Pathogenesis of murine toxoplasmic hydrocephalus

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SUMMARY

The prevailing hypothesis concerning the pathogenesis of toxoplasmic hydrocephalus alleges that (a) parasites invade and destroy the ependymal lining of the lateral ventricles, followed by (b) the sloughing of masses of degenerating ependymal and inflammatory cells leading to obstruction of the ventricular foramina and aqueduct of Sylvius, thereby initiating the hydrocephalus. Our observations in chronically infected mice indicate otherwise. Parasite invasion of the ependyma was not detected; the intraventricular masses of cellular 'debris' contained neither ependymal nor inflammatory cells; and obstruction of the ventricular foramina and/or aqueduct was not seen. As an alternative hypothesis, we suggest the development of hydrocephalus in the infected mice was consequent to severe leptomeningeal inflammation blocking the subarachnoid space and impeding the resorption of cerebrospinal fluid by the arachnoid villi. Narrowing of the aqueduct of Sylvius, when present, was adjudged the result, not the cause of the hydrocephalus, due to compression of the midbrain by the enlarging lateral ventricles.

Key words: *Toxoplasma gondii*, hydrocephalus, meningitis, pathogenesis.

INTRODUCTION

Nya:NYLAR female mice, infected with the Cornell (CS) strain of *Toxoplasma gondii*, develop a progressive wasting disease characterized by pronounced cachexia and numerous physical, physiological, and pathological deficits. During the course of the wasting disease, many pathological changes were detected in the infected Nya:NYLAR mouse brains, particularly in and around the lateral ventricles (Stahl & Turek, 1988; Stahl, Kaneda & Noguchi, 1994). Of these, 'granular ependymitis', intraventricular masses of 'sloughed ependymal and inflammatory cells', periventricular oedema, and ventricular dilatation were conspicuous and frequent findings. It should be noted that in cases of human congenital toxoplasmosis identical pathological changes were described by Wolf & Cowen (1937), Paige, Cowen & Wolf (1942), Callahan, Russell & Smith (1946) and Frenkel & Friedlander (1951); and much of their terminology (in quotation marks, above) was used to describe and characterize our findings.

A presumptive pathogenesis of human congenital toxoplasmic hydrocephalus was formulated by Frenkel (1949) and Frenkel & Friedlander (1951). Frenkel's hypothesis, in brief, is that the ventricular changes begin with the invasion and focal destruction of the ependymal layer by parasites. An ensuing ependymitis then leads to the formation of an ependymal and inflammatory exudate which is sloughed into the ventricles, causing blockage of the foramina of Munro and stenosis of the aqueduct of Sylvius, and the onset of hydrocephalus. However, when this pathogenic outline was applied to our murine model, numerous discrepancies emerged and Frenkel's hypothesis and many of the 'borrowed' descriptive terms had to be revised because (a) neither parasites nor infected cells were detected in the ventricular ependyma, (b) the 'granular ependymitis' proved to be neither an ependymitis nor granular, (c) the masses of 'sloughed inflammatory and ependymal cells' within the ventricles were found to be neither inflammatory nor ependymal in origin and (d) neither the aqueduct of Sylvius nor the ventricular foramina revealed evidence of blockage.

In this paper, we present an updated interpretation of the above-mentioned and other neuropathological observations that have received surprisingly little attention since their original description years ago, and discuss their origin, pathological significance, and putative role in the development of toxoplasmic hydrocephalus.

MATERIALS AND METHODS

Mice

Female Nya:NYLAR albino mice were obtained from an outbred closed colony maintained by the Wadsworth Center for Laboratories and Research, New York State Department of Health. The NYLAR strain originated in 1930 from a single pair

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of albino mice (of unknown origin), and has since been propagated by sibling mating and systematic breeder rotation. Following parabiosis, neither rejection nor intoxication is seen, indicating that the colony is compatible at the H-2 locus (Benson & Abelseth, 1977).

In the present study, a total of 192 mice was used, 120 infected and 72 controls. At biweekly intervals, 10 infected and 6 controls were selected at random for necropsy. Only female mice were used, to avoid morbidity from fighting and to eliminate possible sex-associated differences in host response. At the time of infection, the mice were approximately 10–12 weeks of age and weighed from 18 to 22 g.

Toxoplasma

The Cornell (CS) strain of *T. gondii*, originally obtained from Dr A. Kimball, Cornell University School of Medicine, New York, was maintained in the cyst stage in the brains of chronically infected Nya:NYLAR stock mice. To obtain the large number of cysts necessary for this study, 6 chronically infected Nya:NYLAR stock mice were killed by cervical dislocation and their brains emulsified individually in 4·0 ml of saline in a glass tissue-grinder. The 6 emulsions were pooled and mixed thoroughly. Drops of emulsion were placed on glass slides, cover-slipped, and the number of cysts counted under low power ($\times 100$) in a compound microscope. The volume of emulsion was adjusted with saline to yield the standard mouse-infecting inoculum of 8 cysts/0·5 ml aliquot. The infected brain emulsion was divided into 2 parts; the first portion was used directly to infect the experimental mice, the second portion (for control purposes) was snap-frozen and thawed 3 times in succession, a procedure which has been shown to kill the toxoplasmas but which (in theory) should not affect any viral contaminants (Stahl & Turek, 1988). The purpose of the procedure was to verify that the wasting disease and pathological changes were indeed due to the *T. gondii* infection and not to a contaminant virus (Grimwood, 1985). The control inocula were equal in volume and dilution to the infecting inoculum, and all inoculations were given by i.p. injection.

