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Alzheimer's Disease Cerebrospinal Fluid Antibodies Display Selectivity for Microglia

Investigations with Cell Cultures and Human Cortical Biopsies

A. Dahlström,*[,1] A. McRae,[1] R. Polinsky,[2] L. Nee,[3] B. Sadasivan,[4] and E. A. Ling[5]

[1]*Department of Anatomy and Cell Biology, University of Gotebörg, Medicinaregatan, Göteborg, Sweden;* [2]*Sandoz Pharmaceuticals, East Hanover, NJ;* [3]*National Institute for Neurological Disorders and Stroke, Bethesda, MD;* [4]*Department of Neurosurgery, Tan Tock Seng Hospital Singapore; and* [5]*Department of Anatomy, National University of Singapore, Singapore*

Abstract

Previous investigations demonstrated that the cerebrospinal fluid (CSF) from Alzheimer's disease (AD) patients contains antibodies that recognize specific neuronal populations in the adult rat central nervous system (CNS). These findings suggest a pathogenic role for immunological aberrations in this disorder. To determine if antibodies may provide a means to differentially diagnose the dementias, CSF from a diversified dementia population was screened against the developing rat CNS and a cell culture system. Markings produced by AD CSF were distinctly different from those of vascular dementias (VAD) against the developing rat CNS. More importantly, some AD CSF recognized amoeboid microglia. The recognition of amoeboid microglia by antibodies in AD CSF is particularly interesting since these cells proliferate in response to nervous system disease and also engulf debris. A cell culture technique was developed to allow the rapid screening of CSF antibodies. Patient CSF produced five different types of markings in the cell culture: microglia, glioblasts, fibers, nonspecific, or negative. Correlations with these structures and the diagnosis of four different dementia populations revealed that, in comparison to the other groups, AD CSF displayed remarkable selectivity toward microglial cells. Cortical biopsies from patients suspected to have AD were incubated with the patient's own CSF and that of confirmed AD patients. Both CSF samples recognized microglial cells in the patient's cortical biopsy. The same CSF samples incubated against normal human cortical autopsy or a biopsy from a 3-mo-old child displayed negative immunoreactivity. These three approaches suggest that the presence of CSF microglial antibodies may be a means to distinguish AD patients from other dementias. The results add further support to the widely growing concept that inflammation and similar immune mechanisms may contribute to AD pathogenesis.

Index Entries: Alzheimer's disease; cerebrospinal fluid antibodies; dementia; developing rat; human cortical biopsy; microglia.

*Author to whom all correspondence and reprint requests should be addressed.

Introduction

Surface antigens, once considered only on cells of the immune system, have been unveiled on elements of the central nervous system (CNS) *(1–3)*. These observations set the premise for considering that immunological mechanisms could participate in the pathogenesis of neurodegenerative disorders, such as Alzheimer's disease (AD).

Immunocytochemical approaches have established that hallmark lesions of AD are filled with abnormal proteins *(4–6)*, reactive microglia *(2,7–10)*, and aggregations of lytic scavenger cells *(11,12)* some of which contain phagocytosed amyloid and an arsenal of immune related markers *(13–17)*, all strongly suggesting that immune responses could play an active role in the pathogenesis of this disorder. The presence of immunoglobulins (IgG) is questionable *(18,19)*. However, classical pathway complement proteins are definitely present and fully activated in AD lesions *(14,20,21)*. Recent evidence indicates that microglia secrete complement proteins *(22)* and participate in the formation of amyloid deposits *(23,24)*. These findings add substantial support to the concept that immunological events participate in this disorder.

However, there needs to be a method to determine such responses during the disorder or, even more, before clinical manifestations. Investigations of immunological indices in AD based on blood lymphocytes, immunoglobulins, and albumin levels in the serum and to some degree in the CSF of AD patients, have produced inconsistent findings *(25)*.

Antibrain antibodies have been considered as possible candidates for the detection of immunological events in AD. This has, however, been met with some reluctance since antibrain antibodies are associated with a variety of neurological disorders. There have been attempts to isolate antigens that are selective for AD antibrain antibodies. These approaches have produced a repertoire of antibrain antigens associated with, but not entirely selective for, AD. The fact that antibrain antibodies appear to be directed against a variety of antigens may not be surprising in view of the complexity of the disorder *(26)*.

Previous reports from our laboratory suggest that the developing rat CNS provides a reliable substrate to filter through this vast repertoire of antibrain antigens *(27–29)*. Filtering antibrain antibodies with this substrate has revealed that some AD CSF contain antibodies directed against microglia. In view of the numerous associations between microglia and AD lesions, it does not seem to be a mere coincidence that AD CSF contains antibodies directed against this cell *(29)*.

To determine selectivity between microglial antibodies and AD the following studies have been performed:

1. CSF from a large and diversified patient population was screened with sections of the developing rat CNS and a cell culture system;
2. CSF recognition of microglia in the human brain was examined on AD and normal cortical biopsies; and
3. CSF from at-risk descendants of affected familial AD patients was employed to determine if microglial antibodies may be signal for neurodegenerative processes before the onset of dementia.

To this end, the object of this article will be to summarize these findings and provide reasons to consider that the presence of microglial antibodies may be signals for immune responses implicated in neurodegeneration before dementia or during the early phases.

Methods

Patient Population

In collaboration with neurologists and psychiatrists at hospitals in Göteborg (Sahlgren and Mölndal Hospitals), Linköping (Valla Hospital), and NINDS (Bethesda, MD), CSF samples have been supplied from early stages and throughout advancing stages of dementia. CSF samples from AD patients from 12 families (FAD) with dominantly inherited, histologically verified AD and samples from at-risk descendants of affected family members (AR) were also employed in these investigations *(30)*. According to clinical diagnosis for the patients with dementia disorders, they have been divided into the following groups: Alzheimer and senile dementia (AD/SD), vascular dementias (VAD), and a group consisting of nonspecified dementias (NSD). The patients' disease was diagnosed according to the criteria in the current Diagnostic and Statistical Manual of Mental Disorders (DSM III). Samples from controls (age matched subjects who do not have signs of neurological disorders) and CSF from multiple sclerosis, Parkinson's disease, multiple system atrophy, and pure autonomic failure (referred to as other neurological disorders; OND) have also been included. CSF samples

have been screened on sections of the developing rat and/or fixed neuronal cultures using the procedures described below. Some have been examined on biopsies of human cortical tissue.

