

Up-regulation of the inflammatory cytokines IFN- γ and IL-12 and down-regulation of IL-4 in cerebral cortex regions of APP_{SWE} transgenic mice

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Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disorder, of which the pathogenesis is thought to involve increased β -amyloid (A β) deposition and abnormal immunological responses. To elucidate the mechanisms involved in A β -mediated inflammation, we used immunocytochemistry and in situ hybridization to study the potential role of the cytokines interferon- γ (IFN- γ), interleukin (IL)-12 and IL-4 in transgenic mice APP_{SWE} (Tg2576) that overexpress the human β -amyloid precursor protein gene. Cytokine and cytokine mRNA expression was detected in brain sections from cortical regions at various postnatal ages ranging from 3 to 19 months. High levels of IFN- γ and IL-12 mRNA expression, as well as their protein production, appeared early at 9 months and peaked at 17–19 months in Tg2576 mice. Significantly increased transcripts of IFN- γ and IL-12 genes were found in the reactive microglia and astrocytes surrounding β -amyloid deposits. In accordance with the kinetics of mRNA levels, the expression of IFN- γ and IL-12 at the protein level was positively correlated with age and reached a maximum in 17–19-month-old mice. Both findings suggest a role for the pro-inflammatory cytokines IFN- γ and IL-12 in early disease development and are consistent with microglial activation related to β -amyloid formation. In contrast, transcription and production of IL-4 in brain sections was almost undetectable in transgenic mice up to post-natal ages of 17–19 months. These results suggest a major pro-inflammatory role for IL-12 and IFN- γ in Tg2576 transgenic mice that may provide the association between β -amyloid plaque formation and microglial and astrocyte activation in these animals. These observations call for further studies on the potential role of anti-inflammatory therapeutic strategies for AD. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Alzheimer's disease; APP_{SWE} transgenic mouse; APP; β -amyloid; Cytokines

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most common cause of dementia. AD is characterized neuropathologically by the deposition of extracellular amyloid plaques containing aggregates of the β -amyloid (A β) peptide, as well as by intracellular aggregations of neurofibrillary tangles consisting of paired helical filaments of hyperphosphorylated tau protein (Glennner et al., 1984; Braak and Braak 1998; Selkoe, 1998). AD pathology

also involves selective neuronal cell loss accompanied by cerebrovascular amyloidosis. The mechanisms behind AD have not been completely defined.

An abnormal immunological response and inflammatory cytokines are proposed to be involved in the pathogenesis of a number of neurodegenerative processes (Benveniste 1992; Du, 1999). In AD, immune and inflammatory process related proteins have been implicated as mediators in responses to brain injury. Elevated levels of major histocompatibility complex (MHC) antigen class II (MHC II) molecules and a number of inflammatory mediators associated with β -amyloid deposits have been detected in brains from AD patients. The latter include up-regulation of cytokines, such as interleukin-1 (IL-1) (Griffin et al., 1989), IL-6 (Bauer et al., 1991)

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and tumor necrosis factor- α (TNF- α) (Dickson et al., 1993). Acute phase proteins, such as α 1-antichymotrypsin (Abraham et al., 1990), α 2-macroglobulin (Bauer et al., 1991; Strauss et al., 1992), and up-regulation of several complement proteins are also thought to be involved in the pathogenesis of AD (Eikelenboom et al., 1989, 2000; Eikelenboom and Hoogendijk, 1999; Zhan et al., 1994). Moreover, microglia and astroglial cells surrounding mature plaques in AD brains may further lead to inflammatory neurotoxin production resulting in cell death (Cotter et al., 1999). The neuroimmunological cascade produced by the local immune reaction may play a major contribution to neuronal dysfunction and neurodegeneration.

Although the majority of AD cases occur spontaneously, respectively sporadic, the study of inherited familial cases has provided much insight as to the genetic factors that contribute to the development of AD neurodegeneration. Various mutations in the amyloid protein precursor (APP) and the presenilin genes can lead to early onset, autosomal dominant AD (Hardy, 1997). The presenilin 1 and presenilin 2 genes have been identified as pathogenic loci involved in the majority of early onset AD (Hutton and Hardy 1997).

