Acta Neuropathol (2002) 103:458–468
DOI 10.1007/s00401-001-0491-7

REGULAR PAPER

Andreas Strack · Valérie C. Asensio · Iain L. Campbell
Dirk Schlüter · Martina Deckert

Chemokines are differentially expressed by astrocytes, microglia and inflammatory leukocytes in *Toxoplasma* encephalitis and critically regulated by interferon-γ

Received: 30 July 2001 / Revised, accepted: 30 October 2001 / Published online: 31 January 2002

Abstract The intracerebral formation of inflammatory infiltrates is a complex process, which may be regulated by chemokines. This study defines the kinetics and cellular sources of T cell- and macrophage-attracting chemokines in murine *Toxoplasma* encephalitis (TE) by ribonuclease protection assay, reverse transcription-PCR, in situ hybridization, and immunohistochemistry. Whereas astrocytes were the major source of interferon (IFN)-γ-inducible protein-10 (CRG-2/IP-10) and monocyte chemoattractant protein (MCP)-1, microglia expressed RANTES, monokine induced by IFN-γ (MuMIG) and occasionally CRG-2/IP-10 RNA. Despite being ubiquitously activated, only astrocytes and microglia confined to inflammatory infiltrates expressed chemokine genes. Intracerebral leukocytes transcribed RANTES, MuMIG, and occasionally CRG-2/IP-10 and MCP-1. IFN-γ-deficient mice failed to produce CRG-2/IP-10, MuMIG, RANTES and expressed macrophage inflammatory protein (MIP-1)α, MIP-1β, and MCP-1 mRNA at reduced levels, functionally resulting in a strongly reduced recruitment of leukocytes across the blood-brain barrier and prevented their further invasion of the brain parenchyma. Since T cells are the single source of IFN-γ in TE, these findings indicate that T cells pave the way of leukocytes to parenchymatous parasites via IFN-γ.

Keywords Chemokines · Encephalitis · Astrocyte · Microglia · T cells · Interferon-γ

The last two authors contributed equally to this work.

A. Strack · M. Deckert (✉)
Abteilung für Neuropathologie, Universität zu Köln,
Joseph-Stelzmann-Str. 9, 50931 Köln, Germany
e-mail: neuropatho@uni-koeln.de,
Tel.: +49-221-4785265, Fax: +49-221-4787237

V.C. Asensio · I.L. Campbell
Department of Neuropharmacology,
The Scripps Research Institute, La Jolla, California, USA

D. Schlüter
Institut für Med. Mikrobiologie und Hygiene,
Universitätsklinikum Mannheim, Universität Heidelberg,
Mannheim, Germany

Introduction

Cerebral leukocyte recruitment and accumulation is a key process in the pathogenesis of many inflammatory disorders of the central nervous system (CNS). In this complex process a cascade of cell adhesion molecules, including the intercellular cell adhesion molecule (ICAM)-1 and the vascular cell adhesion molecule (VCAM)-1, which are induced on cerebral blood vessel endothelial cells and mediate recruitment, adherence and transendothelial migration of leukocytes across the blood-brain barrier [13, 52, 62], is critically involved. In addition, chemokines may facilitate this process and the subsequent directional movement of inflammatory leukocytes to the site of the offending pathogen in the brain parenchyma.

Chemokines are defined as small cytokines with potent chemoattractive properties, which selectively promote adhesion and chemotaxis of various leukocyte subtypes and which may also regulate cellular activation and leukocyte effector function [5, 34]. Currently, chemokines are divided into four distinct subfamilies, the CXC, the CC, the CX_3C, and the C family of chemokines [5, 41, 63]. Members of each family exert overlapping chemoattractant specificity for leukocyte populations [63]. Thus, CC chemokines, to which macrophage inflammatory protein (MIP)-1α, MIP-1β, monocyte chemoattractant protein (MCP)-1, and RANTES belong, commonly attract T cells, monocytes and granulocytes, whereas the CXC family members, to which interferon (IFN)-γ inducible protein-10 (CRG-2/IP-10), and monokine induced by IFN-γ (MIG, MuMIG) belong, attract lymphocytes and monocytes, but not neutrophils [4].

A variety of in vitro studies have demonstrated that T cells and macrophages are characterized by specific chemokine and chemokine receptor expression profiles, which are dynamically regulated and depend on the activation state of the cell [4, 6, 10, 60]. Furthermore, isolated

human, rat and murine astrocytes and microglia respond to tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IFN-γ, and lipopolysaccharide (LPS) stimulation with the transcription and secretion of MCP-1, MIP-1α, MIP-1β, RANTES, CRG-2/IP-10, and MuMIG, the pattern depending on the inducing stimulus [7, 19, 24, 25, 26, 29, 38, 39, 61]. Chemokines are also produced in inflammatory CNS disorders including autoimmune and infectious diseases [3, 4, 22, 23, 28, 32, 44, 51, 56, 59]. With respect to parasitic infections, MuMIG and CRG-2/IP10 were induced in the brains of *Toxoplasma gondii*-infected C57BL/6 mice, but not in *Plasmodium yoelii*-infected mice [1]. Interestingly, a recent study demonstrated that CRG-2/IP-10 also controls the immune response to *T. gondii* at various levels, including the regulation of T cell influx to *T. gondii*-infected organs and the magnitude of the effector response in infected tissues [30]. However, the kinetics and cellular sources of a broad panel of chemokines as well as their regulation and specific role in parasitic diseases of the CNS largely remain to be determined.

In murine *Toxoplasma* encephalitis (TE), a chronic persistent infection of the CNS, leukocytes, in particular $CD4^+$ and $CD8^+$ T cells, which are obligatory for an effective anti-parasitic immune response [21, 48, 49, 55], are recruited to the brain and form dense inflammatory infiltrates in intimate association with the parasite [48]. Protection of $CD4^+$ and $CD8^+$ T cells is mainly mediated by IFN-γ, which induces anti-parasitic effector mechanisms and is the key activator of microglia and cerebral endothelium [14, 15, 20, 55]. The crucial role of IFN-γ for immunity to *T. gondii* is illustrated by the rapid fatal course of the infection in IFN-$\gamma^{0/0}$ and IFN-$\gamma R^{0/0}$ mice, which succumb to the disease up to day 10 after infection (p.i.) [14, 47]. In addition, resident brain cells including microglia, astrocytes, vascular endothelial cells, neurons, choroid plexus epithelium and ependyma are activated and differentially contribute to the intracerebral (i.c.) immune response by the induction of immunologically relevant cell surface molecules and by the production of protective cytokines [13, 15, 50].

To gain more insight into the regulation of immune responses in TE, we characterized the cellular sources and the kinetics of the i.c. gene expression profiles of a panel of chemokines including CRG-2/IP-10, MuMIG, MCP-1, RANTES, MIP-1α, and MIP-1β during various stages of murine TE. To precisely characterize the role of IFN-γ for chemokine gene expression in TE, we took advantage of IFN-$\gamma^{0/0}$ mice and compared them to wild-type (WT) mice. Here, we show that astrocytes, microglia, and i.c. leukocytes differentially express chemokines. Importantly, IFN-γ was identified as a major inducer and regulator of chemokines, and, functionally, the absence of IFN-γ resulted in an almost complete abolishment of leukocyte recruitment to the brain parenchyma.

Materials and methods

Animals

Female BALB/c WT mice ($H\text{-}2^d$) at the age of 6–8 weeks were obtained from Harlan-Winkelmann (Borchen, Germany). Breeding pairs of IFN-γ-deficient (IFN-$\gamma^{0/0}$) mice on a BALB/c background were obtained from the Jackson Laboratory (Bar Harbor, Me.) and bred in our animal facility. All animals were kept under conventional conditions in an isolation facility throughout the experiments.

T. gondii infection

Parasites were harvested from the brains of mice chronically infected with a low-virulent strain of *T. gondii* (DX strain). Brain tissue of these animals was dispersed in 0.1 M PBS pH 7.4. The final concentration of the infectious agents was adjusted to a dose of 5 cysts/0.5 ml, which was administered intraperitoneally (i.p.) to the experimental animals.

Experimental procedure and tissue processing

Uninfected and *T. gondii*-infected (days 4, 7, 10, 30, and 60 p.i.) mice were studied. At the respective dates, animals were perfused intracardially with 0.9% saline in deep Metofane (Janssen, Neuss, Germany) anesthesia.

