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Microglial cells qualify as the stimulators of unprimed CD4$^+$ and CD8$^+$ T lymphocytes in the central nervous system

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SUMMARY

The potential of central nervous system (CNS)-derived cells for initiating T cell responses is not known. Using the capacity of unprimed T cells to respond to allogeneic determinants on antigen-presenting cells (APC), we assessed the ability of microglial cells to act as stimulators of primary T cell responses *in vitro*. For this purpose, microglial cells were activated with lipopolysaccharide (LPS), interferon-gamma (IFN-γ), or by phagocytosis of progenitor oligodendrocytes and subsequently tested for their ability to induce a proliferative response of naive, resting T cells. Activated microglial cells induced a significant proliferation of virgin, alloreactive CD4$^+$ and CD8$^+$ T lymphocytes, with a more substantial response of highly purified CD4$^+$ than of CD8-expressing T cells. Phagocytosis activation was the most efficient stimulus to induce this APC competence on microglial cells. By contrast, IFN-γ-pretreated, MHC-expressing astrocytes were unable to induce similar responses of alloreactive CD4$^+$ or CD8$^+$ T cells under the same experimental conditions. Collectively, our data suggest the role of activated microglia as the fully immunocompetent accessory cell population of the CNS.

Keywords microglia phagocytosis priming resting T lymphocytes

INTRODUCTION

The ability of central nervous system (CNS)-resident cells to prime immune responses *in vitro* and *in vivo* is not known. The controversial issue of a brain-inherent capacity to initiate organ-specific immune responses can be regarded as pivotal for our understanding of various CNS immunopathologies, including autoimmune diseases such as multiple sclerosis (MS) [1,2]. In general, organ-specific inflammatory reactions can be initiated by antigen-presenting cells (APC) either localized in the target organ itself or by immune cell priming in the periphery. Using the primary allogeneic mixed-lymphocyte reaction (AMLR) as the appropriate *in vitro* test system, it has been well established that 'professional', bone marrow-derived APC, i.e. B cells, macrophages and in particular dendritic cells, can readily initiate primary responses of CD4$^+$ T lymphocytes or CD8$^+$ effector T cells [3,4]. By contrast, non-haematopoietically derived cells are probably only able to perpetuate, but not to initiate primary T cell responses.

The prerequisite for any presentation of antigens to CD4$^+$ and CD8$^+$ T lymphocytes is the expression of MHC class I and class II determinants on an APC. A characteristic of brain-derived cells, however, concerns the general paucity of MHC expression in the absence of specific stimuli, thereby contributing to the special immune status of the CNS [5,6]. *In vitro*, two glial cell populations can be readily manipulated to express MHC class I and II antigens: astrocytes and microglial cells [7,8]. *In vivo*, the corresponding inducing stimulus for MHC expression in the brain could be either the activity of (peripherally primed) interferon-gamma (IFN-γ)-secreting T cells, or a (viral) infection of the CNS.

A recent report [9] has demonstrated the virtual inability of astrocytes to induce primary T cell responses *in vitro*. This prompted us to focus on the priming capacity of the second major CNS-resident APC population, microglial cells. Like macrophages, microglia are known to secrete a number of cytokines and to present antigens to specific T cells after stimulation *in vitro* and *in vivo* [10,11]. In MS lesions, myelin debris can be frequently detected in microglia and/or brain macrophages, suggesting a very active process of phagocytosis of myelin sheaths [12]. Furthermore, some authors provided evidence that demyelination is a macrophage-mediated process, by demonstrating myelin disruption following *in vitro* interactions between myelin and macrophages [13]. Recently, we have shown that microglial cells can efficiently present myelin basic protein (MBP) to antigen-specific T cells after phagocytosis of myelin-synthesizing oligodendrocytes [14]. Here, we demonstrate that phagocytosis activation and to a lesser extent IFN-γ treatment is able to induce a priming capacity for virgin, alloreactive T lymphocyte responses on microglial cells. Under the same experimental conditions, purified responder

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T cells did not exhibit any reactivity to IFN-γ-activated allogeneic astrocytes. Our results thus give strong evidence that microglial cells, but not astrocytes, can initiate immune responses of unprimed $CD4^+$ and $CD8^+$ T lymphocytes in the CNS.