One of the most widely used animal models for β -amyloid plaques in the cortical regions of the brain is that of the Tg (HuApp695.K670-M671L) 2576 transgenic mouse, which over-expresses human APP with the Swedish double mutation (Hsiao et al., 1996). The Tg2576 transgenic mice reflect in part AD pathology including elevated levels of A β _{1–40} and A β _{1–42}, the presence of amyloid plaques, neuropil abnormalities and microglial activation (Irizarry et al., 1997; Frautschy et al., 1998).

We used Tg2576 mice to elucidate the mechanisms underlying the hypothesis that β -amyloid induces immune activation, as well as to improve the understanding of the role of cytokines in the disease processes of AD. The expression of the cytokines, IFN- γ , IL-12 and IL-4 by immunocytochemistry and in situ hybridization was examined in brain sections from cortical regions of transgenic Tg2576 mice at various postnatal ages ranging between 3 and 19 months.

2. Materials and methods

2.1. Transgenic animal

Two female Tg (HuAPP₆₉₅SWE) 2576 mice (a kind gift from Dr. K. Hsiao) in a hybrid background of C57BL6/SJL and C57B6 males (Bomice and Mollegaard Breeding Laboratories, Ejby, Denmark) were employed to breed a colony of experimental animals. The animals were individually housed in Plexiglas cages (25 × 20 × 15 cm) with free access to rodent chow and water and were maintained on a 12-h light/dark cycle.

Studies were performed on male Tg2576 mice and age-matched male non-transgenic littermates as controls at var-

ious postnatal ages ranging between 3 and 19 months. All mice were born and bred in our own colony except for the animals in the 17–19-month age group which were received as gifts from Pharmacia and Upjohn Kalamazoo (USA) and Merck Sharpe and Dohme (Essex, UK). The transgenic mice used in the study have human APP695 with the double mutation Lys670-Asn and Met671-Leu (K670N, M671L), which was found in a large Swedish family with early onset of AD, inserted into a hamster prion protein (PrP) cosmid vector in which the PrP open reading frame was replaced by that for the variant APP Tg (HuAPP695.K670N-M671L) 2576, as developed by Hsiao et al. (1995, 1996).

2.2. Tissue preparation

Mice were sacrificed by cervical dislocation and the brains were rapidly removed. One hemisphere was immediately frozen in powdered dry ice and stored at -80°C . Frozen brains were sectioned with a cryostat at approximately -20°C . Ten-micrometer saggital sections were collected and thaw-mounted onto Superfrost*Plus glass microscope slides (Menzel-Glaser, Germany) thoroughly air-dried at room temperature and stored at -80°C until use for in situ hybridization and immunohistochemistry.

2.3. In situ hybridization

In situ hybridization (ISH) was performed as described for brain tissue sections (Zhu et al., 1994). Briefly, synthetic oligonucleotide probes (Scandinavian Gene Synthesis AB, Köping, Sweden) were labeled, using ^{35}S -deoxyadenosine-5-(thio)-triphosphate (New England Nuclear, Cambridge, MA, USA) with terminal deoxynucleotidyl transferase (Amersham, Little Chalfont, UK). The oligonucleotide sequences were obtained from GenBank and probes were designed using MacVector software (IBI, New Haven, CT, USA). A constant ratio of the guanine/cytosine content of approximately 60% was employed. The oligonucleotide probes were approximately 48 bases long and checked for absence of palindromes or long sequences of homology within the species against available GenBank data. To increase the sensitivity of the method a mixture of four different probes was used per cytokine. The rat IFN- γ probes (GenBank accession nos. M29315, M29316 and M29317) were complementary to bases 298–345 (exon 1), 80–125 (exon 2), 303–350 (exon 3) and 180–227 (exon 4) (Dijkema et al., 1986). The rat IL-4 probes (GenBank accession no. X16058) corresponded to bases 83–130, (exon 1), 209–256, (exon 2), 270–317 (exon 3) and 331–378 (exon 4) (McKnight et al., 1991). The mouse IL-12 probes (GenBank accession nos. M86771 and M86672) corresponded to bases 147–194, (exon 1) and 595–642 (exon 2), 190–238, (exon 1) and 706–753 (exon 2) (Schoenhaut et al., 1992), respectively. After ISH, emulsion autoradiography slides were developed, and stained with cresyl violet, in order to identify the cell type with expression of the cytokine mRNAs and mounted with

