Karolinska Institutet

Division of Experimental Geriatrics, Department of Neurotec, Huddinge University Hospital
Stockholm, Sweden

Immunomodulation of Cytokine and Chemokine Production in Animal Models of Neuroinflammatory and Neurodegenerative Disorders

Nagat Abbas Ahmed M. Gadeh El Dum

Stockholm 2003

Box 200, SE-171 77 Stockholm, Sweden

ISBN 91-7349-422-4

In loving memory of, Dr Alum ELHuda Abbas

To my father

Abstract

Experimental autoimmune neuritis (EAN) is a $CD4^+$ T cell–mediated autoimmune disease of the peripheral nervous system (PNS) that can be actively induced in susceptible animal species and strains by active immunization with heterogeneous peripheral nerve myelin or its component P2 or P0 proteins or their peptides emulsified in Freund's complete adjuvant. EAN represents an animal model for studying the immunopathogenesis and therapy of Guillain-Barré syndrome (GBS) which is a major inflammatory demyelinating disease of the PNS in humans. The close clinical, histopathological, and electrophysiological similarities between EAN and GBS make EAN an especially suitable model, capable of offering insights into the pathophysiology of GBS. EAN is also considered to represent a general model for studying $CD4^+$-mediated autoimmune diseases.

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most common cause of dementia in the Western world. It is characterised neuropathologically by the deposition of extracellular amyeloid plaques containing aggregates of the amyloid protein β (Aβ) peptide, as well as by intracellular aggregation of neurofibrillary tangles and selective neuronal loss accompanied by cerebrovascular amyloidosis. The mechanism of AD has not been completely defined. The inflammatory cytokines have been implicated as mediators in response to brain injury in AD. Aβ precursor protein APP transgenic mice (Tg2576) are one of the most widely used animal model for Aβ plaques in cortical regions of the brain, which over-expresses human APP with the Swedish double mutation.

Peak numbers of macrophage inflammatory protein (MIP)-1α-positive cells in the sciatic nerve were seen on day 14 post-immunization (p.i.), which coincided with the development of severe clinical signs. Administration of an anti-MIP-1α antibody suppressed clinical signs of EAN and inhibited inflammation and demyelination in the sciatic nerve. Peak numbers of monocyte chemotactic protein (MCP)-1-positive cells in the sciatic nerve were detected on day 7 p.i. (i.e., the onset of clinical EAN). Administration of an anti-MCP-1 antibody caused a delay of onset of EAN. The numbers of MIP-2-positive cells reached a maximum on day 21 p.i. Anti-MIP-2 antibody failed to suppress clinical signs of EAN and inflammation and demyelination in the sciatic nerve.

EAN was strongly suppressed by Rolipram administered twice daily intraperitoneally from day 9 p.i., after onset of clinical EAN, to day 18 p.i., over 10 days. This clinical effect was associated with dose-dependent down-regulation of interferon (IFN)-γ and the chemokines MIP-1α, MIP-2 and MCP-1 as well as up-regulated interleukin (IL)-4 production in sciatic nerve sections from Rolipram-treated EAN rats at the maximum of clinical EAN, i.e., on day 14 p.i. These findings suggest that Rolipram could be useful in certain T cell-dependent autoimmune diseases and inflammatory neuropathies.

ABR-215062, which is a new synthetic immunomodulatory compound derived from Linomide, administered daily subcutaneously from the day of inoculation strongly suppressed EAN in a dose-dependent manner. ABR-215062 reduced the incidence of EAN, ameliorated clinical signs, and inhibited P0 peptide 180-199-specific T and B cell responses and also decreased inflammation and demyelination in the peripheral nerves. The suppression of clinical EAN is associated with inhibition of the inflammatory cytokines IFN-γ and tumor necrosis factor-α as well as the enhancement of the anti-inflammatory cytokine IL-4 in peripheral nerve tissues. The suppressive effects of ABR-215062 on EAN are quite similar to those of Linomide on EAN. These findings suggest that ABR-215062 could be useful in certain T cell-mediated autoimmune diseases.

To elucidate the mechanisms involved in Aβ-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 Tg2576. 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 Aβ deposits. Both findings indicate a role for the pro-inflammatory cytokines IFN-γ and IL-12 in early disease development and are consistent with microglial activation related to Aβ 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 Aβ plaque formation, microglial and astrocyte activation in these animals. These observations call for further studies on the potential role of anti-inflammatory therapeutic strategies for AD.

CONTENTS

LIST OF ORIGINAL PAPERS

This thesis is based on the following articles, which will be referred to in the text by their Roman numerals;

I. Liping Zou, Sigliti-Henrietta Pelidou, **Nagat Abbas**, Georgia Deretzi, Elhard Mix, Marianne Schultzberg, Bengt Winblad, Jie Zhu. Dynamics of production of MIP-1α, MCP-1 and MIP-2 and potential role of neutralization of these chemokines in the regulation of immune responses during experimental autoimmune neuritis in Lewis rats. 1999. **J. Neuroimmunol**. 98:168-175.

II. **Nagat Abbas**, Liping Zou, Sigliti Henrietta Pelidou, Bengt Winblad, Jie Zhu. Protective effect of Rolipram in experimental autoimmune neuritis: protection is associated with down-regulation of IFN-γ and inflammatory chemokines as well as up-regulation of IL-4 in peripheral nervous system. 2000. **Autoimmunity.** 32: 93-99.

III. Liping Zou, **Nagat Abbas**, Inga Volkmann, Inger Nennesmo, Michael Levi, Britta Wahren, Bengt Winblad, Gunnar Hedlund, Jie Zhu. Suppression of experimental autoimmune neuritis by ABR-215062 is associated with altered Th1/Th2 balance and inhibited migration of inflammatory cells into the peripheral nerve tissue. 2002. **Neuropharmacology.** 42: 731-739.

IV. **Nagat Abbas**, Ivan Bednar, Svedberg Marie, David Paterson, Anna Ljungberg, Agneta Nordberg, Bengt Winblad, Eilhard Mix, Jie Zhu Up-regulation of inflammatory cytokines, IFN-γ and IL-12 production in cerebral cortex regions of age related APP transgenic mice, an animal model of Alzheimer`s disease. 2002**. J. Neuroimmunol**. 126: 50-57.

ABBREVIATIONS

Aβ	β-amyloid
AD	Alzheimer´s disease
APP	amyloid precursor protein
APPs	soluble amyloid precursor protein
BNB	blood-nerve barrier
BPM	bovine peripheral nerve myelin
EAE	experimental autoimmune encephalomyelitis
EAN	experimental autoimmune neuritis
ELISA	enzyme-linked immunosorbent assay
FCA	Freund's complete adjuvant
GBS	Guillain-Barré syndrome
IFN	interferon
IL	interleukin
mAb	monoclonal antibody
MCP	monocyte chemoattractant protein-1
MIP	macrophage inflammatory protein
MNC	mononuclear cells
mRNA	messenger ribonucleic acid
NFT	neurofibrillary tangles
NTs	neuropil threads
PBS	phosphate buffered saline
PHA	phytohemagglutinin
PHFs	paired helical filaments
p.i.	post-immunization
PNS	peripheral nervous system
SP	senile plaques
TGF	transforming growth factor
Th	T helper
TNF	tumor necrosis factor

INTRODUCTION

Experimental autoimmune neuritis (EAN) is a $CD4^+$ T cell-mediated demyelinating inflammatory disease of the peripheral nervous system (PNS). EAN can be induced in susceptible animal strains and species by active immunization with peripheral nerve tissue (Waksman and Adams, 1955) or purified peripheral nerve myelin proteins P2 (Kadlubowski and Hughes, 1979; Suzuki et al., 1980), and P0 (Milner et a1, 1987; Adelmann and Linington, 1992; Deretzi et al.,1999; Yan et al., 2001) as well as peripheral myelin protein 22 (PMP-22) (Gabriel, Hughes et al., 1998; Gabriel, Gregson et al., 2000). Synthetic peptides from P2 protein (Shin et al., 1989), including the amino acid sequences 53-78 and 61-70 (Hartung et al., 1988; Olee et al., 1990), or P0 peptides 180-199 and 56-71 (Zou et al., 1999a), together with Freund's complete adjuvant (FCA) also induced EAN induction. EAN can be transferred to naive Lewis rats by myelin peptides, such as syngeneic P2- or P0-specific $CD4^+$ T cells or transfer of myelin-specific T cell lines (Linington et al., 1986; Linington et al., 1992).

Guillain-Barré syndrome (GBS) is a monophasic disease, and constitutes a heterogeneous group of disorders mainly caused by immune-mediated damage of the PNS. GBS can be classified into acute inflammatory demyelinating polyradiculoneuropathy (AIDP), acute motor and sensory axonal neuropathy (AMSAN) and acute motor axonal neuropathy (AMAN) (Van der Meche et al., 2001). The most common form of GBS is AIDP that are found mainly in North America and Europe whereas the other two types are responsible for less than 10% (Hughes et al., 1999). Clinically, GBS is characterized by subacute with pronounced motor weakness, but usually self-limited with a spontaneous recovery starting around 4 weeks after the onset of neurological symptoms.

Etiology of GBS

The etiology of GBS is not fully understood, but there are triggering events that are believed to initiate nerve damage. The etiology of GBS appears to be multifactorial and may essentially include viral and/or bacterial e.g., *Campylobacter jejuni* (*C. Jejuni*), cytomegalovirus (CMV) and Mycoplasma infections (Rees et al., 1995; Hughes, Hadden et al., 1999; Hadden et al., 2001). Another study confirms the role of molecular mimicry in the induction of anti-ganglioside antibodies in GBS patients infected with *C. Jejuni* (Ang et al., 2000). Also, GBS is associated with human immunodeficiency virus infection before development of AIDS (Pardo et al., 2001). Influenza vaccination has also been suspected as a triggering event (Lasky et al., 1998).

EAN shares many of the clinical, immunological, electrophysiological and morphological characteristics of human GBS and serves as a useful model for exploring the pathogenesis and immunotherapy of GBS, as well as for T-cell-mediated autoimmune diseases in general.

Similarities between EAN and GBS

	EAN	GBS
Etiology	Immunization with PNS components	Unknown, but often preceded by viral or bacterial infections
Clinic signs	Ascending peripheral paresis, rarely chronic or relapsing	Ascending peripheral paresis, rarely chronic or relapsing
CSF	Few cells and raised protein levels	Moderate numbers of cell and raised protein level
Histopathlo-gy	Mononuclear cell infiltration, inflammation and demyelination	Mononuclear cell infiltration, inflammation and demyelination
Serum immunoglobulins	Increased	Sometimes increased
Neurophysiology	Conduction slowing to block	Conduction slowing to block

Immunopathogenesis of GBS

There is evidence that abnormal immune reactions may be involved in the pathogenesis of GBS, which is not fully understood (Hartung, 1995). Both cellular and humoral immune reactions are important in the demyelination and nerve damage (Shoenfeld et al., 1996). The inflammatory infiltrates in the PNS are mainly composed of T lymphocytes and macrophages, which are regarded as important effector cells in GBS pathogenesis (Hartung, 1995). The activated T cells and autoreactive antibodies cross the blood-nerve barrier (BNB) and initiate an inflammatory response that causes demyelination and axonal damage. Activated T cells and increased soluble products from activated T cells, such as interleukin (IL)-2 and interferon (IFN)-γ, as well as enhanced expression of HLA-DR and the IL-2 receptor, were found in GBS patients (Hartung el al., 1990). The increased synthesis of pro-inflammatory cytokines such as TNF-α (Hartung, 1993; Sharief and Thompson, 1993), IL-6 (Maimone et al., 1993), IL-1, TNF-β and IFN-γ (Dahle el al., 1997; Elkarim et al., 1998; Zhu el al., 1997; 1998a), as well as of endothelial leukocyte adhesion molecules, implies an important role for these molecules in the pathogenesis of GBS (Oka et al., 1994). T cells, macrophages, IFN-γ and TNF-α may act synergistically in causing peripheral nerve demyelination and axonal degeneration (Giovannoni and Hartung, 1996). Myelin specific antibodies have the ability to contribute to nerve damage, and higher

levels of IgG anti-ganglioside GMI antibodies were often found in GBS patients with *C. jejuni* infection (Walsh et al., 1991). Moreover the immunogenetic background of the patients will influence the development of GBS, since a few patients develop the disease after infection (Hughes et al., 1999). However, the production of IL-4 by cells bearing the Th2 phenotype has a beneficial role in GBS (Dahle et al., 1997).

Treatment of GBS

Plasma exchange is considered the most effective treatment in GBS. Additional combined treatments with intravenous immunoglobulin (IVIg) after plasma exchange showed a better outcome compared with plasma exchange alone (Hadden et al., 2001). Administration of high doses of immunoglobulins showed satisfactory results. However, only 62% of the patients treated with these therapies made a full recovery. Eight percent died, and 13% were left unable to walk without assistance after one year (Rees et al., 1998). Filtration of cerebrospinal fluid (CSF) from GBS patients was reported to be as effective as plasma exchange (Wollinsky et al., 2001). The successful treatment with IFN-β in GBS was recently reported (Creange et al., 2001; Schaller et al., 2001).

Immunopathogenesis of EAN

A simplified schematic overview of mechanisms of the immunopathogenesis of EAN is given in Fig. 1. EAN can be induced in susceptible animal species and different inbred strains can show different susceptibility depending on the MHC repertoire (Dahlman et al., 2001). Interestingly, genes regulating Th1/Th2 differentiation have been reported to support the hypothesis of EAN being a Th1 to induce the disease (Dahlman et al., 2001). After immunization circulating autoreactive myelin-specific T and B cells can cross the BNB. Once the BNB is broken down the nerve myelin components are exposed and become accessible to antibodies. Adhesion molecules and cytokines, such as IL-1, monocyte chemotactic protein (MCP)-1 and macrophage inflammatory protein (MIP)-1α are involved in T cell homing and the migration process of inflammatory cells. Elevated levels of matrix metalloproteinase in the sciatic nerve may participate in disruption of the BNB (Hughes et al., 1998). $CD4^+$ Th1 cells release inflammatory cytokines, such as IFN-γ, which activates macrophages. Macrophages play dual roles in autoimmune neuropathy, being detrimental in attacking nervous tissue through phagocytosis and release of inflammatory factors in the early phase, but also salutary in the late phase, aiding in the termination of the inflammatory process and the promotion of recovery (Kiefer and Hartung

et al., 2001). Myelin-specific antibodies are primarily responsible for causing damage to nerves. Th2 cells also produce cytokines such as IL-4 and IL-10, which, together with transforming growth factor (TGF)-β produced by Th3 cells, can inhibit Th1 cell functions.

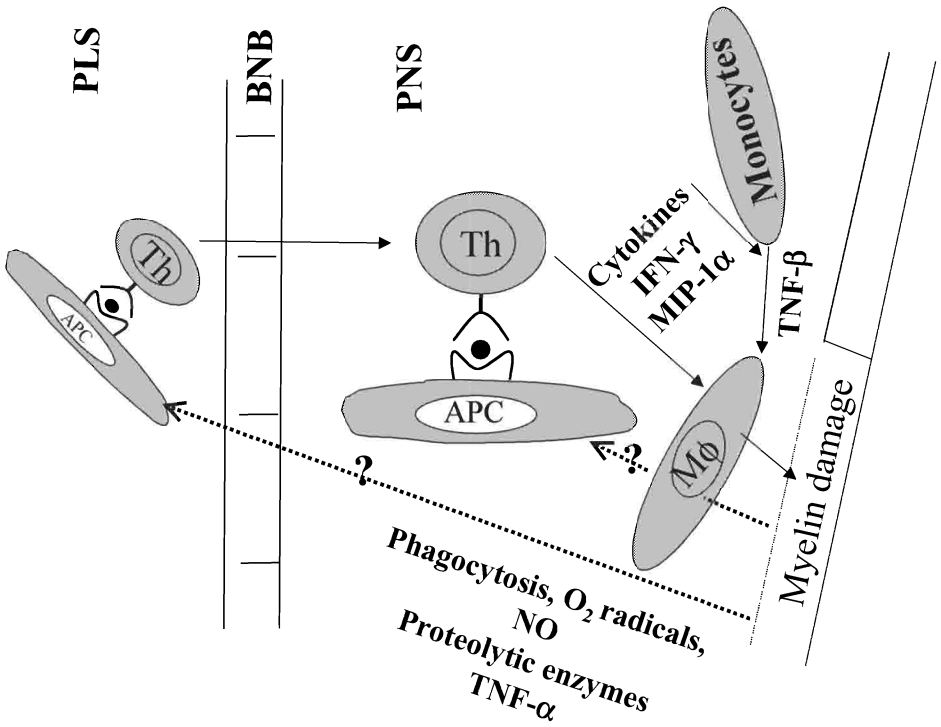


Fig. 1.

Immunopathogenesis of EAN. PLS, peripheral lymphoid system; BNB, blood-nerve barrier; PNS; peripheral nervous system. The lines define promoting effects. The dotted lines indicate the route by which released sequestered antigens will be transported.

Histopathological changes in EAN

During the acute phase of EAN the nerve roots and the peripheral nerves are infiltrated with macrophages, lymphocytes and leucocytes. The myelin sheaths swell and are stripped away by the macrophages (Rosen et al., 1992), which leads to focal demyelination of both nerve roots and nerves (Powell et al., 1991). Deposition of Ig and complement can be found on the myelin sheaths and within the endoneurium due to a breakdown of the BNB (Schmidt et al., 1996). Strong evidence emphasizes the role of inflammation in the subsequent demyelination and axonal degeneration. During the recovery phase of EAN, the process of remyelination of axons

by Schwann cells occurs and can be identified by the presence of myelin sheaths. There are similar pathological changes in humans with GBS (Hartung et al., 1995).

Cytokines in EAN

Cytokines are signal peptides and effector molecules produced by various cells, e.g., epithelial cells, fibroblasts and endothelial cells. Cytokines are pleiotropic, producing a multitude of effects on the growth and differentiation of many cell types; their receptors are expressed on differentiated cells in lymphoid tissues (Janeway and Bottomly, 1994). The production of most cytokines is temporary. They may act in an autocrine fashion (i.e., on the same cell that produces them) or exert paracrine action on cells close by. However, in certain cases cytokines can also act in an endocrine fashion, interacting with target cells elsewhere in the body. Cytokines exert their effects via receptors thereby stimulating cellular activation (Onishi et al., 1998). Most cytokines are multifunctional, and more than one cytokine may act on the same target cells and mediate the same or similar functions (Sterzel et al., 1993). Cytokines form a network by inducing or suppressing the expression of other cytokines and through the synergism or antagonism of two cytokines acting on the same cell. Cytokines may produce profound biological effects (Balkwill et al., 1989; Elias et al., 1992) and may act as essential mediators and activators in inflammation and innate immunity (Feldmann et al., 1996).

The production of cytokines in the peripheral nerves is restricted to resident and recruited macrophages and lymphocytes, mast cells, Schwann cells and possibly, neurons (Creange et al., 1998). Cytokines may be involved in the generation of autoimmune responses in EAN and in the pathogenesis of the disease, which includes damage to myelin, Schwann cells and axons, as well as tissue repair. However, the exact functions of the different cytokines are uncertain. The $CD4^+$ T cells involved in EAN pathogenesis are of the Th1 type. Related pro-inflammatory cytokines, secreted from Th1-type cells, contribute to the tissue damage in EAN by changing the balance between the cells of Th1 and Th2 phenotypes. The levels of IFN-γ-producing cells in lymph nodes and the PNS tissue parallel the clinical course of EAN, implicating Th1-related cytokines in the pathogenesis of EAN (Zhu et al., 1994b; 1998a; Fujioka et al., 1998). Th2 cells suppress cell-mediated autoimmune diseases (Kuchroo et al., 1995; Mosmann and Sad, 1996). However, Th2 cytokines such as IL-10 suppress clinical EAN, and this suppression is associated with down-regulation of Th1 responses and functions (Bai et al., 1997a). On the other hand, Th2 cells produce cytokines such as IL-4, IL-6 and IL-10 that facilitate the production by B cells of antibodies involved in the tissue damage in EAN, suggesting that the relation of pro-inflammatory and Th2-associated cytokines in autoimmune disease is extremely complex. In

general, an adequate balance between Th1 and Th2 responses is necessary for a successful immune response, whereas a disturbed balance may lead to disease.

