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IL-4 Inhibits the Expression of Mouse Formyl Peptide Receptor 2, a Receptor for Amyloid β₁–42, in TNF-α-Activated Microglia¹,²

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Microglia are phagocytic cells in the CNS and actively participate in proinflammatory responses in neurodegenerative diseases. We have previously shown that TNF-α up-regulated the expression of formyl peptide receptor 2 (mFPR2) in mouse microglial cells, resulting in increased chemotactic responses of such cells to mFPR2 agonists, including amyloid β₁–42 (Aβ₄₂), a critical pathogenic agent in Alzheimer’s disease. In the present study, we found that IL-4, a Th2-type cytokine, markedly inhibited TNF-α-induced expression of mFPR2 in microglial cells by attenuating activation of ERK and p38 MAPK as well as NF-κB. The effect of IL-4 was not dependent on Stat6 but rather required the protein phosphatase 2A (PP2A) as demonstrated by the capacity of PP2A small interfering RNA to reverse the effect of IL-4 in TNF-α-activated microglia. Since both IL-4 and TNF-α are produced in the CNS under pathophysiological conditions, our results suggest that IL-4 may play an important role in the maintenance of CNS homeostasis by limiting microglial activation by proinflammatory stimulants. The Journal of Immunology, 2005, 175: 6100–6106.

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2 The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. government.

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4 Abbreviations used in this paper: AD, Alzheimer’s disease; FPR, formyl peptide receptor; siRNA, small interfering RNA; SDF-1α, stromal cell-derived factor 1α; CI chemotaxis index; OA, okadaic acid; PPAR, peroxisome proliferator-activated receptor; iMLF, formyl-methionyl-leucyl-phenylalanine; PP2A, protein phosphatase 2A.

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Materials and Methods

Reagents and cells
IML and LPS were purchased from Sigma-Aldrich. Mouse TNF-α, stromal cell-derived factor 1α (SDF-1α), and IL-4 were purchased from PeproTech. The chemotactic peptide WKYMVm (designated W peptide) was synthesized and purified by the Department of Biochemistry, Colorado State University. The Abs against phospho-ERK1/2, total ERK1/2, phospho-p38 MAPK, and total p38 MAPK were purchased from Cell Signaling Technology. Primary murine microglial cells were isolated from 1-day-old newborn C57BL/6 (wild-type) mice and C57BL/6 Stat6-deficient (Stat6<−/−) mice (a gift from Dr. M. Grusby, Harvard University, Boston, MA). The murine microglial cell line N9 was a gift from Dr. P. Ricciardi-Castagnoli (Università 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 μg/ml 2-ME.

Chemotaxis assays
Chemotaxis assays for microglial cells were performed with 48-well chemotaxis chambers (NeuroProbe) as described previously (11). The results are expressed as the mean ± SD of chemotaxis index (CI), which represents the fold increase in the number of migrated cells counted in three high-power fields (×400) in response to chemotaxants released by 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). For amplification of the mFPR2 gene, primers 5′-TCTACATTCCAGAAGTTGCTG-3′ (sense) and 5′-TTATACCTTCCAGAAGTTGACTTA-3′ (antisense) were designed to yield a 268-bp product. Specific primers for mouse CXCR4 were 5′-GGCTGAAGCGAGTGTTGC-3′ (sense) and 5′-GTTAGAGGTTGACAGTGTAGTGC-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), 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). Briefly, 5 ng of reverse-transcribed cDNA was 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 and consisted of the following: 5′-CCTTA TAGTC TGAGA GAGC CCTGA-3′ (sense), 5′-TGCA GAGGT GAAT AACT-3′ (antisense), and the probe 5′-FAM-TGAGC ATTTG GCTTA ACACCA GTGAT CTAAG C-TAMRA-3′. Detection of mFPR2 and control 18S RNA was performed using TaqMan Universal PCR Master Mix (Applied Biosystems). 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-4 inhibits mFPR2-mediated chemotaxis of TNF-α-activated microglia
We first examined the effect of IL-4 on the chemotactic responses of TNF-α-activated microglia to mFPR2 agonists. Consistent with our previous findings (12), treatment of the murine microglial cell line N9 with TNF-α for 24 h promoted cell migration in response to a synthetic mFPR2 agonist peptide (W peptide) and aB42, an AD-associated peptide (Fig. 1A and data not shown). In contrast, TNF-α treatment down-regulated the chemotactic response of N9 cells to SDF-1α, a chemokine agonist for the receptor CXCR4. In contrast, pretreatment of N9 cells with IL-4 inhibited the effect of TNF-α on promotion of cell chemotaxis in response to mFPR2 agonists. The inhibitory effect of IL-4 was dose dependent and was blocked by a mAb against IL-4 (Fig. 1, A and B). The observations in the N9 cell line were corroborated with primary murine microglial cells in which TNF-α also up-regulated the chemotactic response to mFPR2 agonists (see Fig. 5A), and the effect of TNF-α was inhibited by IL-4. Interestingly, IL-4 failed to reverse the down-regulation of microglial cell responses to SDF-1α caused by TNF-α (Fig. 1A). This is consistent with our previous observation in which IL-4 did not reverse LPS-induced down-regulation of microglial responses to SDF-1α (16). Thus, IL-4 selectively abrogated the capacity of TNF-α to promote the microglial response to mFPR2 agonists.