Developing Rat

Immunocytochemistry

Perinatal albino rats (Sprague-Dawley strain), extending from E18 (embryonic d 18) to P5 (postnatal d 5) were used. Under hypothermia (embryonic rats) or ether anesthesia (postnatal rats), the animals were perfused with 4% paraformaldehyde in 0.1*M* phosphate buffer for 15 min.

VIBRATOME SECTIONS

Following perfusion the brains were removed and fixed in the same fixative for 24 h. Frontal Vibratome® sections (50 μm) were cut from the middle part of the brain and rinsed in PBS.

CRYOSTAT SECTIONS

Following postfixation, the brains were immersed in PBS containing 10% sucrose for at least 48 h before freezing and cryostat sectioning (20 μm) the forebrain. Nonspecific binding was reduced by a 30-min pre-incubation with 5% nonfat milk powder, diluted in PBS. Sections were incubated either with neat CSF or diluted 1:1 with PBS containing 1% normal goat serum and 0.5% Triton X-100, for approx 18 h at room temperature. The immunocytochemistry was performed as previously described *(29)* with the avidin–biotin peroxidase method, and the peroxidase activity was visualized using a glucose oxidase technique *(31)*. Control sections were incubated only with the secondary antibody.

The immunocytochemical stainings were rated in a blinded fashion: The observer did not have knowledge of the clinical diagnosis. The specimens were examined in a Nikon (Tokyo, Japan) Optiphot light microscope and photographed with AGFA 100 ASA film.

Neuronal Cultures

Preparation

Cholinergic neurons were dissected from the medial septum region (MS) of E18 rats. The dissection included the corpus callosum (CC) above the lateral ventricle, as well as the cavum septum pellucidum (CSP), since both regions contain an abundance of proliferating amoeboid microglial cells. The dissected CNS tissue was dispersed in a culture medium and grown as previously described *(32)*. Cells were plated on poly-L-lysine-coated sterile micro-16 well modules affixed to a standard laboratory slide and maintained in sterile conditions for 7 d.

Immunocytochemistry

Cultures were fixed with 4% paraformaldehyde in phosphate buffered saline (PBS, pH 7.4) for 1 h, rinsed, and left overnight in PBS. A 30-min preincubation with 5% nonfat milk powder, diluted in PBS, was performed before addition of CSF samples. CSF samples diluted 1:1 with PBS containing 0.5% Triton X-100 and 1% normal goat serum were incubated on the fixed cultures overnight at room temperature. Cultures were processed for immunocytochemistry *(see above)*.

Scanning Electron Microscopy (SEM)

Cells on cover slips were fixed with 3% glutaraldehyde and 2% paraformaldehyde in 0.1*M* phosphate buffer for 1 h. Postfixation was performed in 1% osmium tetroxide in 0.1*M* phosphate buffer for 30 min. The cultures were then dehydrated in a graded series of alcohol. After critical point drying in liquid nitrogen, the cells on their cover slips were sputter coated with gold in a Balzers (Amsterdam, The Netherlands) SCD 0004 sputter coater and viewed in a Philips (Amsterdam, The Netherlands) 515 scanning electron microscope.

Transmission Electron Microscopy (TEM)

Cultures were grown on the bottom of four well culture dishes. The cultures were fixed with 3% glutaraldehyde and 2% paraformaldehyde in 0.1*M* phosphate buffer for 1 h, rinsed, and then postosmicated for 1 h in 1% osmium tetroxide in 0.1*M* phosphate buffer. The cultures were then dehydrated in a graded series of alcohols and embedded in Epon. The bottom of the culture dishes were cut out and blocks were prepared. Ultrathin sections were prepared and stained in lead citrate only and viewed in a JEOL (Tokyo, Japan) 1200EX electron microscope.

Human Cortical Biopsy

Neurosurgeons at Tan Tock Seng Hospital in Singapore implant ventriculo-peritoneal shunts in patients with dementia and a clinical diagnosis of normal pressure hydrocephalus. If the diagnosis of normal pressure hydrocephalus is correct, the patient will return to normal after the shunt is implanted. In other cases patients remain in a demented state and are considered to have AD. During the operation small cortical biopsies and