IFN-γ in EAN

Among the cytokines that orchestrate cellular interactions during an immune response is IFN-γ a pro-inflammatory cytokine mainly produced by $CD4^+$ Th1 cells and NK cells. IFN-γ exerts many immunoregulatory effects, including activation of macrophages and stimulation of macrophages to release oxygen radicals, which can destroy myelin. IFN-γ promotes T cell homing to the PNS and enhances vascular permeability, thus playing a crucial role in inflammation in both EAN and GBS. IFN-γ induces MHC antigens, particularly MHC class II expression on macrophages, cultured Schwann cells, and adhesion molecules on endothelial cells, macrophages, T cells and Schwann cells (Gold et al., 1995). IFN-γ and TNF-α also up-regulate cytokine-inducible nitric oxide (iNO) synthase mRNA in Schwann cells and precipitate the release of nitrite in a dose-dependent manner (Gold et al., 1996). IFN-γ plays an important role in the pathogenesis of EAN (Schmidt et al, 1992). The levels of IFN-γ parallel the clinical signs of EAN (Zhu et al., 1994a; 1998a; Fujioka et al., 1998). IFN-γ positive cells were found in nerve roots in EAN rats and their levels correlated with levels of MHC class II expression during the course of the disease (Schmidt et al., 1992). IFN-γ receptor–deficient mutant ($IFN\text{-}\gamma R^{-/-}$) mice exhibited later onset of clinical disease associated with less demyelination, supporting the idea that IFN-γ contributes to promote a Th1 cell-mediated immune response (Zhu et al., 2001). Elevated serum levels of IFN-γ have been reported in GBS during the acute phase, indicating that Th1 response may be dominant in GBS (Hohnoki et al., 1998). However, IFN-γ levels in serum from GBS revealed no difference compared to controls (Exley et al., 1994).

IL-4 in EAN

IL-4 is a pleiotropic cytokine with multiplicity biological activities on different cell types. IL-4 is mainly produced by activated Th2 cells, and contributes to regulate B and T cell growth, immunoglobulin secretion and suppress the inflammatory function of macrophages. IL-4 and IFN-γ regulate each other's activities through a feedback mechanism (Paludan, 1998). In EAN, IL-4 mRNA expression in mononuclear cells (MNC) from lymph nodes and spleen cells, in the presence of PNS myelin antigens, was increased during the recovery phase. Nasal administration of rat recombinant IL-4 was associated with low-grade inflammation and milder demyelination within the sciatic nerves (Deretzi et al., 1999). Absence of IL-4 in IL-4 deficient mice with

experimental autoimmune encephalomyelitis (EAE), an analogous T cell-mediated inflammatory demyelinating disease of the central nervous system (CNS), was associated with more severe clinical signs, more extensive pathological changes and higher expression of pro-inflammatory cytokines in the CNS when compared to EAE in wild-type mice, indicating the important role of IL-4 in the modulation and amelioration of the severity of the autoimmune disease (Falcone et al., 1998). Elevated serum levels of IL-4 have been found in GBS of the late phase (Hohnoki et al., 1998).

Chemokines in EAN

Chemokines are a group of small cytokines that are structurally and functionally related and are involved in the directed migration (chemotaxis) and activation of cells, especially phagocytes and lymphocytes, and thereby play an important role in inflammatory responses (Oppenheim et al., 1991; Luster, 1998; Ranshoff et al., 1998). Chemokines form a concentration gradient at the inflammation site (Bleul et al., 1996). The chemokines can be divided into four groups: the C, C-C, C-X-C and C-X_3-C families, based on the position of the first two cysteine residues, which are arranged in two characteristic patterns: C-X-C and C-X_3-C, which characterize the α subfamily, and C-C and C, which characterize the β subfamily (Murphy, 1996). The four groups of chemokines act on different cell types. C chemokines are particularly chemotactic for $CD8^+$ T lymphocytes (Kelner et al., 1994). C-C chemokines, such as MIP-1α and MCP-1, enhance the migration of monocytes/macrophages and T lymphocytes (Schall, 1991), but not neutrophils (Tessier et al., 1997). C-X-C chemokines, such as MIP-2, enhance migration of neutrophils (Spanaus et al., 1997), but not MNC. C-X_3-C chemokines are tethered directly to the cell membrane via a long mucin stalk and induce both adhesion and migration of leukocytes (Nelson and Krensky, 1998). Chemokine production by T cells, macrophages and astrocytes leads to the infiltration of inflammatory cells into the CNS during the acute phase of EAE (Miyagishi et al., 1997). The inflammatory cell infiltrates in the PNS are composed of lymphocytes, macrophages and granulocytes. These cells exert some of their effector and immunoregulatory functions through chemotactic cytokines such as MIP-1α, MIP-2, MCP-1 and the regulated-upon-activation normal T cell expressed and secreted chemokine (RANTES), which belong to a family of small basic pro-inflammatory cytokines. Chemokines are important mediators of inflammation, influencing lymphocyte and granulocyte migration (Ranschoff et al., 1998). Therefore, chemokines play an important role in EAN, in which inflammation is restricted to the PNS and nerve roots, with infiltration by T cells and macrophages (Hartung et al., 1995). Chemokines that act towards T cells and mononuclear phagocytes are upregulated during the clinical course of

EAN (Kieseier et al., 2000). The expression and distribution of CXCR-3 suggest a specific role of this receptor in chemokine mediated lymphocyte traffic into the inflamed PNS tissue and contributes to the pathogenesis of PNS disease (Kieseier et al., 2002). MCP-1 is known to play a significant role in the migration of leukocytes from blood into tissue during inflammatory processes (Karpus and Kennedy, 1997). In addition, MCP-1 attracts T lymphocytes and MNC phagocytes (Rollins et al., 1990). MIP-2 is chemotactic, promotes migration of neutrophils and plays a role in the regulation of angiogenesis during wound repair, inflammation, and the growth of solid tumors (Driscoll et al., 1995). MIP-2 does not influence the migration of macrophages (Driscoll et al., 1995; Feng et al., 1995) or MNC (Spanaus et al., 1997). High-level expression of chemokine mRNAs, including those of IFN-γ inducible protein-10 (IP-10), MCP-1, MIP-1α and RANTES, was seen in the cauda equina of EAN Lewis rats immunized with P2 peptide 53-78 (Fujioka et al., 1999a; b).

Immunotherapy in EAN

Efforts are being made to find therapies that selectively alter inflammatory properties of autoimmune diseases. Several approaches have been proposed for immunotherapy of EAN. The goal of immunotherapy of EAN is to develop an antigen-specific, non-toxic treatment that suppresses the immune response in the target organ of specific inflammatory diseases.

Tolerance

Oral tolerance is a long recognized mechanism of inducing antigen-specific peripheral immune tolerance. Oral or nasal antigen administration offers several important advantages over parenteral immunization including higher efficacy to achieve both mucosal and systemic immunity (Xiao and Link 1997). The induction of tolerance depends upon the route of antigen administration and the different forms of antigens. Oral administration of specific autoantigens results in immune tolerance that is mediated by T cell anergy, activation–induced T cell apoptosis (delation), antibody–mediated suppression and the generation of regulatory cells which mediate active suppression act via the secretion of suppressive cytokines depending on the dose of antigen administered (Kagnoff et al., 1996; Weiner et al., 1997; Komagata and Weiner 2000). Moreover, oral administration of specific autoantigens suppresses the incidence and severity of clinical signs, as well as the pathological changes of other autoimmune disease models (Javed et al., 1996). Administration of P2 peptide 57-81 and P0 peptides 180-199 and 56-71 by the nasal route induced tolerance in EAN, which is associated with down-regulated Th1 responses (Zhu et al.,

1998b; Zou et al., 1999a). Araga and co-workers also showed that a peptide of the bovine P2 protein residue 60-70 induced T cell anergy and prevented EAN in Lewis rats (Araga et al., 1999).

Down-regulation of immune responses by anti-inflammatory cytokines

The neutralization of inflammatory cytokines has been successfully achieved. Administration of anti-IFN-γ antibodies before onset of the disease and pharmacological blockade of IFN-γ synthesis alleviate EAN (Strigård et al., 1989; Tsai et al., 1991). Oral administration of type 1 IFN modulates the severity of EAN, possibly by a reduction in IFN-α/β production (Vriesendorp et al., 1996). Some cytokines such as IL-4, IL-10 and TGF-β have an anti-inflammatory role. Treatment with the anti-inflammatory cytokine IL-4 after the induction of EAN resulted in amelioration of clinical EAN and inhibition of inflammation and demyelination in the PNS (Deretzi et al., 1999). IL-10 is a Th2-type cytokine that suppresses monocyte and Th1 cell functions. Treatment with recombinant human IL-10 (rHuIL-10) can suppress clinical EAN, and this suppression is associated with down-regulation of Th1 responses and macrophage function and up-regulation of Th2 responses (Bai et al., 1997a). Conversely, IL-10 can induce pro-inflammatory effects; the administration of rHuIL-10 was found to worsen EAE or to have no effect on the disease in the mouse (Cannella et al., 1996). Some cytokines with pro-inflammatory activity such as IFN-γ also have anti-inflammatory effects (De Maeyer et al., 1992). Administration of IFN-β is accompanied by a reduction in inflammatory cells in the sciatic nerve; there was a decrease in the migration of inflammatory cells into the peripheral nervous tissue (Zou et al., 1999a; b). In EAN, TGF-β may not only reduce the number of macrophages invading the nerve, but it may also inhibit the function of activated macrophages that have already invaded the peripheral nerves and caused myelin damage (Jung el al., 1994). TGF-β2 holds promise as a therapeutic agent to combat EAN. Neutralizing antibodies to IL-18 can ameliorate EAN by counter-regulation of Th1 response (Yu et al., 2002). Also Kieseier and Hartung (2002) demonstrated that a synthetic antagonist of RANTES can blockage EAN. Other approaches include inhibition of caspases and calpains that are activated by calcium entry into axons. Calpain inhibitors could protect the axons against antibody-mediated complement-dependent injury. These findings encourage the application of these therapeutic approaches in the human diseases GBS and chronic inflammatory demyelinating polyradiculoneuropathy.

Other treatments

Infusion of fresh plasma and IgG immunoadsorption suppressed the clinical signs of chronic EAN (Harvey et al., 1989a; Harvey et al., 1989b). Treatment with the synthetic immunomodulatory compound Linomide, which suppressed several experimental autoimmune diseases (Zhang et al., 1997; Pekarski et. al., 1998; Zhu et al., 1999), is effective at inhibiting the clinical manifestations and histopathological changes of EAN (Bai et al., 1997b; Zhu et al., 1999). Linomide induces a shift towards Th2 cytokines, and may play an important role in the control of T cell-mediated autoimmunity (Diab et al., 1998; Zhu et al., 1999). Treatment with the monoamine reuptake inhibitory anti-depressants clomipramine, imipramine and zimeldine during the clinical course of EAN is also effective. Clomipramine, imipramine and zimeldine suppressed clinical signs and T and B cell response to myelin proteins in EAN (Zhu et al., 1998c). The 5-HT reuptake inhibiting antidepressants also exerted a modulatory effect on MHC class I and II expression in macrophages from EAN rats (Zhu et al., 1994a).

Another phosphodiesterase inhibitor, pentoxifylline (Pox), has been shown to have immunomodulatory effects in vitro and in vivo (Rott et al., 1993). Pox has effects similar to those of Rolipram, such as inhibition of T cell proliferation, Th1-type cytokines and TNF-α production. In addition, the use of immunosuppressive drug Leflunomide halted the progression and markedly reduced the severity of EAN (Korn et al., 2001). An attractive approach has been achieved to protect axons from injury by using sodium channel-blocking pentapeptide (QYNAD) that was elevated in CSF of GBS patients (Brinkmeier et al., 2000). These data suggest that partial sodium channel blockade might be a straightforward and readily applicable clinical therapeutic tool. A number of novel concepts concerning immunotherapy are being developed for the treatment of GBS in particular and T cell-mediated autoimmune disease in general. Some of them are very promising while others are not suitable for clinical application. The therapeutical strategies employed in these studies are based on neutralization or removal of antibodies, inhibition of T cell response and inhibition of the action of macrophage effector molecules, in addition to the effects of immunomodulator and antidepressant drugs.

Alzheimrer`s disease (AD)

Alzheimer's disease (AD) is a neurodegenerative disorder and the most common cause of dementia in the Western world, accounting for 50-60% of all dementia cases (Hardy, 1997). It is characterised neuropathologically by the deposition of extracellular amyloid plaques containing aggregates of the amyloid β (Aβ) peptide, as well as by intracellular aggregation of neurofibrillary tangles and selective neuronal cell loss accompanied by cerebrovascular

amyloidosis. It is clinically characterized by a progressive mental disturbances and personality changes during the course of the disease (Reisberg et al., 1989). The disease affects between 5-10% of the population over the age of 65 and 25-50% over the age of 85 (Morris, 1996).

Neuropathology

Pathologically AD affects different regions of the brain but mainly the hippocampus and neocortex. The abnormal histological lesions are the extracellular and intracellular deposits of insoluble fibrous matter. The extracellular deposits are the plaques and cerebrovascular deposits containing the Aβ peptide (Glenner and wong, 1984). The intracellular deposits are filamentous inclusions found in the cell bodies of neurons known as neurofibrillary tangles (NFT`s) (Grundke Igbal et al., 1986). (NFT`s) affect neurons in specific regions of the brain, particulary the neocortex, the limbic structure (entorhinal cortex, hippocampus and amygdala) as well as the nucleus basalis. Structurally, both NFTs and neuropil threads (NTs) contain insoluble paired helical filaments (PHFs) and straight filaments (Haugh et al., 1986). NFTs are mainly composed of abnormal hyperphosphorylated forms of the microtubule-associated protein tau (Grundke-Igbal et al., 1979). Tau proteins and especially Aβ may eventually elicit local immune responses and inflammation in the brain (Patterson, 1995), with local complements production (McGeer and McGeer 1999a).The neuropathology of AD also includes selective neuronal cell and synapse loss, atrophy of the brain, gliosis and neurotransmitter deficits in the neocortex, hippocampus and amygdala (Braak and Braak, 1991).

Senile plaques (SP)

Senile plaques are associated with extracellular Aβ amyloid deposits and comprise accumuled degenerated neuronal processes, reactive astrocytes and activated microglial. The Aβ peptide found in AD brain plaques and cerebrovasculature is a 39-43 amino acid peptide (Dickson, 1997) that is generated from the amyloid precursor protein (APP) (Glenner and Wong, 1984). Amyloid related plaques in AD brain are classified as diffuse, compact and neuritic (Wisniewski and Weigel, 1994). The classic neuritic plaques are extracellular deposits of fibrillar Aβ, surrounded by dystrophic neurites, astrocytes and actived microglia. Diffuse plaques or preamyloid deposits are more light and almost occur in granular deposits without a clearly fibrillar compacted center (Silverman et al., 1997). Compact plaques are characterized by accumulation of an amyloid core without detectable dystrophic neurites (Dickson, 1997). Plaque formation is also present in the brain of non-demented elderly, but in lower numbers than in AD. However the excessive

presence of compact and neuritic plaques remains the main characteristic feature of AD (Braak and Braak, 1991).

Neuronal loss

The earliest neuronal cell loss in AD brain is that of pyramidal neurons in the entorhinal cortex, layers II and IV as well as in the subiculum and CA1 subfield (Gomez-Isla et al., 1996; West et al., 1994; West and Slomianka, 1998). Other regions with neuronal cell loss are in subcortical nuclei, such as nucleus basalis of Meynert, the locus coeruleus and dorsal Raphé (Vogels et al., 1990). Synaptic loss is detected also in the frontal and parietal cortices, with AD patients showing an average reduction of presynaptic terminal dencity of 45% (Masliah et al., 1991). There is a direct correlation between the extent of synaptic loss, formation of NFT changes and cognitive alterations in AD patients (Gomez-Isla et al., 1996, 1997). The presence of SP and NFT contribute less to the degree of dementia than does the extent of synapse loss (Terry et al., 1991; Heinonen et al; 1995).

Amyloid precursor protein (APP) in AD

Evidence supporting the key role of Aβ in the pathogenesis of AD has arisen from several lines of research most notably the study of various familial alzheimer´s disease (FAD) mutations in the APP as well as the presenilin genes. Levels of total Aβ were elevated early in dementia and strongly correlated with cognitive decline (Näslund et al., 2000). Elevated levels of $A\beta_{42}$ are the predominant and initially depositing form of Aβ in the amyloid plaques that significantly contributes to AD pathogenesis. The Aβ peptide is derived from a proteolytic processing of APP that is in itself a membrane-spanning glycoprotein with a single transmembrane and a large extracellular domain, as well as a short cytoplasmic carboxy-terminus (Kang et al., 1987). APP is an evolutionary conserved glycoprotein that occurs ubiquitously in many different species and tissues (Tu et al., 1992). APP includes three major isoforms, APP_{695}, APP_{751} and APP_{770} (Kang et al., 1987; Tanzi et al., 1987). APP_{695} is the most abundant isoform expressed in neurons. Neurons express the highest levels of APP and secrete the most Aβ peptides (Haass et al., 1992; Tanzi et al., 1993). Both APP_{751} and APP_{770} residue isoforms are expressed in non-neuronal cells such as glial cells and platelets. Other types of cells in the brain that express APP and release Aβ are microglia, endothelial, astrocytes and smooth muscle cells. The function of APP is poorly known. It has been proposed to be involved in neurite outgrowth and cell proliferation (Saitoh et al., 1989; Milward et al., 1992). APP may normally play excitoprotective and neuromodulatory

roles (Mattson et al., 1993). In contrast, Aβ exacerbates the pathogenic processes that gave rise to neurotoxicity related to oxidative stress (Markesbery and Carney 1999). $A\beta_{42}$ is the most likely sources for inflammation in the brains of AD and Downs syndrome cases (Mann and Iwatsubo, 1996). The excessive amounts of Aβ can lead to the formation of intracellular and extracellular amyloid aggregates. Recently it has been demonstrated that $A\beta_{42}$ accumulates inside the cells (Gouras et al., 2000). However, the clearance mechanisms of the secreted Aβ are poorly understood (Hartmann et al., 1997). The majority of AD cases occurs sporadically and constitute about 90%. The remaining 10% of cases are considered as FAD where there are genetic mutations (Tanzi et al., 1996). The first specific genetic cause of AD was identified in APP gene and seems to be responsible for small population of FAD (Goate et al., 1991). The single mutations have been identified at codon 717 of APP (V7171, V717G, V717F) and 716 (1716V) (Goate et al., 1991; Chartier-Harlin et al., 1991; Murrell et al., 1991; Eckman et al., 1997), as well as the Swedish double mutation at codons 670 and 671. These mutations alter APP processing to give an overproduction of Aβ. The mutations at residue 717 increase $A\beta_{1\text{-}42}$ (Suzuki et al., 1994), while the Swedish mutations increase the production of both $A\beta_{1\text{-}42}$ and $A\beta_{1\text{-}40}$ (Cai et al., 1993). Presenilin-1 (PS1) and (PS2) mutations increase the production of $A\beta_{42}$ (Borchelt et al., 1996; Duff et al., 1996; Scheuner et al., 1996). Brain tissues from individuals suffering from Down's syndrome possess an extra copy of the APP gene on chromosome 21 and develop neuropathological changes similar to AD at an early age in life (Selkoe et al., 1994; Iwastsubo et al., 1995).

Inflammation in AD

The deposition of human Aβ in mouse brain tissue might induce a local inflammatory cascade (Mehlhorn et al., 2000). Also the interaction of different inflammatory molecules induces production of other inflammatory mediators such as local upregulation of complement, cytokines, chemokines and acute phase proteins which are likely to significantly exacerbate the pathogenic processes that gave rise to the inflammatory mechanisms in AD brain. Inflammatory molecules are significantly elevated in AD brain and these inflammatory components may either mediate the degeneration or be engaged in repair and regeneration in some cases (McGeer and McGeer 1998; Apelt and Schliebs, 2001).

Cytokine and Chemokine pathways in AD

An abnormal immunological response and inflammatory cytokine production is proposed to be

involved in the pathogenesis of AD (Du et al 1999). The expression of harmful mediaters such as cytokines, IL-1 and tumor necrosis factor (TNF)-α, chemokines, IL-8 and MCP-I and the adhesion molecule ICAM-1 is associated with inflammation or gliosis (Galassoet al., 2000; Rancan et al., 2001). Activated microglia and astrocytes in the brain contribute to brain inflammation partly by secretion of proinflammatory cytokines (Engel et al., 2000). Cytokines play a major role in the initiation, propagation and regulation of immune and inflammatory responses and induce direct actions on neuronal and glial cells during damage, and repair processes (Rothwell and Strijbos 1995; McGeer and McGeer 1999b; Luterman et al., 2000). The pathogenic role of local immune reactions in AD is supported by elevated proinflammatory cytokines, such as IL-1 and IL-6 in cerebrospinal fluid (Blum-Degen et al 1995) and plasma (Licastro et al 2000) of AD patients, as well as an increased oxidative burst (Durany et al 1999; Prasad et al 2000). TNF-α and IL-1β exert their neurotoxicity by upregulation inducible NO synthase (iNOS) (Stoll et al., 2000). The overexpression of IL-1 in AD has led to the suggestion that IL-1 plays a key orchestrating role in plaque evolution and promotes the synthesis and processing of APP. Furthermore, IL-6 may contribute to neuritic plaque formation, since the expression of IL-6 immunoreactivity in microglia has been demonstrated in histological studies in the frontal temporal, parietal cortex and in hippocampus of AD brain associated with diffuse plaques without neuritic pathology (Bauer et al., 1991). IL-6 induces acute phase proteins, increases vascular permeability, lymphocyte activation and antibody synthesis that is associated with CNS destructive and behavioral deficits (Heyser et al., 1997).

