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Interleukin-4 induces proliferation and activation of microglia but suppresses their induction of class II major histocompatibility complex antigen expression

Akio Suzumura [a,*], Makoto Sawada [b], Yasuhiro Itoh [b], Tohru Marunouchi [b]

[a] *Department of Neurology, School of Medicine, Fujita Health University, Toyoake, Aichi 470-11, Japan*
[b] *Division of Cell Biology, Institute for Comprehensive Medical Sciences, Fujita Health University, Toyoake, Aichi 470-11, Japan*

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Abstract

We recently found that microglia, brain macrophages, express interleukin-4 (IL-4) receptor mRNA in vitro. Since IL-4 exhibits a variety of functions on the cells of monocyte-macrophage lineage, we examined the effects of IL-4 on the functions of microglia. Recombinant IL-4 induced the proliferation of microglia in a dose- and time-dependent manner as determined by MTT colorimetric assay, [^{3}H]thymidine uptake and bromodeoxyuridine (BrdU) incorporation. IL-4 also synergistically enhanced the proliferation of microglia with such colony-stimulating factors as IL-3, granulocyte-macrophage colony-stimulating factor (GM-CSF) and macrophage colony-stimulating factor (M-CSF). It also increased acid phosphatase activity and superoxide anion formation by these cells. Despite these positive effects on proliferation and activation, IL-4 suppressed the IFNγ-induced class II MHC antigen expression in these cells. Since these effects of recombinant IL-4 were inhibited by the addition of monoclonal antibody against IL-4 receptors, the effects of IL-4 on microglia appear to be a specific function via IL-4 receptors. Although microglia and astrocytes produce a variety of immunoregulatory cytokines, neither cell produced IL-4 as determined by bioassay or detection of IL-4 mRNA by RT-PCR method. Thus, the exogenous IL-4 may contribute to the accumulation of microglia in or around inflammatory lesions in the central nervous system, and may be involved in the regulatory mechanisms of microglia.

Key words: Microglia; Interleukin-4; Proliferation; Major histocompatibility complex; Glia; Cytokine

1. Introduction

Microglia are one of the three types of glial cells in the central nervous system (CNS). Although their origin is not clear, these cells appear to be from the monocyte-macrophage lineage, and enter the CNS late in the embryonic stage (Del Rio-Hortega, 1932; Imamura et al., 1990). Their phenotypic or functional characteristics are similar to those of monocyte-macrophages (Austin and Gordon, 1981; Perry et al., 1985; Giulian and Baker, 1986; Suzumura et al., 1987). Their growth and functions are regulated by colony-stimulating factors as are the monocyte-macrophages (Sawada et al., 1990; Suzumura et al., 1990; 1991). They produce such monokines as IL-1, IL-6 and tumor necrosis factor (TNF)α (Giulian and Baker, 1986; Sawada et al., 1989, 1992). In addition, microglia can be induced to express class II major histocompatibility complex (MHC) antigens (Suzumura et al., 1987), and reportedly present antigens to T cells in an antigen-specific manner (Righi et al., 1989). Because of their similarity to macrophages, microglia are considered to play a key role in the development of various CNS diseases as inflammatory or immunoregulatory cells. In the case of injury, inflammation or neuronal degeneration, they appear early at the site where they seem to proliferate (Ohmori et al., 1992). An increase in number of microglia leads to the formation of clumps of small nuclei called 'glial star', which is a main histological feature of neuronal loss or CNS tissue damage (Duchen, 1992). Factors in inflammatory products may perhaps induce the proliferation of microglia in these disorders.

IL-4 was initially identified as a B cell growth factor (Haward et al., 1982), being designated as B cell growth

* Corresponding author. Phone (0562) 93 9295; Fax (0562) 93 1856

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factor 1 (Lee et al., 1986). It is a potent B cell growth factor (Paul and Ohara, 1987), a major regulator of the IgG1 and IgE isotype by activated B cells (Coffman et al., 1985; Sideras et al., 1985; Vitetta et al., 1985), and the primary inducer of the low affinity IgE receptor (CD23) on B cells (Defrance et al., 1987). Besides these effects on B cells, IL-4 exerts a regulatory function on macrophages. It activates the tumoricidal function of macrophages (Crawford et al., 1987) and enhances the antigen-presenting cell functions of certain types of macrophage (Zlontnik et al., 1987). However, a suppressive effect of IL-4 on macrophages has also been reported. It suppresses formation of macrophage colonies by M-CSF, and also suppresses the production of monokines by these cells (Essner et al., 1989; Hart et al., 1989; Gautam et al., 1992). It is therefore possible that IL-4 may influence the functions of microglia as well. Recent studies suggest that IL-4 is involved in the pathophysiology of CNS disorders. IL-4 mRNA has been detected in the CNS in the acute phase of viral infections (Moskophidis et al., 1991), toxoplasmic encephalitis (Hunter et al., 1992), experimental allergic encephalomyelitis (Kennedy et al., 1992) and multiple sclerosis (Wucherpfennig et al., 1992).

A variety of immunoregulatory cytokines are produced in the CNS which affect functions of neural cells as autocrine or paracrine mediators, and form a unique network of cytokines (Sawada et al., 1989, 1992; Lieberman et al., 1989; Ohno et al., 1990). However, whether IL-4 is produced in the CNS, or whether it has any effect on neural cells is not known. We recently found that isolated microglia express IL-4 receptor mRNA (Sawada et al., 1993). In this study, we examined the production of IL-4 by glial cells, and examined the effects of IL-4 on the various functions of the isolated microglia in vitro.

2. Materials and methods

2.1. Reagents

Recombinant murine IL-3, IL-4, GM-CSF, IFNγ and human M-CSF, and rat monoclonal antibody against murine IL-4 receptors (IL-4R) were obtained from Genzyme (Boston, MA). Rat monoclonal anti-murine IL-5 antibody was obtained from Endogen (Boston, MA) and used for the control of the IL-4R antibody. The monoclonal antibody against glial fibrillary acidic protein (GFAP) was a gift of Dr. S.U. Kim (British Columbia, Canada). MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) and Nitroblue tetrazolium (NBT) were purchased from Sigma (St. Louis, MO). Lipopolysaccharide (LPS; from *Escherichia coli*) was purchased from Difco (Detroit, MI).

