Differential effects of Th1 and Th2 derived cytokines on NGF synthesis by mouse astrocytes

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Received 3 July 1996; revised version received 4 August 1996

Abstract During the course of brain injury and inflammation there is an increased secretion of neurotrophic substances by astrocytes. We have examined the effect of the Th2-derived cytokine IL-10 and the Th1-derived cytokines Il-2 and IFN-γ on the secretion of NGF by mouse astrocytes. IL-10 induced a dose-dependent increase in NGF secretion which was blocked by anti-IL-10 antibody. In contrast, the Th1-derived cytokines IFN-γ and IL-2 did not induce NGF synthesis. Moreover, INF-γ completely inhibited the increase in NGF secretion induced by IL-10 whereas it had no effect on the induction of NGF by TNF-α. These results indicate that IL-10 similarly to other Th2-derived cytokines may provide a neurotrophic support to injured neurons via the induction of NGF synthesis, whereas the Th1-derived cytokine IFN-γ antagonizes this effect.

Key words: Nerve growth factor; IL-10; IFN-γ; Brain inflammation; Astrocyte

1. Introduction

Pathological conditions in the brain lead to activation of astrocytes and microglia cells in the vicinity of the insult [1]. These cells, as well as various lymphoid cells which infiltrate the CNS, secrete a large number of cytokines which then act on glial and neuronal cells [2]. Reactive astrocytes are also known to synthesize and secrete neurotrophic factors such as NGF [1] which play a role in the survival, growth and differentiation of CNS neurons [3]. Induction of NGF synthesis occurs following brain injury [4] and in response to a variety of cytokines such as FGF, IL-1β, TNF-α [5] and the Th2-derived cytokines IL-4 and IL-5 [6,7].

IL-10 is another Th2-derived factor which was originally identified as a factor which inhibits cytokine synthesis by Th1 clones [8]. IL-10 is produced by T cells, B cells and monocytes and it induces DNA replication and Ig secretion in antigen and anti-CD40 activated B cells [9]. In addition, IL-10 has been reported to enhance the expression of MHC class II [10] and the proliferation of T cells in the presence of IL-2 and IL-4 [11]. IL-10 has been also considered as a negative regulator of immune system activation due to its ability to inhibit cytokine production and IFN-γ-induced MHC class II expression [12] and iNOS induction by IFN-γ [13]. These observations implicate IL-10 as a negative regulator of immune system activation.

In the CNS, IL-10 mRNA has been demonstrated in mice recovered from experimental autoimmune encephalomyelitis [14], during *Listeria* meningitis [15] and in acute Sindbis virus-induced encephalitis [16]. In addition, IL-10 and its receptor are expressed by microglia and astrocytes [17]. In this study we examined the effects of the IL-10 and the Th1-derived cytokines, IL-2 and IFN-γ on the secretion of NGF by astrocytes.

2. Materials and methods

IL-10 and neutralizing anti-IL10 antibody, IL-2, TNF-α and IFN-γ were purchased from Genzyme. Monoclonal anti-NGF and monoclonal anti-NGF conjugated to β-galactosidase were obtained from Boehringer Mannheim and polyclonal anti-rabbit NGF was purchased from Sigma.

2.1. Cell cultures

Primary glial cultures were obtained from cerebral cortices of 2-day old newborn mice as previously described [18]. Under culture conditions, neurons and oligodendrocytes were eliminated and the culture consisted of 95% astroglia cells as determined by GFAP staining.

2.2. NGF assay

For the determination of NGF secretion, astrocytes (2×10^6 cells/ml) were incubated with the appropriate treatments and supernatants were collected every 24 h. NGF levels were assayed using a two-site enzyme-linked immunoabsorbent assay as already described [7]. The detection limit of this assay was about 5 pg/ml.