Necropsy and tissue processing

The mice were lightly anaesthetized with ether, then exsanguinated from the retro-orbital sinus. The skull was opened and the frontal and parietal plates removed with fine iris scissors. The brain was then carefully lifted out with small curved forceps and placed into 10% buffered formalin. After several days fixation of the brain in the formalin, 3 coronal slices approximately 2 mm thick were taken, the first from the cerebrum at the level of the median eminence, the second from the midbrain, midway between the median eminence and the cerebellum, and the third from the cerebellum. The first slice was chosen to permit examination of the lateral and third ventricles; the second, the aqueduct of Sylvius; the third, the cerebellum and fourth ventricle. The tissues were embedded in paraffin and sectioned at a thickness of 5 μm. Several sections were cut and stained with haematoxylin and eosin (H&E) and periodic acid Schiff (PAS). Other sections were used for immunohistochemical studies.

Immunohistochemistry

After deparaffinization of sections in a xylene–ethanol gradient, endogenous peroxidase was quenched by immersion in 0·3% hydrogen peroxide in methanol for 30 min, and non-specific binding blocked by 1% normal rabbit serum in phosphate-buffered saline (PBS; pH 7·4, 30 min). The sections were then incubated overnight at 4 °C with a primary antibody, either rabbit anti-bovine glial fibrillary acidic protein (GFAP), diluted 1:1000, or rabbit anti-toxoplasma antiserum (pooled serum of 2 rabbits infected for 2 months with the CS strain of *T. gondii*), diluted 1:50. Next, a biotinylated goat anti-rabbit IgG, 1:50 dilution, was applied for 1 h at room temperature. Between each step, tissue sections were washed 3 times in PBS. Bound antibodies were visualized by the avidin-biotin-peroxidase complex (ABC) assay using the appropriate Vectastain ABC kit (Vector Laboratories, Burlingame, California, USA) for 1 h at room temperature. The brown reaction product was developed by the application of 3′3-diaminobenzidine tetrachloride (DAB), 10 mg in 20 ml of 0·05 m Tris buffer, pH 7·4, with 0·033 ml of hydrogen peroxide added immediately before use. The sections were counter-stained with haematoxylin, dehydrated and mounted. As controls, the primary antibodies were either omitted or replaced with non-immune serum. The control sections did not show a positive reaction at any time.

RESULTS

Uninfected control mice

In the brains of the control mice, parasites and inflammatory lesions were not encountered. In the dorso-lateral angle of the lateral ventricles, slight ependymal breaks were detected on occasion but, in general, the ependymal layer appeared intact. In the subependymal matrix, underlying the lateral walls of the ventricles, numerous mononuclear cells with darkly-stained nuclei (H&E) invariably were present in every mouse, especially so in the triangular plate region abutting the dorso-lateral angle (Fig. 1).

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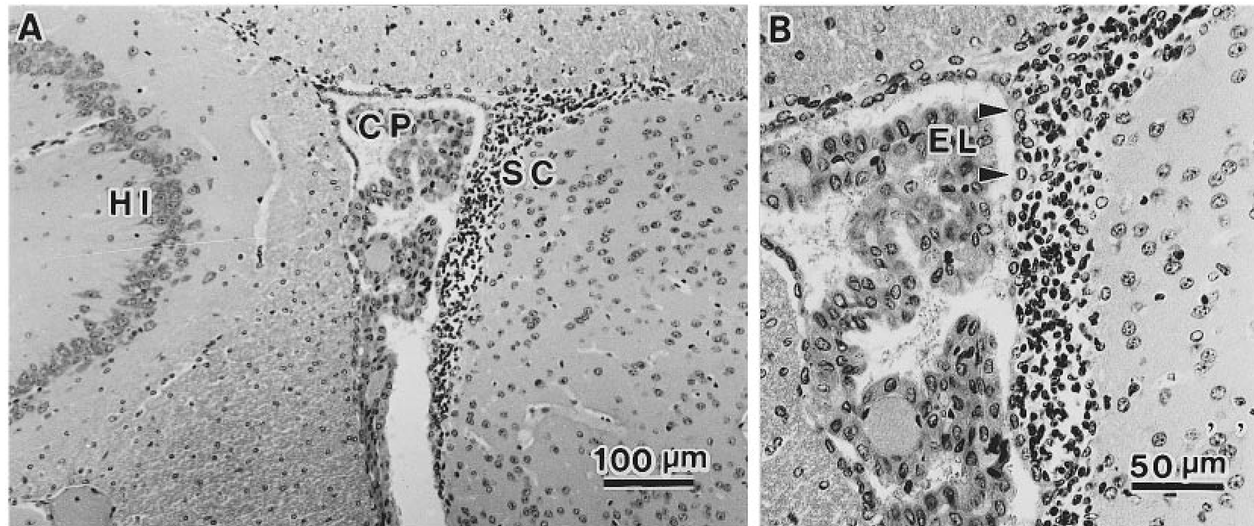

Fig. 1. Lateral ventricle of normal mouse (chronological age, 5 months). (A) Numerous mononuclear subependymal cells (SC) underlying the lateral wall of the ventricle. (HI hippocampus; CP choroid plexus). (B) Higher magnification of the lateral wall of the ventricle. Note the profusion of subependymal cells and the intact ependymal layer (EL).

These subependymal cells were not gathered in nodular aggregates nor were they found within the ventricles. Immunohistochemically, the subependymal cells did not stain for GFAP, nor were large, hypertrophic reactive astrocytes found in the periventricular areas, around blood vessels, or in the meninges. However, sparse numbers of GFAP-positive cells were detected in the corpus callosum and hippocampus of some control mice.

Experimentally infected mice

The first 2 months p.i. Glial nodules and toxoplasma cysts were readily detected in every infected mouse brain, at first in the cerebral cortex then throughout the parenchyma. Occasionally, a cyst was observed developing in close approximation to a glial nodule, although cysts typically were found free of any surrounding inflammatory infiltrate. Concurrently, the infiltration of mononuclear cells into the meninges, interhemispheric commissure, and around small blood vessels (perivascular cuffing) was noted. During the second month p.i., the meningeal inflammation progressively worsened. In the hippocampus, perivascular cuffs were commonplace.