IL-4 inhibits mFPR2 gene expression in TNF-α-activated microglia
We next examined the effect of IL-4 on mFPR2 mRNA expression in N9 cells induced by TNF-α. mFPR2 mRNA was hardly detectable in unstimulated microglial cells (Fig. 1C) and IL-4 by itself did not induce mFPR2 mRNA expression as measured by RT-PCR. TNF-α significantly enhanced mFPR2 mRNA expression in the N9 cells and the effect of TNF-α was markedly inhibited by...
pre-exposure of the cells to IL-4 (Fig. 1C). Real-time PCR was used to more quantitatively measure the changes in mFPR2 mRNA and revealed mFPR2 mRNA to be increased in TNF-α-stimulated N9 microglial cells and to be abrogated by IL-4 (Fig. 1D).

As previously reported, N9 cells also expressed a high level of the gene coding for the SDF-1α (CXCL12) receptor CXCR4, and the expression of CXCR4 mRNA was not affected by TNF-α (Ref. 12 and data not shown). The presence of IL-4 did not cause significant changes in the levels of CXCR4 mRNA in microglial cells. These results suggest that in TNF-α-activated mouse microglial cells, the selective inhibition of cell responses to mFPR2 agonists by IL-4 was associated with reduction of mFPR2 mRNA transcription. Thus, IL-4 appears to antagonize the capacity of TNF-α to induce mFPR2 at both the protein and mRNA levels in microglial cells.

**IL-4 inhibits activation of MAPKs and NF-κB by TNF-α**

To elucidate the mechanistic basis for the effect of IL-4 on TNF-α signaling in microglial cells, we evaluated the capacity of IL-4 to regulate the cell surface expression of TNFRs I and II. N9 microglial cells expressed both TNFRs I and II, with receptor II at a relatively higher level (Fig. 2). TNF-α time-dependently up-regulated both TNFRs I and II on the cell surface of microglia (Fig. 2). In contrast, IL-4 did not cause significant changes in the expression of TNFRs, suggesting that IL-4 might interfere with the signaling cascade down stream of TNF-α receptors in microglial cells, thereby attenuating the induction of mFPR2. The requirement of MAPKs, p38, and ERK1/2 in particular has been well documented for TNF-α activation of mononuclear phagocytes (12, 19, 20). We therefore evaluated whether MAPKs might be potential targets for IL-4 to disrupt the TNF-α signaling cascade in microglial cells. Fig. 3A shows that TNF-α induced a rapid and transient phosphorylation of p38 and ERK1/2 MAPKs in microglial cells (16), and although IL-4 was inactive in enhancing the phosphorylation of MAPKs (Fig. 3A), it significantly reduced the levels of p38 and ERK1/2 phosphorylation stimulated by TNF-α. In addition, IL-4 delayed the time of p38 phosphorylation induced by TNF-α (Fig. 3A). Although IL-4 reduced the phosphorylation of both p38 and ERK1/2 in microglia stimulated by TNF-α, its effect on p38 appeared to be critical for attenuation of mFPR2 expression, since the p38 MAPK inhibitor SB202190, but not the MEK1/2 inhibitor PD98059, decreased TNF-α-induced expression of mFPR2 mRNA (Fig. 3B and Ref. 12).