MATERIALS AND METHODS

Animals

Brown-Norway (BN; MHC haplotype $RT1^n$) and Lewis (LEW; MHC haplotype $RT1^1$) rats were obtained from our own animal breeding facilities.

Cell cultures

Microglial cells were prepared from newborn LEW or BN rat brains as described previously [15]. Briefly, brains were removed from 2–3-day-old rats. After removal of meninges, cerebral tissues were mechanically dissociated in Hanks' medium and then put into culture in DMEM/F12 (GIBCO-BRL, Paisley, UK) containing 10% fetal calf serum (FCS). The flasks were shaken after 10 days of culture, and the supernatant, highly enriched in microglial cells, was seeded in four-well plates (Nunclon, Roskilde, Denmark) at 400 000 cells/well per ml for FACS analysis or in 96-well plates (Costar, Cambridge, MA) at 5000, 10 000, 50 000 cells/well for mixed lymphocyte reaction (MLR) assays. Experiments were started at the same day of isolation.

Astrocyte cultures were prepared from newborn LEW or BN rat brains as described [16]. Cells were cultured in the same medium containing 10% FCS as microglial cells. Astrocytes were used within 14 days of culture and were more than 95% positive for glial fibrillary acidic protein (GFAP). MHC class II expression was induced by adding IFN-γ (50 U/ml) to the culture, as described for microglial cells. Astrocytes were used as stimulator cells for MLR assays in the same way as microglial cells.

Progenitor glial cells were obtained from neonatal LEW rats and were characterized as oligodendrocyte-type 2 astrocyte cells, as described [17]. The cells were cultured in DMEM/F12 medium with 0·5% FCS on coverslips at the same concentrations as microglial cells.

Primary MLR

Total primary MLR were assessed by culturing mesenteric lymph node (LN) T cells on microglial cells. Resting LN T cells were prepared by passing cells through a nylon wool column and a subsequent incubation on anti-rat whole serum-coated culture plates (Jackson Immunoresearch Labs, West Grove, PA). After this treatment, a cell population 99% positive for T lymphocyte markers (CD3) was recovered. Fractionated $CD4^+$ or $CD8^+$ T cells were obtained by a subsequent complement-mediated cytotoxic treatment with an anti-rat CD8 (OX-8, ascites; Chemicon, Temecula, CA) or anti-rat CD4 antibody (W3/25, ascites; Chemicon), respectively. The residual populations were $CD4^+$ T cells or $CD8^+$ T cells with a purity of 98%.

LN T cells (2×10^5, 10^6 cells/well) were added onto 30 Gy irradiated syngeneic or allogeneic stimulator cells consisting of LN cells (5×10^5 cells/well) as primary positive control MLR or microglial cells (5×10^3, 10^4, 5×10^4 cells/well). The stimulator cells were activated or left untreated for 18 h before the MLR assays. Microglial cells were activated with rat recombinant IFN-γ (200 U/ml) (kindly provided by Dr H. Schellekens, TNO Primate Centre, Rijzwijk, The Netherlands) or lipopolysaccharide (LPS; 1 μg/ml) or by phagocytozing predamaged progenitor glial cells (added to the microglia culture at a ratio of 1 : 1) as previously described [14]. Human recombinant IL-2 (hrIL-2) (Sanofi, Labège, France) at 20 U/ml or OX6 antibody (anti-MHC class II antigen) were added to CD8- or CD4-mediated MLR, respectively. The medium used for the MLR was RPMI 1640 supplemented with β-mercaptoethanol, penicillin, streptomycin, glutamine, sodium pyruvate and 10% FCS. Cultures lasted 5 days for total and $CD4^+$ T cell responses and 4 days for $CD8^+$ T cell responses. Proliferation was assayed by incorporation of 0·2 μCi methyl, ^{3}H-thymidine during the last 18 h of culture. The stimulation index (SI) was calculated in allogeneic MLR as:

$$\frac{\text{mean ct/min of lymphocytes cultured on allogeneic microglia}}{\text{mean ct/min of lymphocytes cultured on syngeneic microglia}}$$

The SI for the syngeneic response was calculated by comparing lymphocyte response on activated microglia with that on non-activated microglia.

