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J Immunol 2008; 181:2683-2693; doi: 10.4049/jimmunol.181.4.2683
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Astrocyte gp130 Expression Is Critical for the Control of
Toxoplasma Encephalitis*

Katrin Drögemüller,† Ulrike Helmuth,*, Anna Brunn,† Monika Sakowicz-Burkiewicz,*,†
David H. Gutmann,‡ Werner Mueller,§¶ Martina Deckert,† and Dirk Schlüter²*

Toxoplasma gondii infects astrocytes, neurons and microglia cells in the CNS and, after acute encephalitis, persists within neurons. Robust astrocyte activation is a hallmark of Toxoplasma encephalitis (TE); however, the in vivo function of astrocytes is largely unknown. To study their role in TE we generated C57BL/6 GFAP-Cre gp130fl/fl mice (where GFAP is glial fibrillary acid protein), which lack gp130, the signal-transducing receptor for IL-6 family cytokines, in their astrocytes. In the TE of wild-type mice, the gp130 ligands IL-6, IL-11, IL-27, LIF, oncostatin M, ciliary neurotrophic factor, B cell stimulating factor, and cardiophosphin-I were up-regulated. In addition, GFAP+ astrocytes of gp130fl/fl control mice were activated, increased in number, and efficiently restricted inflammatory lesions and parasites, thereby contributing to survival from TE. In contrast, T. gondii-infected GFAP-Cre gp130fl/fl mice lost GFAP+ astrocytes in inflammatory lesions resulting in an inefficient containment of inflammatory lesions, impaired parasite control, and, ultimately, a lethal necrotizing TE. Production of IFN-γ and the IFN-γ-induced GTPase (IGTP), which mediate parasite control in astrocytes, was even increased in GFAP-Cre gp130fl/fl mice, indicating that instead of the direct antiparasitic effect the immunoregulatory function of GFAP-Cre gp130fl/fl astrocytes was disturbed. Correspondingly, in vitro infected GFAP-Cre gp130fl/fl astrocytes inhibited the growth of T. gondii efficiently after stimulation with IFN-γ, whereas neighboring noninfected and TNF-stimulated GFAP-Cre gp130fl/fl astrocytes became apoptotic. Collectively, these are the first experiments demonstrating a crucial function of astrocytes in CNS infection. The Journal of Immunology, 2008, 181: 2683–2693.

Inflammatory disorders of the CNS are characterized by the recruitment of leukocytes to the brain and the activation of resident CNS cell populations, including astrocytes. In these disorders, pathogenetically important leukocyte populations have been identified and characterized, whereas their interplay with CNS resident cell populations and the selective in vivo function of the latter are poorly defined.

In murine Toxoplasma encephalitis (TE)† the obligate intracellular parasite infects astrocytes, neurons, and microglia cells (1). Control of intracerebral (i.c.) T. gondii is critically dependent on the local IFN-γ-production of CD4 and CD8 T cells and, to a lesser extent, on B cells (2–4). IFN-γ is important for the induction of other protective cytokines, including TNF, and induces protective effector mechanisms in infected cells (5, 6). In vitro studies revealed that IFN-γ-activated astrocytes control T. gondii via the small inducibly expressed GTPase IGTP (7); IGTP−/− mice are unable to control T. gondii and succumb to a nectrotizing TE (8). Interestingly, IFN-γ failed to inhibit the growth of T. gondii in neurons in vitro (9), indicating that in addition to IFN-γ, other factors are required to prevent parasite replication in this particular target cell.

In TE, astrocytes produce the chemokines IFN-γ-inducible protein 10 (IP-10/CRG-2) and MCP-1 and up-regulate the expression of MHC class I molecules (10, 11). In addition, astrocytes produce IL-1α, IL-6, GM-CSF, and PGE2 upon in vitro infection with T. gondii (12). Furthermore, hypertrophic astrocytes surround parasite-associated lesions and, of functional importance, astrocytes deficient in the expression of their major intermediate filament glial fibrillary acid protein (GFAP) have a reduced capacity to restrict T. gondii-associated inflammatory lesions, resulting in an increased i.c. parasite load and a more widespread inflammation (13). Taken together, these findings indicate an immunoregulatory function of astrocytes in TE; however, data are still lacking that depict their decisive function in TE or any other cerebral infections.

The gp130 receptor is an essential ubiquitous signal transducer for members of the IL-6 cytokine family, which includes IL-6, IL-11, IL-27, LIF, oncostatin M (OSM), ciliary neurotrophic factor (CNTF), B cell stimulating factor (BSF)-3, and cardiophosphin-1 (14). Gp130-deficient mice die perinatally and suffer from a more severe phenotype than mice deficient for individual gp130 ligands (for review, see Ref. 14). This also explains why the function of gp130 in inflammatory diseases is poorly characterized, whereas the function of gp130 ligands and coreceptors has been partially characterized. In toxoplasmosis, IL-27Rα, which forms a signaling complex in combination with gp130, plays a crucial protective role by inhibiting an immunopathology mediated by IL-17-producing

*Institut für Medizinische Mikrobiologie, Otto-von-Guericke-Universität, Magdeburg, Germany; †Abteilung für Neuropathologie, Universität zu Köln, Cologne, Germany; ‡Department of Neurology, Washington University School of Medicine, St. Louis, MO 63110; ¶Helmholtz-Zentrum für Infektionsforschung, Braunschweig, Germany; and §University of Manchester, Manchester, United Kingdom.

Received for publication February 25, 2008. Accepted for publication June 7, 2008.

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1 This work was supported in part by grants from the Deutsche Forschungsgemeinschaft (Schl. 3927/1-1) and the Bundesministerium für Bildung und Forschung (ToxNet 01; TP1).

