Journal of NeuroVirology (1999) 5, 95–101

http://www.jneurovirol.com

Chemokine expression in GKO mice (lacking interferon-gamma) with experimental autoimmune encephalomyelitis

Andrzej R Glabinski[1,2], Michelle Krakowski[3], Yulong Han[1], Trevor Owens[3] and Richard M Ransohoff[1]

[1]Department of Neurosciences, NC 30, The Lerner Research Institute, Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, Ohio 44 195, USA; [2]Department of Neurology, Medical University of Lodz, ul. Kopcinskiego 22, 90-153 Lodz, Poland; [3]Montreal Neurological Institute and McGill University, Montreal, Quebec, Canada

Experimental autoimmune encephalomyelitis (EAE) is an inflammatory disease of the central nervous system (CNS) considered to be an animal model for multiple sclerosis (MS). The detailed mechanism that specifies accumulation of inflammatory cells within the CNS in these conditions remains a subject of active investigation. Chemokines including IP-10, GRO-α, MCP-1 are produced in EAE tissues selectively by parenchymal astrocytes, but the regulatory stimuli that govern this expression remain undetermined. The unexpected occurrence of increased EAE susceptibility in Balb/c GKO mice (lacking IFN-γ) offered an opportunity to examine the spectrum of chemokine expression during immune-mediated inflammation in the absence of a single regulatory cytokine. We found that chemokines MCP-1 and GRO-α were upregulated in the CNS of mice with EAE despite the GKO genotype. IP-10, which is highly expressed in the CNS of mice with an intact IFN-γ gene and EAE, was strikingly absent. *In vitro* experiments confirmed that IFNγ selectively stimulates astrocytes for IP-10 expression. These results indicate that IP-10 is dependent upon IFN-γ for its upregulation during this model disease, and document directly that astrocyte expression of chemokines during EAE is governed by pro-inflammatory cytokines.

Keywords: chemotactic factors; demyelinating diseases; type II interferon; mice; knockout

Introduction

Experimental autoimmune encephalomyelitis (EAE) is an inflammatory disease of the central nervous system (CNS) considered to be a prototypic organ-specific immunopathological process and a suitable animal model for the human demyelinating disease, multiple sclerosis (MS). EAE and MS share common histologic features including widespread invasion of the CNS with hematogenous inflammatory cells, arranged in perivascular cuffs and parenchymal infiltrates. The detailed mechanism that specifies accumulation of inflammatory cells within the CNS in this and other conditions remains a subject of active investigation. Prior reports have described increased intrathecal production of inflammatory cytokines, including interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) during the active phase of both MS and EAE (Hofman *et al*, 1986, 1989; Selmaj *et al*, 1991; Sharief and Hentges, 1991; Merrill *et al*, 1992; Renno *et al*, 1995). Such results suggest that inflammatory cytokines may play an important role in the mechanism of disease initiation or progression.

The role of IFN-γ in the pathogenesis of CNS inflammation has been exceptionally cryptic. In some respects, it is clear that IFN-γ functions as a pro-inflammatory cytokine. Most importantly, treatment of MS with IFN-γ led to significant disease worsening (Panitch *et al*, 1987a,b). Further, the direct injection of IFN-γ into normal rat CNS produced inflammation (Simmons and Willenborg, 1990; Sethna and Lampson, 1991). In contrast, intraventricular injection of IFN-γ inhibited EAE in the rat (Voorthuis *et al*, 1990), and administration of anti-IFN-γ antibodies exacerbated EAE (Billiau *et al*, 1988; Duong *et al*, 1994). The discrepant results

Correspondence: RM Ransohoff
Received 3 August 1998; revised 4 September 1998; accepted 9 September 1998

coming from these studies has been rationalized by differential CNS and peripheral actions of IFN-γ (Krakowski and Owens, 1996). In particular, it was proposed that circulating IFN-γ restrains antigen-triggered T lymphocyte proliferation and may promote apoptosis (Krakowski and Owens, 1996). Therefore, it is plausible that elimination of IFN-γ from the circulation would have the effect of upregulating autoaggressive T cells and worsening the EAE disease process. In contrast, intrathecal IFN-γ is considered likely to activate resident microglia and infiltrating macrophage cells, culminating in increased tissue injury and signs (Krakowski and Owens, 1996). Strongly supporting this concept were studies in transgenic mice that expressed IFN-γ in the mature CNS, under control of an oligodendrocyte-specific promoter, and developed demyelination, gliosis and inflammation (Horwitz *et al*, 1997). The timing of exposure to IFN-γ also affects its regulation of autoimmunity, since pretreatment with IFN-γ ameliorated experimental autoimmune neuritis (EAN), a peripheral nervous system analog of EAE; injections of IFN-γ after immunization resulted in more severe EAN (Hartung *et al*, 1990). The pathogenic role of IFN-γ was recently addressed in IFN-γ-deficient (gamma-knockout: GKO) mice, that were generated by targeted disruption of the IFN-γ gene through homologous recombination (Ferber *et al*, 1996; Krakowski and Owens, 1996). In the setting of the EAE-resistant Balb/c background, disruption of the IFN-γ gene led to marked and surprisingly increased sensitivity to induction of EAE (Krakowski and Owens, 1996). Supporting these results, IFN-γ was shown to be a dispensable factor for disease induction in an EAE-sensitive background (B10.PL) (Ferber *et al*, 1996).

