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Opposite Effects of Interferon-γ and Prostaglandin E_2 on Tumor Necrosis Factor and Interleukin-10 Production in Microglia: A Regulatory Loop Controlling Microglia Pro- and Anti-Inflammatory Activities

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Following brain injury, microglial cells produce pro- and anti-inflammatory cytokines, such as tumor necrosis factor (TNF) and interleukin-10 (IL-10). IL-10 provides an efficient autocrine mechanism for controlling microglia activation. To elucidate the mechanisms that regulate the cytokine profile of microglia, we examined the effects of several immunomodulators on IL-10 and TNF production by cultured mouse microglia. Lipopolysaccharide (LPS) was the only inducer of IL-10 and TNF gene expression and secretion. The T helper 1-type cytokine interferon-γ (IFN-γ) induced TNF transcripts, but not TNF secretion, and suppressed LPS-induced IL-10 mRNA and secretion by microglia. Opposite to IFN-γ, the lipid mediator prostaglandin E_2 (PGE_2) enhanced the LPS-induced production of IL-10 and inhibited that of TNF. The effects of PGE_2 on cytokine gene expression and secretion were antagonized by IFN-γ. Agents that increase cAMP levels mimicked the action of PGE_2 on cytokine secretion, indicating the involvement of cAMP-coupled prostaglandin receptors. In conclusion, IFN-γ and PGE_2, two mediators released at inflammatory sites, differentially regulate the production of a proinflammatory and an anti-inflammatory cytokine in microglia. We suggest that the activity and role of microglia in the damaged CNS could be finely tuned by the local concentration ratio of these mediators. J. Neurosci. Res. 56:571–580, 1999.

Key words: central nervous system; cytokines; glia; inflammation; prostanoids

INTRODUCTION

The central nervous system (CNS) is endowed with intrinsic immune cells, microglial cells, which belong to the monocyte/macrophage lineage. Microglial cells are in a resting state until they are activated by CNS trauma, infection, inflammation, or neuronal degeneration (Streit et al., 1988; Giulian, 1992; Kreutzberg, 1996).

The identification of the signals responsible for turning on and off microglia has long been considered an important issue for elucidating mechanisms of immune surveillance and homeostasis in the CNS and for developing pharmacological approaches for limiting CNS inflammation. Microbial components, signals originating from damaged neurons, and cytokines produced by CNS-infiltrating immune cells (e.g., interferon-γ (IFN-γ), TNF, colony-stimulating factors, chemokines) are all likely candidates for triggering microglia activation (Merrill and Benveniste, 1996; Benveniste, 1997; Harrison et al., 1998). On the other hand, a number of immunosuppressors and macrophage deactivators, such as transforming growth factor-β (TGF-β), interleukin-10 (IL-10), IL-4, and prostaglandin E_2 (PGE_2), have been implicated in limiting microglia activation and CNS inflammation (Bogdan and Nathan, 1993; Weissmann, 1993; Merrill and Benveniste, 1996; Minghetti and Levi, 1998). Factors released during neuronal activity (e.g., β-adrenergic agonists and neurotrophins) could also contribute to counteract the inducing effects of proinflammatory mediators on microglia cytokine secretion and major histocompatibility complex molecule expression (Hetier et al., 1991; Neumann et al., 1998).

TNF, a cytokine with proinflammatory, antimicrobial, and antitumoral activities, is produced by reactive microglia in most infectious and noninfectious CNS

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diseases (Tyor et al., 1992; Renno et al., 1995; Mattson et al., 1997). In vitro, microglial cells express TNF mRNA and/or secrete TNF after viral infection or exposure to lipopolysaccharide (LPS) and T cell-derived factors (Frei et al., 1987; Dhib-Jalbut et al., 1994; Renno et al., 1995; Benveniste, 1997). Blockade of TNF in experimental allergic encephalomyelitis (EAE), an animal model of CNS autoimmunity, and recent studies in mice lacking TNF or overexpressing TNF in the CNS support the idea that TNF has a critical role in the initiation and/or progression of the inflammatory processes leading to demyelination and neuronal degeneration (Probert et al., 1997; Körner et al., 1997; and references therein).

IL-10 is a 35-kDa cytokine with immunosuppressive and anti-inflammatory properties. Following brain trauma, local application of IL-10 decreases microglia activation and intracerebral TNF production (Balasingam and Yong, 1996). In vitro, IL-10 inhibits the ability of microglia to function as antigen-presenting cells (Frei et al., 1994; Mizuno et al., 1994; Menèndez-Iglesias et al., 1997) and to secrete IL-1, IL-6, IL-12, TNF, and colony-stimulating factors after stimulation with LPS (Frei et al., 1994; Mizuno et al., 1994; Chao et al., 1995; Aloisi et al., 1997). When overexpressed in transgenic mice (Bettelli et al., 1998), delivered directly to the CNS (Willenborg et al., 1995) or administered peripherally (Rott et al., 1994), IL-10 inhibits the development of EAE. Microglia, infiltrating macrophages, and T cells are all likely sources of IL-10 in the inflamed CNS (Merrill and Benveniste, 1996; Jander et al., 1998), and IL-10 is produced by microglia in vitro (Mizuno et al., 1994; Williams et al., 1996; Sheng et al., 1995). Microglia-derived IL-10 could downregulate CNS inflammation by acting both in an autocrine and paracrine fashion.

The present study was designed to investigate the effects of the prototype T helper (Th) 1 cytokine IFN-γ, a major macrophage stimulator, and of PGE_2, a lipid mediator with immunoregulatory and macrophage-deactivating properties, on the production of IL-10 and TNF by cultured mouse microglia. Since the levels of IFN-γ and PGE_2 are elevated in the brain and cerebrospinal fluid during neuroinflammatory diseases (Fretland, 1992; Olsson, 1995), we also examined whether these two mediators would reciprocally interact in regulating the profile of pro- and anti-inflammatory cytokines produced by activated microglia.