2 Address correspondence and reprint requests to Dr. Dirk Schlüter, Institut für Medizinische Mikrobiologie, Otto-von-Guericke-Universität Magdeburg, Leipziger Strasse 44, 39120 Magdeburg, Germany. E-mail address: dirk.schlueter@medizin.uni-magdeburg.de

3 Abbreviations used in this paper: TE, Toxoplasma encephalitis; BSF, B cell stimulating factor; CNTF, ciliary neurotrophic factor; GFAP, glial fibrillary acid protein; HPF, high power field; HPRT, hypoxanthine phosphoribosyl transferase; i.c., intracerebral; IGTP, IFN-γ-induced GTPase; iNOS, inducible NO synthase; LDH, lactate dehydrogenase; NeuN, neuron-specific nuclear protein; OSM, oncostatin M; WT, wild type.

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Th cells (15). In contrast, mice deficient in IL-6 developed impaired immune responses after infection with *T. gondii*, resulting in an enhanced susceptibility to TE (4, 16). Collectively, these divergent findings indirectly suggest that gp130 plays a regulatory role in inflammatory CNS disorders, but it is unknown whether astrocytes need to be responsive to gp130-stimulating cytokines.

To gain insight into the in vivo function of astrocytes in toxoplasmosis, we generated mice lacking gp130 expression in astrocytes and infected these mice with *T. gondii*. These experiments demonstrate that astrocyte gp130 expression is crucial for the survival of GFAP<sup>+</sup> astrocytes in TE and, of functional importance, that astrocyte loss results in a lethal course of TE.

**Materials and Methods**

**Mice**

C57BL/6 gp130<sup>fl/fl</sup> (17) and C57BL/6 human GFAP-Cre transgenic mice (18) were bred to generate GFAP-Cre<sup>+/−</sup> gp130<sup>−/−</sup> mice. The colony was maintained by the breeding of GFAP-Cre<sup>+/−</sup> gp130<sup>−/−</sup> mice with GFAP-Cre<sup>+/−</sup> gp130<sup>−/−</sup> control mice. The genotype of offspring was determined by PCR of tail DNA using primers for GFAP-Cre and gp130<sup>−/−</sup> published previously (17, 18). C57BL/6 wild-type (WT) mice were obtained from Harlan. Animal care and experimental procedures were performed according to European regulations and approved by state authorities (Landeswaltungsamt Saxony-Anhalt, Halle, Germany).

**Parasites and *T. gondii* infection**

Toxoplasmas of a type II strain of *T. gondii*, DX (19, 20), were grown in vitro in human foreskin fibroblasts in DMEM supplemented with 10% FCS, penicillin (100 U/ml), and streptomycin (100 μg/ml) at 37°C and 5% CO<sub>2</sub>. Parasites were harvested from freshly lysed fibroblasts by centrifuging the tissue culture medium at 50 × g before use for in vitro experiments. For the infection of mice, DX parasites were harvested from the brains of chronically infected NMRI mice. Parasites were adjusted to a concentration of 25 cysts/ml in 0.1 M PBS, and 200 μl were administered i.p. to the experimental animals.

**Histology**

For immunohistochemistry on frozen sections, mice were perfused intracardially with 0.9% NaCl in methoxyflurane anesthesia. Brains were processed and immunohistochemistry for CD45, CD4, CD8, Ly6G, F4/80 Ag, GFAP, neuron-specific nuclear protein (NeuN), inducible NO synthase (iNOS), and *T. gondii* was performed as described previously (10, 21). For the detection of *T. gondii* cysts and tachyzoites, frozen brain sections were stained with bradyzoite-specific mouse anti-BAG1 and tachyzoite-specific mouse anti-P30, respectively (provided by Dr. C. Lüder, University of Göttingen, Göttingen, Germany), followed by a M.O.M. (mouse on mouse) kit (Vector Laboratories). For histology on paraffin sections, anesthetized mice were perfused with 4% paraformaldehyde in PBS and brains were cardially with 0.9% NaCl in methoxyflurane anesthesia. Brains were processed and stained with hemalum-eosin, cresyl violet, Luxol fast blue, periodic acid Schiff stain (PAS) and also used for immunohistochemistry for CD45, CD4, CD8, Ly6G, F4/80 Ag, GFAP, neuron-specific nuclear protein (NeuN), inducible NO synthase (iNOS), and *T. gondii*. The colony was maintained by the breeding of GFAP-Cre<sup>+/−</sup> gp130<sup>−/−</sup> mice with GFAP-Cre<sup>+/−</sup> gp130<sup>−/−</sup> control mice. The genotype of offspring was determined by PCR of tail DNA using primers for GFAP-Cre and gp130<sup>−/−</sup> published previously (17, 18). C57BL/6 wild-type (WT) mice were obtained from Harlan. Animal care and experimental procedures were performed according to European regulations and approved by state authorities (Landeswaltungsamt Saxony-Anhalt, Halle, Germany).

**Isolation of cerebral leukocytes and flow cytometry**

Cerebral leukocytes were isolated from the brains and stained for CD4 T cells, CD8 T cells, microglia, macrophages, B cells, and granulocytes as described previously (22). For intracellular cytokine staining, isolated leukocytes were incubated with 50 ng/ml PMA, 500 ng/ml ionomycin, and GolgiPlug (1 μl/ml; BD Biosciences) containing brefeldin A in MEM-α at 37°C for 4 h. Thereafter, cells were stained with rat anti-mouse CD4-FITC and rat anti-mouse CD8-allophycocyanin, fixed and permeabilized with Cytofix/Cytoperm (BD Biosciences), and stained with rat anti-mouse IL-17-PE or IFN-γ-PE (BD Biosciences). MHC class II expression of microglia was analyzed by staining of isolated cerebral leukocytes with rat anti-mouse I-A/E<sup>-/−</sup> PE, rat anti-mouse CD11b-FITC, and rat anti-mouse CD45-allophycocyanin. Microglia is CD11b<sup>+</sup> and CD45<sup>low</sup> (23). Controls included staining with isotype-matched control Abs. All Abs were obtained from BD Biosciences. Flow cytometry was performed on a FACScan (BD Biosciences), and the data were analyzed with WinMDI or CellQuest software.

