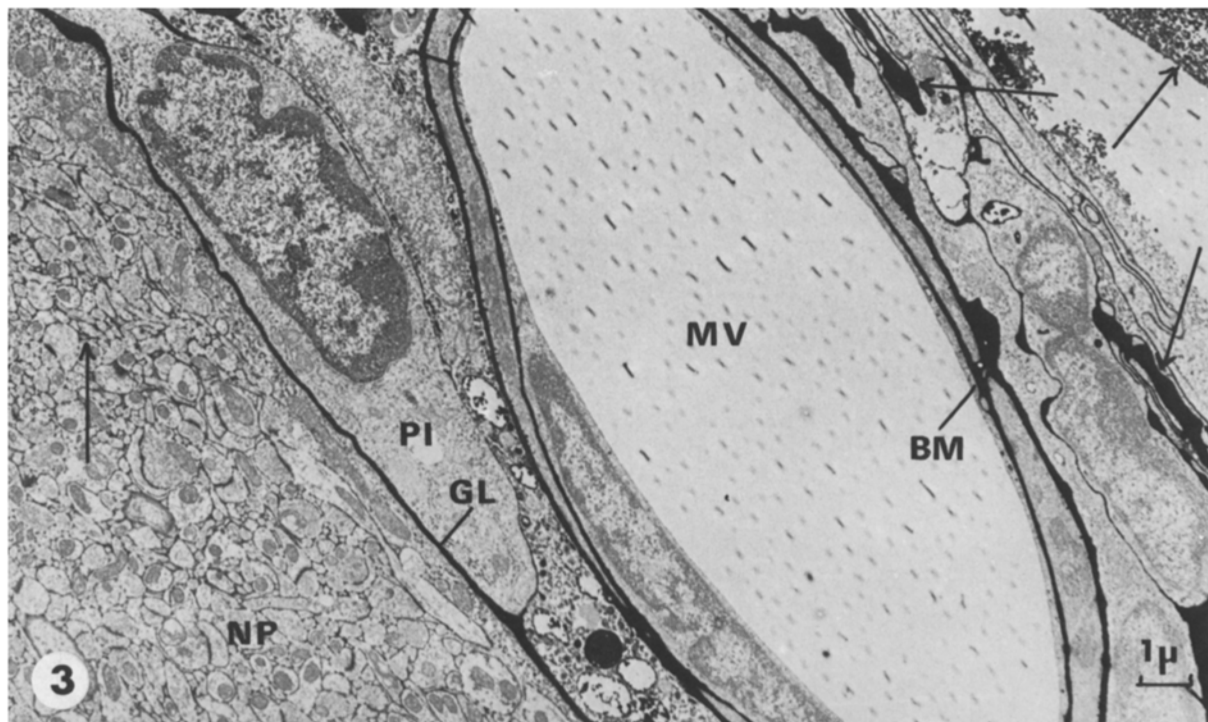


Fig. 3. Part of small blood vessel (*MV*) in the arachnoid space of infected mouse. A pial cell is marked (*PI*) and the superficial neuropil of the cortex (*NP*). The electron-dense reaction product is confined to the space occupied by the basement membrane of the vessel (*BM*), to the glia limitans (*GL*), to the extracellular fluid of the subarachnoid space, and to the neuropil of the superficial cortical layers (*arrows*). ×6,380

perivascular leakage of HRP in the leptomeninges. Few perivascular deposits of reaction product were seen in the brain tissue. In the 1 μm thick plastic sections HRP-reaction product was clearly seen to lie in the subarachnoid space together with large numbers of lymphocytes. In the neuropil, reaction product was only observed around the vessels.

Seven Days After LCMV Infection. In the ten animals examined, a pronounced accumulation of lymphocytes and larger mononuclear cells was seen in the leptomeninges and in the choroid plexus. Lymphocytes were also present in the ventricular system with a tendency to adhere to the ependyma. In some animals of this group, the affection had progressed to the brain tissue, especially to outer cortical layers, hippocampus, etc. Around the vessels and attached to the luminal endothelial membrane, lymphocytes were seen. Only a few macrophages and polymorphous nuclear cells were present. HRP-injected mice displayed the same leakage as that seen in animals 5 days after LCMV inoculation.