MATERIALS AND METHODS

Reagents

Mouse recombinant cytokines used in this study were: IFN-γ (specific activity, 1.1×10^7 U/mg), IL-1α (specific activity, 3.7×10^9 U/mg), IL-1β (specific activity, 4.55×10^9 U/mg), IL-6 (specific activity, 1×10^9 U/mg), leukemia inhibitory factor (LIF, specific activity, 1×10^8 U/mg), and TNF (specific activity, 4×10^7–4×10^9 U/mg), from Genzyme, Cambridge, MA; IL-3, IL-4 (specific activity, 0.5–1×10^6 U/mg), IL-10 (specific activity, 1.7–3.3×10^6 U/mg), and granulocyte-macrophage CSF (GM-CSF, specific activity, 0.67–2.0×10^7 U/mg) from R&D Systems, Abingdon, UK. Purified human TGF-β_1 (specific activity, 2×10^7 U/mg) was purchased from R&D Systems. PGE_2, N^6, 2'-0-dibutyryl-adenosine 3'-5'-cyclic monophosphate (dbcAMP), LPS (Escherichia coli, serotype 026:B6), isoproterenol, and N-monomethyl-arginine were purchased from Sigma Chemical Co., St. Louis, MO.

Cell Cultures

Primary mixed glial cultures were established from the forebrains of 1-day old CD1 Swiss mice, as previously described (Aloisi et al., 1997). In brief, forebrains were carefully freed of meninges, chopped into 0.25-mm sections, and dissociated by mild trypsinization and gentle mechanical disruption with a Pasteur pipette. The cells were seeded into poly-L-lysine (10 μg/ml) coated 175-cm^2 flasks at the density of 4×10^4 cells/cm^2 and grown at 37°C in a 92% air–8% CO_2-humidified atmosphere in DMEM containing 0.45 μm-filtered, 10% FCS Myoclone (Life Technologies, Gaithersburg, MD), 2 mM glutamine, and penicillin (100 u/ml)/streptomycin (100 μg/ml). The medium was replaced after 4 days. After about 10 days in vitro, microglial cells were detached from the astroglial monolayer by rapid and gentle manual shaking of the culture flasks; the supernatants were collected and centrifuged, and the cells were reseeded on plastic surfaces, in the same medium as above, at the density of 5×10^4 cells/cm^2. After 1 hr, the medium was replaced to remove nonadherent cells and microglial cells were allowed to grow for additional 24 hr before the experiments were started. The cellular composition of microglial cultures was ascertained by immunocytochemistry with anti-mouse CD11b (Mac-1 α M chain, IgG2b) monoclonal antibody (Pharmingen, San Diego, CA). Flow cytometric and fluorescence microscope analysis revealed that cultures of microglia contained 90–95% of Mac-1^+ cells (Aloisi et al., 1998).

Determination of Secreted IL-10 and TNF

Microglial cells (1×10^5/well in 48-well plates) were incubated with 0.3 ml of culture medium, either in the absence or presence of cytokines or LPS, for the indicated times. At the end of incubations, supernatants were collected, centrifuged, and stored at −80°C until use. IL-10 and TNF secreted in culture supernatants were quantified using commercially available ELISA from Genzyme specific for mouse IL-10 and TNF-α, respec-

tively. The detection limit for both cytokines was 15 pg/ml.

Semiquantitative Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Total cellular RNA from microglia (1.5–2.0 $\times$ 10^6 cells) was extracted by the guanidinium isothiocyanate-CsCl method (Chirgwin et al., 1979) and quantified. The purity of RNA was checked on ethidium bromide-containing 1% agarose gels in Tris-borate/EDTA buffer.

Reverse transcriptase (RT) was performed using 1 µg of total RNA from unstimulated and cytokine- or LPS-stimulated cells and Moloney Leukemia Virus Reverse Transcriptase system (Life Technologies). The 20-µl reaction mixture contained 4 µl 5× synthesis buffer [0.25 M Tris-HCl (pH 8.3), 0.375 M KCl, 15 mM $MgCl_2$], 0.2 µg of oligo$(dT)_{12–18}$, 1 µl dNTP mix (10 mM), 2 µl 0.1 M DTT, and 200 U M-MLV reverse transcriptase.

To determine the PCR conditions for each set of primers, a fixed amount of total RNA from a LPS-stimulated mouse microglia cell line (BV-2) was reverse transcribed and amplified by using different primers for an increasing number of cycles. The conditions allowing the signal to be in the linear portion of the amplification curve were as follows: 4 µl of each cDNA sample and 30 cycles for IL-10, 8 µl and 20 cycles for TNF, and 4 µl and 25 cycles for the ''housekeeping'' gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). PCR was carried out in 50 µl of reaction mixture containing Taq DNA polymerase buffer (50 mM KCl, 10 mM Tris-HCl (pH 9), 1.5 mM $MgCl_2$), 0.2 mM of each primer and 2U of Taq DNA polymerase (Promega, Madison, WI). A negative control lacking template RNA or RT was included in each experiment.