For immunohistochemistry on frozen sections, reverse transcription (RT)-PCR analysis and ribonuclease protection assay (RPA), brains of five animals per group were dissected and blocks were mounted on thick filter paper with Tissue-Tek O.T.C. Compound (Miles Scientific, Naperville, Ill.), snap-frozen in isopentane (Fluka, Neu-Ulm, Germany) precooled on dry ice, and stored at –80°C.

For flow cytometry analysis of brain-derived leukocytes, the brains of three animals per experimental group were pooled and prepared as described previously [50]. In brief, brain tissue was passed through a 100-mesh stainless steel sieve, and leukocytes were separated by Percoll gradient centrifugation (Amersham-Pharmacia, Freiburg, Germany).

For in situ hybridization five animals per group were perfused intracardially with 4% buffered paraformaldehyde in deep Metofane narcosis. Their brains were dissected and fixed in 4% buffered paraformaldehyde at 4°C for 24 h followed by routine embedding in paraffin.

Monoclonal and polyclonal antibodies

The following antibodies were used: rat anti-mouse CD45 (LCA, clone M1/9.3.4.HL.2), rat anti-mouse I-A(b, d, q haplotypes, clone M5/114.15.2, both from the American Type Culture Collection, ATCC, Manassas, Va.), polyclonal rabbit anti-*T. gondii* antiserum (Biogenex, Duiven, The Netherlands), goat anti-mouse MCP-1 (Santa Cruz Biotechnology, Heidelberg, Germany), peroxidase-linked sheep anti-rat IgG $F(ab')_2$ fragments (Amersham-Pharmacia), peroxidase-conjugated goat anti-rabbit IgG $F(ab')_2$ fragments (Dianova, Hamburg, Germany), biotinylated mouse serum-preadsorbed mouse anti-rat IgG $F(ab')_2$ (Dianova), biotinylated mouse anti-goat IgG $F(ab')_2$ fragments (Dianova), rabbit anti-cow glial fibrillary acidic protein (GFAP, Dako, Hamburg, Germany), peroxidase-linked streptavidin-biotin complex (Dako), biotin-labeled B_4-isolectin GS4 (Sigma, Deisenhofen, Germany), anti-rabbit/anti-mouse avidin-biotinylated horseradish peroxidase complex (ABC-Kit, Vector, Burlingame, Calif.), mouse mAb to neurofilaments (SMI 33, Sternberger Monoclonals, Lutherville, Md.). F4/80-FITC was from Alexis Biochemicals (Grünberg, Germany). Mouse serum preadsorbed phycoerythrin (PE)-conjugated goat anti-rat IgG and avidin PE/Cy5 were from Southern Biotechnology Associates-Biozol (Freising, Germany). For flow cytometry, CD45 was conjugated with biotin by standard labeling procedures.

Immunohistochemistry

Immunohistochemistry was performed on 10-μm frozen sections as described previously [48]. In brief, for the detection of CD45 (LCA), and *T. gondii* an indirect immunoperoxidase protocol using sheep anti-rat IgG $F(ab')_2$ or goat anti-rabbit IgG $F(ab')_2$, respectively, as secondary antibody was employed. In addition, the avidin-biotin complex technique was used for demonstration of F4/80, GFAP, and B_4-isolectin GS4 on 4-μm paraffin sections and for detection of MCP-1 on cryostat sections. Peroxidase reaction products were visualized using 3,3'-diaminobenzidine (Sigma) and H_2O_2 as co-substrate. Sections were lightly counterstained with hemalum (Merck, Darmstadt, Germany).

Flow cytometry analysis

Brain-derived leukocytes were analyzed by double-immunofluorescence staining followed by flow cytometry as described previously [50]. Murine microglia and macrophages were identified by staining with anti-CD45-biotin and anti-F4/80-FITC followed by avidin-PE/Cy5. Microglia is $CD45^{low}$, whereas inflammatory leukocytes are $CD45^{high}$ [50]. Control staining included incubation of brain-derived leukocytes with unlabeled or fluorochrome-labeled isotype-matched control antibodies.

Flow cytometry was performed on a FACScan (Becton Dickinson, Heidelberg, Germany), and the data were analyzed with the CellQuest Software (Becton Dickinson).

Detection of cytokine and chemokine mRNA by RT-PCR

Chemokine (CRG-2/IP-10, MuMIG, MCP-1, RANTES, MIP-1α, MIP-1β) mRNA transcripts as well as IFN-γ and hydroxyphosphoribosyltransferase (HPRT) mRNA expression were analyzed in brain tissue homogenates according to a protocol described in detail previously [15]. Primer and probe sequences for chemokines were as follows: CRG-2/IP-10, 5'-CCACGTGTTGAGATCATTGC-3' (sense), 5'-GCTTACAGTACAGAGCTAGG-3' (antisense), and 5'-TGTGATGGACAGCAGAGAGC-3' (probe); MuMIG, 5'-GAGGAACCCTAGTGATAAGG-3' (sense), 5'-GTAGTCTTCCTTGAACGACG-3' (antisense), and 5'-CCTGCCTAGATCCGGACTCG-3' (probe); MCP-1, 5'-AGAGAGCCAGACGGAGGAAG-3' (sense), 5'-GTCACACTGGTCACTCCTAC-3' (antisense), and 5'-GAGAGAGGTCTGTGCTGACC-3' (probe); RANTES, 5'-GGTACCATGAAGATCTCTGC-3' (sense), 5'-GGGTCAGAATCAAGAAACCC-3' (antisense), and 5'-CTCTCCCTAGAGCTGCCTCG-3' (probe); MIP-1α, 5'-CCTGCTCAACATCATGAAGG-3' (sense), 5'-GAATTGGCGTGGAATCTTCC-3' (antisense), and 5'-TCTGTACCATGACACTCTGC-3' (probe); MIP-1β, 5'-GCAGCTTCACAGAAGCTTTG-3' (sense), 5'-TCTCAGTGAGAAGCATCAGG-3' (antisense), and 5'-CAGACAGATCTGTGCTAACC-3' (probe).

In brief, mRNA was extracted from the brains using an mRNA extraction kit (Pharmacia). After reverse transcription of mRNA using the Superscript RT kit (Life Technologies, Eggenstein, Germany), PCR reactions were carried out in a volume of 30 μl. The PCR reaction conditions were optimized for each set of primers. PCR was performed at different cycle numbers to ensure that amplification occurred in the linear range. PCR products were electrophoresed through an agarose gel and the DNA was transferred to a nylon membrane (Amersham-Pharmacia). Blots were hybridized using specific oligonucleotide probes, which were 3'-end labeled with digoxigenin using a DIG oligonucleotide 3'-end labeling kit (Roche, Mannheim, Germany). A DIG luminescent kit (Roche) was used to visualize the hybridization products.

Ribonuclease protection assay

For RPA, a multiprobe set containing probes against CXC chemokines including CRG-2/IP10, and MuMIG, CC chemokines including MCP-1, MIP-1α, MIP-1β, and RANTES were used to detect the simultaneous expression of multiple chemokine mRNA transcripts as described previously [3]. A fragment of the RPL32 gene served as an internal loading control. In brief, the mRNA samples were hybridized with the labeled probe set at 56°C for 12–16 h. After digestion with RNase A and RNase T1 (Promega, Madison, Wis.), purified protected fragments were denatured and electrophoresed in a standard 6% polyacrylamide/7 M urea/0.5% TBE-buffered sequencing gel. Dried gels were placed on XAR film (Kodak, Rochester, N.Y.) with intensifying screens and were exposed at –70°C.

Quantitation of chemokine mRNA signals obtained by RPA was performed with an imaging densitometer (Scanpack, Biometra, Göttingen, Germany). The relative intensity of bands for each chemokine was determined and compared with the intensity of the autoradiographic band used for the internal control, RPL32. The results were expressed as the degree of increase over chemokine mRNA levels obtained for the chemokines in uninfected animals of the same strain, levels of which were defined as 100%.

In situ hybridization

In situ hybridization was performed according to a protocol described in detail previously [3]. Briefly, paraffin sections were dewaxed and rehydrated in graded alcohols. After pretreatment with 4% buffered formaldehyde, proteinase K and acetic anhydride, slides were dehydrated and dried. CRG-2/IP-10 (762 bp), MuMIG (300 bp), RANTES (250 bp, kindly provided by Dr. T. Lane, Irvine, Calif.) and MCP-1 (170 bp) cDNA fragments were used. Templates for the probes for CRG-2/IP-10, MuMIG, RANTES and MCP-1 were generated by RT-PCR, cloned into the pGEM-4 plasmid and sequenced to confirm their identity with published sequences. ^{33}P-labeled antisense probes were hybridized to the tissue at 55–59°C overnight. After digestion with RNase A (Sigma), slides were washed in decreasing concentrations of SSC.

