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CYTOKINE NETWORK IN THE CENTRAL NERVOUS SYSTEM AND ITS ROLES IN GROWTH AND DIFFERENTIATION OF GLIAL AND NEURONAL CELLS

MAKOTO SAWADA,*† AKIO SUZUMURA‡ and TOHRU MARUNOUCHI*

*Division of Cell Biology, Institute for Comprehensive Medical Science and ‡Department of Neurology, School of Medicine, Fujita Health University, Toyoake, Aichi 470-11, Japan

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Abstract—Cells resident within the central nervous system (CNS) can synthesize, secrete and respond to inflammatory cytokines not only contributing to the responses to injury or immunological challenge within the CNS, but also regulating their own growth and differentiation potential. The actions and cell communication via cytokines in the CNS are designated as the CNS cytokine network, in which microglia and astrocytes play the central roles. To further characterize the CNS cytokine network we investigated the differences in roles of these cells, and found that microglia might contribute to the early phase of cytokine production reaction and that astrocytes might contribute the late phase of the reaction. We also investigated roles of inhibitory cytokines such as TGF β, IL-4, and IL-10, and showed that each might play a distinct role in the inhibitory regulation in the CNS. We summarized our previous report about cellular distribution of cytokine receptors in the CNS cells and discussed their roles in the CNS cytokine network. Finally, we investigated that expression of IL-6 and IL-2 receptors in neuronal and oligodendrocytic differentiation, respectively. From these results, we discussed the features of the CNS cytokine network.

Key words: cytokine network, glial cells, neuronal cells, differentiation.

Cytokines are polypeptidic soluble factors that control the growth and differentiation of cells involved in immune and hematopoietic systems. These factors were initially considered to act on target cells in a cell-type and stage-specific manner. However, it has been shown that their biological actions are pleiotropic, complementary and counteractive; each of them exerts multiple effects on different cells, and different factors can act on the same cell populations to induce similar or opposite effects. Moreover, production of several cytokines is controlled by other cytokines in stimulatory or inhibitory manners and, in some cases, acts as a cascade. Therefore, these complex cytokine actions are referred to as a cytokine network.

Recent evidence suggests that bi-directional communication occurs between cells of the nervous and immune systems. The basis for this communication is the release of cytokines by immunocomponent cells, as well as hormone products of the neuroendocrine system. Cells resident within the central nervous system (CNS) can synthesize, secrete and respond to inflammatory cytokines not only contributing to the responses to injury or immunological challenge within the CNS, but also regulating their own growth and differentiation potential. There are many similarities of cytokine actions between the immune system and the CNS. However, there are also several unique modes of cytokine action in the CNS[26,29,37] (i.e. their target cells, inducing functions on the target cells such as other cytokine production, MHC antigens induction and cell growth control and inducing agents of their production). Therefore, the actions and communication of cytokines in the CNS are designated as the CNS cytokine network.[24]

Microglia, macrophage-like cells in the CNS, are one of the most important cells in the CNS cytokine network. They produce interleukin (IL)-1,[7] IL-5,[26] IL-6,[5,29] tumor necrosis factor-α (TNF-α)[25] and transforming growth factor-β (TGF-β).[45] They also respond to several cytokines such as colony-stimulating factors (CSFs),[20,31,38] IL-4,[37] TGF-β[39] and interferon-γ.[36] Microglial proliferation and other functions are regulated by CSFs including granulocyte–macrophage

†To whom all correspondence should be addressed.

Abbreviations: CNS, central nervous system; CSF, colony-stimulating factor; dbcAMP, dibutyryl cyclic AMP; GM-CSF, granulocyte–macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; M-CSF, macrophage colony-stimulating factor; MHC, major histocompatibility complex; LPS, lipopolysaccharide; TGF, transforming growth factor; TNF, tumor necrosis factor.

colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF) and IL-3.[31,38] GM-CSF and M-CSF are produced by astrocytes in the CNS,[18,20,43] and, therefore, astrocytes may play a role in controlling microglial growth and functions. Astrocytes are another cell population important in the CNS cytokine network and produce a variety of cytokines such as IL-1,[7] IL-5,[26] IL-6,[5,29] CSFs,[18,20,43] TGF-β[45] and TNF-α.[25] There are many similar aspects of cytokine production and responses between microglia and astrocytes, however, the roles in the CNS cytokine network may be different in these cells; microglia may play an important role in the removal of dead cells or their remnants by phagocytosis in brain injury while astrocytes provide structural and environmental support for neurons. There are no clear indications of the difference between the roles of microglia and astrocytes in the cytokine production and response.

Some of these cytokines control the growth, differentiation and activation of cells in the CNS and some modulate growth factor production, major histocompatibility complex (MHC) antigen expression and morphological changes.[19] Their biological functions are mediated by specific receptors, some of which are expressed in the CNS,[15,17,41] and recently cellular localization of cytokine receptors in the CNS has been demonstrated.[24] Neuronal cells and oligodendrocytes as well as microglia and astrocytes expressed several cytokine receptors indicating that certain functions of these cells may also be controlled by some cytokines. However, it is necessary for considerations of the involvement of cytokines in controlling the functions and development of CNS cells to elucidate the functional relationships of cytokines and these cells.

