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Interleukin-6 and α-2-macroglobulin indicate an acute-phase state in Alzheimer's disease cortices

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Recent studies indicated that the formation of a major constituent of Alzheimer's disease (AD) senile plaques, called βA4-peptide, does not result from normal processing of its precursor, amyloid precursor protein (APP). Since proteolytic cleavage of APP inside its βA4 sequence was found to be part of APP processing the formation of the βA4-peptide seems to be prevented under normal conditions. We considered whether in AD one of the endogenous proteinase inhibitors might interfere with APP processing. After we had recently found that cultured human neuronal cells synthesize the most potent of the known human proteinase inhibitors, α-2-macroglobulin (α2M), upon stimulation with the inflammatory mediator interleukin-6 (IL-6) we now investigated whether α2M and IL-6 could be detected in AD brains. Here we report that AD cortical senile plaques display strong α2M and IL-6 immunoreactivity while no such immunoreactivity was found in age-matched control brains. Strong perinuclear α2M immunoreactivity in hippocampal CA1 neurons of Alzheimer's disease brains indicates that neuronal cells are the site of α2M synthesis in AD brains. We did not detect elevated IL-6 or α2M levels in the cerebrospinal fluid of AD patients. Our data indicate that a sequence of immunological events which seem to be restricted to the local cortical environment is part of AD pathology.

Alzheimer's disease; α-2-Macroglobulin; Interleukin-6; Acute phase; Protease inhibitor

1. INTRODUCTION

A peptide of 42–43 amino acids with a high tendency to aggregate, called βA4 protein (βA4), has been identified as a major proteinaceous component of Alzheimer's disease (AD) senile plaques (reviewed in [1–3]). βA4 is derived from a 90–140 kDa precursor, called amyloid precursor protein (APP), which, due to differential splicing of its hn mRNA, is translated into three major protein forms (with 695, 751, and 770 amino acids, respectively) [1–3]. APP is constitutively expressed by neuronal and non-neuronal cells in brains of both healthy individuals and AD patients. A recent study revealed that no differences in the 770:751:695 ratio could be found between normal aged persons and AD patients [4]. This ratio, however, is changed along development and along normal aging [4]. A recently described single point mutation of the APP gene could be found only in a minority of familial AD patients (2 out of 11 investigated cases) while a majority of familial and all of the investigated non-familia AD cases lack this mutation [5].

Studies on APP expression in cell cultures revealed that APP is inserted as a transmembrane protein by the synthesizing cells [1–3]. The βA4 sequence of APP spans from the middle of the membrane to the extracellular part of the APP molecule [1–3]. Normal processing of APP then includes its proteolytic cleavage *inside* the βA4 sequence by a still unknown secretase resulting in the release of the large extracellular, N-terminal part of APP [6,7]. Thus, the formation of βA4 is prevented by normal APP processing. We considered whether in AD one of the endogenous human protease inhibitors might inhibit the normal cleavage of APP and may thus give rise to the breakdown product βA4 which, due to its tendency to aggregate, could then contribute to plaque formation. After we had found that cultured human neuronal cells could be induced to synthesize the most potent of the known human proteinase inhibitors, α-2-macroglobulin (α2M), upon stimulation with the inflammatory mediator interleukin-6 (IL-6) [8], we now immunohistochemically examined AD cortices for the presence of α2M and IL-6.

2. MATERIALS AND METHODS

Formalin-fixed paraffin-embedded sections of different isocortical (frontal, parietal and occipital) and hippocampal areas from 6 cases of AD confirmed by clinical and independent histopathological examination (ages 66, 69, 70, 73, 76 and 78) and brain sections of the same areas from normal aged-matched control persons without any neuropathological diagnosis were immunohistochemically investigated for APP (with monoclonal anti-APP, Boehringer, Mannheim, Germany (not shown), α2M, and IL-6 immunoreactivity. After

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incubation with the primary monospecific rabbit antibodies polyclonal anti-α2M [9] and anti-IL-6 (Genzyme, Boston, MA), immunostaining was performed with the avidin-biotin-peroxidase complex method (Vectastain, Vector Laboratories, Burlingame, CA). Peroxidase activity was developed with diaminobenzidine (Sigma, Deisenhofen, Germany) / H_2O_2. The sections were counterstained with hematoxylin. Control stains were performed separately (i) with non-immune rabbit control serum and showed no or only very low background staining and (ii) by saturating the specific antibodies with their purified antigens (IL-6 or α2M, respectively) prior to immunohistochemistry resulting in abolisment of the immunostaining.

