Ly6C<sup>high</sup> Monocytes Control Cerebral Toxoplasmosis

Aindrila Biswas, Dunja Bruder, Susanne A. Wolf, Andreas Jeron, Matthias Mack, Markus M. Heimesaat and Ildiko Rita Dunay

*J Immunol* 2015; 194:3223-3235; Prepublished online 20 February 2015; doi: 10.4049/jimmunol.1402037

http://www.jimmunol.org/content/194/7/3223

---

**Supplementary Material**

http://www.jimmunol.org/content/suppl/2015/02/20/jimmunol.1402037.DCSupplemental

**References**

This article cites 82 articles, 33 of which you can access for free at: http://www.jimmunol.org/content/194/7/3223.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Ly6C<sup>high</sup> Monocytes Control Cerebral Toxoplasmosis

Aindrila Biswas,* Dunja Bruder,*,† Susanne A. Wolf,‡ Andreas Jeron,* Matthias Mack,§ Markus M. Heimesaat,¶ and Ildiko Rita Dunay*

Cerebral infection with the parasite *Toxoplasma gondii* is followed by activation of resident cells and recruitment of immune cells from the periphery to the CNS. In this study, we show that a subset of myeloid cells, namely Ly6C<sub>high</sub>CCR2<sup>+</sup> inflammatory monocytes that infiltrate the brain upon chronic *T. gondii* infection, plays a decisive role in host defense. Depletion of this monocyte subset resulted in elevated parasite load and decreased survival of infected mice, suggesting their crucial role. Notably, Ly6C<sub>high</sub>CCR2<sup>+</sup> monocytes governed parasite control due to production of proinflammatory mediators, such as IL-1α, IL-1β, IL-6, inducible NO synthase, TNF, and reactive oxygen intermediate. Interestingly, Ly6C<sub>high</sub>CCR2<sup>+</sup> monocytes were also able to produce the regulatory cytokine IL-10, revealing their dual feature. Moreover, we confirmed by adoptive transfer that the recruited monocytes further develop into two distinct subpopulations contributing to parasite control and profound host defense. The differentiated Ly6C<sub>low</sub>CCR2<sup>+</sup>F4/80<sup>int</sup> subset upregulated MHC I and MHC II molecules, suggesting dendritic cell properties such as interaction with T cells, whereas the Ly6C<sub>neg</sub>F4/80<sup>high</sup> cell subset displayed elevated phagocytic capacity while upregulating triggering receptor expressed on myeloid cells-2. Finally, we have shown that the recruitment of Ly6C<sub>high</sub> monocytes to the CNS is regulated by P-selectin glycoprotein ligand-1. These results indicate the critical importance of recruited Ly6C<sub>high</sub> monocytes upon cerebral toxoplasmosis and reveal the behavior of further differentiated myeloid-derived mononuclear cell subsets in parasite control and immune regulation of the CNS. The Journal of Immunology, 2015, 194: 3223–3235.

*Institute of Medical Microbiology, University of Magdeburg, 39120 Magdeburg, Germany; †Immune Regulation Group, Helmholtz Centre for Infection Research, 38124 Braunschweig, Germany; ‡Department of Cellular Neuroscience, Max-Delbrueck-Center for Molecular Medicine, 13092 Berlin, Germany; §Universitätsklinikum Regensburg, Innere Medizin II/Nephrologie-Transplantation, 93042 Regensburg, Germany; and ¶Department of Microbiology and Hygiene, Charité-University Medicine Berlin, 12203 Berlin, Germany

Received for publication August 11, 2014. Accepted for publication January 16, 2015.

This work was supported by the German Research Foundation (Deutsche Forschungsgemeinschaft DU1112/3/1 and SFB854 to I.R.D.).

Address correspondence and reprint requests to Dr. Ildiko Rita Dunay, Institute of Medical Microbiology, University of Magdeburg, Leipziger Strasse 44, 39120 Magdeburg. E-mail address: iildikodunay@gmail.com

The online version of this article contains supplemental material.

Abbreviations used in this article: DC, dendritic cell; iNOS, inducible NO synthase; PSGL-1, P-selectin glycoprotein ligand-1; ROS, reactive oxygen intermediate; TREM2, triggering receptor expressed on myeloid cells-2.

Copyright © 2015 by The American Association of Immunologists, Inc. 0022-1767/15/525/00
We demonstrate that, during cerebral the course of a low-cyst dose-induced chronic *T. gondii* infection. We demonstrate that, during cerebral *T. gondii* infection, Ly6<sup>high</sup>CCR2<sup>+</sup> monocytes along the course of a low-cyst dose-induced chronic *T. gondii* infection. We demonstrate that, during cerebral *T. gondii* infection, Ly6<sup>high</sup>CCR2<sup>+</sup> inflammatory monocytes are recruited to the brain and perform crucial antiparasitic functions. Their specific ablation resulted in decreased survival, suggesting pivotal defense function against the invaders. The infiltrated Ly6<sup>high</sup>CCR2<sup>+</sup> monocytes promoted parasite control through production of proinflammatory molecules, such as IL-1α, IL-1β, IL-6, iNOS, TNF, and ROS. Furthermore, we describe that the recruited Ly6<sup>high</sup>CCR2<sup>+</sup> monocytes differentiated and changed their phenotype and function into two distinct mononuclear cell subpopulations. One subset down-regulated Ly6C, CCR2, and upregulated CD11c, MHC I as well as MHC II (Ly6<sup>b</sup>CCR2<sup>−</sup>CD11c<sup>+</sup>), adopting DC properties. The other myeloid cell subpopulation did not exhibit Ly6C expression, but upregulated F4/80 and triggering receptor expressed on myeloid cells-2 (TREM2), presenting potent phagocytic capacity (Ly6C<sup>int</sup>CCR2<sup>−</sup>F4/80<sup>−</sup>), respectively. Finally, we show that P-selectin glycoprotein ligand-1 (PSGL-1) plays a role in the recruitment of Ly6C<sup>high</sup> monocytes to the CNS. Our results indicate the critical importance of the recruited Ly6C<sup>high</sup> monocytes in the CNS upon chronic *T. gondii* infection, thus further exposing the dynamics and behavior of antiparasitic and inflammatory properties of the mononuclear cell subsets in a murine model of cerebral toxoplasmosis.

### Materials and Methods

#### Animals

All animal experiments were approved by the local authorities according to German and European legislation. Experiments were conducted with adult C57BL/6 female mice (8 wk old; purchased from Janvier, Saint Berthevin, France). Four to five animals per group in up to four independent experiments were investigated.