In the lateral ventricles of some mice, focal disruptions and gaps in the ependymal layer lining the lateral walls were noticed, particularly in the dorso-lateral angle of the frontal horns. There was no evidence of ependymal cell sloughing, nor were infected or pyknotic cells detected within the ependymal layer. In the subependyma, accumulations of mononuclear subependymal cells, indistinguishable from those observed in the control mice, were found gathered in nodules which often protruded into the ventricles. Frequently, clusters of these cells were seen erupting through the ependymal layer into the lumen of the lateral ventricles (Fig. 2). Within the ventricles, the cell clusters were observed either adhering to the ependyma and choroid plexus, or floating free. The choroid plexus appeared normal. In the periventricular tissues, vacuolation and spongy, edematous changes were noted. In the third ventricle, small clumps of cells and shreds of amorphous 'debris' occasionally were detected, but not the ependymal disruptions and subependymal nodules so commonly observed in the lateral ventricles. In some mice, the middle of the third ventricle was distinctly narrowed, as if by lateral compression. Within the periventricular tissues surrounding the third ventricle, glial nodules, microabscesses, toxoplasma cysts, and oedematous changes were present. GFAP immunostaining demonstrated a reactive gliosis in the subependymal tissues bordering the ventricles and in the adjacent hippocampus and corpus callosum. The anti-toxoplasma antiserum stained flakes of amorphous material within many of the glial nodules as well as the developing *T. gondii* cysts.

The aqueduct of Sylvius, in every instance, was patent and free of the cells and poorly defined debris seen within the lateral and third ventricles. Although small ependymal tears were noted in some aqueducts, neither stenosis nor dilatation could be unambiguously identified. In the surrounding periaqueductal tissues, cysts and glial nodules were found on occasion, along with slight oedematous changes and a mild reactive gliosis. In general, however, the aqueducts of the infected mice were comparable to the controls.

The third and fourth months p.i. During these 2 months, the inflammatory reaction further intensi-

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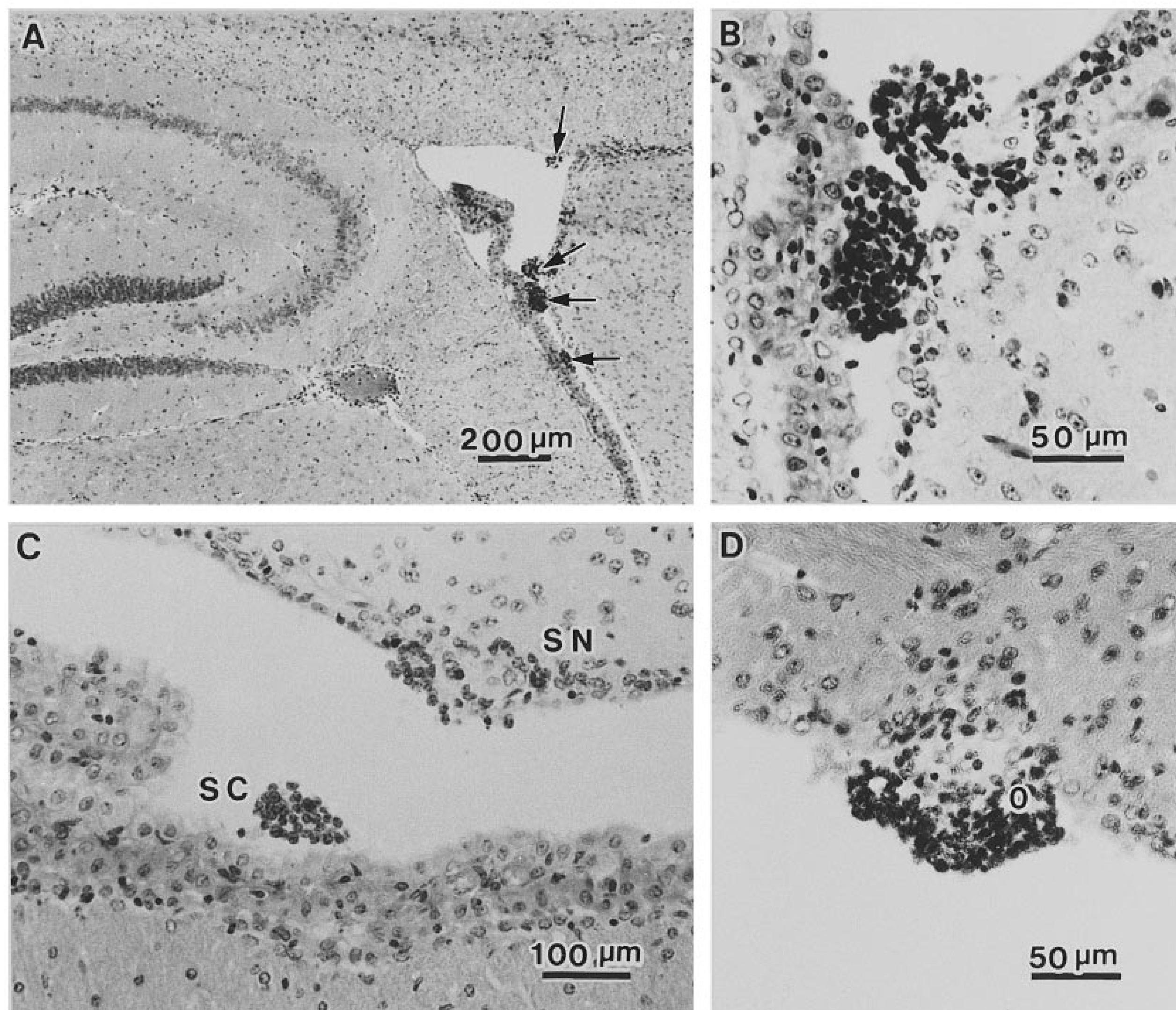