The cDNA samples were amplified using a Perkin-Elmer Cetus (Foster City, CA) DNA thermal cycler machine, under optimized conditions for each set of primers (for IL-10: 30 cycles of denaturation at 94°C for 30 sec, annealing at 59°C for 30 sec, and extension at 72°C for 60 sec; for TNF: 20 cycles of denaturation at 94°C for 30 sec, annealing at 58°C for 30 sec, and extension at 72°C for 45 sec; for GAPDH: 25 cycles of denaturation at 94°C for 40 sec, annealing at 62°C for 40 sec, and extension at 72°C for 60 sec). The primers and probes for mouse IL-10, TNF, and GAPDH were designed according to published sequences (Fransen et al., 1985; Murphy et al., 1993; Di Marzio et al., 1994): IL-10: sense 5'-TCCTTAATGCAGGACTTTAAGGGTTAC-TTG-3', antisense 5'-GACACCTTGGTCTTGGAGCT-TATTAAAATC-3', probe 5'-CGGCTGAGGCGCTGT-CATCGATTTCTCCCC-3' (size of the amplified portion of IL-10 cDNA was 240 bp); TNF: sense 5'-GCGACG-TGGAACTGGCAGAAG-3', antisense: 5'-GGTACAAC-CCATCGGCTGGCA-3', probe: 5'-CAGTTCTATGGC-CCAGACCCTC-3' (size of the amplified fragment of TNF cDNA was 384 bp); GAPDH: sense 5'-CCATG-GAGAAGGCCGGGG-3', antisense 5'-CAAAGTTGT-CATGGATGACC-3', probe 5'-GTGTGAACCAC-GAGAAAT-3' (size of the amplified portion of GAPDH cDNA was 194 bp). Twenty µl of each amplified product were electrophoresed in a 2% agarose gel and transferred to a nylon filter. Filters were then hybridized with a probe end-labeled with γ-ATP^{32}P and polynucleotide kinase. The levels of transcripts were analyzed by high resolution scanning TM425F Phosphorimager (Molecular Dynamics, Ltd., Sunnyvale, CA).

RESULTS

Induction of IL-10 and TNF Production in Cultures of Mouse Microglia

Using specific ELISAs, IL-10 and TNF were undetectable in the supernatants of unstimulated mouse microglial cultures. With the exception of LPS, none of the numerous cytokines tested [IFN-γ (10, 100 U/ml), TNF (50, 500 U/ml), IL-1α (10, 100 U/ml), IL-1β (10, 100 U/ml), IL-3 (10, 20 ng/ml), IL-4 (10, 100 U/ml), IL-6 (100, 250 U/ml), GM-CSF (5, 10 ng/ml), TGF-β_1 (1, 10 ng/ml), and LIF (20 ng/ml)] was effective in inducing secretion of either IL-10 or TNF by microglia. Maximal stimulation of IL-10 and TNF secretion was obtained with 100 ng/ml and 10 ng/ml of LPS, respectively (Fig. 1A). Kinetic analysis of cytokine secretion by microglia showed that both IL-10 and TNF rapidly accumulated in the culture supernatants during the first 8 hr of LPS treatment (Fig. 1B). This temporal pattern differs from that previously reported for macrophages and human microglia, where IL-10 production is delayed relative to that of TNF and other proinflammatory cytokines (Sheng et al., 1995; Donnelly et al., 1995).

RT-PCR analysis revealed that IL-10 and TNF mRNAs were either undetectable or present at very low levels in unstimulated microglia and that both transcripts rapidly accumulated after treatment with LPS (10–100 ng/ml) (Fig. 2A,B). Levels of IL-10 mRNA were increased at 3 hr and remained sustained up to 24 hr. TNF mRNA was highly expressed at 3 hr, but almost undetectable at 24 hr after addition of LPS.

Since T-cell-derived products were shown to stimulate expression of TNF mRNA in microglia (Renno et al., 1995), we examined the levels of TNF and IL-10 transcripts in IFN-γ-treated microglia. IFN-γ (10, 100 U/ml) upregulated expression of TNF mRNA (Fig. 2C) but not of IL-10 mRNA (Fig. 3B). IFN-γ-induced accumulation of TNF mRNA was maximal at 3 hr and sharply decreased thereafter (Fig. 2C). Such rapid decline could account for the inability of IFN-γ to induce TNF protein synthesis and/or secretion.

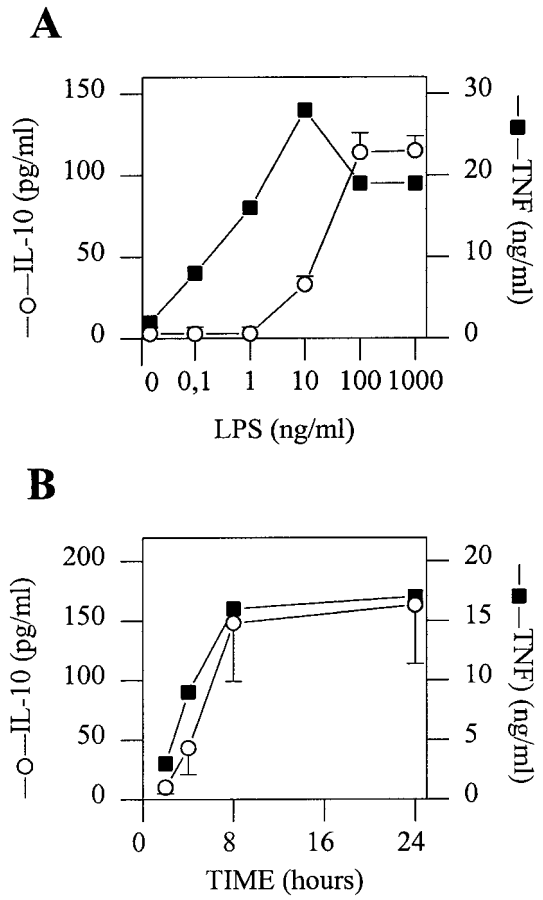

Fig. 1. Dose and time dependence of LPS effect on IL-10 and TNF secretion by microglia. **A:** Microglial cells (1×10^5/well in 48-well culture plates) were incubated for 24 hr in 0.3 ml of control culture medium or with medium containing increasing amounts of LPS. **B:** For kinetics experiments, the cells were incubated with LPS (100 ng/ml) for 2, 4, 8, and 24 hr. IL-10 and TNF-α in culture supernatants were quantified by specific ELISA. IL-10 determinations are expressed as mean values ± SEM from three (A) and five (B) experiments. TNF-α determinations are expressed as mean values from two experiments.