#### Infection

*T. gondii* cysts of type II ME49 strain were harvested from the brains of female NMRI mice strain infected i.p. with *T. gondii* cysts 5–6 mo earlier, as described previously (15). Brains obtained from infected mice were mechanically homogenized in 1 ml sterile PBS, and the cyst numbers were determined using a light microscope. Three cysts were administered i.p. into mice in a total volume of 200 μl. Control mice were mock infected with sterile PBS.

#### Histopathology

Brains were removed and immersed in 4% paraformaldehyde for several days and sectioned coronally at 4 μm. Paraffin-embedded, 4-μm-thick sections were deparaffinized and conventionally stained with H&E. Brain sections were deparaffinized and conventionally stained with H&E. Pri-

#### Parasite number

To determine the total *T. gondii* cyst burden in the brains of infected and anti-CCR2-treated mice, whole brains were obtained and mechanically ho-
mogenized in 1 ml sterile PBS and the total cyst numbers were determined using light microscope. To confirm *T. gondii* cyst numbers, five to six coronal slides per mouse (same brain region) were analyzed by microscopy; *n* = 4 mice per group. The blinded analysis was performed by two researchers.

#### Ab treatment

Ablation of monocytes was performed by administration of anti-CCR2 mAb (clone MC-21; provided by M. Mack, University of Regensburg). Mice were injected with 75 μg (40) Ab i.p. on days 20, 22, 24, 26, and 28 postinfection. Twenty-four hours after the last treatment on day 29, the mice were sacrificed. Rat IgG (eBioscience, San Diego, CA) was used as a control mAb.

#### Cell isolation

Brains were homogenized in a buffer containing 1 M HEPES (pH 7.3) and 45% glucose and then sieved through a 70 μm strainer. The cell suspension was washed and fractionated on 25–75% Percoll gradient (GE Healthcare) for 25 min at 800 × g without brake. The cells in the interphase comprised of mononuclear cells, which were washed with PBS and used immediately for further experiments. Peripheral blood obtained by cardiac puncture was collected in 4% NaHC03. RBC lysis buffer (eBioscience) and then washed twice with ice-cold PBS at 300 × g for 10 min, followed by staining with the desired fluorescent conjugated Abs.

#### Flow cytometry

Mononuclear cells isolated from different organs were incubated with an anti-FcγRIII/II Ab (clone 93) to block unspecific staining. Thereafter, cells were stained with fluorochrome-conjugated Abs against cell surface markers, as follows: CD45 (30-F11), CD11b (M1/70), CD11c (N418), Ly6C (HK.14), F4/80 (BM8), MHCII-H2<sup>d</sup> (28-14-8), MHCII-A1/E (M5/114.15.2), VLA-4 (R1-2), and LFA-1 (M17/4), all purchased from eBioscience; Ly6G (1A8), PSGL-1 (2PH1), and CD62-L (MEL-14) from BD Biosciences; and CX3CR1 (polyclonal goat), Trem2 (237920), and CCR2 (473501) from R&D Systems for 30 min on ice, and then washed twice in 4% paraformaldehyde for 10 min. Matched isotype controls were used to assess the level of unspecific staining.

For intracellular cytokine staining, single-cell suspensions from the brains (5 × 10<sup>6</sup> cells/well) were stimulated in 24-well flat-bottom plates in the presence of Toxoplasma lysate Ag (5 μg/ml), brefeldin A (10 μg/ml), GolgiPlug; BD Biosciences), and monensin (10 μg/ml; BioLegend). After 6 h, cells were incubated with FcR-blocking Ab (clone 93) for 15 min on ice and surface stained for CD45 (30-F11), CD11b (M1/70), Ly6G (1A8), Ly6C (HK.1.4), and CD11c (N418) in FACS buffer for 30 min on ice; washed twice in FACS buffer; and fixed in 4% paraformaldehyde. Cells were then permeabilized using BD Perm/Wash Buffer. To measure cyto-
kine expression, cells were stained with the following Abs: TNF (MP6-XT22), IL-12p60 (C17.8), IL-1α (ALF-161), IL-1β (JNF3), IL-6 (MP-20F3), and IL-10 (JES5-16E3) from eBioscience and iNOS (M-19) from Santa Cruz for 45 min in BD Perm/Wash Buffer.

A total of 100,000 cells was acquired using a flow cytometer (BD FACSCanto II), and flow cytometric data were analyzed using FlowJo software (Version 9.6.4; Tree Star). Matched isotype controls were used to subtract the nonspecific background staining.

#### Detection of reactive oxygen species

Single-cell suspensions from brain after blocking their FcyRs were surface stained for CD45 (30-F11), CD11b (M1/70), Ly6G (1A8), Ly6C (HK.1.4), and CD11c (N418) in FACS buffer for 30 min on ice. ROS production was measured by Total ROS Detection Kit (ENZO, 51011), according to the manufacturer’s instructions.

#### In vitro phagocytosis assay

The isolated mononuclear cells were cultured in 12-well chambers (Nalgé Nunc International) at a density of 4 × 10<sup>5</sup> cells/ml. A total of 50 μl FluoSpheres latex beads were added (Molecular Probes) after 1-h pre-
treatment with cytoclasin D (Sigma-Aldrich), and cells were incubated under standard culture conditions for 8 h. Samples were acquired on flow cytometer (BD FACSCanto II) and analyzed with FlowJo software (Version 9.6.4; Tree Star).

#### Immunofluorescence

Frozen sections were acetone fixed for 10 min and then rehydrated and blocked by incubation for 10 min in diluent (PBS containing 2.0% normal goat serum, 1% BSA, 0.1% gelatin, 0.05% Tween 20, and 0.05% sodium
azide). Sections were incubated in primary Abs (Ly6C clone HK1-4, eBioscience; CCR2, GenWay Biotech) in diluent for 60 min, rinsed in wash buffer (PBS containing 0.5% FBS), and incubated for 60 min with secondary Abs conjugated to Alexa 488 or Alexa 594 (Molecular Probes). For negative controls, primary Abs were substituted by isotype-matched controls of the same species. Sections were rinsed in wash buffer and mounted in Vectashield containing DAPI (Vector Labs, Burlingame, CA) or ProLong Gold containing DAPI (Molecular Probes). Slides were examined with a Zeiss epifluorescence microscope equipped with an AxioCam CCD camera and Axiovision v4.0 software for image capture (Carl Zeiss, Peabody, MA). Images were processed with similar linear adjustments for all samples in Photoshop 4.0 (Adobe, San Jose, CA).

