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Induction of the Formyl Peptide Receptor 2 in Microglia by IFN-γ and Synergy with CD40 Ligand

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Human formyl peptide receptor (FPR)-like 1 (FPR1) and its mouse homologue mFPR2 are functional receptors for a variety of exogenous and host-derived chemotactic peptides, including amyloid β 1–42 (Aβ_{42}), a pathogenic factor in Alzheimer’s disease. Because mFPR2 in microglial cells is regulated by proinflammatory stimuli including TLR agonists, in this study we investigated the capacity of IFN-γ and the CD40 ligand (CD40L) to affect the expression and function of mFPR2. We found that IFN-γ, when used alone, induced mFPR2 mRNA expression in a mouse microglial cell line and primary microglial cells in association with increased cell migration in response to mFPR2 agonists, including Aβ_{42}. IFN-γ also increased the endocytosis of Aβ_{42} by microglial cells via mFPR2. The effect of IFN-γ on mFPR2 expression in microglial cells was dependent on activation of MAPK and 1xB-α. IFN-γ additionally increased the expression of CD40 by microglial cells and soluble CD40L significantly promoted cell responses to IFN-γ during a 6-h incubation period by enhancing the activation of MAPK and 1xB-α signaling pathways. We additionally found that the effect of IFN-γ and its synergy with CD40L on mFPR2 expression in microglia was mediated in part by TNF-α. Our results suggest that IFN-γ and CD40L, two host-derived factors with increased concentrations in inflammatory central nervous system diseases, may profoundly affect microglial cell responses in the pathogenic process in which mFPR2 agonist peptides are elevated. The Journal of Immunology, 2007, 178: 1759–1766.

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of IFN-γ and other proinflammatory cytokines is particularly high in old age (24, 25). In addition, in aged human and rodent brains the level of constitutively produced IFN-γ is significantly higher than in the younger counterparts (26). Therefore, IFN-γ is not only a potent activator of microglial cells and plays an important role in inflammatory CNS diseases but also is also considered a risk factor for AD (27).

To further elucidate the role of IFN-γ in CNS inflammation and immune responses, in this study we investigated the capacity of this Th1 cytokine to regulate the expression and function of mFPR2 in microglia. We report that microglial cells stimulated with IFN-γ expressed high levels of mFPR2 and migrate in response to a variety of mFPR2 agonist peptides including AB42. We additionally observed that IFN-γ treatment enhanced the capacity of microglial cells to uptake AB42 through mFPR2. Furthermore, IFN-γ up-regulated the expression of CD40 on the microglial cell surface, and soluble CD40 ligand (CD40L) markedly increased the effect of IFN-γ on the expression of mFPR2. Our study suggests that IFN-γ and CD40 may profoundly affect microglial cell responses in the pathogenic process of proinflammatory CNS diseases in which mFPR2 agonist peptides are elevated.

Materials and Methods

Reagents and cells

Recombinant murine IFN-γ and recombinant murine soluble (s) CD40L were purchased from PeproTech. A neutralizing anti-TNF-α Ab and iso-type Ab rat IgG isotype were purchased from Endogen. FMLP and LPS were purchased from Sigma-Aldrich. 5-Bromo-2′-deoxyuridine (BrdU) and Brefeldin A were obtained from Calbiochem. The AB42 peptide was from California Peptide Research. Abs specific for total ERK1/2, ERK1/2 phosphorylated (p)- at Tyr202 (p-ERK), total p38 MAPK, p-p38 MAPK, total IκBα and p-IκBα, total Akt, and Akt phosphorylated at Ser473 (p-Akt) were purchased from Cell Signaling Technology. The murine microglial cell line N9 was a gift from Dr. P. Ricciardi-Castagnoli (Università Degli Studi di Milano-Bicocca, Milan, Italy) and was grown in IMDM supplemented with 5% heat-inactivated FCS, 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 50 μM 2-ME. Primary murine microglial cells were isolated from 1-day-old newborn C57BL/6 mice and grown in DMEM supplemented with 10% heat-inactivated FCS, 100 U/ml penicillin, 100 μg/ml streptomycin, 1 M HEPES, 2.5 μg/ml Fungizone, 100 μM nontoxic amino acids, and 5 μg/ml insulin. N9 cells grown to 4 × 10^6 cells per 25-cm² flask were used for experiments except for Western immunoblotting, in which 1 × 10^6 cells/well cultured on 6-well plates were used. Primary microglial cells isolated from newborn mice were cultured at 1.0 × 10^6 cells/ml in 5-mL polypropylene round-bottom tubes (BD Labware) for experiments.