To further our understanding of the CNS cytokine network, in this study we investigated (1) differences in the production of cytokines between microglia and astrocyte, (2) the roles of inhibitory cytokines in the CNS, (3) cytokine receptor distribution in the CNS and (4) changes in cytokine receptor expression during neuronal and glial differentiation.

EXPERIMENTAL PROCEDURES

Cell cultures

Microglia, astrocytes and oligodendrocytes were purified from primary mixed brain cell cultures from neonatal C3H/HeN mice, as described previously.[30,33,34,36] The purity of each glia-enriched culture was determined by indirect immunofluorescence assay with cell-type-specific markers as demonstrated previously.[30,33,34,36] and was considered to be sufficient to assay mRNA expression by RT-PCR as described previously.[24] Glial cells were cultured in Falcon plastic tissue culture dishes (Becton Dickinson, Lincoln Park, NJ) containing Eagle's MEM supplemented with 10% fetal bovine serum, 0.5 μg/ml bovine insulin and 0.2% glucose. A neuronal cell line, N18, was cultured in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum in the presence of 1 mM dibutyryl cyclic AMP (dbcAMP) to induce neuronal differentiation[24] and total RNA was extracted at varying days after induction. A bi-potential glial precursor cell line, OS3, which differentiates into either astrocytes or oligodendrocytes depending on the culture conditions[21] was cultured in plastic tissue culture dishes containing Eagle's MEM supplemented with 10% fetal bovine serum, 0.5 μg/ml bovine insulin and 0.2% glucose.[21] For oligodendrocytic differentiation of OS3 cells, the cells were cultured in 2% serum-containing medium[21] and total RNA was extracted at varying days after induction.

Measurement of biological activity

Activities of IL-1 and IL-6 were determined by bioassay based on cell growth of IL-1- and IL-6-dependent cell lines such as D10 cells[11] (from Dr K. Onozaki, Nagoya City University) and MH60 cells[29] (from Dr T. Hirano, Osaka University, Japan), respectively. TNF-α activity was measured by colorimetric assay of cytotoxicity for L929 cells as described previously.[25] Briefly, overnight culture of L929 cells (2.5×10^4 cells/well) in the 96-well test plate was incubated with 250 ng/well of actinomycin D and various amounts of samples or recombinant TNF (Genzyme, Boston, MA) as a standard. After 24 hr incubation, viable cells were stained by 0.05% of crystal violet for 15 min, washed with PBS-, treated with 0.1% Triton X-100 and disrupted by sonication. The optical density at 620 nm of the solubilized materials was measured by an Immuno Reader J2000 (Inter Med Japan, Tokyo, Japan). The percent killing was calculated with the following equation:

$$\text{percent killing}\,(\%)=[(S-B)/(C-B)]*100,$$

where S means sample O.D., B means blank O.D. from experiment without L929 cells and C means control O.D. from experiment without any cytotoxic factor.

RNA preparation and RT-PCR

After 24 hr of conditioning or varying times of chemical treatments, about 1×10^6 cells were washed twice with PBS-, then used for RNA preparation. Total RNA preparation based on the acid–guanidine–phenol–chloroform extraction method and semiquantitative RT-PCR were performed as described previously.[26,27] The following primers were synthesized and used to detect mouse cytokine receptor mRNA: IL-3 receptor sense and antisense, 1193–1212 and 1602–1621 (Gene Bank, M29855); IL-4 receptor sense and antisense, 573–590 and 1122-1140 (Gene Bank, M29854); IL-5 receptor sense and antisense, 280–297 and 850–869 (Gene Bank, D90205); IL-6 receptor sense and antisense, 245–263 and 728–746 (Gene Bank, X53802); IL-7 receptor sense and antisense, 617–634 and 1143–1162 (Gene Bank, M29697); GM-CSF receptor sense and antisense, 1202–1220 and 1636–1656;[27] and M-CSF receptor sense and antisense, 811–832 and 1231–1248 (Gene Bank, X06368) of respective receptor cDNAs. These primers were useful for semiquantitation of mRNA expression in the RT-PCR method.[26]

Materials

Recombinant cytokines were obtained from Genzyme (Cambridge, MA). Lipopolysaccharide (LPS) from *E. coli* was from Difco (Detroit, MI). dbcAMP was purchased from Boehinger Mannheim Japan (Tokyo, Japan). Actinomycin D was purchased from Sigma (St. Louis, MO). Recombinant molony mouse leukemia virus reverse transcriptase and random primers were obtained from BRL (Bethesda, MD). RNasin and *taq* DNA polymerase were obtained from Promega (Madison, WI).