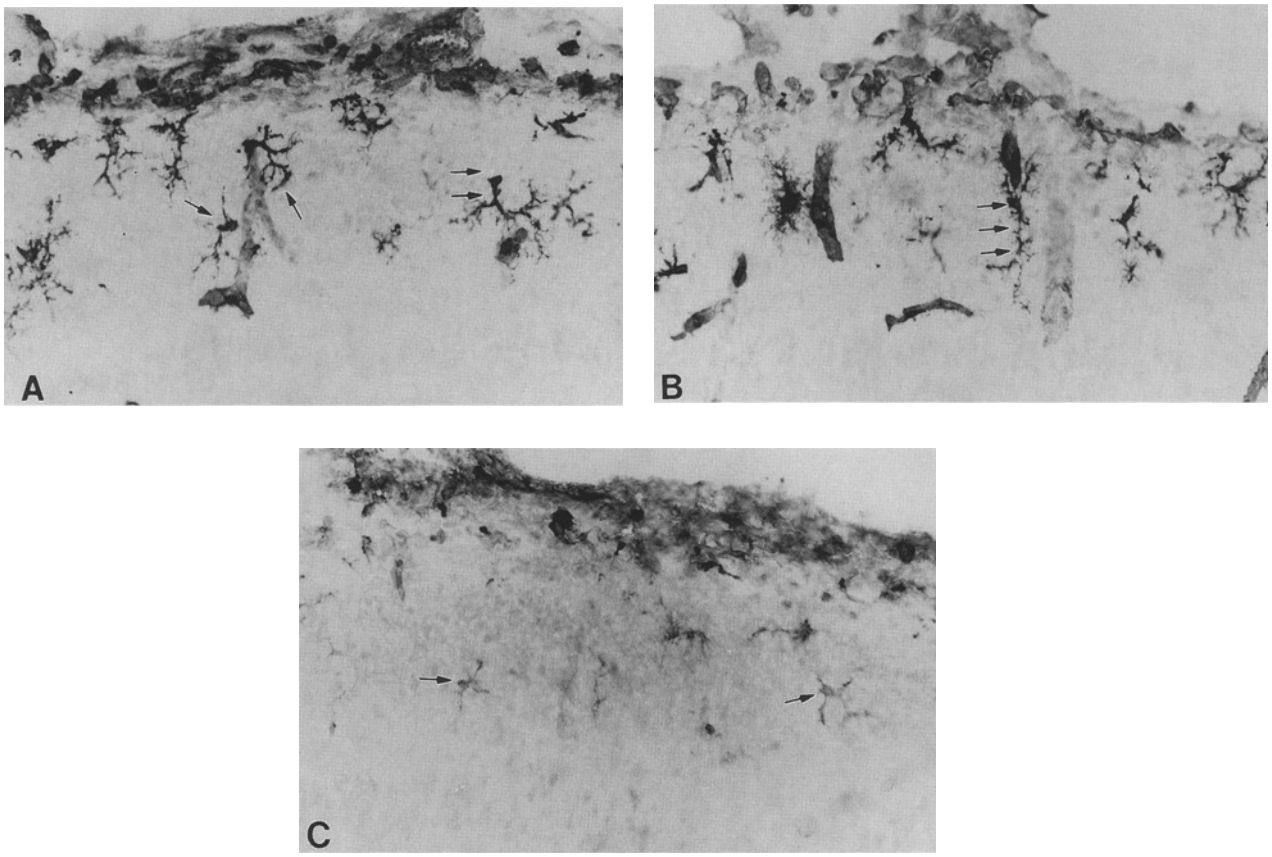

Fig. 1. Photomicrographs of vibratome sections of the cortex from a postnatal d 3 rat brain following incubation with AD CSF from two different AD patients: Sweden **(A, B)** and Singapore **(C)**. CSF-positive microglia showed a diversity of morphological forms, ranging from round to cells with a few stout processes, and also some ramified cells were observed (arrows). Some positive blood vessels with immunoreactive microglia attached to them are also observed (arrowhead). CSF from C recognized microglial cells in his cortical biopsy; ×340.

CSF are removed. The biopsy is immediately fixed in 4% paraformaldehyde. The CSF and biopsy are examined in a blinded fashion. Following cryostat sectioning, the cortical material is incubated with the patient's own CSF or CSF from confirmed AD patients from Sweden. For controls CSF was incubated on a biopsy from a 3-mo-old baby and autopsy material from normal human cortex. Cortical sections incubated only with biotinylated goat-antihuman IgG served as controls for nonspecific binding against human tissue.

Results

Developing Rat

Screening CSF Antibodies

The use of the developing rat as a substrate for CSF antibodies provided interesting and unexpected results. Comparisons of staining produced by CSF within the AD population revealed differences. Some AD CSF recognized fibers whereas others unexpectedly recognized microglial cells *(27)*. As shown in Fig. 1, CSF-positive microglia showed a diversity of morphological forms, ranging from round to cells with a few stout processes. Some ramified cells were also observed. Examination at the ultrastructural level showed that AD CSF immunoreactivity was directed toward the receptor membrane of the microglia *(29)*.

The presence of microglial antibodies was determined in a diversified patient population using CSF from 62 AD, 12 FAD, 31 AR, 37 OND, 11 VAD, and 23 controls. Sections were examined in a blinded fashion and scored for microglia immunoreactivity. Examples of AD CSF microglial staining and negative control staining are depicted in Fig. 2. The data