Electron Microscopy

Uninfected Mice. The fine structure of the leptomeninges, the arterioles, venoles, and intracortical capillaries was as that seen in normal animals. The junctions between adjacent endothelial cells always included an approximately 180 nm wide zonula occludens (tight junction). There were no inflammatory cells inside or outside the vessels. Peroxidase activity was found only inside endothelial cells where it was located in spherical or oblong, possibly tubular, organelles (Fig. 1). The tubular organelles occasionally communicated with the lumen of the vessel.

Seven Days After LCMV Infection. The leptomeninges were the site of severe inflammatory changes dominated by proliferation of pia cells and extravascular accumulation of fluid and mononuclear cells, mostly lymphocytes (Figs. 3, 4). Intravascular lymphocytes, which were adhering to and sometimes in the state of penetrating the vessel wall, were noted in most ultrathin sections (Figs. 5, 6). The ultrastructure of the endothelial cells corresponded to that of endothelial cells in normal mice, just as the tight junctions between neighboring cells were always intact (Figs. 2, 7, 8). The peroxidase activity in endothelial cells was also found in vesicular and tubular structures (Fig. 2). As compared to controls, the number of vesicles and tubules was of the same order of magnitude. Contrary to the controls, the peroxidase activity in the infected animals was also

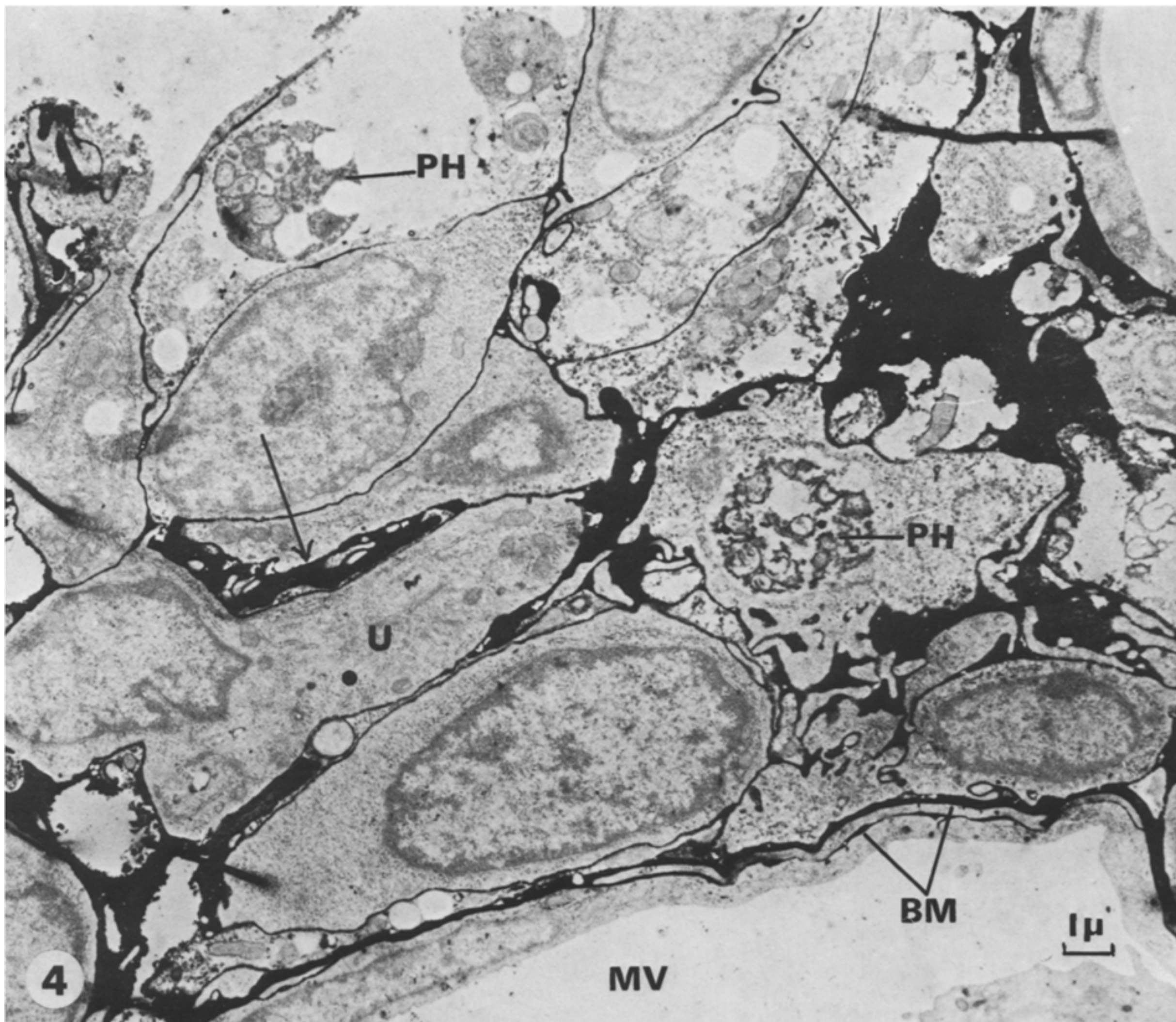


Fig. 4. Part of meningeal vessel (*MV*) from infected mouse. The cells accumulated in the subarachnoid space, are: activated lymphocytes, one of which displays an uropod (*U*), and macrophages with phagocytosed necrotic cells (*PH*). The electron-dense reaction product occurs in the space occupied by the basement membrane (*BM*), and in the extracellular fluid (*arrows*).