Cytofluorographic analysis

Cells were phenotypically characterized by immunostaining. Microglial cells showed positivity with an anti-Mac 1 (OX 42; Seralab, JRH Biosciences, Crawley Down, UK) antibody, but not with anti-GFAP (Boehringer, Mannheim, Germany) antibody. Isolated resting LN T cells were positive for both CD4 and CD8 T cell markers. Isolated microglial cells were able to express elevated levels of MHC class II molecules after IFN-γ stimulation, and synthesize IL-1α after LPS and phagocytosis stimulation. MHC class II expression was monitored using MoAb OX6 (Seralab, JRH Biosciences); IL-1α was detected with the help of a rabbit polyclonal anti-IL-1α antibody (kindly provided by Dr Wollman, INSERM U283, Paris, France) on fixed (4% paraformaldehyde) and permeabilized (0·1% Triton X100) microglial cells. The microglial cells were activated with LPS at 1 μg/ml or by phagocytozing predamaged progenitor oligodendrocytes. In this last activation procedure, microglial cells were detached from coverslips or plastic flasks after coculture experiments by incubating them with cold PBS without Ca^{++} and Mg^{++} as described previously [14]. Five thousand events were analysed on a FACScan (Becton Dickinson).

Statistical analysis

Student's *t*-test was used to compare mean ct/min ± s.e.m. scores of responder lymphocytes on syngeneic or allogeneic stimulator cells. Differences in proliferation were considered significant when $P < 5\%$. Standard deviations were always < 10% mean values. Data are the means of triplicate determinations, and are representative of at least three separate experiments.

RESULTS

Microglial cells induce allogeneic LEW T cell responses

Microglial cells were isolated from mixed primary brain cultures after a culture period of 10 days following explanation [15]. The purity of the resulting cell population was subsequently assessed by FACS analysis; more than 95% of cells

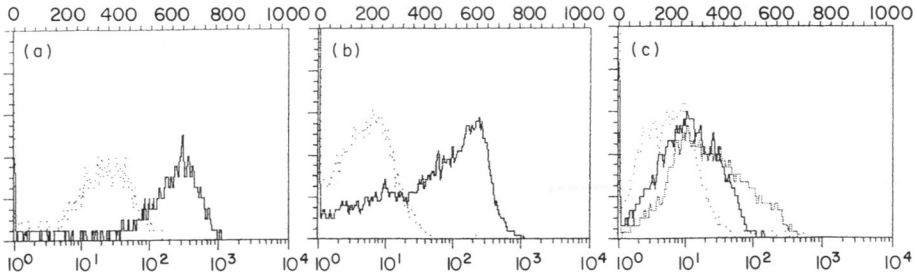

Fig. 1. Microglia phenotype studied by FACS analysis. (a) Surface expression on Mac-1 on isolated microglial cells: dotted line represents isotype control antibody, and solid line represents Mac-1 staining. (b) Surface expression of MHC class II molecules on microglia activated with IFN-γ for 2 days (solid line) or not activated (dotted line). (c) Microglia activated with lipopolysaccharide (LPS) (dashed line) or with phagocytosis of predamaged cells (solid line) or not activated (dotted line) for 18 h were examined for IL-1α production by FACS analysis.

reacted with an anti-Mac-1 antibody and expressed MHC class II antigens after a 2-day stimulation period with IFN-γ, as presented in Fig. 1a,b. Furthermore, microglial cells produced intracytoplasmic IL-1*a* after overnight stimulation with LPS or phagocytosis, as presented in Fig. 1c.

In a first set of experiments, we tried to investigate the potential of microglia to induce primary responses of non-fractionated, purified T lymphocytes. To this end, microglial cells were left unstimulated or were treated by different means (LPS or IFN-γ treatment or activation by phagocytosis) for 18 h, before being used as stimulators in MLR assays.

In the positive control MLR experiment, 2×10^5 to 1×10^6 LEW responder T cells per well proliferated vigorously in the presence of irradiated BN stimulator LN cells (SI = 10 and 51, respectively). When using BN-derived cells as responders, however, as many as 1×10^6 T cells were necessary to obtain a comparable alloreactive proliferation to irradiated LEW LN cells (SI = 50) (Fig. 2). Similar low reactivities of BN-derived T lymphocytes in response to a variety of stimuli (including responses to concanavalin A (Con A), LPS, or MLR assay systems) have already been documented by other investigators [18].