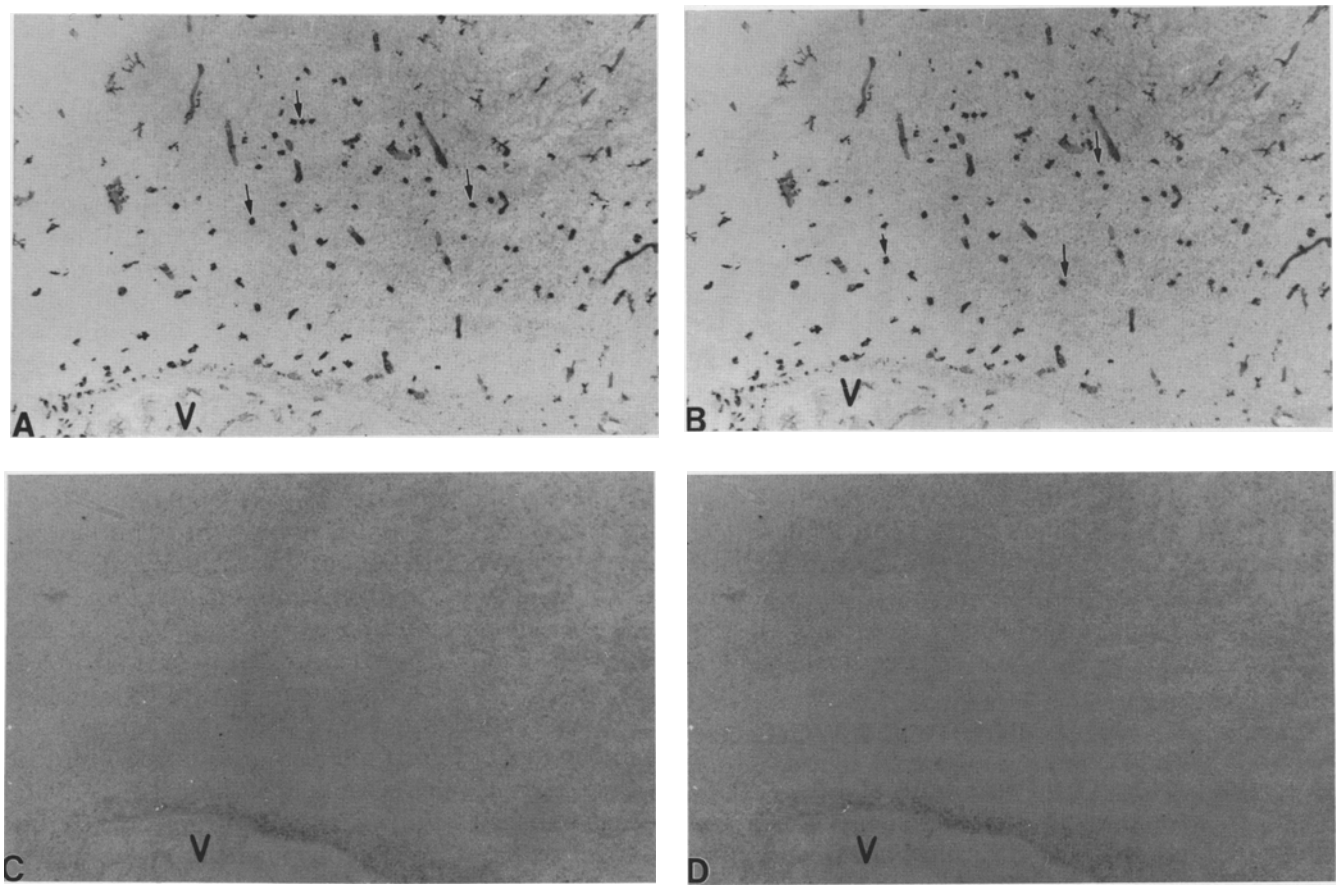

Fig. 2. Photomicrographs of frontal vibratome sections of the corpus callosum from a postnatal d 3 rat. **(A, B)** Incubated with AD CSF marking ramified and amoeboid microglia (arrows). **(C, D)** Incubated with CSF from patients not considered to have AD. Notice the absence of staining in the P3 rat corpus callosum; ×110. Lateral ventricle (V).

revealed that microglia were recognized by 20 AD, 4 FAD, 4 AR, 0 OND, and 1 control. These results indicate that the frequency of recognition of microglia by AD CSF far exceeds that of the others.

Our previous investigations confirmed that the recognition of microglia by AD CSF was independent of the CSF IgG levels *(29)* This excluded the possibility that the immunocytochemical markage is caused by simple binding of Fc parts of the IgG to Fc receptors on the surface of the microglia cells *(33)*. It is significant that CSF from some at-risk relatives recognized amoeboid microglial cells. This was highlighted by the fact that two of these patients subsequently developed AD within 2 yr *(29)*.

Neuronal Cultures

The results obtained by screening CSF antibodies with developmental CNS suggest that further development of this type of methodology may improve diagnostic strategies for different neurological disorders. To rapidly screen CSF antibodies, a cell culture system is currently being employed.

Scanning Electron Microscopy (SEM)

This investigation demonstrated that the majority of the cultured cells were neurons and astrocytic glial cells. Among these were, however, some round cellular elements, ranging from 10–15 μm in diameter. These cells showed typical features of amoeboid microglial cells, comparable to the cell type observed in in vivo SEM studies of the cell type described in perinatal CNS *(34,35)*. They displayed long, slender, filopodial processes, perching on the culture surface (Fig. 3). The soma of the cells also showed blebs.

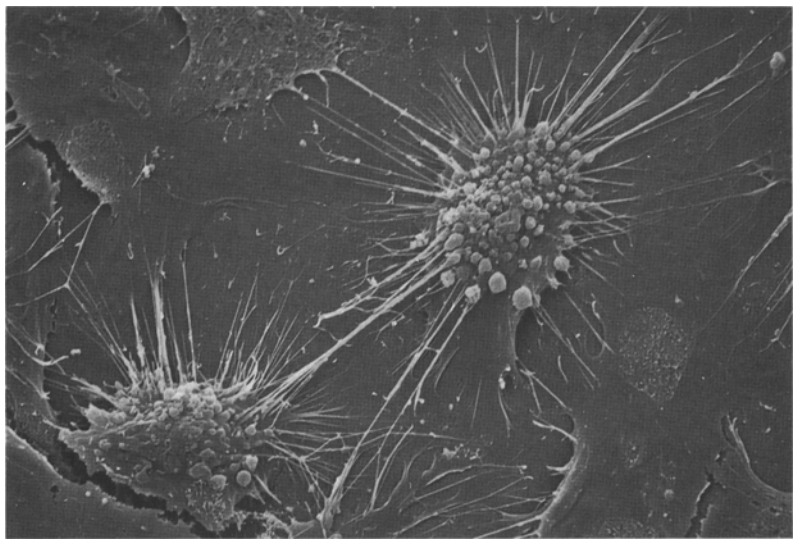

Fig. 3. Scanning electron micrographs of neuronal cultures prepared from the medial septum region of E18 rats. This approach showed the presence of amoeboid microglial cells in the culture. These cells showed typical features of amoeboid microglial cells comparable to the cell type previously described in perinatal cavum septum pellucidum. They displayed long, slender filopodial processes perching on the culture surface. The soma of the cells also showed blebs. These appear to be mitotic microglia; ×2300.

Screening Dementia CSF Antibodies

This study included 197 CSF samples; 82 AD, 26 VAD, 40 NSD, and 49 others. For this part of the investigation the group of NSD patients was divided into NSD and a group named "other." This group included patients diagnosed for depression, confusion, amnesia, cerebrovascular disorders (other than vascular dementia), cerebral infarcts, cerebral trauma, organic brain disorders, multiple scelerosis, Parkinson's dementia, and progressive supra nuclear palsy, as well as 14 normal age-matched subjects. Based on available clinical data, the average duration in years was: AD 4.3 ± 2.5, VAD 3.6 ± 2.5, NSD 3.0 ± 2.1, and "other" 5.7 ± 0.6 (expressed as mean ± SD). Samples from familial AD, their at-risk relatives, or the OND *(see above)* were not included in the data presented. These samples have been assayed with the culture system and identical results to those of the developing rat were obtained.

Immunocytochemical investigations revealed that incubations of the cell cultures with patient CSF produced five different types of staining patterns: microglia, glioblasts, fibers, nonspecific, or negative (Fig. 4). A correlation was made between the recognized structures and the diagnosis. The frequency of recognition of microglia by AD CSF exceeded that of any other patient group in the cell culture system (Fig. 5). The distribution of cells marked by 82 AD CSF samples was: 29 microglia, 30 negative, 8 glioblast, 7 fiber, and 8 nonspecific. In this group of 82 there were 48 patients in the early phases (duration between 1 and 4 yr); 19 of the AD microglial positive samples were in this group. CSF samples from "other" displaying microglial antibodies were from amnesiac and Parkinson's dementia patients. NSD microglial positive samples were from four patients with this diagnosis whereas the other two were from patients who could have AD. Discussions with clinicians revealed that the one VAD patient displaying CSF microglial antibodies may have AD; this was based on the slow progression of this particular patient's dementia.