Activation of the transcription factor NF-κB is the hallmark for the proinflammatory activity of TNF-α (21), and the 5’ flanking region of the mFPR2 gene contains a consensus NF-κB motif (22). We observed that a selective inhibitor of TNF-α- inducible IκBα phosphorylation, BAY 11-7082, blocked TNF-α-induced expression of mFPR2 (Fig. 3B). Furthermore, IL-4 significantly decreased the levels NF-κB-driven luciferase activity and the expression of mFPR2 mRNA in N9 cells stimulated by TNF-α (Fig. 4A). IL-4 also abolished TNF-α induced the activity of AP-1 luciferase reporter in N9 cells stimulated by TNF-α (Fig. 4B). These results indicate that IL-4 disrupts the TNF-α signaling pathway in microglial cells by attenuating activation of MAPKs and important transcription factors which control the expression genes coding for proinflammatory proteins.

**Utilization of phosphatase, but not of Stat6, by IL-4**

Stimulation of the IL-4 receptor complex results in activation of multiple signaling pathways, one of which involves Stat6 (23). We then examined the effect of IL-4 on primary microglial cells isolated from Stat6-deficient (Stat6−/−) mice. In Stat6−/− microglia activated by TNF-α, IL-4 inhibited mFPR2-mediated chemotactic responses equally as well as in wild-type mice (Fig. 5, A and B).
Moreover, TNF-α significantly enhanced mFPR2 mRNA expression in Stat6/−/− microglia and the effect of TNF-α was markedly inhibited by pre-exposure of the cells to IL-4 (Fig. 5C), suggesting that Stat6 is not required for IL-4 to interfere with TNF-α signaling in microglial cells.

To further elucidate the nature of the molecules used by IL-4 to disrupt TNF-α signaling in microglial cells, we evaluated the capacity of IL-4 to activate phosphatase PP2A, which is okadaic acid (OA) sensitive and has been implicated in the dephosphorylation and deactivation of MAPKs. We found that IL-4 rapidly (within 5 min) increased the levels of activated PP2A in microglia (Fig. 6). In contrast, TNF-α only slightly increased the levels of PP2A (Fig. 6A) at late stages of stimulation (15–30 min). In the presence of both TNF-α and IL-4, the peak levels of PP2A appeared at 5 min, indicating that IL-4 accelerated and enhanced the activation of PP2A induced by TNF-α alone (Fig. 6A). In parallel experiments, IL-4 exhibited a similar capacity to modify PP2A activation induced by LPS (Fig. 6B) in association with its attenuation of TLR4 signaling in microglial cells. Fig. 7A shows that although treatment of microglial cells with OA alone did not induce activation of p38, the presence of OA prevented the inhibitory effect of IL-4 on TNF-α-induced p38 phosphorylation. We additionally used PP2A siRNA to “knock down” the expression of PP2A in N9 cells and examined the cell response to IL-4. Transfection of PP2A siRNA decreased the levels of PP2A mRNA (Fig. 7B) in N9 cells and completely reversed the inhibitory effect of IL-4 on mFPR2-mediated cell migration in TNF-α-activated microglia (Fig. 7C). These results indicate that PP2A plays a key role in the “blockade” of TNF-α by IL-4.

Discussion

In this study, we have shown that IL-4 was capable of attenuating the TNF-α-induced signaling cascade and the resultant functional expression of the chemotactic receptor mFPR2 in microglial cells. We additionally demonstrated that IL-4 inhibits TNF-α-induced activation of MAPK and transcription factors. Furthermore, our study revealed that up-regulation of the phosphatase PP2A, in a manner independent of Stat6, may play an essential role in IL-4-mediated attenuation of the TNF-α signaling cascade in microglial cells and the resultant expression mFPR2, the mouse homolog of human FPRL1, a functional receptor for the AD-associated Aβ42 peptide.