2.2. Cell culture

Primary mixed glial cell cultures were prepared as described previously (Suzumura et al., 1984), using newborn C3H/HeN mice (Charles River Japan, Shizuoka, Japan). In brief, after the meninges were carefully removed, the brain was dissociated by passing it through a 320-μm pore nylon mesh. After being washed with Hanks' balanced salt solution, the cell suspension was triturated and plated in 75-cm^2 culture flasks (Falcon 3024, Beckton-Dickinson, NJ) at a density of two brains/flask in 10 ml Eagle's MEM supplemented with 10% fetal calf serum, 5 μg/ml bovine insulin, and 0.2% glucose. Microglia were isolated on the 14th day by the 'shaking off' method as previously described (Suzumura et al., 1987). The purity of the cultures was 97–100% as determined by immunostaining with Mac 1 antibody (Hybritech, San Diego, CA, used in 1:50 dilution) (Sawada et al., 1990; Suzumura et al., 1990, 1991). Astrocyte-enriched cultures were prepared from the primary mixed glial cell cultures by repetitive trypsinization and replating (Suzumura et al., 1988). The purity of the cultures exceeded 95% as determined by indirect immunofluorescence staining with anti-GFAP antibody (Suzumura et al., 1988).

2.3. Production of IL-4 and expression of IL-4 receptors in glial cells

The expression of mRNA for IL-4 and IL-4R was studied by the RT-PCR method as described below. A T cell line, D10N3 (provided by Dr. K. Onozaki, Nagoya City University, Japan), was used as a positive control for the expression of IL-4 mRNA. IL-4 activity in the supernatant was assessed by a bioassay using an IL-2- and IL-4-dependent cell line, CTLL-2, and the MTT colorimetric assay (Sawada et al., 1992). Microglia and astrocyte-enriched cultures were plated in 2.5-cm diameter culture dishes (Falcon 3001, Beckton-Dickinson, Lincoln Park, NJ) at a density of 5×10^5/ml for 24 h with or without 1 μg/ml LPS or 100 U/ml IFNγ. The supernatant was then collected and stored at −70°C until assayed for the IL-4 activity. After remaining cells had been washed three times with PBS, the total RNA was isolated from the cells using the method of Chomczynski and Sacchi (1987). An amount of 1 μg of RNA was used for first-strand cDNA synthesis at 37°C for 90 min with 50 U of recombinant M-MuLV reverse transcriptase (Boehringer-Mannheim, Germany) and 0.2 μg of DNA random hexamers. The reaction was terminated by boiling for 5 min, and 2 μl of the mixture was amplified by 30 PCR cycles with 0.5 units of Taq polymerase (Promega, Madison, WI) and 0.5 μg each of the sense and antisense primers. The thermal cycle profile was as follows:

1 min at 94°C, 1 min at 55°C, and 2 min at 72°C. An aliquot (5 μl) of the PCR mixture was subjected to electrophoresis in 2% agarose gel. The agarose gel was then stained with ethidium bromide and photographed. The primers used are; IL-4 sense, 5′-ATGGGTCTCAACCCCCAGCTAGT, IL-4 antisense, 5′-GCTCTTTAGGCTTTCCAGGAAGTC, IL-4R sense, 5′-CCAGTGGTAATGTGAAGC, IL-4R antisense, 5′-GACAAGTTTTCCAGTGCG.

2.4. Effects of IL-4 on proliferation of microglia

2.4.1. MTT assay

MTT is cleaved by living cells to yield a formazan product. Thus, the measurement of a formazan product using a colorimetric method allows estimation of the relative number of surviving cells. Microglia were plated into wells of a 96-well microtiter plate (Falcon 3072, Beckton-Dickinson, Lincoln Park, NJ) at a density of 2×10^4/100 μl culture medium, and were stimulated with graded concentrations (1–100 U/ml) of IL-4 for 72 h. To examine the time course, microglia were stimulated as above for 24–96 h. An amount of 50 μg/ml MTT solution was added to each well in the final 6 h. The reaction was then halted by adding 150 μl of 0.04 N HCl-isopropanol. After sonication, the optical density (OD_{620nm}) of the solubulized materials was measured by an Immunoreader J200 (Inter Med Japan, Tokyo, Japan). In some experiments, graded concentrations of monoclonal antibody against IL-4 receptors (final dilution $1:10^2$–$1:10^6$) were applied with the stimulants, using the same dilution of rat monoclonal anti-mouse IL-5 antibody as a control.

2.4.2. [^{3}H]thymidine incorporation

Microglia were plated into wells of a 24-well culture plate (Falcon 3047, Beckton-Dickinson, Lincoln Park, NJ) at a density of 1×10^5/500 μl, and were stimulated with 0.1–100 U/ml IL-4 in the presence or absence of 20 U/ml IL-3, 20 U/ml GM-CSF or 10 U/ml M-CSF for 72 h. 1 μCi/well [^{3}H]thymidine was added in the final 12 h. The cultures were then washed three times with PBS. Cells were lysed with 100 μl of 0.2 N NaOH and were precipitated with 20 μl of 50% trichloroacetic acid (TCA). After extensive washing with 5% TCA, the precipitates were collected on glass fiber filter paper. The radioactivity of each paper was counted in a liquid scintillation counter (Beckman, USA).

2.4.3. BrdU uptake by microglia

Parallel cultures were plated onto 14-mm glass coverslips at a density of 5×10^4/ml, and stimulated with the same reagents as above for 72 h. BrdU (10 μg/ml) was added for the final 12 h. Coverslips were then immunolabeled with FITC-labeled anti-BrdU antibody (Beckton-Dickinson, Lincoln Park, NJ, final dilution 1:3). FITC-positive BrdU-incorporated microglia were counted under a fluorescence microscope (Zeiss, Germany), as described (Suzumura et al., 1990, 1991).

