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Interleukin 10 and TNF α Synergistically Enhance The Expression of The G-Protein Coupled Formylpeptide Receptor 2 in Microglia

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Abstract

Microglia are important participants in inflammatory responses in the central nervous system. We previously observed that tumor necrosis factor alpha (TNF α) induces the expression of the formylpeptide receptor mFPR2 on microglial cells. This chemoattractant receptor mediates microglial cell chemotaxis in response to a variety of peptides, including amyloid β peptide (A β ₄₂), a major pathogenic factor in Alzheimer's disease (AD). In search for agents that regulate microglial activation, we unexpectedly found that IL-10 enhanced the expression of mFPR2 on TNF α -activated microglia. This was associated with a markedly increased microglial chemotaxis to A β ₄₂ and its endocytosis via mFPR2. Mechanistic studies revealed that the synergistic effect of IL-10 on TNF α -induction of mFPR2 in microglia was dependent on activation of p38 MAPK. Our results suggest that IL-10 may affect the pathogenic process of AD by up-regulating mFPR2 and thus favoring the recognition and internalization of A β ₄₂ by activated microglial cells.

Keywords

Cytokines; Chemotaxis; Inflammation; Protein Kinases; Neuroimmunology

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Introduction

Microglial cells in the central nervous system (CNS) share characteristics of tissue macrophages (Flaris et al., 1993). Under normal conditions microglial cells are in a resting state, but they become rapidly activated upon contact with pro-inflammatory signals and participate in CNS responses to infection, inflammation, injury and neurodegeneration (Gonzalez-Scarano and Martin-Garcia, 2005). Microglial cells express a number of chemoattractant receptors, migrate in response to selected chemoattractants and accumulate in inflammatory brain lesions where chemoattractants are elevated (Sorensen et al., 1999; Iribarren et al., 2005c). Pro-inflammatory stimuli also induce selected chemoattractant receptors in microglial cells presumably to expand the scope of cell responses in pathological conditions. For instance, mouse microglial cells activated by agonists for Toll-like receptors (TLR) or tumor necrosis factor (TNF) α , show increased expression of a G protein coupled formylpeptide receptor mFPR2 (Chen et al., 2006; Cui et al., 2002b; Cui et al., 2002c; Iribarren et al., 2005a), which is a homologue of human FPRL1. Both, mFPR2 and FPRL1 recognize a diverse array of chemotactic agonists including the bacterial formylated peptide fMLF, HIV-1 envelope protein derived peptides (Le et al., 2001b), as well as the 42 amino acid form of A β peptide (A β ₄₂, a key pathogenic agent associated with the CNS lesions of Alzheimer's disease (AD) (Le et al., 2001a; Tiffany et al., 2001). Activation of mFPR2 or FPRL1 elicits a cascade of signals in phagocytic leukocytes and microglia resulting in increased cell chemotaxis, phagocytosis and release of pro-inflammatory mediators (Le et al., 2002; Le et al., 2001b). Therefore, mFPR2 or FPRL1 may play an important role in host defense and inflammatory responses.

Interleukin 10 (IL-10) is a key regulator of inflammation and immunologic reaction produced by activated monocytes and T cells. Despite its pleiotrophic activity, the main biological function of IL-10 has been best understood for its capacity to inhibit the production of pro-inflammatory cytokines by activated immune cells. Consequently, mice depleted of IL-10 gene overproduce pro-inflammatory cytokines and develop chronic inflammatory diseases (Kuhn et al., 1993). IL-10 may also actively participate in inflammatory and immune responses in the brain, since functional IL-10 receptors are detected in major glial populations, microglia. IL-10 promotes the survival of glia and neurons (Strle et al., 2002). It is therefore not surprising that as a defensive host reaction, the level of IL-10 increases in major CNS diseases, including meningoencephalitis (Deckert et al., 2001), experimental autoimmune encephalomyelitis (EAE) (Cua et al., 2001), trauma (Spera et al., 1998), as well as AD (Apelt and Schliebs, 2001).