IL-12

IL-12 is a heterodimeric cytokine consisting of two disulfide bound subunits, named p35 and p40 based on their approximate molecular weights, which are encoded by genes located on separate chromosomes and are regulated independently (Siburth et al., 1992). The two chains become covalently linked to form the active part p70 heterodimer (Kobayashi et al., 1989). IL-12 activities are mediated through their receptor that is composed of two subunits, namely β1 and β2 (Maurice et al., 1998). IL-12 promotes the development of Th-1 type immune responses and is a powerful inducer of IFN-γ production by T cell and NK cells (Trinchieri, 1998). IL-12 also induces cell-mediated cytotoxicity and exerts co-mitogenic effects on T cells (Storkus, 1998). IL-12 plays an important role in the normal host defense against infection by a variety of intracellular pathogens and is produced by the phagocytic cell: monocyte-macrophages and dendritic cells (Ma et al., 1995), in response to infection by bacteria or parasites. IL-12 is also

confirmed to play a central role in the pathogenesis of inflammatory disorders by shifting the T cell response to the Th1 type (Caspi et al., 1998), and together with IFN-γ is involved in the pathogenesis of several CNS disorders (Navikas and Link, 1996). The role of IL-12 p40 in Multiple Sclerosis (MS) brain lesions has been well documented (Windhagen et al., 1995). Increased numbers of IL-12 p40 mRNA expressing blood MNC in MS patients were reported (Matusevicius et al., 1998; Deretzi et al., 1999). IL-12 is considered as an ideal target for the intervention in the therapy of autoimmune and inflammatory diseases and as an attractive target for immunotherapy in the future.

IFN-γ

The potential role for IFN-γ, to promote neuronal differentiation in cultured cortical and septal neurons has been demonstrated (Erkman et al., 1989; Jonakait et al., 1994). IFN-γ is associated with neurodegeneration in the CNS lesions (Navikas and Link, 1996), and possesses numerous immunoregulatory effects, including activation of microglia and stimulation of microglia to release oxygen radicals, which are linked with toxic effects to the CNS. Additionally, IFN-γ increased vascular permeability, which underlines potential damage in the inflammation accompanying neurodegenerative diseases. Another potential role of IFN-γ is to induce a substantial increase in the expression of MHC class I and class II antigens on astrocytes, oligodendrocytes and microglia (Hirayama et al., 1986; Cogswell et al; 1991). IFN-γ promotes the effects of TNF-α and IL-1 and may exhibit a synergistic biological effect with TNF-α on up-regulation of expression of adhesion molecules on macrophages (Vassalli, 1992). Administration of recombinant IL-12 modulates the effects of IFN-γ (Gately et al., 1994). IFN-γ mediate the expression of MCP-1 on monocytes or on microglia with Aβ (25-35) (Meda et al., 1996). Overproduction of IFN-γ and TNF-α from NK cells could be involved in the progression of neurodegeneration and dementia (Solerte et al., 2000). IFN-γ in combination with TNF-α or IL-1β seems to trigger $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ production by supporting β-secretase cleavage of the immature APP molecule (Blasko et al., 2000). Neurotoxicity induced by LPS or $A\beta_{1\text{-}42}$ plus IFN-γ to rat cortical microglia and neurons cultures results in the production of peroxynitrite as a mediator of the toxicity of activated microglia, which play a major role in AD (Xie et al., 2002).

IL-4

IL-4 is an anti-inflammatory cytokine in CNS disorders that plays a potential role in regulating immune responses in the CNS (Navikas and Link 1996). However, the role of IL-4 in the

neurodegenerative disorders is still unclear. The trophic effects of IL-4 on hippocampal neuronal cultures have been demonstrated and may be mediated by glia-derived factors (Araujo and cotman, 1993). The neuroprotective role of IL-4 against activated microglia has been reported through the inhibition of IFN-γ priming of microglia with a subsequent decrease in the production of TNF-α and nitric oxide (Chao et al., 1993). Recombinant IL-4 inhibited LPS-induced synthesis of TNF-α and IL-6 in human blood tested in vitro (Guzdek et al., 2000). Furthermore, IL-4 down regulates TNF-α and IL-6 production in human peripheral monocytes by decreasing gene expression. This unique property of IL-4 may be important in the regulation of the immune response (Essner et al., 1989). IL-4 and other anti-inflammatory cytokines, such as IL-10 and IL-13, can down-regulates microglial responses to Aβ (Szczepanik et al., 2001). However, the gene expression of the Th2 cytokine transcription factor, GATA-3, correlated with IL-4 and IL-10 in the brains of neonatal but not adult mice (Lovett-Racke et al., 2000). This finding indicated that brain-derived Th2 cytokines play an important role in the CNS development and potentially contribute to the immune-privileged nature of the brain.

The role of Chemokines in AD

The up-regulation of chemkines and chemokine receptors (CCR) has been observed in resident CNS cells in AD brain (Xia and Hyman 1999), indicating that chemokines may contribute to plaque formation associated inflammation and neurodegeneration in the CNS. Up regulation of CXCR2 expression has been found in some dystrophic neuritis in senile plaques (Xia et al., 1997). Moreover, the expression of CCR3 and CCR5 was increased on some reactive microglia in AD, and MIP-1β was also found in reactive astrocytes (Xia et al., 1998). Also, the expression of MCP-1 is associated with mature senile plaques and reactive microglia, but not found in immature senile plaques. It is likely that plaque–associated chemokine production plays a role in the recruitment and accumulation of astrocytes and microglia in senile plaques.

The role of microglia and astrocyte in AD

Activated microglia by neuronal degeneration may participate in local inflammatory cascade that promotes tissue damage and contributes to amyloid plaque formation (Sasaki et al., 1997; McRae et al., 1997; Halliday et al., 2000). The strong age association of AD incidence suggests that there is age–associated increase in microglial activation (Sheng et al., 1998). Furthermore, activated microglia increased the expression of surface antigens, including MHC class I and II, and cell adhesion molecules (Perry, 1994). Although in normal brain microglia play

neurotrophic roles (Streit et al., 2000), activated microglia have been demonstrated to be involved in plaque formation and play neurotoxic effects in vitro by producing a different pro-inflammatory mediators (Haga et al., 1993; Griffin et al., 1995). These inflammatory molecules include cytokines, complement, reactive oxygen, secreted proteases and nitric oxide (Banati et al., 1993). Aβ can induce the activation of microglia to produce inflammatory cytokines and chemokines as well as superoxide free radicals in vitro (Cotman et al., 1996 Mehlhorn et al., 2000). In addition, macrophage colony stimulating factor (M-CSF) strongly augmented Aβ deposits and induced microglial production of pro-inflammatory cytokines and nitric oxide (Vincent et al., 2002). Also elderly human microglia provides a brain endogenous source for a wide range of inflammatory mediators (Lue et al., 2001). Microglia may be an important source of Aβ protein in AD and Aβ production can be augmented by microglia activation in response to an insult and to stimuli such as the bacterial endotoxins. Also there is evidence that microglia itself can process APP and generate Aβ (Bitting et al., 1996). The neurotoxic Aβ(25-35) fragment was found to be the biologically active part that mediates the toxic effects of Aβ(1-40) (Yankner et al., 1990). Treatment with Aβ sequence HHQK has beneficial effects in rats with chronic intraventricular infusion of Aβ which reduces microglia activation, presumably by blocking Aβ interactions with receptors on the microglial cell surface. This finding suggests that activation of microglia by exposure to Aβ may be a crucial step in the initiation of the inflammation seen in AD (Giulian et al., 1998). Recently, it was reported that the suppression of signaling events necessary for microglia activation through vitamin E slows down the inflammatory process in AD patients (Li et al., 2001). Inflammation mediated by activated microglia is an important characteristic of AD pathophysiology and strategies to control this response could provide new therapeutic approaches for the treatment of AD.

Astrogliosis is one of the neuropathological features of AD, and is mostly characterize by an abundance of reactive astrocytes, particularly in association with senile plaques (Pike et al., 1994). Astrocytes may play an important role in the progression of neurodegenerative diseases (Vernadakis et al., 1996). Normally, astrocytes are mainly involved in the functions of ion homeostasis, energy storage in the form of glycogen, catabolism of different toxins, and growth factors to maintain the neurons survival (Tacconi et al., 1998). In response to disease or injury, astrocytes become reactive, producing additional factors as well as cytokines and expressing surface proteins that may enhance axonal regrowth (Ridet et al., 1997). Reactive astrocytes are associated with neuritic degeneration rather than the diffuse (noncongophilic) plaque (Hardy and Allsop, 1991). Both astrocytes and microglia are clustered surrounding the amyloid deposits

(Mark et al., 1996). The astrocytic response was associated with Aβ plaques in vitro in an aggregated structure and causing neurotoxicity (Pike et al., 1994). Furthermore, astrocytes may contribute to impairing the natural ability of microglia to clear plaques. This is consistent with the preferential localization of proteoglycans to mature neuritic plaques. In response to different stimuli, astrocytes are capable to express a wide range of inflammatory mediators, such as the pro-inflammatory cytokines IL-1 and IL-6 (Del Bo et al., 1995).

Animal models of AD

The availability of stable transgenic mouse strains has been advocated as providing important model systems for gathering information about the physiology and the in vivo function of the expressed gene and gene-product, particularly for brain related disorders. There has been enormous progress in generating such mice, as APP or presenilin overexpressing mice that model aspects of the human neurodegenerative diseases. Transgenic mice overexpressing APP have been created, that simulate some of the prominent behaviour and pathogical features of AD (Hsiao, 1997). A transgenic mouse model for AD should mimic the age-dependent accumulation of Aβ plaques, neurofibrillary tangles, neuronal cell death as well as display memory loss and behavioral deficits. However, so far there is no single animal model that can mimic the full range of neuropathological alterations that exists in AD (Hsiao 1998). The APP transgenic mouse models are being comprehensively characterized and offer excellent perspectives for studing the early biochemical and pathological aspects that are not accessible in human AD patients. Multiple transgenic mice may provide an improved approach (Van Leuvan, 2000). Studies of APP or presenilin transgenic mice show there is no requirement for a maturation step in dense core plaque formation. Evidence that Aβ deposition is directed by regional factors and impairment in learning and memory are observed before Aβ deposition. The crossing of APP transgenic mice with mice modified for known AD risk factors may be necessary to view the complete replication of AD (Guenette et al 1999).

Tg2576 mice overexpressing human APP containing the double mutation Lys 670-Asn, Met 671-Leu that was found in a large swedish family with early onset AD (Mullan et al., 1992), and inserted into a hamster prion protein (PrP) gene promoter in C57B6/SJL F1 hybrid mice backcrossed with C57B6 over many generations, show normal spatial memory at three months of age but impairment by 9 to 10 months. Aged Tg2576 mice show a five-fold increase in Aβ(1-40) and a very high increase in Aβ(1-42/43) together with memory deficits (Hsiao et al., 1996). Mice with elevated amounts of Aβ developed numerous amyloid plaques and Aβ deposits in

selected cortical areas and limbic structures (Irizarry et al., 1997). Amyloid plaques appeared to stimulate a cellular inflammatory response in the brain. Both hypertrophic astrocytes and activated microglia surrounded the plaques (Irizarry et al., 1997; Frautschy et al., 1998). Markers for oxidative lipid and glycoxidative damage as well as to antioxidant defence enzymes such as heme-oxygenase and superoxide dismutase were observed in transgenic animals, in a similar way to that found in AD (Pappolla et al., 1998). Absence of neuronal cell loss in the CA1 and loss of synaptic density in hippocampus was not detected in aged mice known to develop memory impairment (Irizarry et al., 1997). However, these mice represent a model of AD caused by the APP Swedish mutations and may not be able to represent the spordic type of AD.

The machnisms of the pathological cascade in AD is presented in the Fig. 2.

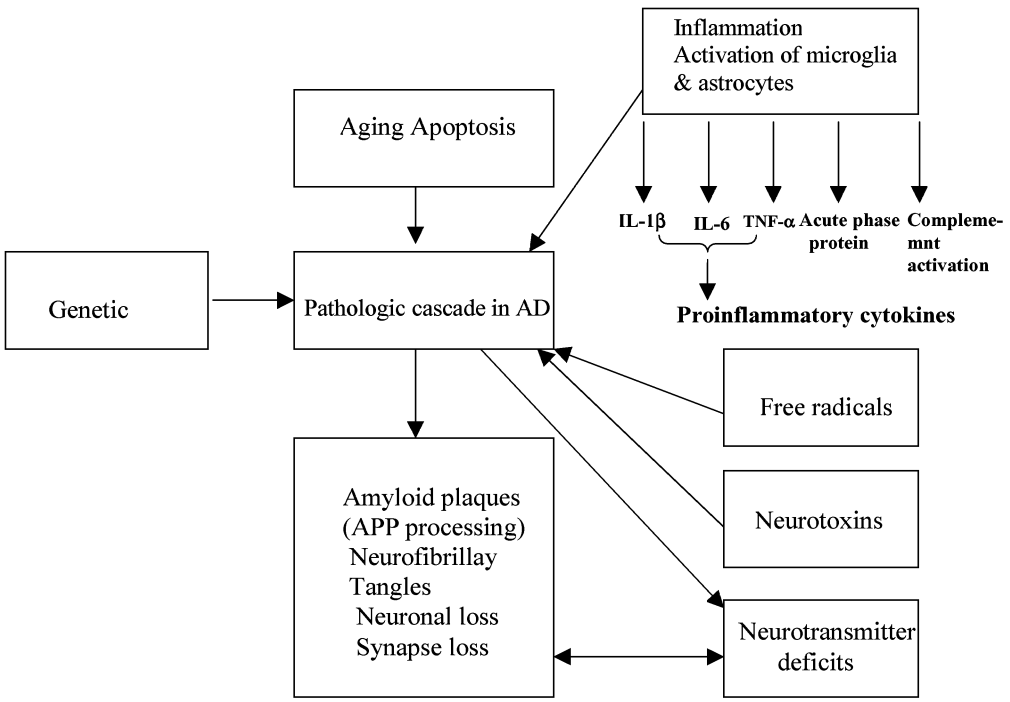


Fig. 2. The pathological cascade in AD.

GENERAL AIMS OF THE THESIS

The general aims of the thesis were to investigate disease mechanisms and therapeutic modulations in human GBS and AD by using EAN and Tg2576 mice as animal models so as to improve understanding of the pathogenesis of inflammation in the PNS and CNS.

The specific aims of this study were:

Paper I.

To define the profile of MIP-1α, MIP-2 and MCP-1 production in the target organ during the course of EAN, and to study the effect of neutralization of MIP-1α, MIP-2 and MCP-1 by in vivo administration of anti-MIP-1α, anti-MIP-2 or anti-MCP-1 antibodies.

Paper II.

To study the therapeutic effect of Rolipram on cytokine and chemokine profiles in sciatic nerves of EAN rats.

Paper III.

To investigate the effect of ABR-215062 on the clinical course of EAN and the histopathological changes as well as immune response in the PNS.

Paper IV

To elucidate the mechanisms involved in Aβ-mediated inflammation and to study the potential roles of IFN-γ, IL-12 and IL-4 in neurodegeneration using transgenic mice (Tg2576).

MATERIALS AND METHODS

Animals

Male Lewis rats, body weight 160-180 g (6-8 weeks old) were purchased from Charles River Co. (Sulzfeld, Germany). And male Tg2576 mice and age matched male non-transgenic littermates as controls at various postnatal ages ranging between 3-19 months old. All mice were born and bred in our own colony except for the animals in the 17-19 month age group that were received as gifts from Pharmacia and Upjohn Inc, Kalamazoo (USA) and Merck Sharpe and Dohme Ltd, Essex, (UK).

Tissue Preparation

Mice were sacrificed by cervical dislocation and the brains rapidly removed. One hemisphere from each animal was fixed in 1% paraformaldehyde/PBS before being used for Aβ immunohistochemistry. The other hemisphere was immediately frozen in powered dry ice and stored at -80°C, until use for in situ hybridization and immunohistochemistry.

Antigens and immunoreagents

Bovine peripheral myelin (BPM) was prepared from the lumbosacral plexus according to the procedure of Norton and Poduslo (1973). The neuritogenic P2 protein peptide, corresponding to amino acids 57-81 of bovine PNS myelin P2 protein (Olee et al., 1988) and the neuritogenic P0 protein peptide, corresponding to amino acids 180-199 of PNS myelin P0 protein (Adelman and Linington, 1992), were synthesized by solid-phase stepwise elongation using a Tecan peptide synthesizer (Multisyntech, Bochum, Germany). Mass-spectrometry showed the expected masses as major components in the spectra. Anti IFN-γ, anti-IL-4 and anti-TNF-α antibodies were provided by the Department of Cytokine Research of the University of Utrecht (Netherlands). The rabbit polyclonal antibodies to MIP-1α, MCP-1 and MIP-2, and the mouse monoclonal antibodies (mAbs) W3/25 (anti-rat CD4, T helper cells), Ox8 (anti-rat CD8, T cytotoxic/suppressor cells), CD3 (anti-rat T cell) and ED1 were purchased from Biosource (Camarillo, Calif., USA) and Serotec (Oxford, UK), respectively. Biotinylated swine anti-rabbit IgG was purchased from Dakopatts (Copenhagen, Denmark), and biotinylated mouse anti-rat IgG were purchased from ams (Frankfurt, Germany). The avidin-biotin peroxidase complex was obtained from Vector Labs (Burlingame, Calif., USA).

Compounds

Rolipram was kindly provided by Scherring AG, Preclin, Drug Research, Berlin, Germany. ABR-215062 and Linomide were a generous gift from Active Biotech Research AB, Lund, Sweden.

Anti-MIP-1α, MCP-1 and MIP-2 antibodies blocking

Anti-MIP-1α, anti-MCP-1 and anti-MIP-2 antibodies were injected subcutaneously into EAN rats at a dose of 30 μg/rat/day from day 0 to day 10 post-immunization (p.i.) (paper I).

Rolipram therapy in vivo

Rolipram therapy was started at the time of onset of clinical signs of EAN, i.e., from day 9 to 18 p.i. Rats were injected intraperitoneally twice daily with 3 mg/kg (0.6 mg/rat/day) or 15 mg/kg (3 mg/rat/day) of Rolipram (paper II).

In vivo treatment with ABR-215062

ABR-215062 was dissolved in PBS and administered by a daily subcutaneous injection from the day of immunization to day 35 p.i. Groups of 10 rats received ABR-215062 at 0.16, 1.6 or 16 mg/kg/day, respectively, while 10 rats received Linomide at 16 mg/kg/day and 10 rats received PBS daily, serving as a sham-treated control group (paper III).

Induction of EAN and assessment of clinical signs

All animals were immunized by injection into both hind footpads with altogether 200 μl of inoculum containing 5 mg of BPM (Paper I) or 230 μg of P2 peptide 57-81 (Paper II) or 100 μg P0 peptide 180-199 (Paper III), and 2 mg *Mycobacterium tuberculosis* (strain H 37 RA; Difco) emulsified in 100 μl saline and 100 μl Freund's incomplete adjuvant (FIA) (Difco). The FIA + *M. tuberculosis* mixture is referred to as Freund's complete adjuvant (FCA). Rats were monitored blindly for clinical signs. Body weights and clinical scores were assessed immediately before immunization (day 0) and every second day thereafter. Severity of paresis was graded as follows: 0, no illness; 1, flaccid tail; 2, moderate paraparesis; 3, severe paraparesis; 4, tetraparesis; intermediate scores of 0.5-grade increments were given to rats with intermediate signs.

Histopathological assessment

After animals were sacrificed, segments of the sciatic nerve close to the lumbar spinal cord were dissected, fixed in 4% para-formaldehyde and embedded in paraffin. Longitudinal sections (5-6 μm, two sections from each sciatic nerve) were stained with hematoxyloin-eosin and with luxol fast blue violet for evaluation of the extent of MNC infiltration and demyelination, respectively. Tissue areas were measured by image analysis and the number of inflammatory cells was counted at ×20 magnifications.

Immunohistochemistry

Segments of the sciatic nerves were dissected and snap-frozen in liquid nitrogen. Cryostat sections (10 μm) were exposed to rabbit anti-rat MIP-1α, anti-MCP-1 or anti-MIP-2 antibodies, and to DB1 (anti-rat IFN-γ), anti-rat IL-4, anti-TNF-α antibodies, as well as to mouse mAbs W3/25 (anti-rat CD4, T helper cells), ED1 (anti-rat macrophage) or CD3 (anti-rat T cell) (Serotec), for double staining. Cryostat sections (10 μm) of the cortical region from Tg2576 and non-transgenic control mice were exposed to mouse monoclonal antibodies (mAb) DBI, anti-mouse IL-4 and anti-mouse IL-12. The sections were stained according to the avidin-biotin technique. (Vecta stain Elite kit, Vector Labs). Peroxidase-substrate solution was added until desired color (yellow) intensity had developed. For double staining, a soluble enzyme immune complex method was used. After the first staining for measurement of MIP-1α, MIP-2, MCP-1, IFN-γ, IL-4 and IL-12 production, the sciatic nerve sections were incubated with mouse mAb ED1, or W3/25 (anti-rat CD4, T helper cells), or CD3. 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), an intermediate filament protein in astrocytes, which were employed 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 in situ hybridization (ISH) staining was detected for the mRNA expression of IFN-γ, IL-4 and IL-12. Omission of the primary antibodies and incubation with an irrelevant mAb served as negative controls. Specificity of the staining was also analyzed on sections of peripheral lymphoid organs. Tissue areas were measured by image analysis and numbers of positive-stained cells and infiltrates counted at ×20 magnifications in the entire section area. The results from both sections were averaged and expressed as cells per 100 mm^2 tissue section.