Effect of IFN-γ on LPS-Induced IL-10 and TNF Production

We next examined the influence of IFN-γ on IL-10 and TNF production by LPS (100 ng/ml)-stimulated microglia. Figure 3 shows that IFN-γ inhibited LPS-induced IL-10 secretion in a dose-dependent manner. A concentration of 0.1 U/ml of IFN-γ was sufficient to inhibit LPS-stimulated IL-10 secretion by about 50%. IL-10 production was completely abolished also when microglial cells were pretreated with IFN-γ (100 U/ml) for 3 hr, then stimulated with LPS alone (data not shown). IL-1α, IL-1β, IL-3, IL-4, TNF, GM-CSF, and TGF-β (concentrations as above) did not affect LPS-induced IL-10 secretion (data not shown). In agreement with the ELISA data, IFN-γ (100 U/ml) almost totally inhibited IL-10 mRNA accumulation in LPS-stimulated microglia, indicating that downregulation of IL-10 production occurred at the transcriptional level (Fig. 3B).

IFN-γ (10, 100 U/ml) did not significantly affect TNF secretion induced by 10 ng/ml of LPS (data not shown). Moreover, we could not detect any increase in TNF mRNA levels when microglial cells were treated simultaneously with these doses of IFN-γ and LPS (0.1–10 ng/ml) (data not shown).

Effect of PGE_2 on Microglial IL-10 and TNF Production

The arachidonic acid metabolite PGE_2 inhibits the synthesis of proinflammatory cytokines and upregulates IL-10 production by monocytes/macrophages (Strassmann et al., 1995; Zhong et al., 1995). We therefore examined the effect of PGE_2 on the LPS-induced secretion of IL-10 and TNF by microglia. PGE_2 did not trigger IL-10 secretion by itself (data not shown), but dose-dependently enhanced IL-10 secretion induced by LPS (100 ng/ml). The maximal potentiating effect (3- to 4.5-fold increase over stimulation with LPS alone) was obtained with 10^{-7} M of PGE_2 (Fig. 4A). Conversely, LPS-induced secretion of TNF was suppressed by PGE_2 in a dose-dependent manner (Fig. 4A).

In agreement with the ELISA data, RT-PCR analysis of mRNA isolated from microglia showed that PGE_2 enhanced the LPS-induced accumulation of IL-10 mRNA (Fig. 4B) and inhibited that of TNF mRNA (Fig. 4C).

IFN-γ Interferes With the Effects of PGE_2 on IL-10 and TNF Production

To test whether IFN-γ and PGE_2 reciprocally affected their divergent activities on IL-10 production, microglia were simultaneously treated for 24 hr with LPS (100 ng/ml), PGE_2 (10^{-7} M), and increasing amounts of IFN-γ (0.1 to 100 U/ml). Figure 5A shows that IFN-γ, in a dose-dependent manner, abrogated the stimulatory effect of PGE_2 on LPS-induced IL-10 production, being active at concentrations as low as 0.1 U/ml. In the presence of IFN-γ (100 U/ml), PGE_2 still suppressed TNF secretion in a dose-dependent manner, but the inhibition was significantly lower than that observed when microglial cells were stimulated with LPS alone (Fig. 5B).

Also at the mRNA level, the effects of IFN-γ were predominant over those of PGE_2. No or only very little IL-10 mRNA was detected in LPS-activated microglia that were concomitantly exposed to IFN-γ (100 U/ml) and PGE_2 (10^{-7} M) (Fig. 6A). At variance with the complete inhibitory effect on LPS-induced TNF tran-