3. RESULTS AND DISCUSSION

Isocortical and hippocampal AD senile plaques, which as expected could consistently be stained for APP (not shown), displayed strong α2M (Fig. 1A) and IL-6 immunoreactivity (Fig. 1B). This was a consistent finding in all investigated AD cases. In addition, large neurons of the AD cortices, mainly in the hippocampal CA1 region, showed a pronounced intracellular, perinuclear α2M stain (Fig. 2A) which was absent in normal aged brains. This points to α2M synthesis in these cells. Furthermore, the bodies of many large neurons, mainly in the isocortical areas, were surrounded by an edge of strong IL-6 immunoreactivity (Fig. 2B) indicating binding of IL-6 to these neurons. Since no antigenic cross-reactivity with other proteins has been reported neither for α2M nor for IL-6, our observations suggest the actual presence of α2M and IL-6 (or of their respective breakdown products) in AD senile plaques. Unspecific staining could be excluded by extensive control studies (i) with several non-immune sera which showed no or only extremely low background staining

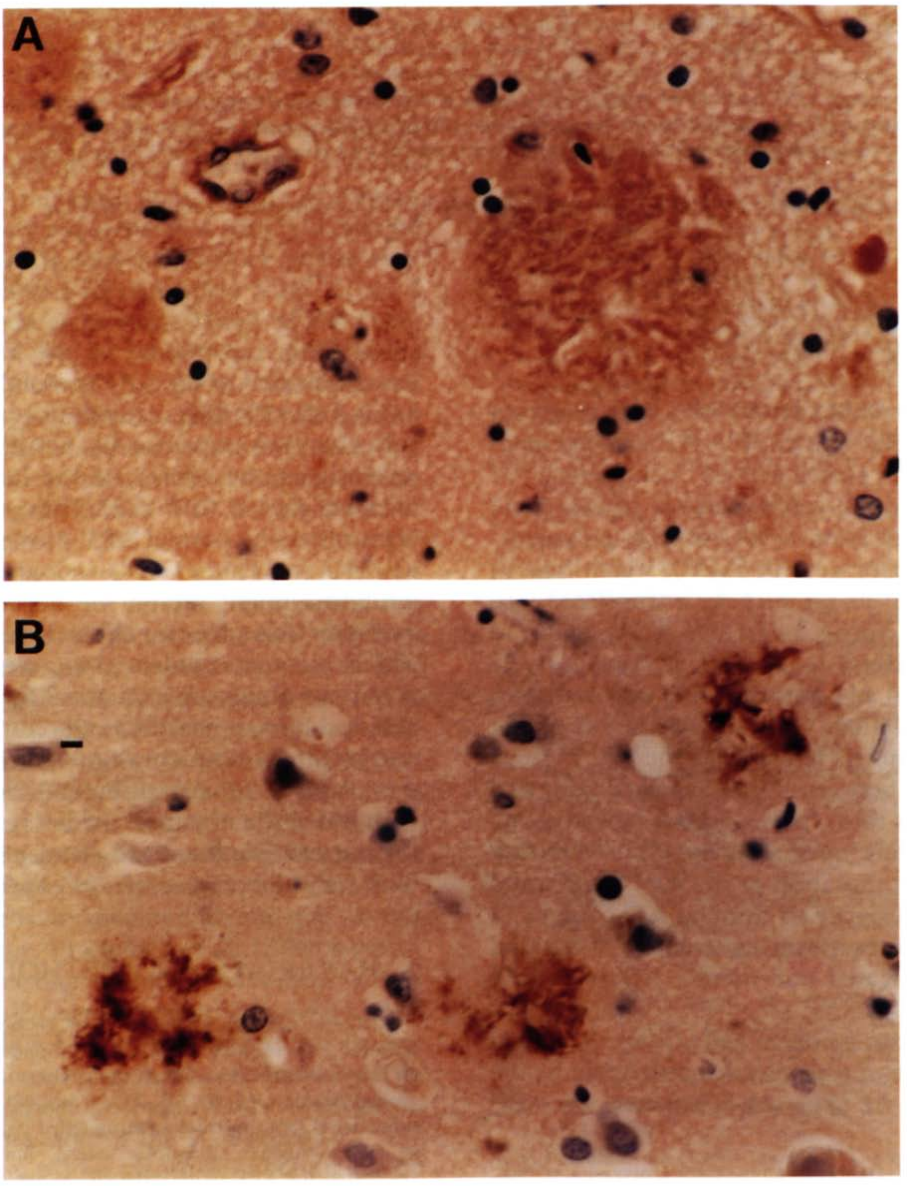

Fig. 1. Immunohistochemical detection of α2M (A) and IL-6 (B) immunoreactivity in AD senile plaques (parietal section, magnification × 512).

(not shown) and (ii) by pre-absorbing the antibodies with their respective antigens prior to the immunohistochemical procedure resulting in complete disappearance of the signals (not shown).

We have previously found that spontaneous α2M synthesis in cultured human neuronal cells is very low but can be strongly induced (more than 20-fold) upon stimulation of the cells with IL-6 in a dose- and time-dependent manner [8]. This observation suggests that the detected immunoreactivities for IL-6 and α2M in AD brains may be functionally connected. In addition to neuronal cells, we previously identified astrocytes as a potential site of α2M synthesis [10]. Recently, we found that α2M may interfere with APP processing [8]. However, it is still unclear whether α2M may affect APP processing directly (e.g. by inhibiting the pro-

