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Fulminant Lymphocytic Choriomeningitis Virus-Induced Inflammation of the CNS Involves a Cytokine-Chemokine-Cytokine-Chemokine Cascade

Jeanette E. Christensen,* Stine Simonsen,* Christina Fenger,† Maria R. Sørensen,* Torben Moos,‡ Jan P. Christensen,* Bente Finsen,† and Allan R. Thomsen2*

Intracerebral inoculation of immunocompetent mice with lymphocytic choriomeningitis virus (LCMV) normally results in fatal CD8+ T cell mediated meningoencephalitis. However, in CXCL10-deficient mice, the virus-induced CD8+ T cell accumulation in the neural parenchyma is impaired, and only 30–50% of the mice succumb to the infection. Similar results are obtained in mice deficient in the matching chemokine receptor, CXCR3. Together, these findings point to a key role for CXCL10 in regulating the severity of the LCMV-induced inflammatory process. For this reason, we now address the mechanisms regulating the expression of CXCL10 in the CNS of LCMV-infected mice. Using mice deficient in type I IFN receptor, type II IFN receptor, or type II IFN, we analyzed the expression of CXCL10 in the CNS of LCMV-infected mice. Using mice deficient in type I IFN receptor, type II IFN receptor, or type II IFN, we analyzed the up-stream regulation as well as the cellular source of CXCL10. We found that expression of CXCL10 initially depends on signaling through the type I IFN receptor, while late expression and up-regulation requires type II IFN produced by the recruited CD8+ T cells. Throughout the infection, the producers of CXCL10 are exclusively resident cells of the CNS, and astrocytes are the dominant expressors in the neural parenchyma, not microglial cells or recruited bone marrow-derived cell types. These results are consistent with a model suggesting a bidirectional interplay between resident cells of the CNS and the recruited virus-specific T cells with astrocytes as active participants in the local antiviral host response. The Journal of Immunology, 2009, 182: 1079–1087.

The CNS is classically considered a site of “immune privilege.” This concept was originally introduced as a result of the now classical finding that allografts were only slowly rejected when introduced into the CNS (1). However, subsequent observations have greatly modified the original concept, and it is now clear that the CNS is not beyond the reach of normal immunological surveillance, but rather that induced responses are subject to an extremely restrictive regulation (2, 3). Such stringent control has probably developed to minimize the risk of debilitating pathology in an organ with limited regenerative potential. Specific features influencing the immune surveillance of the CNS are the blood-brain and blood-cerebrospinal fluid barriers and a relative lack of lymphatic drainage as well as endogenous APCs (2–4). One result of this stringent regulation is that the migration of T lymphocytes into the CNS is kept at a very low level under normal conditions (5). However, during infections and neuroinflammatory diseases such as multiple sclerosis (MS)3 and its animal model, experimental autoimmune encephalomyelitis, a distinct leukocyte infiltration of the CNS can be found and may even be responsible for the related symptoms.

Generally, the recruitment of leukocytes to any organ site is a complex, multistep process, and extravasation as well as the final positioning of the infiltrating cells is tightly regulated through stringent control of the local expression of adhesion molecules and chemokines (6, 7). Similarly, in the CNS there is mounting evidence that certain chemokines/chemokine receptors play a critical role in controlling the pattern of T cell infiltration, and in this respect the pair CXCL10 (IFN-γ-inducible protein 10, IP-10)/CXCR3 has attracted particular attention. Thus, the concentration of CXCL10 has been found to be elevated in the CSF of patients with MS and in active MS lesions (8–10). Additionally, T cells in the lesions express the matching receptor, CXCR3 (9–11). Importantly, expression of CXCL10 in the CNS may also be potentially beneficial to the host, e.g., by attracting effector T cells critically involved in controlling viral infections of the CNS (12–14). CD8+ T cells are often important in the response to viral encephalitides and for a number of years, studies performed by our group have been focused on studying the chemokines and chemokine receptors involved in regulating CD8+ T cell migration during viral infection of the CNS (15–17). These studies along with those of other groups have established that expression of CXCL10 leads to a type I cytokine polarized response with the accumulation of primed CD8+ T cells in the CNS. Indeed, in some cases (lymphocytic choriomeningitis virus (LCMV), West Nile virus, dengue virus; Refs. 12, 13, 15, 16) the outcome of viral infection may be...
decided by the presence or absence of CXCL10-induced CXCR3-dependent CD8+ T cell recruitment to the infected CNS.

