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SPECIAL ISSUE

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Inflammatory mechanisms in Alzheimer's disease

Abstract In recent years many studies have indicated an involvement of inflammatory mechanisms in Alzheimer's disease (AD). Acute-phase proteins such as α1-antichymotrypsin and c-reactive protein, elements of the complement system, and activated microglial and astroglial cells are consistently found in brains of AD patients. Most importantly, also cytokines such as interleukin-6 (IL-6) have been detected in the cortices of AD patients, indicating a local activation of components of the unspecific inflammatory system. Up to now it has remained unclear whether inflammatory mechanisms represent a primary event or only an unspecific reaction to brain tissue damage. Therefore, we investigated whether IL-6 immunoreactivity could be found in plaques prior to the onset of neuritic changes, or whether the presence of this cytokine is restricted to later stages of plaque pathology. We confirmed our previous observation that IL-6 is detectable in a significant proportion of plaques in the brains of demented patients. In AD patients IL-6 was found in diffuse plaques in a significant higher ratio as would have been expected from a random distribution of IL-6 among all plaque types. This observation suggests that IL-6 may precede neuritic changes, and that immunological mechanism may be involved both in the transformation from diffuse to neuritic plaques in AD and in the development of dementia.

Key words Senile plaques · Primitive plaques · Alzheimer's disease · Interleukin-6

Introduction

The underlying pathological process which causes tissue destruction in Alzheimer's disease (AD) is still unknown. Classical histopathological hallmarks of AD are deposits of amyloid and neurofibrillary degeneration of neurons. The presence of these neuropathological signs are part of definitive diagnostic criteria for AD (Khachaturian 1985).

Amyloid pathology

Amyloid deposits are not only found in the brains of AD patients, but also in the majority of nondemented persons above the age of 65 years (Berg et al. 1993; Sparks et al. 1993). Recent studies, some of them with a prospective design, have focused on the correlation between the number of amyloid plaques and the degree of dementia. Most studies showed only a weak correlation between the total number of plaques and the severity of dementia (Crystal et al. 1993), or even no correlation at all (Sparks et al. 1993; Jellinger et al. 1992). All plaques with neuritic pathology are classified as neuritic plaques in contrast to diffuse plaques, which are amyloid deposits in the absence of neuritic degeneration. In contrast to total plaque number, the number of neuritic plaques shows a weak correlation with the degree of dementia (Jellinger et al. 1992)

Neuritic and neurofibrillary pathology in AD

The degree of neuritic degeneration within plaques as well as neurofibrillary and neuritic changes outside plaques appear to correlate closely with the clinical degree of dementia. The neurofibrillary degeneration of neurons shows a characteristic topological progression. Neurofibrillary changes usually start in the entorhinal area, progress to the hippocampal area, and eventually involve the neocortex (Bancher et al. 1993; Braak and Braak 1991). This topological progression is associated closely with the intellectual decline. Studies based on autopsies of patients with a well-documented time course of intellectual decline show that a significant increase in neurofibrillary pathology occurs only in the later stage of dementia

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(Jellinger et al. 1992). These data indicate that neuritic and neurofibrillary changes are a consequence, rather than the cause, of the pathological events underlying AD. The processes which cause neurofibrillary and neuritic changes inside and outside amyloid deposits are unknown.

Synaptic pathology in AD

Synaptic pathology is one of the early histopathological events in AD patients. Functional impairment (Murphy et al. 1993) and loss of synapses which leads to a decrease in cortical synaptic density and to a reduction of cortico-cortical connectivity may represent a primary correlate of dementia in AD (DeKosky and Scheff 1990; Terry et al. 1991). Synaptic pathology may cause both the clinical symptomatology and neurofibrillary pathology.