Due to the potential of IL-10 to act as a down-regulator of inflammatory responses in AD, we examined its capacity to regulate mFPR2 expression in microglia. We unexpectedly observed that despite its inhibition of MHC class II molecules in TNF α -activated microglia, IL-10 enhanced cell expression of mFPR2, which resulted in markedly increased cell chemotaxis in response to A β ₄₂, and subsequent endocytosis of this AD-associated peptide. Our results suggest that IL-10 may selectively promote the interaction of microglial cells with pathogenic agents in the brain and concomitantly limiting potentially detrimental pro-inflammatory and immune responses.

Materials and Methods

Reagents and cells

fMLF was purchased from Sigma-Aldrich (St. Louis, MO). Mouse IFN γ , TNF α , SDF-1 α and IL-10 were purchased from PeproTech (Rocky Hill, NJ). The chemotactic peptide WKYMVm (designated W peptide) was synthesized and purified by the Department of Biochemistry, Colorado State University (Fort Collins, CO). The antibodies against phospho-ERK1/2, total

ERK1/2, phospho-p38 MAPK and total p38 MAPK were purchased from Cell Signaling Technology, Inc. (Beverly, MA). Primary murine microglial cells were isolated from 1-day-old newborn C57BL/6 (WT) mice (Cui et al., 2002c; Iribarren et al., 2005b). The murine microglial cell line N9 was a kind gift from Dr. P. Ricciardi-Castagnoli (Universita Degli Studi di Milano-Bicocca, Milan, Italy). The cells were grown in IMDM supplemented with 5% heat-inactivated FCS, 2 mM glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, and 50 µM 2-mercaptoethanol.

Chemotaxis assays

Chemotaxis assays for microglial cells were performed with 48-well chemotaxis chambers (NeuroProbe, Cabin John, MD) with an incubation period of 90 min at 37°C as described (Cui et al., 2002c). The results are expressed as the mean ($\pm$ SD) of chemotaxis index (CI), which represents the fold increase in the number of migrated cells counted in three high-power fields (X 400) in response to chemoattractants over spontaneous cell migration (to control medium).

RT-PCR and Real Time PCR

Total RNA was extracted from cells with an RNeasy Mini kit and depleted of contaminating DNA with RNase-free DNase (Qiagen, Valencia, CA). For amplification of mFPR2 gene, primers 5'-TCT ACC ATC TCC AGA GTT CTG TGG-3' (sense) and 5'-TTA CAT CTA CCA CAA TGT GAA CTA-3' (antisense) were designed to yield a 268-bp product. Specific primers for mouse CXCR4 were: 5'-GGC TGT AGA GCG AGT GTT GC-3' (sense) and 5'-GTA GAG GTT GAC AGT GTA GAT-3' (antisense) which yield a product of 390 bp. RT-PCR was performed with 0.5 µg of total RNA for each sample (High Fidelity ProSTAR HF System; Stratagene, La Jolla, CA), consisting of a 15 min reverse transcription at 37°C, 1 min inactivation of Moloney murine leukemia virus reverse transcriptase at 95°C, 40 cycles of denaturing at 95°C (45 s), annealing at 55°C (52°C for CXCR4; 45 s), and extension at 72°C (1 min), with a final extension for 10 min at 72°C. Primers for murine β -actin gene were used as controls (Stratagene). Real time PCR was performed by using an ABI-Prism 7700 Sequence Detector (Applied Biosystems, Foster City, CA). Briefly, 5 ng of reverse-transcribed cDNA were used in triplicate samples. The assays were initiated with 2 min at 50°C, 10 min at 95°C, and then 40 cycles of 15 s at 95°C and 1 min at 60°C. Primers and specific probes were obtained from Applied Biosystems (Foster City, CA) and were consisted of the following: 5'-CCTTA TAGTC TTGAG AGAGC CCTGA-3' (sense); 5'-TGCAG GAGGT GAAGT AGAAC TGG-3' (antisense) and the probe 5'-FAM- TGAGG ATTCT GGTC AACA GTGAT TCAAG C - TAMRA-3'. Detection of mFPR2 and control 18S rRNA was performed using TaqMan Universal PCR Master Mix (Applied Biosystems).