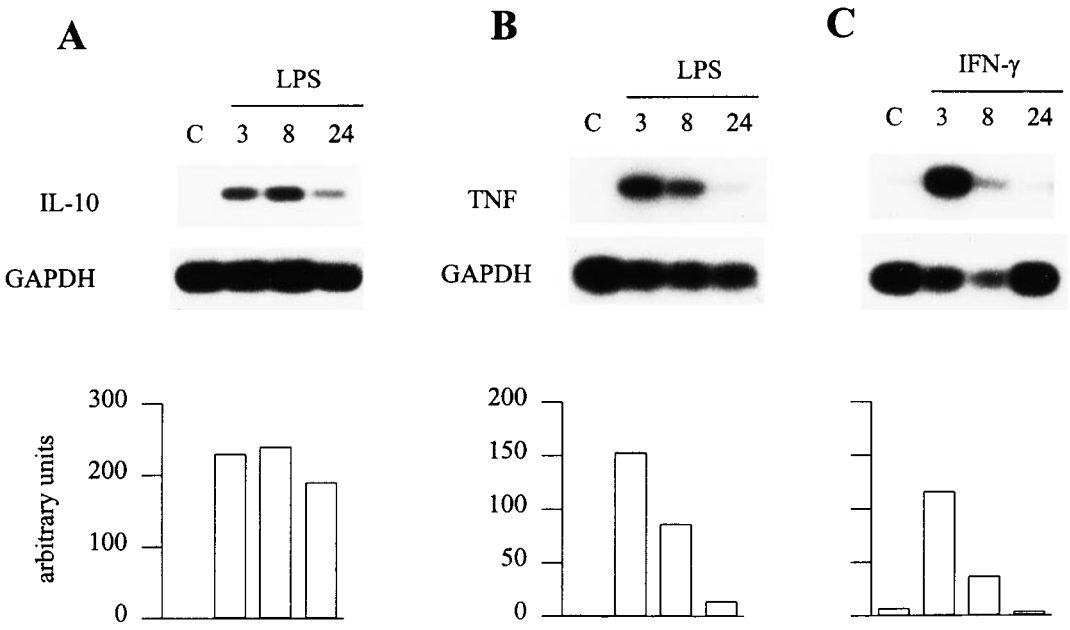

Fig. 2. Semiquantitative RT-PCR analysis of IL-10 and TNF mRNA in cultures of mouse microglia. Microglial cells were treated with control culture medium (C) for 8 hr or with medium containing LPS (100 ng/ml) **(A,B)** or IFN-γ (100 U/ml) **(C)** for 3, 8, and 24 hr. RNA was reverse transcribed and amplified with specific primers for IL-10, TNF, or the ''housekeeping'' gene GAPDH, as described in Materials and Methods. Densitometric analysis of IL-10 and TNF mRNAs normalized to the GAPDH control is shown in the lower panels.

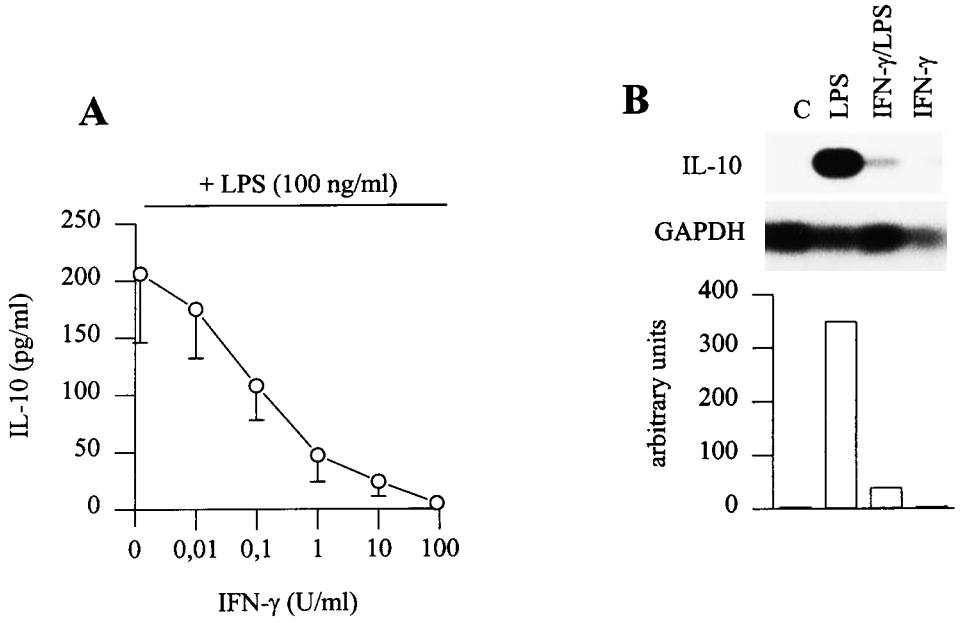

Fig. 3. Inhibitory effect of IFN-γ on LPS-induced IL-10 secretion (**A**) and IL-10 gene expression (**B**). A: Microglial cells were stimulated for 24 hr with LPS (100 ng/ml) in the absence or presence of increasing concentrations of IFN-γ. IL-10 in culture supernatants was quantified by specific ELISA. Mean values ± SEM are from three experiments. B: Semiquantitative RT-PCR analysis of IL-10 mRNA in microglia incubated for 8 hr in control culture medium (C) or in medium containing LPS (100 ng/ml), IFN-γ (100 U/ml), or IFN-γ plus LPS for 8 hr. Densitometric analysis of IL-10 gene expression, as normalized to the GAPDH control, is shown below.