Adaptive transfer of CD11b+Ly6C<sub>high</sub> monocytes

Bone marrow cells were harvested from femora and tibiae of wild-type noninfected mice, lysed with erythrocyte (RBC) lysis buffer (eBioscience), and washed twice with ice-cold PBS at 300 × g for 10 min, before staining with the desired fluorescent conjugated Abs. CD11b<sup>+</sup>Ly6C<sub>high</sub> monocytes were sorted using BD FACSDiva software with Digital Vantage Option from wild-type C57BL/6 bone marrow cells and labeled with 5 μM CFSE (Life Technologies), and 1 × 10<sup>6</sup> cells were injected i.v. into 4-wk T. gondii (ME49)-infected C57BL/6 mice. Forty-eight hours later, the recipient mice were sacrificed and the brain mononuclear cells were isolated and analyzed by flow cytometry.

Anti-PSGL-1 treatment

To assess the transmigration capacity of the cells, 2 ng/kg rat anti-mouse PSGL-1 4RA10 (BioXCell) was administered i.p. every alternate day from day 16 to day 26 postinfection. Twenty-four hours after the last treatment on day 27, the mice were sacrificed. Isotype-matched rat IgG (eBioscience) was administered to the controls.

Statistical analysis

Data were analyzed by Student t test for two groups or one-way ANOVA for several groups, followed by Tukey’s posttest with GraphPad Prism 6 (San Diego, CA). In all cases, results were presented as mean ± SD and were considered significant with p < 0.05.

Results

Myeloid cells are recruited to the brain upon chronic T. gondii infection

Cerebral toxoplasmosis is associated with activation of resident immune cells and peripheral cell recruitment to the CNS; however, the heterogeneity of infiltrating immune cell subsets has not been fully characterized. Accordingly, we investigated the characteristic features and fate of the newly described Ly6C<sub>high</sub> monocytes following extravasation in the brains of infected mice. We observed an ingress of CD45<sup>high</sup> populations in the brains of C57BL/6 mice after 4 wk with a low-dose T. gondii infection (Fig. 1B), whereas, in uninfected controls, the major leukocyte population in the brain was resting resident microglia, (CD45<sup>low</sup>CD11b<sup>+</sup>; Fig. 1A), corroborating previous reports (19, 27, 38). Further phenotyping of the cell subsets (Fig. 1B) subdivided the CD45<sup>high</sup> subset (ungated) comprised mainly of recruited lymphocytes and a CD45<sup>high</sup>CD11b<sup>+</sup> population (upper gate; 8.0 ± 1.06% of the parent population) encompassing myeloid-derived cells, namely monocytes, neutrophils, macrophages, and DCs. Brain resident-activated microglia expressed elevated levels of CD45 upon the infection (CD45<sup>low</sup>CD11b<sup>+</sup> gated, lower; 4.1 ± 0.31% of the parent population).

The Ly6G (IAB) Ab (7, 40) was used to distinguish between Ly6G<sup>+</sup> monocytes (Fig. 1C; 96.0 ± 2.11% of the CD45<sup>high</sup>CD11b<sup>+</sup>) and Ly6G<sup>+</sup> neutrophils (4.0 ± 2.02% of the CD45<sup>high</sup>CD11b<sup>+</sup>). Relative expression of Ly6C and F4/80 was used to further differentiate between the myeloid cell subsets (Fig. 1D). Based on these surface markers, we identified three distinct myeloid cell subpopulations, as follows: Ly6C<sup>high</sup>F4/80<sup>+</sup>, Ly6C<sup>low</sup>F4/80<sup>+</sup>, and Ly6C<sup>neg</sup>F4/80<sup>+</sup>. These data suggest that, during chronic T. gondii infection, alongside with the activation of resident microglia, a heterogeneous population of myeloid cells infiltrates the CNS that can be further divided into three distinct subsets based on their Ly6C and F4/80 expression.

Ly6C<sub>high</sub>CCR2<sup>+</sup> cells localize in the parenchyma during cerebral toxoplasmosis

Immunofluorescence analysis of brain sections of T. gondii–infected mice revealed accumulation of Iba1-positive cells (Fig. 2E) in the meninges as well as in the cortex compared with noninfected controls (Fig. 2A). Closer examination suggested robust cell activation of Iba-positive microglia with less ramification, bigger soma, marked increase of average surface area, and rather amoeboid morphology (Fig. 2I). Importantly, inflammatory foci of infected brains contained Ly6C (Fig. 2F, 2J) and CCR2 (Fig. 2G, 2K)-positive cells, suggesting Ly6C<sup>high</sup>CCR2<sup>+</sup> monocytes (Fig. 2H, 2L). These amoeboid-shaped inflammatory cells were mainly located adjacent to the lesions in the cortex close to the vessels. These observations further indicate that Ly6C<sup>high</sup> CCR2<sup>+</sup> monocytes are recruited to the CNS upon T. gondii infection, which is in accordance with previous studies describing rapid influx of monocytes in the CNS upon inflammatory processes (16, 38, 41).

Ablation of CCR2<sup>+</sup>Ly6C<sub>high</sub> monocytes upon cerebral toxoplasmosis is detrimental

To evaluate the contribution of CCR2<sup>+</sup>Ly6C<sub>high</sub> monocytes in the parasite control, we took advantage of the newly available depleting anti-CCR2 Ab (MC-21) (35, 42, 43). Thus, we applied either anti-CCR2 Ab or isotype control IgG mAb, on alternating days from 20 d postinfection to 28 d postinfection, to two groups of infected mice, respectively. Twenty-four hours after the last Ab treatment, mice were sacrificed and the successful depletion of CCR2<sup>+</sup>Ly6C<sub>high</sub> monocytes in the blood was confirmed by flow cytometry by staining for Ly6C and CD11b (2.0 ± 0.23–0.0% of peripheral blood leukocytes; Fig. 3A, 3B, 3D).

Importantly, we noticed a reduction of recruited myeloid cells (8.0 ± 1.5% to 2.0 ± 0.28%) in the brains of anti-CCR2–treated mice (Fig. 3C, upper panel). This observation was further confirmed with a compelling decrease of Ly6C<sub>high</sub> monocytes (lower panel; 66.5 ± 8.6% to 11.2 ± 6.2%) and a slight decrease in the Ly6C<sub>low</sub> population (30.3 ± 2.7% to 24.2 ± 1.6%). In addition, a rise of the Ly6C<sub>neg</sub> population was detected (4.0 ± 0.7% to 65.0 ± 4.8%) that might have entered the brain before depletion occurred (before day 20). Alterations in the absolute cell numbers revealed a significant reduction of Ly6C<sub>high</sub> (p < 0.001) and Ly6C<sub>low</sub> (p < 0.01) cell subsets. Moreover, there was an increase in the Ly6C<sub>neg</sub> (p < 0.001) cell subset, whereas the microglia compartment remained unaltered (Fig. 3E).