**In vitro culture, DNA isolation, and flow cytometry of astrocytes, microglia, and neurons**

Astrocytes were isolated from 1- to 2-day-old pups and cultivated as published by Frei et al. (24). Neuronal cultures were obtained according to Lenz et al. (25) with slight modifications. Briefly, pregnant females were killed by cervical dislocation at gestational day 18.5. Dissociated cells of each embryonic brain were cultivated in flasks coated with poly-L-lysine in neurobasal medium supplemented with B27 (Invitrogen) and 500 μM l-glutamine. The purity of cultures for astrocytes was ≥80% (with ≥20%

**FIGURE 1.** Up-regulation of IL-6 family cytokine mRNA in TE. The i.c. mRNA transcription of IL-6 family cytokine members was evaluated by RT-PCR in uninfected C57BL/6 mice and C57BL/6 mice at various stages of TE. Data are expressed as the increase of the respective cytokine mRNA of infected over noninfected mice normalized to HPRT expression.

Three mice per group were analyzed and data represent the mean ± SD.
FIGURE 2. Organ and cell-type-specific deletion of gp130 in GFAP-Cre gp130fl/fl mice. A, Deletion of exon 16 of gp130 from various organs of GFAP-Cre gp130fl/fl and gp130fl/fl mice was analyzed by PCR. The gp130Δ product has a size of 400 bp. B, The presence of the GFAP-Cre transgene (850 bp) and the loxP site-flanked exon 16 of gp130 (400 bp) was analyzed by multiplex PCR from DNA isolated from cultivated and FACS-sorted astrocytes, microglia, and neurons and from the total brain of GFAP-Cre gp130fl/fl mice. C, Deletion of exon 16 of gp130 from cultivated and FACS-sorted astrocytes, microglia, and neurons of GFAP-Cre gp130fl/fl (lanes 1) and gp130fl/fl (lanes 2) mice. Total brain (positive control) was from a GFAP-Cre gp130fl/fl mouse. D, Cell surface expression of the gp130 protein (in red) was analyzed by flow cytometry from cultivated astrocytes, microglia, and neurons of GFAP-Cre gp130fl/fl and gp130fl/fl mice, and representative histograms are shown. The percentage of gp130+ and gp130− cells is given for each cell population.
CD11b+ microglia) and that for neurons was 98%, respectively, as determined by immunofluorescence staining for GFAP and neuron-specific class III β-tubulin. To isolate pure astrocytes from astrocyte cultures, cells were harvested from tissue culture and stained with rat anti-mouse CD11b-PE (BD Biosciences). Stained cells were separated into CD11b+ microglia and CD11b- astrocytes, respectively, with a FACSVantage cell sorter (BD Biosciences). DNA was isolated from sorted astrocytes and microglia as well as neurons using a DNA isolation kit (Qiagen). For flow cytometry, mixed astrocyte/microglia cultures were stained with biotin-labeled goat anti-mouse gp130 followed by streptavidin-PE (BD Biosciences) and CD11b-FITC (BD Biosciences). Neurons were stained with biotin-labeled goat anti-mouse gp130 followed by streptavidin-PE. Control included staining with isotype-matched control Abs.

RT-PCR
For RT-PCR, mRNA was isolated from the brains of infected and T. gondii-infected mice (RNasy kit; Qiagen). mRNA was transcribed into cDNA by use of the SuperScript reverse transcriptase kit with oligo(dT) primers (Invitrogen). Semiquantitative PCR for gp130 ligands in TE was performed with cDNA derived from C57BL/6 WT mice. The ratio between the respective cytokine and hypoxanthine phosphoribosyltransferase (HPRT) was calculated per mouse and data are presented as the increase over uninfected mice. Quantitative RT-PCR for IFN-γ, IL-27, IFGβ, MIF, IL-10, and HPRT was performed with cDNA from GFAP-Cre gp130fl/fl and gp130fl/fl mice with a GeneAmp 5700 sequence detection system (Applied Biosystems). Quantitation was performed with the sequence detector software SDS 2.1 (Applied Biosystems) according to the ΔΔCt threshold cycle method (26) with HPRT as housekeeping gene. Data are expressed as the increase of mRNA expression in infected mice over that in noninfected controls of the respective mouse strain. All primers and probes were obtained from Applied Biosystems.

Lactate dehydrogenase (LDH) assay
In vitro cultivated astrocytes were either infected with DX toxoplasmas (multiplicity of infection: 5:1) or stimulated with TNF (30 U/ml; Pepro-Tech) or IFN-γ (100 U/ml from Tebu-bio) for either 24 or 48 h, respectively. Supernatants were harvested, and the LDH level was determined using a commercially available LDH assay (CytoTox96; Promega) on a fluorometer (SpectrafluorPlus, Tecan) according to the manufacturer’s instructions. Data are expressed as the percentage of cytotoxicity calculated by the following formula: experimental LDH release × 100/maximal LDH release.

Statistics
For statistical evaluation of the i.c. parasitic load determined on anti-BAG1 and anti-P30, respectively, stained sections as well as the number of GFAP+ astrocytes on GFAP-immunostained sections in at least 50 high power fields (HPF; original magnification, ×400), randomly selected from all areas of the brain were analyzed per section in three animals per group. Differences were analyzed with the nonparametric Mann-Whitney rank sum test. To test for statistical differences in the survival rate and LDH release, the two-tailed Student’s t test was used. Values of p < 0.05 were accepted as significant. All statistical tests were performed with SigmaStat 3.5 software (Systat Software).

Results
Up-regulation of gp130 ligands in TE
Because the gp130 receptor is essential for the signal transduction of molecules of the IL-6 cytokine family, we analyzed whether the expression of single or multiple members of this family is regulated in TE. The i.c. expression of all molecules analyzed (IL-6, IL-11, IL-27, LIF, cardiotoxin-1, OSM, CNTF, and BSF-3) was strongly up-regulated in acute TE of WT mice with peak levels at day 21 or 28 postinfection (p.i.) (Fig. 1). All molecules were still elevated in chronic TE (day 42 p.i.), although expression of IL-6, LIF, OSM, and BSF declined over time in TE. The observation that cytokines that signal through the gp130 receptor were up-regulated in TE provided the basis to further explore the astrocyte-specific function of gp130 in TE.

