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Supraependymal Cells of Hypothalamic Third Ventricle: Identification as Resident Phagocytes of the Brain

Abstract. *Cells lying on the ventricular surface of the hypothalamic ependyma of the tegu lizard exhibit the pseudopodial and flaplike processes characteristic of macrophages found elsewhere. Since they ingest latex beads, they may be considered a resident phagocytic system of the brain. The importance of ependyma and ventricular phagocytes as a first line of defense against viral invasion of the brain, as well as their role in the pathogenesis of certain virus-related diseases, is suggested by a number of experimental and clinical observations.*

Recent studies of the ventricular surface of the hypothalamus, with the scanning electron microscope, have revealed cells lying free upon the ependyma (*1*). The cells described in these studies were found in both the supraoptic and infundibular recesses of the third ventricle in several species—monkey, cat, mink, rat, and rabbit—but their function was left an open question. The ultrastructural characteristics of a cluster of large cells on the wall of the infundibular recess of the rat suggested to some workers that the cells were neurons (*2*).

One of us (*3*) has found supraependymal cells throughout the major ciliated portion of the third ventricle of the mouse as well as in the nonciliated recess region. These cells exhibit a variety of forms, have one or more pseudopodial processes extending over the adjacent ependyma, have a usual size of 8 to 10 μm (overall range, 5 to 20 μm) and vary widely in number (from few to 20 to 30) and distribution from one brain to another. The association of clusters of these cells with mounds of debris in the mouse suggested that the cells may have a phagocytic function.

This report describes the phagocytic activity of supraependymal cells in the hypothalamic third ventricle of the tegu lizard, *Tupinambis nigropunctatus*, in response to the presence of latex beads injected into the third ventricle and also included in a culture medium. The tegu lizard was used in this study, since we have found that large numbers of supraependymal cells (50 to 150) are predictably located on a nonciliated vertical depression of the third ventricular wall (Fig. 1a). This depression corresponds to the ventricular ependymal organ or paraventricular organ previously described with light microscopy (*4*).

For the study of normal morphology with the scanning electron microscope, the brains of one female and one male tegu lizard were rapidly removed under sodium pentobarbital anesthesia and immersed in a 1.5 percent phosphate-buffered glutaraldehyde fixative (*p*H 7.4). The ventricles were opened immediately to permit rapid fixation, and diencephalic blocks were dissected while submerged in fixative. The blocks were fixed over 24 hours and dehydrated in ethyl alcohol (through a graded series up to 100 percent), which was then replaced with 100 percent amyl acetate. The blocks were critical point dried with liquid CO_2 and coated with gold-palladium in a vacuum evaporator (*5*). Cells were examined and photographed with the use of a JEOL U-3 scanning electron microscope at an accelerating voltage of 20 kv and a usual tilt angle of 20° to 30°.

To investigate phagocytosis, 25.0 μl of Bacto-latex beads, 0.81 μm in diameter (Difco Laboratories, Detroit, Michigan), were injected into the third ventricle of the anesthetized tegu lizard during direct visualization of the roof of the third ventricle. After 15 minutes the brain was removed. One side of the third ventricle was placed in fixative and the other side was placed in a dish of tissue culture medium NCTC-109 (Microbiological Associates, Bethesda, Maryland) to which several drops of the latex bead solution were added. After 15 minutes at room temperature, the incubated side was also placed in fixative. Subsequent fixation, dehydration, and drying followed the procedures described above for the normal controls.

In all specimens examined, supraependymal cells lay scattered singly and in clusters over the ventricular ependymal organ and were seen rarely on the larger ciliated surface of the third ventricle. In brains not exposed to latex beads all the cells were somewhat flattened, ranged in size from 8 to 10 μm, and had a relatively smooth cell body surface, that is, without folds or microvilli (Fig. 1b). All cells had several cytoplasmic extensions over the surrounding ependymal surface. Some extensions were long, narrow, tapering, and branched, while others were broad flaps with occasional branching extensions. The appear-