For this reason, it was of obvious interest to elucidate key aspects concerning the regulation of the expression of CXCL10 in the virus-infected CNS. Using the LCMV intracerebral (i.c.) infection model and various knock-out mouse strains as our model system, we addressed the question of how the expression of CXCL10 is regulated in the virus-infected CNS.

The LCMV model is ideal for studying the molecular mechanisms underlying virus-induced T cell-mediated inflammation of the CNS, because the virus itself is noncytolytic (18), and pathogenesis and death is directly related to the influx of virus-specific CD8+ T cells (19–21). LCMV-induced CNS disease occurs in adult mice inoculated i.c. with the virus, and the infection presents with CD8+ T cell-mediated inflammation of the meninges, the choroid plexus, the ependymal lining of the ventricles and the nearby neuroparenchyma (15, 21). Normal adult mice succumb from this CD8+ T cell-mediated meningoencephalitis between 7 and 10 days after virus inoculation (22). However, in CXCL10- or CXCR3-deficient mice, the accumulation of CD8+ T cells is delayed, despite the generation of a normal T cell response, and unlike wild-type (WT) mice, 50–60% of the former mice do not develop lethal T cell-mediated disease (15–17).

Because production of CXCL10 is known to be positively regulated by IFNs, it was our working hypothesis that expression of CXCL10 in the LCMV-infected CNS was controlled at least in part by members of this broad family of cytokines. Based on the finding that initial LCMV-induced up-regulation of CXCL10 expression coincided with up-regulation of several other IFN-regulated genes, we went on to study various gene-targeted and chimeric mice to more directly address the role of type I and type II IFNs in the regulation of LCMV-induced CXCL10 production.

Our results indicate that LCMV initially triggers the production of CXCL10 through a type I IFN-dependent pathway. Upon recruitment of circulating virus-specific T cells to the infected CNS, Ag-driven local production of IFN-γ serves to substantially up-regulate CXCL10 expression, which in turn leads to the recruitment of more effector T cells. Thus, a cytokine-chemokine-cytokine-chemokine cascade is crucially involved in mediating virus-induced inflammation in the LCMV-infected CNS.

Materials and Methods

Mice

CXCL10, IFN-γR, and IFN-γR-deficient mice were bred locally from breeder pairs originally provided by A. D. Luster (Harvard Medical School, Boston, MA) and The Jackson Laboratory, respectively. IFN-αR-deficient mice on a C57BL/6 background were the progeny of breeder pairs originally provided by H. Pircher and R. Zinkernagel (Universitätspital, Zürich, Switzerland). C57BL/6 WT and matched naive mice were purchased from Taconic Farms, and these mice were always allowed to acclimatize to the local environment for at least a week before entering into experiments; by that time the animals were ~7–9 wk old. Transgenic C57BL/6 mice (TCR-318) expressing a TCR specific for an immunodominant, MHC class I-restricted LCMV epitope (gp33–41) on ~60% of their CD8+ T cells were bred locally from breeder pairs originally provided by H. Pircher and R. Zinkernagel (Universitätspital, Zürich, Switzerland) (23). Animals were housed under controlled (specific pathogen-free) conditions as validated by testing of sentinels for unwanted infections according to Federation of European Laboratory Animal Science Association standards; no such infections were detected. Female mice were used in most experiments, but when both sexes were used, no gender effect was observed. Experiments were conducted according to national guidelines regarding animal experiments.

Virus infection

Mice were infected i.c. with a virus dose of 10^4 LD50 (~200 pfu) of LCMV Traub. LCMV is a noncytolytic virus that causes little if any disease in immunodeficient mice (18, 24). However, intracerebral inoculation of LCMV leads to infection of the CNS, and in adult, immunocompetent mice the result is a severe CD8+ T cell-mediated meningoencephalitis from which the animals succumb around day 8–9 post infection (p.i.) (22).

Adaptive transfer of TCR-318 spleen cells

For cell transfers, spleens were removed from naive TCR-318 transgenic mice sacrificed by cervical dislocation. Single cell suspensions were obtained by pressing the organs through a fine steel mesh, and cells were washed and counted. Three × 10^6 TCR-318 splenocytes were injected i.v. into transgenic syngeneic recipients 1 day before virus challenge. With this number of TCR-318 transgenic cells transferred, donor-derived CD8+ T cells will totally dominate the virus-specific CD8+ T cell response (25).