Flow cytometry

Murine microglia were examined for expression of MHC class II and CD86 by labeling with PE-conjugated monoclonal antibodies (Pharmingen, San Diego, CA). All staining procedures were completed at 4°C in DPBS containing 5 mM EDTA and 1% FCS. After extensive washing, the cells were analyzed using a FACScan flow cytometer (BD Biosciences, Mountain View, CA).

Western immunoblotting

N9 cells were grown in 60 mm dishes until sub-confluency and then were cultured overnight in FCS free medium. After treatment with cytokines at the indicated time points, the cells were lysed with 150 µl ice-cold lysis buffer. The cell lysate was centrifuged at 14,000 rpm (4°C) for 5 minutes and the protein concentration of the supernatant was measured by using BCA Protein Assay System (Pierce, Rockford, IL). Western blotting of phosphorylated ATF-2, ERK1/2 or p38 MAPK was performed according to the manufacturer's instruction using phospho-specific

antibodies. Briefly, proteins were electrophoresed on a 10% SDS-PAGE precast gel (Invitrogen, Carlsbad, CA) under reducing conditions and were transferred onto Immun-Blot PVDF Membrane (BIO-RAD, Hercules, CA). The membranes were blocked with 5% non-fat milk (0.1% Tween-20) in TBS overnight at 4°C and then were incubated with primary antibodies for 3 h at room temperature. After incubation with a horseradish-peroxidase conjugated secondary antibody, the protein bands were detected with a Super Signal Chemiluminescent Substrate (Pierce) and BIOMAX-MR film (Eastman Kodak, Rochester, NY). For detection of total ERK1/2, the membranes were stripped with Restore Western Blot Stripping Buffer (Pierce) followed by incubation with specific antibodies.

Fluorescence confocal microscopy

Cytokine-treated N9 cells grown on chamber slides were treated in the presence or absence mFPR2 agonist W peptide, fucoidan (Sigma) or glycated albumin (albumin), and then were fixed in 4% paraformaldehyde for 10 min at room temperature. The cells were further incubated with A β ₄₂ for different time periods; slides were washed with PBS and incubated with 5% normal goat serum (Sigma) in PBS, 0.05% Tween-20 (PBS-T-NGS), for 1 h to reduce nonspecific binding of antibodies to the cell surface and for cell permeabilization. An anti-A β ₄₂ antibody (Sigma) was applied to the slides, which were further incubated for 1 h at room temperature. After three rinses with PBS, the slides were incubated with FITC-conjugated goat anti-mouse IgG (Pharmingen) in TBS containing 1% BSA for 60 min. After three washes with PBS the slides were stained with propidium iodide (PI) for 20 minutes at room temperature. The slides were mounted with an anti-fade, water-based mounting medium and analyzed under a laser scanning confocal fluorescence microscope (Zeiss LSM410 NLO Meta, Jena, Germany). Excitation wavelengths of 488 (for FITC) and 568 (for PI) nm were used to generate fluorescence emission in green (for A β ₄₂) and red (for nuclei), respectively.

Statistical analysis

All experiments were performed at least three times and the results presented are from representative experiments. For cell migration the significance of the difference between test and control groups was analyzed using the Student's *t* test.