The induction of LEW LN T cell proliferation on BN and LEW stimulator microglial cells is illustrated in Fig. 3. When microglial cells were used in the non-activated state, no lymphoproliferation was detectable. LPS activation of BN and LEW microglial cells induced a transient T cell response with both T cell concentrations employed, but only with 5000 microglial stimulator cells per well employed. When IFN-γ-activated microglial cells were tested, an allogeneic T cell response was demonstrable with 1×10^6 LEW LN responder T cells per well (but not with 2×10^5 cells/well), as shown in Fig. 3b. Interestingly, however, phagocytosis-activated BN-derived microglia induced a reproducible and strong allogeneic T cell response (Fig. 3a) at relatively low responder/stimulator ratios as detected in three separate experiments. With 2×10^5 LEW LN T cells, the SIs ranged from 5·2 to 6·3 and 14·9 with microglial cells used at concentrations of 5000, 10 000 and 50 000 cells/well, respectively. With 1×10^6 LEW LN T cells, this allogeneic response was only an SI of 4·8.

Allogeneic BN LN T cell proliferation with microglial stimulator cells was not observed, although BN LN T cell responses with LEW LN stimulator cells were readily detectable and highly significant.

In parallel, we tested the ability of purified astrocyte cultures to stimulate the proliferation of allogeneic T lymphocytes. Astrocytes of BN and LEW rat origin were stimulated with IFN-γ or left untreated, and were subsequently cocultured with unfractionated BN and LEW T cells. As already noted by others [9], MHC class II-expressing astrocytes were unable to initiate any detectable allogeneic response in our test system (Fig. 4).

Microglial cells induce a syngeneic LEW T cell response

A detectable stimulation of LEW T cell proliferation was also found when syngeneic phagocytosis-activated microglia were used as stimulators. This syngeneic response was significant, although the SIs were much lower than for allogeneic responses, and became evident only when high numbers of stimulator cells were used. Maximal SIs were 7·6 with 50 000 microglial cells/well (Fig. 3a). Such an autologous proliferation was not detected with BN-derived T lymphocytes.

Microglial cells stimulate more vigorous $CD4^+$ than $CD8^+$ allogeneic LEW T cell responses

We next determined whether the T cell priming potential of activated microglia was possibly limited to a certain T cell

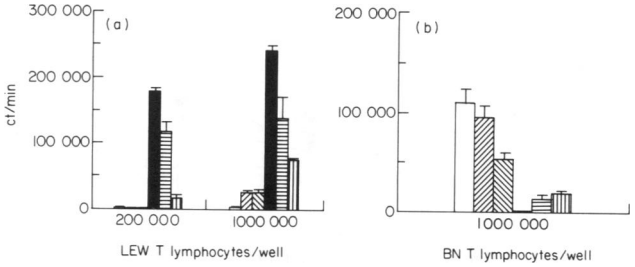

Fig. 2. Positive control mixed lymphocyte reaction (MLR) assays. LEW (a) or BN (b) LN T cells were cultivated with irradiated 5×10^5 lymph node (LN) cells as stimulators. The effectors were non-fractionated LN T cells, $CD4^+$ or $CD8^+$ LN T cells. (a) Lymphoproliferation of 2×10^5 and 10^6 LEW T cells cultured on irradiated BN LN cells. (b) Lymphoproliferation of as much as 10^6 BN T cells on irradiated LEW LN cells. □, T cells on LEW LN; ▨, $CD4^+$ T cells on LEW LN; ▧, $CD8^+$ T cells on LEW LN; ■, T cells on BN LN, ▤, $CD4^+$ T cells on BN LN; □, $CD8^+$ T cells on BN LN.