Bone marrow chimeras

Syngeneic and allogeneic bone marrow chimeras were made using CXCL10−/− and WT mice. Mice were lethally irradiated (9 Gy) in the morning and transplanted i.v. with 20 × 10^6 femur cells from allogeneic donors in the afternoon. Eight weeks later, the mice were infected with virus as described.

Isolation of total RNA for quantitative PCR

Brains from mice deeply anesthetized and exsanguinated were immediately removed, snap frozen in liquid nitrogen, and stored in a liquid nitrogen freezer. Total RNA was extracted from homogenized brains by use of RNeasy midi kit (Qiagen).

Detection of mRNA in the brain by quantitative PCR (Q-PCR)

One microgram of mRNA was reverse transcribed to cDNA using ReverTra Aid First strand cDNA synthesis kit (MBT Fermentas). For Q-PCR reaction, a Brilliant SYBR Green QPCR Mastermix was used according to the manufacturer’s instructions (Stratagene, AH Diagnostics). In brief, the Q-PCR components included Brilliant QPCR master mix, distilled water, ROX reference dye, reverse transcribed cDNA, and the forward and reverse target gene primers (Table I). IFN-α primers detected a consensus sequence covering IFN-α1, 5, 6, 7, and 12. Target gene expression was normalized against the housekeeping genes GAPDH or porphobilinogen. In this article, WT mice infected i.c. 3 or 7 day earlier with LCMV were used as standard curve template. ΔCp(control − sample) refers to the difference in threshold cycle (Ct) between day 0 (control) and day 3, 5, or 7 p.i. (sample). C, reflects the number of cycles it takes to reach a point in which the fluorescent signal is first recorded as statistically significant above background (26).

Quantitative PCR for IFN-regulated genes

To evaluate the expression of IFN-regulated genes we used a RT (2) Profiler PCR array kit from SABiosciences. The applied kit (PAMM-016A) profiles signaling molecules involved in the IFN-α and -β response and IFN-responsive genes. Genes associated with virally induced and intrinsic IFN resistance are included as well. The preparation of cDNA, the running of the assay and the analysis of the results were all performed according to the manufacturer’s instructions.

Quantification of chemokine production from Ag-stimulated CD8+ T cells

CD4+ and MHC class II+ cells were removed from splenocytes by negative selection. Cultures of 2.5 and 5 × 10^6 of the remaining cells (primarily CD8+ cells) were either stimulated with a dominant LCMV MHC
class I restricted epitope (GP33-41) in vitro for 6 h or left unstimulated. Supernatants were harvested and assayed for CXCL10 and CCL3 using a chemokine 5-plex bead immunoassay (BioSource Cat. No. LMC0005) for Luminex 100 system according to the manufacturer’s instructions (Luminexcorp). More than 100 events were acquired per bead set. StarStation Luminex 100 system according to the manufacturer's instructions (Luminexcorp). More than 100 events were acquired per bead set. StarStation

### In situ hybridization and immunohistochemistry

Mice were deeply anesthetized with tribromoethanol (Sigma-Aldrich) and decapitated. Brains were rapidly dissected and frozen in CO2-snow. Subsequently, brains were cut into serial 30-µm cryostat sections, mounted on RNase-free super frost plus glass slides (Hounsen), and stored in sealed boxes at -80°C.

The in situ hybridization technique was performed as described by Lamberts et al. (27). The CXCL10 mRNA was detected by a mixture of two alkaline phosphatase (AP)-labeled DNA probes (5′GGCAGGATAG GCTCGCAGGGATGATTTC 3′ and 5′GGGTGTGTGCGTGGCTTCAC TCCAGTTA 3′ (4 pmol/ml)) both complementary to murine CXCL10 mRNA. The probes were designed by use of Oligo-design software 6.0 and purchased from DNA Technology A/S (Aarhus). Hybridization took place overnight at 37°C and sections were then rinsed. The in situ hybridization signal was developed in a Tris-HCl MgCl2 buffer containing the AP sub -strate, 5-bromo-4-chloro-3-indolyl phosphatase (Sigma-Aldrich) and nitro blue tetrazolium (Sigma-Aldrich) and after 3 days in dark, the staining was documented by showing that 1) sections hybridized with individual CXCL10 probes yielded similar signal to sections hybridized with the probe mixture, 2) the hybridization signal was abolished when hybridizing RNase A (Pharmacia Biotech) digested sections, or when hybridizing sections with a 100-fold excess of the unlabelled probe mixture, and 3) incubation of sections with buffer yielded no signal. Finally, hybridization of parallel sections with an AP-labeled GAPDH probe ensured the overall suitability of the tissue for hybridization.