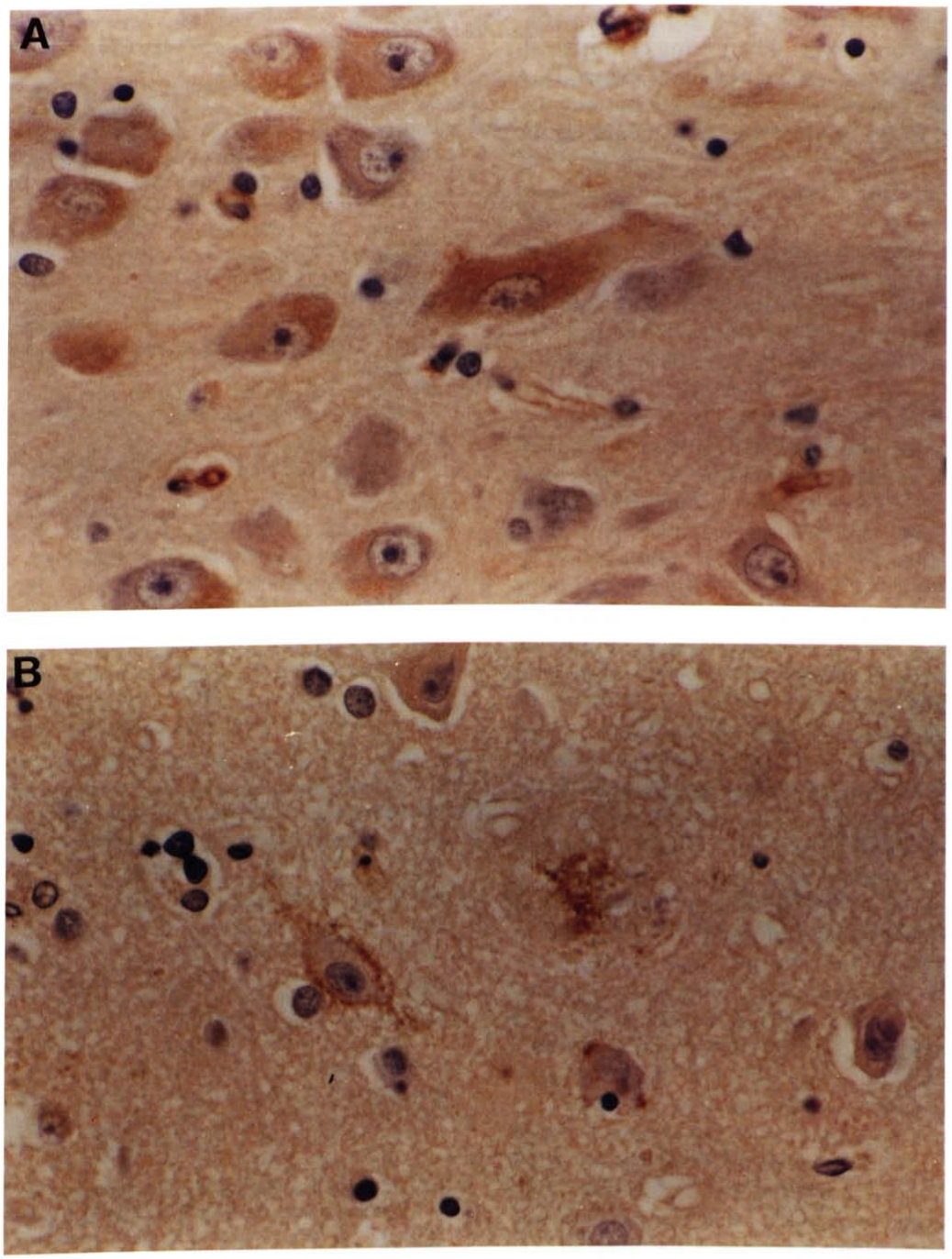

Fig. 2. Immunohistochemical detection of intracellular, perinuclear α2M immunoreactivity in AD brain large hippocampal CA1 neurons (A). Detection of pericellular IL-6 immunoreactivity around a neuronal cell in an AD brain parietal section (B). Note the additional IL-6 staining of a senile plaque in (B) (magnification × 600).

teolytic secretase) or indirectly (e.g. by affecting neuronal cell vitality via other unknown mechanisms).

The peptide IL-6 is one of the endogenous inflammatory mediators or cytokines, which are synthesized by the host upon injury or infection [11,12] (for review see [13,14]). Along with tumor necrosis factor α and interleukin-1, IL-6 induces a complex reaction of the host called the acute-phase response [15]. In the brain, IL-6 can be synthesized by microglia, astrocytes, and endothelial cells [16]. IL-6 synthesis requires previous stimulation of the synthesizing cells, e.g. by bacteria, viruses or immune complexes. When using commercially available IL-6 ELISA's, we did not find elevated IL-6 levels in the cerebrospinal fluid of 10 AD patients investigated so far. In our hands, also α2M could not be detected in the unconcentrated cerebrospinal fluids of AD patients. Therefore expression of IL-6 and α2M seems to be restricted to the intracortical environment. Our data make it necessary to further investigate what may trigger local IL-6 synthesis in AD brains.