Results

IL-10 potentiates mFPR2-mediated chemotaxis of TNF α -activated microglia

We first examined the expression and function of mFPR2 in mouse microglia in the presence or absence of cytokines. In agreement with our previous findings (Cui et al., 2002b), treatment of murine microglial cell line N9 with TNF α promoted cell migration in response to mFPR2 agonists W peptide (W pep) and A β ₄₂ (Fig. 1A). In contrast, TNF α treatment down-regulated the chemotactic response of N9 cells to SDF-1 α a chemokine agonist for the receptor CXCR4. On the other hand, N9 cells pretreated with IL-10, when further stimulated with TNF α exhibited a markedly increased chemotaxis response to the mFPR2 agonists as compared with cells treated with TNF α alone (Fig 1A). The effect of IL-10 was dose dependent and was blocked by a monoclonal antibody against IL-10, excluding potential contaminant(s) in the IL-10 preparations used in this study (Fig. 1B and C). The observations with N9 cell line were corroborated by using primary murine microglial cells in which TNF α also promoted cell chemotaxis in response to mFPR2 agonists (Fig. 1D), and pretreatment of the cells with IL-10 enhanced the effect of TNF α . Interestingly, IL-10 did not restore microglial cell responses to SDF-1 α , which was down-regulated by TNF α (Fig. 1A and C). Thus, IL-10 appears to selectively augment the function of mFPR2 in microglial cells stimulated by TNF α .

IL-10 enhances mFPR2 gene expression stimulated by TNF α in microglia

We next examined whether IL-10 affected mFPR2 mRNA expression in N9 cells stimulated by TNF α . mFPR2 mRNA was hardly detectable in non-stimulated N9 microglial cells (Fig. 2A) and IL-10 by itself did not significantly increase the level of mFPR2 mRNA as measured by RT-PCR. In contrast, TNF α induced mFPR2 mRNA in N9 cells and the effect of TNF α was markedly enhanced by pre-treatment of the cells with IL-10 (Fig. 2A and B). Real-time PCR was used to more quantitatively measure the changes in mFPR2 mRNA and confirmed the increase in mFPR2 mRNA in TNF α -stimulated N9 cells and a synergistic enhancement induced by IL-10 (Fig. 2B). Thus, IL-10 enhances microglial response to TNF α with increased expression of mFPR2 at both the mRNA and functional levels. It is of interest to note that N9 microglial cells expressed both TNF receptors I and II on cell surface. Neither, TNF α alone nor in combination with IL-10 increased the levels of these receptors, suggesting that the effect of these cytokines on mFPR2 expression did not require elevated TNF receptor expression (data not shown).

IL-10 inhibits MHC class II expression in TNF α -activated microglia

The unexpected ability of IL-10 to enhance microglial expression of mFPR2 induced by TNF α prompted us to examine how IL-10 regulates other cell responses to TNF α . By examining MHC class II and CD86, we found that N9 cells expressed low levels of MHC class II, but high levels of CD86 on the cell surface (Fig. 3). IL-10 treatment of the cells for up to 48 h did not change the levels of either MHC class II or CD86 (Fig. 3). In contrast, the level of MHC class II molecule on microglial cells was up-regulated by TNF α or INF γ . Interestingly, preincubation of the cells with IL-10 diminished the effect of both TNF α and INF γ (Fig. 3). On the other hand, CD86 on microglial cells remained at a constant level despite treatment by TNF α or INF γ , and IL-10 did not show any effect. These results indicate that IL-10 differentially regulates microglial cell responses to TNF α , as evidenced by inhibition of MHC class II but increase in mFPR2 expression.