RESULTS

Differences in CNS cytokine production between microglia and astrocytes

To examine the roles of microglia and astrocytes in CNS cytokine production, we investigated the time-course of TNF-α production in microglia and astrocytes under LPS stimulation. After LPS stimulation, TNF-α activity in microglial cultures increased rapidly, reached a maximal level at 3 hr and remained at the maximal level during stimulation (Fig. 1). In astrocyte cultures, however, TNF-α activity gradually increased, reached a maximal level at 5 hr and thereafter decreased to basal level (Fig. 1). These patterns of TNF-α activity in microglia and astrocytes were supported by mRNA expression detected by a semiquantitative RT-PCR method (data not shown).

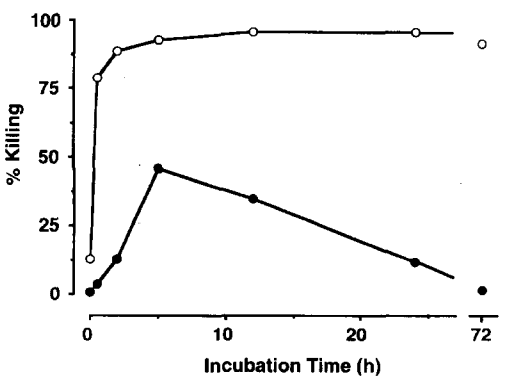

Fig. 1. Production of TNF-α activity in LPS-activated microglia and astrocytes. After 24 hr of conditioning, purified microglia and astrocytes were stimulated by 100 ng/ml of LPS, and centrifuged and supernatant fractions were collected at the indicated times. TNF-α activity was then measured as described in the Experimental Procedures. Open circles and filled circles indicate TNF-α activity in microglial and astrocytic supernatants, respectively. The indicated results were the means of two independent experiments.

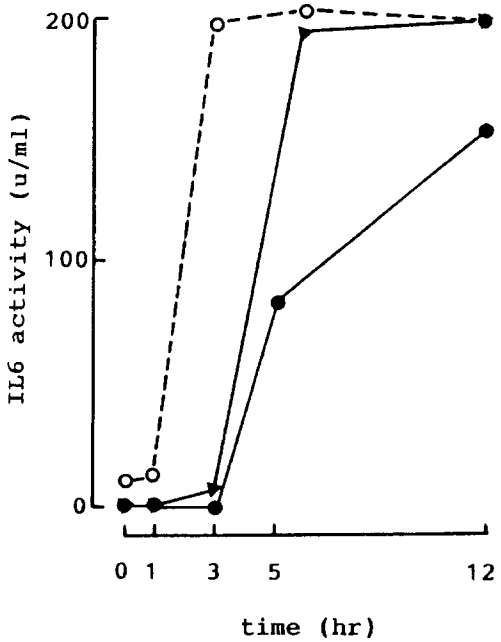

Fig. 2. Production of IL-6 activity in LPS-activated microglia and LPS- and TNF-α-activated astrocytes. After 24 hr of conditioning, purified microglia and astrocytes were stimulated by 100 ng/ml of LPS, or 100 U/ml of TNF-α, and centrifuged and supernatant fractions were collected at the indicated times. IL-6 activity was then measured as described in the Experimental Procedures. Open circles, triangles and filled circles indicate IL-6 activity from LPS-activated microglial, LPS-activated and TNF-α-activated astrocytic supernatant, respectively. The indicated results were typical ones in three independent experiments.

Since TNF-α induced IL-6 activity in astrocytes but not in microglia,[29] we compared the onset of IL-6 production in LPS-stimulated microglia, and LPS- and TNF-α-stimulated astrocytes. As shown in Fig. 2, the onset of IL-6 production was slower in astrocytes than in microglia, and in astrocytes, TNF-α induced IL-6 activity more slowly than LPS.

We also investigated IL-1α expression in astrocytes. Although microglia expressed IL-1α mRNA only after stimulation with LPS, IL-1α transcript expression was observed in astrocytes after IFN-γ and TNF-α treatment (Fig. 3A). The onset and time to reach maximum level were compared in LPS-stimulated microglia, LPS-stimulated astrocytes and IFN-γ-stimulated astrocytes (Fig. 3B). Microglia expressed IL-1-α mRNA under normal culture conditions and the expression reached 90% of maximum 1 hr after LPS stimulation. In astrocytes, IL-1-α mRNA was detected 1 and 0.5 hr after LPS- and IFN-γ-stimulation, respectively, reaching a maximal level at 5 and 3 hr, respectively.

Inhibitory cytokines in the regulation of microglial functions

We investigated the effects of the inhibitory cytokines, such as TGF-β, IL-10 and IL-4 on microglial functions and growth (Table 1). TGF-β suppressed IFN-γ-induced MHC class I/II expression, LPS-activated cytokine production and biochemical functions, and CSF-induced growth. IL-10 decreased LPS-activated cytokine production and biochemical activities, but did not affect CSF-induced growth. IL-4 inhibited IFN-γ-induced MHC class I/II expression but increased growth and LPS-activated biochemical functions.