Fig. 2. (A–D) Lateral ventricles of infected mice and the subependymal cellular response to ependymal damage. (A) Six weeks post-infection. Four clusters of cells (arrows) within the ventricle. (B) Higher magnification of 2 of the subependymal cell clusters; the upper cluster is in the process of being discharged into the ventricular lumen. Note disruption of the ependymal layer. (C) Two months post-infection. Remnants of a subependymal nodule (SN), loss of ependyma, and a small intraventricular cluster of subependymal cells (SC) can be seen. (D) Two months post-infection. Expulsion of the cellular contents of a subependymal nodule into the ventricular lumen. Disruption of the ependymal layer is conspicuous.

fied. The meninges, scattered remnants of which were found despite the disruptive manner in which the brain was removed from the calvarium, were markedly inflamed. The subarachnoid spaces were obliterated by multiple layers of mononuclear inflammatory cells, in places 15–20 cells deep (Fig. 3). Conspicuous perivascular cuffs, consisting of several concentric rings of mononuclear cells, were found throughout the cerebrum. The hippocampus in particular was marked by perivascular cuffing, microglial nodules, and numerous toxoplasma cysts, occasionally causing focal disruption of the pyramidal cell layers. PAS-positive deposits in the walls of blood vessels, resulting in partial or complete occlusion of the lumen, also were seen. These widespread inflammatory lesions persisted until the death of the chronically infected animals.

Rounding of the dorso-lateral angles and dilatation of the lateral ventricles was recognized in many of the chronically infected animals. In the walls of the lateral ventricles, subependymal nodules discharging cells into the ventricles had become a routine finding. Occasionally, the cells within the ventricles were found aggregated in very large clusters. The ependymal discontinuities, particularly those near the dorso-lateral angle of the ventricles, appeared larger and more ragged, and the underlying subependymal and periventricular tissues more oedematous. Within the lateral ventricles, clusters and multilayered rafts of subependymal cells frequently were found ‘ap-

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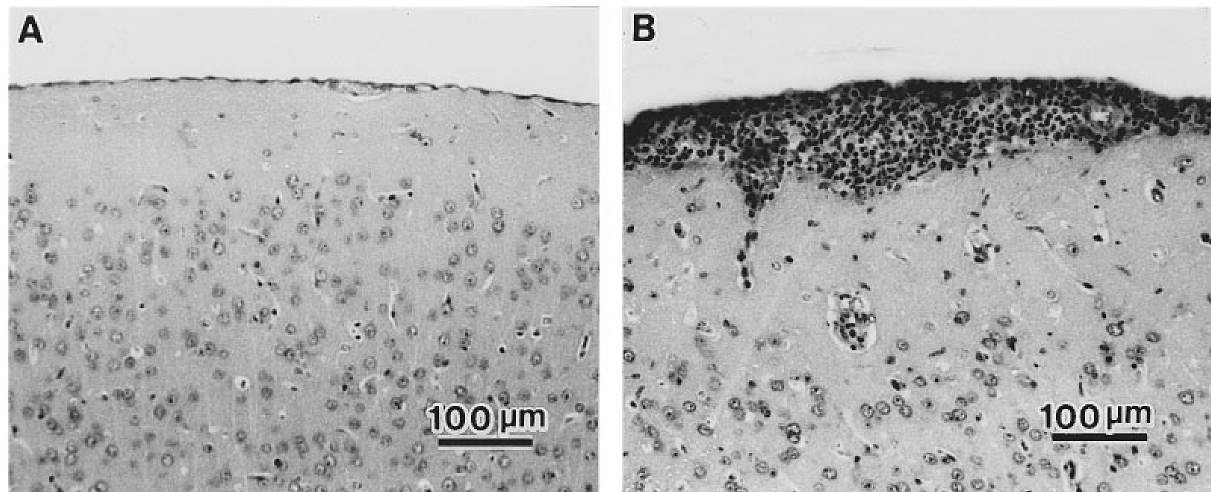

Fig. 3. Leptomeninges of cerebral cortex. (A) Normal mouse. (B) Four months post-infection. Note the florid leptomeningitis and obliteration of subarachnoid space.

