Phenotype and Functions of Brain Dendritic Cells Emerging During Chronic Infection of Mice with *Toxoplasma gondii*

Hans-Georg Fischer, Ursula Bonifas and Gaby Reichmann

*J Immunol* 2000; 164:4826-4834; 
doi: 10.4049/jimmunol.164.9.4826

http://www.jimmunol.org/content/164/9/4826

References

This article cites 46 articles, 28 of which you can access for free at: http://www.jimmunol.org/content/164/9/4826.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
Phenotype and Functions of Brain Dendritic Cells Emerging During Chronic Infection of Mice with *Toxoplasma gondii*¹

Hans-Georg Fischer,²* Ursula Bonifas,* and Gaby Reichmann³

During chronic infection of mice with *Toxoplasma gondii*, gene message for IL-12p40, CD86, and the potassium channel Kv1.3 was detected in brain mononuclear cells, suggesting the presence of dendritic cells (DC) in the CNS. Consistently, cells bearing the DC markers CD11c and 33D1 were localized at inflammatory sites in the infected brain. The number of isolated CD11c⁺ brain cells increased until peak inflammation. The cells exhibited the surface phenotype of myeloid DC by coexpressing 33D1 and F4/80, little DEC-205, and no CD8α. These brain DC were mature, as indicated by high-level expression of MHC class II, CD40, CD54, CD80, and CD86. They triggered Ag-specific and primary allogeneic T cell responses at very low APC/T cell ratios. Among mononuclear cells from encephalitic brain, DC were the main producers of IL-12. Evidence for a parasite-dependent development of DC from CNS progenitors was obtained in vitro: after inoculation of primary brain cell culture with *T. gondii*, IL-12-secreting dendriform cells emerged, and DC marker genes were expressed. Different stimuli elicited the generation and maturation of brain DC: neutralization of parasite-induced GM-CSF prevented outgrowth of dendriform cells and concomitant release of IL-12. IL-12 production was up-regulated by external IFN-γ but was stopped by inhibiting parasite replication. Consistently, DC isolated from GM-CSF-treated brain cell culture were activated to secrete IL-12 by exposure to parasite lysate. In sum, these results demonstrate *T. gondii*-induced expansion and functional maturation of DC in the CNS and, thus, highlight a mechanism that may contribute to the chronicity of the host response. *The Journal of Immunology, 2000, 164: 4826–4834.

¹Abbreviations used in this paper: DC, dendritic cell; TE, toxoplasmic encephalitis; BMNC, brain mononuclear cells; p.i., postinfection; PPD, purified protein derivative; SN, supernatant.

Address correspondence and reprint requests to Dr. Hans-Georg Fischer, Institute for Medical Microbiology and Virology, Heinrich-Heine University, Duesseldorf, Germany. E-mail address: hans-georg.fischer@uni-duesseldorf.de

This work was supported by grants from the Deutsche Forschungsgemeinschaft (SFB194/B11 and RE-1334/2-1).

¹Institute for Medical Microbiology and Virology, Heinrich-Heine University, Duesseldorf, Germany; and ²Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104

Received for publication November 3, 1999. Accepted for publication February 14, 2000.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

© 2000 by The American Association of Immunologists
Materials and Methods

Animals

Female BALB/c and C57BL/10 mice were used at 12–16 wk of age for in situ and ex vivo analyses. Newborn animals of either sex were used for the preparation of brain cell cultures. Mice were bred under specific pathogen-free conditions from breeding stock supplied by Biological Research Laboratories (Füllinsdorf, Switzerland) and The Jackson Laboratory (Bar Harbor, ME), respectively.

Parasites and infection

The DX strain of T. gondii (intraspecies group II) was used. For infection of mice, cysts were harvested from the brains of chronically infected animals. Mice were injected i.p. with cysts, and infection was serologically verified 2–3 wk postinfection (p.i.). For inoculation of brain cell cultures, bradyzoites were prepared as described (23) by mild tissuepization of cell culture cysts. Parasite lysate containing 10 mg/ml protein was prepared from tachyzoites (strain BK) as detailed in (24). Feeder cells and toxoplasmosis were routinely tested for mycoplasma contamination.