2.3. Intracellular cell staining

Cells were stimulated for 16 h with IL-10 (25 ng/ml) in the presence of 0.3 μM monensin. Intracellular staining for NGF was performed according to a modification of the method described by Jung et al. [19]. Briefly, cells (5×10^5/ml) were washed with HBSS and fixed in ice-cold HBSS containing 4% paraformaldehyde (PFA) for 10 min. After two washes in HBSS, cells were suspended. Cells were fixed in methanol for 10 min at 0°C. Following blocking, cells were resuspended in 300 μl of staining buffer (HBSS including 0.1% saponin, 3% BSA and 0.01 M HEPES buffer). After 20 min incubation cells were spun down and incubated with rabbit anti-NGF antibody for 30 min followed by incubation with FITC-conjugated goat anti-rabbit antibody diluted in staining buffer. After washings, cells were analyzed with a FACSscan analyzer and values of % positive cells and mean fluorescence intensity (MFI) were calculated. For calculating non-specific binding, cells were incubated with rabbit control antibodies.

2.4. Protein assay

Protein content of the cells was determined according to the method of Lowry et al. [20] using bovine serum albumin as a standard.

2.5. Statistical analyses

All data were analyzed using Student's *t*-test for comparison of the means.

3. Results

3.1. IL-10 increases NGF secretion

For measuring secreted NGF, cells were treated for the indicated times with various concentrations of IL-10 in the presence and absence of anti-IL-10 antibody. Control untreated cells produced low amounts of NGF. Treatment of

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PII S0014-5793(96)00911-8

the cells with IL-10 induced a dose-dependent increase in NGF production. No changes in the amount of secreted NGF were observed in cells treated with low concentrations of IL-10 (1 ng/ml), however, an increase of 45% was observed with 5 ng/ml IL-10 and a maximal increase of 160% was obtained with a concentration of 25 ng/ml (Fig. 1A). The induction of NGF by IL-10 was inhibited by addition of anti-IL-10 antibody. The time course of IL-10 effect was monitored over the 72 h culture period and supernatants were sampled every 24 h. The effect of IL-10 was already observed after 24 h and plateau levels were obtained after 48 h (Fig. 1B). The effect of IL-10 on NGF induction was not associated with cell proliferation since IL-10 did not increase this parameter but rather exerted a small decrease (data not shown).

3.2. *IL-10 increases intracellular staining for NGF*

To study the effects of IL-10 on NGF synthesis we performed intracellular staining of NGF. Since the effect of IL-10 on NGF secretion was first observed after 24 h, we stained the cells 16 h following treatment with the cytokine. As can be seen in Fig. 2, NGF was synthesized by the majority of the astrocytes except for a small subpopulation of the cells (15%).