2.5. Effects of IL-4 on activation of microglia

Microglia were plated into wells of a 96-well microtiter plate at a density of 2×10^4/100 μl culture medium. They were cultured with graded concentrations of IL-4 (1–100 U/ml). After 3 days in culture, acid phosphatase activity and superoxide anion formation (NBT-reducing activity) were assessed as described previously (Sawada et al., 1990). Results were expressed as mU/well of acid phosphatase activity and nmol formed NBT diformazan for superoxide anion formation (Sawada et al., 1990; Suzumura et al., 1990). All the experiments were done in at least quadruplicate, and the *t*-test was used for the statistical analyses. We also evaluated the effects of IL-4 on the induction of class II MHC antigen expression by IFNγ. For this purpose, microglia were plated in a 2.5-cm culture dish at a density of 5×10^5/ml culture medium, and stimulated with 100 U/ml IFNγ in the presence or absence of 1–100 U/ml IL-4. In some experiments, anti-IL-4R antibody ($1:10^3$ in final dilution) was added with IFNγ and IL-4. After 12 h in culture, they were washed three times with PBS. Total RNA was isolated and the expression of mRNA for class II MHC antigens was examined by the RT-PCR method described above. The following primer was used to detect mRNA for I-A^k-α class II MHC; sense, 5′-AAGAAGGAGACTGTCTGGATGC, antisense, 5′-TGAATGATGAAGATGGTGCCC (Bishop et al., 1988). Microglia were also examined for their surface expression of MHC class II antigens by indirect immunofluorescence and flow cytometric analyses. For this, microglia were stimulated with 100 U/ml IFNγ for 48 h either in the presence or absence of 100 U/ml IL-4. Non-stimulated cultures were used for the control of the effects of IFNγ or IL-4. They were then stained with the mouse monoclonal anti-Ia^k antibody (Meiji Nyugyo, Tokyo, Japan, 1:50 dilution) and FITC-conjugated anti-mouse IgG antibody (Cappel, West Chester, PA, 1:50 dilution), using the monoclonal anti-Ia^d antibody (Meiji Nyugyo, Tokyo, Japan, 1:50 dilution) as the control of the first antibody. The samples were analyzed on an EPICS Elite (Coulter, Hialeah, FL).

3. Results

3.1. Production of IL-4 and expression of IL-4 receptor in glial cells

CTLL-2, known as an IL-2-dependent cell line, was also induced to grow in response to IL-4 as shown in

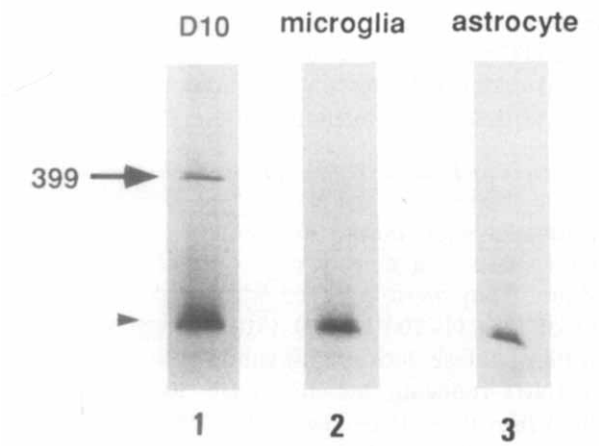

Fig. 1. Expression of IL-4 mRNA in microglia and astrocytes. D10N3 cells used as a positive control express IL-4 mRNA (arrow). Microglia and astrocytes did not express IL-4 mRNA, although the same amount of RNA was subjected to electrophoresis. The lower arrowhead indicates a nonspecific artifact.

the previous paper (Gearing et al., 1991; Mosmann and Fong, 1989). However, the supernatant from astrocytes and microglia, either stimulated or unstimulated, did not induce the growth of CTLL-2, indicating the absence of a sufficient amount of IL-4 as well as that of IL-2 in the supernatant. No mRNA for IL-4 was detected in the glial cells, while it was detected in D-10N3 cell line used as the positive control (Fig. 1). However, mRNA for IL-4 receptor was detected in the microglia (Fig. 2, lane 2). Digestion with restriction enzyme *Pvu*II

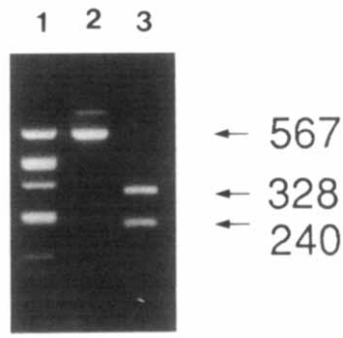

Fig. 2. Expression of IL-4 receptor mRNA in microglia. Lane 1, 0.5 μg of *Hae*III-digested pUC118 as size marker. Microglia expressed IL-4 receptor mRNA (lane 2), which was digested into two bands of the predicted size (328 and 240 bp) by treatment with *Pvu*II.

revealed two bands of the predicted sizes; 328 and 240 bp (Fig. 2, lane 3).