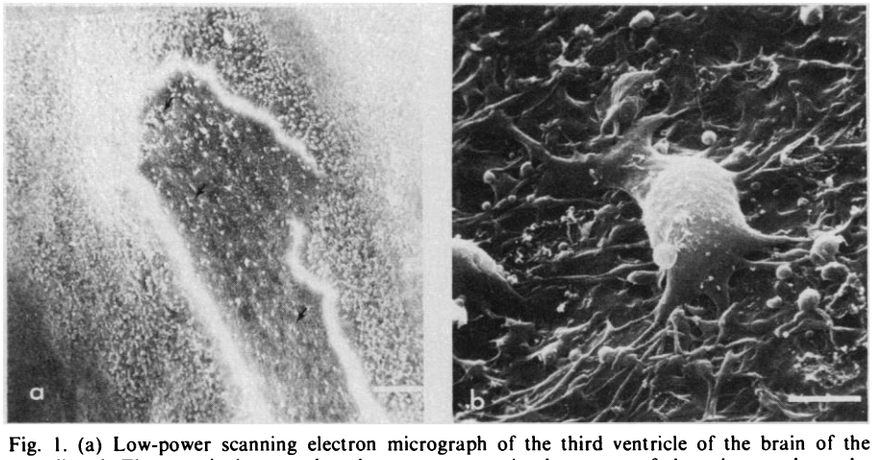

Fig. 1. (a) Low-power scanning electron micrograph of the third ventricle of the brain of the tegu lizard. The ventricular ependymal organ appears in the center of the micrograph as the vertical nonciliated region with a brightly reflecting border. Many supraependymal cells can be seen lying on this organ singly and in clusters (arrows). Cilia are visible on the rest of the ependymal surface. Bar represents 100 μm. (b) Supraependymal cell lying on the ventricular ependymal organ, showing many tapering branching pseudopodial extensions and one broad cytoplastic flap which also branches. The spherical structure lying on the cell body may be a platelet. Bar represents 5 μm.

ance of the cells was similar to that of phagocytic cells reported in other studies (*6*) and similar to some of the supraependymal cells seen in the mouse third ventricle. The ependymal floor on which the cells lie has few cilia and is covered with a network of structures which are identical in appearance with the cytoplasmic processes and flaps of the supraependymal cells.

After exposure to latex beads, the activated cells appeared rounded and developed stubby surface folds and microvilli. Similar cells were not seen in control brains. Latex beads were observed in the process of being phagocytosed, and cell surfaces appeared bumpy as a result of internalized latex particles (Fig. 2). When the region between a cell and bead could be visualized, it could be easily determined whether the bead was beneath the cell membrane or merely lying on the cell surface. The cell body diameter of the phagocytes ranged between 8 and 10 μm, with occasional elongated cells measuring up to 12 μm. Phagocytic cells were found on both sides of the ventricle, that is, after either injection into the ventricle or after injection plus incubation, and constituted between 10 and 25 percent of supraependymal cells. The absence of the large, oval, easily recognizable erythrocytes of lizard blood makes it highly unlikely that the phagocytes viewed were derived from blood leukocytes introduced at the time of the injection.

The results indicate the presence of supraependymal cells with phagocytic properties. Those neither incorporating latex beads nor changing their surface morphology may be inactive phagocytic cells (under the conditions of this experiment) or another cell type such as neurons or glia.

We propose that the ventricles of the brain, like certain other tissues or organs—liver, lung, spleen, peritoneum—have a resident phagocytic system. The association of these cells with mounds of debris in the mouse and their capacity to phagocytose latex beads introduced into the ventricular system of the tegu lizard suggest at least two functions: removal of materials (for example, secretory material and dying cells) resulting from the normal metabolic activities of ependyma and also the ingestion of foreign particles.

Strong experimental evidence for the latter has recently appeared (*7*). Two days after the intracerebral inoculation of mumps virus into suckling hamsters, viral nucleocapsids were visualized first in the ependymal and choroid plexus epithelial cells but not in neurons. On day 4 of infection membrane-limited viral nucleocapsids were found in macrophages lying on the ventricular surface of the ependyma.