In GFAP-Cre gp130fl/fl mice astrocytes and some neurons lack gp130 cell surface expression
To generate mice with a deletion of gp130 in GFAP+ cells, we bred mice expressing the recombinase Cre under the control of a human GFAP promoter (GFAP-Cre mice) (18) with gp130fl/fl mice carrying a loxP site-flanked exon 16 of gp130 (gp130fl/fl mice) (17). Deletion of exon 16, which encodes the transmembrane region of gp130, results in a nonfunctional gp130 molecule. As expected, DNA of all organs from GFAP-Cre+/−/gp130fl/fl mice contained the GFAP-Cre gene and a loxP site-flanked gp130 gene (not shown), and the gp130 gene was exclusively deleted in the CNS of these mice (Fig. 2A). In contrast, gp130 was not deleted in mice lacking the GFAP-Cre transgene (Fig. 2A).

To further determine which cell populations lacked gp130 in the CNS of GFAP-Cre gp130fl/fl mice, we cultivated pure astrocytes depleted of CD11b+ microglia by FACS sorting, CD11b+ microglia and cortical neurons. All of these cell types of GFAP-Cre gp130fl/fl mice carried the GFAP-Cre and gp130fl/fl gene (Fig. 2B). In GFAP-Cre gp130fl/fl mice astrocytes always had a deleted exon 16 of gp130, whereas this deletion was only exceptionally observed in some neuronal cultures and microglia never had deleted a exon 16 of gp130 (Fig. 2C). In neurons of GFAP-Cre gp130fl/fl showing a deletion of exon 16, PCR products were always weakly positive compared with astrocytes, indicating a substantially more effective deletion in astrocytes (Fig. 2C). In contrast, astrocytes, neurons, and microglia of gp130fl/fl mice never deleted exon 16 of gp130 (Fig. 2C). To confirm these findings at the protein level, gp130 cell surface expression was analyzed by flow cytometry (Fig. 2D). The vast majority of cultivated astrocytes of GFAP-Cre gp130fl/fl mice were gp130− (83%), whereas virtually all astrocytes from gp130fl/fl control mice were gp130− (98%). Microglia cells of both strains of mice stained equally weak for gp130. The vast majority of cultivated cortical neurons of GFAP-Cre gp130fl/fl mice were gp130− (84%), but some neurons were gp130+. In gp130fl/fl mice, 96% of neurons were gp130−. In addition, the mean gp130 fluorescence intensity of astrocytes and neurons from GFAP-Cre gp130fl/fl mice was reduced as compared with gp130fl/fl mice (Fig. 2D). Thus, GFAP-Cre driven deletion of gp130 resulted...
in a loss of gp130 cell surface expression in the vast majority of astrocytes and also in a small number of neurons. GFAP-Cre gp130<sup>fl/fl</sup> mice were born in a normal Mendelian ratio and did not show any physical or behavioral abnormalities. At the age of 6 wk, 6 mo, and 12 mo, respectively, GFAP-Cre gp130<sup>fl/fl</sup> mice exhibited macroscopically as well as histopathologically a normal brain architecture, including a normal number and morphology of GFAP<sup>/H11001</sup> astrocytes (Fig. 2, E and F) and neurons (not shown). In addition, the ependyma, which is also GFAP<sup>/H11001</sup>, and the blood-brain barrier, to which astrocytes contribute, were normal (not shown).

Collectively, these findings illustrate that GFAP-Cre gp130<sup>fl/fl</sup> mice were largely devoid of gp130 cell surface expression and did not show abnormalities under physiological conditions, which makes them a suitable tool to study the function of gp130 in astrocytes in disease states.

**T. gondii**-infected GFAP-Cre gp130<sup>fl/fl</sup> mice exhibit an impaired parasite control and die from necrotizing TE

To determine whether astrocyte gp130 expression is important in response to parasitic infection, GFAP-Cre gp130<sup>fl/fl</sup> and gp130<sup>fl/fl</sup> control mice were infected with *T. gondii*. Up to day 40 p.i., 90% of GFAP-Cre gp130<sup>fl/fl</sup> mice succumbed to the infection and the remaining mice died up to day 50 p.i. In contrast, only 15% of gp130<sup>fl/fl</sup> control mice succumbed until day 40 p.i., and 50% were still alive at day 50 p.i. (Fig. 3).

Death of GFAP-Cre gp130<sup>fl/fl</sup> mice was caused by a widespread necrotizing TE, and these animals were unable to control multiplication and spread of *T. gondii* in the brain with the subsequent induction of necrosis (Fig. 4A). In contrast, gp130<sup>fl/fl</sup> mice efficiently prevented parasite replication, resulting in the absence of large areas of parasite-induced tissue necrosis and remarkably smaller lesions containing parasites and inflammatory infiltrates (Fig. 4B). Quantitation of i.c. *T. gondii* further revealed that GFAP-Cre gp130<sup>fl/fl</sup> mice harbored significantly more P30<sup>/H11001</sup> tachyzoites than gp130<sup>fl/fl</sup> mice at both day 14 and day 35 p.i. (Fig. 4C; *p* < 0.01 for both time points). In addition, the number of BAG1<sup>/H11001</sup> cysts was significantly increased in GFAP-Cre gp130<sup>fl/fl</sup> mice at day 14 p.i. (*p* < 0.05), while a tendency toward an increased number did not reach statistical significance at day 35 p.i. (Fig. 4C).