Next, we investigated whether the selective ablation of CCR2<sup>+</sup> Ly6C<sub>high</sub> monocytes had an impact on brain pathology and survival of chronically infected mice. Histological examination of infected anti-CCR2 Ab-depleted mice revealed a higher frequency of inflammatory foci (Fig. 4B, 4D), macrophage 1 Ag<sup>+</sup> microglia, and mononuclear cells, and infiltration of immune cells into the cortex (Fig. 4D, 4F). Notably, we observed increased numbers of T. gondii cyst numbers (Fig. 4H, 4I) when compared with noninfected control brains. Most importantly, depletion of Ly6C<sub>high</sub> monocytes was followed by decreased survival rates, as by day 60 all anti-CCR2–treated mice succumbed due to the infection, whereas all noninfected control mice survived (Fig. 4J). Together these results demonstrate that Ly6C<sub>high</sub>CCR2<sup>+</sup> monocytes carry out fundamental functions in parasite control.
during cerebral toxoplasmosis, and their depletion exacerbates the outcome of the infection.

**Characterization of mononuclear cell subsets upon cerebral T. gondii infection**

The heterogeneity of monocyte–macrophage populations and their multifunctionality in the CNS have been intensively studied recently (33, 35, 38, 44). The different mononuclear cell subsets can be distinguished by their pattern of characteristic surface markers, making their discrimination between the subpopulations a complex assignment (45, 46). Therefore, to perceive the distinct myeloid cell subsets in the brain upon T. gondii infection, we first compared the expression of specific surface markers. Brain mononuclear cells from infected and control mice were isolated, and comprehensive flow cytometry analysis was performed.

Microglia from noninfected and infected mice did not express Ly6C, in contrast to monocytes (Fig. 5A, 5E). CCR2, which is a receptor for monocyte chemokine protein-1 (or CCL2), has been established to play a determining role for inflammatory monocyte egress from bone marrow (6, 32). As expected, Ly6Chigh monocytes notoriously expressed high levels of CCR2, and its expression on Ly6Cint and Ly6Cneg population was lower, whereas CCR2 was absent on microglia. This observation initiated our hypothesis that the Ly6C<sup>int</sup> and Ly6C<sup>neg</sup> subpopulations might have differentiated from the infiltrating Ly6C<sup>high</sup> monocyte subset (Fig. 5I, 5M).

Next, we measured the expression of the chemokine receptor CX<sub>3</sub>CR1. Fractalkine, the ligand of the CX<sub>3</sub>CR1 receptor, is expressed by neurons along with other cells and plays a role in maintaining certain microglial function (47–49). Microglia expressed high amounts of CX<sub>3</sub>CR1, as described previously (30, 34, 35, 38, 50, 51), whereas the receptor on recruited myeloid cells was present in low levels (Fig. 5B, 5F). The F4/80 Ag, which is exhibited by mature macrophages, was expressed predominantly by activated microglia and myeloid-derived Ly6C<sup>neg</sup> cells. The expression intensity of F4/80<sup>+</sup> within the Ly6C<sup>high</sup> and Ly6C<sup>int</sup> population was weaker (Fig. 5J, 5N). We observed a significant upregulation of a common DC marker CD11c on activated microglia, in line with previous studies highlighting their activation status (27). Furthermore, expression of CD11c was high on Ly6C<sup>int</sup> and Ly6C<sup>neg</sup> myeloid-derived cells (Fig. 5C, 5G). The surface marker TREM2 was strongly upregulated on activated microglia and myeloid-derived Ly6C<sup>neg</sup> cells (Fig. 5K, 5O), pointing toward a potentially elevated phagocytic capacity. The Ly6C<sup>high</sup> and Ly6C<sup>low</sup> subpopulations expressed low levels of TREM2, implying little phagocytic activity. Parallel to their ele-

**FIGURE 1.** Myeloid cell recruitment and activation of microglia cells. Leukocytes were isolated from brains of noninfected (A) and T. gondii–infected (B–D) C57BL/6 mice and analyzed by flow cytometry. Following the basic forward light scatter–side light scatter gating, the singlet cells were selected for further characterization. (A) Shows resident microglia (CD11b<sup>+</sup>CD45<sup>low</sup>, lower gate), and (B) shows the percentage of activated microglia (CD11b<sup>+</sup>CD45<sup>int</sup>, lower gate) and the myeloid population (CD11b<sup>+</sup>CD45<sup>high</sup>, upper gate). (C) The myeloid population consists of neutrophils (CD11b<sup>+</sup>Ly6G<sup>+</sup>, upper gate) and monocytes (CD11b<sup>+</sup>Ly6G<sup>−</sup>, lower gate). (D) The monocytes can be further divided according to their expression of Ly6C and F4/80: Ly6C<sup>high</sup>F4/80<sup>int</sup>, Ly6C<sup>int</sup>F4/80<sup>int</sup>, and Ly6C<sup>neg</sup>F4/80<sup>high</sup>. Numbers represent percentage of parent population. Data shown are representative of four individual experiments (n = 4); results are shown as mean ± SD.

**FIGURE 2.** Immunofluorescence staining of microglia and monocytes in brain slides. We show the cortex and the cortical meninges in noninfected control (A–D) and infected (E–L) brains. In the noninfected brain (A), Iba<sup>+</sup> microglia have a ramified morphology, whereas in the infected brain (E) they display a rather amoeboid morphology with bigger soma. The Ly6C and CCR2 staining are negative in the control brains, but positive in the parenchyma of the infected brains (F and G), displaying the recruitment of inflammatory monocytes (H) during T. gondii infection. Five to six coronal slides per mouse were analyzed; n = 4 mice per group. This experiment was repeated three times. Scale bars, 100 μm in (A)–(H) and 25 μm in (I)–(L).
vated CD11c expression, Ly6C<sup>int</sup> cells upregulated primarily the activation markers MHC I and II, indicating their efficacy to initiate adaptive immune responses by Ag presentation. Activated microglia, Ly6Chigh, and Ly6C<sup>neg</sup> resident monocytes (lower gate) in the blood. The coexpression of Ly6C and CCR2 of the inflammatory monocytes (Ly6C<sup>high</sup>) is shown further (A, right). (D) The bar graph represents the percentage of Ly6C<sup>high</sup> in the blood. (E) After the basic forward light scatter–side light scatter and singlet gating, upper plots show the gating of activated microglia (CD11b<sup>+</sup>CD45<sup>int</sup>) and the myeloid population (CD11b<sup>+</sup>CD45<sup>high</sup>) in the brain. Lower plots display the monocyte subsets (from the myeloid gate): Ly6C<sup>high</sup>, Ly6C<sup>int</sup>, and Ly6C<sup>neg</sup>. (E) The bar graphs represent the total cell number of the respective subset in the brain. Numbers in the plots represent percentage of parent population. Data shown are representative of five individual experiments (with n = 4 individual mice); results shown as mean ± SD. Significant differences (**p < 0.01, ***p < 0.001) were determined using the Student t test.