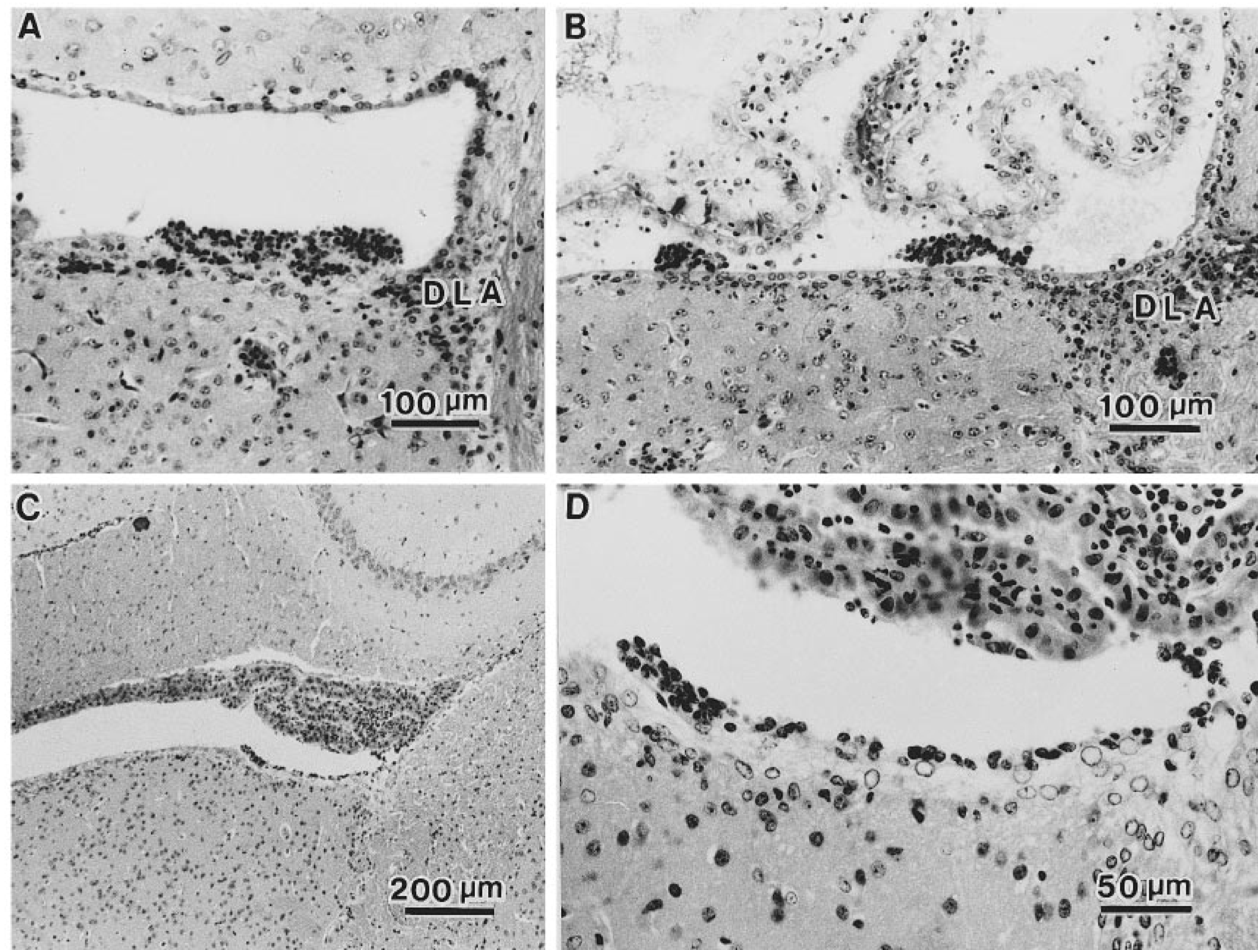

Fig. 4. (A–D) Repair of lateral ventricle ependymal breaches by intraventricular subependymal cells. (A) Three months post-infection. A multilayered raft of subependymal cells covering a large denuded gap in the ependyma. (DLA dorsolateral angle). (B) Four months post-infection. Two clusters of subependymal cells proceeding to sites of ependymal disruption. (DLA dorsolateral angle). (C) Six months post-infection. Subependymal cells arrayed in a protective layer over an area lacking an intact ependyma. (D) Higher magnification of the layer of subependymal cells covering the ependymal breach.

proaching' ependymal gaps, particularly in the dorsolateral angle (Fig. 4A–B). The flexible configuration of many of the intraventricular clusters gave the impression that the mode of progression of the cellular aggregates was by a process of creeping or inching along the ependyma, in worm-like

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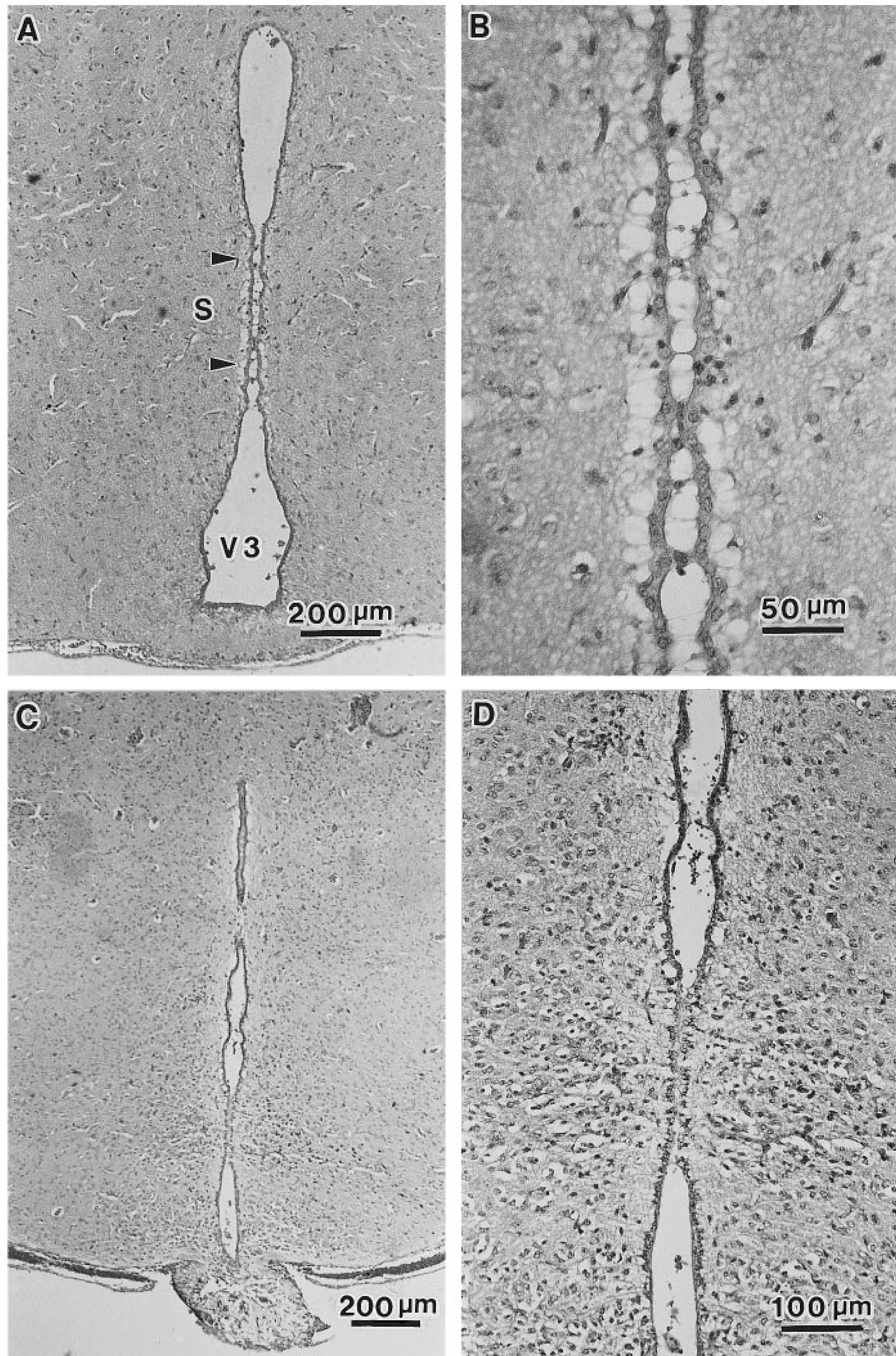