3.2. Effects of IL-4 on proliferation of microglia

The microglia treated with 1–100 U/ml IL-4 for 72 h increased in number; this effects of IL-4 was neutralized by adding anti-IL-4 receptor antibody (Fig. 3), but not by anti-IL-5 antibody used as a control. The mitogenic effects of IL-4 were time- and dose-dependent when assessed by MTT-colorimetric assay (Fig. 4). The effects of each dose of IL-4 were neutralized by adding anti-IL-4 receptor antibody in a dose-dependent manner (Fig. 5). Incorporation of [^{3}H]thymidine was also

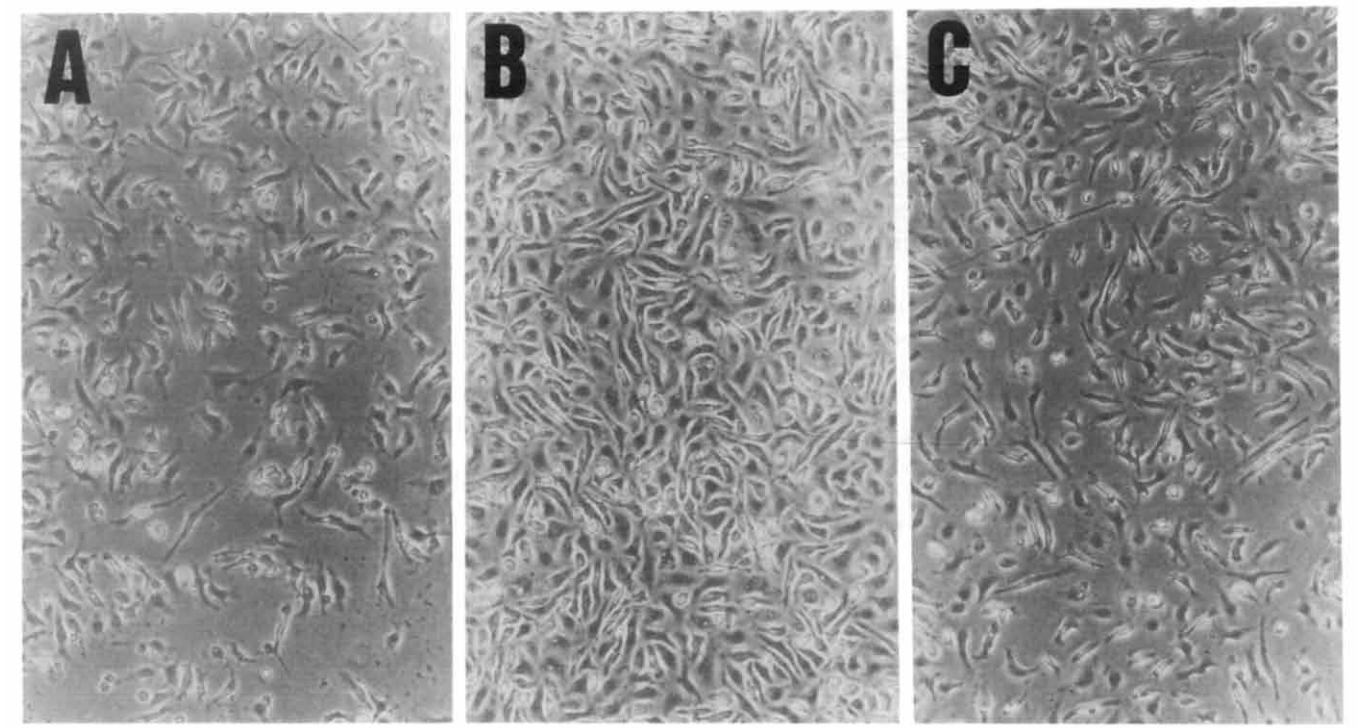

Fig. 3. Phase-contrast photomicrograph of microglia in vitro. Microglia cultured for 3 days without stimulation (**A**), with 100 U/ml IL-4 in the absence (**B**) or presence (**C**) of anti-IL-4 receptor antibody (final concentration $1:10^3$).

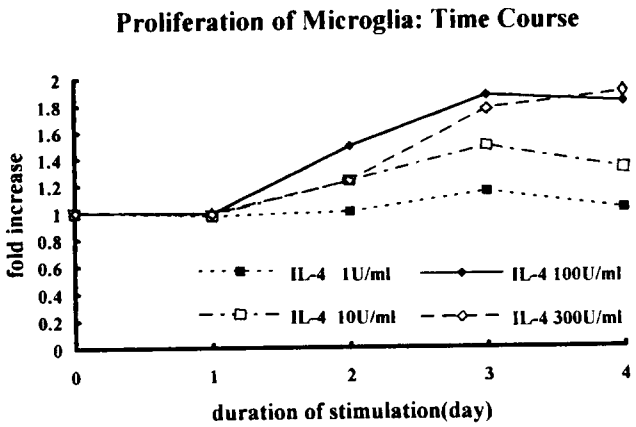

Fig. 4. Time and dose curve of IL-4 on proliferation of microglia. Microglia were treated with 1–300 U/ml IL-4 for up to 4 days. At 1, 2, and 3 days after stimulation, cell growth was measured by MTT colorimetric assay. Fold increase was calculated as (OD_{620} in stimulated culture)/(OD_{620} in unstimulated culture).

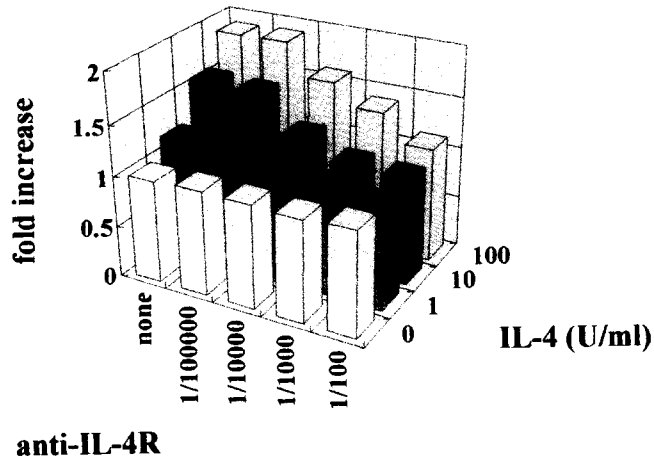

Fig. 5. Growth of microglia as assessed by MTT colorimetric assay. Fold increase was calculated as (OD_{620} in stimulated culture)/(OD_{620} in unstimulated culture). Amounts of 1–100 U/ml IL-4 increased the values of fold increase in a dose-dependent manner. The effects of each dose of IL-4 were neutralized by the addition of anti-IL-4R antibody. The effects of this antibody were also dose-dependent; an amount of 1:100 in final dilution almost completely neutralized the effects of IL-4. Each bar indicates the mean value obtained from triplicate samples in three different experiments. Standard deviations were less than 10% of each mean value.