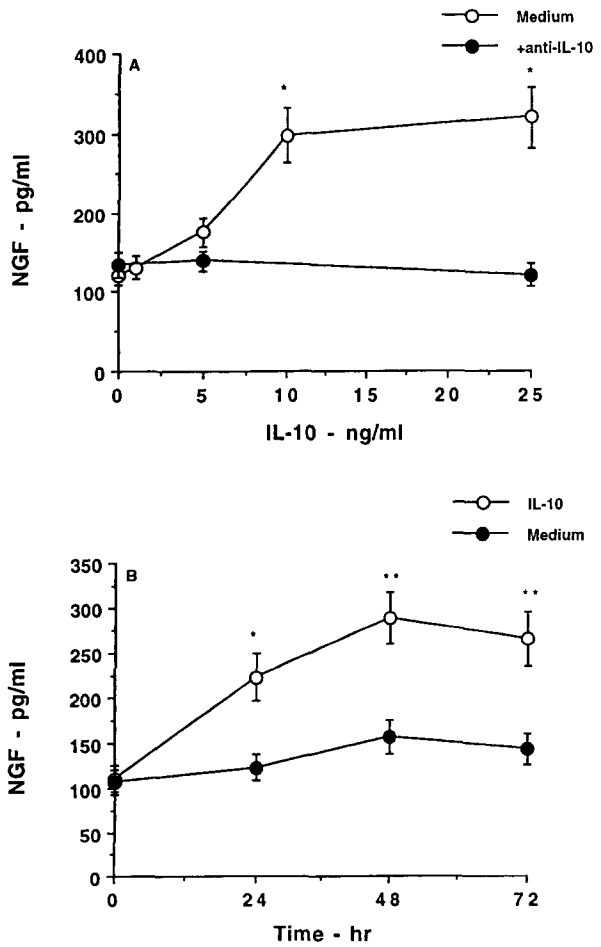

Fig. 1. Effects of IL-10 on NGF secretion. Dose response (A) and kinetics (B) of IL-10 induced NGF secretion in cortical astroglial cells. Cells were incubated with various concentrations of IL-10 for 48 h in the presence or absence of anti-IL-10 (A) or in the presence or absence of IL-10 (25 ng/ml) for various periods of time (B). NGF secretion was determined by EIA. Results represent the means ± S.D. of five separate experiments. $^{*}p < 0.002$ $^{**}p < 0.001$.

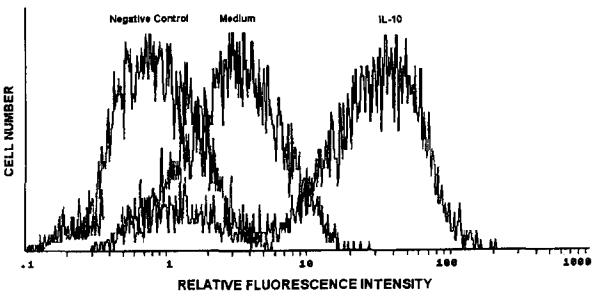

Fig. 2. Cytofluorometric analysis of NGF in IL-10-treated astrocytes. Cells were treated in the presence or absence of IL-10 (25 ng/ml) for 16 h in the presence of monensin (30 nM). Cells were fixed with 4% PFA, permeabilized with 0.1% saponin and stained with anti-NGF antibody. Fluorescence was quantified on a FACS analyzer and values of % positive cells and mean fluorescence intensity were calculated for each histogram. Histograms of a representative experiment out of four are presented.

Using intracellular staining for GFAP it appeared that this population of cells was characterized by low levels of GFAP (data not shown). Treatment of the cells with IL-10 increased the amount of NGF produced by the cells as determined by mean fluorescence values (MFI). Thus, MFI values for control cells was 76.8, whereas that of IL-10-treated cells increased to 158.

3.3. *The Th1-derived cytokines, IL-2 and IFN-γ do not increase NGF secretion*

Based on recent reports and on our current findings it appears that the Th2-derived cytokines IL-5, IL-4 and IL-10 increase NGF production by astrocytes. We therefore examined the effects of the Th1-derived cytokines, IFN-γ and IL-2 on NGF production. Cells treated with IL-2 (10–100 U/ml) or with IFN-γ (10–100 U/ml) up to 72 h did not increase NGF production. Moreover, IFN-γ at concentration over 50 U/ml inhibited the basal levels of NGF production by 35% (data not shown).

3.4. *IFNγ inhibits IL-10 induced NGF secretion*

IL-10 and IFN-γ have been shown to exert antagonistic effects on various functions of lymphoid cells [12,13]. We therefore examined the interaction of these two cytokines on NGF production. Cells were treated concomitantly with IL-10 and IFN-γ and supernatants were analyzed for NGF levels. IFN-γ at a concentration of 50 U/ml inhibited NGF production by 20%. Concomitant treatment of IL-10 and IFN-γ inhibited NGF secretion by 75% (Fig. 3A) whereas pre-treatment of the cells with IFN-γ exerted stronger inhibition of NGF production induced by IL-10 (87%). Similar effects were obtained with the production of NGF by IL-4-treated cells (data not shown).