Unique cytokine profile of myeloid cell subsets in cerebral toxoplasmosis

Consequently, to find out the particular cytokine production of these three distinct myeloid-derived cell subsets, we performed intracellular flow cytometry analysis. We detected that Ly6C<sup>high</sup> monocytes were able to produce high amounts of proinflammatory mediators such as IL-1α, IL-1β, IL-6, TNF, and iNOS (Supplemental Fig. 1G–J). The surface Ag CD11c was weakly expressed on all investigated cell populations in the blood; however, the highest appearance was observed on Ly6C<sup>high</sup> monocytes (Supplemental Fig. 1K, 1L). Similarly, MHC I was upregulated on Ly6C<sup>high</sup> inflammatory monocytes upon T. gondii infection, and MHC II was mainly expressed on resident monocytes during infection (Supplemental Fig. 1M–P).

FIGURE 3. Depletion of CCR2<sup>+</sup>Ly6C<sup>high</sup> monocytes. C57BL/6 mice were infected with T. gondii and from day 20 to day 28 postinfection alternatively treated with either IgG mAb or anti-CCR2 to deplete inflammatory monocytes. After the standard forward light scatter–side light scatter gating, single cells were selected for further characterization. (A left, and B) displays the representative plots to define Ly6C<sup>high</sup> inflammatory monocytes (upper gate), Ly6C<sup>int</sup> neutrophils (middle gate), and Ly6C<sup>neg</sup> resident monocytes (lower gate) in the blood. The expression of CD11c and CCR2 of the inflammatory monocytes (Ly6C<sup>high</sup>) is shown further (A, right). (D) The bar graph represents the percentage of Ly6C<sup>high</sup> in the blood. (E) After the basic forward light scatter–side light scatter and singlet gating, upper plots show the gating of activated microglia (CD11b<sup>+</sup>CD45<sup>int</sup>) and the myeloid population (CD11b<sup>+</sup>CD45<sup>high</sup>) in the brain. Lower plots display the monocyte subsets (from the myeloid gate): Ly6C<sup>high</sup>, Ly6C<sup>int</sup>, and Ly6C<sup>neg</sup>. (E) The bar graphs represent the total cell number of the respective subset in the brain. Numbers in the plots represent percentage of parent population. Data shown are representative of five individual experiments (with n = 4 individual mice); results shown as mean ± SD. Significant differences (**p < 0.01, ***p < 0.001) were determined using the Student t test.

FIGURE 4. Increased parasite load and decreased survival in anti-CCR2–treated T. gondii–infected mice (A–H). Immunohistological representation of control (left panel) and anti-CCR2 (right panel) brains shows more inflammation by H&E staining (D, arrows), increased habitation of mononuclear cells (F), and more parasites (H) in the anti-CCR2 brain. (I) The bar graph is the quantification of the total cyst burden in the control and anti-CCR2 brains. (J) Survival curve of the T. gondii–infected control and anti-CCR2–treated mice was monitored from day 0 until day 60. Five to six coronal slides per mouse were analyzed; n = 4 mice per group. Total cyst load was determined from brain lysates; n = 4 mice per group. The survival experiment was repeated twice for a total of n = 8–10 mice per experiment. Significant differences (*p < 0.05) were determined using Student t test.
ROS secretion was measured explicitly by Ly6C<sup>high</sup> monocytes, suggesting their strong potential to eliminate the parasites (Fig. 6L, 6P). The Ly6C<sup>int</sup> and Ly6C<sup>neg</sup> population produced lesser amounts of cytokines; however, IL-12p40 was mainly produced by Ly6C<sup>int</sup> cells, suggesting their role to shape the adaptive immune system (Fig. 6D, 6I). Activated microglia contributed with low levels of cytokine production to the host defense in this model with low-dose <i>T. gondii</i>–induced chronic infection in the brain. Interestingly, alongside their proinflammatory and antiparasitic functions, Ly6C<sup>high</sup> cells also expressed the immunoregulatory cytokine IL-10 upon in vitro stimulation, implicating their possible dual nature to maintain tissue homeostasis and counterbalance the ongoing CNS inflammation (Fig. 6M, 6Q).

**Ly6C<sup>neg</sup> myeloid cells perform strong phagocytic capacity**

To examine the phagocytic properties of microglia and recruited myeloid cell subsets, we performed ex vivo phagocytosis assay. To this end, the respective cell subsets were isolated, sorted, and then incubated with fluorescent latex beads. Whereas recruited Ly6C<sup>high</sup> and Ly6C<sup>int</sup> cells exhibited low phagocytic capacity (31.1 ± 3.6% and 25.0 ± 2.2%, respectively), Ly6C<sup>neg</sup> cells demonstrated prominent phagocytic ability (80.2 ± 5.6%), alongside with those of activated resident microglia (60.2 ± 1.2%; Fig. 6E).