Selected sections were additionally immunostained for GFAP, SMI 33, *T. gondii* or B_4 isolectin. Thereafter, slides were dipped in Kodak NTB-2 emulsion, exposed at 4°C for 6–21 days, developed and counterstained with hemalum.

Table 1 Kinetics of the intracerebral parasitic load in *Toxoplasma gondii*-infected IFN-$\gamma^{0/0}$ and wild-type mice. The number of *T. gondii* cysts was determined in 100 high power fields in anti-*T. gondii* immunostained sections. Data represent mean ± SEM from three to five mice per group (*p.i* post infection)

Mouse strain	4 days p. i.	7 days p. i.	10 days p. i.	30 days p. i.	60 days p. i.
BALB/c	0.53±0.87	7.26±1.73	21.84±2.24	14.10± 2.63	1.57±1.27
IFN-$\gamma^{0/0}$	4.94±1.65[a]	17.61±1.49[a]	32.09±0.66[b]	–[c]	–[c]

[a]Significantly different from the respective WT value ($P<0.005$)
[b]Significantly different from the respective WT values ($P<0.05$)
[c]Died

Statistical evaluation

For statistical evaluation of the i.c. parasitic load at the various time points of TE, the number of parasites was determined on anti-*T. gondii*-immunostained sections from various brain regions in IFN-$\gamma^{0/0}$ and WT mice. At least 100 high power fields (HPF), which were randomly selected, were analyzed per section in three to five animals per group, and the statistical significance of the differences was evaluated by using the Student's *t*-test. Differences in the number of i.c. inflammatory leukocytes between IFN-$\gamma^{0/0}$ and WT mice were statistically analyzed by Student's *t*-test. *P* values <0.05 were accepted as significant.

Results

Chemokines are differentially expressed in IFN-$\gamma^{0/0}$ and WT mice

Following i.p. infection, both strains developed TE. In accordance with published data [14, 15, 47], IFN-$\gamma^{0/0}$ mice were highly susceptible to acute toxoplasmosis and succumbed up to day 10 p.i., whereas WT mice were resistant. This differential degree of susceptibility was reflected by a significantly increased i.c. parasitic load of IFN-$\gamma^{0/0}$ mice as compared to WT mice during the various stages of TE (Table 1).

To define the i.c. chemokine pattern in TE, the gene expression of a panel of chemokines involved in the attraction predominantly of T cells and macrophages was investigated by RPA and RT-PCR. In the normal brain of both WT and IFN-$\gamma^{0/0}$ mice, chemokine mRNA was not detectable by RPA. During TE of WT animals, CRG-2/IP-10, RANTES, MuMIG, and MCP-1 were transcribed (Fig. 1), while MIP-1α and MIP-1β were not detectable by RPA (data not shown). In close association with increasing numbers of both i.c. parasites as well as i.c. leukocytes, chemokine mRNA levels raised from day 10 p.i. to day 30 p.i. in parallel to progressive TE and declined thereafter (Fig. 1). Thus, in WT mice there was a correlation between disease activity and i.c. chemokine expression during the various stages of acute and chronic TE.

To dissect the role of IFN-γ for the induction of chemokines in TE, IFN-$\gamma^{0/0}$ mice were studied. RPA was completely negative in uninfected and *T. gondii*-infected IFN-$\gamma^{0/0}$ mice and detected only faint signals for CRG-2/IP-10, MuMIG, and MCP-1 in WT mice (Fig. 1) at days 7 and 10 p.i., respectively. Since these findings probably may reflect detection limit of the RPA, we further applied a highly sensitive semiquantitative RT-PCR to avoid missing low levels of chemokine mRNA. In general, RT-PCR exactly paralleled RPA data, but was – as expected – more

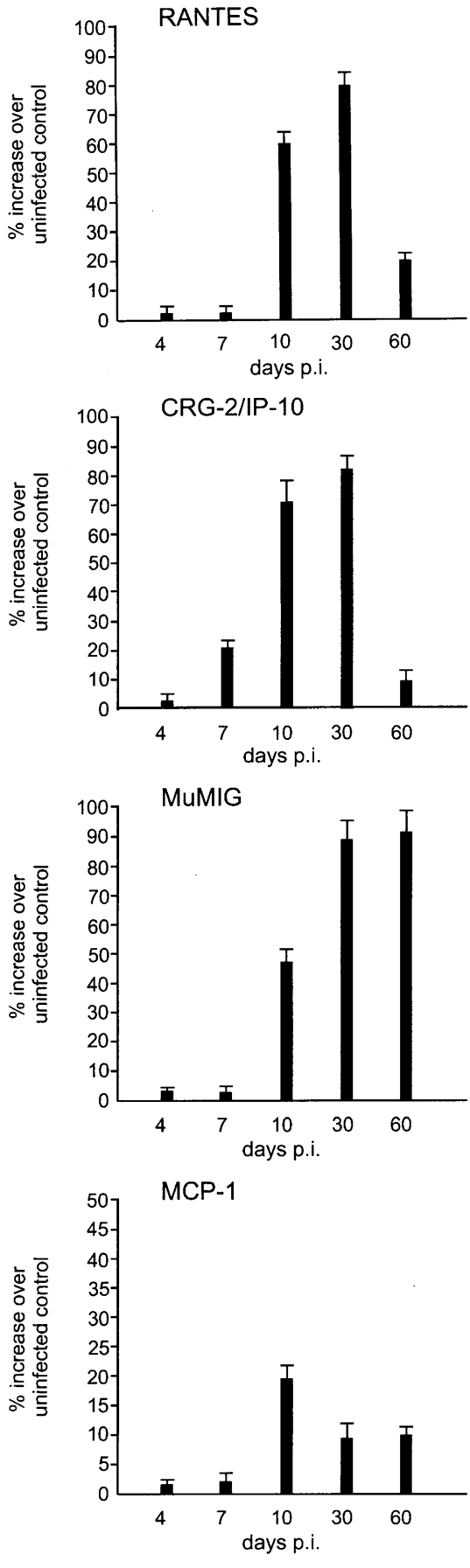


Fig. 1 Chemokine gene expression in the brains of uninfected and *Toxoplasma gondii*-infected WT mice at days 4, 7, 10, 30, and 60 p.i. Data are presented as relative increase of chemokine mRNA levels equalized to RPL32 mRNA levels at the indicated time points over values obtained from brains of uninfected mice of the respective strain, defined as 100%. Data represent the mean ± SEM of two mice in each experimental group. A second experiment yielded identical results (*p.i.* post infection)

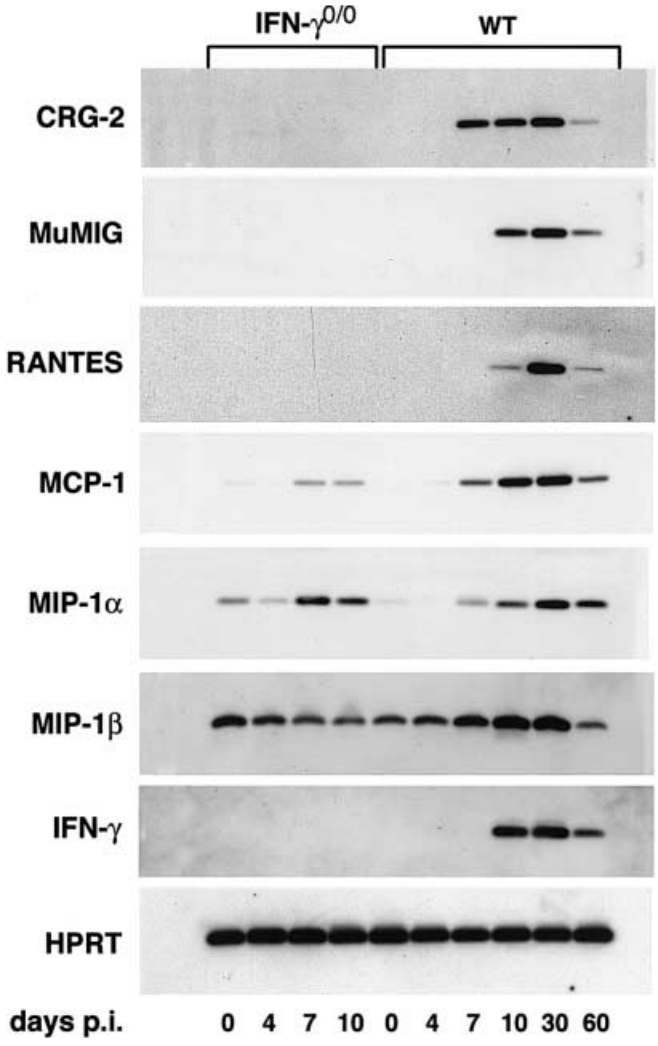


Fig. 2 RT-PCR analysis of chemokine mRNA expression in brain tissue homogenates of uninfected and *T. gondii*-infected IFN-$\gamma^{0/0}$ and WT mice. At the indicated time points, three mice per experimental group were analyzed, and representative autoradiograms are shown (*RT* reverse transcription, *WT* wild type)