Immunohistochemistry

Brains from infected or control mice were mounted in OCT compound (Miles Scientific, Naperville, IL), snap-frozen in prechilled 2-methylbutane, and stored at −70°C. Cryostat sections (5 μm) were thaw-mounted on poly-L-lysine-coated microscope slides and fixed in ice-cold acetone for 10 min. Sections were incubated for 30 min with 0.3% H2O2/0.2 M NaN3 to quench endogenous peroxidase activity before blocking with 10% goat serum (Vector Laboratories, Burlingame, CA) in HBSS. Sections were then stained for 1 h with anti-CD11c mAb N418 (Endogen, Cambridge, MA), DC-specific mAb 33D1 (Biotrend, Cologne, Germany), or hamster anti-FcγR mAb 2.4G2 (PharMingen) to block unspecific binding of primary Abs. Sections were incubated with biotinylated rabbit anti-rat IgG (Vector) or goat anti-hamster IgG (The Jackson Laboratory). Subsequent incubation of the slides with peroxidase-conjugated avidin-biotin complex (Vectastain Elite ABC Kit; Vector) was performed according to the manufacturer’s protocol. Sections were developed with 3,3'-diamino-benzidin (DAB) using the DAB substrate kit (Vector), slightly counterstained with hematoxylin, dehydrated, and mounted.

Preparation of brain mononuclear cells (BMNC)

Mice were transcardially perfused with 2 × 20 ml ice-cold PBS while being kept under deep Metofane (Janssen, Neuss, Germany) anesthesia. The brains were excised, minced in DMEM with 10% FCS, and passed through Ficoll-Paque (Pharmacia, Uppsala, Sweden) and then with washes in PBS. All experiments of infection in vivo were performed with BALB/c mice. Mice were injected i.p. with cysts, and infection was serologically verified 2–3 wk postinfection (p.i.). For inoculation of brain cell cultures, bradyzoites were prepared as described (23) by mild tissuepization of cell culture cysts. Parasite lysate containing 10 mg/ml protein was prepared from tachyzoites (strain BK) as detailed in (24). Feeder cells and toxoplasmosis were routinely tested for mycoplasma contamination.

Isolation of DC and brain macrophages/microglia

Splenocytes or BMNC were depleted of T cells by treatment with anti-CD8 mAb and rat complement; Cedarlane, Hornby, Canada). Then, CD11c+ cells were isolated by immunomagnetic sorting from CD11c+ BMNC using the Dynal MicroBead isolation kit (Cytomation). Cells were resuspended in phenol red-free DMEM supplemented with 10% FCS, glutamine, and 50 μM 2-ME.

Flow cytometry

CD11c+ BMNC were subjected to immunofluorescence staining of mouse DC markers by using the following Abs: hamster anti-CD11c mAb N418 (Endogen), rat anti-DEC-205 mAb NLDC-145 (Dianova, Hamburg, Germany), PE- or FITC-labeled rat anti-F4/80 mAb CI-A3-1 (Dianova), FITC-labeled rat anti-DC mAb 3D1 (Biotrend), PE-labeled mouse anti-CD8a mAb 53-6,7, biotinylated rat anti-I-A/I-E+ mAb 2C9, hamster anti-CD40 mAb HM40-3, biotinylated hamster anti-CD84 mAb 3E2, PE-labeled hamster anti-CD80 mAb 16-10A1, and PE-labeled rat anti-CD68 mAb GL1 (PharMingen). Species and isotype-matched control Abs, extravidin-FITC, and FITC-labeled F(ab’)2 fragments of goat anti-rat or hamster IgG as secondary reagents were from PharMingen or Dianova.