Chemotaxis assays

Chemotaxis assays for microglial cells were performed with 48-well chemotaxis chambers and polycarbonate filters (8-μm pore size) (NeuroProbe) as described (15, 28). The results are expressed as the mean ± SD of the chemotaxis index (CI), which represents the fold increase in the number of migrated cells counted in three high-power fields (× 400) in response to chemotacticants over spontaneous cell migration (to control medium).

RT-PCR

Total RNA was extracted from cells with a RNeasy mini kit and depleted of contaminating DNA with RNase-free DNase (Qiagen). For amplification of the mFPR2 gene, primers 5′-TCTACCATCTCCAGAGTTCTGTTG (sense) and 5′-ATTTCCC (antisense), which yield a product of 514 bp. Mouse CD40 primers 5′-TGTGATGGTGGGAATGGGTCA (sense) and 5′-TTTGATGTGACGCACGACAGCCGATTCCCC (antisense), which yield a product of 514 bp. Mouse CD40 primers were CGCATTGGGCTGTGGTGTGGACACG (sense) and GACGGTGATCAGTGCTGGTGGTGTGGACACG (antisense), which yield a product of 400 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 (45 s), and extension at 72°C (1 min) with a final extension for 10 min at 72°C. All PCR products were resolved by 1.5% agarose gel electrophoresis and visualized with ethidium bromide. For quantitation, gels were scanned and the pixel intensity for each band was determined using the ImageJ program (NIH Image) and normalized to the amount of β-actin.

Fluorescence confocal microscopy

N9 cells were seeded at 2.8 × 10^5 cells/well on eight-well chamber slides (Nalge Nunc International) for 24 h. The cells were then treated at 37°C with IFN-γ or CD40L alone or both in combination for 24 h. Activated N9 cells were further treated in the presence or absence of G protein receptor de-activator pertussis toxin (PTX) for 1 h followed by AB42 (50 μg/ml) for 30 min. The cells were fixed in 2% paraformaldehyde for 20 min at room temperature, washed with PBS, and incubated with 5% normal goat serum (Sigma-Aldrich) in PBS plus 0.05% Tween 20 for 1 h to reduce nonspecific binding of Abs to the cell surface and for cell permeabilization. An anti-AB42 Ab (Sigma-Aldrich) 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 (BD Pharmingen) in TBS containing 1% BSA for 60 min. After three washes with PBS, the slides were stained with propidium iodide (PI) for 20 min 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 LSM510 NLO Meta). Excitation wavelengths of 488 nm (for FITC) and 561 nm (for PI) were used to generate fluorescence emission in green (for AB42) and red (for nuclei), respectively. The intensity of green fluorescence detected for AB42 was analyzed with ImageJ (NIH software).

Flow cytometry

N9 cells or primary mouse microglial cells stimulated with IFN-γ alone or in combination with CD40L were examined for the expression of CD40 by labeling with PE-conjugated mAbs (BD Pharmingen). All staining procedures were completed at 4°C in Dulbecco’s PBS containing 5 mM EDTA and 1% FCS. After extensive washing, the cells were analyzed using a FACScan flow cytometer (BD Biosciences).