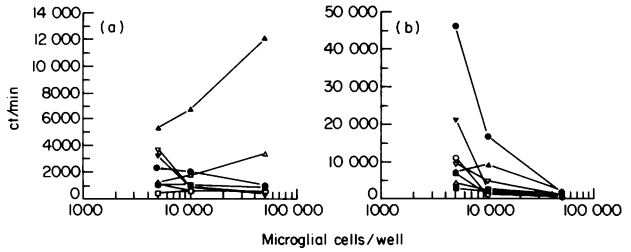

Fig. 3. LEW lymph node (LN) T cell proliferation in response to allogeneic microglial cells. Before the mixed lymphocyte reaction (MLR) assays, microglial cells were stimulated or not for 18 h with lipopolysaccharide (LPS). IFN-γ or by phagocytosis of predamaged progenitor oligodendrocytes and then irradiated. (a) Lymphoproliferation of 2×10^5 LEW LN T cells cultivated with microglia. (b) Lymphoproliferation of five times more T lymphocytes (10^6 LEW LN T cells) cultivated with microglia. Closed symbols indicate lymphoproliferation on BN microglia, and open symbols indicate lymphoproliferation on LEW microglia. ■, No activation; ▲, phagocytosis; ▼, LPS; ●, IFN-γ; □, no activation; △, phagocytosis; ▽, LPS; ○, IFN-γ.

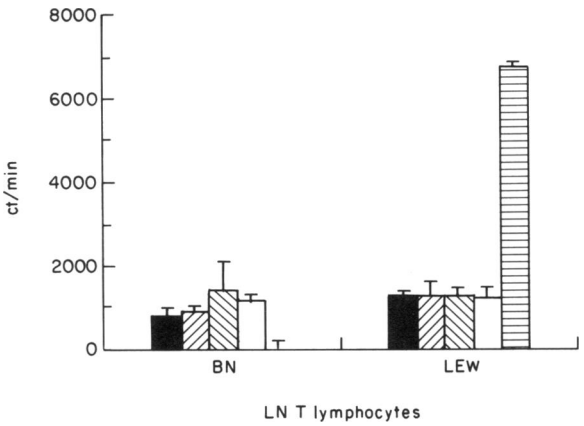

Fig. 4. Inability of astrocytes to stimulate an allogeneic response of lymph node (LN) unfractionated LN T lymphocytes. LEW or BN T lymphocytes (2×10^5) were cocultured on 10^4 astrocytes freshly isolated from primary culture of LEW or BN rat brains. Before the mixed lymphocyte reaction (MLR) assays, astrocytes were stimulated or not for 18 h with IFN-γ. The proliferation of LEW LN T lymphocytes on BN activated microglia are presented as positive allogeneic T lymphocyte response. ■, BN; ▨, BN + IFN; ▧, LEW; □, LEW + IFN; ▤, BN microglia.

subset. To this end, highly purified $CD4^+$ or $CD8^+$ T lymphocytes were separately used as responders. The purity of the two subpopulations was high (more than 98%), with no detectable numbers of $CD4^+$ T cells in the $CD8^+$ cell population, or *vice versa* (data not shown).

$CD4^+$ LEW T cells displayed an allogeneic response when cocultured with microglial cells activated either with IFN-γ or by phagocytosis. Results obtained with 2×10^5 LEW $CD4^+$ LN T cells are presented in Fig. 5a (when employing 1×10^6 $CD4^+$ LN T cells, proliferative responses were less significant; data not shown). The highest SI values for phagocytosis- or IFN-γ-activated microglia were 5·6 and 4·1, respectively. This $CD4^+$ T cell-mediated proliferation could be specifically blocked by the addition of antibodies directed against rat MHC class II antigen (data not shown). When using purified $CD4^+$ T lymphocytes, no detectable syngeneic T cell reactivity was observed, even with very high numbers of responder cells.

Furthermore, we could likewise detect a stimulation of LEW $CD8^+$ T cell proliferation with BN microglial stimulator cells, as shown in Fig. 5b,c. Both phagocytosis- and IFN-γ-activated BN microglia induced LEW lymphoproliferation in the absence of exogenous IL-2 (maximal SI = 7·0) (Fig. 5b). The addition of recombinant IL-2 did not further augment the response (Fig. 5c). This contrasts with the MLR control assay with LN cells as stimulators, where the cytokine could markedly enhance the proliferative response (SI without IL-2 = 2·8; with IL-2 = 7·0). Allogeneic $CD8^+$ T cell responses were also detected with higher numbers of responder cells (1×10^6 T lymphocytes/well), both in the presence and absence of exogenous hrIL-2 (data not shown).