Because the control of *T. gondii* in astrocytes is dependent on IGTP (7), we determined whether IGTP expression was reduced in GFAP-Cre gp130<sup>fl/fl</sup> mice. However, the increased parasitic load was paralleled by an increased IGTP expression in the *T. gondii*-infected brains of GFAP-Cre gp130<sup>fl/fl</sup> mice compared with control

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**FIGURE 4.** Increased parasite burden and expression of IGTP in TE of GFAP-Cre gp130<sup>fl/fl</sup> mice. A and B, The strongly increased parasite and cyst number in GFAP-Cre gp130<sup>fl/fl</sup> mice (A) is partially associated with tissue necrosis (asterisk) and free *T. gondii* Ag (arrow) within the necrotic brain tissue. In gp130<sup>fl/fl</sup> mice, cyst number is low and tissue necrosis is absent (B). Note that in both strains of mice the parasites are accompanied by an inflammatory infiltrate. Anti-*T. gondii* immunostaining and slight counterstaining with hemalum were used; original magnification, ×100. C, The i.c. parasite load was quantitated by counting the number of BAG1<sup>/H11001</sup> cysts and P30<sup>/H11001</sup> tachyzoites in a 100 HPF per mouse in anti-BAG1 and anti-P30 stained brain sections, respectively. The mean ± SD is shown. *, *p* < 0.05; **, *p* < 0.01. D, The mRNA expression of IGTP was analyzed by quantitative RT-PCR from GFAP-Cregp130<sup>fl/fl</sup> and gp130<sup>fl/fl</sup> mice. Data are expressed as the increase of the respective cytokine mRNA from infected over noninfected mice and were normalized to HPRT expression. In C and D, three mice per group and time point were analyzed and the data represent the mean ± SD. One of two experiments is shown.
mice (Fig. 4D). These results indicate that a theoretically conceivable insufficient production of IGTP is not responsible for the impaired pathogen control of GFAP-Cre gp130fl/fl mice.

**TE results in loss of astrocytes and a widespread inflammation in GFAP-Cre gp130fl/fl mice**

To further assess the impact of gp130 on astrocyte reactions and pathology in TE, a detailed immunohistochemical study was performed. In uninfected mice, the number of GFAP$^+$ astrocytes was identical in GFAP-Cre gp130fl/fl and gp130fl/fl mice (Fig. 5A). During TE, the number of astrocytes declined in inflammatory lesions of GFAP-Cre gp130fl/fl mice (Fig. 5A). Outside the inflammatory lesions, astrocytes of both strains of mice did not differ in number after infection (Fig. 5A). Inflammatory lesions of *T. gondii*-infected gp130fl/fl control mice contained strongly activated, hypertrophic astrocytes with increased GFAP expression close to inflammatory infiltrates (Fig. 5, B and F).

**FIGURE 5.** Reduced number of GFAP$^+$ astrocytes in TE of GFAP-Cre gp130fl/fl mice. A, The i.c. number of GFAP$^+$ cells was quantitated by counting the number of positive cells per 100 HPF in anti-GFAP-stained brain sections of uninfected (day 0) and *T. gondii*-infected (days 21 and 35 p.i.) GFAP-Cre gp130fl/fl and gp130fl/fl mice. Three mice were analyzed per time point. The mean ± SD is shown. *, *p < 0.05; **, *p < 0.01. B–F and I, The number of GFAP$^+$ cells is strongly reduced in parasite-associated inflammatory parenchymal lesions of a *T. gondii*-infected GFAP-Cre gp130fl/fl mouse (D, E, and F) as compared with a gp130fl/fl mouse (B, C, and F). The arrow in the GFAP-Cre gp130fl/fl mouse (D) points to a *T. gondii* cyst not associated with GFAP$^+$ astrocytes. In addition to parenchymal astrocytes, the numbers of perivascular GFAP$^+$ astrocytes are strongly reduced in a *T. gondii*-infected GFAP-Cre gp130fl/fl mouse (E) as compared with a gp130fl/fl mouse (C). B–E, Anti-GFAP immunostaining of brain sections at day 35 p.i. and slight counterstaining with hemalum are shown; original magnification: ×100 (B and D), ×200 (C and E). F and I, Anti-GFAP (FITC; green) immunostaining is shown; original magnification, ×200. White circles demarcate an inflammatory infiltrate. Exposition times: F, 900 ms; I, 2000 ms. G and J, The number of S100$^+$ cells is strongly reduced in parasite-associated inflammatory parenchymal lesions of a *T. gondii*-infected GFAP-Cre gp130fl/fl mouse (J) as compared with a gp130fl/fl mouse (G) at day 35 p.i. Note the staining for the S100 protein in astrocytes (arrows) as well as in oligodendrocytes (arrowheads) and macrophages (white asterisks in G). In G and J, anti-S100 protein immunostaining of brain sections at day 35 p.i. and slight counterstaining with hemalum are shown; original magnification, ×100. H and K, Numbers and morphology of neurons are normal in the brain of *T. gondii*-infected GFAP-Cre gp130fl/fl and gp130fl/fl mice. Anti-NeuN immunostaining of cerebral cortex of a *T. gondii*-infected mouse at day 35 p.i. and slight counterstaining with hemalum are shown; original magnification, ×100.
and also perivascularly (Fig. 5C). With ongoing infection, astrocyte activation and astrogliosis further increased, and the numbers of GFAP+ cells gradually increased over time in inflammatory lesions of gp130fl/fl mice. In contrast, astrogliosis was absent from GFAP-Cre gp130fl/fl mice (Fig. 5, D, E, and I) and only a few GFAP+ astrocytes were associated with parenchymal (Fig. 5, D and I) or perivascular (Fig. 5E) inflammatory lesions. Frequently, these astrocytes had crinkled processes and a condensed nucleus indicative of apoptosis (Fig. 5E). These alterations progressed over time, and at day 35 p.i. the large inflammatory lesions of GFAP-Cre gp130fl/fl mice were nearly completely devoid of astrocytes (Fig. 5F). Only at the border of the lesions were some activated GFAP+ astrocytes present. Staining for the S100 protein, which is strongly expressed by astrocytes and also by some activated macrophages and oligodendrocytes, confirmed the development of astrogliosis in gp130fl/fl control mice (Fig. 5G) and the loss of astrocytes in inflammatory lesions of GFAP-Cre gp130fl/fl mice (Fig. 5J).