Fig. 5. (A-D) Stenosis of the third ventricle (V3) in hydrocephalic mice. (A) Two months post-infection. There is no evidence that the observed stenosis (S) is due to blockage by cellular debris. (B) Higher magnification of the stenotic area. The narrowing appears due to lateral compression of the third ventricle. Glial bridging and periventricular oedema are visible. (C) Four months post-infection. Marked stenosis and occlusion of the third ventricle, apparently the result of lateral compression. (D) Higher magnification of the lower stenotic area indicates that the occlusion is due to apposition of the lateral walls of the ventricle, not blockage by cellular debris.

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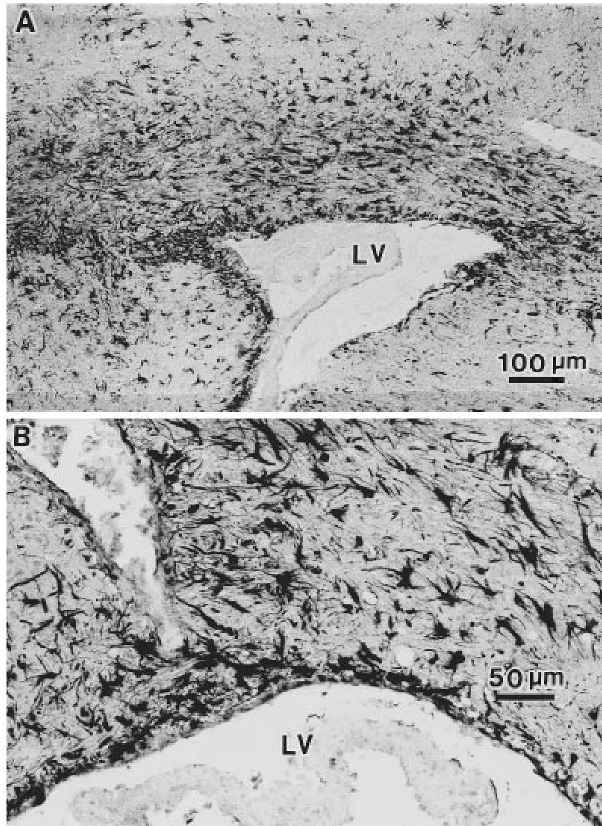

Fig. 6. Periventricular reactive gliosis, demonstrated by glial fibrillary acidic protein (GFAP) immunohistochemistry. (A) Four months post-infection. The lateral ventricle (LV) is enclosed in a dense mat of reactive astrocytes. (B) Six months post-infection. Dorsal aspect of lateral ventricle (LV). There is no abatement of the intense reactive gliosis.

fashion. Upon reaching a disrupted area, the cell clusters spread out to form a covering, ependyma-like layer (Fig. 4C–D). Oedema of the corpus callosum overlying the dorsolateral angle was marked, as was a reactive gliosis within the corpus callosum and hippocampus.

Within the lumen of the third ventricle, large floating aggregates of cells occasionally were encountered, apparently having entered via the foramina of the lateral ventricles. Blockage of the foramina by these intraventricular masses of cells was not observed. Stenotic changes in the third ventricle, ranging from narrowing to complete occlusion due to apposition of the walls of the ventricle (Fig. 5), were seen in some of the infected mice. The narrowing appeared to be the result of lateral compression, rather than blockage of the lumen by cellular debris. In other mice, the third ventricle was dilated.

The aqueduct of Sylvius, in coronal section, continued to be difficult to evaluate. Apparent dilatation of the aqueduct, with ependymal stretching and tearing, was seen in some mice; in others the aqueduct appeared compressed, although occlusion was not observed. Within the aqueducts, the floating clusters of cells and subependymal nodules, so commonly seen in the ventricles, were not encountered. Despite the aqueductal alterations, the periaqueductal tissues generally revealed only slight edematous and gliotic changes.

The fifth and sixth months p.i. During these terminal 2 months, the chronically infected animals exhibited severe wasting and many mice became partially paralysed or moribund.

Enlargement of the lateral ventricles, ependymal disruption, intraventricular clusters of cells, and periventricular spongiform oedema, especially marked in the dorso-lateral angle and overlying corpus callosum, were constant findings. Within the lateral ventricles, 'inchworm' clusters and rafts of subependymal cells were observed approaching and covering the ependymal breaches in the dorso-lateral angle. The third ventricle in a few mice was greatly dilated, with a discontinuous ependyma, subependymal eruptions, and cells and debris within the lumen. In other mice, the third ventricle was strongly compressed, resulting in lengthy areas of stenosis and occlusion. In the periventricular tissues of all the infected mice, toxoplasma cysts, microabscesses, oedematous changes, and enlarging zones of necrosis and cavitation were common.

The aqueducts revealed changes somewhat more pronounced than seen previously. In coronal section, some aqueducts appeared compressed. Shreds of intraluminal debris and ependymal infoldings were detected on occasion, but actual blockage of the lumen by debris, cells, or glial hypertrophy was not observed. In a few sagittal sections, the entire length of the aqueduct visible in the tissue preparation was narrowed, not just in a small, discrete focus, further testifying to compression rather than physical obstruction. Histologically, occlusion and loss of patency of these narrowed aqueducts was not seen. In contrast, the aqueducts in other mice were dilated, and some had lost portions of their ependymal lining. The periaqueductal tissues generally revealed only slight oedema and a mild reactive gliosis, even when the aqueduct showed pronounced changes.