In this report, we describe the expression of three astrocyte-derived chemokines in the CNS tissues of GKO mice with EAE. Expression of the same chemokines was also examined in the lymphoid compartment. Chemokines (chemoattractant cytokines) act selectively towards various populations of leukocytes. Both expression and intervention studies have recently demonstrated that chemokines are involved in the pathogenesis of EAE (Karpus *et al*, 1995). We previously provided evidence that chemokines amplify, rather than initiate inflammation during EAE (Glabinski *et al*, 1995b). In EAE tissues, we found that chemokines are produced either by parenchymal astrocytes (MCP-1, IP-10, GRO-α) or by infiltrating leukocytes (RANTES, MIP-1α) and it was proposed that chemokine expression by parenchymal astrocytes was regulated by the pro-inflammatory cytokine products (IFN-γ, TNF-α, IL-1, and others) that were elaborated by infiltrating perivascular leukocytes (Glabinski *et al*, 1995a,b; 1997; Tani *et al*, 1996).

We report that astrocyte-derived chemokines were dramatically upregulated in GKO mice at the onset of clinical EAE, as observed previously in wild-type mice. Chemokines expressed in this fashion included Monocyte Chemoattractant Protein (MCP)-1 and Growth Regulated Oncogene (GRO)-α, which are chemoattractants respectively for monocytes and neutrophils. These results indicated that IFN-γ was dispensable for induction of chemokines in the CNS of animals with EAE. Interestingly, there was a selective deficiency in the expression of one chemokine mRNA, encoding the gamma-interferon inducible peptide, 10 kDa (IP-10), which was not expressed in the CNS of GKO mice during EAE. *In vitro* experiments confirmed that IFN-γ selectively stimulates astrocytes to express IP-10. These results indicate that IP-10 is dependent upon IFN-γ for its upregulation during this model disease, and document directly that astrocyte expression of chemokines during EAE is governed by pro-inflammatory cytokines.

Results

Chemokine expression in the CNS of GKO and SJL mice

Expression of three astrocyte-derived chemokines (MCP-1, GRO-α, IP-10) in the CNS of unmanipulated (normal) GKO mice was at or below the level of detection by sensitive and specific RT–PCR dot-blot hybridization assay (Figure 1a, b and c and Figure 2a). GKO mice immunized with MBP, but without symptoms of EAE, also expressed chemokines at a very low level (Figure 1a, b and c). During EAE, expression of two chemokines (MCP-1 and GRO-α) in the CNS of GKO mice was elevated (P=0.09 and P=0.04 respectively). The elevation exceeded that observed during EAE in susceptible SJL mice (Figure 1a and b). IP-10 was not elevated in GKO mice during EAE (Figure 1c), but was significantly elevated in control SJL mice with EAE when compared with normal animals (P=0.02). IP-10 expression during EAE in SJL animals was also significantly higher than in GKO animals with EAE (P=0.04) (Figure 1c).

Chemokine expression was analyzed within 3 days of EAE onset, because our previous experiments showed that chemokines are transiently upregulated during symptomatic first attacks of disease (Glabinski *et al*, 1995b). Maximal accumulation of MCP-1 and GRO-α mRNA was observed in spinal cord on day one after EAT onset, while peak chemokines expression in brain occurred on day three (data not shown). This time course of chemokine expression in spinal cord and brain was identical to that described previously in SJL mice with acute EAE (Ransohoff *et al*, 1993).

Chemokine expression in the CNS and liver of GKO mice after systemic IFN-γ administration

Intravenous injection of IFN-γ to GKO mice with EAE markedly upregulated hepatic chemokine