Isolation of MNC from lymph nodes

The popliteal and inguinal lymph nodes were removed under aseptic conditions and cell suspensions prepared by grinding through a wire mesh. The cells were washed three times in culture medium before being suspended to 2 x 10^6 MNC/ml culture medium, consisting of Iscove's modification of Dulbecco's medium (Flow Lab, Irvine, UK) supplemented with 1% (v/v) MEM (Flow), 50 IU penicillin, 60 μg/ml streptomycin (Gibco, Paisley, UK), 2 mM glutamine (Flow) and 3% normal human AB^+ serum without mercaptoethanol.

Lymphocyte proliferation assay

200 μl aliquots of MNC suspensions were cultured in triplicates in round-bottomed 96-well polystyrene microtitre plates (Nunc, Copenhagen, Denmark). For specific lymphocyte stimulation, 10 μl aliquots of P0 peptides 180-199 were added to cultures at a final concentration of 10 μg/ml. This concentration had optimal stimulatory effects as assessed in preliminary experiments. Triplicate wells without antigen served as background controls. After 60 h of incubation, cells were pulsed with ^{3}H-methylthymidine (1 mCi/well; Amersham, Little Chalfot, UK) and cultured for an additional 12h. ^{3}H-methylthymidine incorporation was measured in a liquid β-scintillation counter. The results were expressed as counts per minute (cpm) per culture.

Measurement of levels of IFN-γ and TNF-α in supernatants

IFN-γ and TNF-α productions were measured from 1 ml of cultures containing 2 x 10^6 MNC, which were stimulated with P0 peptide 180-199 at a final concentration of 10 μg/ml. The levels of IFN-γ and TNF-α were determined using ELISA as described as paper III. Capture mAb and detecting polyclonal antibody reactive with rat IFN-γ and TNF-α were produced, and the specificity of antibodies were examined and did not show cross-reactivates with various other cytokines (Bakhiet et al., 1997). In order to quantify supernatant cytokines, standard curves were obtained simultaneously by incubating different known concentrations of IFN-γ and TNF-α (CLAI, Utrecht, The Netherlands) for 60 min at RT in wells pre-coated with anti-cytokine mAb. Development of the plate was performed as described above and the absorbances measured from the standard concentration of cytokines were used to plot cytokines standard curves using computer software. The absorbance obtained for the specimens were automatically converted to pg/ml by the computer from the standard curve.

Determination of P0 peptide 180-199 specific IgG antibodies

Serum was obtained from blood samples at day 16 p.i. Purified P0 peptide 180-199 was coated onto ELISA plates at 10 μg/ml in a volume of 100 μl/well. After 3 washings, samples were diluted to 1:400 with PBS applied to the wells and incubated for 2 h at RT. After another 3 washes, biotinylated mouse anti-rat IgG (1:4000, ams) was added and incubated for 2 h at RT. Three further washes were followed by incubation with avidin-biotin alkaline phosphatase complex (Vector Labs) for 1 h at RT. The reaction was visualized with p-nitrophenyl phosphate substrate (Sigma, St. Louis, Mo., USA) and read at 405 nm using an ELISA reader

ISH to detect cytokine mRNA expression for IFN-γ, IL-4 and IL-12

ISH was performed as described for brain tissue sections (Zhu et al., 1994b). Briefly, synthetic oligonucleotide probes (Scandinavian Gene Synthesis AB, Köping, Sweden) were labelled, using ^{35}S deoxyadenosine-5-(thio)-triphosphate (New England Nuclear, Cambridge, MA) with terminal deoxynucleotidyl transferase (Amersham, Little Chalfont, U.K.). The oligonucleotide sequences were obtaind from the GenBank and probes were designed using MacVector software (IBI, New Haven, CT, USA). After emulsion autoradiography, slides were developed, and stained with cresyl violet. Coded slides were analyzed by dark field microscopy at 10x magnification. The results were expressed as numbers of cytokine mRNA per 100 mm^2 brain tissue sections. 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 control. This control probe was used in parallel with the cytokine probe on sections from each specimen, without revealing any positive cells

Statistical analysis

Differences between pairs of groups were tested by Student's t-test. Differences between the different groups were evaluated by one factor analysis of variance (ANOVA). The level of significance was set to $p<0.05$.

Ethics

Both the EAN model in Lewis rats and the transgenic (Tg2576) mice studies were approved by the South Stockholm Research Animal Ethics Committee, Huddinge County Court, Stockholm, Sweden.

RESULTS

The effects of anti-inflammatory agents on EAN (Papers I-II)

Dynamics of production of chemokines and their potential role in EAN (Paper I)

Dynamics of the expression of the chemokines MIP-1α, MCP-1 and MIP-2 were determined in the sciatic nerve of EAN rats. Additionally, the effect of neutralizing chemokine antibodies on the clinical course of EAN and on chemokine expression was investigated. Rats immunized with BPM + FCA developed clinical signs of EAN around day 8 p.i. and pronounced clinical signs at day 14 p.i., followed by gradual recovery until day 36 p.i. Rats injected with FCA only did not develop any clinical signs of EAN. The highest numbers of MIP-α positive cells in the sciatic nerve were seen on day 14 p.i., correlating with the development of severe clinical signs. The MIP-1α positive cells were scattered in the perineurium and endoneurium of the sciatic nerves of EAN rats. Administration of anti-MIP-1α antibodies delayed the appearance of the clinical signs of EAN by 2 days, significantly suppressed the clinical signs from the onset to the recovery phase, inhibited inflammation and demyelination and reduced the numbers of macrophages in the sciatic nerve (**Fig. 3**). The numbers of MIP-α positive cells were significantly higher in EAN rats compare to FCA-treated control rats on day 14 p.i. The maximum numbers of MCP-1 positive cells in the sciatic nerve were detected on day 7 p.i., and most MCP-1 positive cells were scattered in the perineurium. Administration of anti-MCP-1 antibodies caused a delay of onset of clinical EAN and inhibited the clinical signs. Four of the 6 EAN rats receiving anti-MCP-1 antibodies showed the same degree of inflammatory cell infiltration and demyelination in the sciatic nerve as sham-treated EAN rats. Only 2 rats revealed less inflammation and demyelination. The numbers of MIP-2 positive cells reached a maximum on day 21 p.i. The MIP-2 positive cells were scattered in the perineurium and endoneurium. Anti-MIP-2 antibodies failed to suppress both the clinical signs of EAN and the inflammation and demyelination in the sciatic nerve. Only the administration of anti-MIP-1 antibody resulted in a significant reduction in the numbers of MIP-1α- and ED1-positive cells in the sciatic nerve. The subcutaneous administration of anti-chemokine antibodies modulated the clinical course of EAN, mainly reducing the duration and severity of the disease.

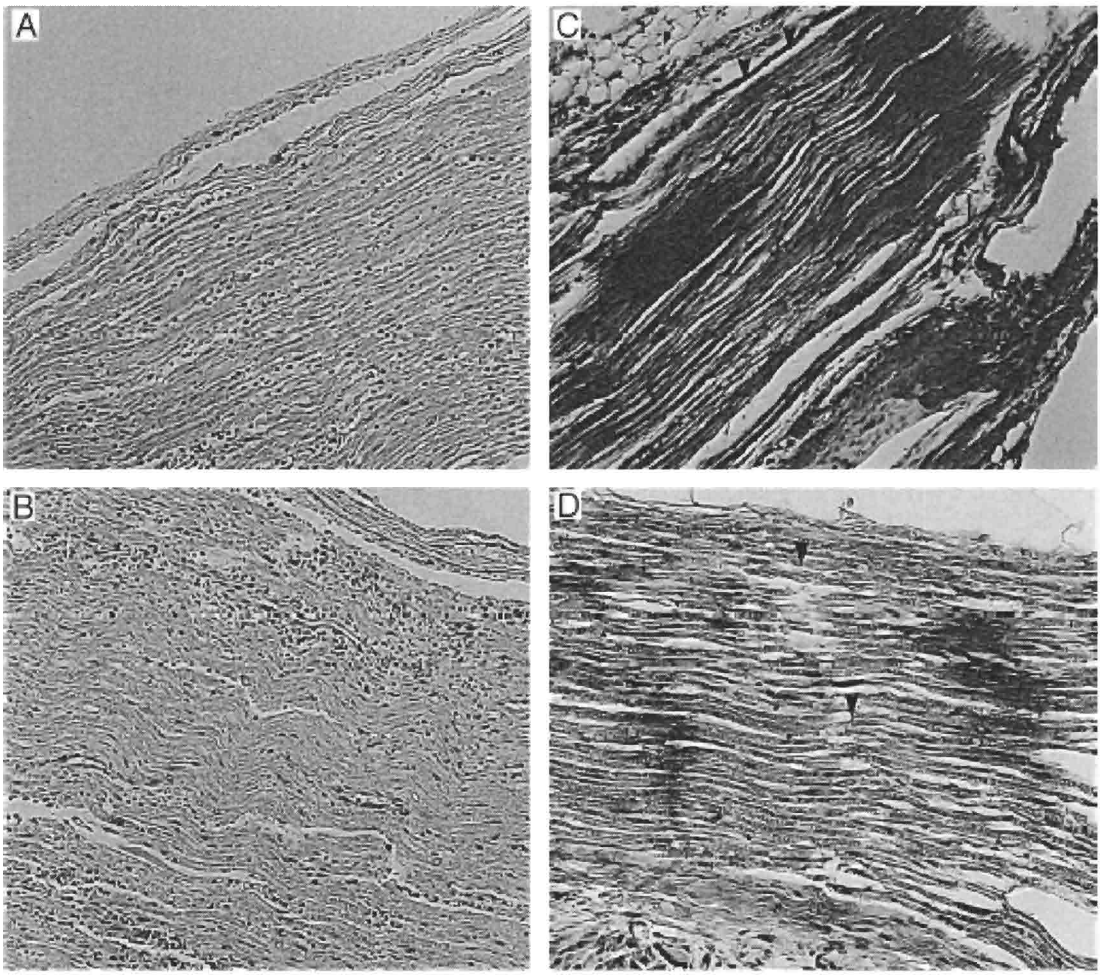


Fig. 3. Micrographs of sections of the sciatic nerve from EAN rats 14 days p.i. stained with hematoxylin (A, B) or Luxol fast blue (C, D), low-grade inflammation (A) and minimal demyelination (C) is observed in the sciatic nerve from the EAN rats treated subscutaneously with anti-MIP-1α antibodies. Inflammatory infiltrates composed of macrophage and lymphocytes (B) as well as severe regional demyelination (D) are observed in the sciatic nerve from sham-treated EAN rats(x 200).

Protective effect of Rolipram on EAN (Paper II)

Administration of Rolipram was carried out twice daily for 10 days from day 9 to 18 p.i., i.e., when rats exhibited clinical signs of EAN. In rats treated with Rolipram at two different doses, 3 mg/kg (0.6 mg/rat/day) or 15 mg/kg (3 mg/rat/day), clinical symptoms of EAN were reduced compared to the control EAN rats treated with saline/Cremophor. After 4 days of treatment, significant differences in clinical scores were observed with both doses of Rolipram when compared to control EAN rats. Histopathological evaluation revealed extensive infiltration of macrophages and lymphocytes in the sciatic nerve of control EAN rats at day 14 p.i. However,

there were fewer inflammatory cells in the sciatic nerve of Rolipram-treated EAN rats. There was significantly higher production of IFN-γ in the sciatic nerve of control EAN rats than in EAN rats treated with either dose of Rolipram. The high dose of Rolipram more strongly suppressed IFN-γ production than the low dose ($p<0.01$). Chemokine production was significantly higher in the control EAN rats than in rats treated with the low dose of Rolipram. Similar patterns of chemokine production were detected in EAN rats treated with the high dose of Rolipram. Cytokine- and chemokine-positive cells were scattered within the endoneurium and perineurium of the sciatic nerve. The production of IL-4 in EAN rats treated with either dose of Rolipram was increased compared to control EAN rats injected with saline/Cremophor. The high dose of Rolipram increased the production of IL-4 at the maximum of clinical EAN more strongly than the low dose ($p<0.001$).

ABR-215062 suppresses clinical EAN and alters Th1/Th2 balance in EAN (Paper III)

EAN induced in Lewis rats by inoculation with peripheral nerve myelin P0 peptide 180-199 and FCA was strongly suppressed by ABR-215062 administered daily subcutaneously from the day of immunization to day 35 p.i. ABR-215062 delayed the onset of clinical EAN by 2-8 days and reduced the clinical signs of the disease (**Fig. 4**). These clinical effects were dose-dependent. ABR-205062 at doses 16, 1.6 and 0.16 mg/kg/day reduced the incidence of EAN by 20%, 30% and 60%, respectively. There was no significant difference between the highest dose of ABR-215062 and the same dose of Linomide. Both ABR-215062 or Linomide on day 16 p.i. gave a significant reduction in infiltration of macrophages, lymphocytes and granulocytes in the sciatic nerves. A lower grade of inflammation was detected in rats injected with the higher dose of ABR-215062 than in rats injected with the lower dose **(Figs. 5 & 6)**. ABR-215062 strongly reduced the demyelination and significantly suppressed and inhibited P0 peptide 180-199-specific T and B cell responses and significantly suppressed T cell proliferation. The suppression of clinical EAN is associated with inhibition of the inflammatory cytokines IFN-γ and TNF-α in the sciatic nerves (**Fig. 7**) as well as in the supernatants and up-regulation of the anti-inflammatory cytokine IL-4 in the periphery nerve tissues in a dose-dependent manner. The suppressive effects of ABR-215062 on EAN are quite similar to the effects of Linomide.

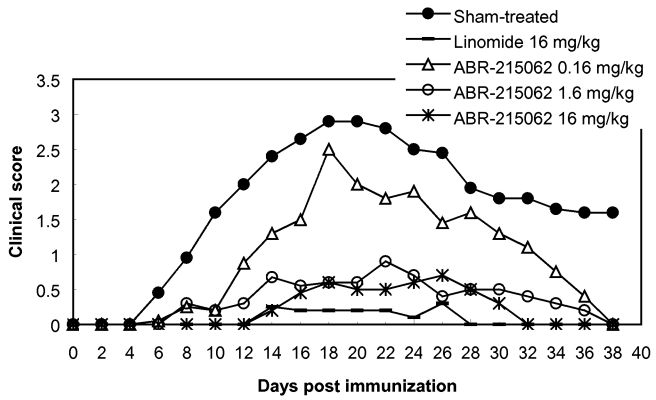
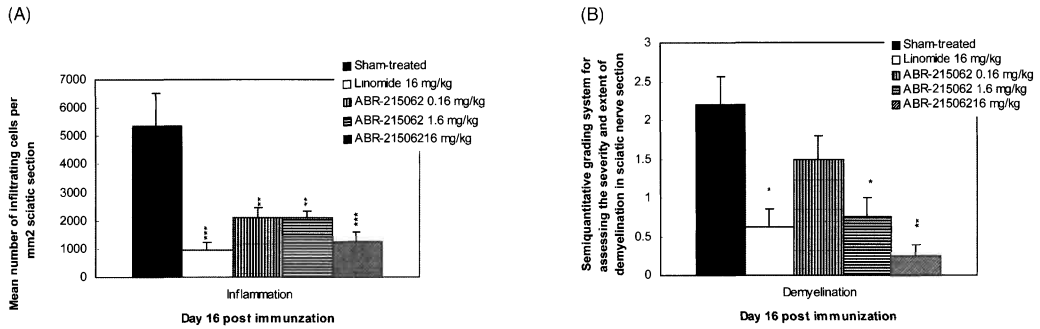


Fig. 4. Clinical scores of EAN rats (n = 50). EAN was induced in Lewis rats by immunisation on day 0 with P0 peptide 180-199 plus Freund's complete adjuvant. Rats received ABR-215062 at doses of 0.16 (n = 10), 1.6 (n = 10), and 16 mg/kg/day (n = 10) and Linomide 16 mg/kg/day (n = 10) from day 0 to 35 post immunisation by the subcutaneous route. Control rats (n = 10) received PBS only. Mean values are depicted.

Inflammation **Demyelination**



Figs. 5 and 6 (Figs. 3A and B in paper III). Inflammatory infiltrates and regional demyelination in sciatic nerve sections from EAN rats (5 rats for each group). Only low-grade inflammation and milder regional demyelination within PNS were detected in rats treated with 16 mg/kg/day of ABR-215062 as analysed on day 16 p.i. and as compared to sham-treated control. Mean values and SEM are depicted. P values refer to comparisons between EAN rats receiving different doses of ABR-215062 or 16 mg/kg/day of Linomide and the sham-treated control EAN rats receiving PBS only.* $p< 0.05$; ** $p<0.01$; *** $p<0.001$.

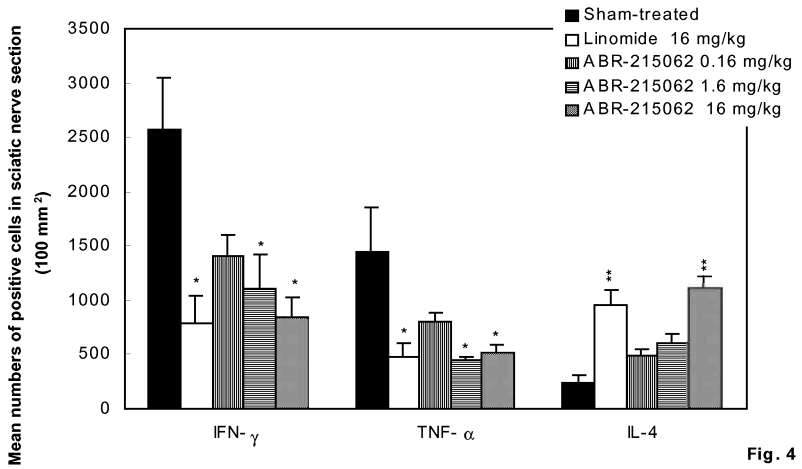


Fig. 7 (Fig. 4 in paper III). Mean numbers of cytokine expressing cells in sciatic nerve sections as measured on day 16 p.i. from EAN rats receiving ABR-215062 at different doses (0.16, 1.6 and 16 mg/kg/day), Linomide at 16 mg/kg/day or PBS (n = 5, respectively). Mean values and SEM are depicted. P values refer to comparisons between EAN rats receiving ABR-215062 or Linomide and sham-treated control EAN rats receiving PBS only *p<0.05, ** p <0.01.

The role of IFN-γ, IL-12 and IL-4 production in neurodegeneration (Paper IV)

Elevated numbers of IFN-γ mRNA expressing cells from Tg2576 mice in brain sections were detected at the age of 3-4 months and the levels remained elevated when evaluated at the ages of 9 and 11 months. The level was further increased at the age of 17-19 months (**Fig. 8**). 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. IFN-γ mRNA producing cells were observed around β-amyloid plaques in brain sections of Tg2576 mice. The production of IFN-γ positive cells in brain sections of Tg2576 mice at the ages of 17-19, 11, 9 and 3-4 months were higher. In comparison, a few numbers of IFN-γ positive cells were detected in brain sections from non-transgenic mice at the same ages. The patterns of IFN-γ expression approximately paralleled with IFN-γ mRNA expression in brain sections from cerebral cortex.

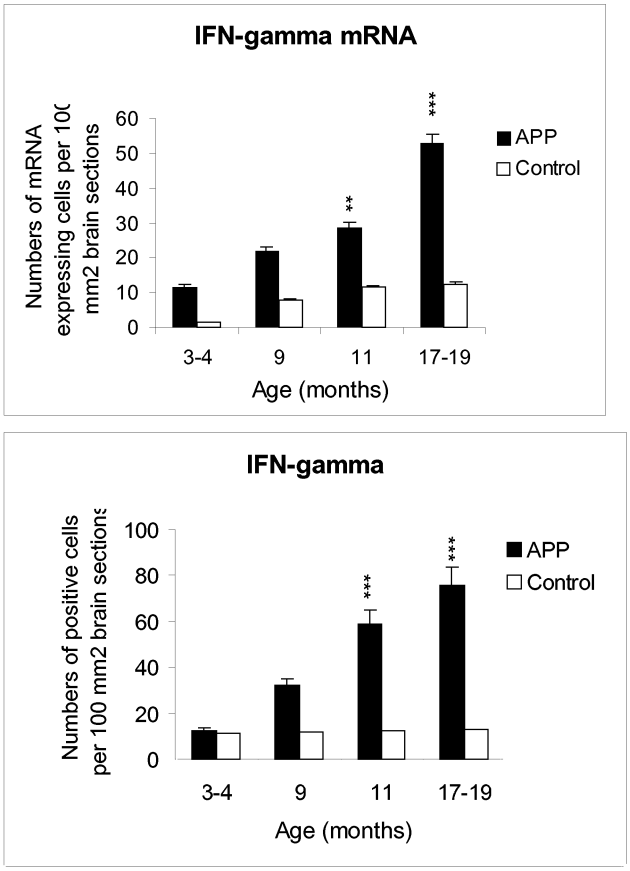


Fig. 8. Numbers of cells expressing IFN-γ mRNA (upper) and IFN-γ protein (down) as detected by in situ hybridization and immunohistochemistry, respectively. The figure shows mean numbers and SEM of positive cells per 100 mm^2 brain sections from cortical regions of transgenic Tg2576 mice and non-transgenic 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 positive numbers of IL-12 mRNA expressing cells were detected in brain sections from Tg2576 mice at post-natal age 9 months and gradually increased at 11 months. The higher expression cells of IL-12 mRNA were observed at the age of 17-19 months. While lower levels and no detectable changes of IL-12 mRNA expressing cells were detected in non-transgenic littermates (**Fig. 9**). IL-12 expression was observed in areas close to β-amyloid plaques in cortical brain sections. The increased levels of IL-12 positive cells were found in Tg2576 paralleled with increasing of the ages, when compared with age-matched non-transgenic littermates.