increase by doses of IL-4 (Fig. 6). As we previously reported, GM-CSF and M-CSF induce the proliferation of microglia (Suzumura et al., 1990, 1991). In the present study the mitogenic effect of IL-4 was additive to the effects each of GM-CSF, M-CSF and IL-3 (Fig. 6). Unstimulated microglia rarely incorporated BrdU into their nuclei. However, the percentage of BrdU-positive microglia increased in accordance with the amount of IL-4 added. 1–200 U/ml IL-4 significantly increased the number of BrdU-positive microglia ($P < 0.001$) as compared to those in unstimulated microglia (Fig. 7), while only 100 U/ml of IL-4 induced a statistically significant increase ($P < 0.001$) in the GM-CSF-

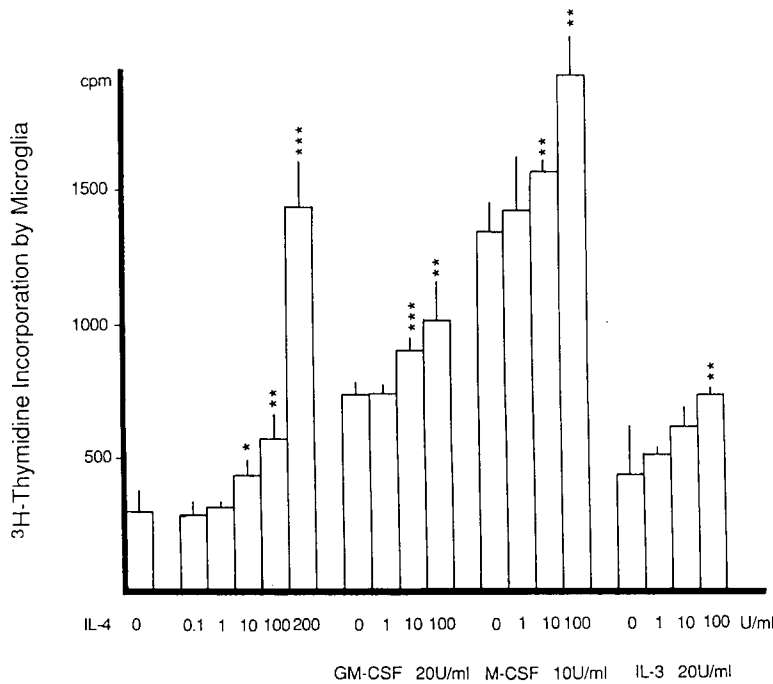

Fig. 6. Incorporation of [^{3}H]thymidine by microglia. IL-4 was applied at the indicated doses to microglia culture in the absence or presence of GM-CSF, M-CSF or IL-3. Open columns indicate mean values of triplicate samples in two different experiments ($n = 6$). Bars indicate standard deviations. * $P < 0.02$, ** $P < 0.01$, *** $P < 0.001$ as compared to each non-stimulated, GM-CSF-, M-CSF-, and IL-3-stimulated control without IL-4, by Student's t-test.

stimulated group, as compared to the non-IL-4-stimulated control in each group. Again, IL-4 acted synergistically with GM-CSF on the proliferation of microglia in vitro (Fig. 7). These BrdU-positive microglia were usually ameboid (Fig. 8). Some of these IL-4-stimulated microglia were multinucleated (Fig. 8C). About one-third of these multinuclei were labeled with anti-BrdU antibody (data not shown).

3.3. Effects of IL-4 on activation of microglia

IL-4 dose-dependently increased acid phosphatase activity and superoxide anion formation (NBT-reducing activity) of the microglia (Fig. 9). These effects of IL-4 on microglia were almost completely neutralized by the addition of anti-IL-4 receptor antibody (used in $1:10^3$ dilution) (Fig. 9). Despite the positive effects of IL-4 on the activation of microglia, IL-4 suppressed the induction of class II MHC antigen expression by IFNγ (Fig. 10). The fluorescence histogram of controls, either IFNγ-stimulated cultures or IFNγ + IL-4-stimulated culture stained with a control antibody (anti-Ia^d), were almost identical to that of non-stimulated culture stained with an appropriate anti-Ia^k antibody (Fig. 10, left). This indicates the absence of surface expression of class II MHC antigen on unstimulated microglia. Class II MHC antigen expression on microglia was induced in most cells when treated with 100 U/ml IFNγ (Fig. 10). However, the addition of 100 U/ml IL-4 decreased the expression of class II MHC antigens. The suppression observed with IL-4 was almost completely neutralized by the addition of anti-IL-4 receptor antibody (data not shown). Doses of 10–100 U/ml IL-4 effectively suppressed the class II MHC antigen expression induced by 100 U/ml IFNγ (data not shown). This suppression of the induction of class II MHC antigen expression was confirmed by the decrease in mRNA expression (Fig. 11). Unstimulated microglia did not express class II MHC antigen mRNA