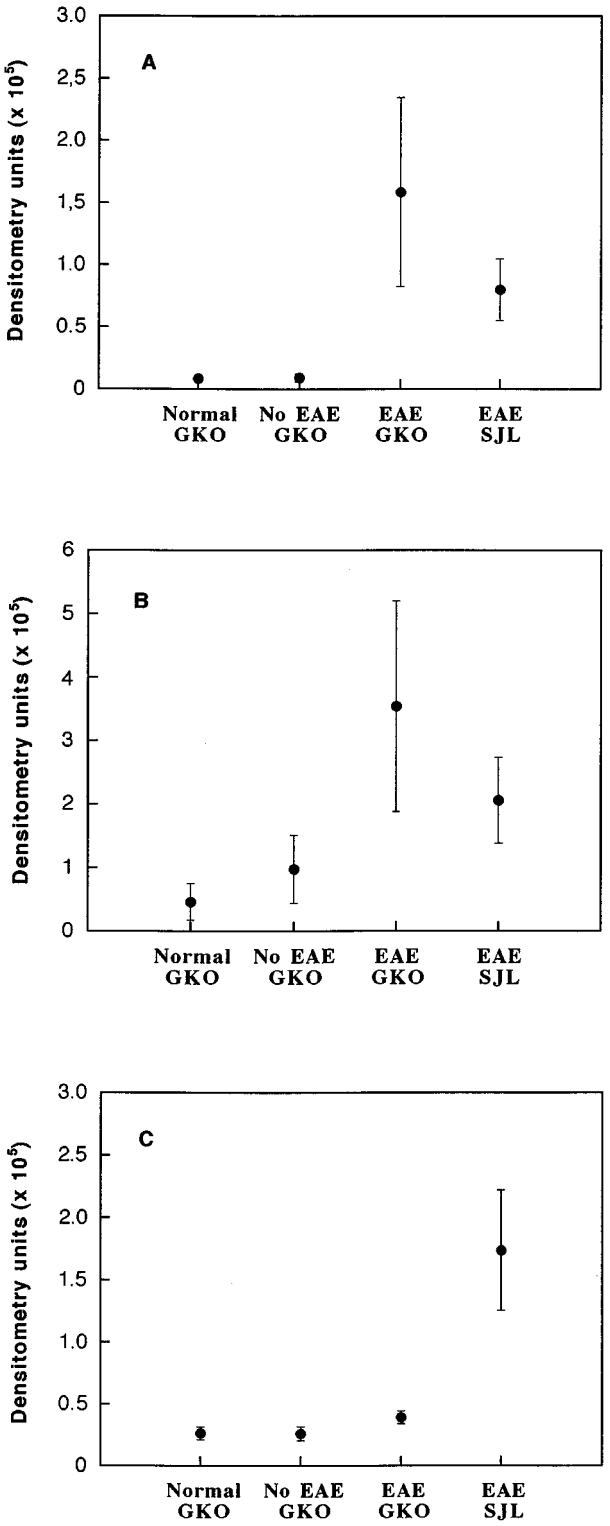

Figure 1 Chemokine expression in the CNS (Experiment I). Each dot represents mean chemokine mRNA accumulation in brains and spinal cords of mice in indicated groups +/− standard error of the means (s.e.; n=4–6). Data are presented in arbitrary PhosphorImager densitometry units. (**a**) MCP-1, (**b**) GRO-α and (**c**) IP-10. Normal: unmanipulated animals; No EAE: immunized animals without clinical and histological EAE; EAE: animals with EAE (2–3 day of disease).

expression (Figure 2b), but elevated only equivocally mean CNS accumulation of MCP-1, GRO-α and IP-10 mRNAs (Figure 2a).

Chemokine expression in the peripheral compartment of GKO mice

Hepatic expression of chemokines MCP-1, GRO-α and IP-10 in GKO mice was low and was not upregulated during EAE (Figure 2b). In two cases, we also analyzed expression of chemokines in peripheral lymph nodes of GKO mice during EAE (data not shown). MCP-1 and GRO-α were elevated in lymph nodes of GKO mice with EAE; IP-10 expression remained near the level of detection (data not shown).

Chemokine expression by mouse primary astrocytes

We analyzed chemokine expression by cultured murine primary astrocytes using RNase protection