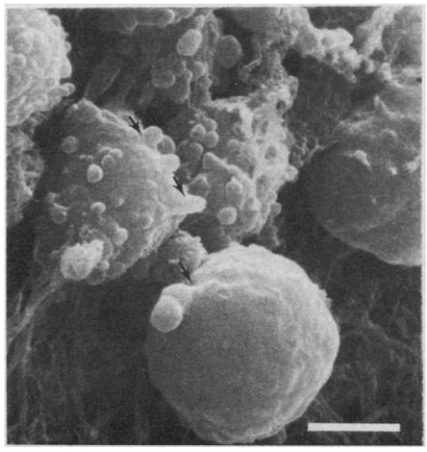

Fig. 2. Supraependymal cells on the ventricular ependymal organ fixed for scanning electron microscopy 30 minutes after injection of latex beads into the third ventricle of the anesthetized lizard. Fifteen minutes after the ventricular injection, the block bearing the third ventricular wall was incubated in culture medium with latex beads for 15 minutes. Latex beads can be seen attached to, and in the process of phagocytosis by, the cells (arrows). Irregular or "bumpy" cell surfaces (see cell at lower edge of micrograph) are due to the presence of internalized latex particles. Bar represents 5 μm.

When nonneuroadapted strains of mumps virus were used, the viral particles remained restricted to ependyma, choroid epithelium, and macrophages and disappeared after 8 days. With neuroadapted strains virion formation by budding from the surfaces of these cells was seen and virions were also found within the macrophages as well as within neurons both adjacent to and distant from infected ependyma.

While Wolinsky *et al.* (*7*) appear to be the first to have noted actively phagocytosing cells on the ependymal surface of infected animals, it had been known that, following inoculation by various routes, certain viruses—mumps, influenza A, parainfluenza 2, measles, Ross River—invade and infect ependyma before the rest of the brain and, depending on host age, virus dose and strain, and other factors, may consistently cause generalized ependymitis, desquamation of ependymal cells, stenosis of the aqueduct of Sylvius, and hydrocephalus (*8*). Experimental data suggest that in natural infections a major pathway of virus spread to the brain is from the blood into cerebrospinal fluid through the choroid plexus (*9*).

It thus appears that in some virus infections, the ependyma and supraependymal macrophages constitute a first line of defense against neuronal invasion, probably both by direct inactivation of viruses and by immobilizing them until specific antibodies are formed, as has been demonstrated for macrophages which monitor other body compartments—blood, lymph, peritoneal cavity—and control entry of viruses to other organs—lung, liver, spleen (*10*). The role of macrophages has been further clarified by work demonstrating that the genetically determined susceptibility of certain strains of mice as well as the age-dependent susceptibility of newborn mice to certain virus infections (hepatitis, yellow fever, B arboviruses, herpes, influenza) is related to properties of macrophages. In susceptible animals, the peritoneal or hepatic (depending on the route of inoculation) macrophages have been shown to support viral growth and multiplication and subsequent spread to parenchymal cells, whereas in the nonsusceptible host, virus becomes inactivated within the macrophages (*11*).

The potential significance of these findings for human central nervous system disease processes is clear in view of a number of clinical observations. Herndon *et al.* (*12*) have reported finding with electron microscopy ependymal cells with cytoplasmic inclusions of paramyxovirus-like nucleocapsid material in the cerebrospinal fluid of each of six patients with mumps virus meningitis, thus establishing that mumps virus in humans may cause infection and shedding of ependymal cells. They review the reports of five children in whom hydrocephalus developed following mumps infection. In a survey of 100 consecutive adult human brains, Johnson and Johnson (*13*) found that 65 percent had granular ependymitis resembling that found in experimental animals. These findings suggest that ependymitis, usually without serious sequelae, may be a not uncommon complication of mumps or other ordinary virus infections. The importance of age-dependent susceptibility for serious consequences of virus infections in humans is indicated by the destructive effects on the brain that may result from fetal and perinatal infections with rubella, herpes-simplex, group B Coxsackie, and Eastern and Western equine encephalomyelitis viruses and cytomegalovirus (*14*).

Thus, while in some virus infections ependyma and macrophages will inactivate and immobilize virus and prevent encephalitis, in others these cells may permit growth and replication of virus with subsequent development of acute encephalitis as well as of slow or chronic infections. Furthermore, ependyma and macrophages may provide a reservoir for prolonged replication of virus, a process which may be asymptomatic, as in the cases described by Johnson and Johnson (*13*), or may have serious sequelae. Allison (*15*) has found vaccinia virus persisting in macrophages for

periods up to 1 year after experimental infection, and suggests that the persisting viral antigen within macrophages may provide the stimulation for continuing antibody formation in states of prolonged or lifelong immunity to such virus diseases as smallpox, measles, and yellow fever.