When in situ hybridization for CXCL10 mRNA and immunohistochemistry for glial fibrillary acidic protein (GFAP) protein were combined (27), sections were subjected to the standard in situ hybridization procedure, except that development was arrested after 1–1.5 h to reduce the amount of chromogenic signal. Sections were then rinsed and incubated with monoclonal Alexa 488-conjugated mouse-anti-GFAP IgG1 (5 µg/ml) (A21204, Invitrogen) or mouse IgG1 isotype control (5 µg/ml) in TBS-buffer for 2 h. After a final rinse in TBS, sections were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) nuclear staining. Using PhotoShop software, the bright field pictures of the chromogenic in situ hybridization signal was color inverted and the in situ signal was recolored with red before being merged with pictures of the Alexa 488 and DAPI staining.

In the case of double immunohistochemistry for CXCL10 protein and GFAP or CD11b (27), sections were fixed in 4% paraformaldehyde, rinsed in TBS-buffer, and then incubated with purified goat-anti-CXCL10 IgG (0.5 µg/ml) (AF4666A, R&D Systems) alone or with monoclonal rat-anti-CD11b IgG2b (1.7 µg/ml) (MCA711, Serotec) overnight at 4°C. Parallel sections were incubated with goat IgG (0.5 µg/ml) (DakoCytomation) or rat IgG2b isotype control (1.7 µg/ml) (Nordic BioSite). After another rinse, sections were incubated with Alexa 594-conjugated donkey-anti-goat IgG (10 µg/ml) (A10581, Invitrogen) and monoclonal Alexa 488-conjugated mouse-anti-GFAP IgG (5 µg/ml) or Alexa 488-conjugated donkey-anti-rat IgG (10 µg/ml) (A21208, Invitrogen) in TBS buffer plus 10% bovine se-

### Statistical analysis

Quantitative results were compared using the Mann-Whitney U test. A p-value of <0.05 was considered as evidence of statistical significance.

### Results

Expression of CXCL10 is up-regulated in the CNS during i.c. LCMV infection and correlates with expression of IFN-regulated 2′,5′-oligoadenylate synthetase 1A (OAS)

Our working hypothesis was that the expression of CXCL10 in the brains of mice infected i.c. with LCMV reflects the induction of type I and/or type II IFNs.

As the first approach to test this assumption, we compared the kinetics of CXCL10 expression with that of 2′,5′-OAS, which is a classical IFN-regulated gene (28), important for the antiviral state in IFN-treated cells (29). Expression of this molecule may therefore serve as a convincing surrogate marker for the fact that IFNs have been induced and functionally expressed.

WT mice were infected intracerebrally with LCMV virus, and on days 3, 5, and 7 p.i., we determined the mRNA levels for CXCL10 and 2′,5′-OAS in the brain by use of Q-PCR. As previously shown (15, 16, 30) there is a slight, but significant increase in CXCL10 expression in virus-infected mice on day 3 p.i. as compared with mice inoculated with PBS (Fig. 1A). With time, CXCL10 expression gradually become more pronounced, and very high expression is found on day 7 p.i. coinciding with maximal CD8+ T cell mediated local inflammation (22). Similar to the expression of CXCL10, there is an early, significant expression of 2′,5′-OAS on day 3 p.i. followed by further increases on days 5 and 7 p.i. Notably, the increase in expression of 2′,5′-OAS over time is not as marked as that regarding expression of CXCL10. However, this may reflect differences in the responsiveness of the two genes to type I vs type II IFNs.

The presence of 2′,5′-OAS strongly indicates that IFNs have been produced, but does not reveal any details regarding amount and type of IFN. Therefore, to address these issues, we determined mRNA levels for IFN-α, β, and γ in the same LCMV-infected brains as evaluated above.