Entellan (Merck, Darmstadt, Germany). Coded slides were analyzed by dark field microscopy at $10\times$ magnification. The results were expressed as numbers of cytokine mRNA per 100 mm^2 brain tissue sections. The cells were judged as positive when containing >15 silver grains per cell with a star-like distribution over the cytoplasm. In cells judged negative, the grains were scattered randomly over the cell and not distributed in a star-like fashion. The cellular distribution of the grains was checked under light microscopy at $20\times$ and/or $40\times$ magnification. There were no difficulties in differentiating between cytokine mRNA positive and negative cells. Variation between duplicates was $<10\%$. For all cytokines evaluated, a sense probe with the nucleotide sequence for rat IFN- γ exon 4 was always used as negative control. This control probe was used in parallel with the cytokine probe on sections from each specimen, without revealing any positive cells.

2.4. Immunohistochemistry

Cryostat sections ($10\ \mu\text{m}$) of the cortical region from 5 Tg2576 and 5 non-transgenic control mice were exposed to mouse monoclonal antibodies (mAb) DB1 (anti-rat IFN- γ) (Biomedical Primate Research Centre, Rijswijk, The Netherlands), anti-mouse IL-4 and anti-mouse IL-12 (Innogenetics, Ghent, Belgium), as well as anti- β amyloid (clone 6F3D) (Dako, High Wycombe, UK). The sections were stained with the avidin–biotin technique (Vectastain Elite Kit; Vector Labs, Burlingame, CA, USA). Peroxidase-substrate solution was added until the desired color (yellow) intensity had developed. Omission of the primary antibodies served as negative controls. For double staining, a soluble enzyme immune complex method was used. After the first staining for measurement of IFN- γ , IL-4 and IL-12 production, the brain sections were incubated with mouse mAb ED1 (anti-rat macrophage/microglia) (Serotec, Oxford, UK) or polyclonal antibodies to glial fibrillary acidic protein (GFAP) (Dako, Glostrup, Denmark), an intermediate filament protein in astrocytes, which were applied to identify activated astrocytes. Alkaline phosphatase–anti-alkaline phosphatase (APAAP) complex was added and alkaline phosphatase red substrate (Vecta stain) applied to give a rose-red color end product on a single-stained cell and a brown product on a double-stained cell. To identify the cell type, immunohistochemistry combined with ISH staining was detected for the mRNA expression of IFN- γ , IL-4 and IL-12. Tissue areas were measured by image analysis and the numbers of positive stained cells were counted at $20\times$ magnification in the entire section area. The results were averaged and expressed as cells per 100 mm^2 tissue section.

2.5. Statistical methods

Differences between the four groups were tested by one-factor analysis of variance (ANOVA). All significance tests were two-sided.

3. Results

IFN- γ and IL-12 mRNA and protein positive cells were scattered within the cortex region of the brain sections of Tg2576 mice and produced mainly in cells with the size and morphology corresponding to microglia (Fig. 1). After double staining, the brain sections of 17–19-month-old Tg2576 mice revealed a co-localization of IFN- γ or IL-12 and ED1 (microglia) (Fig. 2a) or GFAP (activated astrocytes) (Fig. 2b) immunoreactivity, indicating that IFN- γ and IL-12 are expressed by reactive microglia and astrocytes, respectively. β -Amyloid deposits were found in the cerebral cortex and hippocampus of 11-month-old Tg2576 mice in general (Fig. 2c). IL-4 staining was also restricted to microglia and astrocytes with no staining of neurons (data not shown).

3.1. IFN- γ expression

Elevated numbers of IFN- γ mRNA expressing cells were detected in the brain sections of Tg2576 mice at the age of 3–4 months with a mean number of 11.6 per 100 mm^2 tissue section (Fig. 3a). The levels remained elevated, when evaluated at the ages of 9 and 11 months (21.99 and 28.88, respectively, per 100 mm^2). The level was further increased at the age of 17–19 months (52.94 per 100 mm^2 tissue section), when compared with levels seen in mice at ages 3–4, 9 and 11 months ($p<0.001$ for all comparisons).