In view of the recently demonstrated association between virus infections and demyelinating diseases in human beings and laboratory animals (*16*), however, we may suspect also a pathogenetic role for ependyma and macrophages. While in some diseases the demyelination appears to be the result of a direct cytopathic effect of virus upon oligodendroglial cells, in others immunopathological mechanisms appear to be responsible for myelin destruction. As reservoirs for viral replication, ependyma and macrophages may provide antigenic stimulation for continuing antibody formation as well as a continuing supply of virus for infecting and altering oligodendroglia and myelin membranes.

Investigation of macrophage function thus far has been done mainly on peritoneal macrophages, since they are easily accessible in large numbers and also because a resident macrophage system of the ventricles of the brain has not heretofore been described or proposed. Our findings, taken together with information accumulating about the pathological sequelae of viral invasions of the brain and about macrophage-viral infections in other tissues, indicate another host system requiring further investigation in the attempt to understand pathogenetic mechanisms of certain acute and chronic central nervous system diseases (*17*).

RUTH BLEIER
RALPH ALBRECHT
JUDITH A. F. CRUCE*
Departments of Neurophysiology and *Bacteriology, University of Wisconsin, Madison 53706*

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* Present address: Department of Anatomy, Johns Hopkins School of Medicine, Baltimore, Maryland.

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Strain Differences During Intraventricular Infusion of Norepinephrine: Possible Role of Receptor Sensitivity

Abstract. *Two rat strains previously shown to differ with respect to behavioral activity, regional brain tyrosine hydroxylase activity, and norepinephrine-elicited accumulation of adenosine 3′,5′-monophosphate exhibited differential behavioral responsiveness during the intraventricular infusion of norepinephrine. The results are interpreted in terms of differential catecholamine receptor sensitivity.*

Recently we reported an inverse relationship between the behavioral activity of several strains of rats and their respective levels of midbrain and neostriatum tyrosine hydroxylase activity (*1*). This relationship appeared to conflict with the alleged role of central catecholamines (CA) in behavioral arousal (*2, 3*). However, we proposed a possible explanation for this apparent disparity based on the findings that experimental manipulations alleged to impair adrenergic transmission augment central catecholaminergic biosynthetic capacity (*4, 5*) and the converse (*6, 7*). On the basis of these results we suggested that, for the strains examined, both enzyme activity and behavior are influenced by the level of adrenergic transmission. That is, the strain having a high level of functional transmission would manifest relatively reduced enzyme activity and elevated behavioral arousal. The opposite would be expected for a strain with comparatively lower synaptic activity. We further speculated that the primary factor responsible for the differences in transmission might be receptor sensitivity (*1*).

Skolnick and Daly (*8*) tested this hypothesis by measuring norepinephrine (NE)-elicited accumulation of adenosine 3′,5′-monophosphate (cyclic AMP) as an index of receptor sensitivity. Evidence suggests that cyclic AMP is intimately associated with neuronal transmission in both the central (*9*) and peripheral (*10*) nervous systems, and in particular that an adenylate cyclase system may, in fact, be the adrenergic receptor itself (*11*). Comparing the combined midbrain-striatal slices of four strains, Skolnick and Daly found a high positive correlation between NE-elicited accumulation of cyclic AMP and the levels of behavioral activity obtained in our study ($r = .99$; $P < .001$). These results support our hypothesis of a direct relationship between the behavioral activity of the various strains and their corresponding adrenergic receptor sensitivity.

We also studied the effects of intraventricular infusion of NE and reported that NE produces a dose-related increase in the behavioral activity of rats (*3, 12*). To ascertain the mechanism by which NE exerts this behavioral effect, we examined the NE-induced behavioral activation under various pharmacological conditions (*13, 14*). Prior treatment with 6-hydroxydopamine (alleged to selectively destroy central CA neurons) markedly potentiated the response to infused NE. Prior treatment with desmethylimipramine (reported to prevent the uptake inactivation of NE) did not alter the effects of NE on behavior. These results suggest a postsynaptic rather than presynaptic mediation of the NE-induced behavioral activation (*13*). Additional evidence for this conclusion stems from the results obtained with intraventricularly infused amphetamine, a drug believed to affect behavior through the release of CA's. The behavioral excitation elicited by amphetamine appears to be de-