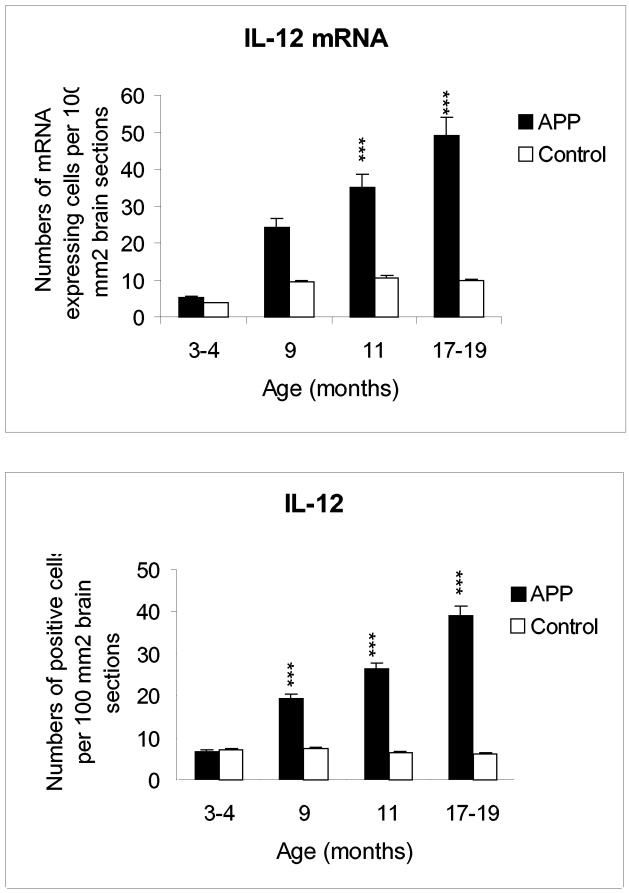


Fig. 9. Numbers of cells expressing IL-12 mRNA (upper) and IL-12 protein (down) as detected by in situ hybridization and immunohistochemistry, respectively. The figure shows mean numbers and one SD of positive cells per 100 mm^2 brain sections from cortical regions of transgenic Tg2576 mice and non-transgenic control mice at various postnatal ages. P values refer to comparisons between transgenic Tg2576 mice and non-transgenic control mice. *** $p< 0.001$.

IL-4 mRNA expressing cells was detected in brain sections of Tg2576 mice, was significantly lower, in contrast, strikingly increased numbers of IL-4 mRNA expressing cells were found at different postnatal ages studied in non-transgenic mice. The highest expression of IL-4 mRNA expressing cells were observed at 9 months of non-transgenic mice, thereafter, the expression gradually declined to (at 11 months) and (at 17-19 months), respectively. Higher levels of IL-4 positive cells were also found in non-transgenic mice from various postnatal ages 9, 11 and 17-19 months. The mean numbers of IL-4 positive cells increased at 9 and 11 months,

to maximum numbers at 17-19 months in non-transgenic mice, in comparison few numbers of IL-4 positive cells were observed in Tg2576 mice (**Fig. 10**), suggesting that no immunoreactivity for IL-4 was detectable in these mice, when compared with age-matched-non transgenic littermates. IFN-γ, IL-12 mRNA and protein positive cells were scattered within the cortex region of the brain sections of Tg2576 mice and produced mainly in cell with the size and morphology corresponding to microglia. After double immunohistochemistry staining for IFN-γ, IL-12, microglia or GFAP in brain sections of 17-19-months Tg2576 mice revealed a co-localization of IFN-γ or IL-12 and microglia or GFAP immunoreactivity clearly indicating that IFN-γ and IL-12 are expressed by reactive microglia and astrocytes.

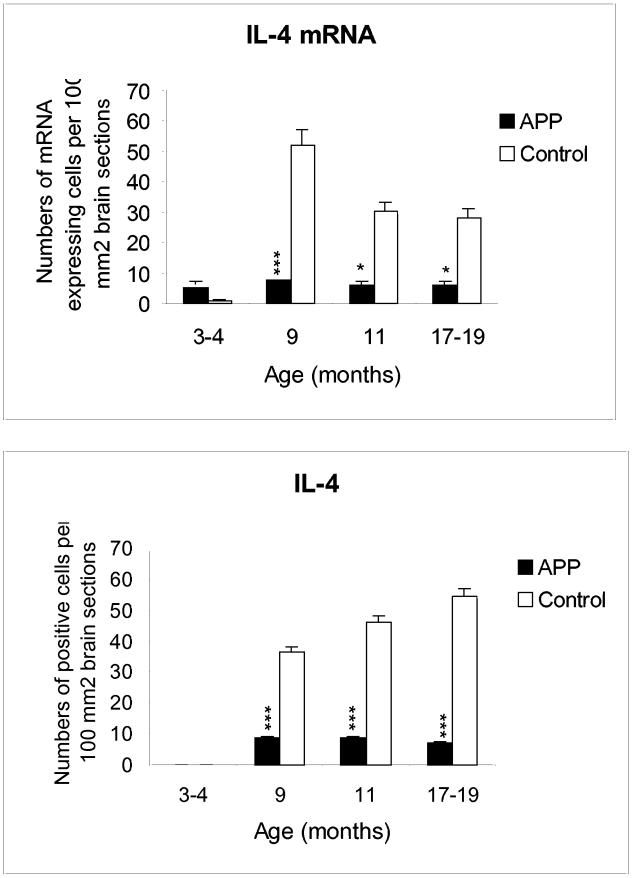


Fig. 10. Numbers of cells expressing IL-4 mRNA (upper) and IL-4 protein (down) as detected by in situ hybridization and immunohistochemistry, respectively. The figure shows mean numbers and one SD of positive cells per 100 mm^2 brain sections from cortical regions of transgenic Tg2576 mice and non-transgenic control mice at various postnatal ages. P values refer to comparisons between transgenic Tg2576 mice and non-transgenic control mice. * $p<0.05$; *** $p<0.001$.

DISCUSSION

The EAN model is useful for studying the immunopathogenesis and immunotherapy of GBS. Both GBS and EAN are prototypes of T cell-mediated autoimmune diseases affecting the PNS. Perivascular accumulation of macrophages and T lymphocytes in the PNS, and high levels systemically of PNS myelin antigen-reactive T cells are characteristic features of both diseases, thereby suggesting a pathogenic role for immunoregulatory cytokines and chemokines. Anti-inflammatory cytokines are potent regulators of immune responses and as such represent potentially powerful therapeutic agents in several autoimmune diseases (Navikas et al., 1996; Zhu et al., 1998b).

In EAN, the inflammatory infiltrates are composed of lymphocytes, macrophages and granulocytes. One critical step in the migratory process of inflammatory cells across tight endothelial junctions is the release of chemokines providing the necessary chemotactic signals for migration (Stuve et al., 1996). The inflammatory cells in the PNS exert some of their effector and immunoregulatory functions through chemotactic cytokines such as MIP-1α, MIP-2, MCP-1 and RANTES, which belong to pro-inflammatory cytokines. Our data have demonstrated this point. We showed that MIP-1α production in the sciatic nerve roughly paralleled the clinical signs of EAN. Administration of anti-MIP-1α antibodies suppressed the clinical symptoms of EAN and inhibited the infiltration of T cells and macrophages into the PNS.

The mechanism of inhibition of EAN seems to be directly related to neutralization of MIP-1α in the PNS, and probably in the peripheral lymphatic system, too. The beneficial effect of anti-chemokine antibodies was found to reside in an inhibitory effect on the migration of blood-derived MNC across the blood-brain barrier (BBB). The transendothelial migration of inflammatory cells is a crucial event in the pathogenesis of inflammatory lesions in the PNS (Hartung, 1995) and is obviously inhibited by anti-chemokine antibodies. Furthermore, the importance of inhibiting MIP-1α production may be due to the fact that MIP-1α is chemotactic for $CD4^+$ and $CD8^+$ T cells. MIP-1α was shown to attract only T cells activated by mAbs to CD3, but not unstimulated lymphocytes. Thus, MIP-1α seems to preferentially recruit activated T cell subsets during the immune response (Taub et al., 1993). Although anti-MCP-1 failed to suppress the clinical severity of EAN, it was observed to delay the onset of the clinical signs and inhibited the early manifestations of EAN. We think that a higher dose of anti-MCP-1 antibodies may be required to block MCP-1 completely, since the levels of MCP-1 production were higher than that of MIP-1α and MIP-2.

The therapy of GBS still presents an important clinical challenge. The current immunomodulatory treatments are only effective for slightly more than half of the patients (Rees et al., 1998). Prevention and treatment of EAN is an important guide to identify an effective immunotherapy for GBS. Rolipram, which is a type IV phosphodiesterase inhibitors, and ABR-215062, the latter a second generation compound of Linomide (roquinimex, LS-2616) have both shown therapeutic effects in a series of models for autoimmune disease (Sommer et al., 1995), including EAN (Zou et al., 2000). The therapeutic effects, including suppressed antigen-driven T cell clone proliferation (Pette et al., 1999), down-regulation of the inflammatory cytokines TNF-α and IFN-γ and up-regulation of the inhibitory cytokine IL-10 in the CNS (Yoshikawa et al., 1999), and reduction of BBB permeability have been demonstrated (Folcik et al., 1999). In the present study, Rolipram treatment resulting in inhibition of clinical EAN was associated with down-regulated Th1 cytokine and up-regulated Th2 cytokine production. Rolipram is a specific type IV phosphodiesterase inhibitor that blocks TNF-α synthesis by increasing intracellular cAMP, which leads to a shift from Th1 to Th2 cytokines (Lacour et al., 1994). Therefore, a Rolipram-generated balance between expression of the Th1 and Th2 types of cytokines may be an optimal goal for inhibiting severe autoimmune disease (Kunzendorf et al., 1998).

The new immune modulator, ABR-215062, suppress EAN in a dose-dependently mannar and dramatically reduces the incidence and the severity of EAN symptoms. This suppression of clinical EAN is associated with reduced inflammation and demyelination of the sciatic nerves and down-regulated PNS antigen-induced B cell responses. Treatment with ABR-215062 also resulted in a down-regulation of the Th1 cytokines IFN-γ and TNF-α, as well as a marked increase in the production of the Th2 cytokine IL-4 in the sciatic nerves. ABR-215062 may act by affecting T cell differentiation and by shifting the cytokine profile from a Th1 to a Th2 cytokine pattern, an effect that was also previously shown for Linomide (Diab et al., 1998; Zhu et al., 1999). As in other organ-specific autoimmune diseases, the balance between pro- and anti-inflammatory cytokines may determine the outcome of an autoimmune attack in EAN and GBS. There is evidence for a relation between clinical activity of EAN and Th1 cytokines, consistent with a disease-promoting role for these cytokines. Elevation of TGF-β1, IL-4 and IL-10 is related to recovery from EAN. Therapeutic interventions in EAN should probably focus upon inhibiting the production of Th1 cytokines, and enhancing that of TGF-β and Th2 cytokines. The simple strategy of shifting the cytokine profile from a disease-promoting Th1-like pattern to a disease-protecting Th2-like pattern (Liblau et al., 1995) may not apply to most autoimmune diseases (McFarland, 1996). Recent therapeutic trials with parenteral and oral administration of antigen aimed to induce

autoantigen tolerance have instead worsened disease in EAE and insulin-dependent diabetes mellitus, respectively (Genain et al., 1996; Blanas et al., 1996). Possible reasons for failure of these treatments could be (1) induction of cytotoxic CD8+ T cells rather than of CD8+ TGF-β-producing suppressor T cells, (2) activation of B cells producing tissue-damaging rather than tissue-protective antibodies, or (3) induction of disease-promoting Th0 cells rather than of a Th1/Th2 shift.

Recent studies have clearly documented that Th1/Th2/Th3 cytokines are differentially up regulated during various clinical phases of EAN and GBS. These observations indicate that the role of cytokines in immune regulation and autoimmune disease is more complex than a simple Th1-Th2 dichotomy would suggest. New treatments must be sought to counteract this complex cytokine imbalance. Treatment with antibodies that selectively target certain proinflammatory cytokines, as well as with immunomodulatory preparations that promote cytokines that beneficially influence the disease course, should be the focus of future therapeutic trials.

In GBS, aggravation of clinical signs is correlated with augmented levels of IL-2, IL-6 and TNF-α. TGF-β1, on the other hand, may have a role in terminating the pathological immune response in GBS. These observations also indicate that the role of cytokines in immune regulation and autoimmune disease is highly complex. Clinicians must carefully monitor the effect of new treatments directed at counteracting a cytokine imbalance in GBS. There are several different mechanisms of selective cytokine induction and inhibition, which contribute to the maintainance of physiological Th1/Th2 cytokine balance. Examples of regulatory parameters are:

(1) Cytokine receptors: The IFN-γ receptor β chain is expressed on Th2 cells, but not on Th1 cells (Pernis et al., 1995). On the other hand, Th1 cells express IL-12 receptors upon activation, but Th2 cells do not (O'Garra and Murphy, 1996).

(2) Co-stimulatory molecules: B7-1 and B7-2 molecules induced differentially Th1 or Th2 responses in EAE (Thompson, 1995; Kuchroo et al., 1995).

(3) Intracellular signal pathways: Th1 cell activation required augmentation of intracellular IP3 and calcium, whereas Th2 cells were activated independently of elevation of intracellular IP3 and calcium (Gajewski et al., 1990). IL-12 activated Th1 cells via the JAK2-STAT4 pathway, whereas IL-4 activated Th2 cells via STAT6 (Romagnani, 1996). The second messenger substance cAMP down-regulated Th1 responses, but not Th2 responses (Adorini and Sinigaglia, 1997).

It is a task for the future to develop effective strategies for manipulation of cytokine expression in a way such that autoimmune reactions are suppressed in vivo. Examples of cytokine

"manipulators" that have successfully applied in animal models of autoimmune demyelinating diseases include:

1. Anti-inflammatory cytokines, anti-cytokine antibodies and cytokine antagonists (Yu et al., 2002).
2. Anti-Th1 cell antibodies (Kuchroo et al., 1995).
3. Hormones or hormone agonists that increase intracellular cAMP, e.g., adrenaline (Adorini and Sinigaglia, 1997) and isoproterenol (Chelmicka-Schorr et al., 1989).
4. Phosphodiesterase inhibitors, e.g. pentoxifylline (Constantinescu et al., 1996).

The neuroimmunological cascade that is initiated by the deposition of Aβ fibrils in the brain contributes to neuronal dysfunction and is involved in Aβ induced immune activation. A role for inflammatory cytokines has been proposed in the pathogenesis of AD (Du et al 1999). In our study, both IFN-γ and IL-12 transcription and production were markedly enhanced with increased age in the cortical brain regions of Tg2576 mice in reactive microglia and astrocytes surrounding Aβ deposits. A role for inflammation in AD has been postulated since IFN-γ and IL-12 may play an inflammatory role in Aβ formation and microglial as well as astrocyte activation. The mechanisms by which Aβ mediates local inflammation and the role of cytokines in AD are not fully understood. However, experimental and clinical studies indicate impairments in both humoral and cellular immunity and elevated proinflammatory cytokines were found in an animal model of AD as well as in AD patients (Blum-Degen et al 1995; Popovic et al 1998; Licastro et al 2000). IFN-γ possesses multiple immunoregulatory effects that are regulated and modulated by IL-12 (Gately et al., 1994). 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 et al., 1998). Moreover, both IFN-γ and IL-12 are involved in the pathogenesis of several CNS disorders (Navikas and Link, 1996). There are abundant experimental data that microglia may play a significant role in the progression of AD (Gonzalez-Scarano and Baltuch 1999; McGeer and McGeer 1999a). The deposition of human Aβ in a mouse brain tissue environment might also induce a local inflammatory cascade (Mehlhorn et al., 2000). Aβ is a toxic protein in vitro. Therefore, it could be assumed that the long-term production of Aβ 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 (Bednar et al., 2002). Aβ deposition may influence immunoregulatory circuits through amplification of naturally existing suppressor/regulatory networks. The early inflammation in Tg2576 mice brain might promote Aβ plaque formation or vice versa.

Although the role of the anti-inflammatory cytokine IL-4 has been associated with several CNS disorders (Navikas and Link, 1996), its role in neurodegenerative disorders remains unclear. IL-4, IL-10 and IL-13 differentially modulate microglial responses to Aβ(1-42) and may play a role in the inflammation pathology observed surrounding senile plaques (Szczepanik et al., 2001). In our study, IL-4 expression 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 finding may be due to the influence of IFN-γ on IL-4 production, as a result of the interaction between Th1-Th2 in the immune system.

The use of anti-inflammatory drugs might inhibit both the onset and the progression of AD (McGeer and McGeer, 1999b). In a previous epidemiological study the prevalence of AD was reduced by 40-50% in persons using anti-inflammatory drugs. Future studies should focus on new strategies including development of new agents and identify the dosage and duration of therapy necessary for a protective or therapeutic effect in AD.

CONCLUSIONS

Paper-I

MIP-1α, and MCP-1 may play a role in the immunopathogenesis of EAN and MIP-1α-induced trafficking of inflammatory cells can be inhibited by anti-MIP-1α antibodies. Further elucidation of the regulation and co-ordination of MIP-1α and MCP-1 production may lead to new therapeutic strategies in GBS

Paper-II

Rolipram strongly suppresses EAN in Lewis rats. Suppression is associated with down-regulation of the pro-inflammatory cytokine IFN-γ and of the chemokines MIP-1α, MIP-2 and MCP-1, as well as up-regulation of the anti-inflammatory cytokine IL-4, in the sciatic nerves. These effects indicate that Rolipram may mediate its effects by regulation of cytokines and chemokines. The observed effects for Rolipram are of interest since EAN also serves as a model for $CD4^+$ T cell-mediated experimental autoimmune diseases in general.

Paper-III

The development of EAN is successfully suppressed in a dose-dependent manner by treatment with the new immunoregulator ABR-215062. ABR-215062 counteracts EAN by suppressing production of the Th1 pro-inflammatory cytokines IFN-γ and TNF-α, as well as increasing IL-4 production in the PNS tissues. ABR-215062 inhibits migration of inflammatory cells into the PNS tissue and reduces demyelination of the nerve. The drug is a potential candidate for effective treatment of autoimmune diseases.

Paper-IV

The deposition of Aβ might induce a local inflammatory cascade, including activation of microglia and astrocytes as well as production of pro-inflammatory cytokines such as IL-12 and IFN-γ. These results call for the development of anti-inflammatory therapeutic strategies for AD.

ACKNOWLEDGEMENTS

I am grateful to all the people who have contributed to the accomplishment of this thesis and I would like to take this opportunity to thank all of you especially:

My supervisor **Associate Professor Zhu Jie**, for excellent guidance in the field of EAN and Alzheimer disease, fruitful discussions, encouragement during my work and valuable advice. And last but not least, helping me to complete this thesis.

Professor Bengt Winblad, my co-supervisor, for giving me the opportunity to work in his Department and for generous support.

ProfessorÅke Seiger, head of the Dept. for his respectful attitude towards PhD-students, improving the financial support and always being their creating a good atmosphere with his gererous idea, encouragement and for help whenever needed.

Associate Professor Richard Cowburn, thanks for your encouragement, support and also instilling in me a spirit to be willing to accomplish this thesis.

Associate Professor Marianne Schultzberg, for valuable scientific discussions during the time I spent in your lab.

Professor Agenta Nordberg and her group, thanks for good collaboration, deep scientific knowledge and fruitful discussions.

My co-authors and collaborators for fruitful collaborations.

Senior scientists in the lab for supporting a creative scientific atmosphere: **Abdu Adem, Nenad Bogdanovic, Atiqul Islam, Erik Sundström, Janne Näslund, Eirikur Benedikz, Abdu Ahmed, Maria Ankarcrona, Jing-Jing Pei and Ivan Bednar.**

All the present and former doctorand fellows at the division: **Charlotta E., Angela, Amelia, Monika, Susanne, Angel, Homa, Li-Ping, Alisabet, Catharina, Åsa, Mircea, Hanna S., Anna B., Anna N., Anne, Annika, Bogdan, Ezra, Halinder, Takeshi, Linda, Malahat, Sara, Tatjana, Dorota, Beata, An Wen-Lin, Shunwei, Chaorui, lena, Maria, Helen, Hanna L., Marina, Roya and Nahid** who is always a dear friend being a constant reminder to improve my pronunciation of the names.