sensitive (Fig. 2). Importantly, RT-PCR confirmed the total lack of the IFN-γ-inducible chemokines CRG-2/IP-10 and MuMIG as well as RANTES from the brains of IFN-$\gamma^{0/0}$ mice (Fig. 2). A weak constitutive expression of MIP-1α was noticed in both IFN-$\gamma^{0/0}$ and WT mice with a further up-regulation in TE of both strains of mice (Fig. 2). In contrast, levels of constitutively expressed MIP-1β were not further up-regulated following infection with *T. gondii* in IFN-$\gamma^{0/0}$ mice, whereas they increased in TE of WT mice. In parallel to the disease activity surviving WT mice down-regulated MIP-1α and MIP-1β back to levels of constitutive expression at day 60 p.i. MCP-1 was slightly induced in the brains of IFN-$\gamma^{0/0}$ mice, levels of which were below WT mice.

Both resident brain cells and inflammatory leukocytes express chemokine RNA in TE and are characterized by specific chemokine profiles

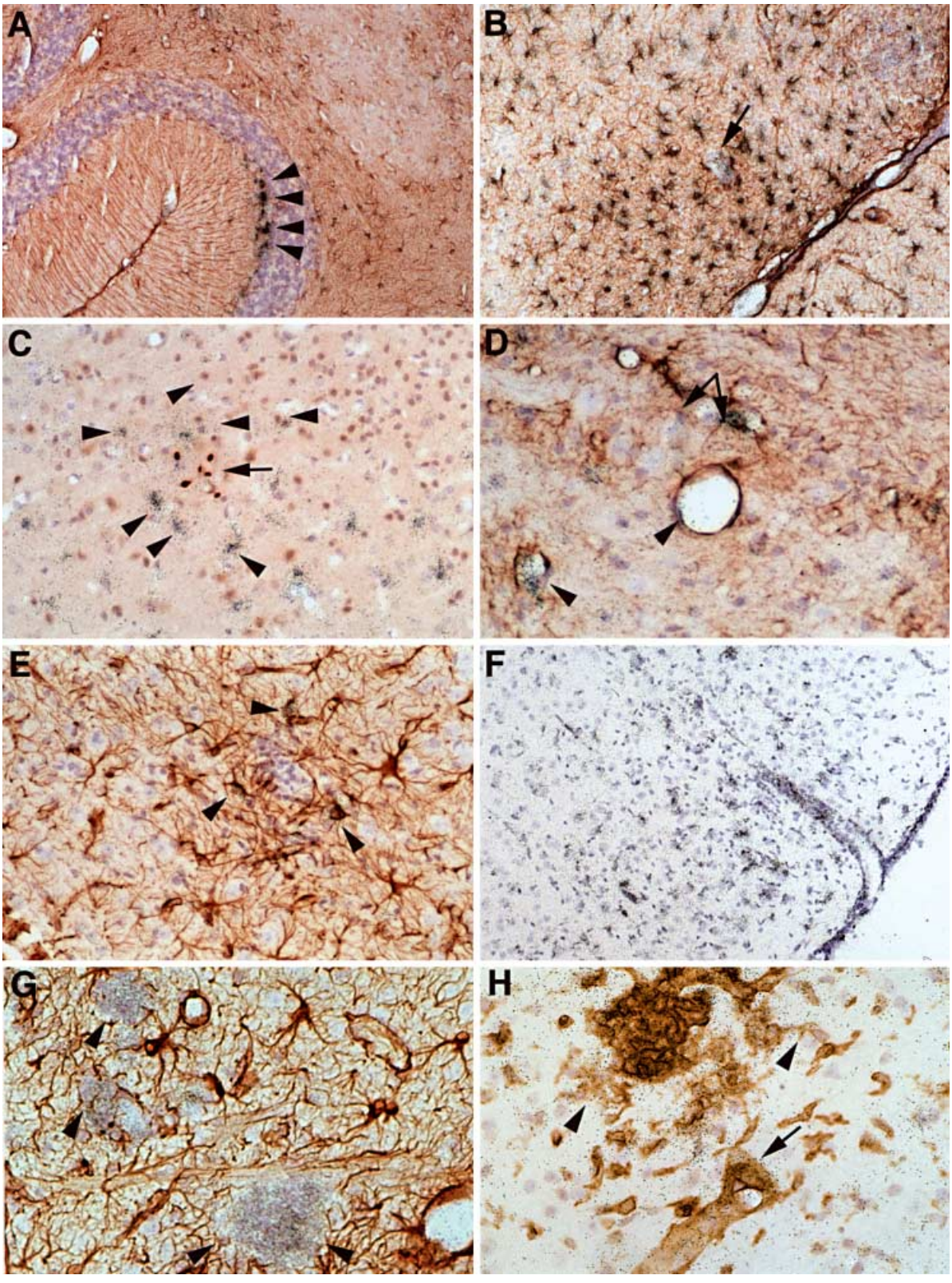
To identify the cellular sources of CRG-2/IP-10, MuMIG, RANTES, and MCP-1 gene expression and to analyze their relation to inflammatory infiltrates and the parasite, combined in situ hybridization and immunohistochemical studies were performed.

Since IFN-$\gamma^{0/0}$ mice succumbed to the infection up to day 10 without a significant transcription of chemokines (Fig. 2), chemokine RNA expression in these animals was not amenable to a topographical study, and, thus, we focused on WT mice. Among the various resident brain cell populations, astrocytes and microglia were the only cell types contributing to the i.c. chemokine gene expression. There was no detectable chemokine RNA expression of neurons, oligodendrocytes, cerebral blood vessel endothelium, ependyma, and choroid plexus.

In the normal brain, chemokine-expressing cells were not identified by in situ hybridization.

At day 10 p.i., astrocytes were focally activated as evidenced by the formation of cells with long, extended cellular processes and an up-regulation of GFAP close to *T. gondii* antigen, leptomeningeal and perivascular inflammatory infiltrates. In situ hybridization located CRG-2/IP-10 RNA to these activated astrocytes and to leptomeningeal and perivascular leukocytes. In addition, Bergmann glia of the cerebellum in the vicinity of leptomeningeal