found outside the endothelial cells, i.e., in the extracellular space of the vessel wall and outside the vessels. In the vessel walls the activity was confined to the space normally occupied by the basal membrane surrounding pericytes and smooth muscle cells (Figs. 2–8), and it was found in the space between adjacent endothelial cells, except for the zone of tight junctions (Figs. 7, 8). Occasionally, peroxidase activity was also present as a thin coat on the luminal surface membrane of endothelial cells (Fig. 7). Outside the vessels, a strong peroxidase activity was found in the abundant intercellular fluid which was accumulated in the leptomeninges between pial cells, mononuclear cells, and lymphocytes (Figs. 3, 4), and to some extent also in the intercellular space of the most superficial cortical layers (Figs. 3, 9). Cortical endothelial cells also contained peroxidase activity in the intracytoplasmic vesicles and tubules, but in these vessels there was extracellular perivascular activity only occasionally (Fig. 2).

The inflammatory cells were located in the subarachnoid space involving the arachnoidea and pia as well, but never deeper than the basal membrane of the glia limiting the cortex. The majority of cells were lymphocytes, but phagocytosing mononuclear cells, probably also pial cells, and a few neutrophil granulocytes were always present. The mononuclear cells and pial cells could be recognised because they contained peroxidase positive cytoplasmic vesicles, which were only occasionally observed in lymphocytes. Necrotic cells and cell debris were frequently noted in the leptomeninges (Figs. 3, 4). Virus-like particles were never observed. Single lymphocytes or clusters of