Signs of inflammation in AD

Inflammatory mechanisms represent a third pathogenic element underlying AD. Activated astrocytes and microglia are found regularly in plaques, but have been regarded as an unspecific reaction of the tissue to plaque formation (McGeer et al. 1993; Dickson et al. 1993). Acute-phase proteins, such as α_1-antichymotrypsin, were immunohistochemically demonstrated in plaques (Abraham et al. 1988). Acute-phase proteins are synthesized in response to a variety of different stimuli which disturb the homeostasis of the tissue. Studies with primary neural cell cultures have shown that astrocytes and microglia synthesize acute-phase proteins (Higuchi et al. 1994). Complement proteins have also been detected in plaques (McGeer et al. 1989). Complement proteins which may be synthesized by astroglia are able to form membrane attack complexes and may be able to destroy neuritic processes.

The cytokine interleukin-6 (IL-6) is the most important inducer of acute-phase proteins (Baumann and Gauldie 1994; Bauer 1989). Outside the central nervous system IL-6 is produced mainly by macrophages in response to bacterial or viral stimulation and stimulates the synthesis of acute-phase proteins and of antibodies. However IL-6 also plays a very important part in noninfectious chronic inflammatory diseases such as chronic polyarthritis (Bauer and Herrmann 1991). In HIV encephalopathy and other infections of the central nervous system elevated amounts of IL-6 are found in the cerebrospinal fluid (Gallo et al. 1989; Helfgott et al. 1989; Laurenzi et al. 1990). In the brain IL-6 is synthesized by astroglial cells and microglia in response to different stimuli including neurotransmitters (Gottschall et al. 1994; Norris and Benveniste 1993; Maimone et al. 1993; Minami et al. 1991).

In a previous study we demonstrated the presence of IL-6 and c-reactive protein, an acute-phase protein, immunohistochemically in plaques of brains from patients with AD. Plaques in brains of nondemented elderly persons did not exhibit IL-6 immunoreactivity (Bauer et al. 1991; Strauss et al. 1992). These findings have been confirmed by Wood et al. (1993) who found elevated levels of IL-6 and c-reactive protein in the brains of AD patients, but not in the brains of nondemented elderly control persons.

In order to understand the role of IL-6 in the progression of tissue destruction, it is important to know whether IL-6 is present in early lesions or appears after vast tissue destructions. If IL-6 is involved early in the destructive process, it should already be found in areas with early pathological changes.

IL-6 in early stages of plaque formation in AD

We investigated whether IL-6 is present already in diffuse plaques or only in later stages of plaque formation. Tissues from ten autopsy cases with clinically diagnosed and neuropathologically confirmed AD were used for this histological study. We used serial sections in order to first identify the plaque stage by the Bielschowsky silver staining (Yamamoto and Hirano 1986) and then applied immunohistochemistry with antibodies against IL-6 (polyclonal antibody against human IL-6, Genzyme, 1:50) on adjacent sections. This procedure allowed us to determine whether IL-6 immunoreactivity shows a tendency to be present in early or late stages of plaque formation. The plaque type of the respective IL-6 positive plaques was determined by comparing IL-6-immunoreactive spots and silver-stained plaques on consecutive sections. The distribution of plaque morphology of IL-6 positive plaques was expressed in percentage of all Il-6 positive plaques. As previously, we did not find IL-6 immunoreactivity in plaques of nondemented cases. In contrast, brains of AD patients displayed IL-6 positive plaques (Fig. 1). A detailed analysis of the distribution of IL-6 immunoreactivity showed that IL-6 immunoreactivity was rare in classical plaques and absent in compact plaques (Fig. 2). In the brains of AD patients 55% of all lesions were neuritic plaques, 44% diffuse and 1% compact. However, most of the IL-6 positive plaques were diffuse (71%) and the remaining IL-6 positive plaques were neuritic plaques (29%). This means that IL-6 is more than 1.5 times more often found in diffuse plaques than would be expected from a random distribution among all plaque types. Thus, IL-6 seems to be associated with early pathological changes in AD indicating that the induction of IL-6 is an early event in the neurodegenerative cascade and may precede neuritic degeneration.