Transmission Electron Microscopy (TEM)

AD CSF mainly stained microglia; however, there were a few samples that recognized glioblasts. Previous immunoelectron microscopic studies confirmed that AD CSF recognized the receptor membrane of microglia *(29)*. Therefore, it appeared of interest to characterize AD CSF immunoreactivity toward glioblasts at the ultrastructural level. The

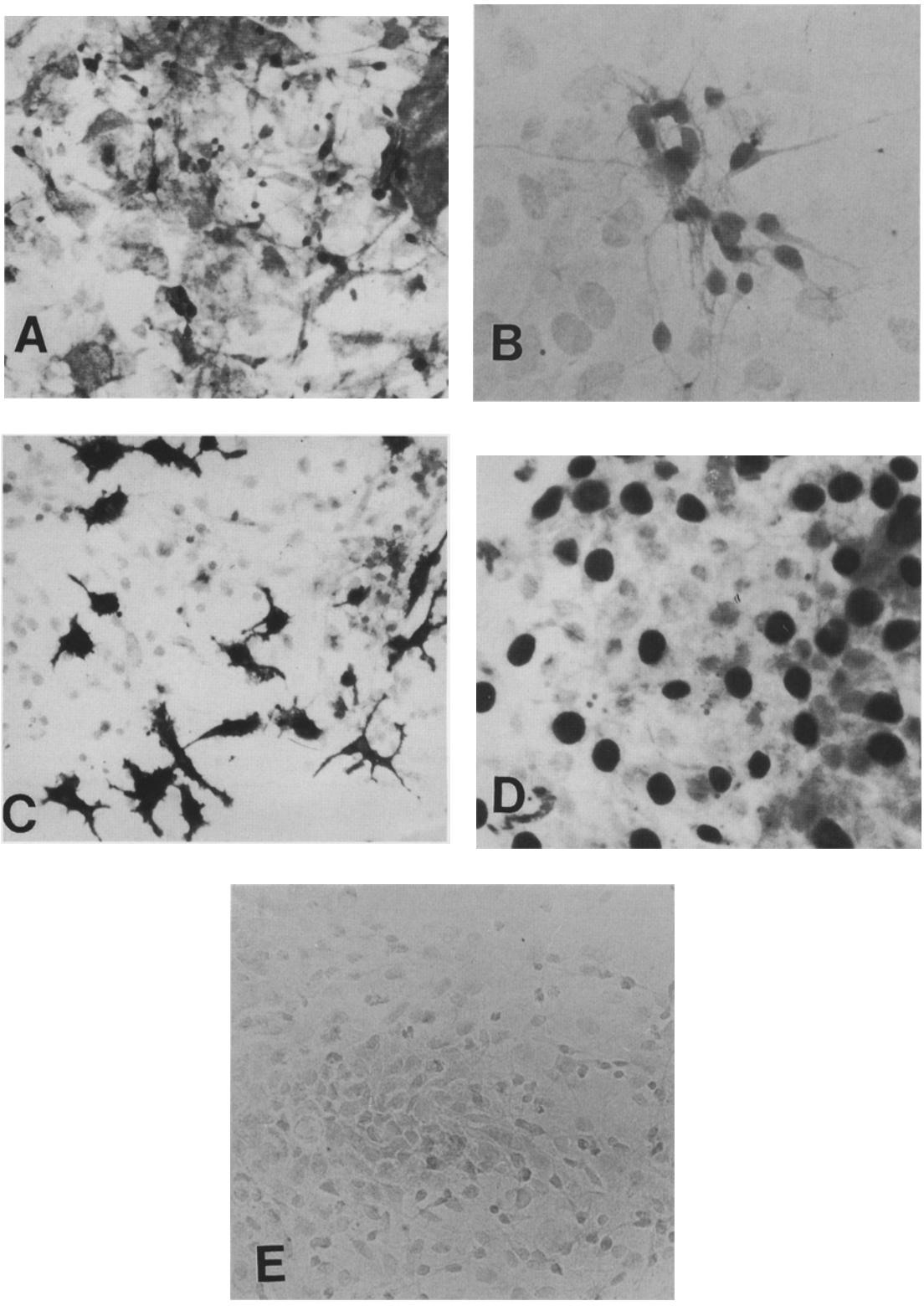

Fig. 4. Photomicrographs of cholinergic cultures depicting five different types of markings produced by patient CSF. **(A)** Nonspecific; **(B)** fibers; **(C)** microglia; **(D)** glioblasts; **(E)** negative. These markings were employed to establish data presented in Fig. 5.

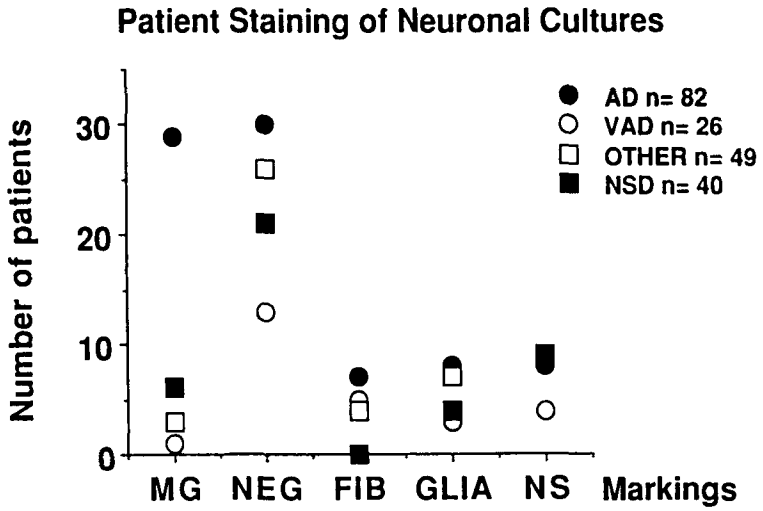

Fig. 5. Graphic presentation of the distribution of markings produced by 197 CSF samples from AD, VAD, NSD, or "other" (*see text* for explanation) screened against cholinergic neuronal cultures. The graph points out the selectivity of microglial antibodies in the CSF of AD patients. As indicated, this is the only recognized cell that allows a clear-cut distinction to be made between AD and the other types of dementias.