α-2-Macroglobulin (α2M) is the most potent of the known human proteinase inhibitors (reviewed in [17]). This protein is mainly synthesized by the liver both in man and in the rat. In the rat, hepatic α2M synthesis is strongly induced by the cytokine IL-6 resulting in elevated serum levels upon injury or infection [18–21]. Therefore, in this species α2M belongs to the so-called acute-phase proteins. In man, α2M serum levels do not increase during the acute-phase response why constitutive expression by the liver is assumed [17]. However, our recent finding that α2M synthesis can be strongly induced in human neuronal cells upon incubation with IL-6 [8] provides evidence that α2M may behave as an acute-phase protein also in man, at least in the central nervous system.

In the central nervous system, important events such as neuronal cell migration during brain development and neurite outgrowth after tissue damage have been found to depend on a precarious balance between proteases and their inhibitors (reviewed in [22]). An important role of α2M for brain development and neuronal plasticity is suggested by the finding of transient α2M expression in the developing brain [23,24]. Since (abortive) neurite sprouting is consistently found around AD senile plaques, the detection of α2M in plaques may be of special significance. In contrast to AD brains, we could hardly detect any α2M immunoreactivity in cortices of age-matched normal brains (not shown) confirming a previous report by other investigators [25].

In conclusion, a sequence of immunological events starting with IL-6-induced cortical synthesis of α2M and possibly ending with altered APP processing may be hypothesized. At least, there is now clear evidence that a kind of acute-phase response is involved in AD pathology.

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