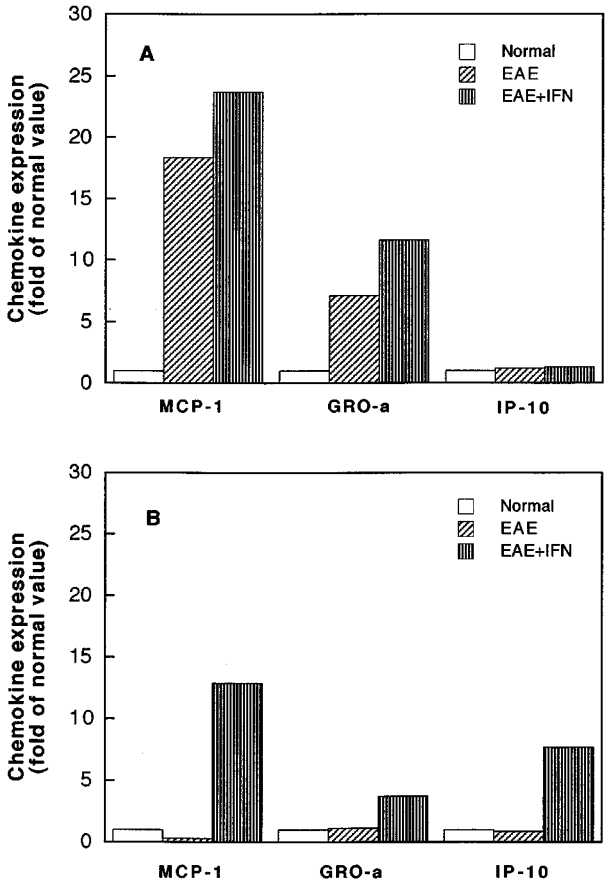

Figure 2 Chemokine expression in the CNS (**a**) and liver (**b**) of GKO mice before and after systemic IFN-γ administration (Experiment II). Each bar represents mean chemokine mRNA accumulation in indicated groups (CNS: n=4–8, liver: n=1–4). For each chemokine, expression is presented as fold mean value in unmanipulated (normal) animals. Normal: unmanipulated animals, EAE: animals with clinical signs of EAE (1–3 day of disease); EAE+IFN: animals with EAE (day 2) injected with murine IFN-γ 4 h before sacrifice.

assay (RPA). Unstimulated astrocytes did not express chemokine message (Figure 3). After stimulation of cultured astrocytes with IFN-γ for 8 or 16 h IP-10 was the only chemokine mRNA detected (Figure 3). When astrocytes were stimulated by TNF-α for the same period of time they expressed several chemokines including IP-10, MCP-1 and MIP-1α (Figure 3).

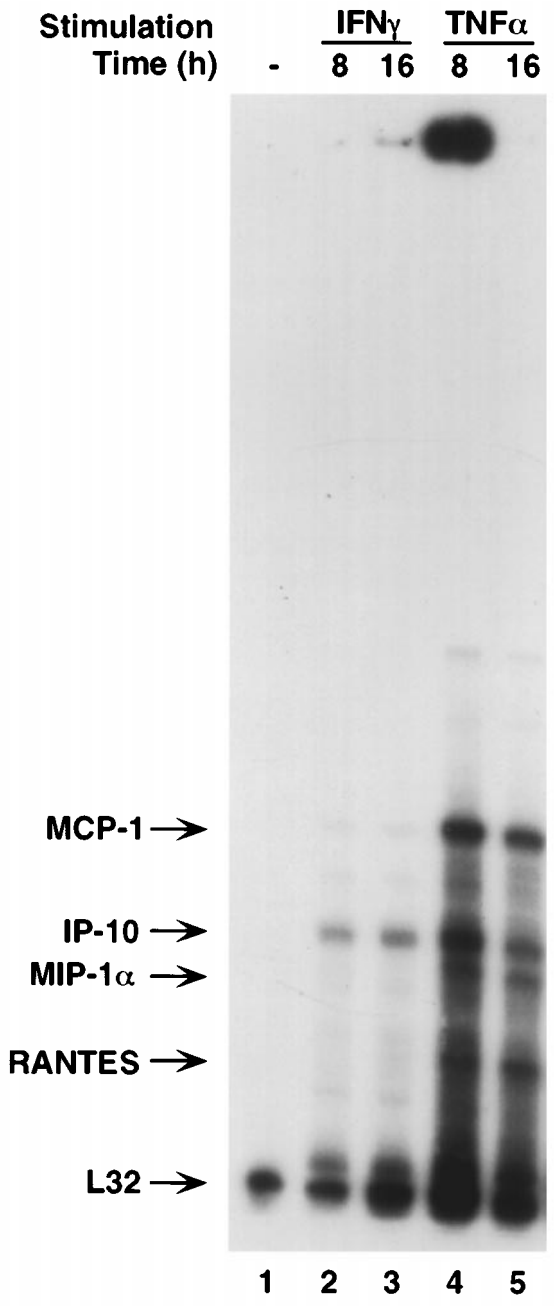

Figure 3 Chemokine expression by cultured primary astrocytes. Astrocytes were cultured and treated with cytokines as described in Materials and methods. Ten micrograms of total cellular RNA was subjected to RPA. Astrocytes (lane 1) were reserved as controls or stimulated with IFN-γ for 8 h (lane 2) or 16 h (lane 3) or with TNF-α for 8 h (lane 4) or 16 h (lane 5).

Discussion

The goal of this study was to address the role of the pro-inflammatory cytokine IFN-γ in regulating intrathecal chemokine expression during EAE. We previously performed studies of the kinetics and cellular sources of chemokines during the EAE process and obtained results suggesting that chemokine expression within the CNS compartment was driven by pro-inflammatory cytokines which in turn were produced by infiltrating leukocytes (Glabinski *et al*, 1995a,b; 1997).