Western immunoblotting

N9 cells grown to 1 × 10^6 cells/well in 6-well plates were cultured overnight in FCS-free medium and stimulated with 10 ng/ml IFN-γ and 1 μg/ml CD40L, each in combination. LPS (300 ng/ml) was used as a positive control. The cells were lysed with 1 × SDS sample buffer (62.5 mM Tris-HCl (pH 6.8), 2% SDS, 10% glycerol, and 50 mM DTT), sonicated for 15 s, and then heated at 100°C for 5 min. The cell lysate was

![FIGURE 1.](http://www.jimmunol.org/)

Up-regulation of mFPR2 mRNA in murine microglial cells by IFN-γ. N9 cells (4.0 × 10^5 cells per 25-cm² flask) were incubated with various concentrations of IFN-γ for 24 h (A) or with IFN-γ at 10 ng/ml for the indicated time intervals, and cells treated with LPS (300 ng/ml) for 36 h were used as a control (B). Total RNA was extracted and examined for mFPR2 mRNA expression by RT-PCR. C. Primary microglial cells (1.0 × 10^6 cells/ml) were stimulated with 10 ng/ml IFN-γ for 24 h and the mRNA was examined for mRNA of mFPR2. The RT-PCR products were electrophoresed on a 1.5% agarose gel and visualized with ethidium bromide staining. The density of product bands was measured by ImageJ (NIH software) with normalization against β-actin bands. *Significantly increased cell migration (p < 0.05) as compared with medium control.
FIGURE 2. mFPR2 agonist-induced chemotaxis of microglial cells stimulated by IFN-γ. N9 cells (4.0 × 10^6 cells per 25-cm² flask) (A and B) or primary mouse microglial cells (1.0 × 10^6 cells/ml) (C and D) were pretreated with 10 ng/ml IFN-γ for 24 h. The cells were examined for migration in response to IMLF (10⁻⁵ M) or Aβ42 (50 μg/ml). E, LPS (100 ng/ml) or IFN-γ (10 ng/ml) preincubated with polymyxin B (PolyB; 10 μg/ml) for 1 h at 37°C was used to stimulate N9 cells for 24 h at 37°C, and the cells were then examined for migration in response to IMLF (10⁻⁵ M). The results are expressed as the CI, representing the fold increase in cell migration in response to chemoattractants vs medium control. *: Significantly increased cell migration (p < 0.05) as compared with medium control (A–D); #: significantly reduced cell responses (p < 0.05) as compared with cells treated with LPS alone (E).

centrifuged at 12,000 rpm (4°C) for 5 min, and the protein concentration of the supernatant was measured by the Micro BCA protein assay system (Pierce). Proteins (50 μg for each sample) were resolved by 10% SDS-PAGE (Invitrogen Life Technologies) and transferred onto nitrocellulose membranes (Bio-Rad). The membranes were blocked for 2 h at room temperature in 3% nonfat milk prepared in Tris-buffered saline-T (10 mM Tris-HCl (pH 7.5), 150 mM NaCl, and 0.1% Tween 20) and probed with primary mouse microglial cells (1.0 × 10⁶ cells/ml) (Fig. 2, A and B). Similar results were obtained with primary murine microglial cells (Fig. 2, C and D).

To ascertain the purity of IFN-γ, we tested polymyxin B, an LPS inhibitor, and found that it did not affect the capacity of IFN-γ to induce the expression of functional mFPR2 in microglial cells. In contrast, polymyxin B inhibited the effect of LPS on mFPR2 induction (Fig. 2E). Thus, the capacity of IFN-γ to induce mFPR2 in mouse microglial cells was not dependent on contaminating LPS.

Involvement of MAPK in the up-regulation of mFPR2 in microglial cells by IFN-γ

Because it has been reported that IFN-γ activates MAPKs and NF-κB in microglial cells (29, 30), we assessed their role in IFN-γ.
induction of mFPR2. Fig. 3 shows that the level of mFPR2 mRNA induced by IFN-γ/H9253 was significantly reduced in microglial cells when the cells were pretreated with SB202190, a p38 MAPK inhibitor, the MEK-ERK1/2 inhibitor PD98059, or the highly selective IκB-α phosphorylation inhibitor BAY117082 (Fig. 3, A–C). This was accompanied by attenuated cell migration in response to mFPR2 agonist (Fig. 3, D–F). These results suggest that p38 and ERK1/2 MAPKs as well as NF-κB are important mediators for IFN-γ to induce the expression of functional mFPR2 in microglial cells.