In contrast, IFN-γ did not inhibit the production of NGF induced by another cytokine, TNF-α. Thus, treatment of the cells with IFN-γ either concomitantly with or prior to TNF-α did not induce significant inhibition in NGF production (Fig. 3B).

4. Discussion

Astrocytes are known to exert distinctive neurotrophic effects which are considered to be mediated by cell surface

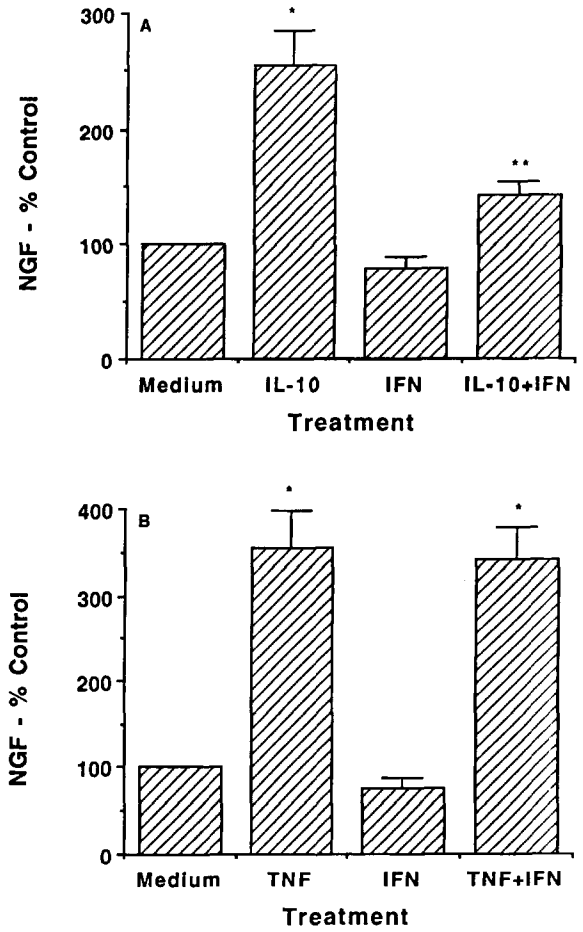

Fig. 3. Effect of combined treatment with IFN-γ and IL-10 or TNF-α on NGF secretion. Effects of combined treatments of IL-10 and IFN-γ (A) or TNF-α and IFN-γ (B) on NGF secretion. Cells were incubated for 48 h with the indicated treatments and NGF secretion was determined by EIA. Results represent the means ± S.D. of three separate experiments. $^{*}p < 0.001$, $^{**}p < 0.005$.

molecules, the extracellular matrix and by neurotrophic factors such as NGF [21]. NGF synthesis is induced in various pathological instances in the CNS such as hypoxic injury [22] fimbria-fornix lesions [23], and seizures [24]. In addition, studies of signals involved in the regulation of NGF synthesis have shown that inflammatory stimuli contribute significantly to increased NGF synthesis [25]. Indeed, cytokines such as TNF-α [26], IL-1, IL-6 and TGF-β [27] increase NGF synthesis in peripheral tissues and in rat cortical astrocytes. Moreover, in a recent study Bracci-Laudiero et al. [28] found a correlation between NGF levels in the CSF of patients with multiple sclerosis and the stage of the disease.

We found that IL-10 induced NGF synthesis/secretion in cortical astrocytes. These results are similar to those reported for other Th2-derived cytokines, IL-4 and IL-5 [6,7]. IL-10 can be produced by either activated astrocytes and microglia [17] or by peripheral lymphoid cells which infiltrate the CNS. Indeed, IL-10 has been associated with pathological instances in the CNS including experimental autoimmune encephalitis [14], *Listeria* meningitis [15] and acute Sindbis virus-induced encephalitis [16].