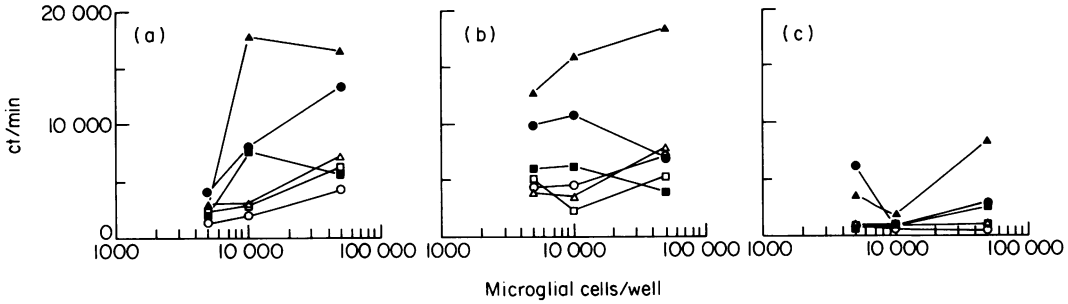

Fig. 5. LEW lymph node (LN) $CD4^+$ or $CD8^+$ T cell proliferation in response to allogeneic microglial cells. Microglial cells were stimulated or not for 18 h before mixed lymphocyte reaction (MLR) assays. Different stimulations were used: phagocytosis of predamaged progenitor oligodendrocytes and IFN-γ. In these experiments, lipopolysaccharide (LPS) activation is not shown because it could not induce alloantigen-presenting function on microglia. After irradiation, microglial cells were used as stimulators of $CD4^+$ or $CD8^+$ lymphocytes. $CD4^+$ T cells were cultivated for 5 days, $CD8^+$ T cells were cultivated for 4 days including radiolabelled thymidine pulse. (a) Lymphoproliferation of 2×10^5 $CD4^+$ LEW T cells cultivated on microglial cells. Closed symbols and open symbols indicate lymphoproliferation on BN and LEW microglial cells, respectively. (b,c) Lymphoproliferation of 2×10^5 $CD8^+$ LEW T cells cultivated on microglial cells. Cultures have been performed without adding hrIL-2 (b) or with hrIL-2 (c). Again, closed symbols and open symbols indicate lymphoproliferation on BN and LEW microglial cells, respectively. ■, No activation; ▲, phagocytosis; ●, IFN-γ; □, no activation; △, phagocytosis; ○, IFN-γ.

In conclusion, microglia were able to prime both $CD4^+$ and $CD8^+$ allogeneic responder T cells, after activation by phagocytosis or treatment with IFN-γ. No autologous response of separated $CD4^+$ and $CD8^+$ T lymphocytes to syngeneic microglial stimulators was found.

Microglial cells do not induce detectable allogeneic $CD4^+$ and $CD8^+$ T cell proliferation with BN responder cells

As already noted for non-fractionated BN LN T cells, $CD4^+$ BN T cell proliferation was similarly not inducible when BN responder cells were confronted with allogeneic microglial cells, although the same cell population was clearly inducible by allogeneic stimulator LN cells (SI = 6·5) (Fig. 2b). Purified $CD8^+$ BN T cells likewise did not respond when cocultivated with allogeneic microglial cells. When employing allogeneic LN cells, a slight proliferation was detectable (SI = 2·7, Fig. 3b). We conclude that the inability of non-fractionated BN T cells to respond to allogeneic microglial cells was not due to any kind of inhibitory effect of either the $CD4^+$ or the $CD8^+$ T cell subset, but can most probably be attributed to the general low reactivity of BN-derived T lymphocytes.

DISCUSSION

In our study, we tried to elucidate the inherent capacity of the brain to initiate immune reactions by assessing the potential of microglial cells to prime responses of naive T lymphocytes. Our data clearly demonstrate that activated microglia are able to initiate a primary T cell response in an allogeneic MLR assay system. These results suggest a prominent role of activated microglia as the initiators of local immune responses within the brain, and provide a new basis for the understanding of certain CNS immune diseases, such as different forms of virus-mediated encephalomyelitis or MS, as locally initiated inflammatory reactions.