Already on day 3 p.i. we observed a small, but consistent, increase in the expression of IFN-β in LCMV-infected mice compared with PBS-injected controls (Fig. 1C). Minimal expression of type I IFNs was noted in sham-injected mice, probably as a consequence of the inoculation trauma. By day 5 and 7 p.i., increasing levels of IFN-α and in particular IFN-β was seen (Fig. 1C). However, at all time-points studied, the relative increase in expression

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**Table I. Primer sequences used for Q-PCR**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>2′,5′-OAS</td>
<td>Forward: 5′-CTT TGA TGT CCT GGG TCA TGT-3′</td>
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<tr>
<td></td>
<td>Reverse: 5′-CTC GCT GAA GCA GCT AGT AAG-3′</td>
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<tr>
<td>CXCL10</td>
<td>Forward: 5′-CTA ACA CTT GGG CAT AGT AGG-3′</td>
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<td>Reverse: 5′-CTC AGG AGC AGT TGA UGA CAT CTC C-3′</td>
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<td>IFN-α</td>
<td>Forward: 5′-GCT AGG TCT GCT TCT GTC ATG-3′</td>
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<td>Reverse: 5′-CTC AGG TAC ACA GTG ATG TGG-3′</td>
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<td>IFN-β</td>
<td>Forward: 5′-AAC AGG TGC ATC CTC CAC GCT GCC-3′</td>
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<td>Reverse: 5′-GTC AGG AGC AGT TGA UGA CAT CTC C-3′</td>
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<td>IFN-γ</td>
<td>Forward: 5′-AAC GCT ACA CAC TGC ATC TGG G-3′</td>
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<td>Reverse: 5′-GCC TGT GCA CTA ACA CAC ACC-3′</td>
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<tr>
<td>PBGD</td>
<td>Forward: 5′-GGT GAG AGC AGT TGA UGA CAT CTC C-3′</td>
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<td>Reverse: 5′-GGG TCA TCT TCT GCA CCA T-3′</td>
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<td>GAPDH</td>
<td>Forward: 5′-CAA TTT GTC CAT CCT GGA G-3′</td>
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<td>Reverse: 5′-GAT GCC TGC TTC ACC ACC-3′</td>
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expression is known to be influenced by IFNs. In addition, this anal-
tically to analysis of IFN-regulated molecular pathways, we ob-


tained results (Fig. 2) that clearly support the assumption that IFN


tended expression was almost completely absent in IFN-

A 

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2′5′-OAS

C

IFN-α and IFN-β

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type I IFNs are the dominating IFN types during the early phase of the host response.

During the acquired immune response, increased expression of CXCL10 is linked to T cell recruitment and production of IFN-γ

In Fig. 1D, an exceptionally marked increase in the level of expression of IFN-γ was found to take place between day 5 and 7 p.i., which could imply that IFN-γ produced by the initially recruited virus-specific T cells exerted a critical positive feedback on the expression of CXCL10 during the acquired immune response. To directly investigate this assumption, brains from T cell-deficient, nude mice, and IFN-γ−/− mice infected i.c. 7 days earlier were analyzed for CXCL10 mRNA. As can be seen in Fig. 5A, neither IFN-γ−/− nor nude mice showed any substantial expression of CXCL10 compared with WT, convincingly suggesting that IFN-γ secreted from T cells recruited to the virus-infected brain induce CXCL10 expression. With the exception of some residual expression of 2′,5′-OAS, probably reflecting the activity of type I IFNs (cf. Fig. 1), a similar expression pattern was observed with regard to this gene. This finding underscores that type I IFNs do not suffice for the sustained expression and the late up-regulation of CXCL10 observed in LCMV-infected mice (Fig. 5B).

Ag-stimulated CD8+ T cells do not secrete CXCL10 in vitro

The ability of Ag stimulated, LCMV-specific effector CD8+ T cells to produce CXCL10 was next evaluated. Splenocytes from LCMV-infected mice were negatively selected, removing CD4+ and MHC II+ cells. The remaining cells were stimulated with a dominant MHC class I restricted LCMV epitope for 6 h, and the supernatants were next analyzed for the presence of CXCL10 protein. CD8+ and MHC II+ cells were removed from splenocytes isolated from WT mice infected i.v. with LCMV 9 days earlier (WT plus LCMV) or uninfected controls (WT). The remaining cells were either stimulated with a dominant LCMV MHC I restricted epitope for 6 h (+ pep) or left unstimulated. The supernatants were harvested and the presence of CXCL10 (A) and CCL3 (B) protein was assayed using a Multiplex Bead Immunoassay. Splenocytes from uninfected WT mice served as controls. Results from duplicate culture of cells from two to three mice/group are presented. N.D. = not detectable.