In contrast, there were no significant changes in the numbers of IFN- γ mRNA expressing cells in the cerebral cortex from non-transgenic mice of 3–19 months. The mean numbers of IFN- γ mRNA expressing cells in non-transgenic mice of the ages of 3–4, 9, 11 and 17–19

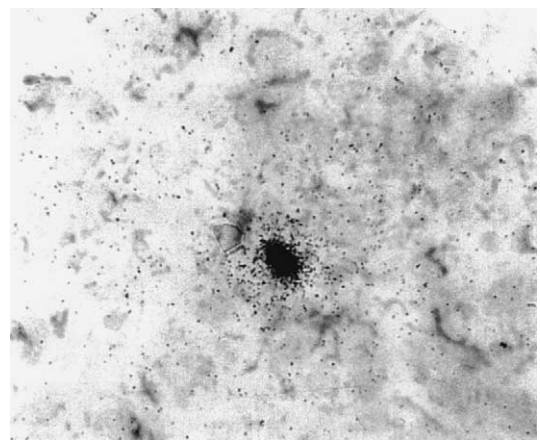


Fig. 1. Autoradiogram of in situ hybridization for IFN- γ mRNA of brain sections from cortical regions of a 17-month-old transgenic Tg2576 mouse. One IFN- γ mRNA hybridizing cell is shown at magnification of $\times 400$ to illustrate the evaluation of positive cells against a negative background.

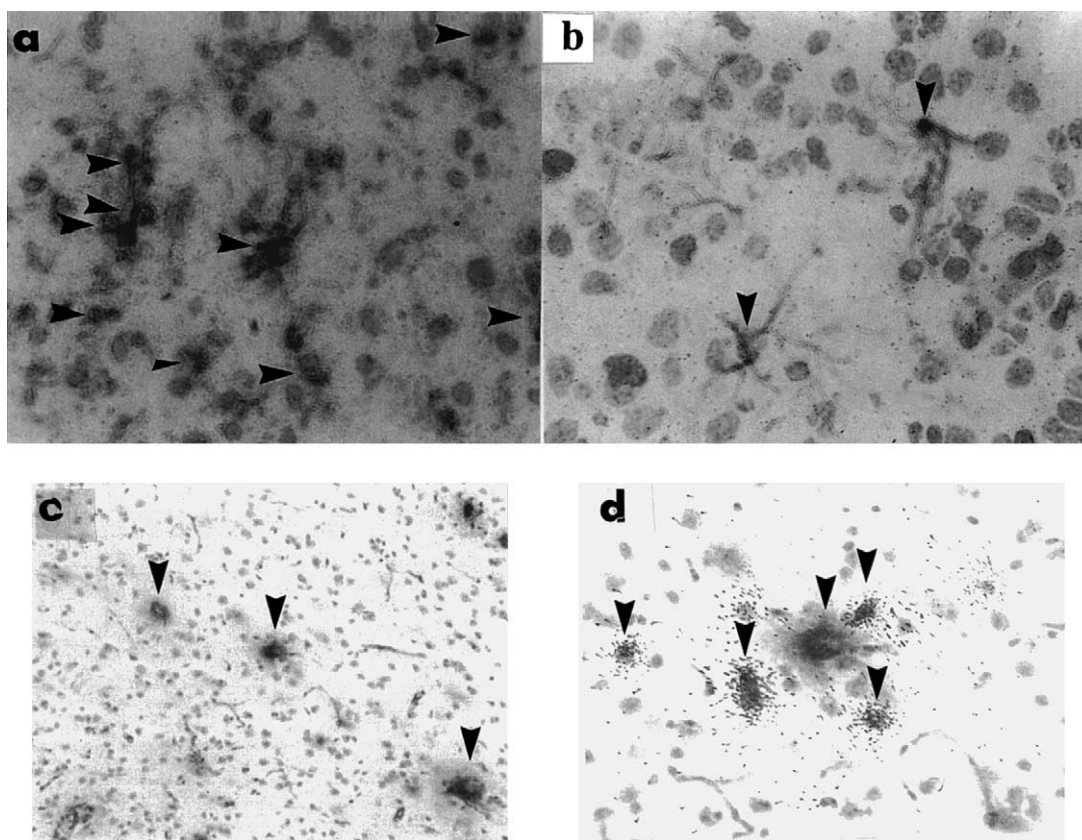


Fig. 2. A double staining for (a) IFN- γ and microglia (ED1) (\blacktriangledown), (b) IL-12 and astrocytes (GFAP) (\blacktriangledown) as well as for (d) IL-12 mRNA expression and β -amyloid deposits (\blacktriangledown); and (c) β -amyloid deposits (\blacktriangledown) detected by immunohistochemistry in the brain sections from cortical regions of 17-month-old transgenic Tg2576 mice (for β -amyloid deposits in 11-month-old mouse) reveal a co-localization of IFN- γ and microglia, IL-12 and astrocytes immunoreactivity (all magnification at $\times 200$) as well as IL-12 mRNA expression around β -amyloid plaques at magnification of $\times 400$.