IL-10 enhances the level of MAP kinase activation induced by TNF α

We then attempted to elucidate the mechanistic basis for the effect of IL-10 on microglial cell response to TNF α . N9 microglial cells expressed both TNF receptor (TNFR) I and II, which were up-regulated by TNF α treatment (data not shown). IL-10 did not significantly change the levels of TNFR expression by non-stimulated cells or cells treated with TNF α , suggesting that IL-10 might affect the signaling cascade down stream of TNFRs in microglial cells. We therefore evaluated whether MAPKs, which are essential for TNF α activation of myeloid cells (Winston and Riches, 1995), might be the targets for IL-10 to increase the efficiency of TNF α signal transduction in microglial cells. Fig. 4A shows that TNF α induced a rapid and transient phosphorylation of p38 and ERK1-2 as well as ATF-2, a p38 controlled signaling molecule in microglial cells (Koistinaho and Koistinaho, 2002). Pretreatment of the cells with IL-10 significantly enhanced the level of MAPK and ATF-2 phosphorylation (Fig. 4A) induced by TNF α . In fact, IL-10 prolonged the duration of p38 and ERK activation while accelerating the kinetics of ATF-2 phosphorylation induced by TNF α (Fig. 4A). IL-10 by itself only induced a weak, if any, MAPK activation in microglia (Fig. 4A). In addition, the effect of IL-10 was blocked by a monoclonal antibody against IL-10 (Fig. 4B) and the phosphorylation of p38 was blocked by the p38 inhibitor SB202190 (Fig. 4B). Although IL-10 enhanced the phosphorylation of both p38 and ERK1/2 in microglia stimulated by TNF α , its effect on p38 appeared to be critical for mFPR2 transcription, since the p38 MAPK inhibitor SB202190, but not the MEK1/2 inhibitor PD98059, completely inhibited TNF α -induced expression of mFPR2 mRNA (Fig. 4C and ref. (Cui et al., 2002b)). Moreover, we observed that a selective inhibitor of I κ B α phosphorylation BAY 11-7082, blocked TNF α -induced expression of mFPR2 (Fig. 4B). Surprisingly, IL-10 significantly decreased the levels of NF κ B-driven luciferase activity

(data not shown), suggesting that the capacity of IL-10 to promote mFPR2 expression in by TNF α activated N9 cells may not correlate with NF- κ B activation.

IL-10 enhances A β ₄₂ up-take by TNF α -activated microglia

Microglia are key immune effector and phagocytic cells in the CNS (Flaris et al., 1993) and have been shown to ingest A β peptides and this process may contribute either to the clearance of A β ₄₂ or to its fibrillary deposition presumably determined by the burden of A β ₄₂ (Cui et al., 2002a;Iribarren et al., 2005c). Since in our previous study with human macrophages, we found that interaction of A β ₄₂ with the human mFPR2 analogue FPRL1 resulted in internalization of the ligand/receptor complexes (Yazawa et al., 2001) and since mouse microglial cells activated by TLR agonists showed increased ingestion of A β ₄₂ via mFPR2 (Chen et al., 2006;Iribarren et al., 2005a), we evaluated A β ₄₂ internalization in N9 mouse microglia treated with TNF α and IL-10. Stimulation of microglial cells with TNF α for 24 h significantly increased the levels of A β ₄₂ up-take as visualized by confocal microscopy (Fig. 5A). The up-take of A β ₄₂ required mFPR2 because pre-incubation of microglial cells with the mFPR2 agonist W peptide (Le et al., 1999), and Pertussis toxin, an inhibitor of G α i protein, that deactivates mFPR2 diminished the effect of TNF α . In addition, glycated albumin and fucoidan, ligands for other two purported A β ₄₂ receptors, the receptor for advanced glycation end products (RAGE) (Yan et al., 1996), and scavenger receptor (El Khoury et al., 1996), respectively, did not interfere with A β ₄₂ up-take by TNF α -activated microglia (Fig. 5). On the other hand, after pre-incubation of microglial cells with IL-10, subsequent treatment with TNF α markedly enhanced the capacity of the cells to up-take A β ₄₂, consistent with the effect of IL-10 on up-regulation of mFPR2. These results indicate that IL-10 and TNF α not only synergistically stimulated the expression of mFPR2 as a mediator of microglial cell chemotaxis in response to A β ₄₂, but also drastically enhanced the capacity of microglial cells to up-take A β ₄₂ via mFPR2.

Discussion

In this study we have shown that IL-10 was capable of enhancing TNF α -induced functional expression of the chemoattractant receptor mFPR2 in microglial cells through a p38 MAPK pathway. As a consequence, IL-10 and TNF α not only synergistically promoted the chemotactic responses of microglia to A β ₄₂, but also further increased their capacity to internalize A β ₄₂ via mFPR2. IL-10 concomitantly reduced the effect of TNF α on up-regulation of MHC class II molecules. Therefore, IL-10 selectively regulates the effects of a pro-inflammatory cytokine on microglial cells and favors cell interaction and internalization of a noxious agent associated with AD.