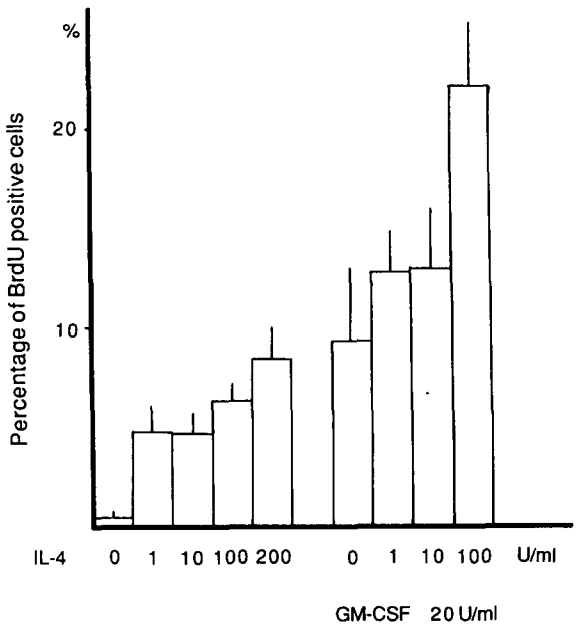

Fig. 7. BrdU incorporation by microglia. Percentage of BrdU-positive microglia in the cultures stimulated by the indicated doses of IL-4 or IL-4 plus 20 U/ml GM-CSF. Open columns indicate mean values obtained from quadruplicate coverslips ($n = 4$). Bars indicate standard deviations. 1–200 U/ml of IL-4 significantly increased the number BrdU-positive cells ($P < 0.001$), while only 100 U/ml of IL-4 induced a statistically significant increase ($P < 0.001$) in the GM-CSF-stimulated group, as compared to non-IL-4-stimulated control in each group.

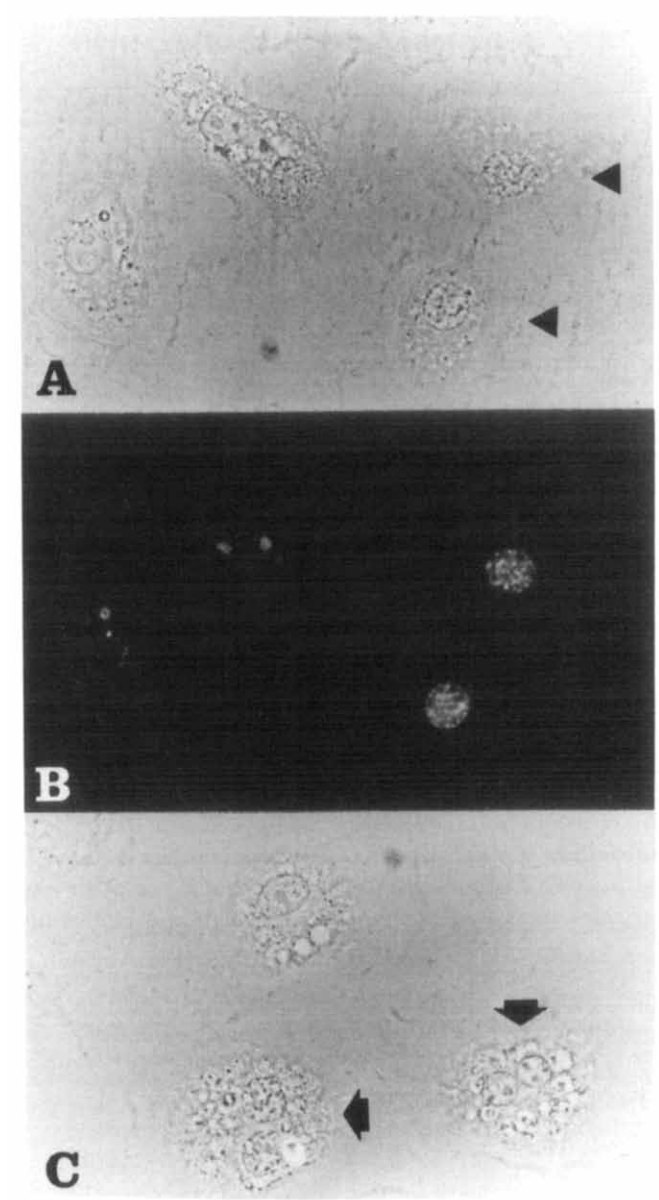

Fig. 8. BrdU-positive microglia in IL-4 stimulated culture. Viewed with phase (**A, C**) and fluorescein optics (**B**). Two ameboid microglia (arrowhead) incorporated BrdU into their nuclei. Some of these ameboid microglia were multinucleated (arrow).

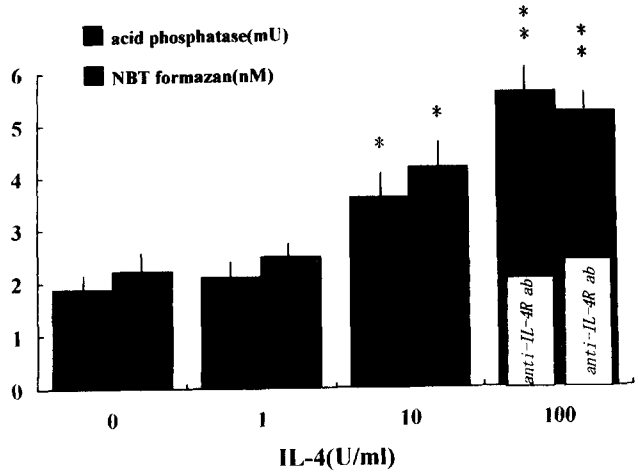

Fig. 9. Acid phosphatase activity and superoxide anion formation in microglia treated with indicated doses of IL-4. Columns indicate mean values of acid phosphatase activities (mU/ml) and superoxide anion formation expressed as nmol of formed NBT diformazan. Bars indicate standard deviations ($n = 9$). * Significant increase ($P < 0.001$) as compared to unstimulated control. ** significant increase ($P < 0.001$) as compared to the culture stimulated with 10 U/ml IL-4.

(Fig. 11, lane 1). IFNγ induced the expression of class II MHC antigen mRNA, while the addition of 10–100 U/ml IL-4 suppressed it. Addition of anti-IL-4R antibody ($1:10^3$ final concentration) reversed this suppressive effect of IL-4. IL-4 itself did not induce class II MHC antigen mRNA in microglia (Fig. 11).