Possible mechanisms of IL-6 activation

The factors which are responsible for IL-6 synthesis in brains of AD patients are still unknown. IL-6 may be synthesized by microglial cells and astrocytes (Gottschall et al. 1994; Norris and Benveniste 1993). Difficulties in the regulation of IL-6 expression in older persons may represent one reason for an elevated basal IL-6 expression (Ershler 1993). We have recently been able to confirm previ-

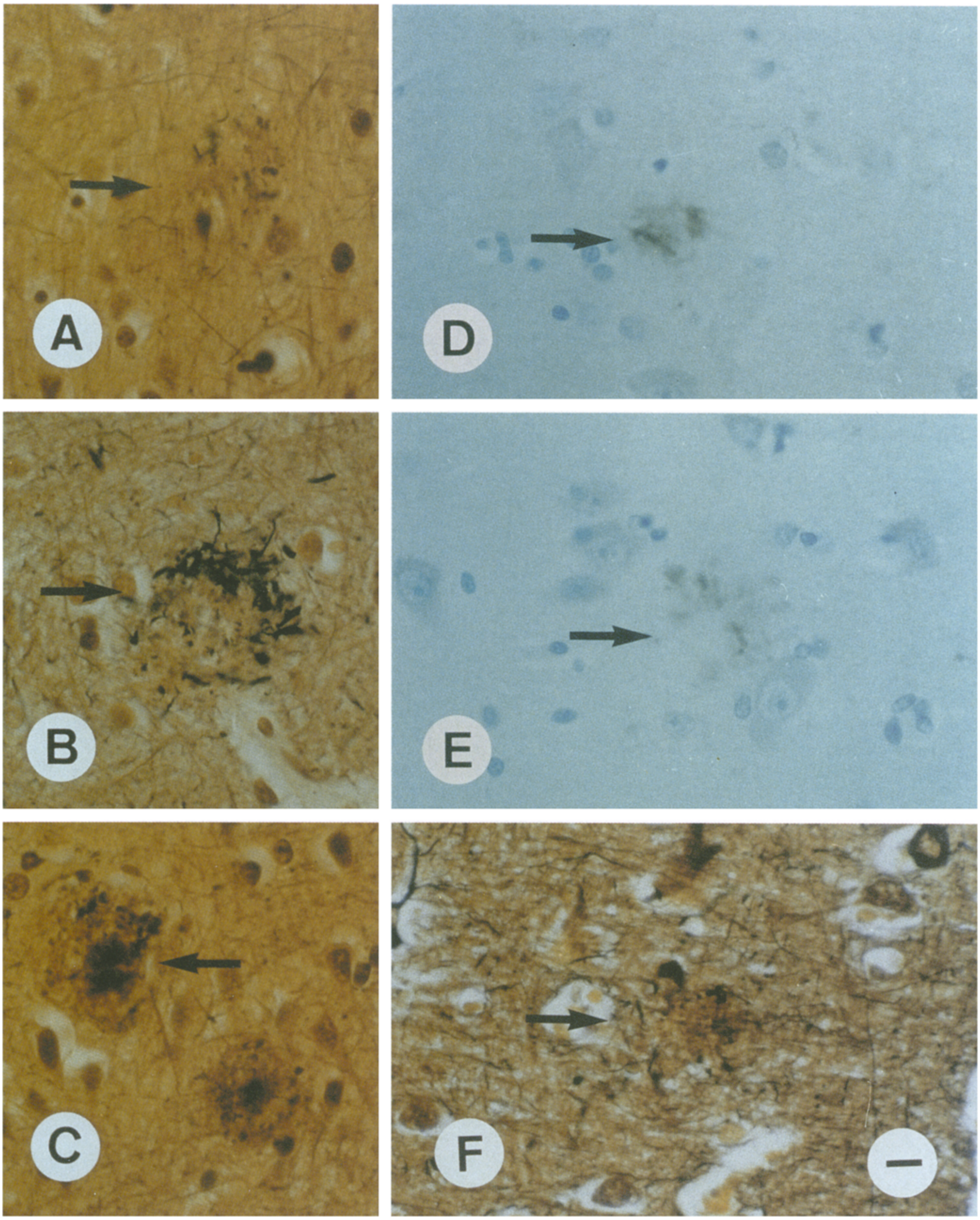

Fig. 1 A–F Histological sections of Alzheimer's disease (AD) cortices. In **A, B** and **C** staining with the Bielschowsky silver method. **A** diffuse plaque with condensed amorphous material (*arrow*). **B** Primitive plaque with black-stained distorted neurites (*arrow*). **C** Classical plaque with a dense core (*arrow*). **D, E** and **F** are serial sections. **D** Immunohistochemical staining with antibodies against interleukin-6 (IL-6) shows an IL-6 positive plaque (*arrow*). **E** Staining with antibodies against the amyloid-precursor protein shows that the plaque contains the amyloid-precursor protein (*arrow*). **F** Bielschowsky silver staining. The plaque shows mainly signs of a diffuse plaque(*arrow*). *Bar* in **F** represents 10 μm

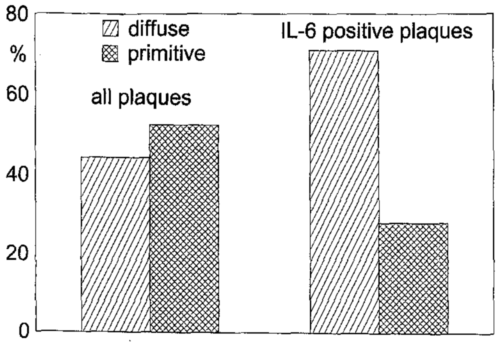

Fig. 2 Morphology and IL-6 immunoreactivity in AD cases. *Left columns*: distribution of all plaques according to diffuse or primitive plaque type. Classical and compact plaques were less than 4% and are not shown. *Right columns*: distribution of IL-6 positive plaques according to plaque type. The greater number of IL-6 positive plaques are diffuse

ous studies showing that basal IL-6 plasma levels rise with age (Hager et al. 1994). Besides age-related changes in IL-6 expression, various neurotransmitters, such as epinephrine, norepinephrine and glutamatergic agonists, are able to stimulate IL-6 syntheses (DeRijk et al. 1994; Maimone et al. 1993, Minami et al. 1991; Norris and Benveniste 1993). Changes in cholinergic and catecholaminergic neurotransmission are known in AD, which may modulate immunological parameters and not only neurotransmission.