The unexpected occurrence of increased EAE susceptibility in Balb/c GKO mice offered an opportunity to examine the spectrum of chemokine expression during immune-mediated inflammation, in the absence of a single regulatory cytokine. We observed that MCP-1 and GRO-α were upregulated in the CNS of mice with EAE despite the GKO genotype. IP-10, which is highly expressed in the CNS of mice with an intact IFN-γ gene and EAE, was strikingly absent. Our results are consistent with previous reports of the regulation of these chemokines, both *in vivo* and *in vitro*. MCP-1, which was highly expressed in the absence of IFN-γ, is known to be responsive both to TNF-α and IL6 (Ping *et al*, 1996; Romano *et al*, 1997). GRO-α was expressed at higher levels in the CNS of GKO mice with EAE than it was in the wild-type EAE controls. This finding is consistent with recent reports that IFN-γ inhibits the induction of GRO-α by inflammatory stimuli such as lipopolysaccharide (LPS) (Ohmori and Hamilton, 1994).

We observed that cultured primary astrocytes selectively express IP-10 after stimulation with IFNγ. This result is consistent with the observation *in vivo* that IP-10 is not expressed during AEA in mice lacking IFN-γ. When cultured astrocytes were stimulated with TNF-α mRNA for several different chemokines was expressed. Data presented in this report indicate that IP-10 expression *in vivo* during EAE depends on the presence of IFN-γ. We excluded the trivial possibility that the IP-10 locus was itself defective in GKO mice, by showing dramatic upregulation of IP-10 expression in the livers of mice that received systemic IFN-γ. This result is consistent with a previous report from Narumi and colleagues demonstrating up-regulation of hepatic IP-10 expression 4 h after systemic injections of IFN-γ (Narumi *et al*, 1992). In mice with cerebral lymphocytic chorionmeningitis virus (LCMV) infection, IP-10 mRNA accumulation was increased in animals lacking IFN-γ, indicating that antiviral host responses presumably direct IP-10 expression through type I IFNs (Asensio and Campbell, 1997). Therefore, the presence of IP-10 in LCMV-associated inflammatory brain pathology of GKO mice and its absence in EAE of GKO mice (current report) indicate that IFN-γ is essential for CNS IP-10 expression in EAE. The influence of the selective

absence of IP-10 on EAE pathogenesis in GKO mice cannot be addressed in these experiments because of profound changes in the experimental system, caused by the lack of IFN-γ.

It was recently shown that infusions of IP-10 antisense oligonucleotides reduced acute EAE severity in Lewis rats (Wojcik *et al*, 1996). Our current results indicate that IP-10 expression is not essential for EAE, but we do not exclude a significant role for this chemokine in disease pathogenesis.

We and others have reported that parenchymal astrocytes express chemokines in the brains of rodents with EAE, CNS injury and cerebral ischemia (Ransohoff *et al*, 1993; Kim *et al*, 1995; Glabinski *et al*, 1995b; 1996; 1997; Gourmala *et al*, 1997). This report, demonstrating dependence of astrocyte IP-10 expression on IFN-γ is the first documentation that astrocytes directly respond *in vivo* to endogenous inflammatory cytokines. This observation supports the concept that astrocytes serve as facultative components of the intrinsic immune/inflammatory apparatus of the CNS.

Materials and methods

Animals

Interferon-γ knock out (GKO) mice, obtained from Genetech (Dalton *et al*, 1993), were originally generated on a 129/J (H-2^b) background, then crossed to C57BL/6J (H-2^d) and back-crossed five times to Balb/c (H-2^d). Hemizygous (GKO+/$-$) mice that were intercrossed at the Montreal Neurological Institute (MNI) and progeny were screened by DNA polymerase chain reaction (PCR) for neomycin and IFN-γ (Goes *et al*, 1995). This breeding protocol generated mice that were homozygous (GKO $-$/$-$) or hemizygous (GKO +/$-$) for the disrupted IFN-γ allele or that expressed IFN-γ normally (GKO +/+), all on the same background. Balb/c mice were purchased from Charles River Canada (St. Constant, Quebec, Canada) and SJL/J (H-2^s) from Harlan-Sprague Dawley (Indianapolis, IN, USA). Mice were housed under specific pathogen-free conditions at MNI. Housing and experimental protocols were approved by the McGill University Animal Care Committee.

Induction and monitoring of EAE

EAE was induced by two subcutaneous injections, 1 week apart, of bovine myelin basic protein (MBP) (Cheifetz *et al*, 1984) (400 mg) in complete Freund's adjuvant (CFA) (Difco, Detroit, MI, USA) (50 mg Mycobacterium tuberculosis per injection). Mice were monitored and weighed daily, and assigned severity scores as follows: 0, no symptoms; 1, flaccid tail; 2, moderate hind limb paresis, clumsiness; 3, severe paresis or unilateral hind limb paralysis; 4, complete hind/fore limb paralysis; or 5, moribund (Renno *et al*, 1995).