I would like to thank my Chinese group, **Yu Zhu, Zhiguo, Bao Lei, Ruisheng Duan and Qinyang Wu,** for creating a friendly atmosphere and pleasant time with friendly discussions

The administration staff: **Ulla Hernlund, Maria Roos, Ulla Fahlgren, Agneta Lindahl, Monica Wikström, Kristina Lundh, Inger Lind and Siw Lundin**

Thanks **Gladys** for your nice flowers at the coffee room, **Mikael Gradin, Esa** and my sincere gratitude to **Akke Bengtsson** for helping whenever needed, mainly with my audora email. My gratitude extended to **Inga, Eva Britt, Hullan, Mo Li-Li, Lena, Bitti and Charlotte,** for always being helpful with every thing with a nice smiling.

Professor Anders Björkman and his group (My former malaria group), for friendly discussions, pleasant time we spent together and even though I am still longing to malaria research. Special thanks to my past members of our research group : **Anna Färnert, Bwijo Bwijo, Zul Premji and Gadir**.

Professor Wretlind Bengt and his group at the institute of Microbiology, Pathology and Immunology (IMPI) Huddinge University Hospital.

Colleaques and friends, the Dept. of Clinical Microbiology and the Dept. of Infectious Diseases, at Uppsala University Hosiptal.

Professor Moiz Bakhiet, for valuable scientific discussion, constat support and splendid attention to my studies in Sweden.

Associate Professor Maha Mustafa, **Ragaa Eltayeb** (Musgut), and **Alyaa Mousa** for your precious friendship and pleasant time we spent in Sweden. **Associate professor Mohammed Eltom**, my sincere gratitude, appreciation for your great help, support and encouragement during the earlier contact when I was planning to come to Sweden to pursue my dream.

Kerstin and Stig, thanks for your oppenness, generousity, wanderful time, and support for giving me a home when I came to Uppsala the first time.

My friends at the former Dept. of Clinical Microbiology Uppsala University Hosiptal, **Suzanna Bergman, Björn Herrmann and Charlotte**.

My sincere gratitude to the **Sudanese researchers** and friends at **Huddinge Hospital** for their extremely generous help and being there whenever needed, as well as the **Sudanese community in Stockholm** and **Uppsala** for the nice time we spent in Sweden.

I am especially grateful to **Leif Engqvist** for your gentle caring spirit, humility, support and also my appreciation for your help whenever needed, thanks for all.

Christa Nyblom, for your precious friendship, thanks for all care, constant support and help.

Last, but not least, my family, thanks to my **father, Agba** and her lovely children in our home, for making our life joyful and for the wanderful happiness they give us. My sisters, **Huda, Abier, Afaf, Nagwa**, and my brothers **Naji and Ogba**, for their generous love, care, constant support and especially my father for his love, unconditional kindness, for believing in my ability to accomplish my goals whatever they might be.

My brothers in-law, **Essam, Yousif, yousif Atuke**

My gratitude is also extended to all the relatives and my sincere friends, Dr. **Salwa Elsir** Dr. **Mariam Abo-Garga**, Dr. **Faiza Saad Omer, Mona Gelender**, **Zienab, Jassmine, Enaam Sherief, Suad El Hassan** and to all the staff members and colleagues at the **Dept. of Clinical Microbiology, Central Lab. Omdurman Teaching Hosiptal Sudan.**

The studies were supported by grants from the Swedish Research Council (K1999-71X-013133-01A, K1999-99P-012720-02B, K2000-71X-13133-02B, K2000-99P-12720-03A, K2001-16X-13133-03A, K2001-99PU-12720-04B, K2002-16X-13133-04B and K2002-99PU-12720-05A)

and funds from Åke Wibergs foundation, Kapten Artur Erikssons Stiftelse, Konung Gustaf V:s och Drottning Victorias foundation, Loo and Hans Ostremans foundation, the Swedish Medical Association, the Swedish Municipal Pension Institute and SADF Foundation and funds from Active Biotech Research AB, Lund, Sweden, Gun och Bertil Stohnes foundation, Eirs 50-year foundation and Alzheimer foundation.

References

Adelmann M, Linington C. Molecular mimicry and the autoimmune response to the peripheral nerve myelin P0 glycoprotein. Neurochem. Res. 17:887-9, 1992.

Adorini L, Sinigaglia F. Pathogenesis and immunotherapy of autoimmune diseases. Immunol. Today. 18: 209-211, 1997.

Ang CW, Endtz H. Ph, Jacobs BC, Laman JD, de Klerk MA, van der Meché FGA and van Doorn PA. Campylobacter jejuni lipopolysaccharides from Guillain-Barré syndrome patients induce IgG anti-GM1 antibodies in rabbits. J. Neuroimmunol. 104:133-138, 2000.

Apelt J, Schliebs R. Beta-amyloid-induced glial expression of both pro-inflammatory and anti-inflammatory cytokines in cerebral cortex of aged transgenic Tg2576 mice with Alzheimer plaque pathology. Brain. Res. 894:21-30, 2001.

Araga S, Kishimoto M, Doi S, Nakashima K. A complementary peptide vaccine that induces T cell anergy and prevents experimental allergic neuritis in Lewis rats. J. Immunol. 163:476-82, 1999.

Araujo DM, Cotman CW. Trophic effects of interleukin-4 -7 and -8 on hippocampal neuronal cultures: potential involvement of glial-derived factors. Brain. Res. 600: 49-55, 1993.

Bai XF, Zhu J, Zhang GX, Kaponides G, Höjeberg B, van der Meide PH, Link H. IL-10 suppressesexperimental autoimmune neuritis and down-regulates TH1-type immune responses. Clin. Immunol. Immunopathol. 83:117-26, 1997a.

Bai XF, Shi FD, Zhu J, Xiao BG, Hedlund G, Link H. Linomide-induced suppression of experimental autoimmune neuritis is associated with down-regulated macrophage functions. J. Neuroimmunol. 76:177-84,1997b.

Bakhiet M, Diab M, Zhu J, Lindqvist L, Link H. Potential role of autoantibodies in the regulation of cytokine responses during bacterial infections. Infect. Immun. 65:3300-3, 1997.

Balkwill FR, Burke F. The cytokine network. Immunol. Today. 10:299-303, 1989.

Banati RB, Gehrmann J, Schubert P, Kreutzberg GW. Cytotoxicity of microglia. Glia. 7:111-8, 1993.

Bauer J, Strauss S, Schreiter-Gasser U, et al. Interleukin-6 and alpha-2 -macroglobulin indicate an acute-phase state in Alzheimer's disease cortices. FEBS. Lett. 825:111-114, 1991.

Bednar I., Paterson D, Marutle A, Pham T, Svedberg M, Hellström-Lindahl E, Mousavi M, Zhang X, Court J, Morris C, Perry E, Mohammed A, Nordberg A. Selective nicotinic receptor consequences in APP_{sw} transgenic mice. Mol. Cell. Neurosci. 20:354-365, 2002.

Bitting L, Naidu A, Cordell B, Murphy GM, Jr. Beta-amyloid peptide secretion by a microglial cell line is induced by beta-amyloid-(25-35) and lipopolysaccharide. J. Biol. Chem. 271:16084-16089, 1996.

Blanas E, Carbone FR, Allison J, Miller JFAP, Heath WR Induction of autoimmune diabetes by oral administration of autoantigen. Science. 274:1707-1709, 1996.

Blasko I, Veerhuis R, Stampfer-Kountchev M, Saurwein-Teissl M, Eikelenboom P, Grubeck-Loebenstein B. Costimulatory effects of interferon-gamma and interleukin-1beta or tumor necrosis factor alpha on the synthesis of Abeta1-40 and Abeta1-42 by human astrocytes. Neurobiol. Dis. 7:682-9, 2000.

Bleul C, C Farzan, M Choe, H, Parolin C, Clark-Lewis I, Sodroski J, Springer T. A, The lymphocyte chemoattractant SDF -Is a ligand for Lester/fusin and blocks HIV-1 entry. Nature. 382: 829-33, 1996.

Blum-Degen D, Muller T, Kuhn W, Gerlach M, Przuntek H, Riederer P. Interleukin-1 beta and interleukin-6 are elevated in the cerebrospinal fluid of Alzheimer's and de novo Parkinson's disease patients. Neurosci. Lett. 29:17-20, 1995.

Borchelt DR, Thinakaran G, Eckman CB, Lee MK, Davenport F, Ratovitsky T, Prada CM, Kim G, Seekins S, Yager D, Slunt HH, Wang R, Seeger M, Levey AI, Gandy SE, Copeland NG, Jenkins NA, Price DL, Younkin SG, Sisodia SS. Familial Alzheimer's disease-linked presenilin 1 variants elevate Abeta1-42/1-40 ratio in vitro and in vivo. Neuron. 17:1005-13, 1996.

Braak H, and Braak E. Neuropathological stageing of Alzheimer-related changes. Acta. Neuropathol. 82:239-259, 1991.

Brinkmeie H, Aulkemeyer P, Wollinsky K H, & Rüde R. An endogenous pentapeptide acting as a sodium channel blocker in inflammatory autoimmune disorders of the central nervous system. Nature. Med. 6:808-811, 2000.

Cai XD, Golde TE, Younkin SG. Release of excess amyloid beta protein from a mutant amyloid beta protein precursor. Science. 259:514-6, 1993.

Cannella B, Gao YL, Brosnan C, Raine CS. IL-10 fails to abrogate experimental autoimmune encephalomyelitis. J. Neurosci. Res. 45:735-46, 1996.

Caspi R.R. IL-12 in autoimmunity. Clin. Immunol. Immunopathol. 88:4-13,1998.

Chao CC, Molitor TW, Hu S. Neuroprotective role of IL-4 against activated microglia. J. Immunol. 151:1473-81, 1993.

Chartier-Harlin MC, Crawford F, Houlden H, Warren A, Hughes D, Fidani L, Goate A, Rossor M, Roques P, Hardy J, et al. Early-onset Alzheimer's disease caused by mutations at codon 717 of the beta-amyloid precursor protein gene. Nature. 353:844-6, 1991.

Chelmicka-Schorr E, Kwasniewski MN, Thomasn BE, Arnason BGW. The β-adrenergic agonist isoproterenol suppresses EAE in Lewis rats. J. Neuroimmunol. 25:203-207, 1989.

Cogswell JP, Zeleznik-Le N, Ting JP. Transcriptional regulation of the HLA-DRA gene. Crit. Rev. Immunol. 11:87-112, 1991.

Constantinescu CS, Hilliard B, Lavi E, Ventura E, Venkatesh V, Rostami A Suppression of experimental autoimmune neuritis by phosphodiesterase inhibitor pentoyifylline. J. Neurol. Sci 143:14-18, 1996.

Cotman CW, Tenner AJ, Cummings BJ. beta-Amyloid converts an acute phase injury response to chronic injury responses. Neurobiol. Aging. 17:723-31, 1996.

Creange A, Lefaucheur JP, Authier FJ, Gherardi RK. Cytokines and peripheral neuropathies. Rev. Neurol. (Paris) Abstract. 154:208-16, 1998.

Creange A, Chazaud B, Plonquet A, Sharshar T, Poron F, Sonnet C, Raphaël C, and Gherardi RK. IFN-B decreases adhesion and transmigration capacities of lympocytes in Guillain-Barré syndrome. Neurology. 57:1704-1706, 2001.

Dahle C, Ekerfelt C, Vrethem M, Samuelsson M, Ernerudh J. T helper type 2 like cytokine responses to peptides from P0 and P2 myelin proteins during the recovery phase of Guillain-Barré syndrome. J. Neurol. Sci. 153:54-60, 1997.

Dahlman E, Wallström HJ, Luthman H, Olsson T and Weissert R. Polygenic control of autoimmune peripheral nerve inflammation in rat. Neuroimmunol. 119:166-174, 2001.

De Maeyer E, De Maeyer-Guignard J. Interferon-gamma. Curr. Opin. Immunol. 4:321-6, 1992.

Del Bo R, Angeretti N, Lucca E, De Simoni MG, Forloni G. Reciprocal control of inflammatory cytokines and β amyloid production in cultures. Neurosci. Lett. 188:70-74, 1995.

Deretzi G, L P Zou, S H Pelidou, I Nennesmo, M Levi, B Wahren, E Mix , J Zhu. Nasal administration of recombinant rat IL-4 ameliorates ongoing experimental autoimmune neuritis and inhibits demyelination J. Autoimmun.12:81-89, 1999.

Diab A, Levi M, Wahren B, Deng GM, Bjork J, Hedlund G, Zhu J. Linomide suppresses acute experimental autoimmune encephalomyelitis in Lewis rats by counter-acting the imbalance of pro-inflammatory versus anti-inflammatory cytokines. J. Neuroimmunol. 85:146-54, 1998.

Dickson DW. The pathogenesis of senile plaques. J. Neuropathol. Exp. Neurol. 56:321-39, 1997.

Driscoll K E, Hassenbein DG, Howard BW, Isfort RJ, Cody D, Tindal MH, Suchanek, M, and Carter JM. Cloning expression and functional characterization of rat MIP-2: a neutrophil chemoattractant and epithelial cell mitogen. J. Leukoc. Biol. *58*:359-64, 1995.

Du ZY, Li XY. Inhibitory effects of indomethacin on interleukin-1 and nitric oxide production in rat microglia in vitro. Int. J. Immunopharmacol. 21:219-225, 1999.

Duff K, Eckman C, Zehr C, Yu X, Prada CM, Perez-tur J, Hutton M, Buee L, Harigaya Y, Yager D, Morgan D, Gordon MN, Holcomb L, Refolo L, Zenk B, Hardy J, Younkin S. Increased amyloid-beta42(43) in brains of mice expressing mutant presenilin 1. Nature. 383:710-3, 1996.

Durany N, Munch G, Michel T, Riederer P. Investigations on oxidative stress and therapeutical implications in dementia. Eur. Arch. Psychiatry. Clin. Neurosci. 249:Suppl 3:68-73, 1999.

Eckman CB, Metha ND, Crook R, et al. A new pathogenic mutation in the APP gene (1716V) increases the realtive proportion of A beta 42(43). Hum. Mol. Gene. 6:2087-2089, 1997.

Elias JA, Zitnik RJ. Cytokine-interactions in the context of cytokine networking. Am. J. Respir. Cell. Mol. Biol. 7:365-367, 1992.

Elkarim RA, Dahle C, Mustafa M, Press R, Zou LP, Ekerfelt C, Ernerudh J, Link H, Bakhiet M. Recovery from Guillain-Barré syndrome is associated with increased levels of neutralizing autoantibodies to interferon-gamma. Clin. Immunol. Immunopathol. 88:241-48, 1998.

Engel S, Schluesener H, Mittelbronn M, et al. Dynamic of microglial activation after human traumatic brain injury are revealed by delayed expression of macrophage related protein MRP8 and MRP14. Acta. Neuropatho. 100:313-322, 2000.

Erkman L, Wuarin L, Cadelli D, Kato AC: Interferon induces astrocyte maturation causing an increase in cholinergic properties of cultured human spinal cord cells. Dev. Biol. 132:375-88, 1989.

Essner R, Rhoades K, McBride WH, Morton DL, Economou JS. IL-4 down-regulates IL-1 and TNF gene expression in human monocytes. J. Immunol. 142:3857-61, 1989.

Exley AR, Smith N, et al. TNF-α and other cytokines in Guillain-Barré syndrome. J. Neurol. Neurosurg. Psychiatry. 57:1118-20, 1994.

Falcone M, Rajan AJ, Bloom BR, Brosnan CF. A critical role for IL-4 in regulating disease severity in experimental allergic encephalomyelitis as demonstrated in IL-4-deficient C57BL/6 mice and BALB/c mice. J. Immunol. 160:4822-30, 1998.

Feldmann M, Brennan F, M Maini R N. Role of cytokines in rheumatoid arthritis. Annu. Rev. Immunol. 14:397-440, 1996.

Feng L, Xia Y, Yoshimura T, Wilson C B. Modulation of neutrophil influx in glomerulonephritis in the rat with anti-macrophage inflammatory protein-2 (MIP-2) antibody. J. Clin. Invest. *95*:1009-17, 1995.

Folcik V A, Smith T, O´Bryant S, Kawczak J A, Zhu B, Sakurai H, Kajiwara A, Staddon J M, Glabinski A, Chernosky A L, Tani M, Johnson J M, Tuohy V K, Rubin L L, Ransohoff R M. Treatment with BBB022A or rolipram stabilizes the blood-brain barrier in experimental autoimmune encephalomyelitis: an additional mechanism for the therapeutic effect of type IV phosphodiesterase inhibitors. J. Neuroimmunol. 97:119-128, 1999.

Frautschy SA, Yang F, Irrizarry M, Hyman B, Saido TC, Hsiao K, Cole GM. Microglial response to amyloid plaques in APPsw transgenic mice. Am. J. Pathol. 152:307-317, 1998.

Fujioka T, Jimi T, Hilliard BA, Ventura ES, Rostami. 1998. The expression of cytokine mRNA in the cauda equina of Lewis rats with experimental allergic neuritis. J. Neuroimmunol. 15:223-9, 1998.

Fujioka T, Kolson DL, Rostami AM. Chemokines and peripheral nerve demylination. J. Neuroimmunol. 1:27-31, 1999a.

Fujioka T, Purev E, Rostami A. Chemokine mRNA expression in the cauda equina of Lewis rats with experimental allergic neuritis. J. Neuroimmunol. 97:51-9. 1999b.

Gabriel CM, Hughes RA, Moore SE, Smith KJ, and Walsh FC. Induction of experimental autoimmune neuritis with peripheral myelin protein.-22. Brain. 121:1895-1902, 1998.

Gabriel CM, Gregson NA and Hughes RA. Anti-PMP22 antibodies in-patients with inflammatory neuropathy. J. Neuroimmunol. 104(2):139-146, 2000.

Gajewski TF, Schell SR, Fitch FW. Evidence implicating utilization of different T cell receptor-associated signaling pathways by Th1 and Th2 clones. J. Immunol. 144:4110-4120, 1990.

Galasso JM, Liu Y, Szaflaski J, et al. Monocyte chemoattractant protein-1 is a mediator of acute excitotoxic injury in neonatal rat brain. Neuroscience. 101:737-744, 2000.

Gately MK, Warrier RR, Honasoge S, Carvajal DM, Faberty DA, Connanghton SE, Anderson TD, Sarmiento U, Hubbard BR, Murphy M. Administration of recombinant IL-12 to normal mice enhances cytolytic lymphocyte activity and induces production of interferon gamma in vivo. Int. Immunol. 6:157-167, 1994.

Genain CP, Abel K, Belmas N, Villinger F, Rosenberg DP, Linington C, Raine CS, Hauser SL. Late complications of immune deviation therapy in a non human primate. Science. 274:2054-2057, 1996.

Giovannoni G, Hartung HP. The immunopathogenesis of multiple sclerosis and Guillain-Barré syndrome. Curr. Opin. Neurol. 9:165-77, 1996.

Giulian D, Haverkamp LJ, Yu J, Karshin W, Tom D, Li J, Kazanskaia A, Kirkpatrick J, Roher AE. The HHQK domain of beta-amyloid provides a structural basis for the immunopathology of Alzheimer's disease. J. Biol. Chem. 273:29719-26, 1998.

Glenner GG, Wong CW. Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochem. Biophys. Res. Commun. 120: 885-90, 1984.

Goate A, Chartier-Harlin MC, Mullin M, et al. Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. Nature. 349:704-706, 1991.

Gold R, Toyka KV, Hartung HP. Synergistic effect of IFN-gamma and TNF-alpha on expression of immune molecules and antigen presentation by Schwann cell. Cell. Immunol. 165:65-70, 1995.

Gold R, Zielasek J, Kiefer R, Toyka KV, Hartung HP. Secretion of nitrite by Schwann cells and its effect on T cell activation in vitro. Cell. Immunol. 168:69-77, 1996.

Gomez-Isla T, Price JL, McKeel DW, Jr., Morris JC, Growdon JH, Hyman BT. Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer's disease. J. Neurosci. 16:4491-500, 1996.

Gomez-Isla T, Hollister R, West H, Mui S, Growdon JH, Petersen RC, Parisi JE, Hyman BT. Neuronal loss correlates with but exceeds neurofibrillary tangles in Alzheimer's disease. Ann. Neurol. 41:17-24, 1997.

Gonzalez-Scarano and Baltuch. Microglia as mediators of inflammatory and degenerative diseases. Annu. Rev. Neurosci. 22:219-240, 1999.

Goss JR, O'Malley ME, Zou L, et al. Aastrocytes are the major source of nerve growth factor up-regulation following traumatic brain injury in the rat. Exp. Neurol. 149:301-309, 1998.

Gouras GK, Tsai J, Naslund J, Vincent B, Edgar M, Checler F, Greenfield JP, Haroutunian V, Buxbaum JD, Xu H, Greengard P, Relkin NR. Intraneuronal Abeta42 accumulation in human brain. Am. J. Pathol. 156:15-20, 2000.