Cell cultures

Cells were surface stained according to standard procedures. In samples with anti-rat secondary reagents, cells had been preincubated with rat anti-FcγR mAb 2,4G2 (PharMingen) to block unspecific binding of primary Ab. Propidium iodide was added in the final wash to label dead cells. For flow cytometry using a FACSScan (Becton Dickinson, Heidelberg, Germany), samples were gated on CD11c+ live cells. For each sample, 106 events were acquired and analyzed using the Lysys II software.

Ag presentation assays

The APC function of CD11c+ spleen or brain cells was measured in T cell proliferation assay with the CD4+ T cell line LNC-2 (26) specific for purified protein derivative (PPD) of tuberculin. Tests were performed in A2 microtiter tissue culture plates (Costar, Cambridge, MA) using IMDM with 5% FCS and glutamine. In a total volume of 100 μl, irradiated APC stimulated from 103 to 105 and cocultured with 104 LNC-2 T cells in the presence or absence of 60 μg/ml PPD (Behringwerke, Marburg, Germany). Replicate wells additionally contained 30 μg/ml of anti-I-A+ mAb MDK-6 or control mouse IgG. Test cultures were pulsed with [3H]Thymidine (7.4 kBq/well) during the last 18 h of 3-day incubation. Results from liquid scintillation counting are given as mean cpm of triplicate test cultures ± SD.

The capacity of isolated DC to stimulate naive T cells was measured in a primary allogeneic MLR. CD11c+ splenocytes and BMNC of BALB/c origin were added in graded doses to 105 C57BL/10 T cells in A2 microtiter wells (100 μl). The T cells had been prepared by passing spleen cell suspensions through a nylon wool and an Ab-coated T cell isolation column (R&D Systems, Minneapolis, MN) to eliminate surface Ig and FcR-positive cells. The resulting cell preparation contained >99% CD3+ cells. Five days after starting test cultures, the T cell proliferation was measured via [3H]thymidine uptake as described above.

In vitro challenge by T. gondii of brain cells

Primary cultures prepared from newborn mouse brains (23) were inoculated with T. gondii bradyzoites (strain DX) at a host-cell-to-parasite ratio of 10/1 when the cell layer reached confluence. Control cultures were left untreated. As indicated, 100 U/ml IFN-γ (Genzyme, Cambridge, MA), 10 μg/ml pyrimethamin (Roche, Reinach, Switzerland), 10 μg/ml of neutralizing anti-GM-CSF mAb MP1-31G6, or control rat IgG (Endogen) was added. During 14 days of continued culture, samples of the supernatant (SN) were collected and tested by ELISA for IL-12.

Measurement of cytokine secretion

Brain cells or splenocytes were seeded into microtiter wells at a density of 2.5 × 106 cells/well in medium with FCS. After 24 h of incubation, the samples of the culture SN were collected. Herein, as in samples from brain cell cultures, IL-12 (p40 + p75), IL-10, and GM-CSF were quantified by using specific sandwich ELISA (Genzyme). Recombinant mouse cytokines served as standard. Assays had a minimum sensitivity of 7 (IL-12) or 5 pg/ml (IL-10, GM-CSF). Values represent means ± SD from triplicate SN productions.

RNA extraction and RT-PCR

Total RNA was extracted by the guanidinium thiocyanate method from brain cells (2 × 106/sample) or T. gondii parasites (104). Reverse transcription of poly(A)+ mRNA was performed using oligo(dT) primers and Moloney murine leukemia virus reverse transcriptase (Clontech, Palo Alto, CA). Synthesized cDNA was used as template in PCR. Gene-specific primers and internal probes were designed according to published sequences: for DEC-205, 5′-ACTGGAAAGCGGAGAACCCACTGTTGAAAA-3′ (sense), 5′-TGCTTGCCCATCGGACCATTC-3′ (antisense), and 5′-GTCCATGTAACCCATTTCATTTTACAAAGCAG-3′ (probe); for IL-12...
FIGURE 1. Expression of DC-associated marker genes by BMNC. BMNC were prepared from naive mice and from mice infected for 30 days with T. gondii. Total RNA was extracted from cells pooled from three to five mice and was tested by RT-PCR and Southern blot for transcripts of Kv1.3, IL-12p40, CD86, and DEC-205. Parasite RNA served for control. As an internal control, the G3PDH gene message was analyzed simultaneously. Similar results were obtained in two additional experiments.