A consensus is now emerging that microglial cells, but not astrocytes, are the major APC population within the CNS. Compelling evidence from a variety of histological examinations suggests that microglia, but not astrocytes, express readily detectable amounts of class II antigens *in situ*. These data have been obtained both in clinical studies on MS and other CNS immune disorders, and in experimental animal models for CNS diseases, notably in experimental allergic encephalomyelitis (EAE) (reviewed in [1]). A rationale for this lack of MHC class II expression on astrocytes, but not microglial *in situ*, has recently been provided by the demonstration of an inhibitory effect of neuron-derived factors on class II inducibility on astrocytes, but not on microglia [19]. In line with these results, Sedgwick and colleagues [9] have recently demonstrated the virtual inability of class II-expressing astrocytes to initiate a primary T cell response. The data from their detailed study show that astrocytes could initiate a weak, but strictly IL-2-dependent $CD8^+$ T cell allogeneic response, but completely failed to prime $CD4^+$ T cell immune reactions. As therefore a prominent role of astrocytes as initiators of inflammation can be excluded, the initiator of CNS immune activity has to be either resident brain macrophages—i.e. microglial cells—or peripheral macrophages, concomitantly invading the brain with autoreactive or virus-specific T lymphocytes.

Our work indicates a stronger stimulation of $CD4^+$ than of $CD8^+$ T cell responses by allogeneic microglial cells. This result might have significance in the context of CNS autoimmune diseases, such as EAE or post-infectious encephalomyelitis syndrome, where the major effector cell population has been shown to belong to the $CD4^+$ subset [20,21]. The responses of naive $CD8^+$ lymphocytes were less pronounced, although independent of the addition of exogenous IL-2. Such an IL-2 independence of rat $CD8^+$ T cell reactivity to allogeneic stimuli has already been described by others [22]. A brain-inherent priming capacity for cytotoxic $CD8^+$ lymphocyte responses is evidently of major interest in the context of infections with viruses, which might either *per se* exhibit a strict tropism for neural cells, or which might have been selected for 'CNS-tropic' variants in the course of a systemic infection.

Phagocytosis-activated microglial cells induced a low syngeneic T lymphoproliferation. This syngeneic reactivity was demonstrable with LEW T cells, but not with BN T cells. This non-antigen-specific response underlines the efficiency of the stimulation of microglial cells by phagocytosis. It should be emphasized that the allogeneic and syngeneic responses were not due to particular properties of oligodendrocyte-astrocyte progenitors, as comparable results were obtained with non-CNS-derived cells used for phagocytosis treatment (data not shown). A comparable weak autologous proliferation of T cells was not detected after LPS or IFN-γ activation of microglial stimulators, although the same agents were efficient inducers of MHC class I and II antigen expression. This suggests that the mechanism by which phagocytosis activation induces syngeneic or allogeneic antigen presentation is probably by a modulatory effect on the synthesis of certain costimulatory molecules, rather than by a differential effect on MHC expression itself. In murine macrophages, IL-1α has been demonstrated to be one of the major costimulators inducing alloantigen presentation [23]. However, variations in IL-1 synthesis cannot fully account for the high efficiency of phagocytosis-activated microglia, as LPS-activated microglia—displaying higher levels of IL-1α production than phagocytosis-activated microglia—were considerably less efficient. Certainly, the exact elucidation of the mechanism responsible for the induction of a T cell priming capacity on microglial cells awaits further studies.

In conclusion, our data demonstrate that microglial cells exhibit the property to initiate independently both $CD4^+$ and $CD8^+$ T cell responses after appropriate stimulation *in vitro*. The precise molecular mechanisms responsible for this induction of a priming capacity in microglia remain to be elucidated. As a prime candidate for the *in vivo* activation of glial cells, viral infections of the CNS have to be envisaged. In such a scenario, a massive infection of microglial cells, as is suspected to occur, for example, during HTLV-1 invasion of the brain [24], might be the crucial step in the initiation of CNS-specific T cell (auto-) immunity. However, the property of the CNS to initiate a cellular immune defence against invading microorganisms might be paralleled by its increasing susceptibility to initiate a tissue-specific immune dysfunction, i.e. its vulnerability to autoimmune disease. The widely used rodent models of adjuvant EAE by-pass the question of the physiological side of T cell priming in CNS autoimmunity. An obvious, but often disregarded, discrepancy between these experimental models and naturally occurring CNS autoimmunity is the fact that in the latter, a massive T cell priming by myelin depots in peripheral lymphoid organs does not occur. Local T cell priming by activated microglial cells might be envisaged as a

possible missing link between these models and the corresponding forms of (virus-triggered) CNS autoimmunity in humans, such as MS or post-infectious encephalomyelitis syndrome.

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