identification of glioblasts was confirmed in cells not treated with immunoreaction (Fig. 6A) Some AD CSF specifically stained the identified glioblast that contains a nucleus with patchy chromatin material. The scanty cytoplasm was characterized by abundant free and polyribosomes. Other organelles included isolated cisternae of rough endoplasmic reticulum and a small Golgi apparatus with dilated saccules. The AD CSF immunoreactivity was localized in the cytoplasmic matrix but not associated with any particular organelle (Fig. 6B). Our investigation at the light microscopic level considered that this patient's CSF only marked glioblasts; however, as shown in Fig. 7A, the sample recognized both glioblasts and microglia. There may be a degree of specificity of AD CSF toward glioblast populations in the culture. As shown in Fig. 7B, AD CSF stained two glioblasts but failed to recognize one that was in between the two positive ones.

Human Cortical Biopsy

To further examine the selectivity of microglial antibodies for AD, a study was performed using human cortical biopsies from patients undergoing shunt operations (Methods). Two samples have marked microglial cells on sections of the developing rat (Fig. 1C) and in their own biopsies (Fig. 8A). Further clinical evaluation of the patients displaying microglial antibodies revealed that they did not respond to the shunt operation and were considered to have AD. It is noteworthy that CSF from confirmed AD patients from Sweden stained the microglial cells of the cortical biopsies from the two positive patients. Results from one of these patients is depicted in Fig. 8B. Incubations of positive AD CSF, both from Singapore and Sweden, on autopsy material of normal human cerebral cortex or a cortical biopsy from a 3-mo-old baby displayed an absence of staining (Fig. 8C,E). Antihuman IgG-incubated human cortical tissues were negative (Fig. 8D,F).

Discussion

The initial association between antibrain antibodies and cerebral failure dates about three decades ago *(36)*. However, since this observation the relationship between antibrain antibodies and dementia has taken a roller coaster ride. Investigators have argued against the existence of antibrain antibodies *(37,38)*, and others have suggested that their presence is insignificant *(39)*. The role of antibrain antibodies in dementia was diluted even more by concepts considering them as age-linked phenomena *(40–43)*. Observations that brain reactive antibodies occur in the serum of patients with a number of neurological disorders *(44)*, especially those in which cognition is impaired, revitalized to some degree the antibody–dementia association *(45–47)*.

Efforts to identify antigens recognized by antibrain antibodies has strengthened the concept that IgG species could serve as a reflection of an active

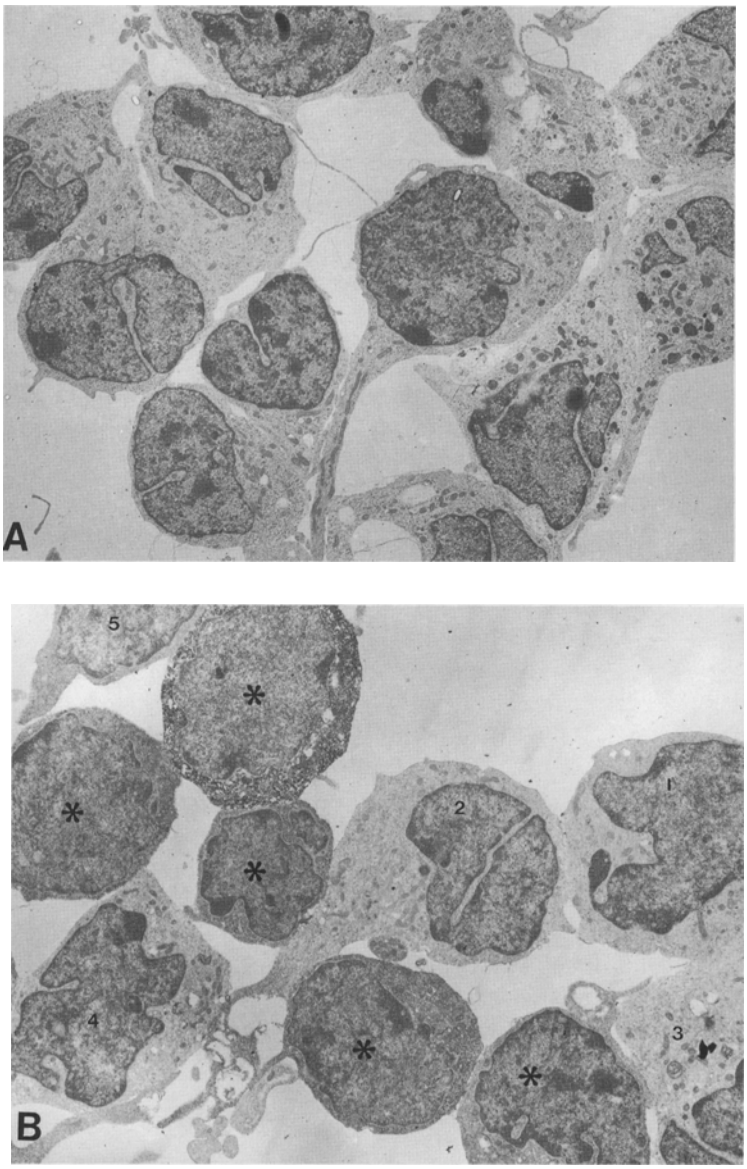

Fig. 6. Immunoelectron micrographs of glioblasts in culture without immunoreaction **(A)** or following incubations with AD CSF **(B)**. (A) Low magnification of glioblasts in culture. The nuclei are deeply infolded with patchy chromatin materials. Their scanty cytoplasm is filled with free and polyribosomes. Some of the cells display slender cytoplasmic processes; ×4025. (B) A mixture of AD CSF positive (asterisks) and AD CSF negative (numbers) glioblasts; ×6667.

immune response in AD. Reports indicate that antibrain antigens of AD include cholinergic elements *(48–50)*, neurofilaments *(51)*, thyroglobuline *(52)*, and vascular proteoglycans *(53)*. All of these antigens have a relationship to AD and auto antibodies produced could be considered detrimental. In fact, AD serum antibodies have a deleterious action toward cholinergic neuronal elements *(54)*. Cognitive impairment in AD could be associated with an IgG-induced neuronal dysfunction since rats immunized with cholinergic antigens display memory deficits *(55)*. Unfortunately, the long list of antibrain antigens found in AD has been employed as a source of argument against their significance *(2)*.