Detection of brain DC in mouse TE

Cellular immunity controls T. gondii infection in the CNS (20); however, it is still unclear which APC direct the intracerebral T cell response. In mononuclear cells prepared from mouse brain, an up-regulation of IL-12p40 and CD86/B7.2 and an induction of Kv1.3 was observed during the chronic stage of infection (Fig. 1). Transcripts for DEC-205, a surface marker of several DC populations (3–5, 27) that has also been found on brain endothelial cells (28), were detected in BMNC from both infected and naive animals. Although none of these parameters alone compellingly indicates the presence of DC, their coincidence raised the question whether DC-like cells exist in the brain during TE. This hypothesis was tested by immunohistochemical staining of mouse brain for the DC markers CD11c and 33D1. Neither molecule was expressed in the brains of naive mice (Fig. 2, A and D) or during acute infection at day 7 p.i. (data not shown). However, in the brains of mice infected for 5 wk with T. gondii, CD11c was detected at inflammatory foci in the brain parenchyma and perivascular cuffs (Fig. 2, B and C). Labeling of 33D1 resulted in a congruent pattern, thus confirming the localization of brain DC in TE (Fig. 2, E and F). In combination, these data demonstrate the presence of DC in the T. gondii-infected encephalitic brain.

Kinetic of intracerebral DC number during chronic brain infection

To isolate DC from the brain, BMNC were depleted of T cells and immunomagnetically sorted for CD11c+ cells. Based on such a protocol, the intracerebral DC population was estimated during the course of T. gondii infection. As shown in Fig. 3A, considerable yields of CD11c+ BMNC were obtained from day 15 p.i., with numbers increasing until day 35 p.i. Simultaneously, the mean proportion of CD11c+ cells within BMNC rose from <1% up to 10%. At maximum, the number of isolated CD11c+ cells per brain reached 106 and thus exceeded that of CD11c+ cells prepared by
the same technique from normal spleen. At 5 mo p.i., the number of total BMNC had dropped near background level (p < 0.1 compared with normal brains), whereas the number of CD11c+ brain cells still remained elevated (p < 0.04). As one parameter for the course of TE in BALB/c mice, the brain cyst load was monitored, revealing a peak cyst number in week 4 p.i. (Fig. 3B). The comparable kinetics of the numbers of cysts, BMNC, and DC in the T. gondii-infected CNS suggest a correlation between the inflammatory process and the emergence of DC. To obtain sufficient numbers, in additional experiments, CD11c+ BMNC were isolated between day 30 and day 43 p.i. unless otherwise stated.

**Cell surface phenotype of DC from T. gondii-infected brain**

DC freshly isolated from infected brain showed the typical morphology with thin membrane projections. Unlike CD11c-CD11b+ brain macrophages/microglial cells, most of CD11c+ BMNC remained nonadherent during incubation overnight and kept their dendritic shape. Flow cytometric analysis of CD11c+ BMNC from chronically infected mice confirmed the coexpression of the myeloid DC-restricted surface molecule 33D1 by >90% of isolated cells (Fig. 4). Consistently, a majority of >80% stained positive for the monocyte/macrophage marker F4/80. In contrast, the lymphoid-related marker CD8α was lacking, and DEC-205 was only expressed on ~10% of cells (Fig. 4). By exhibiting this phenotype, the DC population isolated from T. gondii-infected brain apparently belongs to the myeloid DC subset.

To assess the expression of cell surface molecules involved in APC/T cell interaction, these brain DC were stained for MHC class II, ICAM-1/CD54, and the costimulatory ligands CD40, CD80/B7.1, and CD86/B7.2. All of these markers were broadly expressed (Fig. 4), indicating that the population of CD11c+ cells from infected brain consists of terminally differentiated mature DC.