Microglial activation is an essential component in the pathogenesis of AD, Parkinson's disease (Hirsch et al., 2003), multiple sclerosis, AIDS dementia (Minagar et al., 2002), and brain trauma caused by stroke (Pocock et al., 2002). Our previous studies showed that murine microglial cells in a resting state express very low levels of mFPR2 (Cui et al., 2002c), a receptor that recognizes a diverse array of chemotactic agonists (Cui et al., 2002c;Tiffany et al., 2001;Ying et al., 2004). When stimulated with TNF α or a plethora of TLR agonists (Chen et al., 2006;Cui et al., 2002c;Iribarren et al., 2005a), microglial cells expressed high levels of mFPR2 transcripts and became responsive to mFPR2-specific agonists (Cui et al., 2002b;Cui et al., 2002c), suggesting that the presence of pro-inflammatory stimuli may promote microglial responses in CNS diseases in which agonists for mFPR2 are elevated. This is in agreement with the notion that pro-inflammatory and injurious insults in the brain may exacerbate neurodegenerative diseases, in particular AD, in which microglial cells are in an activated state and their responses to A β peptides trigger the release of neurotoxins. Therefore, inhibition of inflammatory responses has been considered as one of the therapeutic strategies for AD. In fact, a number of studies have shown that selected non-steroidal anti-inflammatory drugs were

beneficial in retarding the onset of AD dementia by inhibition of microglial cell responses to amyloid peptides as well as by a reduction in the production of A β peptides by neuronal cells (Hardy and Selkoe, 2002; Hull et al., 2002).

Host derived anti-inflammatory cytokines also have the capacity to maintain microglial cell homeostasis by reducing their responses to pro-inflammatory stimulants. For instance, the type 2 cytokine IL-4 has been shown to “disrupt” LPS signaling through induction of an okadaic acid (OA)-sensitive phosphatase PP2A, which may rapidly dephosphorylate MAPKs activated by LPS in microglia (Cui et al., 2002c; Iribarren et al., 2005b; Pahan et al., 1998; Shanley et al., 2001). Another anti-inflammatory cytokine, the transforming growth factor β 1 (TGF β 1), also plays an important role in regulating pro-inflammatory responses in the CNS. In experimental AD models, while over-expression of both human TGF β 1 and amyloid precursor protein genes in mice causes a general activation of microglial cells in the brain and a reduced deposition of A β peptides in parenchymal plaques (Wyss-Coray et al., 2001), intra-cerebroventricular administration of TGF β 1 together with A β peptides increased A β peptide accumulation and deposition in rodents (Frautschy et al., 1996). Interestingly, similar to IL-4, TGF β 1 was capable of attenuating LPS-induced signaling cascade in microglial cells and inhibiting the subsequent expression of the A β ₄₂ receptor mFPR2 (Le et al., 2004). These results suggest that a precise temporal-spatial balance of pro- and anti-pro-inflammatory signals in the brain may determine the course of AD pathogenesis.