FIGURE 4. Up-regulation of CD40 in microglial cells by IFN-γ. A and B, N9 cells (4.0 × 10^6 cells per 25-cm² flask) were stimulated with 10 ng/ml IFN-γ, 1 μg/ml CD40L, or in combination for 3–6 h and then examined for the mRNA of mouse CD40. The RT-PCR products were electrophoresed and visualized with ethidium bromide. The density of the RT-PCR product bands was measured by ImageJ (NIH software) with normalization against β-actin bands. *, Significantly increased mCD40 mRNA (p < 0.05) as compared with medium control; #, significantly increased mCD40 mRNA (p < 0.05) as compared with cells treated with IFN-γ or CD40L alone for 3 h (A). Murine primary microglial cells (1.0 × 10^6 cells/ml) were stimulated with 10 ng/ml IFN-γ, 1 μg/ml CD40L, or in combination for 6 h and then examined for surface expression of CD40 by flow cytometry (B). C, N9 cells (4.0 × 10^6 cells per 25-cm² flask) cultured in the presence or absent of IFN-γ (10 ng/ml) for the indicated time intervals were examined for surface expression of CD40 by flow cytometry. The results are presented as mean fluorescence intensity (MFI) and percentage of positive cells in histograms.

FIGURE 5. Synergistic effect of IFN-γ and CD40L on induction of mFPR2 mRNA in microglial cells. A and B, N9 cells (4.0 × 10^6 cells per 25-cm² flask) were stimulated in the presence or absence of IFN-γ (10 ng/ml), CD40L (1 μg/ml), or LPS (300 ng/ml) for 24 h (A) or for different time periods (B). C, Mouse primary microglial cells were incubated with IFN-γ (10 ng/ml), CD40L (1 μg/ml), or in combination for 6 h. Total RNA of the cells was extracted and examined for mFPR2 mRNA by RT-PCR. The RT-PCR products were electrophoresed and visualized with ethidium bromide. The density of the RT-PCR product bands was measured by ImageJ (NIH software) and normalized against β-actin. *, Significantly increased mFPR2 mRNA (p < 0.05) as compared with cells treated with IFN-γ alone. D, N9 cells (4.0 × 10^6 cells per 25-cm² flask) treated with IFN-γ (10 ng/ml) for a total of 6 h in the presence of CD40L for different times (hours) followed by the measurement of mFPR2 mRNA expression with RT-PCR. E, Mouse primary microglial cells (1.0 × 10^6/ml) incubated with IFN-γ (10 ng/ml), CD40L (1 μg/ml), or in combination for 6 h were examined for migration in response to fMLF (10^-5 M). The results are expressed as a CI representing the fold increase in cell migration in response to chemoattractants vs medium control. *, Significantly increased cell migration (p < 0.05) as compared with cells treated in the absence of cytokines; #, significantly increased cell migration (p < 0.05) as compared with cells treated with IFN-γ alone.
IFN-γ and CD40L synergistically increase CD40 expression by microglia

Because IFN-γ has been reported to increase the expression of CD40 by microglial cells, which, in turn, are further activated by CD40 to produce proinflammatory cytokines, we examined whether CD40 participates in the regulation of mFPR2. We found that, consistent with the previously published results (31–34), nonstimulated N9 microglial cells expressed low levels of CD40 mRNA and cell surface CD40 protein, but the expression was progressively increased after stimulation with IFN-γ as well as CD40L. As shown in Fig. 4, CD40 mRNA was rapidly up-regulated by IFN-γ or CD40L alone at 3 h after stimulation (Fig. 4A). The effect was pronounced when IFN-γ and CD40L were used in combination. This was accompanied by an up-regulated cell surface expression of CD40 (Fig. 4B). The effect of IFN-γ alone was delayed and a significantly up-regulated cell surface CD40 was seen at 12 h (Fig. 4C). These results suggest that although CD40L and IFN-γ each enhances the expression of CD40 in microglial cells, their combination results in a more rapid up-regulation. In contrast, unlike CD40L, the expression of CD14 in microglial cells was not changed by treatment with IFN-γ or CD40L at the time points tested in this study (data not shown), indicating their preferential regulatory activity.