Fig. 3A–H Topographical analysis of chemokine RNA expression in *Toxoplasma* encephalitis of WT mice. **A** Activated Bergmann glia (*arrowheads*), which extend their cellular processes towards the surface of the cerebellum, express CRG-2/IP-10 RNA at day 10 p.i.. Combined in situ hybridization for CRG-2/IP-10 and anti-GFAP immunostaining, slight counterstaining with hemalum. **B** Activated cortical astrocytes close to an inflammatory infiltrate (*arrow*) are strongly CRG-2/IP-10 RNA positive at day 30 p.i. Combined in situ hybridization for CRG-2/IP-10 and anti-GFAP immunostaining, slight counterstaining with hemalum. **C** A cluster of *T. gondii* cysts (*arrows*) is intimately associated with CRG-2/IP-10 RNA-positive cells (*arrowheads*) at day 30 p.i. Combined in situ hybridization for CRG-2/IP-10 and anti-*T. gondii* immunostaining, slight counterstaining with hemalum. **D** Expression of MCP-1 RNA in activated astrocytes in the vicinity of a blood vessel (*arrows*) at day 10 p.i. *Arrowheads* point to astrocytes extending with their cellular processes onto the blood vessel wall. Combined in situ hybridization for MCP-1 and anti-GFAP immunostaining, slight counterstaining with hemalum. **E** MCP-1 RNA expression of activated astrocytes (*arrowheads*) surrounding a leukocytic infiltrate at day 30 p.i. Combined in situ hybridization for MCP-1 and anti-GFAP immunostaining, slight counterstaining with hemalum. **F** MuMIG RNA expression of numerous leukocytes, which are part of cortical and meningeal infiltrates at day 30 p.i. In situ hybridization for MuMIG, slight counterstaining with hemalum. **G** Expression of RANTES RNA by leukocytes (*arrowheads*) within inflammatory infiltrates, which are surrounded by strongly activated astrocytes at day 30 p.i. Probing for RANTES, anti-GFAP immunostaining, slight counterstaining with hemalum. **H** $GS4^+$ macrophages and activated microglial cells (*arrowhead*) strongly express RANTES RNA at day 30 p.i. *Arrow* depicts a RANTES expressing perivascular cell. Probing for RANTES, anti-GS4 immunostaining, slight counterstaining with hemalum. **A** ×90; **B** ×110; **C**, **D**, **G** ×180; **E** ×150; **F** ×75; **H** ×300


A
B
C
D
E
F
G
H

and small infiltrates in the brain parenchyma expressed CRG-2/IP-10 RNA (Fig. 3a).

At day 30 p.i., astrocytes were strongly activated throughout the brain. However, although the number of astrocytes expressing CRG-2/IP-10 RNA had increased, CRG-2/IP-10 expression was confined to astrocytes surrounding inflammatory infiltrates and to astrocytes extending their cellular processes onto the endothelium of blood vessels, which were associated with leukocytes (Fig. 3b). In addition, leukocytes adhering to cerebral endothelium, in the leptomeninges, in the choroid plexus and in the ependymal lining of the ventricular wall as well as within perivascular and *T. gondii*-associated infiltrates were strongly positive for CRG-2/IP-10 RNA (Fig. 3b). Interestingly, tachyzoites were always, and cysts often, surrounded by CRG-2/IP-10 RNA-positive leukocytes and astrocytes (Fig. 3c). In addition to astrocytes, microglia were ubiquitously activated as evidenced by a generalized induction of MHC class II antigen; however, microglia contributed only exceptionally to the CRG-2/IP-10 RNA expression. Within perivascular and *T. gondii*-associated infiltrates, some $GS4^+$ macrophages were CRG-2/IP-10 RNA positive, which, however, expressed much lower levels than astrocytes.

MCP-1 RNA was expressed by a number of astrocytes as part of the blood-brain barrier, in the vicinity of inflammatory infiltrates and close to parasites expressed MCP-1 RNA at days 10 and 30 p.i. in WT mice (Fig. 3d, e). In addition, some leukocytes adhering to cerebral endothelium and as part of infiltrates were MCP-1 RNA positive and also expressed MCP-1 protein (Fig. 4).

MuMIG was transcribed by leukocytes in the leptomeninges and within perivascular infiltrates including $GS4^+$ macrophages and microglia (Fig. 3f). Astrocytes were consistently MuMIG negative.

RANTES RNA overlapped with i.c. leukocytes, in particular within inflammatory infiltrates (Fig. 3g). In addition, single leukocytes scattered throughout the brain also, but only weakly, expressed RANTES RNA. $GS4^+$ macrophages and a large number of microglial cells either within inflammatory infiltrates or in close contact with parasites accounted for most of the RANTES RNA expression (Fig. 3h). In contrast, astrocytes did not express RANTES RNA (Fig. 3g). Many, but not all, *T. gondii* cysts were surrounded by RANTES-positive cells.

Thus, in TE astrocytes, microglia and leukocytes recruited to the brain differentially express chemokine RNA. Furthermore, although brain parenchymal cells are strongly and ubiquitously activated throughout the brain, chemokine expression clearly was locally restricted to those cells residing perivascularly, i.e., in the vicinity of the blood-brain barrier, or in association with the offending parasite.

Impaired recruitment and i.c. movement of leukocytes in *T. gondii*-infected IFN-$\gamma^{0/0}$ mice

TE of WT animals was characterized by a recruitment of leukocytes to the CNS. However, in IFN-$\gamma^{0/0}$ mice the number of inflammatory leukocytes was significantly reduced as compared to WT mice ($P<0.05$).

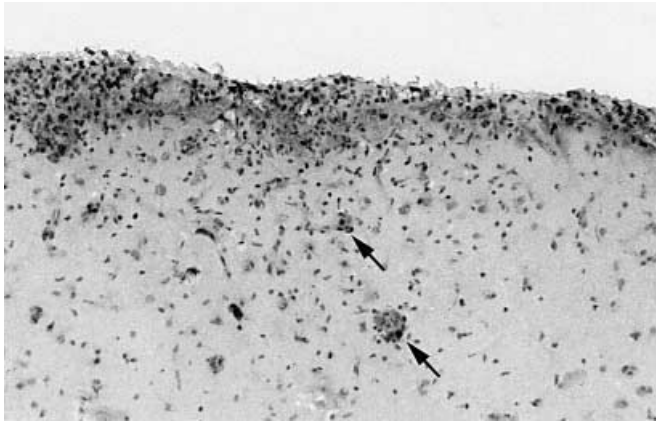


Fig. 4 Immunohistochemical analysis of MCP-1 expression in a WT mouse. Leukocytes as part of meningeal and perivascular (*arrows*) infiltrates express MCP-1 at day 30 p.i. Anti-MCP-1 immunostaining, slight counterstaining with hemalum, ×200

Histopathology showed that in WT mice leukocytes moved to the meninges and the perivascular space starting at day 7 p.i. However, their further movement within the brain parenchyma depended on signaling via IFN-γ. Up to day 10 p.i., inflammatory $CD45^+$ leukocytes had already moved into the brain parenchyma of WT animals, where they had formed small, parasite-associated infiltrates. In marked contrast, IFN-$\gamma^{0/0}$ mice failed to recruit leukocytes to the site of parasites, and only occasionally single leukocytes were detectable in the brain parenchyma of these animals, although the number of i.c. parasites most strongly increased in the brains of these animals (Table 1).

At later time points, i.e., 60 days p.i., the efficient control of *T. gondii* in WT mice was paralleled by decreasing numbers of i.c. leukocytes. Thus, the i.c. parasitic load, the number of i.c. inflammatory leukocytes and levels of i.c. chemokines strongly correlated.

Discussion

This study demonstrates that a panel of chemokine genes is induced during the acute and chronic stage of TE, the levels of which are directly correlated with disease activity. In addition, astrocytes, microglia and inflammatory leukocytes are identified as the cellular sources of the i.c. chemokines. Furthermore, in TE chemokine transcription is critically regulated by IFN-γ, the major mediator of protective immunity against *T. gondii* [20, 55], and, functionally important, the absence of IFN-γ signaling impairs the recruitment of leukocytes to the brain and prevents the i.c. movement of leukocytes to parenchymatous parasites.

Simultaneously with parasite invasion of the CNS, there was a coordinate recruitment of leukocytes to the brain as well as i.c. chemokine transcription including MIP-1α, MIP-1β, MCP-1, CRG-2/IP-10, RANTES, and MuMIG. This pattern of i.c. produced chemokines may

profoundly influence the composition of the infiltrates. CRG-2/IP-10, MuMIG, and RANTES as well as MIP-1α, MIP-1β, and MCP-1 preferentially recruit $CD4^+$ and $CD8^+$ T cells as well as macrophages [8, 37, 46, 58], the major constituents of the inflammatory infiltrates in TE ([48] and this study). CRG-2/IP-10, which has no effect on resting T cells, and MuMIG promote the recruitment of activated T lymphocytes [5, 12, 36], and, moreover, RANTES preferentially attracts activated T cells with a memory phenotype [45]. In fact, only activated and memory T cells are recruited to the *T. gondii*-infected brain ([43], and M. Deckert and D. Schlüter, unpublished observation).