CXCL10 originates from resident cells of the CNS

To investigate whether CXCL10 mRNA derives from resident cells of the CNS or from hematopoietic, non-T cells recruited during the inflammatory response, syngeneic, and mixed bone marrow chimera were generated using CXCL10−/− and WT mice. Eight weeks after transplantation, all the inflammatory cells recruited to the infected brain will be of donor origin (T cells and macrophages) whereas the radio resistant cells of the CNS are of recipient type. Chimeras were then infected with LCMV virus, and CXCL10 levels analyzed on day 3 p.i. when an acquired immune response was observed.
response is not yet induced, and on day 7 p.i. when T cell-dependent inflammation is at its maximum. A similar pattern of CXCL10 expression was found on days 3 and 7 p.i. Thus, irrespective of the genotype of the bone marrow-derived cells, only recipients with radioresistant CNS cells of WT genotype showed substantial CXCL10 expression (Fig. 8), thus strongly indicating that expression of IFNs stimulates resident cells of the brain to produce and secrete CXCL10.

Astrocytes are the main producers of CXCL10 in the CNS proper

To define further the type(s) of cells in the brain that produces CXCL10 in response to LCMV infection, relevant brain sections from LCMV-infected WT mice were hybridized with probes detecting CXCL10 mRNA and Abs detecting CXCL10, the astroglial marker GFAP and the microglial marker CD11b.

As can be seen in Fig. 9A, very few CXCL10 mRNA expressing cells were present in the meningeal membranes and in the neural parenchyma as shown for the corpus callosum (CC) (arrow) on day 3 p.i. This is in contrast to the situation on day 7 p.i., at which time multiple intensely labeled cells were detected both in perimeningeal region (arrow), the periventricular zones (arrow), the choroid plexus and CC (arrow) (Fig. 9B). CXCL10 mRNA expressing cells were present at the site of injection both on day 3 and 7 p.i. (data not shown). As expected, in situ hybridization or immunohistochemistry of sections from WT mice and CXCL10−/− mice injected with PBS 3 and 7 days earlier yielded no signal of CXCL10 outside the injection-region (data not shown).

FIGURE 7. CXCL10 production is restored in IFN-γ−/− mice reconstituted with T cells. Three × 10⁶ TCR-transgenic splenocytes were transferred into IFN-γ−/−, IFN-γ−/−R−/−, and WT mice. The next day all mice were infected i.c. with LCMV, and on day 5 p.i. mRNA was isolated from the brains. The expression of mRNA for CXCL10 (A) and 2′,5′-OAS (B) was determined by Q-PCR. Brains from PBS-injected mice served as controls. Medians and ranges of four mice/group are presented. *, p < 0.05 relative to infected WT mice. n.s. = not significantly different from WT mice. Results are representative of two similar experiments.

FIGURE 9. Localization of CXCL10 producing cells in the CNS of WT mice as a function of time. Brain sections from C57BL/6 mice infected with LCMV 3 (D3) or 7 (D7) days earlier were analyzed by in situ hybridization using CXCL10 probes. Scale bar (A and B), 100 μm. Results are representative of four mice/group.

FIGURE 8. CXCL10 is predominant expressed in resident cells of the CNS. Syngeneic and allogeneic bone marrow chimeras were generated using CXCL10−/− and WT mice. Eight weeks after bone marrow reconstitution, chimeric mice were infected i.c. with LCMV virus. On days 3 and 7 p.i. brains were removed, mRNA extracted, and the level of mRNA for CXCL10 was determined by Q-PCR. Medians and ranges of 3–4 mice/group are represented. *, p < 0.05 relative to reconstituted WT mice. Similar results were obtained in mice infected 15 wk after reconstitution.