An important functional consequence of astrocyte loss was that T. gondii-associated inflammatory lesions were much larger and less well confined in GFAP-Cre gp130fl/fl mice, resulting in a more widespread inflammation (Figs. 4, A and B and 5, F and I).

Neurons did not develop pathology in T. gondii-infected GFAP-Cre gp130fl/fl mice (Fig. 5, H and K), and the numbers of NeuN+ neurons did not differ between GFAP-Cre gp130fl/fl and gp130fl/fl mice.

In summary, these findings indicate the following: 1) gp130 expression prevented loss of astrocytes in inflammatory lesions; and 2) loss of astrocytes results in an inefficient containment of inflammatory lesions and impaired parasite control resulting in a lethal necrotizing TE.

Normal leukocyte recruitment and movement as well as microglia activation in TE of GFAP-Cre gp130fl/fl mice

Because astrocytes are an important source of chemokines in TE (11), the loss of astrocytes close to the blood-brain barrier and in inflammatory foci might cause an impaired recruitment and i.c. homing of leukocytes to inflammatory foci in GFAP-Cre gp130fl/fl mice. In fact, the mRNA transcription of MCP-1, which is (as is that of IP-10) predominantly but not exclusively produced by astrocytes in TE, was reduced during the induction of TE (day 7 p.i.) but not thereafter (days 14 and 35 p.i.; Fig. 6A). The IFN-γ inducible chemokine IP-10 was induced with equal strength in...
GFAP-Cre gp130<sup>fl/fl</sup> and gp130<sup>fl/fl</sup> mice at day 7 p.i.; however, thereafter IP-10 was more prominently produced in GFAP-Cre gp130<sup>fl/fl</sup> mice (Fig. 6B). Of functional importance, both noninjected (day 0) and T. gondii-infected GFAP-Cre gp130<sup>fl/fl</sup> and gp130<sup>fl/fl</sup> mice (days 7, 21, and 35 p.i.) recruited similar numbers of CD4 T cells, CD8 T cells, B cells, macrophages, and granulocytes to the brain (Fig. 6C). In addition, histopathology showed that GFAP-Cre gp130<sup>fl/fl</sup> animals attracted inflammatory CD4<sup>+</sup> T lymphocytes to T. gondii-associated lesions in the brain parenchyma as efficiently as gp130<sup>fl/fl</sup> mice (data not shown).

Microglia, the brain resident macrophage population, was normally activated in TE of GFAP-Cre gp130<sup>fl/fl</sup> and gp130<sup>fl/fl</sup> mice as indicated by an equal induction of MHC class II Ag expression (Fig. 6D). Furthermore, both strains similarly expressed iNOS (Fig. 6E), which inhibits the replication of T. gondii in microglia cells (27).

These findings illustrate that the regional loss of astrocytes and the altered expression of MCP-1 and IP-10 in GFAP-Cre gp130<sup>fl/fl</sup> mice did not inhibit the recruitment and i.c. movement of leukocytes in TE.

**GFAP-Cre gp130<sup>fl/fl</sup> mice exhibit increased IFN-γ but reduced IL-27 expression**

Because astrocyte-derived cytokines might regulate the i.c. parasite-specific T cell response, we investigated whether the loss of astrocytes altered the IFN-γ production of i.c. T cells. Both, i.c. CD4 and CD8 T cells of GFAP-Cre gp130<sup>fl/fl</sup> mice produced ∼2-fold more IFN-γ protein as revealed by intracellular IFN-γ staining and flow cytometry (Fig. 7A). In addition, quantitative RT-PCR confirmed that GFAP-Cre gp130<sup>fl/fl</sup> mice transcribed more IFN-γ mRNA than gp130<sup>fl/fl</sup> mice (Fig. 7B). These results indicate that the major T cell-derived protective cytokine is produced in increased amounts, most probably in response to a higher amount of stimulating T. gondii Ag, in GFAP-Cre gp130<sup>fl/fl</sup> mice.

Recent studies have shown that astrocytes produce IL-27 in TE and that this cytokine is important for suppressing development of the lethal immunopathology mediated by IL-17-producing CD4 T cells (15). Although IL-27 mRNA production was equally increased in both strains of T. gondii-infected mice at days 7 and 14 p.i., we observed that GFAP-Cre gp130<sup>fl/fl</sup> mice transcribed lower IL-27 mRNA levels in terminally ill T. gondii-infected GFAP-Cre gp130<sup>fl/fl</sup> mice at day 35 p.i. (Fig. 7C), indicating that the loss of astrocytes reduced the sources of IL-27-producing cells in GFAP-Cre gp130<sup>fl/fl</sup> mice. However, the frequency of IL-17-producing CD4 T cells was not increased in GFAP-Cre gp130<sup>fl/fl</sup> mice as compared with control mice, and only few CD4 T cells were IL-17<sup>+</sup> at day 35 p.i. (Fig. 7D) in accordance with previously published data from C57BL/6 WT mice (15). In CD4<sup>+</sup> cells from GFAP-Cre gp130<sup>fl/fl</sup> mice, there was a modest increase in IL-17<sup>+</sup> cells as compared with control mice (0.30 vs 0.16%; Fig. 7D). However, these frequencies were reduced as compared with IL-17<sup>+</sup> CD4 T cells from IL-27Ra<sup>−/−</sup> mice with TE (5% IL-17<sup>+</sup> CD4 T cells) (15).

**In vitro gp130 expression of astrocytes is crucial for the survival of astrocytes after infection with T. gondii and stimulation with TNF**

To further define the function of astrocytic gp130 in response to inflammatory stimuli, we cultivated astrocytes of GFAP-Cre...
gp130<sup>fl/fl</sup> and gp130<sup>fl/fl</sup> mice and infected these cells with <i>T. gondii</i>. At both 24 and 48 h p.i. most GFAP-Cre gp130<sup>fl/fl</sup> astrocytes had died, whereas astrocyte death was not evident in gp130<sup>−/−</sup> astrocytes (Fig. 8A). In addition, stimulation with TNF induced a significantly increased cytotoxicity in GFAP-Cre gp130<sup>fl/fl</sup> astrocytes (Fig. 8A).