Cytokine receptor expression in CNS cells

We measured the expression of mRNA for receptors for IL-3, IL-4, IL-5, IL-6, IL-7, GM-CSF and M-CSF in mouse neuronal line cells, microglia-, astrocyte-, and oligodendrocyte-enriched cultures by semiquantitative RT-PCR method, and summarized the results (Table 2). Higher levels of IL-3, IL-7 and GM-CSF receptor mRNA expression were observed in microglia as compared to those in other cells. Expression of IL-3 and IL-4 receptor mRNAs were observed in microglia and oligodendrocytes, and IL-6 receptor mRNA expression was detected in the microglia,

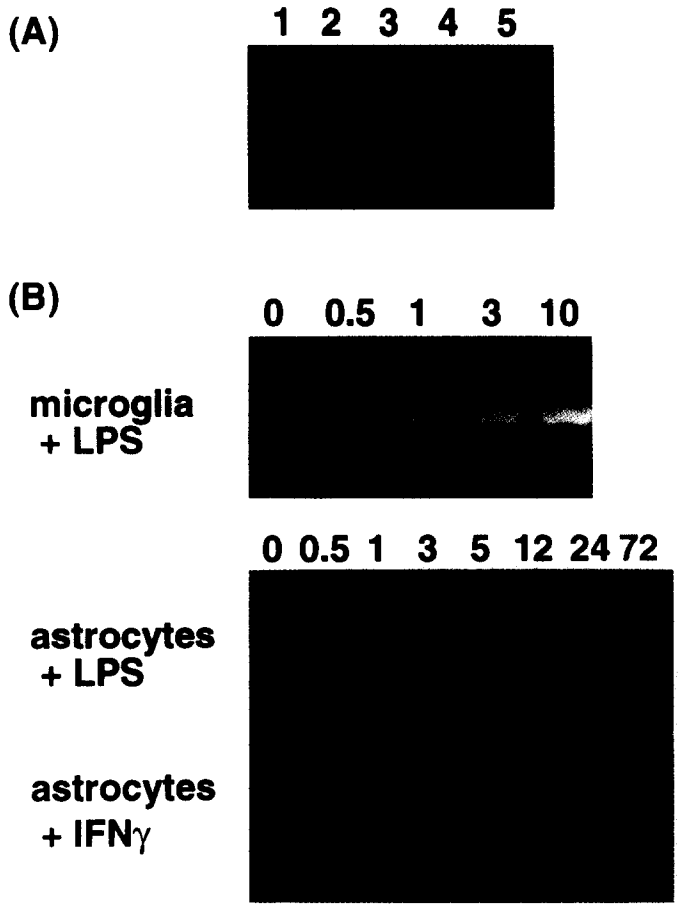

Fig. 3. (A) Effects of various treatments on astrocytic IL-1-α mRNA expression. Astrocytes were treated without (lane 1) or with LPS (100 ng/ml, lane 2), TNF-α (100 U/ml, lane 3), dbcAMP (1 mM, lane 4), and IFN-γ (100 U/ml, lane 5) for 12 hr (B) Time-dependent expression of IL-1-α mRNA in LPS-activated microglia (upper), LPS-activated (middle) and IFN-γ-activated astrocytes (lower). After 24 hr of conditioning, purified microglia and astrocytes were stimulated by 100 ng/ml of LPS or 100 U/ml of IFN-γ. Total RNA was prepared from microglia and astrocytes at the indicated times after stimulation and analyzed by semiquantitative RT-PCR.

Table 1. Inhibitory cytokines in the CNS

	TGF-β*	IL-10†	IL-4‡
IFN-γ-dependent			
MHC antigen expression	↓	N.D.	↓
LPS-activated			
Cytokine production	↓	↓	→
Biochemical activity	↓	↓	↑
Growth	↓	→	↑

↓, decrease. ↑, increase. →, no change. N.D., not determined.
*Ref. 39; †*Neurochem. Res.* (1994), in press; ‡Ref. 37.

astrocytes and N18 neuronal cells. Expression of IL-7 and M-CSF receptor mRNA was detected in microglia, astrocytes and oligodendrocytes. Expression of GM-CSF receptor mRNA was detected in all cells examined, while IL-5 receptor mRNA was not detected in any of the cells examined.

Cytokine receptors involved in neuronal and oligodendrocytic differentiation

Although untreated N18 cells did not express IL-6 receptor mRNA, those treated with dbcAMP were positive for this transcript as mentioned above. Therefore, we determined IL-6 mRNA level as a function of time after dbcAMP treatment. dbcAMP-treated N18 cells stopped proliferating and

Table 2. Relative expression levels of each cytokine receptor mRNA in neural cells

	IL-3R	IL-4R	IL-5R	IL-6R	IL-7R	GM-CSFR	M-CSFR
Microglia	+++	++	−	+++	+++	+++	++
Astrocytes	−	−	−	++	+	++	++
Oligodendrocytes	++	++	−	−	+	+	+++
N18	−	−	−	+	−	+	−
N1E115	−	−	−	−	−	+	−

Relative levels of expression were determined using a TIAS 2000 Image Analyzer and are indicated as follows:
+++, high (more than respective control β-actin level)
++, moderate (comparable to respective control β-actin level)
+, low (1/3 to 1/10 of respective control β-actin level)
−, not detected (less than 1/10 of respective control β-actin level).
IL-3R, IL-3 receptor; IL-4R, IL-4 receptor; IL-5R, IL-5 receptor; IL-6R, IL-6 receptor; IL-7R, IL-7 receptor.