In contrast to the adult CNS, which was employed in most of the previous studies, the developing rat brain and cultures appear to be better substrates to screen CSF antibodies. The first significant results

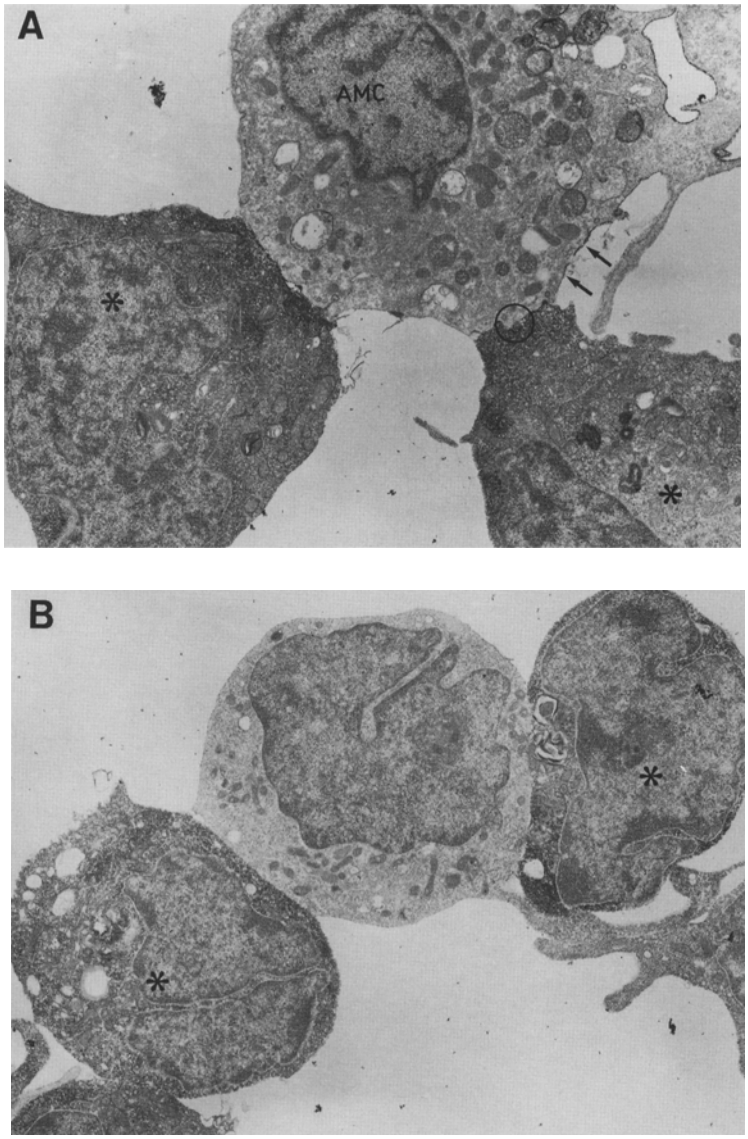

Fig. 7. Immunoelectron micrographs of glioblasts following incubations with AD CSF. **(A)** Immunoreactivity of two glioblasts (asterisk) and an amoeboid microglia cell (AMC). Note that in the former the immunoreactivity is in the cytoplasmic matrix whereas in the latter the immunoreactivity is localized at the plasma membrane (arrowheads). Close structural contacts occur at the sites circled; ×8750. **(B)** An immunonegative cell in the center is flanked by two AD CSF immunoreactive glioblasts; ×8750.

were noticeable immunocytochemical differences between stainings produced by AD CSF and VAD CSF *(27,28)*. Previous results indicated that stainings produced by AD and VAD CSF on adult rodent CNS were indistinguishable *(27,28)*. Second, within the AD patients there were differences in structure recognition. A few recognized glioblasts. The immunoelectron microscopic results suggest a certain selectivity toward glioblast populations. The significance of immunoreactivity toward this cell needs to be determined. Other samples recognized fibers, produced a nonspecific reaction, or no reaction at all. However, as shown in Fig. 5, the comparisons demonstrate that CSF from other neurological disorders recognize these structures to same degree as AD CSF. The only distinguishing cell type was the microglia. As shown in Fig. 5, this cell was recognized at much greater frequency by AD CSF