CD40L and IFN-γ synergistically promote the expression and function of mFPR2 in microglial cells

We then examined the function of CD40 expressed by IFN-γ stimulated microglial cells. Fig. 5A shows cross-linking of the low levels of CD40 on nonstimulated microglia by its soluble ligand (CD40L) did not induce the expression of mFPR2 mRNA, nor did CD40L treatment promote the chemotaxis responses of the cells to mFPR2 agonist peptides. However, CD40L markedly enhanced the expression of mFPR2 by microglial cells incubated with IFN-γ for 6 h, when the effect of IFN-γ on mFPR2 gene expression was at a low level (Fig. 5B). The same results were observed in experiments with primary mouse microglial cells (Fig. 5C). We also found that pretreatment of microglial cells with IFN-γ for 2 h followed by CD40L for 4 h was sufficient to significantly enhance the expression of mFPR2 in microglial cells (Fig. 5D), in association with increased cell chemotaxis (Fig. 5E).

CD40L and IFN-γ synergistically promote the uptake of Aβ42 peptide by microglia through mFPR2

As our previous studies showed that microglial cells activated by TLR agonists increased their capacity to uptake Aβ42 peptides via mFPR2 (14, 15), we therefore investigated whether cells treated by IFN-γ in the presence or absence of CD40L also might increase Aβ42 ingestion. Fig. 6 shows that N9 microglial cells treated with IFN-γ alone increased their capacity to endocytose Aβ42 peptide as measured by confocal microscopy (Fig. 6A). In contrast, whereas CD40L alone slightly increased the levels of Aβ12 fluorescent in microglial cells, its combination with IFN-γ resulted in a markedly enhanced Aβ12 localization in the cytoplasmic compartment of microglial cells as shown by significantly increased values of the fluorescence intensity (FI) for cell-associated Aβ12 and increased percentage of Aβ12-positive cells (Fig. 6, B and C).
The results were confirmed with primary microglial cells (Fig. 6, D and E). The ingestion of \( \Delta \beta_{32} \) by microglial cells stimulated with IFN-\( \gamma \) or in combination with CD40L was significantly inhibited by PTX, an inhibitor of Go, protein-coupled receptors, or by another mFPR2 agonist, W pep (Fig. 6 and data not shown), suggesting that mFPR2 is involved in \( \Delta \beta_{32} \) uptake by microglia activated by IFN-\( \gamma \) alone or in combination with CD40L. Interestingly, a slightly increased \( \Delta \beta_{32} \) uptake by microglial cells treated with CD40L alone was not inhibited by PTX, suggesting that CD40L may increase the expression of other putative \( \Delta \beta_{32} \) binding molecules that might weakly assist in cell uptake of \( \Delta \beta_{32} \) (35).

**Enhanced activation of MAPKs and I\( \kappa \)B-\( \alpha \) in microglial cells stimulated by IFN-\( \gamma \) and CD40L**