FIGURE 10. Astrocytes and meningeal and ependymal cells are the major CXCL10 producers in the neural parenchyma during LCMV infection of CNS. Brains from WT mice infected with LCMV 7 days earlier were analyzed by in situ hybridization and immunohistochemistry. A–C, In situ hybridization detecting CXCL10 mRNA. D, Double staining by in situ hybridization for CXCL10 mRNA and immunohistochemistry for GFAP protein. E and F, Double immunohistochemistry for CXCL10 protein and either GFAP protein (E) or CD11b protein (F). Scale bar (A and B), 20 μm; C–F, 50 μm. Results are representative of four mice/group.
Further analyses evaluating which cells of the CNS that express CXCL10 mRNA on day 7 p.i., showed that CXCL10 is expressed by the cells forming the meningeal membranes (arrow heads), ependymal cells (arrow heads, here shown around the 3 ventricle (3V)), and glial-like cells (arrows) as can be seen in Fig. 10, A–C, respectively.

To determine the identity of the involved glial-like cells, double staining with in situ hybridization for CXCL10 mRNA and immunohistochemistry for GFAP protein was performed. This approach revealed that CXCL10 mRNA (arrows) is expressed by the cells forming the meningeal membranes (arrow heads), ependymal cells (arrow heads, here shown around the 3 ventricle (3V)), and glial-like cells (arrows) respectively.

One may wonder why type I IFNs seemingly contribute to the inflammatory response in one situation (the LCMV infection), while acting as an anti-inflammatory modulator in another (EAE). Clearly the two situations are quite different. Thus, we evaluate the effect very early after a viral challenge, before the induction of an adaptive T cell response. Moreover, in our case the target cells for the action of type I IFNs are resident cells of the CNS. This is in contrast to the situation in the EAE model, where type I IFNs seemingly act on the recruited myeloid cells to down-modulate the local T cell-induced effector response (38). From this comparison, it is tempting to propose that the temporal and cellular context plays a critical role in determining the direction of the effects induced by type I IFNs in the CNS. Thus, acute expression of these cytokines as part of the early, innate response involving the resident cells including CXCL10 producing astrocytes may act to initiate the inflammatory response, while sustained expression during ongoing inflammation may reduce the production of inflammatory mediators (including CXCL10) by the recruited cells. Whether this suggestion is correct or not, with the appearance of a strong adaptive CD8$^+$ T cell response, type I IFNs no longer suffice as positive regulators of CXCL10 in the LCMV-infected CNS, and instead type II IFN becomes the key inducer of CXCL10 expression. Whether type I IFNs actually contribute to reduce inflammation at this stage, unfortunately cannot easily be addressed experimentally in this model, as the course of the LCMV infection is fundamentally altered in IFN-$
\alpha$R$^{-/-}$ mice.

Our results in vitro and in vivo clearly document that the T cells are not themselves important producers of CXCL10. This is in contrast to the local production of CCL3, which is predominantly produced by Ag-stimulated CD8$^+$ T cell as demonstrated both in vitro and in vivo (32). However, the local secretion of type II IFN from activated CD8$^+$ T cells seems to induce resident cells of the CNS to express high amounts of CXCL10. This conclusion is supported by our analysis of mixed bone marrow chimeras, which revealed that the genotype of radioresistant, non-bone marrow-derived cells within the CNS determines whether CXCL10 is expressed in the CNS or not. Moreover, detailed histochecmical analysis discloses that the predominant producers of CXCL10 within the neural parenchyma express the phenotypic marker of astrocytes, but not of microglial cells. These observations confirm and extend our previous study by Aisenhofer et al. (42), which, however, failed to identify the pivotal role of type I IFNs in inducing early CXCL10 expression (35). Moreover, although an association between type II IFN and sustained expression of CXCL10 was noted in the former study (42), the precise cellular relationship has not previously been worked out.

As the expression of CXCL10 in certain areas of the neural parenchyma (Ref. 42 and present report) closely matches the regions in which CD8$^+$ T cells are prominent in lethally infected WT mice, but absent in partly resistant CXCL10- or CXCR3-deficient mice (15, 16), our results support the following hypothesis regarding the events leading to fatal LCMV-induced CNS diseases. In the early stages of i.c. LCMV infection local production of type I IFN is required for limited expression of CXCL10 in meningeal cells and a few astrocytes. This expression suffices for recruitment of some of the first few
Regulation of CXCL10 in LCMV-induced CNS inflammation

The authors have no financial conflict of interest.

Disclosures

References