An important part of the present study relates to the identification of the cellular sources of CRG-2/IP-10, MuMIG, RANTES, and MCP-1. Both resident brain cells and leukocytes recruited to the brain contributed to chemokine gene expression, and each cell population was characterized by a specific chemokine profile. Among brain cells transcription of chemokine genes was tightly regulated and confined to astrocytes and microglial cells, although activation of a variety of resident brain cells including astrocytes, microglia, endothelium, choroid plexus epithelium, ependyma, and neurons is a hallmark of murine TE [48]. Immediately upon parasite infection of the CNS, blood vessel-associated astrocytes responded with the induction of CRG-2/IP-10 and MCP-1 genes. Interestingly, astrocytes, which form an integral part of the blood-brain barrier, do not express cell adhesion molecules in murine TE, whereas cerebral blood vessel endothelial cells show an inverse reaction pattern characterized by the expression of cell adhesion molecules, but not of chemokine genes [13]. These data indicate that the various constituents of the blood-brain barrier might exert highly specific functions during leukocyte attraction to i.c. parasites, which is a complex finely tuned cascade of events, and that their gradually induced, concerted action is highly important for leukocyte recruitment to the *T. gondii*-infected brain. First, leukocytes bind to cerebral endothelial cells, which express ICAM-1 and VCAM-1 in TE [13]. This binding may be profoundly reinforced by the chemokine-strengthening properties of leukocyte binding to ICAM-1 and VCAM-1 [11, 33, 36, 40, 53]. This step is followed by astrocyte-mediated induction of leukocyte transmigration across the blood-brain barrier via their chemokine production, as has been demonstrated in an in vitro model of the blood-brain barrier [61]. As an additional amplification loop of the i.c. immune response, microglia in the vicinity of parasites contribute to the i.c. chemokine production in full-blown encephalitis to further direct leukocytes close to the offending pathogen. Thus, chemokine gene expression may play an important role in the mechanisms of leukocyte recruitment to the CNS and their further i.c. migratory behavior.

Remarkably, microglia did not respond with MuMIG and RANTES transcription before development of full-blown encephalitis. This is of note, since microglia are rapidly activated in the early stages of TE as evidenced by their immediate expression of MHC class I and II antigens, ICAM-1, and LFA-1 [13] and are regarded as the immunological guardian of the CNS; therefore, these observations point to diverse functions of various i.c. cell populations.

The observation of a differential pattern and kinetics of chemokine gene expression by resident brain cells during TE extends previous experiments in lymphocytic choriomeningitis virus (LCMV) infection and mouse hepatitis virus (MHV). In these experimental models, astrocytes were also described as the major source of CRG-2/IP-10 [2, 4, 32, 44]. As observed in our study, in these experiments CRG-2/IP-10 expression topographically overlapped with areas harboring the offending pathogen. However, in contrast to TE, in LCMV infection ependymal, choroid plexus, and meningeal cells also expressed CRG-2/IP-10 cells. In addition, in experimental autoimmune encephalomyelitis (EAE) and multiple sclerosis, astrocytes, microglia, and endothelial cells produced a variety of chemokines including MCP-1, MIP-1α, MIP-1β, RANTES, and CRG-2/IP-10 [22, 23, 28, 51, 59]. The pattern of chemokine transcription described in these autoimmune disorders differed from our findings in TE and from those in MHV and LCMV infection. For example, the induction of CRG-2/IP-10 by astrocytes in an acute, self-limited model of EAE was an early temporary event [56], whereas CRG-2/IP-10 expression of astrocytes persisted into the chronic stages of TE. The differential chemokine expression in the various models illustrates that the pathogenic stimulus and the underlying disease significantly affect i.c. chemokine gene expression. This is supported by the observation that in the aforementioned models of inflammatory CNS disorders an individual brain cell population never expresses all of the chemokines which may principally be induced in these cell types in vitro by stimulation with cytokines [7, 19, 22, 24, 25, 26, 38, 39, 61].

In TE, chemokine gene expression was locally restricted to meningeal, perivascular, and parasite-associated infiltrates, although leukocytes were not only part of infiltrates, but were also scattered throughout the brain, and astrocytes as well as microglia were ubiquitously activated. This regionally accentuated chemokine expression may be the result of locally increased cytokine levels, in particular of IFN-γ, TNF-α, and IL-1β, which are produced by $CD4^+$ and $CD8^+$ T cells, macrophages, microglia, and astrocytes surrounding blood vessels and parasite-associated infiltrates during TE [50]. In this respect, it is particularly interesting that IFN-γ and TNF-α stimulate chemokine expression of resident brain cells [38]. Of note, chemokine production of human polymorphonuclear leukocytes induced by infection with *T. gondii* is also partially dependent on TNF [9]. In TE, the cytokine-induced chemokines may, in contrast, enhance local immune reactions by influencing T cell signaling events and T cell cytokine production [31, 36]. In fact, in vitro, T cells responded to T cell receptor stimulation in the presence of MIP-1α by an enhanced IFN-γ production [29]. From these findings it may be concluded that in TE both inflammatory leukocytes, which are the major producers of RANTES and MuMIG and also contributed to CRG-2/IP-10 and MCP-1 RNA ex-

pression, as well as brain resident cells, regulate the i.c. immune response – at least in part – by a dynamic interplay of chemokines and cytokines.

This assumption is formerly proved by our illustration of an abrogated transcription of CRG-2/IP-10, MuMIG, and RANTES and markedly reduced mRNA levels of MIP-1α, MIP-1β, and MCP-1 in IFN-$\gamma^{0/0}$ mice. These findings are in accordance with in vitro studies, which have demonstrated the capacity of IFN-γ to induce MuMIG and CRG-2/IP-10 [17, 18, 35]. However, in systemic toxoplasmosis MuMIG, but not CRG-2/IP-10 mRNA induction strictly required IFN-γ [1]. Interestingly, these findings indicate the existence of organ-specific requirements for chemokine induction and regulation, a finding which has also been suggested by Amichay et al. [1], who compared various models of infection between IFN-$\gamma^{0/0}$ and WT mice. Moreover, in TE, the absence of IFN-γ and the resulting impaired chemokine gene induction had profound functional consequences for the i.c. immune response. A markedly reduced number of inflammatory leukocytes was recruited to the brain of IFN-$\gamma^{0/0}$ mice, and a careful topographical analysis revealed that inflammatory leukocytes were trapped in the perivascular space and failed to migrate to *Toxoplasma* residing in the deep brain parenchyma. Obviously, the weak IFN-γ-independent expression of MCP-1, MIP-1α, and MIP-1β was only sufficient to attract leukocytes just beyond the blood-brain barrier, whereas the further i.c. movement of leukocytes, in particular of antigen-specific $CD4^+$ and $CD8^+$ T cells, which are the major cell populations mediating parasite control, to the pathogen required the coordinate induction of a variety of IFN-γ-dependent chemokines. Furthermore, CRG-2/IP-10 provides an important functional link between IFN-γ production and T cell effector function, since it also regulates proliferation of antigen-specific $CD4^+$ and $CD8^+$ T cells as well as the number of cytotoxic T cells. The important role of this IFN-γ-inducible chemokine is impressively illustrated by the increased mortality and elevated parasitic load in mice treated with an CRG-2/IP-10-neutralizing antibody [30].

These data and our findings in murine cerebral toxoplasmosis on the strong IFN-γ dependency of antigen-specific T cell influx are in remarkable contrast to the role of IFN-γ in EAE. In EAE of IFN-$\gamma^{0/0}$ mice, RANTES und MCP-1 transcription was also significantly reduced as compared to WT mice, but conversely MIP-2 und TCA-3 transcription was up-regulated [57]. In our model of TE, such an up-regulation of MIP-2 and TCA-3 did not occur (unpublished observation). Moreover, in contrast to TE the recruitment and i.c. movement of inflammatory leukocytes was unimpaired in EAE of IFN-$\gamma^{0/0}$ mice [57]. These findings underscore that in the CNS the underlying disorder critically determines the chemokine pattern of individual cell populations, the functional role of IFN-γ for both chemokine induction as well as for the recruitment and further movement of inflammatory leukocytes in the target tissue.

We also attempted to answer the intriguing question as to the cell population responsible for initiation of chemoattraction; however, even during the early induction phase of TE, which was closely correlated to the onset of chemokine transcription, both leukocytes recruited to the brain as well as activated astrocytes contributed to chemokine transcription. Therefore, it was virtually impossible to answer this open question with certainty.