months were 1.38, 7.78, 11.42 and 12.29, respectively, per 100 mm² tissue section. Comparing the numbers of IFN- γ mRNA expressing cells in Tg2576 mice with non-transgenic mice, there was a significantly higher expression of IFN- γ mRNA expressing cells in the cerebral cortex of Tg2576 mice ($p < 0.001$ for 17–19 months; $p < 0.01$ for 11 months). IFN- γ mRNA expressing cells were observed around the β -amyloid plaques in the brain sections of Tg2576 mice.

The mean numbers of IFN- γ positive cells in the brain sections of Tg2576 mice at the ages of 17–19, 11, 9 and 3–4 months were 75.89, 58.92, 32.05 and 12.38, respectively, per 100 mm² (Fig. 3b). There was a significant difference between the IFN- γ expression in Tg2576 mice of 17–19 and 9 months, 17–19 and 3–4 months as well as 11 and 3–4 months ($p < 0.001$ for all comparisons). In contrast, a few numbers of IFN- γ positive cells were detected in the brain sections from non-transgenic control mice at the same ages. There was significantly higher IFN- γ expression in Tg2576 mice of 17–19 and 11 months, as compared to age matched non-transgenic littermates ($p < 0.001$ for both comparisons). The patterns of IFN- γ

expression approximately paralleled that of IFN- γ mRNA expression in brain sections.

3.2. IL-12 expression

Elevated numbers of IL-12 mRNA expressing cells were detected in the brain sections of Tg2576 mice at the age of 9 months with a mean number of 24.21 per 100 mm² tissue section (Fig. 4a). The numbers of IL-12 mRNA expressing cells increased to 35.0 per 100 mm² at the age of 11 months. The maximum mean numbers of IL-12 mRNA expressing cells (mean 49.0 per 100 mm²) were observed at the age of 17–19 months. Differences over the ages were significant for IL-12 mRNA expressing cells (17–19 vs. 9; 17–19 vs. 3–4; 11 vs. 3–4 months, $p < 0.001$; 9 vs. 3–4 months, $p < 0.05$). Lower levels and no changes over time of IL-12 mRNA expressing cells were noticed in non-transgenic littermates. The mean numbers were 3.81, 9.42, 10.59 and 9.66, respectively, per 100 mm² for the ages of 3–4, 9, 11 and 17–19 months. The IL-12 mRNA expression was observed in areas close to the β -amyloid plaques in the cortical brain sections (Fig. 2d).