The significance of the IL-10 effect on NGF secretion should be considered in view of the possible roles which Th2-derived cytokines play in the CNS during pathological conditions. It has been suggested that the expression of type-2 cytokines in the CNS increases antibody production, decreases cell-mediated immunity and therefore reduces the development of inflammation and neuropathology [16]. The ability of IL-10 as well as other Th2-derived cytokines to increase NGF synthesis may contribute to the increased neuronal support and reduced neurotoxicity associated with type-2 response in the CNS. Moreover, NGF has been shown to induce proliferation and immunoglobulin secretion by B lymphocytes via functional receptors [29,30]. Thus, the increased synthesis/secretion of NGF by Th2-derived cytokines can also contribute to the B-cell derived antibody response by acting directly on these cells.

In contrast to IL-10 effects, Th1-derived cytokines such as IL-2 and IFN-γ did not induce NGF secretion. Moreover, IFN-γ reduced the basal level of NGF secreted by astrocytes and completely inhibited NGF synthesis induced by IL-10 whereas it did not inhibit the effect of TNF-α. Inhibitory effects of IFN-γ on NGF secretion were reported by Awatsuji et al. [6] and were associated with the antiproliferative effect of IFN-γ. The effects of both IL-10 and IFN-γ on NGF secretion in our cells cannot be attributed to cell proliferation since both cytokines were used in concentrations that did not induce significant changes in astrocyte proliferation.

Our results suggest antagonistic effects of Th1 and Th-2 derived cytokines on NGF synthesis by astrocytes. Indeed, these two subsets of CD4 T cells are functionally distinct and frequently act in an antagonistic manner, a factor which determines the outcome of some infectious and autoimmune diseases [31,32]. In the CNS, astrocytes and microglia appear to express functional receptors to these cytokines [7,17,33,34] and similar antagonistic interactions have also been shown to exist in these cells. Thus, IL-4 antagonizes the induction of NO production by IFN-γ in microglia cells [35] and both IL-4 and IL-10 inhibits the expression of MHC class II in these cells [36]. Similarly, IL-10 suppresses the MHC class II and Ag-dependent proliferative response of T cells in the presence of APC [37].

Inflammatory processes in the CNS are associated with a variety of pathological conditions such as traumatic injury, ischemia, AIDS related dementia and Alzheimer's disease [38] thus emphasizing the importance of understanding the mechanisms of this response and its contribution to exacerbation or recovery of tissue injury. Our results indicate that the induction of NGF by IL-10 may be an important component in the function of Th2-derived cytokines as suppressors of inflammation and neurotoxicity.

References

[1] Eddleston, M. and Mucke, L. (1993) Neuroscience 54, 15–36.
[2] Giuilan, D. and Lachman, L.B. (1985) Science 228, 497–499.
[3] Houlgatte, R., Mallat, M., Brachet, P. and Prochiantz, A. (1989) J. Neurosci. Res. 24, 143–152.
[4] Yang, K., Mu, X.S., Xue, S.S., Perez-Polo, J.R. and Hayes, R.L. (1995) J. Neurosci. Res. 42, 571–578.
[5] Yoshida, K. and Gage, F.H. (1992) Brain Res. 569, 14–25.
[6] Awatsuji, H., Furukawa, Y., Hirota, M., Murakami, Y., Nii, S., Furukawa, S. and Hayashi, K. (1993) J. Neurosci. Res. 34, 539–545.
[7] Brodie, C. and Goldreich, N. (1994) J. Neuroimmunol. 55, 91–97.
[8] Fiorentino, D.F., Bond, M.W. and Mosmann, T.R. (1989) J. Exp. Med. 170, 2081–2097.