Microglia plays a critical role in CNS diseases. In fact, activation of microglia is an essential component in the pathogenesis of AD, Parkinson’s disease (24), multiple sclerosis, AIDS dementia (25), and brain trauma caused by stroke (26). Our previous studies showed that murine microglial cells in the resting state express very low levels of mFPR2 (11), a receptor that recognizes a diverse array of chemotactic agonists, including not only Aβ42 but also the...
bacterial formyl peptide fMLF, HIV-1 envelope protein-derived peptides, and a neuroprotective peptide humanin (8, 10, 11). When stimulated with LPS or TNF-α, microglial cells expressed high levels of mFPR2 transcripts and became responsive to mFPR2-specific agonists (11, 12), suggesting the presence of proinflammatory stimuli may promote microglial expression of mFPR2 and the subsequent cell responses in CNS diseases in which agonists for mFPR2 are elevated (27, 28). This is in agreement with the notion that proinflammatory 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 Aβ peptides trigger the release of neurotoxins (8). Therefore, anti-inflammatory strategy has been used as one of the therapeutic approaches to AD. This notion was supported by studies in which selected non-steroidal anti-inflammatory drugs were documented as beneficial in retarding the onset of AD dementia by inhibiting microglial cell responses to amyloid peptides as well as by reducing the production of Aβ peptides by neuronal cells (29, 30).

IL-4 has been reported to inhibit cytokine production by LPS-activated macrophages and microglia (17, 18). In support of these observations, we found that IL-4 potently reduced the production of IL-6 by LPS-stimulated microglia (data not shown). However, we could not detect increased production of major pro- or anti-inflammatory cytokines by TNF-activated microglia in the presence or the absence of IL-4, and RNase protection assays and RT-PCR failed to identify increased cytokine transcripts in microglial cells stimulated for up to 12 h by either IL-4, TNF-α, or both (data not shown). Therefore, neither TNF-α nor IL-5 appear to be significant inducers of cytokines in microglial cells under our experimental condition. We however found that IL-4 potently enhanced the levels of transcription factor peroxisome proliferator-activated receptors (PPAR)γ in microglial cells, which is in agreement with observations with macrophages (31). PPARγ and its ligands are important regulators of immune and inflammatory responses (32) by inhibiting genes coding for proinflammatory proteins, presumably through NF-κB and AP-1 family members. In microglial cells, activation of PPARγ has been reported to retain cells in a quiescent phenotype, suggesting its potentially protective role in neuroinflammation (33, 34). Whether IL-4-induced PPARγ participates in reduction of TNF-α-induced mFPR2 in microglial cells is currently unclear and merits further investigation.

Our present study revealing the capacity of the type of cytokine IL-4 to “disrupt” TNF-α signaling in microglial cells and inhibit the expression of a chemoattractant receptor for Aβ 42 and other...
peptide agonists associated with infectious diseases suggests yet another anti-inflammatory strategy with a host-derived cytokine. Although a number of anti-inflammatory activities have been reported for IL-4 on activated microglia, including inhibition of the expression of cyclooxygenase 2, inducible NO synthase, and TNF-α as well as other proinflammatory cytokines (17, 18), the mechanistic basis for the inhibitory effect of IL-4 on the microglial response to proinflammatory molecules such as TNF-α has not been well defined. In our study, the p38 MAPK inhibitor SB202190 markedly diminished mFPR2 expression induced by TNF-α in microglial cells, indicating a key role of this MAPK in the TNF-α-mediated signaling cascade.