Nevertheless, the central role of IFN-γ for i.c. chemokine transcription suggests that $CD4^+$ und $CD8^+$ T lymphocytes, which are the single sources of IFN-γ in TE [41] and which are essential for immunity to *T. gondii* [21, 54], are the critical regulators of chemokine expression in TE. Moreover, these data indicate that T cells pave their own way and the way of other important inflammatory leukocytes, i.e., macrophages, to the parasite in the brain parenchyma by their IFN-γ production. A critical role of T cells for the i.c. chemokine expression has also been shown in LCMV-infected athymic nude mice, which were devoid of i.c. chemokines [2]. In addition, Lane et al. [32] observed a correlation between $CD4^+$ T cells, i.c. RANTES expression, and the recruitment of immune cells to the brain in MHV infection, and Rajan et al. [42] noticed a strong regulatory effect of γδ T cells on i.c. chemokine production and disease activity in murine EAE.

In conclusion, the present study defines the pattern and the kinetics of chemokine expression in TE and illustrates that IFN-γ regulates the i.c. anti-parasitic immune response at various important levels including i.c. chemokine expression as well as the recruitment and movement of inflammatory leukocytes, thereby contributing to the prevention of further multiplication and dissemination of the parasite within the brain.

Acknowledgements The authors thank A. Rang and O. Schmidt for expert technical assistance, Dr. V. Hans for statistical help and H.U. Klatt for help with photographs. We are grateful to Dr. T. Lane for the gift of the plasmid containing RANTES cDNA. This study was supported by the Deutsche Forschungsgemeinschaft to M.D. (grant De 485/6-2) and NIH Grant MH 50426 to I.L.C. A. S. was a recipient of a scholarship from the Boehringer-Ingelheim Foundation. V.C.A. is a post doctoral fellow of the National Multiple Sclerosis Society.

References

1. Amichay D, Gazzinelli RT, Karupiah G, Moench TR, Sher A, Farber JM (1996) Genes for chemokines MuMIG and CRG-2 are induced in protozoan and viral infections in response to IFN-gamma with patterns of tissue expression that suggest nonredundant roles in vivo. J Immunol 157:4511–4520
2. Asensio VC, Campbell IL (1997) Chemokine gene expression in the brains of mice with lymphocytic choriomeningitis. J Virol 71:7832–7840
3. Asensio VC, Campbell IL (1999) Chemokines in the CNS: plurifunctional mediators in diverse states. Trends Neurosci 22: 504–512
4. Asensio VC, Kincaid C, Campbell IL (1999) Chemokines and the inflammatory response to viral infection in the central nervous system with a focus on lymphocytic choriomeningitis virus. J Neurovirol 5:65–75
5. Baggiolini M (1998) Chemokines and leukocyte traffic. Nature 392:565–568

6. Baggiolini M, Dewald B, Moser B (1994) Interleukin-8 and related chemotactic cytokines-CXC and CC chemokines. Adv Immunol 55:97–179
7. Barna BP, Pettay J, Barnett GH, Zhou P, Iwasaki K, Estes ML (1994) Regulation of monocyte chemoattractant protein-1 expression in adult human non-neoplastic astrocytes is sensitive to tumor necrosis factor (TNF) or antibody to the 55-kDa TNF receptor. J Neuroimmunol 50:101–107
8. Biddison WE, Cruikshank WW, Center DM, Pelfrey CM, Taub DD, Turner RV (1998) $CD8^+$ myelin peptide-specific T cells can chemoattract $CD4^+$ myelin peptide-specific T cells: importance of IFN-inducible protein 10. J Immunol 160:444–448
9. Bliss SK, Marshall AJ, Zhang Y, Denkers EY (1999) Human polymorphonuclear leukocytes produce IL-12, TNF-alpha, and the chemokines macrophage-inflammatory protein-1 alpha and -1 beta in response to *Toxoplasma gondii* antigens. J Immunol 162:7369–7375
10. Bonecchi R, Bianchi G, Bordignon PP, D'Ambrosio D, Lang R, Borsatti A, Sozzani S, Allavena P, Gray PA, Mantovani A, Sinigaglia F (1998) Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. J Exp Med 187:129–134
11. Carr MW, Alon R, Springer TA (1996) The C-C chemokine MCP-1 differentially modulates the avidity of beta 1 and beta 2 integrins on T lymphocytes. Immunity 4:179–187
12. Charles PC, Chen X, Horwitz MS, Brosnan CF (1999) Differential chemokine induction by the mouse adenovirus type-1 in the central nervous system of susceptible and resistant strains of mice. J Neurovirol 5:55–64
13. Deckert-Schlüter M, Schlüter D, Hof H, Wiestler OD, Lassmann H (1994) Differential expression of ICAM-1, VCAM-1 and their ligands LFA-1, Mac-1, CD43, VLA-4, and MHC class II antigens in murine Toxoplasma encephalitis: a light microscopic and ultrastructural immunohistochemical study. J Neuropathol Exp Neurol 53:457–468
14. Deckert-Schlüter M, Rang A, Weiner D, Huang S, Wiestler OD, Hof H, Schlüter D (1996) Interferon-gamma receptor-deficiency renders mice highly susceptible to toxoplasmosis by decreased macrophage activation. Lab Invest 75:827–841
15. Deckert-Schlüter M, Bluethmann H, Kaefer N, Rang A, Schlüter D (1999). Interferon-gamma receptor-mediated but not tumor necrosis factor receptor type 1- or type 2-mediated signaling is crucial for the activation of cerebral blood vessel endothelial cells and microglia in murine Toxoplasma encephalitis. Am J Pathol 154:1549–1561
16. Deckert-Schlüter M, Buck C, Schlüter D (1999) Kinetics and differential expression of heat-stable antigen and GL7 in the normal and *Toxoplasma gondii*-infected murine brain. Acta Neuropathol 98:97–106
17. Farber JM (1992) A collection of mRNA species that are inducible in the RAW 264.7 mouse macrophage cell line by gamma interferon and other agents. Mol Cell Biol 12:1535–1545
18. Farber JM (1993) HuMig: a new human member of the chemokine family of cytokines. Biochem Biophys Res Commun 192: 223–230
19. Fisher SN, Vanguri P, Shin HS, Shin ML (1995) Regulatory mechanisms of MuRantes and CRG-2 chemokine gene induction in central nervous system glial cells by virus. Brain Behav Immun 9:331–344
20. Gazzinelli RT, Hakim FT, Hieny S, Shearer GM, Sher A (1991) Synergistic role of $CD4^+$ and $CD8^+$ T lymphocytes in IFN-gamma production and protective immunity induced by an attenuated *Toxoplasma gondii* vaccine. J Immunol 146:286–292
21. Gazzinelli R, Xu Y, Hieny S, Cheever A, Sher A (1992) Simultaneous depletion of $CD4^+$ and $CD8^+$ T lymphocytes is required to reactivate chronic infection with *Toxoplasma gondii*. J Immunol 149:175–180
22. Glabinski AR, Tani M, Strieter RM, Tuohy VK, Ransohoff RM (1997) Synchronous synthesis of alpha- and beta-chemokines by cells of diverse lineage in the central nervous system of mice with relapses of chronic experimental autoimmune encephalomyelitis. Am J Pathol 150:617–630
23. Glabinski AR, Tuohy VK, Ransohoff RM (1998) Expression of chemokines RANTES, MIP-1alpha and GRO-alpha correlates with inflammation in acute experimental autoimmune encephalomyelitis. Neuroimmunomodulation 5:166–171
24. Guo H, Jin YX, Ishikawa M, Huang YM, van der Meide PH, Link H, Xiao BG (1998) Regulation of beta-chemokine mRNA expression in adult rat astrocytes by lipopolysaccharide, proinflammatory and immunoregulatory cytokines. Scand J Immunol 48:502–508
25. Hayashi M, Luo Y, Laning J, Strieter RM, Dorf ME (1995) Production and function of monocyte chemoattractant protein-1 and other beta-chemokines in murine glial cells. J Neuroimmunol 60:143–150
26. Hurwitz AA, Lyman WD, Berman JW (1995) Tumor necrosis factor alpha and transforming growth factor beta upregulate astrocyte expression of monocyte chemoattractant protein-1. J Neuroimmunol 57:193–198
27. Karpus WJ (1999) Chemokine regulation of inflammatory-mediated nervous system diseases. J Neurovirol 5:1–2
28. Karpus WJ, Lukacs NW, McRae BL, Strieter RM, Kunkel SL, Miller SD (1995) An important role for the chemokine macrophage inflammatory protein-1 alpha in the pathogenesis of the T cell-mediated autoimmune disease, experimental autoimmune encephalomyelitis. J Immunol 155:5003–5010
29. Karpus WJ, Lukacs NW, Kennedy KJ, Smith WS, Hurst SD, Barrett TA (1997) Differential CC chemokine-induced enhancement of T helper cell cytokine production. J Immunol 158:4129–4136
30. Khan IA, MacLean JA, Lec FS, Casciotti L, DeHaan E, Schwartzman JD, Luster AD (2000) IP-10 is crucial for effector T cell trafficking and host survival in *Toxoplasma gondii* infection. Immunity 12:483–494
31. Kopydlowski KM, Salkowski CA, Cody MJ, Rooijen N van, Major J, Hamilton TA, Vogel SN (1999) Regulation of macrophage chemokine expression by lipopolysaccharide in vitro and in vivo. J Immunol 163:1537–1544
32. Lane TE, Asensio VC, Yu N, Paoletti AD, Campbell IL, Buchmeier MJ (1998) Dynamic regulation of alpha- and beta-chemokine expression in the central nervous system during mouse hepatitis virus-induced demyelinating disease. J Immunol 160:970–978
33. Laudanna C, Campbell JJ, Butcher EC (1996) Role of Rho in chemoattractant-activated leukocyte adhesion through integrins. Science 271:981–983
34. Luster AD (1998) Chemokines – chemotactic cytokines that mediate inflammation. N Engl J Med 338:436–445
35. Luster AD, Unkeless JC, Ravetch JV (1985) Gamma-interferon transcriptionally regulates an early-response gene containing homology to platelet proteins. Nature 315:672–676
36. Moser B, Loetscher M, Piali L, Loetscher P (1998) Lymphocyte responses to chemokines. Int Rev Immunol 16:323–344
37. Murphy WJ, Tian ZG, Asai O, Rotter P, Heny M, Strieter RM, Kunkel SL, Longo DL, Taub DD (1996) Chemokines and T lymphocyte activation. II. Facilitation of human T cell trafficking in severe combined immunodeficiency mice. J Immunol 156:2104–2111
38. Oh JW, Schwiebert LM, Benveniste EN (1999) Cytokine regulation of CC and CXC chemokine expression by human astrocytes. J Neurovirol 5:82–94
39. Peterson PK, Hu S, Salak-Johnson J, Molitor TW, Chao CC (1997) Differential production of and migratory response to beta chemokines by human microglia and astrocytes. J Infect Dis 175:478–481