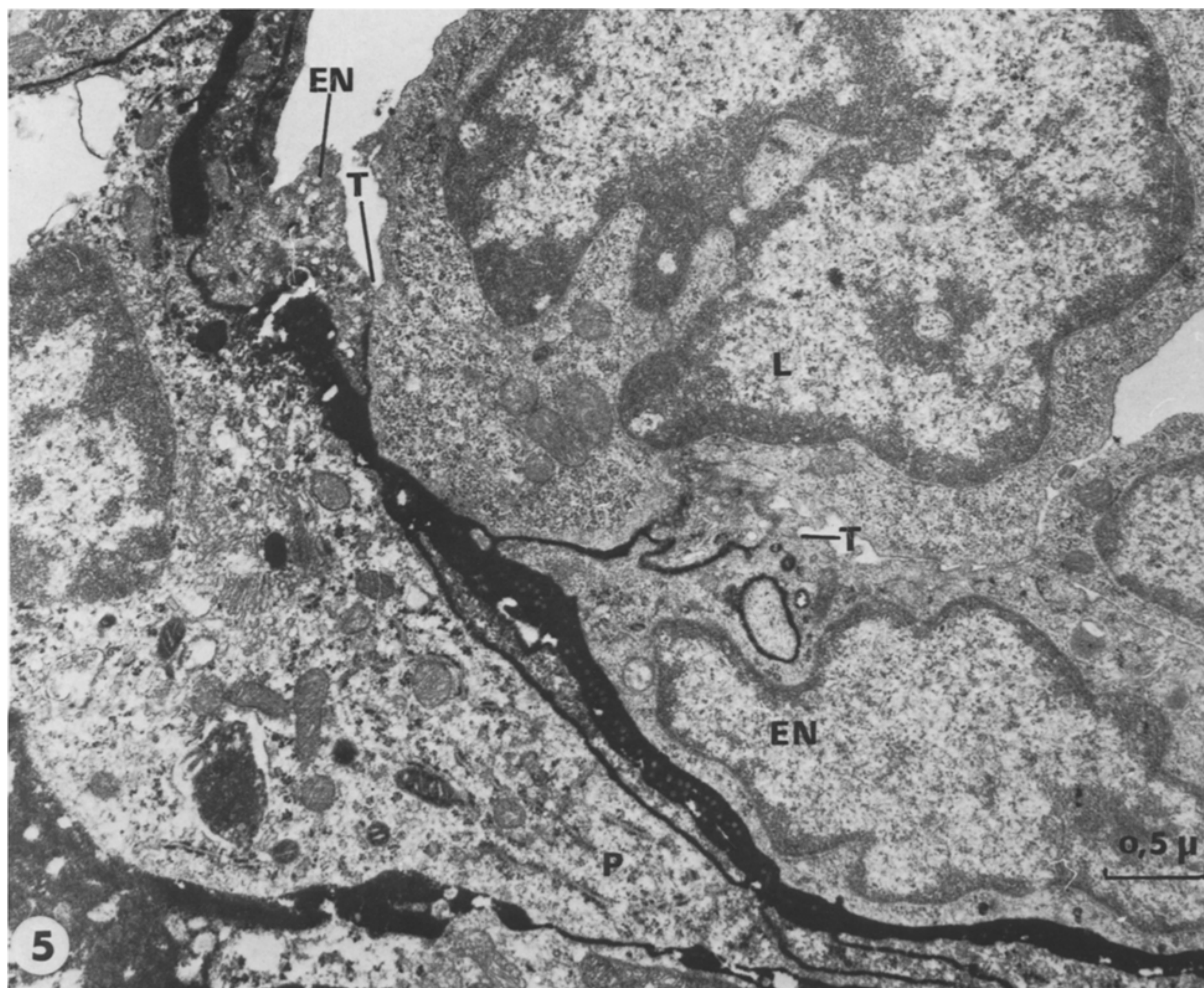


Fig. 5. Infected mouse. A lymphocyte (*L*) is seen to penetrate the endothelial cell (*EN*) of a meningeal vesel. A tight junction (*T*) is formed between the lymphocyte and the endothelial cell. Peroxidase reaction product in present in the space occupied by the basement membrane surrounding the proliferating pericyte (*P*). ×27,800

lymphcytes, which were attached to the luminal surface of endothelial cells, were noted in all specimens from infected animals (Figs. 5, 6). They were of the type "small" lymphocytes, having an eccentrically placed nucleus and few cytoplasmic organelles, such as mitochondria, a small Golgi region, and a varying number of empty-looking vesicles. Some of these lymphocytes had a fully developed uropod. Lymphocytes penetrating the vascular wall were noted frequently. At the site of penetration there were always tight junctions formed by the opposed membranes of adjacent cells (Fig. 5).

Quantitative Autoradiography

As shown in Table 1, there were no statistically significant differences between the AIB concentrations in kidney, heart, and left ventricle blood from the three experimental groups. A large spread in values for liver tissue was observed: two animals had concentrations about 1,500 nCi/g, and two had values within normal range.

From the digitized pictures of the brain autoradiograms it was observed that except for subpial and perichoroidal tissue, the brain concentrations of AIB were not elevated in either controls or infected mice. If the blood concentrations were subtracted from the brain concentrations, a significant increase in area as well as in content of AIB was observed in the LCM animals as compared to the controls and sham-injected animals (Fig. 10).

Discussion

The fatal outcome of murine LCMV-induced choriomeningitis is fundamentally dependent of the T-cell competence of the host animal (Cole et al. 1972). It is