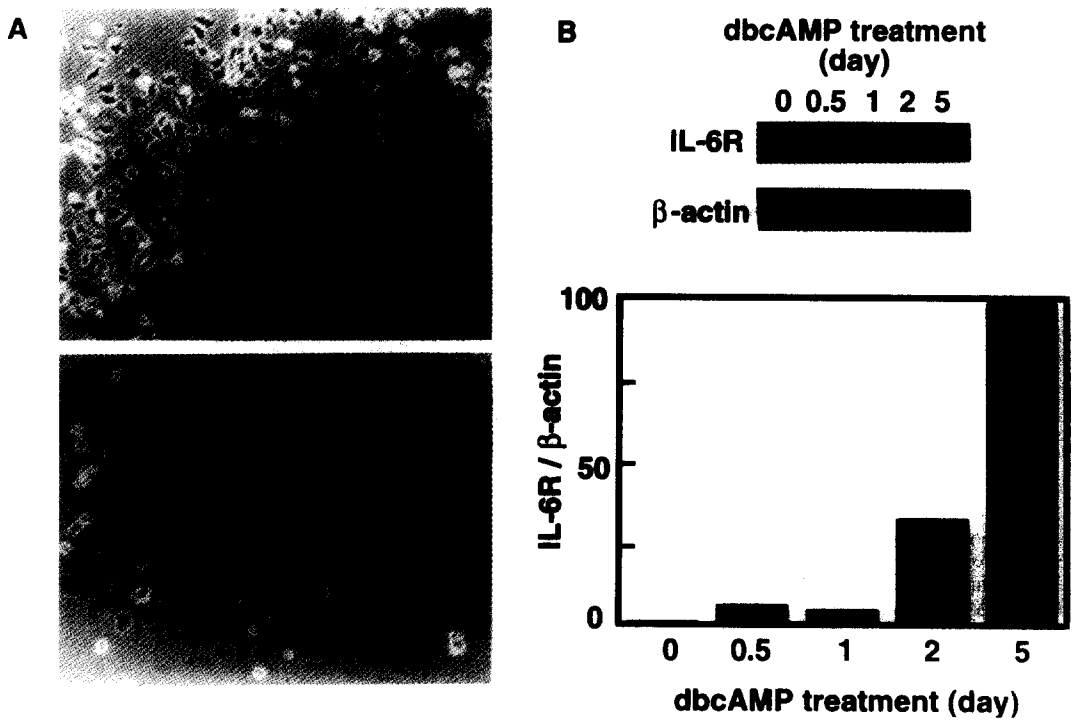

Fig. 4. (A) Morphological changes in N18 cells treated with dbcAMP. N18 cells were treated without (upper) or with 1 mM dbcAMP for five days (lower). (B) Time-dependent expression of IL-6 receptor mRNA in dbcAMP-treated N18 cells. Total RNA was prepared from N18 cells at the indicated times after dbcAMP stimulation and analyzed by semiquantitative RT-PCR. Lower panel indicates results of semi-quantitative analysis. Columns indicate the mean values of the ratio of IL-6 receptor mRNA/β-actin mRNA, calculated from the respective values obtained with the TIAS-2000 image analyzer.

were induced to differentiate into neuronal cells which had bipolar long neurites (Fig. 4A). During this process, IL-6 receptor mRNA was expressed and increased in a time-dependent manner (Fig. 4B).

As shown in Table 2, purified oligodendrocytes expressed the IL-2 receptor. Therefore, we investigated changes in IL-2 receptor mRNA expression during oligodendrocytic differentiation in OS3. In the presence of 10% serum, very low levels of IL-2 receptor mRNA were detected and these increased gradually after serum was decreased to 2% (Fig. 5).

DISCUSSION

Roles of microglia and astrocytes in the CNS cytokine network

We have previously reported that there were differences in the agents inducing IL-6 and in the onset of IL-6 gene expression and production between astrocytes and microglia.[29] We also reported

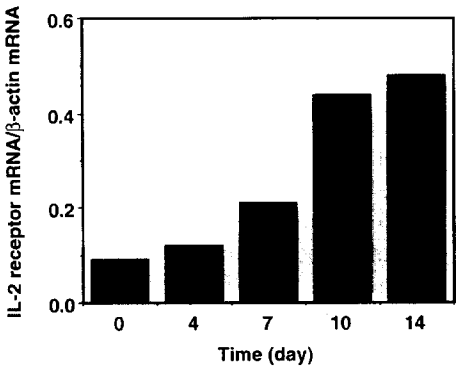

Fig. 5. Time-dependent expression of IL-2 receptor α chain mRNA in OS3 cells cultured in 2% serum. Total RNA was prepared from OS3 cells at the indicated times after medium change and analyzed by semiquantitative RT-PCR. Columns indicate the mean values of the ratio of IL-2 receptor mRNA/β-actin mRNA, calculated from the respective values obtained with the TIAS-2000 image analyzer.