**FIGURE 5.** Phenotypic characterization of mononuclear cell populations in the brain. (A–P). To measure the expression of surface and activation markers by leukocytes in the brain, infected and noninfected controls were analyzed by flow cytometry. A similar gating strategy was followed as in Fig. 1A–D. (A–D and I–L) Representative histograms showing expression levels of surface markers by cell population. Bars mark the cells positive for the particular marker. Numbers above bars display the percentage of cells positive for the marker of the concerned population: resident microglia (CD11b<sup>+</sup>CD45<sup>low</sup>), activated microglia (CD11b<sup>+</sup>CD45<sup>int</sup>), inflammatory monocytes (CD11b<sup>+</sup>CD45<sup>high</sup>Ly6G<sup>−</sup>Ly6C<sup>high</sup>), Ly6C<sup>int</sup> monocytes (CD11b<sup>+</sup>CD45<sup>high</sup>Ly6G<sup>−</sup>Ly6C<sup>int</sup>), Ly6C<sup>neg</sup> monocytes (CD11b<sup>+</sup>CD45<sup>high</sup>Ly6G<sup>−</sup>Ly6C<sup>neg</sup>), isotype control, tinted (B, D, K, and L). (E–H and M–P) Bar graphs represent the median fluorescence intensity for the specific marker median fluorescence intensity (MFI) ± SD (n = 4). Data are representative of four independent experiments. One-way ANOVA analysis followed by Tukey’s post hoc test was performed for multiple comparisons (*p < 0.05, **p < 0.01, ***p < 0.001). Act. microglia, activated microglia; ns, not significant; Res. microglia, resident microglia.
FIGURE 6. Cytokine production and phagocytic capacity of the cell subsets in the brain. (A–D and F–Q) Intracellular cytokine production was analyzed in cells isolated from brains of noninfected and infected mice after in vitro stimulation with Toxoplasma lysate Ag. The cells were gated as shown in the representative plots of Fig. 1A–D. (A–D and J–M) Representative histograms showing cytokine expression by cell population. Bars mark the cells positive for the particular cytokine. Numbers above bars display the percentage of cells positive for the cytokine of the respective population: resident microglia (CD11b+CD45low), activated microglia (CD11b+CD45int), inflammatory monocytes (CD11b+CD45highLy6G−Ly6Chigh), Ly6Cint monocytes (CD11b+CD45highLy6G−Ly6Cint), Ly6Cneg monocytes (CD11b+CD45highLy6G−Ly6Cneg), isotype control, tinted. (F–I and N–Q) Bar graphs represent the median fluorescence intensity (MFI) of the respective fluorochrome for a particular cytokine, MFI ± SD (n = 4). Data are representative of four independent experiments. (E) The uptake of fluorescent latex beads by brain mononuclear cells ex vivo was measured by flow cytometry. After the standard forward light scatter–side light scatter gating and singlet gating, CD11b+ cells were further gated, as shown in Fig. 1. Bar graph shows the percentage of phagocytosis of the latex beads by the respective population. Data are representative of three experiments with n = 4; results are shown as mean ± SD. One-way ANOVA analysis followed by Tukey’s post hoc test was performed for multiple comparisons (*p < 0.05, **p < 0.01, ***p < 0.001). Act. microglia, activated microglia; ns, not significant; Res. microglia, resident microglia.
Ly6C<sup>high</sup> cells infiltrate and differentiate in the brain upon adoptive transfer

To confirm that recruited Ly6C<sup>high</sup> monocytes further differentiate to the previously described Ly6C<sup>int</sup> and Ly6C<sup>neg</sup> cells following extravasation in the CNS, we conducted adoptive transfer experiments. To address this, we injected 1 × 10<sup>6</sup> sorted, CFSE-labeled Ly6C<sup>high</sup>CD11b<sup>+</sup> cells isolated from the bone marrow of wild-type mice i.v. into T. gondii-infected recipient mice at day 28 postinfection. The cells were injected when the ongoing inflammation had already affected the blood-brain-barrier permeability and the recruitment of the inflammatory cells to the CNS reached its peak (data not shown). Notably, CFSE-labeled Ly6C<sup>high</sup> cells were found in the CNS as early as 24 h after the transfer (data not shown). Forty-eight hours after the transfer, Ly6C<sup>high</sup> cells (27.2 ± 3.2% of CD11b<sup>+</sup>CFSE<sup>+</sup> gate) downregulated Ly6C and apparently differentiated into Ly6C<sup>int</sup> (32.0 ± 1.6% of CD11b<sup>+</sup>CFSE<sup>+</sup> gate) and Ly6C<sup>neg</sup> cells (41.0 ± 2.0% of CD11b<sup>+</sup>CFSE<sup>+</sup> gate; Fig. 7B). These data suggest that Ly6C<sup>high</sup> monocytes are recruited to the brain upon T. gondii infection, in which a subset of them loses their Ly6C expression and further generates the Ly6C<sup>int</sup> and Ly6C<sup>neg</sup> subsets.

Migration of CCR2<sup>+</sup>Ly6C<sup>high</sup> monocytes to the brain is PSGL-1 dependent

Selective leukocyte homing to the site of infection has been shown to be dependent on chemokines and distinct adhesion molecules (52–55). To evaluate whether any particular adhesion molecule plays a role in CCR2<sup>+</sup>Ly6C<sup>high</sup> monocyte recruitment to the CNS in toxoplasmosis, we measured the expression of L selectin (CD62L), LFA-1, and PSGL-1 on Ly6C<sup>high</sup> monocytes.

CD62L, which is known to be important for leukocyte rolling on the inflamed endothelium (56, 57), was highly expressed on Ly6C<sup>high</sup> inflammatory monocytes in the periphery, but was downregulated upon entry to the brain. Resident monocytes in the blood expressed only low levels of CD62L (Fig. 8A, 8D, 8G, 8J). LFA-1 was expressed both in the periphery and in the brain by the recruited monocytes as well as on Ly6C<sup>int</sup> and Ly6C<sup>neg</sup> cells in the CNS. Resident monocytes in the blood upregulated LFA-1 upon T. gondii infection, similarly to resident microglia cells in the brain (Fig. 8B, 8E, 8H, 8K). PSGL-1 expression on the Ly6C<sup>high</sup> inflammatory monocytes was significantly higher compared with resident cells in the blood and in the CNS (Fig. 8C, 8F, 8I, 8L).

Hence, to test whether the high PSGL-1 levels detected on the surface of Ly6C<sup>high</sup> monocytes had a functional role in their migration to the CNS, mice were treated i.p. with anti–PSGL-1 Ab from day 16 to day 26 after T. gondii infection. After 24 h of the last Ab application, we observed an increased proportion of Ly6C<sup>high</sup> monocytes in the peripheral blood (Fig. 9A, 9B; 3.1 ± 0.6% to 5.4 ± 0.92%) of the parent population of the treated mice, implying that, in the absence of PSGL-1, sufficient transmigration does not occur and cells accumulate in the blood. In the brains of infected anti-PSGL-1-treated mice, we observed a reduction in the percentage (40.6 ± 1.67% to 25.2 ± 2.1%) of the CD45<sup>high</sup>CD11b<sup>+</sup>Ly6G− population and in the total cell numbers within the Ly6C<sup>high</sup> compartment (Fig. 9C, lower panel), 9D, respectively. No significant differences were detected in the frequencies and total numbers of other cell subsets (Fig. 9C, lower panel). The inhibition of Ly6C<sup>high</sup> cell recruitment to the brain was markedly diminished (p < 0.01; Fig. 9E), confirming that PSGL-1 is an important mediator for monocyte homing to the CNS.

Discussion

Due to the recent discovery of several novel characteristic markers and transcription factors, our knowledge of the myeloid cell heterogeneity has advanced extensively (26, 30, 33, 36, 38, 41, 45, 58, 59). Previous studies have suggested that, alongside the resident microglia, recruited mononuclear cells are important to control CNS inflammation and (different) CNS infections (16, 27, 36, 37, 39). However, differences in the experimental setups have made it difficult to precisely correlate results and determine the relative importance of the definitive cell subpopulations during the inflammatory processes and infections.