**Allostimulatory activity and presentation of foreign Ag**

As definitive criterion for being classified as DC, the isolated brain cells were tested by MLR for their capacity to stimulate alloreactive naive T cells. Graded numbers of CD11c+ cells from T. gondii-infected brain or normal spleen were incubated with a fixed number of allogeneic T cells. Fig. 5A shows that 100 brain DC per 10^5 T cells could already trigger a substantial response the level of which continuously increased with rising stimulator cell concentrations. In comparison, normal spleen DC proved less efficient at low cell numbers but induced a higher level of T cell proliferation at doses >2500 cells/well. This differential activity of both DC populations was observed in all experiments.

The capacity of brain DC to present soluble Ag was similarly evaluated by incubation of CD11c+ BMNC from T. gondii-infected mice with the PPD-specific CD4+ T cell line LNC.2. Splenic DC from naive mice served as reference APC. As is evident from Fig. 5B, the brain DC were excellent APC inducing maximum T cell proliferation at a ratio of already 1/100 T cells, whereas the reference DC were about 10 times less effective. The T cell response was Ag-specific and dependent on MHC-restricted
and for comparison, CD11c<sup>+</sup> splenocytes from naive mice were tested as APC. T cell responses were measured by [<sup>3</sup>H]thymidine incorporation. Data are shown as mean ± SD from triplicate test cultures. Background proliferation of APC or T cells alone was <120 cpm. Similar results were obtained in four additional experiments. A, Stimulation of alloreactive T cells. In MLR, titrated numbers of brain DC or splenic DC from BALB/c mice (H-2<sup>b</sup>) were cocultured for 5 days with 10<sup>5</sup> purified T cells from C57BL/10 mice (H-2<sup>b</sup>). B, Presentation of soluble Ag to PPD-specific T cell line LNC2. In the presence of 60 μg/ml PPD, titrated numbers of BALB/c-derived brain DC or splenic DC were incubated for 3 days with 10<sup>5</sup> syngeneic LNC2 T cells. Controls without Ag yielded <100 cpm.

FIGURE 5. T cell proliferative responses induced by brain DC against alloantigen or PPD. CD11c<sup>+</sup> BMNC from mice infected for 7 wk with <i>T. gondii</i> and, for comparison, CD11c<sup>+</sup> splenocytes from naive mice were tested as APC. T cell responses were measured by [<sup>3</sup>H]thymidine incorporation. Data are shown as mean ± SD from triplicate test cultures. Background proliferation of APC or T cells alone was <120 cpm. Similar results were obtained in four additional experiments. A, Stimulation of alloreactive T cells. In MLR, titrated numbers of brain DC or splenic DC from BALB/c mice (H-2<sup>b</sup>) were cocultured for 5 days with 10<sup>5</sup> purified T cells from C57BL/10 mice (H-2<sup>b</sup>). B, Presentation of soluble Ag to PPD-specific T cell line LNC2. In the presence of 60 μg/ml PPD, titrated numbers of BALB/c-derived brain DC or splenic DC were incubated for 3 days with 10<sup>5</sup> syngeneic LNC2 T cells. Controls without Ag yielded <100 cpm.

Brain DC are the predominant IL-12 producers in TE

Production of IL-12 by brain DC ex vivo was monitored during the course of <i>T. gondii</i> infection and compared with that by other BMNC. Cytokine release by constant numbers of isolated cells was quantified by ELISA after 24 h of incubation. As summarized in Fig. 6A, CD11c<sup>+</sup> BMNC from naive mice secreted little IL-12 equal to the IL-12 release by splenic DC. Compared with this background level, IL-12 production by brain DC rose upon infection to a >20 times higher peak value on day 43 p.i. At later time points, the amounts of IL-12 decreased; however, about 1 year p.i., IL-12 levels were still significantly (<i>p</i> < 0.001) higher than those produced by cells from uninfected mice. Considering the minute number of brain DC months after infection (Fig. 3A), these findings imply a long-lasting presence of few, but activated, DC in the chronically infected brain. As is evident from Fig. 6A, brain DC are the most important source of IL-12 among BMNC because relatively low amounts of IL-12 were produced by the CD11c<sup>−</sup> cell population (<i>p</i> < 0.001). To compare the cytokine profiles of brain DC and brain-associated macrophages/microglial cells in TE, CD11b<sup>+</sup> cells were enriched from CD11c<sup>−</sup> BMNC and were tested against the CD11c<sup>+</sup> cells for secretion of IL-10 and IL-12. As shown in Fig. 6B, brain DC were confirmed to be the principal IL-12 producers by releasing ~10 times more of this cytokine than brain macrophages/microglia which, conversely, proved superior in production of IL-10 (<i>p</i> < 0.001).