To elucidate the mechanistic basis for the synergistic effect of CD40L and IFN-\( \gamma \) on the induction of mFPR2 in microglial cells, we evaluated the activation of signaling pathways involving MAPKs and I\( \kappa \)B-\( \alpha \), a key molecule controlling the activation of NF-\( \kappa \)B. In N9 cells treated with IFN-\( \gamma \) alone, phosphorylation of p38 was detected at 1 h, whereas activated ERK1/2 and I\( \kappa \)B-\( \alpha \) were detected at 6 h (Fig. 7A). IFN-\( \gamma \) did not induce detectable levels of phosphorylated JNK in microglial cells (data not shown). In contrast, microglial cells treated with CD40L alone showed detectable phosphorylation of p38, ERK1/2, and I\( \kappa \)B at 1 h. Furthermore, microglial cells pretreated with IFN-\( \gamma \) for 3 h followed by stimulation with CD40L showed a more rapid phosphorylation of p38, ERK1/2 (15 min), and I\( \kappa \)B-\( \alpha \) (30 min) (Fig. 7B). It is interesting to note a unique pattern of phosphorylated vs nonphosphorylated I\( \kappa \)B-\( \alpha \) species in microglial cell stimulated by both IFN-\( \gamma \) and CD40L, presumably caused by the rapid degradation and de novo synthesis of the I\( \kappa \)B-\( \alpha \) species. These results indicate that IFN-\( \gamma \) and CD40L synergistically activate important signaling molecules involved in promoting the expression of mFPR2 in microglial cells.

**Involvement of TNF-\( \alpha \) in the induction of mFPR2 by IFN-\( \gamma \) and CD40L**

Because IFN-\( \gamma \) has been reported to induce TNF-\( \alpha \) expression in a variety of cell types including microglia and, more importantly, since our previous study showed that TNF-\( \alpha \) (18, 36–38) is a potent inducer of mFPR2 in microglial cells (16), we assessed whether TNF-\( \alpha \) is involved in the effect of IFN-\( \gamma \) on mFPR2 expression. We found that a TNF-\( \alpha \)-neutralizing Ab reduced the level of mFPR2 expression in both a microglial cell line (29 ± 4%) and in primary microglial cells (70 ± 3%) stimulated by IFN-\( \gamma \) (Fig. 8, A and B). The anti-TNF-\( \alpha \) Ab also partially inhibited the synergistic induction of mFPR2 by IFN-\( \gamma \) in combination with CD40L (37 ± 4%) (Fig. 8C). These results confirmed the previously reported capacity of IFN-\( \gamma \) to induce the production of TNF-\( \alpha \) by microglial cells and indicate that TNF-\( \alpha \) contributes to the induction of mFPR2 by IFN-\( \gamma \) and its synergy with CD40L in microglial cells.

**Discussion**

In this study, we identified IFN-\( \gamma \) as an inducer of functional mFPR2 in murine microglial cells. The effect of IFN-\( \gamma \) is further increased by cross-linking CD40, which is up-regulated on the cell surface of IFN-\( \gamma \)-activated microglial cells. This is the first demonstration of the synergistic regulation by two host-derived factors of the G protein-coupled receptor mFPR2 in microglial cells. As a
increased levels of IFN-γ is a classical T and NK cell cytokine produced mainly in the peripheral tissues during inflammatory and immune responses, its immunoreactivity and gene expression have been detected in human sensory neurons in the CNS (22). In addition, it has been reported that human glial cells and rat astrocytes have the capacity to produce IFN-γ after stimulation with proinflammatory cytokines (20, 21, 37). Alternatively, under proinflammatory and immunological conditions in the CNS, T lymphocytes, which are capable of crossing the blood-brain barrier (BBB), may become a major source of IFN-γ. In fact, multiple pathogenic factors are capable of disrupting the integrity of BBB, allowing for the entry of T cells to the brain parenchyma in response to locally produced chemoattractants, mainly chemokines produced by activated astrocytes and microglial cells (27, 39). It is interesting to note that increased levels of IFN-γ are detected in the hippocampus of aged rodents (26) as well as in the CNS of senior normal human subjects, who comprise the principal risk population that succumbs to neurodegenerative diseases, in particular AD (24, 27, 40). Thus, under pathophysiological and the aging process, IFN-γ is readily available in the CNS as an inducer of mFPR2, which mediates the chemotactically microglial cells to multiple microbial and host-derived chemotactic agonist peptides, including AD-associated Aβ12 (5, 9, 41).