previously that sensitivity in production of TNF-α to LPS was different in microglia and astrocytes.[25] Thus, we speculated that microglia and astrocytes might play distinct roles in the CNS cytokine production. In this paper, we examined the time-course of TNF-α production in microglia and astrocytes under continuous stimulation by LPS and found the differences between microglia and astrocytes in TNF-α production (Fig. 1). The results suggested that astrocytes were desensitized under continuous stimulation with LPS, and the signal in astrocytes to induce TNF-α expression and production was turned off, while that in microglia was not and they responded to LPS continuously.

The slower onset of cytokine production in astrocytes than in microglia was observed in the onset of IL-6 production (Fig. 2). Thus, IL-6 production by astrocytes and microglia appear to be regulated by different intracellular mechanisms or different promoter/enhancer elements of the IL-6 gene, the existence of which has been reported previously.[32,42,46] Furthermore, different regulatory mechanisms were involved in IL-6 expression in response to LPS and TNF-α even in the same cell, because the onset of IL-6 production in astrocytes was different between LPS- and TNF-α stimulations (Fig. 2). This speculation is consistent with the previous suggestion that different nuclear factors and enhancer elements induce IL-6 gene expression in response to LPS and TNF-α.

We also investigated IL-1-α expression in astrocytes, and found the similar pattern of difference in IL-1-α production between microglia and astrocytes (i.e. onset, duration and spectra of the inducing agents).

These results suggest that the response of CNS cytokine expression can be divided into three types based on the onset of production; early, intermediate and late responses. Microglia may be involved in reaction of the early response when activated. With the same stimulation as microglial activation, astrocytes produce cytokines at intermediate onset. It is possible that late response may include astrocytic responses stimulated by cytokines such as IL-1 and TNF-α, which are produced in early and/or intermediate responses.

We found that responses of astrocytic production of cytokines such as TNF-α and IL-1-α was desensitized (Fig. 1 and Fig. 3B). This may be involved in the termination of cytokine production in addition to the action of the inhibitory cytokines as discussed below.

It has been reported that the agents inducing cytokines are cell-type-specific; in IL-6 production, IL-1 and TNF-α for fibroblasts,[16] LPS for monocyte–macrophages[1] and phorbol esters and phytohemagglutinin[12] for T-cells. Since microglia are thought to be of the monocyte–macrophage lineage, our results are consistent with those of the previous report.[1] However, astrocytes had a mixed type of IL-6 response, suggesting they may be classified as a new category of IL-6-producing cells.

Inhibitory cytokines in the CNS cytokine network[37,39]

There are mechanisms of inhibitory regulation in the peripheral immune system which control overactivity of immune reactions including overproduction of cytokines. Cytokines involved in this regulation are TGF-β, IL-10 and, in part, IL-4. TGF-β reduces macrophage activation such as

IFN-γ-induced MHC class I/II expression and microbicidal function.[3,44] IL-10 down-regulates activated production of several cytokines by lymphocytes and macrophages.[13] IL-4 has been reported to have a suppressive effect on several functions of macrophages.[4,6,9] Therefore, we investigated the effects of these three cytokines on microglial functions and growth (Table 1). The results suggested that these inhibitory cytokines might have distinct roles in regulation of the CNS cytokine network. TGF-β seemed to be a totally inhibitory factor which suppressed all functions and growth of microglia, while IL-10 seemed to exert inhibitory regulation on cytokine production. IL-4 seemed to be an inhibitory factor for microglial immune function, but to be an activating factor for microglial growth and phagocytic functions. These factors may control microglial growth and functions in different pathophysiological conditions.

Cytokine receptors in the CNS

The biological functions of cytokines are mediated by specific receptors. The cDNAs of many cytokine receptors that operate in the immune and hematopoietic systems have been cloned[40] and some are expressed in the CNS.[15,17,41] Recently, we demonstrated the cellular localization of cytokine receptors in the CNS.[24] Here, we summarized and reviewed the results concerning the expression of mRNA for receptors for IL-3, IL-4, IL-5, IL-6, IL-7, GM-CSF and M-CSF in mouse neuronal line cells, microglia-, astrocyte-, and oligodendrocyte-enriched cultures (Table 2). Microglia expressed mRNA for receptors of IL-3, IL-4, IL-6, IL-7, GM-CSF and M-CSF. Astrocytes were positive for receptors of IL-6, IL-7, GM-CSF and M-CSF. Oligodendrocytes were positive for receptors of IL-3, IL-4, IL-7, GM-CSF and M-CSF. Neuronal cells expressed receptors of IL-6 and GM-CSF with very low levels.