The role of bone marrow–derived CCR2<sup>+</sup>Ly6C<sup>high</sup> monocytes was investigated under various infectious conditions in the periphery, as well as in the CNS. We have previously shown that inflammatory monocytes control acute T. gondii infection in the ileum, by producing antimicrobial mediators (6, 7). Also, during Listeria monocytogenes infection, the absence of Ly6C<sup>high</sup> monocytes leads to rapid death of mice, demonstrating their important role in host defense (32, 60, 61). Furthermore, we recently observed that Ly6C<sup>high</sup> monocytes are substantially involved in brain inflammation and immune cell recruitment to the CNS, leading to experimental cerebral malaria upon Plasmodium ANKA infection (our unpublished observations). In cases of viral encephalitis, Ly6C<sup>high</sup> monocytes infiltrate the CNS and, although contributing to viral clearance, they also induce significant immunopathology (16, 62). The opposing beneficial and detrimental nature of Ly6C<sup>high</sup> monocytes, which seemingly depends on the type of infection, warrants further characterization to understand their intricate behavior.

In the chronic phase of T. gondii infection, parasites persist in cysts within immune-privileged sites (15). The latent stage is associated with marginal inflammation and cell recruitment to the CNS, which is necessary to provide adequate IFN-γ levels, the major driving force for parasite control. In the murine models of chronic infection, the characteristics of T cell subsets (24, 63–65), specific mononuclear cells, for example, resident microglia and APCs, have been extensively studied. In contrast, the role of the newly described myeloid cell subsets is controversial and thus requires further investigation (19–21). Microglia cells have been shown to eliminate parasites in an IFN-γ–dependent manner, in addition to their efficient phagocytic capacity and cytokine production (19, 20, 66, 67). Some early studies suggest that brain DCs differentiate from local resident cells, whereas others have proposed a peripheral hematopoietic cell origin upon cerebral toxoplasmosis (27, 39, 68).

In this study, we have elucidated the nature of the recruited myeloid cells within the brain upon cerebral toxoplasmosis. We

![Image](http://www.jimmunol.org/Downloadedfromhttp://www.jimmunol.org/images/A72852.png)
found that, upon low-dose infection with *T. gondii*, myeloid-derived Ly6C high cells infiltrated the brain and expressed specific surface markers CD45 highCD11b+Ly6G 2 Ly6ChighF4/80 int. In addition, based on their Ly6C and F4/80 expression, there were two more CD45 + myeloid populations present in the CNS, namely Ly6C intF4/80 int and Ly6C negF4/80 high.

In our previous studies, we detected that CCR2 2/2 mice were extremely sensitive to *T. gondii* infection, with 90% of mice succumbing in the acute phase of infection. We therefore concluded that CCR2 + Ly6Chigh monocytes were necessary to control the acute *T. gondii* infection and to limit the small intestinal pathology (6). The remaining 10% of infected mice displayed elevated parasite numbers in the CNS, suggesting that these cells might also be involved in the chronic phase of the infection (6). Thus, to study the role of CCR2 2/2 Ly6C high monocytes in the chronic stage, the depletion of this particular cell subset was necessary to perform after the infection overcame the acute phase and parasites were present in the CNS. Previously, we compared the effect of commonly used Abs to ablate inflammatory monocytes and addressed the concerns surrounding the expression of Gr1, Ly6C, and Ly6G, using the available depletion strategies of neutrophil granulocytes and inflammatory monocytes (7). In these earlier studies, we used the Gr-1–specific RB6-8C5 Ab; however, the application of this Ab led to a depletion of both Ly6C highLy6G 2 Ly6Chigh monocytes, as well as Ly6C highLy6G + neutrophils (7). As control, we used the 1A8 Ab that reacts specifically to Ly6G and depletes solely neutrophils (40). Importantly, our results confirmed that Ly6C high monocytes are crucial in the control of the acute *T. gondii* infection, and that Ly6G + neutrophils rather contributed to the immunopathology (7).

In the current study, taking advantage of a new depletion strategy of Ly6C highCCR2 + monocytes, we applied anti-CCR2 Ab (MC-21), which selectively depletes CCR2 + inflammatory monocytes (69, 70). We observed an increase in immunopathology and ele-
vated parasite numbers in the CNS during cerebral toxoplasmosis. Most importantly, mice were unable to control the parasites and succumbed to infection. This finding strongly suggests that Ly6ChighCCR2+ monocytes play a critical role in governing the chronic phase of Toxoplasma infection.

Due to the differential expression of Ly6C and F4/80 among the myeloid compartment, we characterized the phenotype of the recruited CCR2+Ly6Chigh monocytes, Ly6Cneg and Ly6Cneg myeloid-derived cells, and resident microglia. Therefore, we conducted comprehensive surface and intracellular stainings using highly specific markers. CCR2 expression was the highest on the newly recruited Ly6Chigh cells, and was lower on Ly6Cint and Ly6Cneg cells. The chemokine receptor CX3CR1 was predominately expressed on microglia and was absent on Ly6Chigh monocytes, in accordance with previous studies (31, 33, 34, 38, 71). The mature macrophage marker F4/80 was expressed mainly by activated microglia and by Ly6Cneg cells, suggesting their macrophage-like nature. Microglia upregulated CD11c upon infection-induced activation, as previously reported (27). CD11c expression was also elevated in Ly6Cint and Ly6Cneg cells, pointing toward their DC phenotype. Interestingly, MHC I and II were most intensely expressed on the surface of Ly6Cint cells, implying that these cells may represent the previously characterized brain DCs (27). These results proved that the recruited myeloid cell populations are different from the resident microglia.

Comparing the cytokine profile between the resident and recruited mononuclear cell subsets in the CNS revealed that Ly6Chigh monocytes produced the highest levels of proinflammatory molecules, such as IL-1α, IL-1β, IL-6, TNF, and iNOS. These results indicate that monocytes are essential to control T. gondii infection in the brain, similar to our previous findings in the periphery (6, 7). Additionally, the elevated ROS production was observed exclusively by the Ly6Chigh monocytes, further suggesting their critical role in the host defense arsenal. This proinflammatory signature of Ly6Chigh cells was reaffirmed in cerebral viral infections (62), and in other parasitic models (72, 73). IL-12p40 secretion was the highest by Ly6Cint cells, well in line with their CD11c and MHC expression, suggesting their capability to initiate adaptive immune responses. In comparison with CD11b+ CD11b+ myeloid-derived cells, the activated resident microglia contributed to lesser extent to the secretion of proinflammatory mediators in cerebral toxoplasmosis. Remarkably, in addition to their antiparasitic capacity, Ly6Chigh cells were also able to secrete the regulatory cytokine IL-10, suggesting a dual function of limiting pathogen expansion and regulating detrimental immunopathology in the CNS. Our observations are consistent with a recent study by Grainger et al. (72), reporting similar dual features of Ly6Chigh monocytes in the acute T. gondii infection model. Additionally, this study also described that monocytes regulate neutrophil function by secreting PGE2, hence contributing to our understanding of their complex functions. Such decisive roles played by the Ly6Chigh cells during the resolution of inflammation, tissue regeneration, and debris clearance were also seen in models of autoimmunity and neurodegeneration (26, 36, 37).