In our study, a relatively weaker effect of IFN-γ on the induction of mFPR2 in microglial cells at early stages (6 h) of incubation was markedly increased by the soluble ligand for CD40. In non-stimulated microglial cells, CD40L showed little activity on the expression of mFPR2, which may be attributable to the low level of expression of CD40 by such cells. In contrast, IFN-γ enhanced surface expression of CD40 by microglial cells, which then became responsive to CD40L by further increasing mFPR2 at both mRNA and functional levels. Consequently, IFN-γ and CD40L synergistically promoted the microglial cell chemotaxis to Aβ12 peptides and their internalization via mFPR2.

The CD40/CD40L dyad mediates a broad spectrum of inflammatory and immune responses in diseases including AD (42–44). In a coculture model of primary neurons and microglia, CD40 cross-linking by soluble CD40L or an anti-CD40 Ab potently promoted the neurotoxicity of microglial cells induced by Aβ via the production of proinflammatory mediators (45). Interestingly, in this model, microglial cells were incubated in the presence of IFN-γ, suggesting its “priming” effect on cell responses to CD40L. This is in agreement with our present study in which CD40 only exhibited its activity on mFPR2 induction in microglial costimulated by IFN-γ. In addition to activated microglia, CD40 was also detected on microvascular in the brain of AD patients (46), suggesting that CD40 may play an important role in orchestrating inflammatory responses in CNS diseases. To support the biological relevance of CD40 in the CNS, CD40L has been detected immunocytochemically in astrocytes in the brains of aged human subjects and AD patients. Likewise, in the brains of mice carrying the mutant amyloid precursor protein and presenilin 1, transgenes that develop AD-like pathology, as well as in rat injury models (47, 48), CD40L was expressed by “reactive” astrocytes. Therefore, under inflammatory conditions, astrocytes, in addition to the T lymphocytes potentially infiltrating brain parenchyma due to breached BBB, provide essential signals for activation of CD40, which is up-regulated on activated microglial cells.

Our findings that IFN-γ alone induces mFPR2 in microglia and its synergism with CD40 cross-linking may have important pathophysiological significance in the disease process of AD. In the AD brain, microglia accumulate at plaque lesions containing high levels of Aβ12 (5, 9). Although microglial cells are believed to mediate the “indirect” neurotoxicity of Aβ peptides by secreting toxins, they may play an essential role in phagocytosing and processing Aβ peptides (14, 15, 49–52). In culture, microglia isolated from human AD brains migrate to aggregated Aβ peptides and are capable of removing Aβ deposits. Consistent with this, cultured rat microglia interact with Aβ peptides, which become localized on the cell surface as well as in phagosome-like intracellular vesicles (53). In in vivo experiments, Aβ peptides injected into rat striatum are rapidly phagocytosed by microglia (50), followed by degradation and clearance (52). In mouse AD models, immunization with Aβ peptides (54, 55) or with Abs against Aβ (56) resulted in the reduction of Aβ deposits, apparently mediated by activated microglia. Thus, microglial uptake of Aβ peptides may represent a host defense to eliminate undesirable irritants in the CNS. However, microglial ingestion of Aβ peptides may also present the dilemma of a “double-edged” sword in that, as a consequence of prolonged exposure, Aβ peptides taken by human mononuclear phagocytes formed fibrillar aggregates in the cytoplasmic compartment and increased the apoptotic death of the cells (11, 12, 15). It is therefore possible that the capacity of microglial cells or brain macrophages to eliminate or to promote deposition of Aβ peptides may be determined by the Aβ peptide burden in the brain and the duration of cell exposure. In this context, a fine tuning of microglial cell responses in the inflammatory milieu of AD will be crucial for maximizing the beneficial host responses while minimizing the detrimental consequences that may favor the progression of the disease. Further studies are therefore warranted to more clearly elucidate the biological significance of IFN-γ and CD40 synergy in microglial activation in CNS diseases to facilitate the development of more effective therapeutic agents for AD and other inflammatory CNS diseases.

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