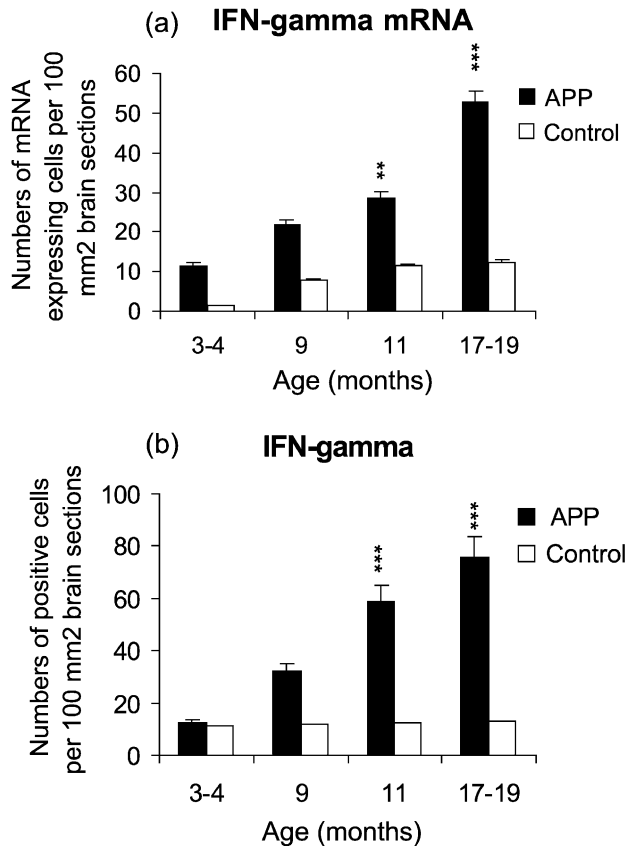


Fig. 3. Numbers of cells expressing IFN- γ mRNA (a) and IFN- γ protein (b) as detected by in situ hybridization and immunohistochemistry, respectively. The figure shows the mean numbers and SEM of positive cells per 100 mm² brain sections from cortical regions of transgenic Tg2576 mice and non-transgenic control mice at various postnatal ages. *P* values refer to comparisons between transgenic Tg2576 mice and non-transgenic control mice. ** *p* < 0.01; *** *p* < 0.001.

Elevated levels of IL-12 positive cells were found in Tg2576 mice using immunohistochemistry. The mean numbers were 6.75, 19.38, 26.46 and 39.29, respectively, per 100 mm² for the ages of 3–4, 9, 11 and 17–19 months (Fig. 4b). The differences for IL-12 expression between the different age groups in Tg2576 mice were significant (17–19 vs. 11; 17–19 vs. 9; 17–19 vs. 3–4; 11 vs. 3–4 and 9 vs. 3–4 months, *p* < 0.001 for all comparisons). When compared with age-matched non-transgenic littermates, differences at 9, 11 and 17–19 months investigated were significant (*p* < 0.001 for all comparisons).

3.3. IL-4 expression

The data of IL-4 mRNA expression and protein production are presented in Fig. 5. A low number of IL-4 mRNA expressing cells were detected in the brain sections of Tg2576 mice at all ages investigated (Fig. 5a). The mean numbers were 5.25, 7.8, 5.95 and 5.97, respectively, per 100 mm² for 3–4-, 9-, 11- and 17–19-month-old mice.

In contrast, strikingly increased numbers of IL-4 mRNA expressing cells were found in 9–19-month-old non-transgenic mice. The highest numbers of IL-4 mRNA expressing cells (mean 52.0 per 100 mm²) were observed in 9-month-old non-transgenic mice (9 vs. 17–19; 9 vs. 11, *p* < 0.05 for both comparisons; 9 vs. 3–4 months, *p* < 0.001). Thereafter, the numbers declined to 30.1 (at 11 months) and 28.1 (at 17–19 months), respectively, per 100 mm².

High levels of IL-4 positive cells were also found in non-transgenic mice at 9–19 months investigated by immunohistochemistry (Fig. 5b). In contrast to the mRNA levels, the mean numbers of IL-4 positive cells increased from 36.46 to 45.99, respectively, per 100 mm² at 9 and 11 months, to maximum numbers of 54.46 per 100 mm² at 17–19 months (17–19 vs. 9; 17–19 vs. 3–4; 11 vs. 3–4; 9 vs. 3–4 months, *p* < 0.001 for all comparisons). In Tg2576 mice, almost no immunoreactivity for IL-4 was detectable. When compared with age-matched non-transgenic littermates, dif-