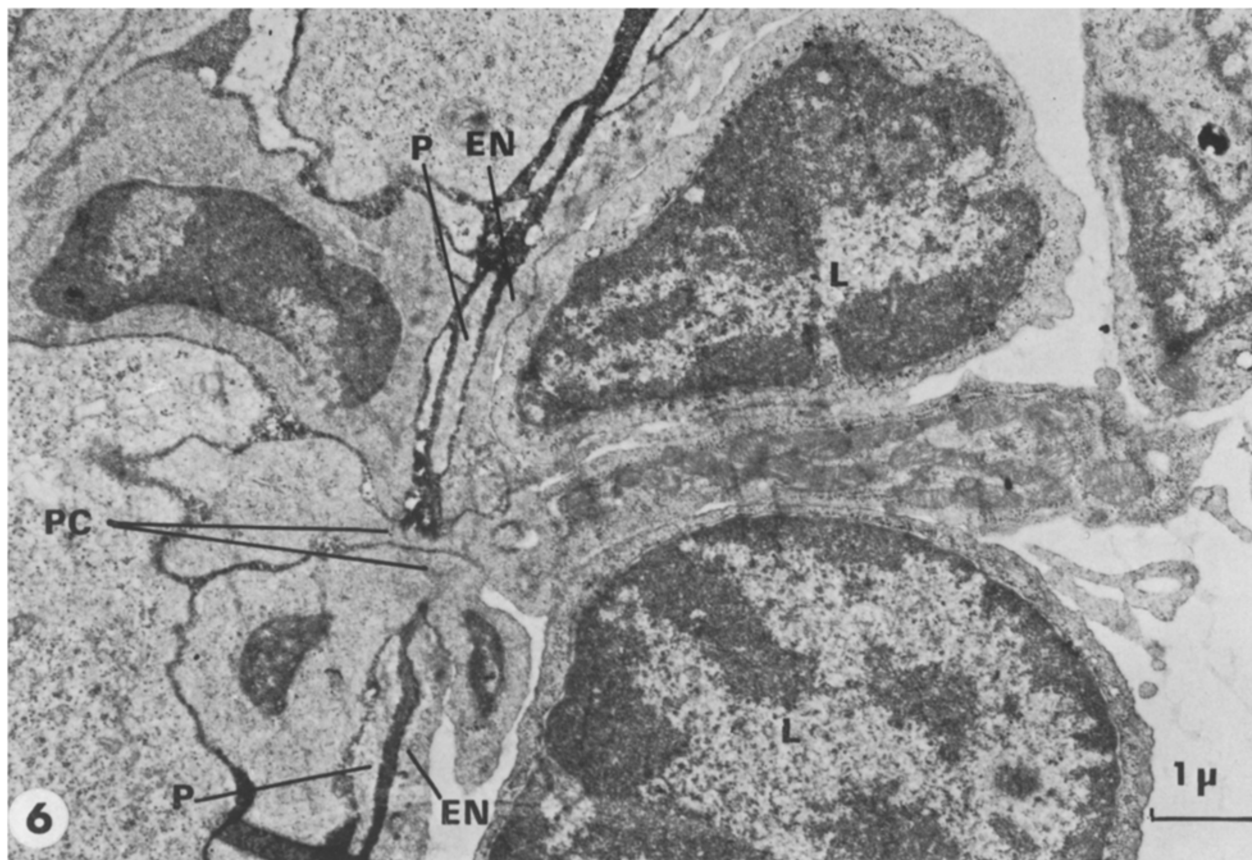
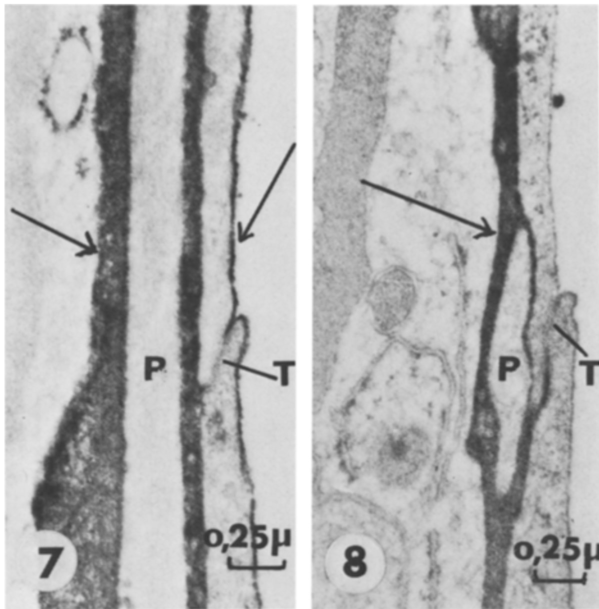


Fig. 6. Two cells penetrating the wall of a meningeal vessel in an infected mouse. Endothelial cell cytoplasm and pericyte cytoplasm are marked *EN* and *P*, respectively. *L* denotes lymphocytes inside the vessel and *PC* the cytoplasm of two cells penetrating the wall. ×13,700

generally accepted, therefore, that the pathogenesis of the LCM disease involves an immunologically specific conflict, whereas the pathologic and pathophysiologic consequences remain obscure.