The meninges and cerebral vasculature remained heavily inflamed. GFAP immunostaining revealed a dense mat of reactive astrocytes in the periventricular areas, corpus callosum, and hippocampus (Fig. 6). The toxoplasma antiserum did not reveal the presence of parasites or parasitic antigen within the ependyma or the intraventricular cell clusters.

DISCUSSION

Hydrocephalus can result from (1) obstruction to the outflow of CSF within the ventricular system, either by blockage of the ventricular foramina or stenosis of the aqueduct of Sylvius, (2) blockage of the arachnoid villi and subarachnoid spaces in the

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meninges, thereby impeding the resorption of CSF and (3) the overproduction of CSF by the choroid plexus (Friede, 1989). To date, the pathogenesis of toxoplasmic hydrocephalus has been almost exclusively attributed to the first of the above possibilities, that of obstruction of the ventricular foramina and stenosis of the aqueduct by an inflammatory exudate and glial hypertrophy (Wolf & Cowen, 1937; Paige *et al*. 1942; Frenkel, 1949; Frenkel & Friedlander, 1951). It was conjectured that blood-borne infected white cells entered the lateral ventricles via the choroid plexus. Within the lateral ventricles, parasites were thought to invade the ependyma and cause focal destruction, denudation, and inflammation in the ependymal layer, followed by the sloughing of dead and degenerating ependymal and inflammatory cells into the ventricular lumen. This necrotic cellular debris would then (supposedly) lodge in the foramina of Munro and, in concert with periforaminal glial hypertrophy, block either or both of the ventricular exits or, if swept into the aqueduct of Sylvius, cause stenosis and occlusion of the aqueduct via physical blockage compounded by ependymal erosion, inflammatory oedema, and intense periaqueductal gliosis. This proposed sequence of events has been widely accepted as the basis of toxoplasmic hydrocephalus. However, this hypothesis, in which ependymitis and aqueductal stenosis play central roles, appears to be seriously flawed in several respects. To begin with, the terminology used to describe many of the original observations needs to be redefined, especially for the changes occurring in and around the lateral ventricles. One such is the condition termed 'granular ependymitis', a commonly seen pathological change in the ependyma, which may develop after any infectious or irritative process (Johnson & Johnson, 1972; Duchen, 1992). Ependymal nodules develop throughout the ventricular system and consist of aggregations of subependymal cells bulging into the ventricles, some of which have broken through the covering layer of ependyma. Because similar ependymal lesions were detected in the enlarged ventricles of infants with congenital toxoplasmosis (Wolf & Cowan, 1937; Paige *et al*. 1942; Callahan *et al*. 1946; Frenkel, 1949), 'granular ependymitis' was proposed as the principal lesion underlying stenosis of the aqueduct and the ensuing hydrocephalus. The numerous dark-staining mononuclear 'inflammatory' cells disrupting the ependyma, and the 'sloughed necrotic debris' observed within the ventricles clearly formed the basis of this line of reasoning. However, our initial acceptance of this premise (i.e. ependymal inflammation), had to be abandoned after comparable numbers of morphologically identical mononuclear cells were found in the subependyma of the uninfected control mice as well. A survey of the literature on the embryology of the mouse brain revealed that these subependymal mononuclear cells were not inflammatory cells but astrocyte precursors, remnants of the germinal cells that lined the walls of the developing ventricles and populated the brain during embryogenesis (Jacobson, 1991). During foetal development, the subependymal germinal zone gradually diminishes but, in rodents, it persists in the walls of the lateral ventricles into adulthood (Smart, 1961). In postnatal mice, convincing evidence has been presented that the proliferation, aggregation and entry of these germinal cells into the ventricles represents an attempt to repair ependymal damage (Bruni *et al*. 1985; Collins & Fairman, 1990; Collins & Goulding, 1992). Our observations indicate that the intraventricular clusters were not composed of dead and degenerating ependymal and inflammatory cells, but were viable germinal cells engaged in purposeful, reparative activity. As Sarnat (1995) has very succinctly explained, 'the term 'granular ependymitis' is inaccurate and draws misleading inferences of inflammation, hence it is best avoided and the more generic descriptive term 'subventricular (subependymal) glial nodules' may be substituted'.

The other major tenet of the hypothesis (stenosis of the aqueduct of Sylvius and/or foraminal obstruction) also must be reconsidered. If indeed aqueduct stenosis is the cause of post-infective hydrocephalus, then the following conditions (formulated by Masters, 1977) should hold. (a) Narrowing of the aqueduct should precede the development of hydrocephalus, (b) dilatation of the aqueduct and lateral and third ventricles should appear rostral to the presumed area of stenosis, (c) there should be a clear distinction between stenosis caused by obstruction versus narrowing caused by external compression and (d) evidence of blockage of CSF flow and/or absorption distal to the aqueduct should **not** be present. After an extensive review of the literature concerning post-infective hydrocephalus, Masters (1977) concluded that few, if any, of the above criteria have ever been met.

In an analysis of a series of 12 case reports of congenital toxoplasmic hydrocephalus, 11 were found to have marked ventricular enlargement (Masters, 1977). However, in every case, the aqueduct was found to be patent with several even being described as enlarged. Another case report (Ribierre, Couvreur & Canetti, 1970) showed a normal-sized aqueduct with a thin glial membrane across the lumen, but with no difference in the size of the aqueduct on either side of the membrane. In a review of 70 cases of congenital toxoplasmosis (9 personal and 61 from the literature), Weber (1983) reported hydrocephalus as a major pathological alteration. Weber searched for signs of aqueductal stenosis and/or blockage of the ventricular foramina to corroborate their presumptive role in the aetiology of hydrocephalus, but with very limited success. The lack of confirmatory evidence led Weber to concede

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that hydrocephalus was very difficult to explain in those cases where both the aqueduct and the ventricular foramina appeared normal, and that other factors must also be considered. Meningeal inflammation, although recognized as a common cerebral lesion, inexplicably was not considered. From the literature, therefore, it is clear that stenosis of the aqueduct of Sylvius is not a consistent feature of toxoplasmic hydrocephalus. Nevertheless, toxoplasmosis is still being cited as a cause of aqueduct stenosis (Remington, McLeod & Desmonts, 1995; Volpe, 1995). In the present study, apparent narrowing of the aqueduct was detected in some of the infected mice, but overt blockage by masses of cellular debris and glial hypertrophy was not observed. Narrowing of the aqueduct did not precede the development of hydrocephalus, dilatation of the aqueduct rostral to a stenosed area was not seen, and evidence of blockage of CSF flow outside the ventricular system could not be discounted.