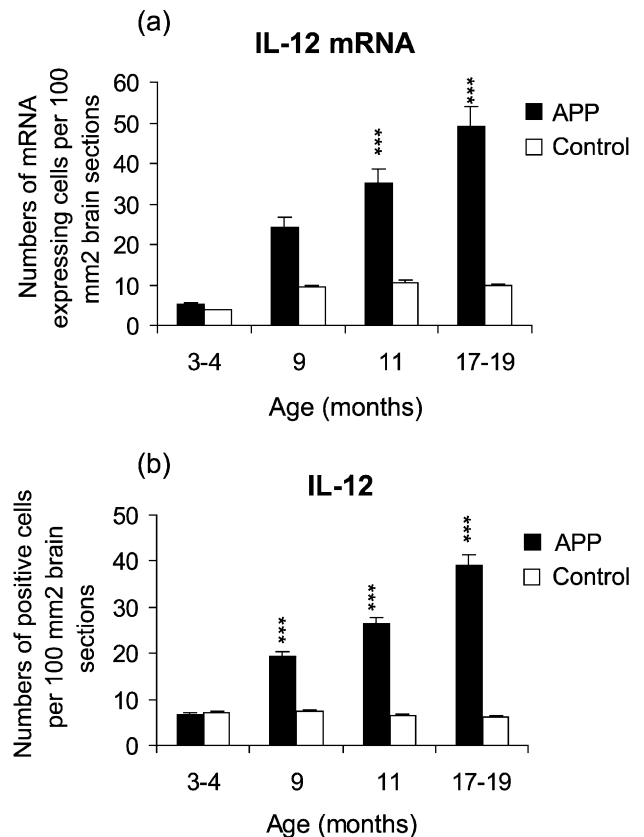


Fig. 4. Numbers of cells expressing IL-12 mRNA (a) and IL-12 protein (b) as detected by in situ hybridization and immunohistochemistry, respectively. The figure shows the mean numbers and one SD of positive cells per 100 mm² brain sections from cortical regions of transgenic Tg2576 mice and non-transgenic control mice at various postnatal ages. *P* values refer to comparisons between transgenic Tg2576 mice and non-transgenic control mice. *** *p* < 0.001.

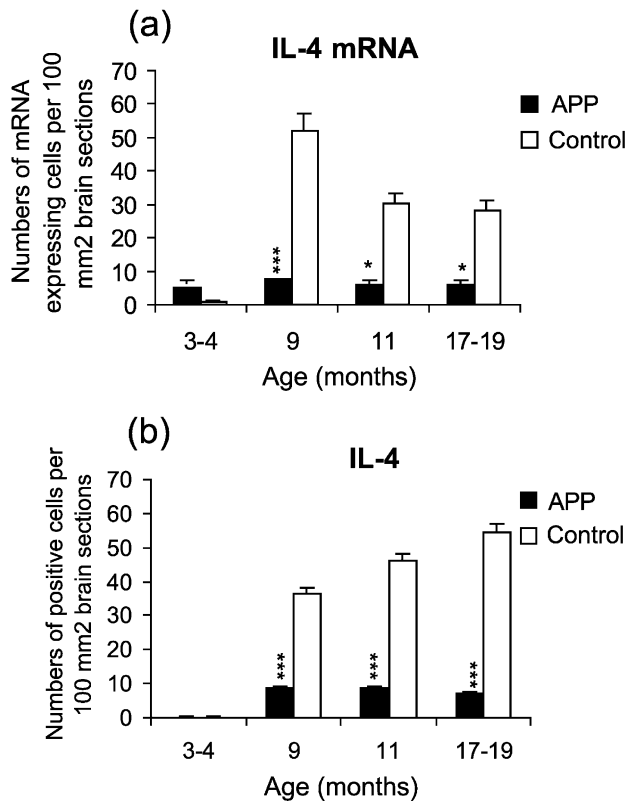


Fig. 5. Numbers of cells expressing IL-4 mRNA (a) and IL-4 protein (b) as detected by in situ hybridization and immunohistochemistry, respectively. The figure shows the mean numbers and one SD of positive cells per 100 mm² brain sections from cortical regions of transgenic Tg2576 mice and non-transgenic control mice at various postnatal ages. *P* values refer to comparisons between transgenic Tg2576 mice and non-transgenic control mice. * *p* < 0.05; *** *p* < 0.001.

ferences at 9, 11 and 17–19 months investigated were significant (*p* < 0.001 for all comparisons).

4. Discussion

In this study, we show that both IFN- γ and IL-12 transcription and production are markedly enhanced with increased age in the cortical brain regions of Tg2576 mice in reactive microglia and astrocytes surrounding β -amyloid deposits. Therefore, IFN- γ and IL-12 may play an inflammatory role in amyloid plaque formation and microglial as well as astrocyte activation. The increase of the difference and degree of significance between transgenic Tg2576 mice and wild-type control mice suggests a perpetual process of inflammation in a basically degenerative process in the nervous system.

The mechanisms by which β -amyloid mediates local inflammation and the role of cytokines in AD are not fully understood. However, it has been demonstrated that inflammatory molecules are uniquely present or significantly elevated in the AD brain and that inflammation may be a necessary component of AD pathogenesis (Rogers et al.,

1996; McGeer and McGeer 1998; Apelt and Schliebs, 2001). The deposition of human β -amyloid in a mouse brain tissue environment might induce a local inflammatory cascade (Mehlhorn et al., 2000). The actions of cytokines have been associated with many neurodegenerative and inflammatory disorders in the central nervous system (CNS) (Rothwell and Strijbos, 1995; Zhao and Schwartz, 1998). Cytokines play a major role in the initiation, propagation and regulation of immune and inflammatory responses and exert direct actions on neuronal and glial cells during damage, proliferation and repair processes (Rothwell and Strijbos 1995; McGeer and McGeer 1999a; Luterman et al., 2000). β -Amyloid is a toxic protein in vitro. Therefore, it could be assumed that the long-term production of β -amyloid in vivo in Tg2576 mice might induce brain inflammation, including activation of glial cells and production of pro-inflammatory cytokines, as well as alterations in cholinergic neurotransmission (Auld et al., 1998; Bednar et al., 2002, in press).