In addition to IL-4 and TGF β 1, IL-10 is yet another key mediator of anti-inflammatory host responses (Mosmann, 1994). It is well known that exposure of mononuclear phagocytes and dendritic cells to IL-10 inhibits the production of pro-inflammatory cytokines, reactive oxygen and nitrogen intermediates, as well as antigen presentation induced by pro-inflammatory stimulants. In vivo, IL-10 protects mice from endotoxin shock by preventing excessive production of pro-inflammatory cytokines (van der Poll et al., 1995). Thus, one of the key properties of IL-10 is to turn off a pro-inflammatory signaling cascade that may result in multiple organ injury such as in the case of acute respiratory disease syndrome. IL-10 has also been reported to inhibit microglial cell response to pro-inflammatory cytokines such as TNF α , by as yet unknown mechanisms (Wu et al., 2005). To support the notion of IL-10 as a “turn-off” signal in microglia, our present study showed that IL-10 potently inhibited MHC class II molecules induced by TNF α . Interestingly, our present study revealed an unexpected biological function of IL-10, i.e. it further promoted the expression of mFPR2 in microglial in response to TNF α . Consequently, microglial cells stimulated by both IL-10 and TNF α showed markedly increased chemotaxis in response to mFPR2 agonist peptides and their capacity to phagocytose A β ₄₂, as compared with cells stimulated with TNF α alone. Our study further demonstrated that IL-10 enhanced the levels of MAPK and ATF-2 phosphorylation induced by TNF α . The observation that p38 MAPK plays a key role in the induction of mFPR2 in microglial cells by TNF α suggests that IL-10, despite its lack of significant activity on p38 phosphorylation, primes the cells to respond to TNF α in a more rapid and efficient manner. It should be noted that some earlier studies showed that IL-10 is capable of up-regulating the expression of chemokine receptors in human macrophages stimulated by LPS, and simultaneously IL-10 down-regulated the expression of chemokine ligands (Sozzani et al., 1998; Wang et al., 1994). It was therefore postulated that IL-10 has a unique property of limiting the production of excessive and thus potentially detrimental inflammatory mediators by host cells, while preparing the cells to be in a ready state with proper expression of chemoattractant receptors.

The capacity of IL-10 to promote selective microglial cell response to TNF α by synergistically up-regulating the expression of mFPR2 may have significant implication in the pathogenic process of AD. It has been postulated that in the AD-affected brain, microglia accumulate at lesions containing high levels of A β ₄₂ (Rogers and Lue, 2001). This process was thought to

be an important host response to an aberrantly produced noxious agent as shown by serial three-dimensional reconstruction of A β plaques in animal model of AD in which A β deposits were enveloped by microglial processes (Stalder et al., 2001). In addition, microglial cells have been documented to phagocytose fibrillary aggregates of A β (Ard et al., 1996; Bard et al., 2000; Paresce et al., 1996) and to break isolated senile plaques *in vitro* (DeWitt et al., 1998). Furthermore, active immunization with A β peptides (Lombardo et al., 2003; Schenk et al., 1999), or passive immunization with antibodies to A β (Wilcock et al., 2003), results in substantial removal of A β deposits, which apparently is mediated by microglia. It is therefore plausible that appropriately enhanced microglial interaction with A β peptides may have the beneficial effects on their clearance thus reducing their deposition and subsequent damage to neurons. In this context, the capacity of IL-10 to inhibit MHC class II while increasing mFPR2 in TNF α -activated microglia suggests a unique model for developing novel therapeutic strategies for AD.

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Fig 1.

The effect of IL-10 on chemotaxis of TNFα-activated microglia. **(A)** N9 cells were incubated with 10 ng/ml IL-10 at 37°C for 15 min, followed by addition of TNFα (100 ng/ml) for 24 h. The cells were then examined for migration in response to the mFPR2 agonist W peptide (1 μM), Aβ₄₂ (17 μM), and the chemokine SDF-1α (100 ng/ml). **(B)** N9 cells were incubated with different doses of IL-10 for 15 min, followed by addition of TNFα (100 ng/ml) for 24 h. The cells were then examined for migration in response to the mFPR2 agonist W peptide (1 μM). **(C)** IL-10 (10 ng/ml) was pre-incubated with an anti-IL-10 monoclonal antibody (10 μg/ml) (αIL-10) or a control IgG (10 μg/ml) for 1 h at 37°C, before being added to N9 cells. The cells were then incubated with TNFα (100 ng/ml) for 24 h before evaluation of chemotaxis induced by W peptide (1 μM) and the chemokine SDF-1α (100 ng/ml). **(D)** Primary mouse microglial cells were incubated with 10 ng/ml IL-10 at 37°C for 15 min, followed by addition of TNFα (100 ng/ml) for 24 h. The cells were then examined for migration in response to the mFPR2 agonist W peptide (1 μM), Aβ₄₂ (17 μM), and the chemokine SDF-1α (100 ng/ml). The results are expressed as chemotaxis index (CI) representing fold increase in cell migration in response to chemoattractants over the baseline migration (to medium). * Indicates statistically significant ($p < 0.01$) increase in cell migration compared to un-stimulated cells. # indicates significantly reduced ($p < 0.01$) cell migration compared to cells treated with TNFα.