[9] Itoh, K. and Hirohata, S. (1995) J. Immunol. 154, 4341–4350.
[10] Go, N.F., Castla, B.B., Barrett, R., Kastelein, R., Dang, W., Mosmann, T.R., Moore, K.W. and Howard, M. (1990) J. Exp. Med. 172, 1625–1631.
[11] MacNeil, I.A., Suda, T., Moore, K.W., Mosmann, T.R. and Zlotnik, A. (1990) J. Immunol. 145, 4167
[12] de Waal Malefyt, R., Adams, J., Bennett, B., Figdor, C.G. and De Vries, J.E. (1991) J. Exp. Med. 174, 1209
[13] Cunha, F.Q., Moncada, S. and Liew, F.Y. (1992) Biochem. Biophys. Res. Commun. 182, 1155–1159.
[14] Kennedy, M.K., Torrance, D.S., Picha, K.S. and Mohler, M. (1992) J. Immunol. 149, 2496–2505.
[15] Frei, K., Nadal, D., Pfister, H.-W. and Fontana, A. (1993) J. Exp. Med. 178, 1255–1262.
[16] Wesselingh, S.L. and Griffin, D.E. (1994) Semin. Virol. 5, 457–463.
[17] Mizuno, T., Sawada, M., Marunouchi, T. and Suzumura, A. (1994) Biochem. Biophys. Res. Commun. 205, 1908–1915.
[18] Brodie, C. (1995) Neurosci. Lett. 186, 5–8.
[19] Jung, T., Schauer, U., Heusser, C., Neumann, C. and Rieger, C. (1993) J. Immunol. Methods 159, 197–207.
[20] Lowry, D.H., Rosenbrough, J.C., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 93, 265–275.
[21] Lindsay, R.M. (1987) Nature 80–82.
[22] Lorez, H., Keller, F., Ruess, G. and Otten, U. (1989) Neurosci. Lett. 98, 339–344.
[23] Weskamp, G., Gasser, U.E., Dravid, A.R. and Otten, U. (1986) J. Neurosci. Res. 20, 403–410.
[24] Lauterborn, J.C., Isackson, P.S. and Gall, C.M. (1994) Exp. Neurol. 125, 22–40.
[25] Weskamp, G. and Otten, U. (1987) J. Neurochem. 48, 1779–1786.
[26] Hattori, A., Tanaka, E., Murase, K., Ishida, N., Chatani, Y., Tsujimoto, M., Hayashi, K. and Kohno, M. (1993) J. Biol. Chem. 268, 2577–2582.
[27] Gadient, W., Cron, K.C. and Otten, U. (1990) Neurosci. Lett. 117, 335–340.
[28] Bracci-Laudiero, L., Aloe, L., Levi-Montalchini, R., Buttinelli, C., Schilter, D., Gillessen, S. and Otten, U. (1992) Neurosci. Lett. 147, 9–12.
[29] Brodie, C. and Gelfand, E.W. (1994) J. Neuroimmunol. 52, 87–96.
[30] Brodie, C. and Gelfand, E.W. (1992) J. Immunol. 148, 3492–3497.
[31] Liew, F.Y. (1989) Immunol. Today 10, 40
[32] Powrie, F. and Mason, D. (1990) J. Exp. Med. 172, 1701
[33] Sawada, M., Suzumura, A. and Marunouchi, T. (1995) Int. J. Dev. Neurosci. 13, 253–264.
[34] Yong, V.W., Tejada-Berges, T., Goodyer, C.G., Antel, J. and Yong, F.P. (1992) Glia 6, 269–280.
[35] Chao, C.C., Molitor, T.W. and Hu, S. (1993) J. Immunol. 151, 1473–1481.
[36] Suzumura, A., Sawada, M., Itoh, Y. and Marunouchi, T. (1994) J. Neuroimmunol. 53, 209–218.
[37] Frei, K., Lins, H., Schwerdel, C. and Fontana, A. (1994) J. Immunol. 152, 2720–2727.
[38] Perry, V.H., Bell, M.D., Brown, H.C. and Mtyszak, M.K. (1995) Curr. Opin. Neurobiol. 5, 636–641.