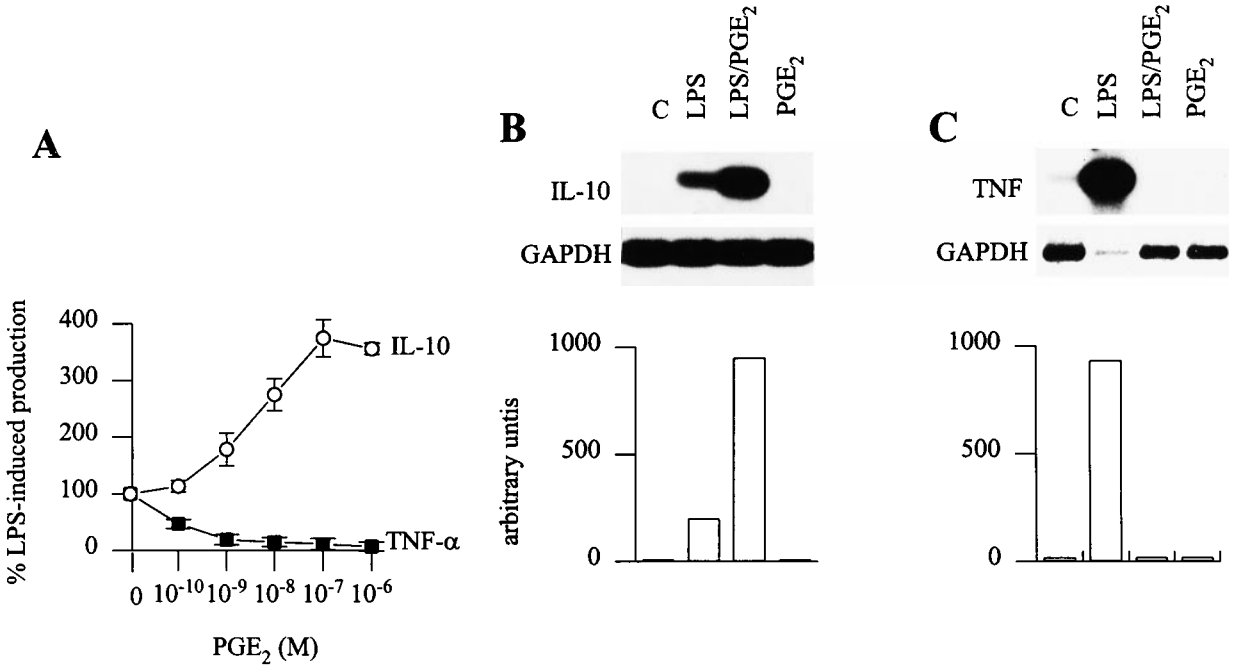

Fig. 4. Effect of PGE_2 on LPS-induced IL-10 and TNF-α secretion and gene expression. **A:** Microglial cells were treated for 24 hr with LPS (100 ng/ml) in the absence or presence of increasing amounts of PGE_2. IL-10 and TNF-α in culture supernatants were quantified by ELISA. Data are expressed as percentage of the response seen in cultures treated with LPS alone. Mean values ± SEM are from three experiments. **B,C:** Semiquantitative RT-PCR analysis of IL-10 and TNF mRNA in microglia incubated for 8 hr in control culture medium (C) or in medium containing LPS (100 ng/ml), PGE_2 (10^{-7} M), or PGE_2 plus LPS. Densitometric analysis of IL-10 and TNF gene expression, as normalized to the GAPDH control, is shown in the lower panels.

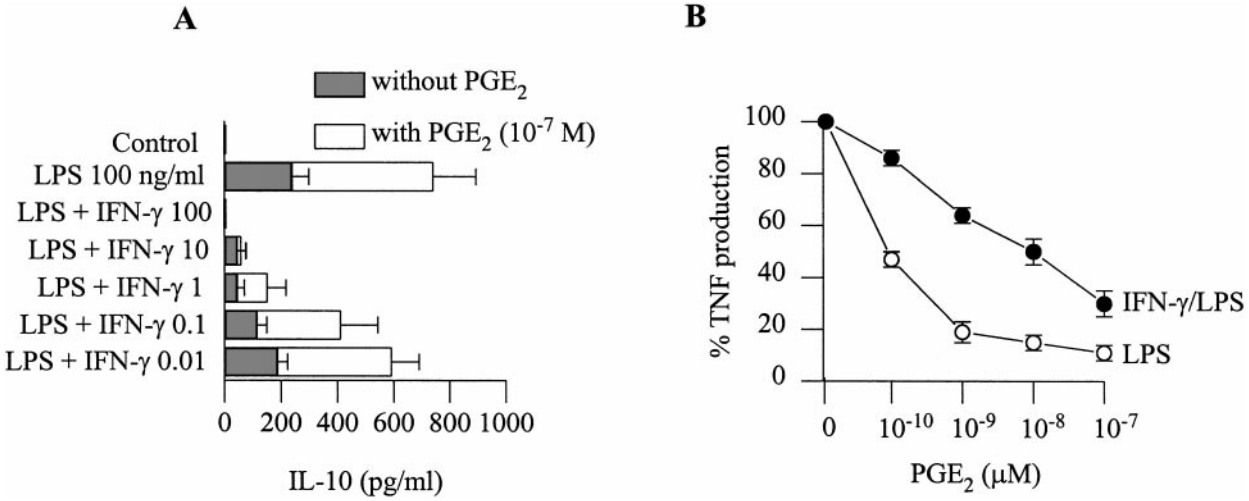

Fig. 5. IFN-γ counteracts the effects of PGE_2 on LPS-induced IL-10 and TNF secretion by microglia. **A:** Microglial cells were incubated for 24 hr in control medium or in medium containing LPS (100 ng/ml) or LPS plus increasing concentrations of IFN-γ, either in the absence or presence of PGE_2 (10^{-7} M). IL-10 in culture supernatants was quantified by ELISA. **B:** Microglial cells were incubated for 24 hr with LPS (100 ng/ml) or LPS plus IFN-γ (100 U/ml) in the presence of increasing concentrations of PGE_2. TNF in culture supernatants was quantified by ELISA. Data are expressed as percentage of the response seen in cultures treated with LPS or LPS plus IFN-γ. Mean values ± SEM are from three experiments.

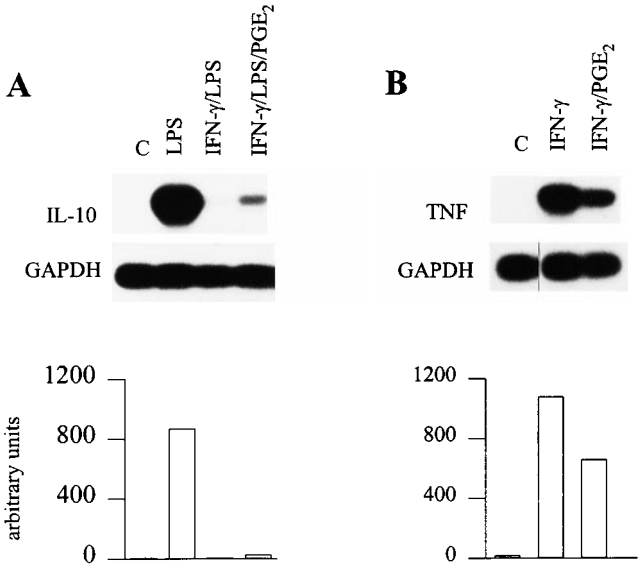

Fig. 6. IFN-γ counteracts the effects of PGE_2 on IL-10 and TNF gene expression. **A:** Semiquantitative RT-PCR analysis of IL-10 mRNA in microglia incubated for 8 hr in culture medium without (C) or with LPS (100 ng/ml), IFN-γ (100 U/ml)/LPS, or IFN-γ/LPS plus PGE_2 (10^{-7} M). **B:** Semiquantitative RT-PCR analysis of TNF mRNA in microglia incubated for 3 hr in culture medium without (C) or with IFN-γ (100 U/ml) or IFN-γ plus PGE_2 (10^{-7} M). Densitometric analysis of IL-10 and TNF gene expression, as normalized to the GAPDH control, is shown in the lower panels.

scripts, PGE_2 only partially (by 40–50%) reduced IFN-γ-induced TNF mRNA accumulation (Fig. 6B).