IL-3, GM-CSF and M-CSF are growth factors for monocyte lineage cells including microglia.[38] These cytokines induce microglia to proliferate, activate their biological activity and change their shape.[31,35] We demonstrated that microglia expressed mRNAs encoding IL-3, GM-CSF and M-CSF receptors at relatively high levels as compared to those in other cells (Table 2). This result was compatible with our previous finding that immunoreactivity for the *c-fms* protein, an M-CSF receptor, was localized on isolated microglia.[31] We found that oligodendrocytes also expressed mRNAs for IL-3, GM-CSF and M-CSF receptors, but further studies are needed to elucidate the functions of these cytokines on oligodendrocytes. We also found that dbcAMP-induced neuronal line cells to express GM-CSF receptor mRNA. This finding was supported by the evidence that GM-CSF induces neurite outgrowth activity in primary neurons.[14]

IL-4, IL-5, IL-6 and IL-7, which have been found to be growth and/or differentiation factors for B-cells, are multipotent cytokines some of which control the function of neural cells. IL-4 modulates microglial growth and functions,[37] IL-4 receptor mRNA was found in microglia and oligodendrocytes and IL-5 is produced by microglia and astrocytes,[26] and induces NGF production by astrocytes.[10] Therefore, it is possible that astrocytes express the IL-5 receptor. However, no detectable IL-5 receptor mRNA was observed in neural cells examined under our experimental conditions. IL-6 is also produced by microglia and astrocytes,[29] and has been shown to modulate microglial function (unpublished observation), neuronal cell survival and differentiation.[8] IL-6 receptor mRNA was observed in microglia and astrocytes at high levels and dbcAMP-induced N18 neuronal cells at a very low level. IL-7 receptor mRNA was observed in three types of glial cells, but its function in the CNS cells has not yet been determined.

Although their functions are not fully elucidated, they may be involved in the CNS cytokine network. Ligands for some receptors are produced in the CNS and, therefore, may interact with their own receptors on the CNS cells and modulate cellular activity, proliferation and differentiation in both autocrine and paracrine fashions. The other cytokine receptors whose ligands are not produced in the CNS may be involved in communication between cells of the nervous and immune systems. Both types of humoral communication may be crucial for developmental or pathological processes in the CNS.

Cytokine receptors in neuronal and glial cells development

It seems that cytokines in the CNS might contribute to CNS development because they regulate growth and differentiation of neural cells *in vitro*. Thus, expression of their receptors was thought to change during cellular differentiation. Therefore, we investigated mRNA expression of cytokine