In the periaqueductal tissues, a reactive gliosis and some oedematous changes usually were present, but distinctly less intense than that observed around the lateral ventricles. Occasionally, glial nodules and toxoplasma cysts were observed developing near the walls of the aqueduct, but we assume this was fortuitous and not related to aqueductal pathology.

As for blockage of the foramina of Munro, we have no evidence that this occurred. In earlier reports by other investigators, whenever clumps of cells were detected in the inferior angle of the ventricles, they usually were described as being ependymal and inflammatory detritus blocking the ventricular foramina (Paige *et al*. 1942; Frenkel, 1949). However, in the present study, mitigating against possible foraminal blockage was the frequent appearance of cellular aggregates within the third ventricle, attesting to the ability of these germinal cell clusters to successfully traverse the foramina of Munro. In addition, we found that enlargement of the lateral ventricles was always bilateral. If foraminal obstruction by debris and glial hypertrophy does indeed occur, we wonder why unilateral enlargement was not detected occasionally. It seems unlikely that the foramina of both lateral ventricles would consistently become blocked.

Several investigators have contended that aqueductal stenosis is a secondary phenomenon due to external compression of the midbrain by the enlarging lateral ventricles, with subsequent kinking or pinching of the aqueduct (Williams, 1973; Masters, Alpers & Kakulas, 1977; Nugent, Almefty & Chou, 1979). In addition, Williams (1991) described how, during the early stages of hydrocephalus, the enlarging lateral ventricles also cause the caudal, downward displacement of the midbrain and third ventricle, thereby compressing the third ventricle both vertically and from side to side. This compression can lead to the formation of an adhesion across the middle of the third ventricle. The foramina of Monro are similarly compressed from side to side so that occasionally they may appear to be occluded. In a prescient early case report (Paige *et al*. 1942), the aqueducts in 2 hydrocephalic infants were described as narrowed by external compression, but still patent throughout their length. Nevertheless, the authors concluded that both cases of hydrocephalus were due to 'occlusion of the interventricular foramens and stenosis of the aqueduct of Sylvius'! In an analogous study on viral hydrocephalus in hamsters (Nielson & Baringer, 1972), stenosis of the third ventricle was a prominent finding. Within days after infection, meningitis developed, and glial cell proliferation in the subependyma and protrusion of glial nodules into the lumen of the ventricles and aqueduct were observed. In the third ventricle, in particular, opposing surfaces became adherent after the narrowed ventricular lumen seemingly was bridged by tufts of gliovascular cells. It was therefore adduced that the stenotic changes in the third ventricle (and aqueduct) led to the development of the hydrocephalus. That the hydrocephalus may have been responsible for the stenotic changes was not considered. In our study, although aqueductal changes generally were unremarkable in the toxoplasma-infected mice, the third ventricle frequently showed areas of distinct narrowing and even occlusion. Histologically, it was apparent that the stenosis was not due to internal blockage by cellular debris, but rather the result of compression and narrowing of the third ventricle, plus glial hypertrophy wherever the walls of the third ventricle met in apposition. According to William's (1991) lucid and comprehensive explanation, the anatomical alterations engendered by the dilating lateral ventricles very likely were responsible for the stenotic changes observed in the third ventricle of the infected mice.

The crucial question then is: if not aqueductal stenosis, what did the infected mice have in common that would lead to the development of hydrocephalus? The obvious answer is the florid leptomeningitis. The meningeal changes in murine toxoplasmosis have received comparatively little attention, doubtlessly due in part to the typically crude manner of removal of the brain from the calvarium. In our study, we were fortunate to find sizeable meningeal remnants which revealed the obliteration of the subarachnoid spaces and arachnoid villi by the marked inflammation. Although leptomeningeal inflammation was shown early on to be a prominent feature of toxoplasmic encephalitis (Wolf & Cowen, 1937; Paige *et al*. 1942), only once has it been mentioned as a possible factor in the pathogenesis of toxoplasmic hydrocephalus (Callahan *et al*. 1946). In the intervening 5 decades since then, in numerous papers and review articles, 'leptomeningitis' and

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'meningoencephalitis' invariably have been reported as constant and prominent findings in CNS toxoplasmosis. However, when considering the pathogenesis of toxoplasmic hydrocephalus, the meningeal inflammation is glossed over and aqueduct stenosis is dutifully invoked as the major villain.

We suggest that the severe leptomeningitis observed in every infected mouse, which undoubtedly interfered with the resorption of CSF by the arachnoid villi, was the immediate cause of the hydrocephalic changes. The toxoplasmic hydrocephalus did not originate in the ventricular system but in the grossly inflamed meninges. Further, we propose that the disruptive changes in the ependyma and dorso-lateral angle of the lateral ventricles, the associated periventricular oedema, the assemblages of mononuclear cells in subependymal glial nodules, and the entry of these cells into the ventricles are subsequent to dilatation of the lateral ventricles. Likewise, narrowing of the aqueduct and third ventricle is secondary to compression and caudal herniation of the midbrain by the expanding lateral ventricles, rather than to primary occlusion of the interventricular CSF channels by cellular debris and glial hypertrophy.

Finally, Master's (1977) admonition that there is little value in concentrating on the histology of the aqueduct in examples where the leptomeninges and arachnoid villi have not been comprehensively examined is an appropriate footnote to this discussion on hydrocephalus.

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