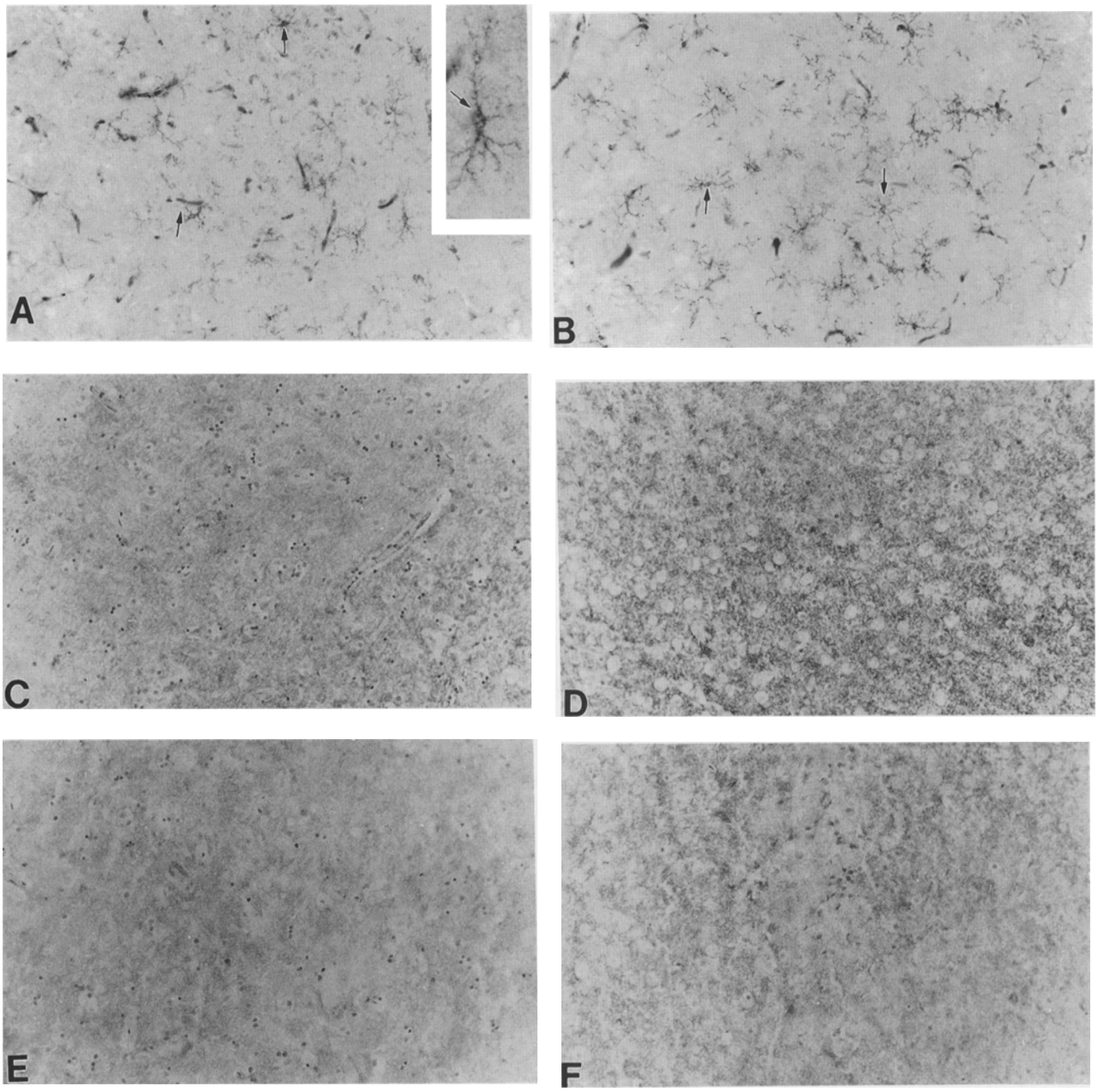

Fig. 8. Photomicrographs of frontal cryostat sections of human cortical biopsies or autopsy. **(A)** Staining of microglial cells (arrows and inset) of human cerebral cortex (biopsy) using the patient's own CSF. The intensity of microglial staining is similar to that produced by the confirmed Swedish AD patient in (B). This patient is considered to have AD; ×170. Inset shows staining of ramified microglia ×440. **(B)** Staining of microglia cells of a human cerebral cortical biopsy by a confirmed AD patient from Sweden. Notice the intense staining of ramified microglial cells (arrows) by AD CSF ×170. **(C)** Absence of staining of microglial cells of human cerebral cortex from a normal adult (autopsy) brain using AD CSF from (B) ×110. **(D)** Same material as in (C) incubated with biotinylated goat antihuman IgG. Note the absence of staining. **(E)** AD CSF from (B) incubated against a cortical biopsy from a normal 3-mo-old child. Notice the lack of staining; ×170. **(F)** Same material as (E) following incubations with biotinylated goat antihuman IgG. Note the absence of staining; ×170.

compared to any group. CSF from a Parkinson's dementia patient recognized microglia, and this could be significant since lesions in this disorder are considered to resemble those of AD patients *(56)*.

Most reactive microglial positive CSF samples also stain brain vessels (Fig. 1). Antibodies to vascular heparan sulfate proteoglycan (HSPG) have been reported in the serum of AD patients *(53)*. Work in progress is aimed at establishing if these particular CSF antibodies are also directed against vascular HSPG. It is notable that both HSPG and microglia participate in the early events of amyloid plaque formation in AD *(57,58)*.

The presence of microglial antibodies in some AR patients and amnesiacs suggests that they could be potential signals of neurodegeneration before the onset of dementia. This appears to be in agreement with an evolving hypothesis suggesting that microglia play an active role in neurodegeneration before the onset of dementia *(22)*. It is established that microglia respond to inflammation and move into the brain to clear out debris *(34,59–62)*. It could be that when microglia move into the brain in response to inflammation they also secrete complement proteins, which can kill cells when the proteins are appropriately activated, since they appear to be in the plaques of AD brains and deposit amyloid; thereby damaging healthy neurons until dementia occurs *(22)*.

Of the AD patients displaying microglial antibodies, 70% of these patients were in the early phases of the disorder, which further suggests that microglial antibodies may be signals for early neuronal degeneration. In the absence of CT scan data it is not possible to correlate the presence of microglial antibodies with the magnitude of neuronal degeneration. Correlations of this type are needed to strengthen the relationship between microglial antibodies and the degree of neuronal degenerative in AD.

Prolonged neuronal degeneration could be considered as a factor influencing antibody production. However, in our previous investigations we were unable to demonstrate a relationship between the presence of antibrain antibodies and the duration or the severity of disorders *(27)*. In the present study, CSF from a patient having his disorder for 26 yr marked glioblasts, whereas CSF from another one having his disorder for 15 yr produced nonspecific markings. These results seem to indicate that the production and the specificity of antibrain antibodies do not depend on the duration of the disorder.

Autopsy material has confirmed the presence of reactive microglia in the hallmark lesions of AD brains *(2,7–10)*. Our findings showing that AD CSF recognized reactive microglia in their own cortical biopsies strongly suggest that microglia are reactive throughout the progression of the disorder. It would be inconceivable to take a cortical biopsy from every patient and run CSF against it to examine for microglial antibodies. However, it is possible to remove CSF and with the methodologies described in this article establish the presence of microglial antibodies. Although small in number, the samples from this study add further support to our concept that CSF microglial antibodies may be potential sources to distinguish AD from other neurological disorders.

In light of the hypothesis suggesting that immune mechanisms play a role in the pathogenesis of AD, it is pertinent that administration of anti-inflammatory drugs slows down the progression of AD *(3,22)*. Our results suggest that microglial antibodies may be signals for early phase immune mechanisms. If this is the case, then administration of anti-inflammatory drugs on finding CSF microglial antibodies might interrupt AD in its early stages or even prevent its development in at-risk descendants.

Acknowledgments

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