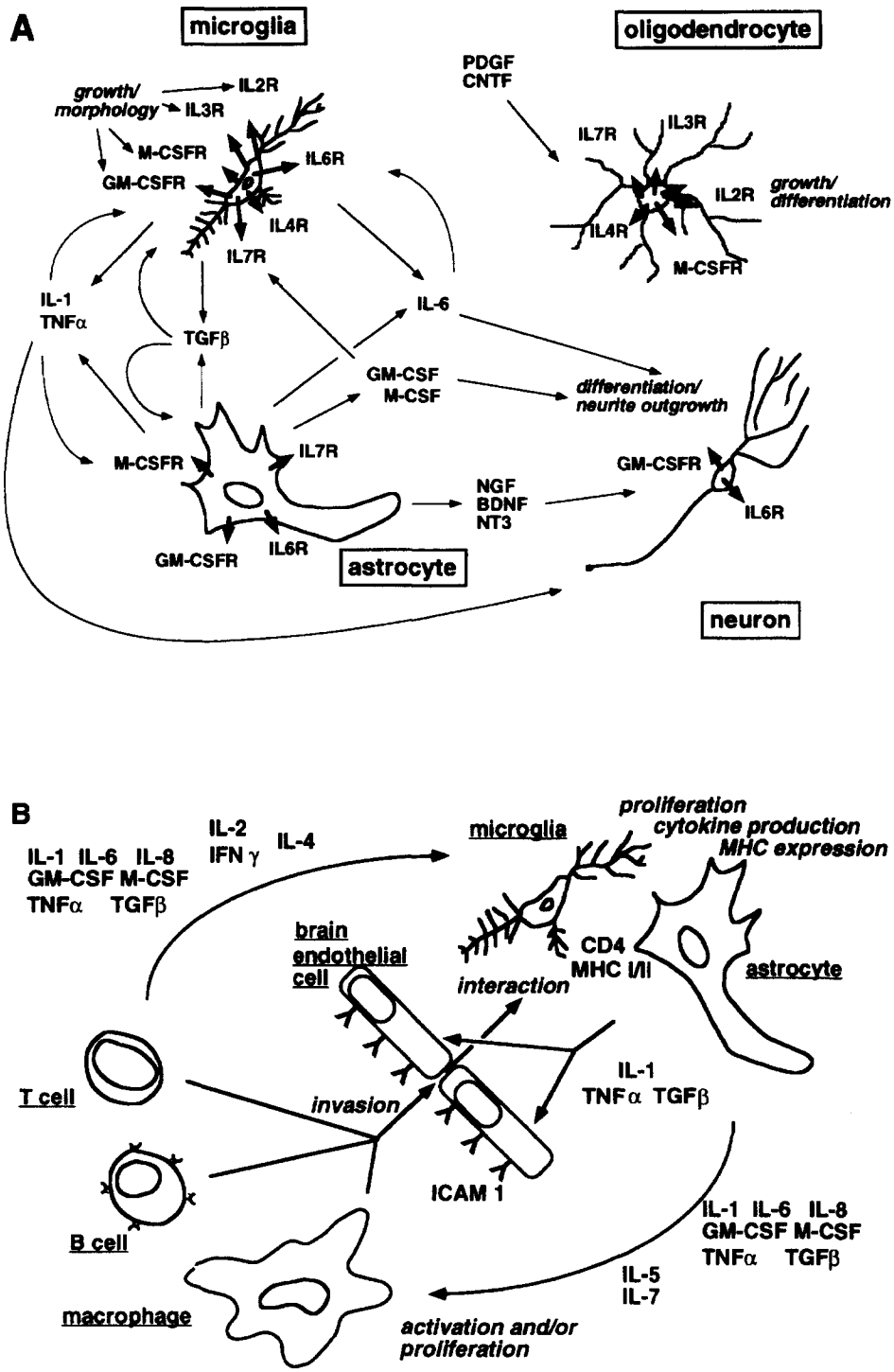

Fig. 6. Schematic diagrams of the CNS cytokine network. (A) Roles in regulation of growth and differentiation of neural cells, and production of cytokines in the CNS. (B) Interaction with the immune system.

receptors and showed changes in expression of IL-6 and IL-2 receptors during neuronal and oligodendrocytic differentiation, respectively.

During N18 cell differentiation by dbcAMP, IL-6 receptor mRNA was expressed and increased in a time-dependent manner (Fig. 4B). We also found that IL-6 had a regulatory role in neuronal differentiation and cell survival during dbcAMP treatment (unpublished observation). This was supported by the evidence that IL-6 exerts both neurite outgrowth activity and cell survival effects on primary neurons[8] and PC12 cells.[23]

OS3 cells are bi-potential glial cells.[21] While they have morphological similarity to type-2 astrocytes and express an astrocytic marker, GFAP, when cultured in the presence of 10% serum, they undergo morphological transformation, decrease astrocytic phenotype and express the oligodendrocytic markers galactoserebroside (GalC) and O4 antigen when cultured in the presence of 2% serum or in some chemically defined media.[21] This process seems to correlate the oligodendrocyte differentiation *in vivo*.[21] IL-2 receptor mRNA expression increased gradually during oligodendrocytic differentiation in OS3 (Fig. 5). As shown in Table 2, purified oligodendrocytes expressed the IL-2 receptor. IL-2 has been reported to act as a mitogen on immature oligodendrocyte progenitor cells,[2] to stop the proliferation of mature oligodendrocyte progenitors[22] and to induce differentiation of oligodendrocytes to mature myelin basic protein-positive forms.[2] This suggests the possibility that the IL-2 receptor may involve oligodendrocyte differentiation.

CONCLUSION

There are two aspects of roles of the CNS cytokine network, one of which is regulation of growth and differentiation of neural cells and production of cytokines in the CNS (Fig. 6A). Microglia and astrocytes play central roles in the CNS cytokine network and they produce a variety of cytokines, some of which seem to be unique, while others are complementary and redundant. Since microglia are involved in early responses, they may initiate the CNS cytokine reactions. Astrocytes are involved in intermediate/late responses and, thus, may expand the CNS cytokine reactions. These two types of cells control their cell activities including growth, viability, cytokine production, morphological transformation and differentiation in paracrine and/or autocrine fashions. Cytokines produced by these cells may also contribute to the growth and differentiation of other CNS cells, including neurons and oligodendrocytes.

Another aspect of the CNS cytokine network is its interaction with the immune system (Fig. 6B). Since the majority of cytokines are produced by immune cells, it is possible that immune cells may also control the growth and function of CNS cells. For example, T-cell factors, GM-CSF and IL-3 enhance microglial growth,[20,38] and IFN-γ induces MHC antigen expression on microglia and astrocytes.[36] Moreover, other T-cell factors such as IL-4[37] and TGF-β[39] have been shown to control microglial proliferation or functions positively and negatively. Therefore, the control of microglial growth by T-cell factors are one of important methods of regulation of CNS cells by peripheral immune cells. In addition to this, cytokines produced in the CNS can control immune cell functions. For example, microglia and astrocytes can produce IL-5 but none of the CNS cells express its receptor. Therefore, it is possible that CNS-derived IL-5 may control immune cells within and/or out of the CNS. These communications may be important for brain-immune interactions and some aspects may play crucial roles in certain pathological conditions such as AIDS–dementia complex and multiple sclerosis.[28]

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