SJL mice were used as a controls for Balb/c-GKO mice because the two mouse strains display comparable clinical and histopathological disease intensity (Krakowski and Owens, 1996). In particular, it was previously reported that homozygous GKO $-$/$-$ mice (backcrossed to BALB/c background) exhibit similar incidence rate (70%), day of onset (20 days post-immunization) and disease severity (maximal score around 4) as SJL/J mice (Krakowski and Owens, 1996). Heterozygous GKO +/$-$ mice showed a similar low incidence rate and delayed time of disease onset after immunization as Balb/c-GKO +/+ mice but higher clinical score (Krakowski and Owens, 1996). Balb/c-GKO +/+ mice are resistant to EAE induction and therefore could not be directly compared with strain-matched GKO littermates for CNS chemokine expression.

IFN-γ treatment

10 000 U of recombinant murine IFN-γ (Genentech) in PBS was diluted to a total volume of 200 uL in sterile PBS and injected into a tail vein of mice. Mice had previously been immunized with MBP as described and had exhibited symptoms for 2 days. Four hours after injection of IFN-γ, animals were sacrificed and tissue isolated as described below.

Tissue isolation and RNA preparation

Animals were lethally anesthetized (Somnotol, 4.45 ml/kg body mass) (MT Pharmaceuticals, Cambridge, Ontario, Canada) then perfused through the heart with ice cold, sterile PBS and CNS tissue removed. Brains and spinal cords were analyzed separately.

Total RNA was isolated from homogenized tissue using TRIZOL (Gibco/BRL) and yield quantitated by spectrophotometry. Samples were resuspended in H_2O and stored at -20°C until use.

Reverse transcription polymerase chain reaction (RT–PCR)

First strand cDNA was synthesized using 1 μg of total cellular RNA. Reverse transcription with AMV reverse transcriptase (Boehringer Mannheim, Indianapolis, IN, USA) was performed as previously described for IP-10, MCP-1 and GRO-α, using gene-specific backward primers (Glabinski *et al*, 1995b). The product of this reaction was amplified by polymerase chain reaction (PCR) using Taq DNA polymerase (Boehringer Mannheim, Indianapolis, IN, USA). Before amplification, cDNA templates were denatured at 94°C for 3 min and then amplified. PCR conditions were as follows: 20 cycles (94°C, 2 min; 60°C, 2 min; 72°C, 2 min). PCR cycle numbers that provided a linear relation between PCR products and cDNA input were determined empirically as previously described (Glabinski *et al*, 1995b). Under these conditions, this assay is semiquantitative (Ransohoff *et al*, 1997). Moreover, chemokine mRNA expression,

determined by this method, exhibits a robust correlation with tissue levels of chemokine protein, measured by ELISA (Glabinski *et al*, 1996). Negative technical controls (without RT) were used for each set of reactions. Amplification of tubulin transcripts confirmed intact RNA in all samples and analysis of the reaction products on ethidium bromide-stained gels demonstrated freedom from genomic DNA contamination.

Dot-blot hybridization analysis of chemokine cDNA
PCR reaction products were denatured (2 M NaOH, 1 M Tris/HCl, pH 8.0, 80°C, 15 min, diluted in $6 \times$ SSC (3 fourfold dilutions) and transferred to nylon membrane (GeneScreen, Dupont, Boston, MA, USA) using a vacuum blotter (V&P Scientific, San Diego, CA, USA), UV cross-linked (Stratalinker 1800, Stratagene, La Jolla, CA, USA) and air-dried. Membranes were hybridized with radiolabeled probes and washed at high stringency as described. Hybridization signal was quantitated with a PhosphorImager (Molecular Dynamics, Sunnyville, CA, USA). We previously showed that in this assay, hybridization signal intensity for individual samples obtained by Southern blotting and dot-blot hybridizations were identical (Glabinski *et al*, 1995b).

RNase protection analysis of chemokine expression by primary cultured astrocytes
Astrocytes were purified from P0–P3 mouse brain by differential adhesion and were maintained on 12-mm PLL coated coverslips as previously described (Zhou and Miller, 1998). This protocol yields cells 99% GFAP positive. They were stimulated with murine IFN-γ (500 U/ml) or murine TNF-α (50 ng/ml) for 8 or 16 h. Controls were left without stimulation. After stimulation total cellular RNA was collected using Trizol (Gibco-BRL) according to manufacturer's instruction. Ten milligrams of RNA was used for RNase protection assay (RPA). Murine chemokine template set for RPA was a gift from Dr I Campbell (Scripps Research Institute, La Jolla, CA, USA). The assay was performed as previously described (Asensio and Campbell, 1997). Hybridization signal was quantitated with a PhosphorImager (Molecular Dynamics, Sunnyville, CA, USA).

Statistical analysis
Results were analyzed using the Wilcoxon rank sum test for nonparametric data, after evaluation by Kolmogorov-Smirnov test excluded normal distribution. $P < 0.05$ was considered significant.