Neurodegeneration in IL-6 transgenic mice

The potential of IL-6 to contribute to a neurodegenerative cascade has recently been demonstrated in a transgenic mouse model. Campbell et al. created transgenic mice bearing a construct of the IL-6 cDNA under the control of the SV40- and GFAP-promotor. This led to a brain-specific overexpression of IL-6 in astrocytes. These animals showed cerebral abnormalities with astrogliosis, neurodegeneration of hippocampal neurons and reduction of dendritic arborization. The decrease in arborization of pyramidal neurons in this model shows that synaptic plasticity is seriously affected by overexpression of IL-6. Thus, the presence of elevated levels of IL-6 may in fact be pathogenic and may induce morphological alterations including synaptic pathology.

Possible therapeutic approaches

If inflammatory mechanisms are important for the progression of dementia, anti-inflammatory drugs should be beneficial and should influence the clinical course of AD. Several retrospective studies have shown an inverse correlation of the use of anti-inflammatory drugs and onset and progression of dementia (Breitner et al. 1994; Rich et al. 1995). However, there is only one small prospective double-blind study on anti-inflammatory treatment of AD (Rogers et al. 1993). This study documented an attenuated decline of intellectual capacity in the patients treated with indomethacin for 6 months. Further studies are necessary to evaluate the benefit of anti-inflammatory therapy in AD (Aisen and Davis 1994).

Conclusion

IL-6 appears to be a specific element of AD and is absent in the brains of nondemented elderly individuals. Our data show that inflammatory mechanisms are involved in the pathogenesis of AD. IL-6 can be found in plaques prior to the onset of neuritic degeneration indicating an early involvement of inflammatory mechanisms in the neurodegenerative cascade.

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