TUNEL staining of astrocytes revealed that infection with <i>T. gondii</i> as well as stimulation with TNF induced a significantly increased number of apoptotic TUNEL<sup>+</sup> astrocytes in GFAP-Cre gp130<sup>fl/fl</sup> mice (Fig. 8B). Interestingly, combined TUNEL and anti-<i>T. gondii</i> staining revealed that predominantly noninfected astrocytes were TUNEL<sup>+</sup>, whereas <i>T. gondii</i>-infected astrocytes were largely protected from apoptosis (Fig. 8, C and D), which is compatible with an antiapoptotic activity of this obligate intracellular parasite in infected cells (28, 29). The number of infected cells did not differ between astrocytes of GFAP-Cre gp130<sup>fl/fl</sup> and gp130<sup>fl/fl</sup> mice at 24 and 48 h p.i. (Fig. 8E). In addition, treatment with IFN-γ equally reduced the number of infected astrocytes in both strains without affecting the cytotoxicity induced by parasite infection (Fig. 8F).

Together with the observation that in vivo astrocytes are lost in inflammatory lesions, these findings indicate that astrocyte gp130-expression may have a general function in protecting astrocytes from death under inflammatory conditions.

**Discussion**

The present study provides direct evidence that activated GFAP<sup>+</sup> astrocytes are crucial components of the immune response in cerebral toxoplasmosis and that gp130 expression of astrocytes is required for their survival in inflammatory lesions of TE.

Under physiological conditions a deletion of astrocytic gp130 cell surface expression in C57BL/6 mice did not influence the number and distribution of astrocytes. With respect to the time point of gp130 deletion in our mutant, it should be stressed that the GFAP-Cre transgene becomes active at approximately day 14.5 of embryogenesis (18) and, thus, before this time point the development of astrocytes occurs under intact gp130 signaling. In addition, mice with conditional gp130 deletion in all cells after birth and conventional gp130<sup>−/−</sup> mice on a mixed 129/Sv background harbored normal astrocyte numbers (17, 30). However, after backcrossing to an ICR background, these conventional gp130<sup>−/−</sup> mice were moderately impaired in their astrocyte differentiation (31). In another conventional gp130<sup>−/−</sup> mouse of unknown genetic background, astrocyte development was nearly completely abolished (32). These findings indicate that, depending on the genetic background, complete loss of gp130 expression on all cells from the very beginning of embryogenesis may result in defective astrocyte differentiation, whereas deletion of gp130 in astrocytes at later stages of embryogenesis or after birth is not critical for the development of astrocytes.

In vitro, infection with <i>T. gondii</i> induced apoptosis in GFAP-Cre gp130<sup>fl/fl</sup> astrocyte cultures. Importantly, <i>T. gondii</i>-infected astrocytes, but not surrounding noninfected ones, were protected from death under inflammatory conditions.
from apoptosis, which extends previous observations that this obligate intracellular parasite actively suppresses apoptosis of its host cells to ensure its own survival (28, 29). Based on these findings, apoptosis of GFAP-Cre gp130\textsuperscript{fl/fl} astrocytes in T. gondii-infected cultures is most probably caused indirectly by secreted immune mediators. In fact, T. gondii-infected and LPS-stimulated astrocytes produce several cytokines including IL-1, TNF, and GM-CSF (12). Our finding that TNF induces apoptosis of GFAP-Cre gp130\textsuperscript{fl/fl} astrocytes directly demonstrates that gp130 is required to counteract the proapoptotic function of TNF. The observation that IFN-\(\gamma\) inhibited parasite multiplication without reducing the apoptosis of GFAP-Cre gp130\textsuperscript{fl/fl} astrocytes further illustrates that apoptosis of GFAP-Cre gp130\textsuperscript{fl/fl} astrocytes is not linked to parasite number and replication.

A variety of cytokines, including TNF, are produced in TE (33) and may contribute to the loss of GFAP-Cre gp130\textsuperscript{fl/fl} astrocytes in vivo. In TE, exceptional astrocytes with a condensed nucleus and cell shrinkage were detectable in GFAP-Cre gp130\textsuperscript{fl/fl} mice, indicating that these single cells underwent apoptosis. However, we could not detect TUNEL\textsuperscript{+} astrocytes, which may be explained by the slow but continuous loss of astrocytes over weeks in GFAP-Cre gp130\textsuperscript{fl/fl} mice and the rapid phagocytosis of apoptotic cells. In good agreement with an antiapoptotic function of gp130, survival of cardiomyocytes after biomechanical stress (34) and survival of oligodendrocytes after spinal cord injury (35) are gp130 dependent. In addition, apoptosis of gp130-deficient hepatocytes is increased after in vivo stimulation with LPS (36).

In TE, the loss of astrocytes resulted in a widespread diffuse encephalitis in GFAP-Cre gp130\textsuperscript{fl/fl} mice, and the failure to contain and restrict the inflammatory lesion was paralleled by an impaired control of T. gondii. These findings are reminiscent of TE in GFAP\textsuperscript{−/−} mice, in which inflammatory lesions were also less well restricted and parasite numbers were increased (13). However, GFAP\textsuperscript{−/−} mice survived TE, because astrocytes were not lost and inflammatory lesions were relatively better locally restricted as compared with GFAP-Cre gp130\textsuperscript{fl/fl} mice.