Parasite-triggered generation of functional DC from brain cells via induction of GM-CSF

To elucidate brain-specific cellular interactions involved in parasite-induced development of DC, we used an in vitro system that mimics CNS infection by <i>T. gondii</i>. After inoculation of primary brain cell culture with the DX strain of <i>T. gondii</i>, formation of both parasite cysts and lytic foci starts from day 5 p.i. (29). In parallel, we observed that dendriform cells emerged and proliferated on the cellular interaction because blocking with anti-I-A<sup>d</sup> mAb completely abrogated the proliferation triggered by an optimal number of APC (data not shown).

IL-12 secretion upon toxoplasmic challenge of brain cells correlates with tachyzoite proliferation and is synergistically enhanced by IFN-γ

The impact of parasite replication on brain DC development and function was investigated by treatment of infected primary cultures with sulfadoxin/pyrimethamin. At the concentrations used to block <i>T. gondii</i> growth in vitro, both drugs affect neither the cytokine response of brain cells to LPS nor the development of DC in GM-CSF-treated primary culture. Administration of sulfadoxin/
pyrimethamine to infected brain cells produced, with a 48-h delay, no further increase in the IL-12 level of culture SN (Fig. 8C). From day 9 of the 2-wk test period, the level of accumulative cytokine production was significantly reduced compared with the IL-12 release by infected but untreated cells (p < 0.05). Whether the proliferating stage of T. gondii, the tachyzoite, induces functional maturation of already DC-committed brain cells was tested by exposure of GM-CSF-grown brain cells to tachyzoite lysate. As shown in Fig. 9, parasite challenge of these brain-derived immunocytes requires endogenous GM-CSF to provide an efficient costimulus. Moreover, GM-CSF accelerated and enhanced the IL-12 response in parasitized brain DC and brain macrophages/microglial cells isolated from T. gondii-infected mice at week 6 p.i. Aliquots of immunomagnetically sorted CD11c<sup>+</sup> and CD11c<sup>-</sup> CD11b<sup>+</sup> cells were tested for cytokine secretion during subsequent 24 h of incubation. Comparable results were observed in three experiments.

**Discussion**

It is generally accepted that the parenchyma of the normal CNS lacks DC, whereas MHC class II<sup>+</sup> dendriform cells have been found outside the blood-brain barrier in the cranial meninges (30) and at the choroid plexus (31, 32). Similar cells have been detected also within the brain parenchyma in experimental autoimmune encephalomyelitis and delayed-type hypersensitivity lesions (33, 34). However, their phenotype and whether they functionally qualify as DC are still unresolved. By immunohistochemistry based on DC markers in combination with ex vivo phenotyping and demonstration of DC functions, the present study provides stringent evidence that the brain can indeed harbor DC and that brain cells generate DC upon challenge by T. gondii. In the brains of mice chronically infected with this parasite, a population of myeloid-related DC emerges at perivascular and parenchymatic sites of inflammation and persists in an activated state for months.