Acknowledgements

This work was supported by the NIH-NINDS (grant 1RO1-NS32151 to RMR), NIH-FIC (grant 1RO3 TW00784-01A1 to RMR), the Williams Family Fund for Multiple Sclerosis Research (RMR), The Medical Research Council of Canada (TO) and The Multiple Sclerosis Society of Canada (TO). Authors thank Dr Iain L Campbell (Scripps Research Institute) for providing RPA template set and Dr Lixin Zhou for assistance with the tissue cultures.

References

Asensio VC, Campbell IL (1997). Chemokine gene expression in the brains of mice with lymphocytic choriomeningitis. *J Virol* **71:** 7832–7840.

Billiau A, Heremans H, Vandekerckhove F, Dijkmans R, Sobis H, Meulepas E, Carton H (1988). Enhancement of experimental allergic encephalomyelitis in mice by antibodies against IFN-gamma. *J Immunol* **140:** 1506–1510.

Cheifetz S, Moscarello M, Deber C (1984). NMR investigation of the charge isomers of bovine myelin basic protein. *Arch Biochem Biophys* **233:** 151–160.

Dalton D, Pitts-Meek S, Keshav S, Figari I, Bradley A, TA S (1993). Multiple defects of immune cell function in mice with disrupted interferon-gamma gene. *Science* **259:** 1739–1742.

Duong T, Finkelman F, Singh B, Strejan G (1994). Effect of anti-interferon-gamma monoclonal antibody treatment on the development of experimental allergic encephalomyelitis in resistant mouse strains. *J Neuroimmunol* **53:** 101–107.

Ferber I, Brocke S, Taylor-Edwards C, Ridgway W, Dinisco C, Steinman L, Dalton D, Fathman C (1996). Mice with a disrupted IFN-gamma gene are susceptible to the induction of experimental autoimmune encephalomyelitis. *J Immunol* **156:** 5–7.

Glabinski A, Tani M, Aras S, Stoler M, Tuohy V, Ransohoff R (1995a). Regulation and function of central nervous system chemokines. *International J Dev Neurosci* **13:** 153–165.

Glabinski A, Tani M, Tuohy VK, Tuthill R, Ransohoff RM (1995b). Central nervous system chemokine gene expression follows leukocyte entry in acute murine experimental autoimmune encephalomyelitis. *Brain Behav Immun* **9:** 315–330.

Glabinski A, Tani M, Strieter R, Tuohy V, Ransohoff R (1997). Synchronous synthesis of α- and β-chemokines by cells of diverse lineage in the central nervous system of mice with relapses of experimental autoimmune encephalomyelitis. *Am J Pathol* **150:** 617–630.

Glabinski AR, Tani M, Balasingam V, Yong VW, Ransohoff RM (1996). Chemokine monocyte chemoattractant protein-1 (MCP-1) is expressed by astrocytes after mechanical injury to the brain. *J Immunol* **156:** 4363–4368.

Goes N, Sims T, Urmson T, Vincent D, Ramassar V, Holloran P (1995). Disrupted MHC regulation in the IFN-gamma knockout mouse. Evidence for three states of MHC expression with distinct roles for IFN-gamma. *J Immunol* **155:** 4559–4566.

Gourmala NG, Buttini M, Limonta S, Sauter A, Boddeke HW (1997). Differential and time-dependent expression of monocyte chemoattractant protein-1 mRNA by astrocytes and macrophages in rat brain: effects of ischemia and peripheral lipopolysaccharide administration. *J Neuroimmunol* **74:** 35–44.

Hartung H, Schafer B, van der Meide P, Fierz W, Heininger K, Toyka K (1990). The role of interferon-gamma in the pathogenesis of experimental autoimmune disease of the peripheral nervous system. *Ann Neurol* **27:** 247–257.

Hofman F, von Hanwehr R, Dinarello C, Mizel S, Hinton D, Merrill J (1986). Immunoregulatory molecules and IL-2 receptors identified in multiple sclerosis brain. *J Immunol* **136:** 3239–3247.

Hofman FM, Hinton DR, Johnson K, Merrill JE (1989). Tumor necrosis factor identified in multiple sclerosis brain. *J Exp Med* **170:** 607–612.

Horwitz M, Evans C, McGavern D, Rodriguez M, Oldstone M (1997). Primary demyelination in transgenic mice expressing interferon-gamma. *Nature Med* **3:** 1037–1041.

Karpus WJ, Lukacs NW, McRae BL, Strieter RM, Kunkel SL, Miller SD (1995). An important role for the chemokine macrophage inflammatory protein-1α in the pathogenesis of the T cell-mediated autoimmune disease, experimental autoimmune encephalomyelitis. *J Immunol* **155:** 5003–5010.

Kim JS, Gautam SC, Chopp M, Zaloga C, Jones ML, Ward PA, Welch KMA (1995). Expression of monocyte chemoattractant protein-1 and macrophage inflammatory protein-1 after focal cerebral ischemia in the rat. *J Neuroimmunol* **56:** 127–134.