Griffin WS, Sheng JG, Roberts GW, Mrak RE. Interleukin-1 expression in different plaque types in Alzheimer's disease: significance in plaque evolution. J. Neuropathol. Exp. Neurol. 54:276-81, 1995.

Grundke-Igbal I, Igbal K, Tung YC, Quinlan M, Wisniewsky HM, Binder Li. Abnormal phosphorylation of the microtubule-associated protein t (tau) in Alzheimer cytoskeletal pathology. Proc. Natl Acad Sci. 83:4913-4917, 1986.

Grundke-Iqbal I, Johnson AB, Wisniewski HM, Terry RD, Iqbal K. Evidence that Alzheimer neurofibrillary tangles originate from neurotubules. Lancet. 1:578-80, 1979.
Guenette SY, Tanzi RE. Progress toward valid transgenic mouse models for Alzheimer´s disease. Neurobiol. Aging. 20:201-211, 1999.
Guzdek A, Stalinska K, Guzik K, Koj A. Differential responses of hematopoietic and non-hematopoietic cells to anti-inflammatory cytokines: IL-4, IL-13 and IL-10. J. Physiol. Pharmacol. 51:387-99, 2000.
Hadden RD, Gregson NA: Guillain--Barre syndrome and Campylobacter jejuni infection. Symp. Ser. Soc. Appl. Microbiol. 30:145S-54S, 2001
Haga S, Ikeda K, Sato M, Ishii T. Synthetic Alzheimer amyloid beta/A4 peptides enhance production of complement C3 component by cultured microglial cells. Brain. Res. 601:88-94, 1993.
Halliday G, Robinson SR, Shepherd C, Kril J. Alzheimer's disease and inflammation: a review of cellular and therapeutic mechanisms. Clin. Exp. Pharmacol. Physiol. 27:1-8, 2000.
Hardy J, Allsop D. Amyloid deposition as the central event in the aetiology of Alzheimer's disease. Trends. Pharmacol. Sci. 12:383-8, 1991.
Hardy J. Amyloid, the presenilins and Alzheimer's disease. Trends. Neurosci. 20:154-159, 1997.
Hartmann T, Bieger SC, Bruhl B, et al. Distinc sites of intracellular production for Alzheimer's disease A beta 40/42 amyloid peptides. Nat. Med. 3:1016-1020, 1997.
Hartung HP, Heininger K, Schäfer B, Fierz W, Toyka KV. Immune mechanisms in inflammatory polyneuropathy. Ann. NY. Acad. Sci. 540:122-161, 1988.
Hartung HP, Hughes RA, Taylor WA, Heining K, Reiners K, Toyka KV. T cell activation in Guillain-Barré syndrome and in MS: elevated serum levels of soluble IL-2 receptors. Neurology. 40:215-218, 1990.
Hartung HP, Immune-mediated demyelination. Ann. Neurol. 33:563-567, 1993.
Hartung HP, Pathogenesis of inflammatory demyelination: implications for therapy. Curr. Opin. Neurol. 8:191-9, 1995.
Harvey GK, Pollard JD, Schindhelm K, McLeod JG. Experimental allergic neuritis: effect of plasma infusions. Clin. Exp. Immunol. 76:452-7, 1989a.
Haass C, Schlossmacher M, Hung AY, Vigo-Pelfrey C, Mellon A, Ostazewski B, LieberburgI, Koo EH, Scenk D, Teplow D, Selkoe DJ. Amyloid B-peptide is produced by culture cells during normal metabolism. Nature. 359:322-325, 1992.
Harvey GK, Schindhelm K, Pollard JD. IgG immunoadsorption in experimental allergic neuritis: effect on antibody levels and clinical course. J. Neurol. Neurosurg. Psychiatry. 52:865-70, 1989b.
Haugh MC, Probst A, Ulrich J, Khan J, Anderton BH. Alzheimer´s neurofibrillary tangles contain phosphorylated and hidden neurofilament epitope. J. Neurol. Neurosurg. Psychiatry. 49:1213-1220, 1986.
Heinonen O, Soinonen H, Sorvari H, Kosunen O, Paljarvi L, Koivisto E, Riekkinen PJS. Loss of synaptophysin-like immunoreativity in the hippocampal formation is an early phenomenon in Alzheimer's disease. Neuroscience. 64:375-384, 1995.
Heyser CJ, Masliah E, Samimi A, Campbell IL, Gold LH. Progressive decline in avoidance learning paralleled by inflammatory neurodegeneration in transgenic mice expressing interleukin 6 in the brain. Proc. Natl. Acad. Sci. 94:1500-5, 1997.
Hirayama M, Yokochi T, Shimokata K, Iida M, Fujiki N. Induction of human leukocyte antigen-A,B,C and -DR on cultured human oligodendrocytes and astrocytes by human gamma-interferon. Neurosci. Lett. 72:369-74, 1986.
Hohnoki K, Inoue A and Chang-SK. Elevated serum levels of IFN-γ, IL-4 and TNF-α unelevated serum levels of IL-10 in patients with demyelinating diseases during the acute stage. J. Neuroimmunol. 87:27-32, 1998.

Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S, Yang F, Cole G. Correlative memory deficits, Aβ elevation and Amyloid plaques in trangen mice. Science.274:99-102, 1996.

Hsiao KK. From prion diseases to Alzheimer's disease. J. Neural. Transm. Suppl. 49:135-44, 1997.

Hsiao K. Transgenic mice expressing Alzheimer amyloid precursor proteins. Exp. Gerntol. 33:883-887, 1998.

Hughes PM, Wells GM, Clements JM, Gearing AJ, Redford EJ, Davies M, Smith KJ, Hughes RA, Brown MC, and Miller KM. Matrix metalloproteinase expression during experimental autoimmune neuritis. Brain. 121:481-494, 1998.

Hughes RAC, Hadden RDM, Gregson NA and Smith KJ. Pathogenesis of Guillain Barré syndrome. J. Neuroimmunol. 100(1-2):74-97, 1999.

Irizarry HM, McNamara M, Fedorchak K, Hsiao K, Hyman BT. APPSw transgenic mice develop age-related Aβ deposits and neuropil abnormalities, but no neuronal loss in CA1. J. Neuropathol. Exp. Neurol. 56:173-182, 1997.

Iwatsubo T, Mann DM, Odaka A, Suzuki A, Ihara Y. Amyloid beta protein (A beta) deposition: A beta 42(43) precedes A beta 40 in down syndrome. Ann. Neurol. 37:294-299, 1995.

Janeway CA, Bottomly K. Signals and signs for lymphocyte responses. Cell. 76:275-85, 1994.

Javed NH, Gienapp I, Cox K, Whitacre CC. Oral tolerance in experimental autoimmune encephalomyelitis: specificity of peptide-induced oral tolerance. Ann. NY. Acad. Sci. *778*:393-4, 1996.

Jonakait GM, Wei R, Sheng ZL, Hart RP, Ni L. Interferon-gamma promotes cholinergic differentiation of embryonic septal nuclei and adjacent basal forebrain. Neuron. 12:1149-59, 1994.

Jung S, Schluesener HJ, Schmidt B, Fontana A, Toyka KV, Hartung HP. Therapeutic effect of transforming growth factor-beta 2 on actively induced EAN but not adoptive transfer EAN. Immunology. 83:545-51, 1994.

Kadlubowski M, Hughes RAC. Identification of the neuritogen for experimental allergic neuritis. Nature 277:140-1, 1979.

Kagnoff M. Oral tolerance: mechanism and possible role in inflammatory joint disease. Bailliere. Clinical. Rheumatology. 10: No-1, 1996.

Kang J, Lemaire HG, Unterbeck A. The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. Nature. 325:733-736, 1987.

Karpus W J, Kennedy K. J, MIP-1alpha and MCP-1 differentially regulate acute and relapsing autoimmune encephalomyelitis as well as Th1/Th2 lymphocyte differentiation. J. Leukoc. Biol. 62:681-7, 1997.

Kelner GS, Kennedy J, Bacon KB, Kleyensteuber S, Largaespada DA, Jenkines NA, Copeland NG, Bazo JF, Moore KW, Schall TJ, Zlotnik A. Lymphotactin: a cytokine that represents a new class of chemokine. Science. 266:1395-1399, 1994.

Kiefer R, Kieseier BC, Stoll G, and Hartung HP. The role of macrophages in immune-mediated damage to the peripheral nervous system. Progress in Neurobiolo. 64:109-127, 2001.

Kieseier BC, Kim K, Stefan J, Heidrun P, Klaus V, Toyka, Richard MR and Hans-PH. Sequential expression of chemokines in experimental autoimmune neuritis. J. Neuroimmunol. 110:121-129, 2000.

Kieseier BC, Tani M, Mahad D, Oka N, Ho T, Woodroofe N, Griffin JW, Toyka KV, Ransohoff RM, Hartung HP. Chemokines and chemokine receptors in inflammatory demyelinating neuropathies: a central role for IP-10. Brain. 125:823-834, 2002.

Kobayashi M, Fitz L, Ryan M, Hewick RM, Clark SC, Chan S, Loudon R, Sherman F, Perussia B, Trinchieri G. Identification and purification of natural killer cell stimulatory

factor (NKSF), a cytokine with multiple biologic effects on human lymphocytes. J. Exp. Med. 170:827-45, 1989.
Komagata Y, and Weiner HL. Oral tolerance. Reviews immunogenetics. 2:61-73, 2000.
Korn T, Toyka K, Hartung HP, and Jung S. Suppression of experimental autoimmune neuritis by leflunomide. Brain. 124:1791-1802, 2001.
Kuchroo VK, Das MP, Brown JA, Ranger AM, Zamvil SS, Sobel RA, Weiner HL, Nabavi N, Glimcher LH. B7-1 and B7-2 co-stimulatory molecules activate differentially the Th1/Th2 developmental pathways: application to autoimmune disease therapy. Cell. 80:707-718, 1995.
Kunzendorf U, Tran TH, Bulfone-Paus S. The Th1-Th2 paradigm in : law of nature or rule with exceptions. Nephrol. Dial. Transplant. 13:2445-8, 1998.
Kusunoki S. Antiglycolopid antibody in inflammatory neuropathy. Rinsho. Shinkeigaku. 35:1370-1372, 1995.
Lacour M, Arrighi JF, Muller KM, Carlberg C, Saurat JH, Hauser C. cAMP up-regulates IL-4 and IL-5 production from activated CD4+ T cells while decreasing IL-2 release and NF-AT induction. Int. Immunol. 6:1333-1343, 1994.
Lasky T, Gina J, Terracciano DO, Magder L, Koski CL, Ballesteros M, Nash D, Clark S, Haber P, Paul DS, Lawrence BS, and Robert TC. The Guillain-Barré Syndrome and the 1992–1993 and 1993–1994 Influenza Vaccines. New. England. J. of medicine. 339(25): 1797-1802, 1998.
Li Y, Liu L, Barger SW, Mrak RE, Griffin WS. Vitamin E suppression of microglial activation is neuroprotective. J. Neurosci. Res. 66:163-70, 2001.
Liblau RS, Singer SM, McDevitt HO. Th1 and Th2 CD4+ T cells in the pathogenesis of organ-specific autoimmune diseases. Immunol. Today. 16:34-38, 1995.
Licastro F, Pedrini S, Caputo L, Annoni G, Davis LJ, Ferri C, Casadei V, Grimaldi LM. Increased plasma levels of interleukin-1, interleukin-6 and alpha-1-antichymotrypsin in patients with Alzheimer's disease: peripheral inflammation or signals from the brain? J. Neuroimmunol. 103:97-102, 2000.
Licastro F, Pedrini S, Ferri C, Casadei V, Govoni M, Pession A, Sciacca FL, Veglia F, Annoni G, Bonafe M, Olivieri F, Franceschi C, Grimaldi LM. Gene polymorphism affecting alpha1-antichymotrypsin and interleukin-1 plasma levels increases Alzheimer's disease risk. Ann. Neurol. 48:388-91, 2000.
Linington C, Wekerle H, Meyermann R. T lymphocyte autoimmunity in peripheral nervous system autoimmune disease. Agents. Actions. 19:256-65, 1986.
Linington C, Lassmann H, Ozawa K, Kosin S, and Mongan L. Cell adhesion molecules of the immunoglobulin supergene family as tissue-specific autoantigens: induction of experimental allergic neuritis (EAN) by P0 protein-specific T cell lines. Eur. J. Immunol. 22:1813-7, 1992.
Lovett-Racke AE, Smith ME, Arredondo LR, Bittner PS, Ratts RB, Shive CL, Forsthuber TG, Racke MK. Developmentally regulated gene expression of Th2 cytokines in the brain. Brain. Res. 870:27-35, 2000.
Lue LF, Walker DG, Rogers J. Modeling microglial activation in Alzheimer's disease with human postmortem microglial cultures. Neurobiol. Aging. 22:945-56, 2001.
Luster AD, Chemokines-chemotactic cytokines that mediate inflammation. New. Engl. J. Med. 338:436-445, 1998.
Luterman JD, Haroutunian V, Yemul S, Ho L, Purohit D, Aisen PS, Mohs R, Pasinetti GM. Cytokine gene expression as a function of the clinical progression of Alzheimer disease dementia. Arch. Neurol. 57:1153-1160, 2000.
Ma X, D´Kubin M, Andrea A, Aste-Amezaga M, Satori A, Monterio J, Showe L, Wysocka M, Trinchieri G. Production of interleukin –12. Res. Immunol.146:432-438, 1995.
Maimone D, Annunziata P, Simone IL, Livrea P, Guazzi GC. Interleukin-6 levels in the cerebrospinal fluid and serum of patients with Guillain-Barré syndrome and chronic inflammatory demyelinating polyradiculoneuropath. J. Neuroimmunol. 47:55-61, 1993.

Mann D, Iwatsubo T. Diffuse plaque in the cerebellum and corpus striatum in Down's syndrome contain amyloid beta protein (A beta) only in the form of A beta 42(43). Neurodegeneration. 5:115-120, 1996.

Mark RJ, Blanc EM, Mattson MP. Amyloid beta-peptide and oxidative cellular injury in Alzheimer's disease. Mol. Neurobiol. 12:211-24, 1996.

Markesbery WR, and Carney JM. Oxidative alterations in Alzheimer's disease, Brain Pathol. 67:133-146, 1999.

Masliah E, Teryy RD, Alford M, et al. Cortical and sub cortical patterns of synaptophysin like immunoreactivity in Alzheimer's disease. Am. J. Pathol. 138:235-246, 1991.

Mattson MP, Cheng B, Culwell AR, et al. Evidence for excitoprotective and intraneuronal calcium-regulating roles for secreted forms of the beta-amyloid precursor protein. Neuron. 10: 243-254, 1993.

Matusevicius D, Kivisakk P, Navikas V, Soderstrom M, Fredrikson S, Link H. Interleukin-12 and perforin mRNA expression is augmented in blood mononuclear cells in multiple sclerosis. Scand. J. Immunol. 47:582-90, 1998.

Maurice K, Gately LM, Renzetti, JM, Alvin SS, et al. The IL-12/ IL-12-receptor system: role in normal and pathologic immune responses. Annu. Rev. Immunol. 16: 495-521, 1998.

McFarland HF, Complexities in the treatment of autoimmune disease. Science. 274:2037-2038, 1996.

McGeer EG, McGeer PL. The importance of inflammatory mechanisms in Alzheimer disease. Exp. Gerontol. 33:371-378, 1998.

McGeer PL, McGeer EG. Inflammation of the brain in Alzheimer's disease: implications for therapy. J. Leukoc. Biol. 65:409-415, 1999a.

McGeer EG, McGeer PL. Brain inflammation in Alzheimer disease and the therapeutic implications. Curr. Pharm. Des. 5:821-836, 1999b.

McRae A, Dahlstrom A, Ling EA. Microglial in neurodegenerative disorders: emphasis on Alzheimer's disease. Gerontology. 43:95-108, 1997.

Meda L, Bernasconi S, Bonaiuto C, Sozzani S, Zhou D, Otvos L, Jr., Mantovani A, Rossi F, Cassatella MA. Beta-amyloid (25-35) peptide and IFN-gamma synergistically induce the production of the chemotactic cytokine MCP-1/JE in monocytes and microglial cells. J. Immunol. 157:1213-8, 1996.

Mehlhorn G, Hollborn M, Schliebs R. Induction of cytokines in glial cells surrounding cortical beta-amyloid plaques in transgenic Tg2576 mice with Alzheimer pathology. Int. J. Dev. Neurosci. 18:423-431, 2000.

Milner PA, Lovelidge CA, Taylor WA, Hughes RAC. P0 myelin protein produces experimental allergic neuritis in Lewis rats. J. Neurol. Sci. 79:275-85, 1987.

Milward EA, Papadopoulos R, Fuller SJ, Moir RD, Small D, Beyreuther K, Masters CL. The amyloid protein precursor of Alzheimer's disease is a mediator of the effects of nerve growth factor on neurite outgrowth. Neuron. 9:129-37, 1992.

Miyagishi R, Kikuchi S, Takayama C, Inoue Y, Tashiro K. Identification of cell types producing RANTES, MIP-1 alpha and MIP-1 beta in rat experimental autoimmune encephalomyelitis by in situ hybridization. J. Neuroimmunol. 77:17-26, 1997.

Morris JC. Classification of dementia and Alzheimer's disease. Acta .Neurol. Scand. Suppl. 165:41-50, 1996.

Mossman TR, Sad S. The expanding universe of T cell subsets: Th1 Th2 and more. Immunol. Today. 17:138-146, 1996.

Mullan M, Houlden H, Windelspecht M, Fidani L, Lombardi C, Diaz P, Rossor M, Crook R, Hardy J, Duff K, et al. A locus for familial early-onset Alzheimer's disease on the long arm of chromosome 14, proximal to the alpha 1-antichymotrypsin gene. Nat. Genet. 2:340-2, 1992.

Mullan M: Familial Alzheimer's disease: second gene locus located. Bmj. 305(6862): 1108-9, 1992.
Murphy P M. Chemokine receptors: cloning strategies. Methods. 10:104-18, 1996.
Murrel J, Farlow M, Ghetti B, Benson MD. A mutation in the amyloid precursor protein associated with hereditary Alzheimer's disease, Science. 254:97-99, 1991.
Navikas V, Link H. Review, cytokines and the pathogenesis of multiple sclerosis. J. Neurosci. Res. 45:322-333, 1996.
Nelson P J, Krensky A M. Chemokines lymphocytes and viruses: what goes around, comes around. Curr. Opin. Immunol. 10:265-270, 1998.
Norton WI, Poduslo SE. Myelination in rat nerve: Method of myelin isolation. J. Neurochem. 21:749-57, 1973.
Näslund J, Haroutunian V, Mohs R, Davis KL, Davies P, Greengard P, Buxbaum JD. Correlation between elevated levels of amyloid beta-peptide in the brain and cognitive decline. Jama. 283:1571-1577, 2000.
O'Garra A, Murphy K. Role of cytokines in development of Th1 and Th2 cells. Chem. Immunol. 63:1-13, 1996.
Oka N, Akiguchi I, Kawasaki T, Ohnishi K, Kimura J. Elevated serum levels of endothelial leukocyte adhesion molecules in Guillain-Barré syndrome and chronic inflammatory demyelinating polyneuropathy. Ann. Neurol. 35:621-624, 1994.
Olee T, Powell HC, Brostoff SW. New minimum length requirement for a T cell epitope for experimental allergic neuritis. J. Neuroimmunol. 27:187-90, 1990.
Olee T, Powers JM, Brostoff SWA. T cell epitope for experiment allergic neuritis. J. Neuroimmunol. 19:167-73, 1988.
Onishi M, Nosaka T, Kitamura T. Cytokines receptors: structures and signal transduction. Int. Rev. Immunol. 16:617-34, 1998.
Oppenheim JJ, Zachariae CO, Mukaida N, Matsushima K. Properties of the novel proinflammatory supergene intercrine cytokine family. Annu. Rev. Immunol. 9:617-48, 1991.
Paludan SR. Interlukin-4 and interferon-gamma: the quintessence of a mutual antagonistic relationship. Scand. J. Immunol. 45:5:459-68, 1998.
Pappolla MA, Chyan YJ, Omar RA, et al. Evidence of oxidative stress and in vivo neurotoxicity of beta –amyloid in a transgenic mouse model of Alzheimer's disease: a chronic oxidative paradigm for testing antioxidant therapies in vivo. Am. J. Pathol. 152:871-877, 1998.
Pardo CA, Mcarthur JC and Griffin JW. HIV neuropathy: insights in the pathology of HIV peripheral nerve disease. J. of the peripheral Nervous System. 6:21-27, 2001.
Patterson PH. Cytokines in Alzheimer's disease and multiple sclerosis. Curr. Opin. Neurobiol. 5:642-6, 1995.
Pekarski O, Bjork J, Hedlund G, Andersson G. The inhibitory effect in experimental autoimmune encephalomyelitis by the immunodulatory drug Linomide (PNU-212616) is not mediated via release of endogenous glucocorticoids. Autoimmunity. 28:235-41, 1998.
Pernis A, Gupta S, Gollob KJ Garfein E, Coffman RL, Schindler C, Rothman P. Lack of interferon gamma reptor beta chain and the prevention of interferon gamma signaling in Th1 cells. Science. 269:245-247, 1995.
Perry VH. Modulation of microglia phenotype. Neuropathol. Appl. Neurobiol. 20:177, 1994.
Pette M, Muraro PA, Pette DF, Dinter H, McFarland HF, Martin R. Differential effects of phosphodiesterase type 4-specific inhibition on human autoreactive myelin-specific T cell clones. J. Neuroimmunol. 98:147-156, 1999.
Pike CJ, Cummings BJ, Monzavi R, Cotman CW. Beta-amyloid-induced changes in cultured astrocytes parallel reactive astrocytosis associated with senile plaques in Alzheimer's disease. Neuroscience. 63:517-531, 1994.