A large body of studies suggests that PP2A plays a major role in down-regulation of the ERK MAPK pathway and probably acts at multiple points in the signal transduction cascade. In vitro, PP2A dephosphorylates and inactivates MEK1 and ERK family kinases (35, 36), and the activity of both kinases is restored after treatment of cells with the PP2A inhibitor OA (37, 38). It has been reported that inhibition of PP2A by interaction with SV40 small t-Ag resulted in enhanced activation of the stress-activated MAPK pathways in association with increased tumor cell growth and invasiveness (39). Phosphatases are activated by a number of cellular stimulants (40, 41). For instance, IL-4 was also shown to inhibit CD40 ligand-induced activation of MAPK in macrophages (42), and the effect of IL-4 was attributed to its potential activation of phosphatases. In our study, using OA as “knocking down” the expression of PP2A by siRNA in microglial cells reversed the inhibitory effect of IL-4 on TNF-α signaling, providing strong evidence for the importance of PP2A in the capacity of IL-4 to disrupt TNF-α-induced microglia activation. In support of this notion, IL-4 significantly increased the levels of the catalytic PP2A protein in microglial cells. Moreover, IL-4-induced PP2A may also play a major role in IL-4-mediated inhibition of LPS signaling via TLR4 in microglial cells (Ref. 15 and Fig. 6B). It has been suggested that OA-sensitive protein phosphatases PP1/PP2A may cause rapid dephosphorylation of MAPKs induced by proinflammatory stimulants to maintain a balanced cell response in stress conditions (18, 34). Thus, IL-4 may provide protection against deleterious effects of proinflammatory stimuli in the CNS by maintaining a balanced microglial cell response through activation of phosphatase PP2A in particular. Further elucidation of the mechanisms by which IL-4 protects microglial cells should be beneficial for the design of therapeutic approaches to neurodegenerative diseases in which inflammatory responses exacerbate the pathogenic processes.

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**Disclosures**

The authors have no financial conflict of interest.
References

1. Selkoe, D. J. 1999. Translating cell biology into therapeutic advances in Alzhei-

2. Eikelenboom, P., J. M. Rozemuller, and F. L. van Muiswinkel. 1998. Inflamma-
tion and Alzheimer’s disease: relationships between pathogenic mechanisms and

H. E. Genselmann. 1999. Insights into the neurodegenerative process of Alzhei-
mer’s disease: a role for mononuclear phagocyte-associated inflammation and


peptide receptor like 1 (FPRL1/MFPR2) in mononuclear phagocyte responses in


formyl peptide receptor-like 1 (FPRL1) in inflammatory aspects of Alzheimer’s

8. Tiffany, H. L., M. C. Lavigne, Y. H. Cui, J. M. Wang, T. L. Leto, J. L. Gao, and
P. M. Murphy. 2001. Amyloid-β induces chemotaxis and oxidant stress by acting
at formylpeptide receptor 2, a G protein-coupled receptor expressed in phago-

C. C. Li, and J. M. Wang. 2001. β amyloid peptide (Aβ1-42) is internalized via the
G-protein coupled receptor FPRL1 and forms fibrillar aggregates in macroph-

10. Ying, G., P. Irinbarren, Y. Zhou, W. Gong, N. Zhang, Z. X. Yu, Y. Le, Y. Cui,
and J. M. Wang. 2004. Humanin, a newly identified neuroprotective factor, uses the
G-protein coupled formylpeptide receptor-like-1 as a functional receptor. J.

11. Cui, Y., Y., Y. Le, W. Gong, P. Proost, J. Van Damme, W. J. Murphy, and
J. M. Wang. 2002. Bacterial lipopolysaccharide selectively up-regulates the func-
tion of the chemotactic peptide receptor formyl peptide receptor 2 in murine

12. Cui, Y., Y. Le, X. Zhang, W. Gong, K. Abe, R. Sun, J. Van Damme, P. Proost,
and J. M. Wang. 2002. Up-regulation of FPRL2, a chemotactic receptor for amy-
loid β 1-42 (Aβ1-42), in murine microglial cells by TNFa. Neurobiol. Dis. 10:

23–35.

Biol. 73: 209–212.

15. Abbas, N., I. Bednar, E. Mix, S. Marie, D. Paterson, A. Ljungberg, C. Morris,
W. Jungblut, C. Thomas, A. Ljungberg, C. Morris, D. Paterson, A. Ljungberg,
and C. Morris. 2003. The role of formyl peptide receptor like 1 (FPRL1/MFPR2)
in mononuclear phagocyte responses in Alzheimer’s disease. Immunol. Res. 31:
165–176.