4. Discussion

This study demonstrated that microglia express functional IL-4 receptors, but not produce IL-4. Receptors for IL-4 are expressed by various types of hematopoietic and non-hematopoietic cells (Ohara and Paul, 1987; Lowenthal et al., 1988). In the neural cells, the presence of IL-4 receptors on a neuroblastoma cell line or a brain stroma cell line has been shown by receptor–ligand binding analysis (Lowenthal et al., 1988). However, it was not known which type of cells in the CNS express IL-4R, and whether or not these receptors function. It has recently been shown that IL-4 affects the survival of hippocampal neurons in culture (Araujo and Cotman, 1993). Since the number of GFAP-positive astrocytes and Mac 1-positive microglia was also increased by the IL-4 treatment, these authors suggested that the neurotrophic effects of IL-4 may be indirect via factors elaborated by glial cells (Araujo and Cotman, 1993). IL-4 has been shown to increase the expression of nerve growth factor (NGF) mRNA and the secretion of NGF by astrocytes (Awatsuji et al., 1993). It has also been shown that IL-4 suppresses TNFα and nitric oxide production by activated microglia (Chao et al., 1993). These studies strongly suggest an effect of IL-4 on the glial cells and the presence of IL-4R on such cells. More recently, in an investigation of the expression of mRNA for various cytokine receptors in isolated glial cells and neuronal cell line by the RT-PCR method, we found that microglia, but not the neuronal cell lines, expressed the IL-4 receptor mRNA in vitro (Sawada et al., 1993).

Recombinant IL-4 induced the proliferation of microglia as determined by MTT colorimetric assay, [^{3}H]thymidine uptake and BrdU incorporation. Since the mitogenic effects of IL-4 was neutralized by adding anti-IL-4 receptor monoclonal antibody, IL-4 seemed to exert its action by binding with the IL-4 receptors on microglia. As we demonstrated previously (Sawada et al., 1990; Suzumura et al., 1990, 1991), microglia respond to such colony-stimulating factors as GM-CSF and M-CSF and proliferate in vitro. These cytokines

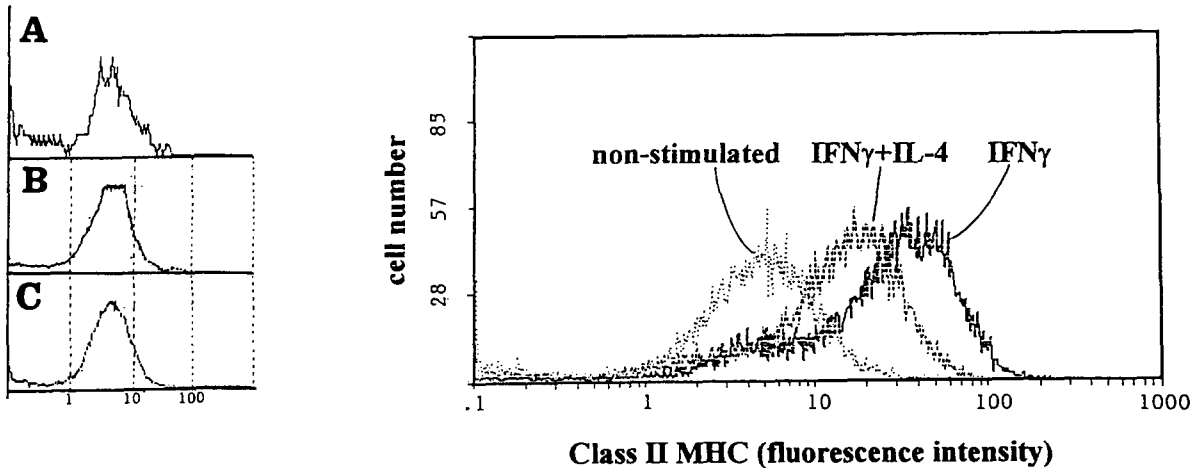

Fig. 10. Expression of class II MHC antigens on microglia. Left: Fluorescence histogram of controls, either IFNγ-stimulated cultures or IFNγ + IL-4-stimulated culture stained with control antibody; anti-Ia^d (A, B), were almost identical to that of non-stimulated culture stained with appropriate anti-Ia^k (C). The histogram represents fluorescence intensity on x-axis and cell number in y-axis. Right: The fluorescence intensity at the peak of the histogram in IFNγ-stimulated, IFNγ + IL-4-stimulated and non-stimulated was 35, 17 and 5.2, respectively.

are the only ones reported to induce the proliferation of microglia in vitro. IL-4 synergistically increased the proliferation of microglia with these colony-stimulating factors. In human monocyte-macrophages, IL-4 reportedly suppressed the mitogenic effects of GM-CSF, but not those of M-CSF, although IL-4 itself did not induce proliferation in these cells (Akagawa and Takasuka, 1992). Since microglia and astrocytes do not produce IL-4 in vitro, and since IL-4 mRNA is not detected in the normal brain (Wucherpfennig et al., 1992), the IL-4-induced proliferation of microglia may occur during an interaction with the invading T cells. Under these conditions, astrocyte-derived M- or GM-CSF may act synergistically with IL-4 to induce further proliferation of microglia. Although the M- or GM-CSF-induced proliferating microglia are rod-shaped, as we have shown previously (Suzumura et al., 1990, 1991), the IL-4-induced proliferating microglia were ameboid and sometimes had multiple nuclei (Fig. 8). An increased number of elongated rod-shaped microglia are observed in certain CNS infectious diseases (Schmidt and Gonyea, 1976), while numerous ameboid microglia are observed in CNS inflammatory lesions. Microglia nodules or increased number of multinucleated giant microglia have been observed in patients with viral encephalitis or those with AIDS-dementia complex (Sharer, 1992). In these inflammatory lesions, IL-4 may influence the accumulation of ameboid microglia or the formation of multinucleated cells. Indeed, IL-4 activity has been detected in the cerebrospinal fluid of animals with acute lymphocytic choriomeningitis virus infection (Moskophidis et al., 1991). IL-4 mRNA has been detected in the lesions of acute experimental allergic encephalomyelitis (Kennedy et al., 1992) as well as those of acute multiple sclerosis, but not in chronic lesions of the disease (Wucherpfennig et al., 1992). It is therefore possible that IL-4 plays a role in the proliferation of microglia in inflammatory lesions in the CNS.