Popovic M, Caballero-Bleda M, Puelles L, Popovic N. Importmance of immunological and inflammatory processes in the pathogenesis and therapy of Alzheimer´s disease. Int. J. Neurosci. 95:203-236, 1998.

Powell HC, Myers RR, Mizisin AP, Olee T, and Brostoff SW. Response of the axon and barrier endothelium to experimental allergic neuritis induced by autoreactive T cell lines. Acta Neuropathol. 82:364-377, 1991.

Prasad KN, Hovland AR, Cole WC, Prasad KC, Nahreini P, Edwards-Prasad J, Andreatta CP. Multiple antioxidants in the prevention and treatment of Alzheimer disease: analysis of biologic rationale. Clin. Neuropharmaco.. 23:2-13, 2000.

Rancan M, Otto VI, Hans VH, et al. Up-regulation of ICAM-1 and MCP-1 but not of MIP-2 and sensorimotor deficit in response to traumatic axonal injury in rats. .J. Neurosci. Res. 63:438-446, 2001.

Ranschoff R M, Tani M, Do chemokines mediate leukocyte recruitment in post-traumatic CNS inflammation? Trends. Neurosci. 21:154-159, 1998.

Rees JH, Soudain SE, Gregson NA, Hughes RA. Campylobacter Jejuni infection and Guillain-Barré Syndrome. N. Engl. J. Med. 23:333 21, 1374-9, 1995.

Rees JH, Thompson RD, Smeeton NC, Hughes RA. Epidemiological study of Guillain-Barre syndrome in south east England. J. Neurol. Neurosurg. Psychiatry. 64:74-78, 1998.

Reisberg B, Ferris SH, de Leon MJ, Kluger A, Franssen E, Borenstein J, Alba RC. The stage specific temporal course of Alzheimer's disease: functional and behavioral concomitants based upon cross-sectional and longitudinal observation. Prog. Clin. Biol. Res. 317: 23-41, 1989.

Ridet JL, Malhotra SK, Privat A, Gage FH. Reactive astrocytes: cellular and molecular cues to biological function. Trends. Neurosci. 20:570-7, 1997.

Rollins B J, Yoshimura T, Leonard E J, Pobe J S. Cytokine-activated human endothelial cells synthesize and secrete a monocyte chemoattractant, MCP-1/JE, Am. J. Pathol. 136:1229-33, 1990.

Romagnani S. Development of Th 1- or Th 2-dominated immune responses: what about the polarizing signals?, Int. J. Clin. Lab. Res. 26:83-98, 1996.

Rosen JL, Brown MJ, Rostami A. Evolution of the cellular response in P2-induced experimental allergic neuritis. Pathobiology 60:108-12. 1992.

Rothwell NJ, Strijbos PJLM. Cytokines in neurodegeneration and repair. Int. J. Devl. Neurosci.13:179-185, 1995.

Rott O, Cash E, Fleischer B. Phosphodiesterase inhibitor pentoxifylline, a selective suppressor of T helper type 1 - but not type 2-associated lymphokine production, prevents induction of experimental autoimmune encephalomyelitis in Lewis rats. Eur. J. Immunol. 23:1754-175, 1993.

Saitoh T, Iimoto D. Aberrant protein phosphorylation and cytoarchitecture in Alzheimer's disease. Prog. Clin. Biol. Res. 317:769-80, 1989.

Sasaki A, Yamaguchi H, Ogawa A, Sugihara S, Nakazato Y. Microglial activation in early stages of amyloid beta protein deposition. Acta. Neuropathol. 94:316-22, 1997.

Schade FU, Schudt C. The specific type III and IV phosphodiesterase inhibitor zardaverine suppresses formation of tumor necrosis factor by macrophages. Eur. J. Pharmacol. 230:9-14, 1993.

Schall T J. Biology of the RANTES/SIS cytokine family, Cytokine. 3:165-83, 1991.

Schaller B, Alexander JR, Andreas JS. Successful treatment of Guillain-Barré Syndrome with combined administration of Interferon-beta-1a and intravenous immunoglobulin. European Neurolo. 46:3:167-168, 2001.

Scheuner D, Eckman C, Jensen M, et al. Secreted amyloid B-protein similar to that in the senile plaque of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutation linked to familial Alzheimer's disease, Nature Med. 2:864-870, 1996.

Schmidt B, Stoll G, van der Meide P, Jung S, Hartung Hp. Transient cellular expression of gamma- interferon in myelin-induced and T-cell line mediated experimental autoimmune neuritis. Brain. 115:1633-1646, 1992.

Schmidt BKV, Kiefer RT, Full J, Hartung HP, Pollard J. Inflammatory infiltrates in sural nerve biopsies in Guillain-Barré syndrome and chronic inflammatory demyelinating neuropathy. Muscle Nerve. 19:474-87, 1996.

Selkoe DJ. Amyloid beta-protein precursor: new clues to the genesis of Alzheimer's disease. Curr. Opin. Neurobiol. 4:708-16, 1994.

Sharief MK, Thompson EJ, Elevated serum levels of tumor necrosis factor α in Guillain Barré syndrome. Ann. Neurol. 33:591-596, 1993.

Sheng JG, Mrak RE, Griffin WS. Enlarged and phagocytic, but not primed, interleukin-1 alpha-immunoreactive microglia increase with age in normal human brain. Acta. Neuropathol. (Berl) 95:229-34, 1998.

Shin HC, McFarlane EF, Pollard JD, Watson EG. Induction of experimental allergic neuritis with synthetic peptides from myelin p2 protein. Neurosci. Lett. 102:309-312, 1989.

Shoenfeld Y, George J, Peter J B. Guillain-Barre as an autoimmune disease. Int. Arch. Allergy. Immunol. 109:318-26, 1996.

Siburth D, Jabs EW, Warrington JA, Li X, Lasota J, Laforgia S, Kelleher K, Huebner K, Wasmuth JJ, Wolf SF. Assigment of genes encoding a unique cytokine (IL-12) composed of two unrelated submits to chromosomes 3 and 5. Genomics. 14:59-62, 1992.

Silverman W, Wisniewski HM, Bobinkski M, et al. Frequency of stages of Alzheimer's-related lesions in different age categories. Neurobiol. Aging. 18:377-379, 1997.

Solerte SB, Cravello L, Ferrari E, Fioravanti M. Overproduction of IFN-gamma and TNF-alpha from natural killer (NK) cells is associated with abnormal NK reactivity and cognitive derangement in Alzheimer's disease. Ann. NY. Acad. Sci. 917:331-40, 2000.

Sommer N, Löschmann PA, Northoff GH, Weller M, Steinbrecher A, Steinbach JP, Lichtenfels R, Meyermann R, Riethmuller A, Fontana A, Dichgans J, Martin R. The antidepressant Rolipram suppresses, cytokine production and prevents autoimmune encephalomyelitis. Nat. Med. 1:244-248, 1995.

Spanaus K S, Nadal D, Pfister H W, Seebach J, Widmer U, Frei Gloor S, Fontana A. C-XC and C-C chemokines are expressed in the cerebrospinal fluid in bacterial meningitis and mediate chemotactic activity on peripheral blood-derived polymorphonuclear and mononuclear cells in vitro. J. Immunol. 158:4, 1956-64, 1997.

Sterzel R B, Schulze-Lohoff E, Marx M, Cytokines and mesangial cells. Kindney Int. Suppl. 39:26-3I, 1993.

Stoll G, Jander S, Schroeter M. Cytokines in CNS disorders: neurotoxicity versus neuroprotection. J. Neural. Transm. Suppl. 59:81-9, 2000.

Storkus WJ, Tahara H, Lotze MT. IL-12. In:Thomson A (ed) the cytokine Handbook. Academic Press, San Diego, pp 390425, 1998.

Streit WJ. Microglial response to brain injury: a brief synosis. Toxicol. Pathol. 28:28-30, 2000.

Strigård K, Holmdahl R, van der Meide PH, Klareskog L, Olsson T. In vivo treatment of rats with monoclonal antibodies against gamma interferon: effects on experimental allergic neuritis. Acta. Neurol. Scand. 80:201-7, 1989.

Stuve O, Dooley NP, Uhm JH, Antel JP, Francis GS, Williams G, Yong WV. Interferon β-1b decreases the migration of T lymphocytes in vitro effects on matrix metalloproteinase-9. Ann Neurol. 40:853-863, 1996.

Suzuki M, Kitamura K, Uyemura K, Ogawa Y, Ishihara Y, Matsuyama H. Neuritogenic activity of peripheral nerve myelin proteins in Lewis rats. Neurosci. Lett. 19:353-8, 1980.

Suzuki N, Cheung TT, Cai X-D, et al. An increased percentage of long amyloid β protein secreted by familial B protein precursor (BAPP 717). Science. 264:1336-1340, 1994.

Szczepanik AM, Funes S, Petko W, Ringheim GE. IL-4, IL-10 and IL-13 modulate A beta (1-42)-induced cytokine and chemokine production in primary murine microglia and a human monocyte cell line. J. Neuroimmunol. 113:49-62, 2001.

Tacconi MT. Neuronal death: is there a role for astrocytes? Neurochem. Res. 23:759-65, 1998.

Taffet SM, Singhel KJ, Overholtzer JF, Shurtleff SA. Regulation of tumor necrosis factor expression in a macrophage-like cell line by lipopolysaccharide and cyclic AMP. Cell. Immunol. 120:291-300, 1989.

Tanzi RE, Gusella JF, Watinkd PC, et al. Amyloid β protein gene: cDNA, mRNA distribution and genetic link age near the Alzheimer locus. Science. 235:880-884, 1987.

Tanzi RE, Wenninger JJ and Kim BT. Cellular specificity and regional distribution of amyloid beta protein precursor alternative transcripts are unaltered in Alzheimer's disease hippocampal formation. Mol. Brain Res. 18:246-254, 1993.

Tanzi RE, Kovacs DM, Kim TW, Moir RD, Guenette SY, Wasco W. The gene defects responsible for familial Alzheimer's disease. Neurobiol. Dis. 3:159-68, 1996.

Taub D D, Conlon K, Lloyd AR, Oppenheim J J, Kelvin D J. Preferential migration of activated $CD4^+$ and $CD8^+$ T cells in response to MIP-1α and MPI-1β. Science. 260:355-358, 1993.

Terry RD, Malish E, Salmon DP, Butters N, DeTeresa R, Hill R, Hansen LA, Katzman R. Physical basis of cognitive alteration in Alzheimer's disease: synapse loss in the major correlate of cognitive impairment. Ann. Neurol. 30:572-580, 1991.

Tessier PA, Naccache PH, Clark-Lewis I, Gladue RP, Neote KS, McColl SR, Chemokine networks in vivo: involvement of C-X-C and C-C chemokines in neutrophil extravasation in vivo in response to TNF-alpha. J. Immunol. 159:3595-3602, 1997.

Thompson CB. Distinct roles for the co-stimulatory ligands B7-1 and B7-2 in T helper cell differentiation? Cell. 81:979-982, 1995.

Trinchieri G: Proinflammatory and immunoregulatory functions of interleukin-12. Int. Rev. Immunol. 16:365-96, 1998.

Tsai CP, Pollard JD, Armati PJ. Interferon-gamma inhibition suppresses experimental allergic neuritis: modulation of major histocompatibility complex expression of Schwann cells in vitro. J. Neuroimmunol. 31:133-45, 1991.

Tu GF, Southwell BR, Schreiber G. Species specificity and development patterns of expression of the beta amyloid precursor protein (APP) gene in brain, liver and choroid plexus in birds. Comp. Biochem. Physiol. B. 101:391-398, 1992.

Van der Meché FGA, Van Doorn PAJ. Meulstee J, Jennekens FGI. Diagnostic and Classification Criteria for the Guillain-Barré Syndrome Euro. Neurolo. 45:3:133-139, 2001.

Van Leuven F. Single and multiple transgenic mice as models for Alzheimer's disease. Prog. Neurobiol. 61:305-12, 2000.

Vassalli P. The pathophysiology of tumor necrosis factors. Annu. Rev. Immunol. 10: 411-52, 1992.

Vernadakis A. Glia-neuron intercommunications and synaptic plasticity. Prog. Neurobiol. 49:185-214, 1996.

Vincent VA, Selwood SP, Murphy GM, Jr.: Proinflammatory effects of M-CSF and A beta in hippocampal organotypic cultures. Neurobiol. Aging. 23:349-62, 2002.

Vogels OJ, Broere CA, ter Laak HJ, ten Donkelaar HJ, Nieuwenhuys R, Schulte BP. Cell loss and shrinkage in the nucleus basalis Meynert complex in Alzheimer's disease. Neurobiol.

Vriesendorp FJ, Flynn RE, Khan M, Pappolla MA, Brod SA. Oral administration of type I interferon modulates the course of experimental allergic neuritis. Autoimmunity. 24:157-65, 1996.

Waksman BH, Adams RD. Allergic neuritis: an experimental disease of rabbit induced by the injection of peripheral nervous tissue and adjuvant. J. Exp. Med. 102:213-25, 1955.

Walsh FS, Cronin M, Koblar S, Doherty P, Winer J, Leon A, Hugh R.A.C. Association between glycoconjugate antibodies and Campylobacter infection in patients with Guillain- Barré syndrome. J. Neuroimmunol. 34:43-51, 1991.

Weiner HL. Oral tolerance: immune mechanisms and treatment of autoimmune diseases. Immunol. Today. 18:335-43, 1997.

West MJ, Coleman PD, Flood DG, Troncoso JC. Differences in the pattern of hippocampal neuronal loss in normal ageing and disease. Lancet. 344(8925):769-72, 1994.

West MJ, Slomianka L. Total number of neurons in the layers of the human entorhinal cortex. Hippocampus. 8:69-82, 1998.

Windhagen A, Newcombe J, Dangond F, Strand C, Woodroofe MN, Cuzner ML, Hafler DA. Expression of costimulatory Alzheimer's molecules B7-1 (CD80), B7-2 (CD86), and interleukin 12 cytokine in multiple sclerosis lesions. J. Exp. Med. 182:1985-96, 1995.

Wisniewski HM and Weigel J. Beta-protein fibrillogenesis and neuritic plaques. In by D.B. Calne. ed. Neurodegenerative. diseases. Philadelphia. Sanders. 83-95, 1994.

Wollinsky KH, Hülser P–J, Brinkmeier H, Aulkemeyer P, Bössenecker W, Huber–Hartmann K–H, Rohrbach P, Schreiber H, Weber F, Kron M, Büchele G, H-H. Mehrkens H–H, Ludolph AC, and Rüdel R. CSF filtration is an effective treatment of Guillain–Barré syndrome: a randomized clinical trial. Neurology. 57:774-780, 2001.

Xia M, Qin S, McNamara M, Mackay C, Hyman BT. Interleukin-8 receptor B immunoreactivity in brain and neuritic plaques of Alzheimer's disease. Am. J. Pathol. 150:1267-74, 1997.

Xia MQ, Qin SX, Wu LJ, Mackay CR, Hyman BT. Immunohistochemical study of the beta-chemokine receptors CCR3 and CCR5 and their ligands in normal and Alzheimer's disease brains. Am. J. Pathol. 153:31-7, 1998.

Xia MQ, Hyman BT. Chemokines/chemokine receptors in the central nervous system and Alzheimer's disease. J. Neurovirol. 5:32-41, 1999.

Xiao BG and Link H. Short analytical review: mucosal tolerance: a two edged sword to prevent and treat autoimmune diseases. Clin. Immunol. and immunopathol. 85:119-128, 1997.

Xie Z, Wei M, Morgan TE, Fabrizio P, Han D, Finch CE, Longo VD. Peroxynitrite mediates neurotoxicity of amyloid beta-peptide1-42 and lipopolysaccharide-activated microglia. J. Neurosci. 22:3484-92, 2002.

Yan WX, Archelos JJ, Hartung HP and Polard JD. P0 protein is a traget antigen in chronic inflammatory demyelinating polyradiculoneuropathy. Annals of Neurolo. 50:3:286-92, 2001.

Yankner BA, Duffy LK, Kirschner DA. Neurotrophic and neurotoxic effects of amyloid beta protein: reversal by tachykinin neuropeptides. Science. 250:279-282, 1990.

Yoshikawa M, Suzumura A, Tamaru T, Takayanagi T, Sawada M. Effects of phosphodiesterase inhibitors on cytokine production by microglia. Mult. Scler. 5:126-133, 1999.

Yu S, Chen Z, Mix E, Zhu SW, Winblad P, Ljunggren HG, and zhu J. Neutralizing antibodies to IL-18 ameliorate experimental autoimmune neuritis by counter-regulation of autoreactive Th1 responses to reripheral myelin antigen. J. Neuropathol. Exp. Neurol. 61:614-22, 2002.

Zhang GX, Yu LY, Shi FD, Xiao BG J, Bjork J, Hedlund G, Link H. Linomide suppresses both Th1 and Th2 cytokine in experimental autoimmune myasthenia gravis. J. Neuroimmunol. 73:175-182, 1997.

Zhu J, Bengtsson BO, Mix E, Thorell LH, Olsson T, Link H. Effect of monoamine reuptake inhibiting antidepressants on major histocompatibility complex expression on macrophages in

normal rats and rats with experimental allergic neuritis (EAN). Immunopharmacology. 27:225-44, 1994a.

Zhu J, Mix E, Olsson T, Link H. Cellular mRNA expression of interferon-g, interleukin 4 and transforming growth factor-ß by rat mononuclear cells stimulated with peripheral nerve myelin antigens in experimental allergic neuritis. Clin. Exp. Immunol. 98:306-12, 1994b.

Zhu J, Bai XF, Mix E, Link H. Cytokines dichotomyin the peripheral nervous tissues influences the outcome of experimental allergic neuritis:Dynamic of mRNA expression for IL-1β, IL-6, IL-10, IL-12, TNF-α, and TNF-β and cytolysin. Clin. Immunol. Immunopath. 84:85-94, 1997.

Zhu J, Mix E, Link H. Cytokine production and the pathogenesis of experimental autoimmune neuritis and Guillain-Barré syndrome. J. Neuroimmunol. 84:40-52, 1998a.

Zhu J, Deng G M, Levi M, Wahren B, Diab A, van der Meide, P H, Link H. Prevention of experimental autoimmune neuritis by nasal administration of P2 protein peptide 57-81. J. Neuropathol. Exp. Neurol. 57:291-301, 1998b.

Zhu J, Bengtsson BO, Mix E, Ekerling L, Thorell LH, Olsson T, Link H. Clomipramine and imipramine suppress clinical signs and T and B cell response to myelin proteins in experimental autoimmune neuritis in Lewis rats. J. Autoimmun. 11:319-27, 1998c

Zhu J, Bai XF, Hedlund G, Bjork J, Bakhiet M, Van Der Meide PH, Link H. Linomide suppresses experimental autoimmune neuritis in Lewis rats by inhibiting myelin antigen-reactive T and B cell responses. Clin. Exp. Immunol. 115:56-63, 1999.

Zhu Yu, Ljunggren H-G, Mix E, Li H-L, Meide PVD, Elhassan MA, Winblad B, Zhu J, Suppression of Autoimmune Neuritis in IFN-γ Receptor-Deficient Mice. Experiment. Neurolo. 169:472-478, 2001.

Zielasek J, Jung S, Gold R, Liew FY, Toyka KV, Hartung H. Administration of nitric oxide synthase inhibitors in experimental autoimmune neuritis and experimental autoimmune encephalomyelitis. J. Neuroimmunol. 58:81-8, 1995.

Zou Lp, DH Ma, M Levi, B Wahren, L Wei. E Mix, PH. van der Meide, H Link, J Zhu. Antigen-specific immunosuppression: nasal tolerance to P0 protein peptides for the prevention and treatment of experimental autoimmune neuritis in Lewis Rats J. Neuroimmunol. 94:109-121, 1999a.

Zou LP, DH Ma, L Wei, PH van der Meide, E Mix, J Zhu. IFN-β suppresses Axperimental Autoimmune Neuritis in lewis rats by inhibiting the migration of inflammatory cells into peripheral nervous tissue. J. Neuro. Rese. 56:123-130, 1999b.

Zou LP, G Deretzi S-H Pelidou, M Levi, Br Wahren, C Quiding, P van der Meide, J Zhu. Rolipram suppresses experimental autoimmune neuritis and prevents relapses in Lewis rat Neuropharmacology. 39:324-333, 2000.