40. Piali L, Weber C, LaRosa G, Springer TA, Clark-Lewis I, Moser B (1998) The chemokine receptor CXCR3 mediates rapid and shear-resistant adhesion-induction of effector T lymphocytes by the chemokines IP10 and Mig. Eur J Immunol 28:961–972
41. Prieschl EE, Kulmburg, PA, Baumruker T (1995) The nomenclature of chemokines. Int Arch Allergy Immunol 107:475–483
42. Rajan AJ, Asensio VC, Campbell IL, Brosnan CF (2000) Experimental autoimmune encephalomyelitis in the SJL mouse: effect of gamma delta T cell depletion on chemokine and chemokine receptor expression in the central nervous system. J Immunol 164:2120–2130
43. Reichmann G, Villegas EN, Craig L, Peach R, Hunter CA (1999) The CD28/B7 interaction is not required for resistance to *Toxoplasma gondii* in the brain but contributes to the development of immunopathology. J Immunol 163:3354–3362
44. Sasseville VG, Smith MM, Mackay CR, Pauley DR, Mansfield KG, Ringler DJ, Lackner AA (1996) Chemokine expression in simian immunodeficiency virus-induced AIDS encephalitis. Am J Pathol 149:1459–1467
45. Schall TJ, Bacon K, Toy KJ, Goeddel DV (1990) Selective attraction of monocytes and T lymphocytes of the memory phenotype by cytokine RANTES. Nature 347:669–671
46. Schall TJ, Bacon K, Camp RD, Kaspari JW, Goeddel DV (1993) Human macrophage inflammatory protein alpha (MIP-1 alpha) and MIP-1 beta chemokines attract distinct populations of lymphocytes. J Exp Med 177:1821–1826
47. Scharton-Kersten TM, Wynn TA, Denkers EY, Bala S, Grunvald E, Hieny S, Gazzinelli RT, Sher A (1996) In the absence of endogenous IFN-gamma, mice develop unimpaired IL-12 responses to *Toxoplasma gondii* while failing to control acute infection. J Immunol 157:4045–4054
48. Schlüter D, Löhler J, Deckert M, Hof H, Schwendemann G (1991) Toxoplasma encephalitis of immunocompetent and nude mice: immunohistochemical characterisation of *Toxoplasma* antigen, infiltrates and major histocompatibility complex gene products. J Neuroimmunol 31:185–198
49. Schlüter D, Deckert-Schlüter M, Schwendemann G, Brunner H, Hof H (1993) Expression of major histocompatibility complex class II antigens and levels of interferon-gamma, tumour necrosis factor, and interleukin-6 in cerebrospinal fluid and serum in *Toxoplasma gondii*-infected SCID and immunocompetent C.B-17 mice. Immunology 78:430–435
50. Schlüter D, Kaefer N, Hof H, Wiestler OD, Deckert-Schlüter M (1997) Expression pattern and cellular origin of cytokines in the normal and *Toxoplasma gondii*-infected murine brain. Am J Pathol 150:1021–1035
51. Simpson JE, Newcombe J, Cuzner ML, Woodroofe MN (1998) Expression of monocyte chemoattractant protein-1 and other beta-chemokines by resident glia and inflammatory cells in multiple sclerosis lesions. J Neuroimmunol 84:238–249
52. Steffen BJ, Butcher EC, Engelhardt B (1994) Evidence for involvement of ICAM-1 and VCAM-1 in lymphocyte interaction with endothelium in experimental autoimmune encephalomyelitis in the central nervous system in the SJL/J mouse. Am J Pathol 145:189–201
53. Stein JV, Rot A, Luo Y, Narasimhaswamy M, Nakano H, Gunn MD, Matsuzawa A, Quackenbush EJ, Dorf ME, Adrian UH von (2000) The CC chemokine thymus-derived chemotactic agent 4 (TCA-4, secondary lymphoid tissue chemokine, 6Ckine, exodus-2) triggers lymphocyte function-associated antigen 1-mediated arrest of rolling T lymphocytes in peripheral lymph node high endothelial venules. J Exp Med 191:61–76
54. Suzuki Y, Remington JS (1988) Dual regulation of resistance against *Toxoplasma gondii* infection by Lyt-2^+ and Lyt-1^+, L3T4^+ T cells in mice. J Immunol 140:3943–3946
55. Suzuki Y, Orellana MA, Schreiber RD, Remington JS (1988) Interferon-gamma: the major mediator of resistance against *Toxoplasma gondii*. Science 240:516–518
56. 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
57. Tran EH, Prince EN, Owens T (2000) IFN-gamma shapes immune invasion of the central nervous system via regulation of chemokines. J Immunol 164:2759–2130
58. Uguccioni M, D'Apuzzo M, Loetscher M, Dewald B, Baggiolini M (1995) Actions of the chemotactic cytokines MCP-1, MCP-2, MCP-3, RANTES, MIP-1 alpha and MIP-1 beta on human monocytes. Eur J Immunol 25:64–68
59. Van Der Voorn P, Tekstra J, Beelen RH, Tensen CP, Van Der Valk P, De Groot CJ (1999) Expression of MCP-1 by reactive astrocytes in demyelinating multiple sclerosis lesions. Am J Pathol 154:45–51
60. Ward SG, Bacon K, Westwick J (1998) Chemokines and T lymphocytes: more than an attraction. Immunity 9:1–11
61. Weiss JM, Downie SA, Lyman WD, Berman JW (1998) Astrocyte-derived monocyte-chemoattractant protein-1 directs the transmigration of leukocytes across a model of the human blood-brain barrier. J Immunol 161:6896–6903
62. Wong D, Prameya R, Dorovini-Zis K (1999) In vitro adhesion and migration of T lymphocytes across monolayers of human brain microvessel endothelial cells: regulation by ICAM-1, VCAM-1, E-selectin and PECAM-1. J Neuropathol Exp Neurol 58:138–152
63. Zlotnik A, Morales J, Hedrick JA (1999) Recent advances in chemokines and chemokine receptors. Crit Rev Immunol 19:1–47