Microglia exhibit a variety of functions on the development of pathological conditions in the CNS either as inflammatory cells or immunoregulatory cells. They may play a critical role on the development of immune-mediated inflammatory lesions in the CNS as antigen-presenting cells (Hickey and Kimura, 1988; Righi et al., 1989), and function as effector cells by secreting soluble factors including cytokines (Giulian, 1987; Sawada et al., 1989). Cytokines can influence microglial functions. IFNγ induces the expression of class II MHC antigen expression in microglia (Suzumura et al., 1987) and induces antigen-presenting activities (Righi et al., 1989). IL-1 reportedly induces IL-6 synthesis in microglia of the human embryo (Sebire et al., 1993). M-CSF activates acid phosphatase activity and superoxide anion formation by these cells (Sawada et al., 1990). In this study, we showed that IL-4 enhances acid phosphatase activity and superoxide anion formation, but suppressed the expression of class II MHC antigens induced by IFNγ. These findings suggest that IL-4 may upregulate the functions of microglia as inflammatory cells but suppress the functions as immunoregulatory cells. Although the precise functions of IL-4 on

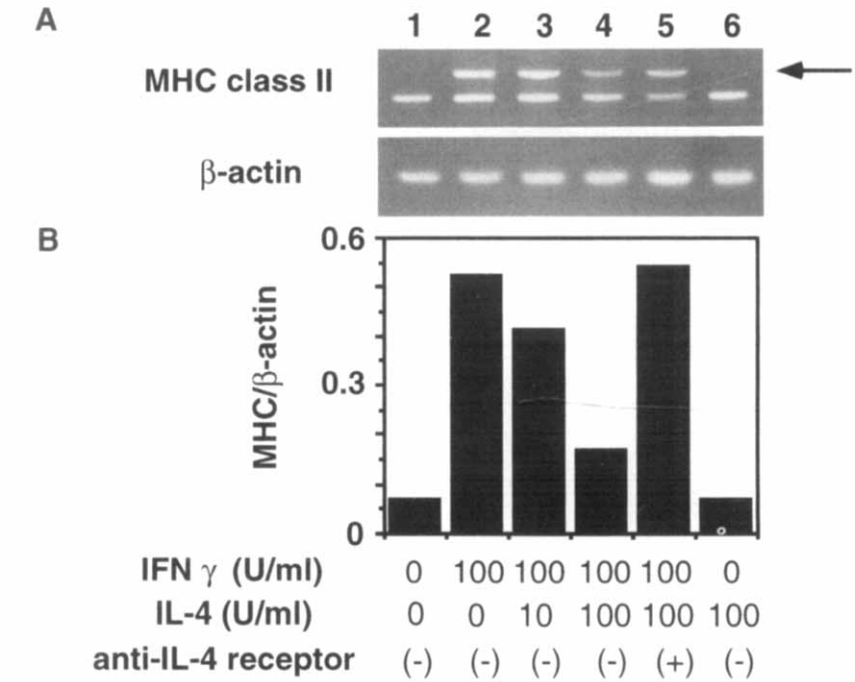

Fig. 11. Expression of mRNA for class II MHC antigens in microglia. (**A**) RT-PCR analyses for expression of class II MHC antigen mRNA and β-actin mRNA in microglia treated with IFNγ and/or IL-4, and anti-IL-4R antibody. (**B**) Closed columns indicate mean values of the ratio of class II MHC mRNA/β-actin mRNA, calculated by each value obtained from the TIAS-200 image analyzer.

microglia in the development of disease require further elucidation, since IL-4 was detected only in acute CNS lesions (Moskophidis et al., 1991; Hunter et al., 1992; Kennedy et al., 1992; Wucherpfennig et al., 1992), it is possible that IL-4 may influence the acute phase of inflammatory CNS lesions by affecting microglial functions. These effects of IL-4 on activities of cells of monocytic lineage may differ according to the species or the type of cells, since human IL-4 reportedly inhibits the production of superoxide, but enhances the acid phosphatase activity of human mononuclear phagocytes (Abramson and Gallin, 1990).

IL-4 reportedly upregulates the expression of class II MHC antigen in murine peritoneal macrophages in a manner different from that of IFNγ (Cao et al., 1989). However, in our experiments, IL-4 did not induce class II MHC antigens on naive microglia, and suppressed the IFNγ-induced class II MHC antigen expression in microglia. Since microglia do not usually express class II MHC antigens in unstimulated cultures (Suzumura et al., 1987) or the normal brain in vivo (Imamura et al., 1990), they may respond to IL-4 in a different manner than macrophages which constitutively express class II MHC antigens. Our results seem to be compatible with the current findings which suggest that T helper cells 1 and 2 and their secretory products may function in a distinct fashion on macrophages to either promote or suppress nonspecific inflammatory functions. IL-4, acting synergistically with IL-10 or TGFβ, inhibits the IFNγ-induced production of reactive nitrogen oxides by peritoneal macrophages, and also suppresses the killing of schistosomula by IFNγ-treated macrophages (Oswald et al., 1992). IL-4 also suppressed the IFNγ- and/or IL-2-induced expression of the cytokine gene in murine peritoneal macrophages (Gautam et al., 1992). We previously showed that TGFβ suppresses the IFNγ-induced expression of class II MHC antigen in microglia (Suzumura et al., 1993). In contrast to IL-4, TGFβ suppresses all microglial functions, including enzyme activity, cytokine production, and CSF-induced proliferation in vitro. Thus, IL-4 may be another negative regulator in the CNS cytokine network that regulate the functions of microglia differently as compared with TGFβ.

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