Previous studies have unequivocally shown that control of T. gondii is impaired in conventional IL-6\textsuperscript{−/−} mice and that these mice succumb to chronic TE (4, 16) and develop a more severe ocular toxoplasmosis (37). However, the mechanism leading to the inefficient parasite control in IL-6\textsuperscript{−/−} mice is still controversial. Whereas Suzuki et al. (4) reported a reduced i.c. production of IFN-\(\gamma\) mRNA and smaller numbers of inflammatory cells, Jebsari et al. (16) observed increased IFN-\(\gamma\) production and an equally strong i.c. inflammatory immune response. In addition, Lyons et al. (37) detected a more severe inflammation and increased TNF-\(\alpha\) mRNA production in the retina of IL-6\textsuperscript{−/−} mice. Although in none of the previous reports were astrocyte reactions studied in detail, the histopathological data presented in these studies did not provide evidence for a loss of astrocytes in IL-6\textsuperscript{−/−} mice. Additionally, the following should be stressed: 1) all IL-6 cytokine family members signal via gp130 and may therefore compensate for each other; and 2) all known ligands of gp130 are up-regulated in TE (Fig. 1), implying that not a single cytokine but rather the combination of these cytokines guarantees astrocyte survival. Interestingly, CNTF and LIF are important for preventing the loss of oligodendrocytes but not of astrocytes in experimental autoimmune encephalomyelitis (38, 39). This indicates that individual IL-6 family members are important for the survival of brain resident cells in inflammatory CNS disorders but that they act differentially on individual CNS cell populations.

In TE, the permanent presence of i.c. IFN-\(\gamma\)-producing T cell infiltrates, which induce the production of protective IgTP in astrocytes, is required for the control of i.c. persisting T. gondii (3, 7, 8). Importantly, the production of IFN-\(\gamma\) and IgTP in TE of GFAP-Cre gp130\textsuperscript{fl/fl} mice was even increased in response to the higher amount of parasitic Ag. Moreover, microglia cells/macrophages were activated in GFAP-Cre gp130\textsuperscript{fl/fl} mice and produced normal amounts of iNOS, which mediates the IFN-\(\gamma\)-induced toxoplasmatic activity in these cell types (27). In addition, IFN-\(\gamma\) effectively induced a toxoplasmatic effect in GFAP-Cre gp130\textsuperscript{fl/fl} astrocytes in vitro. Collectively, these findings may imply that the impaired control of T. gondii in GFAP-Cre gp130\textsuperscript{fl/fl} mice is not caused by an insufficient control of the parasite in astrocytes or microglia but rather in neurons. Neurons are a major target cell of T. gondii tachyzoites in the CNS. In addition, cysts are exclusively detectable in neurons (40). Thus, especially the increased number of cysts in GFAP-Cre gp130\textsuperscript{fl/fl} mice indirectly suggests that the control of T. gondii is impaired in neurons. The mechanisms leading to tachyzoite-to-bradyzoite conversion and the control of T. gondii bradyzoites in neurons are unresolved. Previously, we demonstrated that IFN-\(\gamma\) and TNF alone are insufficient to induce toxoplasmatic activity in neurons in vitro (9). Potentially, in vivo additional factors derived from surrounding astrocytes must be supplied to infected neurons for the induction of antiparasitic activity in this cell population. Currently, it is intensively discussed whether astrocyte-derived products of the kynurenic acid pathway affect the neuronal activity and the behavior of T. gondii-infected hosts (41). Because astrocytes produce IDO, a major constituent of the kynurenic acid pathway with antitoxoplasmatic activity in astrocytes (42), the loss of astrocyte-derived products of the kynurenic acid pathway may result in an anatomically restricted impaired intraneuronal control of T. gondii. In GFAP-Cre gp130\textsuperscript{fl/fl} mice astrocyte loss may prevent such an indirect effect, resulting in impaired parasite control, excessive tissue necrosis, and death. The assumption that astrocytes either directly or indirectly play a role in the control of T. gondii in neurons is further supported by the predominant location of the enhanced parasite replication and tissue necrosis in the cortex, which contains numerous neurons.

In addition, the recruitment and homing of leukocytes to i.c. parasites, the numbers of i.c. inflammatory leukocytes including T cells, and the mRNA transcription of MCP-1 and IP-10 were normal in T. gondii-infected GFAP-Cre gp130\textsuperscript{fl/fl} mice. Thus, the partial loss of astrocytes at the blood-brain barrier and within inflammatory lesions was not critical for the recruitment, movement, and persistence of inflammatory leukocytes in the T. gondii-infected brain. However, these inflammatory leukocytes could not compensate for the loss of astrocytes and the inefficient local restriction of the encephalitis, finally resulting in insufficient parasite control and death of GFAP-Cre gp130\textsuperscript{fl/fl} mice. The more widespread inflammatory response may also contribute to death by disturbing important physiological functions of the brain. In this context it is noteworthy that IL-27-producing astrocytes may be important to suppress an immunopathology mediated by IL-17-producing CD4 T cells (15). In fact, IL-27 mRNA levels were reduced in the TE of GFAP-Cre gp130\textsuperscript{fl/fl} mice but IL-17 production of CD4 T cells was not increased, indicating that IL-17-mediated immunopathology did not occur.

The observation that a minor fraction of cultivated neurons of GFAP-Cre gp130\textsuperscript{fl/fl} mice was gp130\textsuperscript{−/−} is in agreement with reports from other transgenic mutants with GFAP-Cre-driven deletion of loxP flanked genes, which also showed a deletion of the loxP site-flanked gene in some neurons (18). This is caused by the development of some GFAP\textsuperscript{−/−} cells into neurons during embryogenesis (43). Interestingly, synapsin-Cre gp130\textsuperscript{fl/fl} mice with a neuron-specific deletion of gp130 survived beyond day 50 after infection with T. gondii, when all GFAP-Cre gp130\textsuperscript{fl/fl} mice have already succumbed. Thus, the severe course of TE in GFAP-Cre gp130\textsuperscript{fl/fl} mice is not caused by the deletion of gp130 in neurons.
In conclusion, a selective functional analysis of astrocytes revealed that this cell population is critically regulated via gp130 and plays a central role with respect to the outcome of cerebral infectious diseases.

Acknowledgments

The expert technical assistance of Annette Sohnkind, Nadja Schlüter, and Elena Fischer is gratefully acknowledged.

Disclosures

The authors have no financial conflict of interest.

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