Effects of PGE_2 on Cytokine Production Are Mimicked by cAMP-Elevating Agents

Many of the effects of PGE_2 are known to be mediated by cAMP (Kammer, 1988) and functionally active EP2 receptors, linked to protein kinase A activation, are present on rodent microglia (Minghetti et al., 1997). We therefore tested the effects of a cAMP elevating agent and of the permeable cAMP analogue dbcAMP on LPS- and IFN-γ/LPS-induced IL-10 and TNF secretion. The β-adrenergic agonist isoproterenol potentiated the inducing effect of LPS (100 ng/ml) on IL-10 secretion in a dose-dependent manner, the maximal effect being obtained at the concentration of 10^{-7} M (Fig. 7A). In agreement with previous data (Hetier et al., 1991), isoproterenol inhibited LPS-induced TNF secretion by microglia (Fig. 7A). Consistent with these findings, dbcAMP (0.1 and 1 mM) enhanced LPS-stimulated IL-10 secretion by about 2.5-fold and almost completely inhibited TNF production (data not shown from two independent experiments).

When microglial cells were stimulated with LPS in the presence of increasing concentrations of IFN-γ and isoproterenol (10^{-7} M), IFN-γ strongly reduced the potentiating effect of isoproterenol on LPS-induced IL-10 secretion (Fig. 7B). IFN-γ also counteracted the inhibitory effect of isoproterenol on LPS-induced TNF secretion (data not shown).

DISCUSSION

The present study describes the divergent effects of IFN-γ and PGE_2 on the production of IL-10 and TNF by LPS-activated microglia. IFN-γ enhances TNF and suppresses IL-10 production, whereas PGE_2 affects the two cytokines in the opposite way. Moreover, when microglia are exposed simultaneously to IFN-γ and PGE_2, the effects of IFN-γ appear to overwhelm those of PGE_2. These findings highlight the complexity of the interactions between different mediators in regulating the cytokine profile of macrophages/microglia in an inflammatory environment.

IFN-γ, a cytokine secreted predominantly by $CD4^+$ lymphocytes of the T helper 1 subset and NK cells, primes microglia for secretion of proinflammatory cytokines (e.g., IL-12) (Benveniste, 1997; Aloisi et al., 1997). It also stimulates microglial cells to function as antigen-presenting cells and to release reactive oxygen and nitrogen intermediates (Frei et al., 1987; Colton et al., 1994; Benveniste, 1997; Minghetti et al., 1996). As shown here, IFN-γ is a potent downregulator of IL-10 gene expression and secretion by LPS-activated mouse microglia, and its effect was not shared by a number of other cytokines. IL-10 was shown to limit macrophage/microglia activation and proinflammatory cytokine production in an autocrine way (Frei et al., 1994; Chao et al., 1995). It is therefore possible that suppression of IL-10 production contributes to the activating properties of IFN-γ. Our preliminary, unpublished experiments indicate that the inhibitory effect of IFN-γ on IL-10 production is not mediated by nitric oxide, which is a major product of IFN-γ- and IFN-γ/LPS-activated microglia (Chao et al., 1992; Minghetti et al., 1996). This is supported by the finding that IFN-γ suppresses IL-10 secretion also when microglia are treated with N-monomethyl-arginine, an inhibitor of nitric oxide production.

While there is consensus that the cerebral levels of IFN-γ and other proinflammatory cytokines (including TNF) are elevated during the induction phase or peak of EAE and decrease thereafter (Renno et al., 1995; Olsson, 1995), nonconcordant data have appeared on the expression of IL-10 transcripts. IL-10 mRNA levels or the number of IL-10-producing cells in the CNS (which