Krakowski M, Owens T (1996). Interferon-gamma confers resistance to experimental allergic encephalomyelitis. *Eur J Immunol* **26:** 1641–1646.

Merrill J, Kono D, Clayton J, Ando D, Hinton D, Hofman F (1992). Inflammatory leukocytes and cytokines in the peptide-induced disease of experimental allergic encephalomyelitis in SJL and B10.PL mice. *Proc Natl Acad Sci USA* **89:** 574–578.

Narumi S, Wyner L, Stoler M, Tannenbaum C, Hamilton T (1992). Tissue specific expression of murine IP-10 mRNA following systemic treatment with interferon-γ. *J Leukocyte Biol* **52:** 27–33.

Ohmori Y, Hamilton TA (1994). IFN-gamma selectivity inhibits lipopolysaccharide-inducible JE/monocyte chemoattractant protein-1 and KC/GRO/melanoma growth-stimulating activity gene expression in mouse peritoneal macrophages. *J Immunol* **153:** 2204–2212.

Panitch HS, Hirsch RL, Haley AS, Johnson KP (1987a). Exacerbations of multiple sclerosis in patients treated with gamma interferon. *Lancet* **1:** 893–895.

Panitch HS, Hirsch RL, Schindler J, Johnson KP (1987b). Treatment of multiple sclerosis with gamma interferon: exacerbations associated with activation of the immune system. *Neurology* **37:** 1097–1102.

Ping D, Jones PL, Boss JM (1996). TNF regulates the in vivo occupancy of both distal and proximal regulatory regions of the MCP-1/JE gene. *Immunity* **4:** 455–469.

Ransohoff R, Tani M, Glabinski A, Chernosky A, Krivacic K, Peterson J, Chien H, Trapp B (1997). Chemokines and chemokine receptors in model neurological pathologies: molecular and immunocytochemical approaches. *Methods Enzymol* **287:** 319–348.

Ransohoff RM, Hamilton TA, Tani M, Stoler MH, Shick HE, Major JA, Estes ML, Thomas DM, Tuohy VK (1993). Astrocyte expression of mRNA encoding cytokines IP-10 and JE/MCP-1 in experimental autoimmune encephalomyelitis. *FASEB J* **7:** 592–602.

Renno T, Krakowski M, Piccirillo C, Lin JY Owens T (1995). TNF-alpha expression by resident microglia and infiltrating leukocytes in the central nervous system of mice with experimental allergic encephalomyelitis. Regulation by Th1 cytokines. *J Immunol* **154:** 944–953.

Romano M, Sironi M, Toniatti C, Polentarutti N, Fruscella P, Ghezzi P, Faggioni R, Luini W, van Hinsbergh V, Sozzani S, Bussolino F, Poli V, Ciliberto G, Mantovani A (1997). Role of IL-6 and its soluble receptor in induction of chemokines and leukocyte recruitment. *Immunity* **6:** 315–325.

Selmaj K, Raine CS, Canella B, Brosnan CF (1991). Identification of lymphotoxin and tumor necrosis factor in multiple sclerosis lesions. *J Clin Invest* **87:** 949–954.

Sethna M, Lampson L (1991). Immune modulation within the brain: recruitment of inflammatory cells and increased major histocompatibility antigen expression following intracerebral injection of interferon-gamma. *J Neuroimmunol* **34:** 121–132.

Sharief M, Hentges R (1991). Association between tumor necrosis factor-alpha and disease progression in patients with multiple sclerosis. *N Engl J Med* **325:** 467–471.

Simmons RD, Willenborg DO (1990). Direct injection of cytokines into the spinal cord causes autoimmune encephalomyelitis-like inflammation. *J Neuro Sci* **100:** 37–42.

Tani M, Glabinski AR, Tuohy VK, Stoler MH, Estes ML, Ransohoff RM (1996). In situ hybridization analysis of glial fibrillary acidic protein mRNA reveals evidence of biphasic astrocyte activation during acute experimental autoimmune encephalomyelitis. *Am J Pathol* **148:** 889–896.

Voorthuis J, Uitdehaag B, De Groot C, Goede P, van der Meide P, Dijkstra C (1990). Suppression of experimental allergic encephalomyelitis by intraventricular administration of interferon-gamma in Lewis rats. *Clin Exp Immunol* **81:** 183–188.

Wojcik WJ, Swoveland P, Zhang X, Vanguri P (1996). Chronic intrathecal infusion of phosphorothioate or phosphodiester antisense oligonucleotides against cytokine responsive gene-2/IP-10 in experimental allergic encephalomyelitis of Lewis rat. *J Pharmacol Exp Ther* **278:** 404–410.

Zhou L, Miller R (1998). Antibody-mediated oligodendrocyte cell death requires an astrocyte-derived cosignal. *J Neurosci Res* **52:** 137–148.