As indicated by the yield of DC purified from brain tissue, the DC number in situ reflects the degree of CNS inflammation during all phases of chronic toxoplasmosis. There are four potential scenarios that may account for the observed 50- to 100-fold expansion of brain DC upon onset of brain infection: 1) DC develop from blood monocytes infiltrating into the inflamed CNS tissue; a monocyte-to-DC differentiation pathway recently has been shown (13, 35); 2) meningeal DC are recruited in very large numbers to inflammatory foci beyond the blood-brain barrier; 3) perivascular MHC class II<sup>+</sup> macrophages which function as APC in the brain (36, 37) proliferate and differentiate to DC and migrate to inflammatory sites even deep in the parenchyma; and 4) brain DC develop locally from intracerebral progenitors or resident microglia due to signals from the T. gondii-infected neural environment. Prerequisites for the latter, most intriguing possibility are the existence of a myeloid precursor cell pool in the brain as demonstrated in the mouse (38) and a parasite-triggered pathway by which such brain cells expand and differentiate to DC. The first evidence supporting this hypothesis was obtained in a previous study that showed the generation of immature DC in GM-CSF-treated brain cells (17). In addition, secretion of cytokines critical in DC differentiation, like GM-CSF, IL-1α, and TNF-α, is induced by T. gondii in glial cells (23). Accordingly, GM-CSF expression is induced in the brains of infected mice at day 15 p.i. (39). These findings were complemented with the present data, which demonstrate that the development and maturation (in terms of IL-12 production) of brain DC from parasitized glial cells requires endogenous GM-CSF but can already occur in the absence of T cells. A final determination of the origin of brain DC and their possible migration route(s) and destiny remains an issue of further research, which may be done by utilizing congenic chimera models and prelabeled cells.

The CD11c<sup>+</sup> 33D1<sup>+</sup> F4/80<sup>+</sup> CD8α<sup>-</sup> DEC-205<sup>-/-</sup> phenotype of the cells isolated from brain resembles that of their counterparts from brain cell culture (17). Thus, brain-derived DC correspond to the myeloid subset of mouse DC and are related to macrophages/microglia but differ from those by exhibiting the DC-restricted markers CD11c and 33D1. Due to difficulties in discriminating rat CD11b and CD11c by mAb, brain DC could have been recognized...
as inflammatory macrophages in the rat systems commonly studied (33, 37, 40, 41). The ontogenetic relationship of both cell populations renders a microglial or monocytic origin of brain DC to be most likely.

With the definitive detection of brain DC, a novel type of APC is identified for the CNS. Because the known brain APC, namely astrocytes, microglial cells, and other CNS macrophages, require IFN-γ for their function (for review, see Ref. 16), the presence of DC in the inflamed brain solves the “hen and egg” dilemma of intracerebral T cell activation before IFN-γ is produced. Upon isolation, brain DC are often found clustered with T lymphocytes (M. Sahm and H.G. Fischer, unpublished observations), indicating an APC activity in situ. Similar aggregates have been reported on MHC class II⁺ rat microglia in brain graft-vs-host disease (41). A role for brain DC as amplifiers of T cell responses in TE is suggested by their strong expression of MHC class II, CD40, and B7

FIGURE 7. Emergence of immature DC and expression of DC-associated marker genes in brain cells upon toxoplasmic challenge. Primary cultures from mouse brain were inoculated with the DX strain of T. gondii and continued for another 2 wk. A, Morphology of DC developing in T. gondii-infected brain cell culture. From day 5 p.i. when formation of parasite cysts started, loosely adherent veiled cells emerged on the glia layer. Phase contrast, bar = 20 µm. B, Expression of DC marker genes. At days 7 and 14 p.i., total RNA was extracted from infected brain cell cultures and tested by RT-PCR and Southern blot for transcripts of Kv1.3, IL-12p40, CD86, DEC-205, and parasite stage markers SAG1 and SAG4. RNA from uninfected brain cells and from T. gondii parasites were used as controls. As internal control, the G3PDH gene message was analyzed simultaneously. Similar results were obtained in two additional experiments.