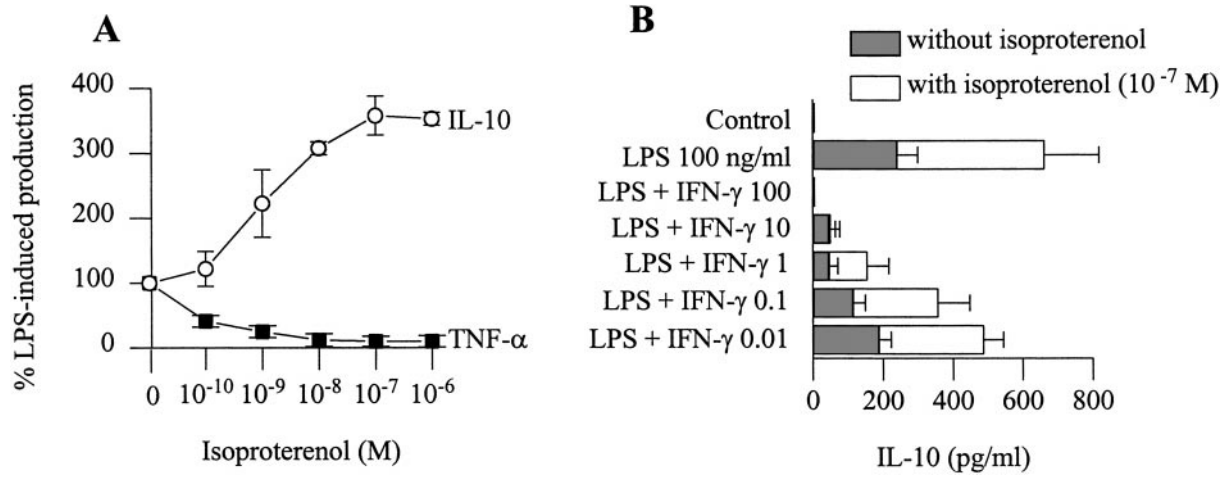

Fig. 7. Effect of isoproterenol on IL-10 and TNF-α secretion by LPS-stimulated microglia (**A**) and on IL-10 secretion in the presence of IFN-γ (**B**). A: Microglial cells were treated for 24 hr with LPS (100 ng/ml) in the absence or presence of increasing amounts of isoproterenol. IL-10 and TNF-α in culture supernatants were quantified by ELISA. Data are expressed as percentage of the response seen in cultures treated with LPS alone. B: Microglial cells were incubated for 24 hr in control medium or in medium containing LPS (100 ng/ml) or LPS plus increasing concentrations of IFN-γ, either in the absence or presence of isoproterenol (10^{-7} M). IL-10 in culture supernatants was quantified by ELISA. Mean values ± SEM are from three experiments.

include microglia) were found to be increased only during the recovery phase of EAE (Kennedy et al., 1992; Issazadeh et al., 1995), at early phases of the disease (Tanuma et al., 1997; Jander et al., 1998), or at no time during the disease (Di Rosa et al., 1998). These discrepancies could be due to the different protocols used to induce EAE or to the different sensitivity of the methods employed to detect IL-10 (RT-PCR, in situ hybridization, or ELISAs) in CNS tissue or CNS-infiltrating cells. Based on the inhibitory effect of IFN-γ on IL-10 gene expression in microglia (this study) and macrophages (Donnelly et al., 1995), we propose that these discrepancies might also be explained by differences in the amount of intracerebrally produced IFN-γ.

Sustained intracerebral TNF production by macrophages/microglia could contribute to CNS inflammation and dysfunction by promoting immune cell recruitment, macrophage/microglia activation, and demyelination (Renno et al., 1995; Probert et al., 1997). In this study we show that IFN-γ induces TNF mRNA but not TNF secretion. In agreement with previous findings, LPS is the only strong inducer of TNF gene expression and secretion. It is possible that IFN-γ is not a sufficient stimulus for inducing TNF protein synthesis. Alternatively, IFN-γ could induce TNF protein synthesis but not stimulate the activity of the metalloproteinase which facilitates the secretion of TNF by cleavage of its membrane-bound form (Black et al., 1997). So far, we could not distinguish between these two possibilities, as we found no good antibody to detect membrane-bound or intracellular TNF protein in either LPS- or IFN-γ-activated microglia.

Opposite to IFN-γ, the arachidonic acid metabolite PGE_2 inhibits several microglia/macrophage functions, including the expression of MHC class II and adhesion/costimulatory molecules and the synthesis of proinflammatory cytokines, such as IL-1 and IL-12 (Caggiano and Kraig, 1998; Menèndez-Iglesias et al., 1997; Aloisi et al., 1997). PGE_2 also inhibits microglia neurotoxic activity (Théry et al., 1994) and ability to produce NO (Minghetti et al., 1996). Consistent with the macrophage deactivating activities of PGE_2, we show here that PGE_2 inhibits TNF but upregulates IL-10 gene expression and secretion by LPS-stimulated microglia. These results indicate that the anti-inflammatory effect of PGE_2 on microglia may be mediated in part by an autocrine feedback mechanism involving IL-10.

PGE_2 levels are increased intracerebrally in rats during recovery from EAE (Khoury et al., 1994) and in the cerebrospinal fluid of patients with MS (Fretland, 1992). Moreover, a stable PGE_2 analogue suppresses EAE (Reder et al., 1994), suggesting that PGE_2 has a modulatory role in neuroinflammatory CNS diseases. In the inflamed CNS, intracerebral PGE_2 synthesis may be sustained by infiltrating immune cells as well as by activated microglia and astrocytes (Minghetti and Levi, 1998). Our in vitro finding that IFN-γ strongly reduces the effects of PGE_2 on microglia cytokine secretion raises the possibility that intracerebrally produced PGE_2 might be less effective as a macrophage/microglia downregulator, and therefore as an anti-inflammatory agent, when high amounts of IFN-γ are released by activated T cells in the CNS microenvironment.

As the effects of PGE_2 on IL-10 and TNF-α were mimicked by cAMP elevating agents and PGE_2 was previously shown to enhance cAMP accumulation in microglia (Patrizio et al., 1996) via activation of EP2 receptors (Minghetti et al., 1997), it is likely that the prostaglandin acted through the elevation of cAMP levels. A recent study has shown that the IL-10 gene contains a cAMP-responsive element (Platzer et al., 1995). In addition, both LPS and IFN-γ reduce the accumulation of intracellular cAMP in rat microglia by stimulating type IV phosphodiesterase (Patrizio et al., 1996). These observations support the hypothesis that stimulation of cAMP breakdown may at least partly account for the ability of IFN-γ to prevent or reduce the effects of PGE_2 and cAMP elevating agents on microglia cytokine secretion.

In conclusion, the present findings delineate a regulatory loop of microglia secretory activities based on the differential regulation of pro- and anti-inflammatory cytokines by PGE_2 versus IFN-γ, and suggest that the balance between these two mediators may play a role in controlling the state of microglia activation during immune-mediated CNS inflammation.

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