Using a quantitative auroradiographic method for the study of the BBB function based on the vascular permeability for i.v. injected ^{14}C-labeled AIB (Blasberg et al. 1980) and a qualitative LM and EM method demonstrating the extravasation of the protein-bound HRP (Westergaard 1977), we have clearly shown an abnormal function of the BBB in the leptomeninges during acute LCM disease. The question whether the increased vascular permeability is accomplished by opening of tight junctions between epithelial cells, as is seen with HRP after hyperosmolar mannitol infusion (Nagy et al. 1979), or by enhanced vesicular transport across the cerebral endothelium, as is described in different experimental conditions (Westergaard 1977; Vorbrodt et al. 1981) is not clearly answered in our study. It should be emphasized, however, that there were neither signs of necrosis of vascular endothelial cells nor extravasation of erythrocytes in the preparations from the diseased animals. This, in connection with the finding that the distribution of endothelial vesicles showing peroxidase activity was the same and their number only to a minimal extent increased in the virus-inoculated mice as compard with the controls, might suggest an opening of tight junctions.

Using radioiodinated bovine serum albumin (BSA), Doherty and Zinkernagel (1974) obtained results supporting their theory of cerebral edema in LCM disease. They also reported that i.v. injected Evans blue penetrates into the brain parenchyma, and they described obliteration of the foramen magnum and narrowing of the lateral ventricles eventually resulting from the postulated brain edema. However, their calculations on and interpretations of the experiments with BSA have recently been questioned seriously. With only small amounts of blood remaining in the cerebral vessels, their results could have been obtained without any dysfunction of the BBB (Camenga et al. 1977). Furthermore, attempts to demonstrate edema by comparing wet to dry weight ratios of brains from LCMV-infected animals and controls failed to reveal differences (Camenga et al. 1977). Our experiments with the murine LCM model clearly demonstrate a leakage of



Figs. 7, 8. Tight junctions (*T*) of endothelial cells are devoid of peroxidase reaction product. This product can be found on the luminalol surface of the endothelial cell and in the space occupied by the basement membranes (*arrows*). *P* denotes pericytes. ×27,800

the meningeal vessels, a leakage which was present in the brain parenchyma only to an inappreciable extent. The presence of intercellular HRP reaction product observed by us in the most superficial layers of the cortex can therefore most probably be explained as the result of diffusion of the tracer from deposits in the subarachnoid space. Moreover, the cortical HRP gradient observed in our preparations is found to be in accordance with the diffusion rate for this compound as determined earlier (van Deurs et al. 1978). Also, a distension of the extracellular space gradually extending from the vessels in the neuropil, as demonstrated after cold lesions in rats and cats (Ikuta et al. 1983), has not been observed in our preparations.

Two main explanations of the death of mice suffering from LCM disease are (1) the existence of brain edema with concomitant compression of the brain and brain stem and (2) an epileptiform seizure disorder leading to compromised blood circulation and brain anoxia (Walker et al. 1977).

Our results do not support the first of these explanations, and even though the animals often die during convulsive seizures, these seizures seem to be a symptom rather than the actual cause of death. Thus, Walker et al. (1977) published experiments in which anticonvulsant treatment prolonged the survival of the diseased animals by about 2 days. These results were easily confirmed in our laboratory. With diazepam in doses of 5 mg/kg b.w. twice a day, survival was prolonged, convulsions were prevented, and yet death inevitably occurred (Marker, unpublished results). This outcome contrasts with the theory of the epileptiform seizures being the direct cause of death in lymphocytic choriomeningitis.