Fig 2.

The effect of IL-10 on mFPR2 gene expression by microglia. N9 cells were cultured in the presence of IL-10 (10 ng/ml) at 37°C for 15 min, followed by addition of $TNF\alpha$ (100 ng/ml). After incubation at 37°C for 12 h, total RNA was extracted and examined for mFPR2 gene expression by RT-PCR (A) and Real-time PCR (B). (A) The RT-PCR products were electrophoresed on agarose gel and visualized with ethidium bromide staining. In RT-PCR the fold increase in stimulated versus un-stimulated cells after normalization with the β -actin transcripts are shown as “Densitometry”. (B) In real time PCR, arbitrary units were used to indicate the fold difference in stimulated versus un-stimulated cells after normalization with the β -actin transcripts.

Fig 3.

The effect of IL-10 on the expression of MHC class II and CD 86 molecules on activated microglia. N9 cells, cultured for 24 h at 37°C in the presence or absence of IL-10 (10 ng/ml) and TNF α (100 ng/ml), or IL-10 and INF γ , were examined for surface expression of MHC class II and CD86 by flow cytometry. The results are presented as percentage of positive cells and mean fluorescence intensity (MFI) in histograms.

Fig 4.

The activation of MAPK by TNF α in microglial cells and the effect of IL-10. **(A)** N9 cells were cultured in the presence of IL-10 (10 ng/ml) at 37°C for 15 min, followed by TNF α (100 ng/ml). The cells were lysed at the indicated time points and proteins were measured for phosphorylated p38, ATF-2 and ERK1/2. Anti-pan p38, ATF-2 and ERK antibody was used to indicate the loading of total cell proteins. **(B)** IL-10 (10 ng/ml) was pre-incubated with an anti-IL-10 monoclonal antibody (10 μ g/ml) (α IL-10) or a control IgG (10 μ g/ml) for 1 h at 37°C, before being added to N9 cells. The cells were then incubated with TNF α (100 ng/ml) for 15 minutes 37 °C. In addition, some samples of N9 cells were cultured in the presence of the p38 inhibitor SB202190 (20 μ M) for 30 min at 37°C before stimulation with TNF α (100 ng/ml) for 15 minutes. The cells were lysed and proteins were measured for phosphorylated p38. Anti-pan p38 antibody was used to indicate the loading of total cell proteins. **(C)** N9 cells were cultured in the presence of the MEK inhibitor PD98059 (50 μ M), p38 inhibitor SB202190 (20

μM) or I κ B inhibitor BAY 11-7082 (20 μM) for 30 min at 37 °C before stimulation with TNF α (100 ng/ml) for 6 h. Total cell RNA was extracted and examined for mFPR2 gene expression by RT-PCR.

Fig 5. The effect of IL-10 on A β_{42} up-take by microglia. **(A)** N9 cells placed on chamber slides were cultured in the presence or absence of TNF α (10 ng/ml) for 24 h at 37 °C. The cells were then incubated with W peptide (W pep) (1 μ M), **Pertussis toxin (PTX)**, fucocidan (F) or glycated albumin (GA) for 30 min before further incubation with A β_{42} (10 μ M) for 24 h. The slides were stained by a monoclonal antibody anti-A β_{42} (green) and propidium iodide (red) then were evaluated by confocal microscopy. **(B)** N9 cells placed on chamber slides were cultured in the presence or absence of TNF α (10 ng/ml) or IL-10 (10 ng/ml) for 24 h at 37 °C. The slides were stained by an anti-A β_{42} monoclonal antibody (green) and propidium iodide (red), and then were evaluated by confocal microscopy. The results are presented as relative units of mean green fluorescence intensity (MFI) in bars calculated by using the ImageJ software.