FIGURE 8. GM-CSF dependency of T. gondii-induced IL-12 release by brain cells: effects of external IFN-γ or anti-parasite treatment. Primary brain cell cultures were inoculated with T. gondii, and during subsequent 2 wk of incubation, the cytokine content in the SN was measured in 2- or 3-day intervals. Values are means ± SD from triplicate test cultures. Similar results were obtained in a second set of experiments. Cytokine production by uninfected controls over time was <10 pg/ml IL-10 and <80 pg/ml IL-12. A, Kinetics of IL-10 and IL-12 release. B, Effect of neutralization of GM-CSF on the parasite-triggered production of IL-12. Parallel to inoculation of primary brain cell cultures with T. gondii, anti-GM-CSF mAb MP1-31G6 (10 µg/ml) or control rat IgG1 was added. Ab concentrations were maintained during test period. Data shown are from the same experiment as in A. C, Effects of IFN-γ and of inhibiting parasite growth on IL-12 production. Infected cultures were treated either with IFN-γ (100 U/ml) on day 1 p.i. or with sulfadoxin (20 µg/ml) plus pyrimethamin (1 µg/ml) on day 5 p.i., or they remained untreated. In uninfected IFN-γ or sulfadoxin/pyrimethamin (S/P)-treated controls, IL-12 levels were <120 pg/ml.
A matured cells in the brain DC as confirmed by their IL-12 secretion by spleen DC. This is probably due to a higher proportion of fully optimal APC doses, these brain DC proved superior to normal cells. In stimulating primary or secondary T cell responses at sub-(40) but comparable to human microglial cells (42), the DC from T. gondii-infected mouse brain triggered the proliferation of T cells. In stimulating primary or secondary T cell responses at sub-optimal APC doses, these brain DC proved superior to normal spleen DC. This is probably due to a higher proportion of fully matured cells in the brain DC as confirmed by their IL-12 secretion (see Fig. 6A). Both populations were purified via the β2-integrin CD11c, which is expressed as a pan-DC marker also on immature DC (3, 43).

A notable feature of brain DC in TE was their high-level production of IL-12 ex vivo coupled with a low production of IL-10. Co-isolated microglia/brain macrophages exhibited a converse profile in secretion of these cytokines. Evidence from mice suffering from experimental autoimmune encephalomyelitis or injected with LPS indicates that microglia-like cells in the inflamed CNS tissue express IL-12p40 (44, 45). Because activated astrocytes can antagonize microglial IL-12 release (46) and IL-12 production by brain DC was measured on purified cells, our approach perhaps detects a functional capability masked in vivo. However, IL-12 was also secreted by cells developing in parasitized brain cell culture adjacent to astrocytes (Fig. 8), which would suggest that IL-12 production of brain DC is an intrinsic function that distinguishes them from CNS macrophages. The long-lasting presence of activated DC in the infected brain might contribute to the chronicity of the intracerebral cellular response in TE.

The parasite itself is involved, via induction of GM-CSF, in the generation of brain DC and, via soluble tachyzoite components, in their terminal maturation. Whether both effects are triggered by the same or by different parasite molecules remains unclear. To date, two cytokine-inducing activities of T. gondii have been distinguished biochemically and functionally (24, 47). Although IFN-γ is not crucial for the IL-12 response of brain DC in vitro (Fig. 8) or of splenic DC in vivo (48), it synergistically up-regulates DC activation by T. gondii. Thereby, the parasite acts in an LPS-like manner according to the conventional model of DC stimulation (49, 50). Finally, the dependency on parasite proliferation of IL-12 secretion in parasitized brain cells implies a feedback loop by which the host response can adapt to the actual parasite burden during persistent infection.

In conclusion, the present data prove the existence of functional DC in the T. gondii-infected mouse brain and reveal a mechanism by which CNS tissue can generate DC in response to parasite challenge. This unique potential gives a novel meaning to the traditional concept of the brain as an “immunoprivileged” organ.

Acknowledgments

We thank John Delamarer and Edgar Schmitt for providing Abs and T cells, Karin Buchholz for technical assistance, Nicole Nischik and Stefan Stachelhaus for preparing Toxoplasma lysate, and James Alexander for critical reading of the manuscript.

References

BRAIN DENDRITIC CELLS IN TOXOPLASMIC ENCEPHALITIS