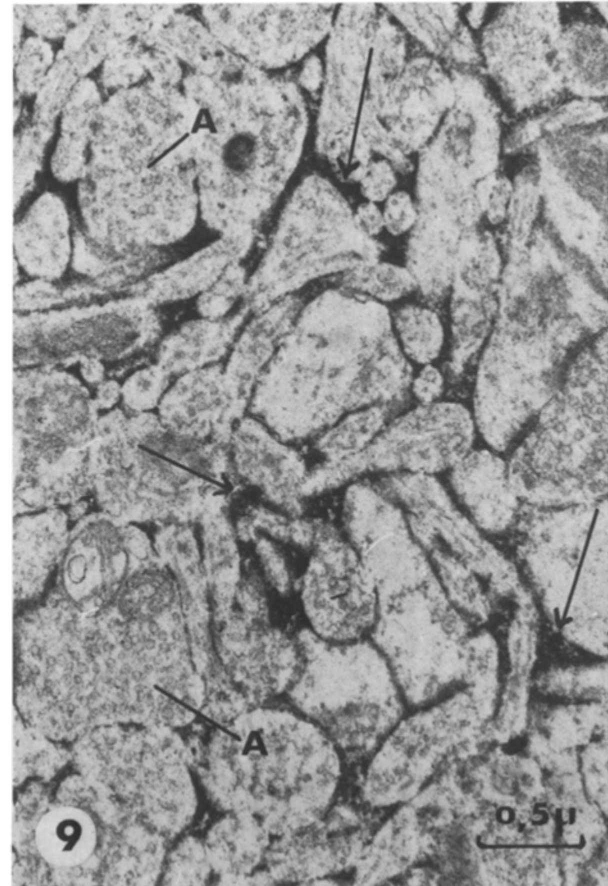


Fig. 9. Neuropil from the most superficial cortical layer from infected mouse. *A* denotes axon terminal with synaptic vesicles. Peroxidase reaction product is located in the extracellular space only (*arrows*). ×27,800

Table 1. Autoradiographic determination of ^{14}C-AIB concentration (nCi/g) in liver, kidney, heart, and left ventricle blood

	Liver	Kidney	Heart	Left ventricle blood
A[a] ($n = 4$)	561 ± 37	1,444 ± 60	130 ± 32	130 ± 24
B[b] ($n = 4$)	973 ± 540	1,442 ± 94	95 ± 34	176 ± 40
C[c] ($n = 4$)	574 ± 79	1,378 ± 70	162 ± 31	165 ± 19
	NS	NS	NS	NS

[a] Controls
[b] Injected intracerebrally LCM virus
[c] Sham-injected
NS = Not significant

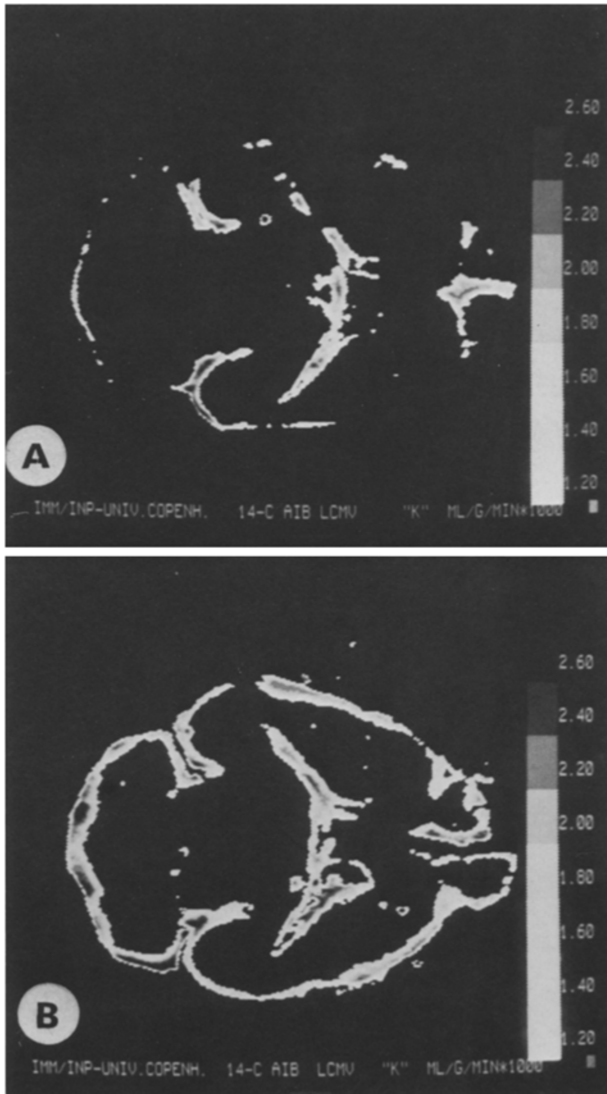


Fig. 10A, B. ^{14}C-AIB autoradiograms of horizontal sections of the mouse brain. **A** Control brain. **B** LCMV – 7 days after infection. There is increased permeability to AIB in all leptomeningeal regions and around the choroid plexus. Scale units represent value of "K_{in}", the transfer for constant (ml/g/min × 1,000) × 7.5

Our findings of intravascular lymphocytes attached to the luminal surface of the endothelial cells in brains of mice suffering from LCM disease has, to our knowledge, not been reported before, although it has been demonstrated in experimental allergic encephalomyelitis (Lampert and Carpenter 1965; Åström et al. 1968), and following mechanical ablation of the cortical projections of thalamic nuclei (Matthews and Kruger 1973).