We also found that activated microglia alongside with Ly6Cneg myeloid-derived cells displayed phagocytic potential, whereas
Ly6<sub>high</sub> and Ly6<sub>int</sub> cells were less capable in this activity. The F4/80 and particularly the TREM2 expression of those cells followed the same pattern, as described recently for the latter molecule (49, 74–77), suggesting that the activated microglia has the capacity to engulf invading microorganisms and dead tissue remains during the ongoing inflammation (49, 76–79). The current paradigm suggests that monocytes do not substantially contribute to tissue-resident macrophages under stable state conditions; rather, resident macrophages and microglia in the CNS develop in the embryonic stage (38). However, during infection and inflammation, macrophage-like cells can differentiate from infiltrating Ly6<sub>high</sub> inflammatory monocytes. The diverse behavior of the monocyte-derived macrophages was demonstrated in experimental autoimmune uveitis, in which the kinetics of CX<sub>3</sub>CR<sub>1</sub><sup>low</sup>Ly6C<sup>+</sup> and CX<sub>3</sub>CR<sub>1</sub><sup>high</sup>Ly6C<sup>−</sup> changed along the course of the disease (36). Such heterogeneity was also shown in an acute model of colitis, in which the recruited CX<sub>3</sub>CR<sub>1</sub><sup>int</sup>Ly6<sub>high</sub> monocytes promoted inflammation, but over time gave rise to a CX<sub>3</sub>CR<sub>1</sub><sup>int</sup>Ly6<sub>low</sub> population that displayed all the trademarks of a DC (35).

In cerebral toxoplasmosis, the conversion of monocytes to brain DCs upon recruitment was also proposed by a previous study (27). Confirming their plasticity, we also detected that recruited monocytes in the brain further differentiate into two distinct population CD11c<sup>+</sup>Ly6<sub>high</sub> brain DCs and Ly6<sub>low</sub>F4/80<sup>+</sup> macrophages and carry out particular functions in parasite clearance.

Blood monocytes are comprised of two distinct populations, the inflammatory CX<sub>3</sub>CR<sub>1</sub><sup>low</sup>CXCR2<sup>+</sup>Ly6<sub>high</sub> and the patrolling CX<sub>3</sub>CR<sub>1</sub><sup>high</sup>CXCR2<sup>−</sup>Ly6<sub>low</sub> cells. Correspondingly, in our current experiments, CX<sub>3</sub>CR<sub>1</sub><sup>low</sup>CXCR2<sup>+</sup>Ly6<sub>high</sub> monocytes and CX<sub>3</sub>CR<sub>1</sub><sup>high</sup>CXCR2<sup>−</sup>Ly6<sub>low</sub> were present in the blood, and, upon Toxoplasma infection, the common DC marker CD11c and the MHC I molecule were upregulated on the surface of Ly6<sub>high</sub> monocytes, suggesting modified activation status even before reaching the site of infection.

Leukocyte transmigration in the blood through the blood-brain barrier upon cerebral inflammation is dependent on adhesion molecules and their receptors. Inflammatory monocytes use the CCR2–CCL2 axis to egress from the bone marrow, but the mechanism of crossing the blood-brain barrier is poorly understood (6, 78). Therefore, we investigated the involvement of certain key molecules in the recruitment of Ly6<sub>high</sub> inflammatory monocytes to the CNS. CD62L (L-selectin), which assists immune cells to enter different tissues (56, 57), was highly expressed on Ly6<sub>high</sub> monocytes in the blood, but was downregulated upon their entry into the CNS. Consistent with previous studies, the CD62L expression was low on resident monocytes and microglia (38). LFA-1, which can interact with P- and E-selectin on activated endothelium or with CD62L on monocytes, suggests modified activation status even before reaching the site of infection.

The dependence on PSGL-1 for lymphocyte migration is well established (56, 57, 79–82). However, to date, the function of PSGL-1 in monocyte recruitment has not been sufficiently addressed. One previous study described the role of PSGL-1 in Ly6<sub>high</sub> monocyte homing to the site of atherosclerosis in blood vessels of mice (54). This study revealed that Ly6C<sup>high</sup> monocytes, which are PSGL-1<sup>−/−</sup> and CD62L<sup>+</sup>, preferentially interacted with P- and E-selectin on activated endothelium or with CD62L on a rolling/adherent leukocyte under flow by secondary tethering. However, the authors speculated that other adhesion molecules such as LFA-1 and VCAM-1 (ligand VLA-4) may not be key factors in monocyte homing, as their ligands were expressed at lower levels on Ly6<sub>high</sub> cells. Furthermore, other studies detected PSGL-1–dependent monocyte migration in the periphery during Leishmania major infection, tumor metastasis, and thrombus formation (48, 81, 82). Thus, to our knowledge, we investigated for the first time PSGL-1–dependent monocyte recruitment to the CNS. Treatment of mice with anti–PSGL-1 Ab revealed a significant inhibition of Ly6<sub>high</sub> cell recruitment to the brain upon chronic T. gondii infection, confirming that PSGL-1 is critical for monocyte homing to the CNS.

Altogether, our findings combined with emerging evidence from other murine models further highlight the plasticity of recruited Ly6<sub>high</sub> monocytes. Cerebral T. gondii infection leads to cytokine production by the inundated Ly6<sub>high</sub> monocytes, which play an influential role in the protection of the inflamed brain. The Ly6<sub>high</sub> monocytes further give rise to Ly6<sub>int</sub> and Ly6<sub>neg</sub> subsets and perform divergent functions such as Ag presentation and phagocytosis. Thus, monocytes and their descendants play multifaceted functions to control cerebral toxoplasmosis. In conclusion, our findings indicate that, during cerebral T. gondii infection, Ly6<sub>high</sub> inflammatory monocytes infiltrate the CNS and differentiate into phenotypically and functionally distinct cell subsets and carry out pivotal functions to control the chronic stage.

Acknowledgments

We thank Dana Zablter for excellent technical assistance.

Disclosures

The authors have no financial conflicts of interest.

References

5. Lambert, H., N. Hitziger, I. Dellacasa, M. Svensson, and A. Barragan. 2006. Induction of dendritic cell migration upon Toxoplasma gondii infection poten-