In a previous study, it was shown that IL-1 β was the only cytokine induced in reactive astrocytes around β -amyloid plaques in the cerebral cortex and hippocampus of 14-month-old Tg2576 mice (Mehlhorn et al., 2000). Our results show that high levels of IFN- γ and IL-12 production appear early in the cerebral cortex (at 9 months) as compared to β -amyloid deposits (at 11 months, in general) in Tg2576 mice. Furthermore, increased IFN- γ and IL-12 production also occurs in the cerebral cortex of 17–19-month-old Tg2576 mice with reactive microglia and astrocytes surrounding β -amyloid deposits. The reason for the different results may be owing to the use of different methodologies for detecting cytokine production.

IFN- γ possesses multiple immunoregulatory effects, including activation of macrophages/microglia and stimulation of macrophages to release oxygen radicals, which are toxic substances to the CNS. IFN- γ enhances vascular permeability, thereby playing a crucial role in inflammation accompanying neurodegenerative diseases. Other effects of IFN- γ include promotion of TNF- α and IL-1 production and synergistic effects with TNF- α on expression of immune molecules and antigen presentation by microglia. The effects of IFN- γ are regulated and modulated by IL-12 (Gately et al., 1994).

IL-12 is a heterodimeric cytokine that promotes the acquisition of a Th1 cytokine profile (Storkus et al., 1998). IL-12 plays an important role in the normal host defense against infection by different intracellular pathogens. IL-12 is also thought to have a central role in the pathogenesis of inflammatory disorders by shifting T cell response to the Th1 type (Caspi, 1998). IFN- γ and IL-12 are involved in the pathogenesis of several CNS disorders (Navikas and Link, 1996).

We hypothesized that β -amyloid may activate microglia and astrocytes to up-regulate proinflammatory cytokine production, such as IL-12 and IFN- γ , which promotes TNF- α and IL-1 production. β -Amyloid deposits may

influence immunoregulatory circuits through amplification of naturally existing suppressor/regulatory networks. It is still unclear as to the nature of the relationship between β -amyloid deposits and inflammatory cytokine production in the brains of Tg2576 mice. The two might interact with each other during the neurodegenerative process. Early inflammation in Tg2576 mice brain might promote β -amyloid plaque formation or vice versa.

IL-4 is an anti-inflammatory cytokine in several CNS disorders (Benveniste, 1992; Navikas and Link, 1996). The role of IL-4 in neurodegenerative disorders is still unclear. However, IL-4 and other anti-inflammatory cytokines, such as IL-10, differentially regulate microglial responses to β -amyloid in primary murine microglia and a human monocyte cell line, which indicates that IL-4 may play a role in the inflammation pathology observed surrounding senile plaques (Szczepanik et al., 2001). In the present study, IL-4 expression/production in the brain sections of Tg2576 mice did not reveal an induction. In contrast, IL-4 expression is suppressed as compared to control mice. This may result from a negative effect of IFN- γ on IL-4 production, as is known from the Th1–Th2 interaction of lymphocytes in the immune system.

A number of epidemiological studies suggest that the prevalence of AD is reduced by 40–50% in persons using anti-inflammatory drugs. Anti-inflammatory drugs might inhibit both the onset and the progression of AD (McGeer and McGeer, 1999b). However, a major disappointment is the broad chasm between the progress made in diagnostic methods and the low effectiveness of therapy in AD (Domzal, 1999). Furthermore, future studies should focus on the development of new agents and identify the dosage and duration of therapy necessary for a protective or therapeutic effect in AD.

In conclusion, β -amyloid plaque formation and microglia and astrocyte activation in Tg2576 mice mediate an inflammatory response. This might call for the development of anti-inflammatory therapeutic strategies for AD.

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