In our experiments, such lymphocytes were not only seen to be attached to endothelial cells but also to penetrate the vascular wall, always by forming tight junctions with the opposed membranes of adjacent cells. The cells were small lymphocytes, and their general appearance was compatible with that of T cells. Lymphocytes of varying morphology have been observed very recently penetrating vascular endothelium in LCM-diseased mice (Schwendemann et al. 1983). The compatibility of the adhering and penetrating lymphocytes with T cells observed in our preparations was even more striking since some of the cells had a fully developed uropod. It should be stressed, however, that an immunochemical identification has not yet been carried out. On the other hand, the cellular exudate in LCM-diseased mice has been shown to be rich in virus-specific cytotoxic T cells (Zinkernagel and Doherty 1973).

It is also noteworthy in this context that LCM virions have been demonstrated in great numbers in choroidal epithelial cells of such animals, and that necrosis of these cells was present although LCMV is known to be a predominantly non-cytopathogenic agent (Walker et al. 1977; Schwendemann et al. 1983).

Since all the animals in our experiments have been initially fixed by perfusion, and the vessels were therefore generally empty, the observed attachment of lymphocytes to the endothelial surface must be rather firm. Therefore, the question naturally arises as to whether this attachment is of an immunologically specific nature. Recently, a hypothesis has been advanced which suggests that the vascular endothelial cells play a central role in the development of cell-mediated immune reactions, and that interaction between sensitized lymphocytes and antigen-presenting endothelial cells is an initial phenomenon which leads to the production of different lymphokines mediating the accumulation of cellular exudate and local inflammation (Burger et al. 1981; Burger and Vetto 1982).

In their search for an explanation of the adhering white cells to the endothelial cells in inflammation, Wisniewski et al. (1983) demonstrated the presence of viral antigen on the luminal surface and in the cytoplasm of endothelial cells. These findings were made in ferrets infected with an SSPE strain of measles virus, and the authors suggest that the antigen in the endothelial cells may be the site of interaction with sensitized lymphoid cells.

In this context it may be of interest that recent, but unpublished, experiments in our laboratories do show the presence of LCMV antigen in the cytoplasm of endothelial cells in some small vessels in virus-infected areas of the brain.

The extension of our knowledge of the basic pathogenesis in the LCM disease presented by Walker and coworkers (Walker et al. 1977; Camenga et al. 1977) raises objections to some previously advanced

theories, while it is in accordance with others. Their findings do not support the hypothesis of the generation of brain edema, and by treating the diseased animals with anticonvulsant drugs they obtained results which were confirmed by us, and which contrasted with the idea of convulsive seizures being the cause of death. However, they demonstrated numerous undifferentiated mononuclear cells in the choroid plexus, huge amounts of LCMV virions in the epithelial cells of the plexus, and frequent necrosis of these cells. Since they also claim that there was no correlation between the amount of virus antigen in the cells on one side, and cell damage on the other, these latter findings are in complete accordance with the hypothesis that cytotoxic cells destroy antigen-presenting cells of the choroid plexus and thus disturb the regulation of the composition of CSF (Doherty and Zinkernagel 1974; Marker et al. 1976; Schwendemann et al. 1983).

Our findings do not speak in favor of brain edema to any appreciable extent either. However, the demonstration of mononuclear cells, morphologically compatible with T cells, attached to the luminal surface of the endothelial cells of the vessels in the meninges, penetrating the vascular wall, and constituting a predominant part of the cellular exudate is in line with the above theory.

On the other hand, we have clearly shown the presence of a new element in the pathogenesis of the LCM disease, i.e., a very pronounced increase in vascular permeability reflecting a serious BBB dysfunction. This pathologic condition may very well lead to an unphysiologic or even fatal composition of the CSF, much more effectively and rapidly than a T-cell-mediated injury of the epithelial cells of the plexus choroideus. The apparently general extent of the increased vascular permeability demonstrated in the meninges may suggest a diffusible mediator. One or more lymphokines could be secreted by T cells after specific binding to the LCMV antigen, and the presence of activated macrophages and neutrophils might speak in favor of this possibility.

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