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Critical Requirement of CD11b (Mac-1) on T Cells and Accessory Cells for Development of Experimental Autoimmune Encephalomyelitis

Daniel C. Bullard,* Xianzhen Hu,† Trenton R. Schoeb,* Robert C. Axtell,‡ Chander Raman,‡ and Scott R. Barnum§

Mac-1 (CD18/CD11b) is a member of the β2-integrin family of adhesion molecules and is implicated in the development of many inflammatory diseases. The role of Mac-1 in the development of CNS demyelinating diseases, including multiple sclerosis, is not understood, and Ab inhibition studies in experimental allergic encephalomyelitis (EAE), the animal model for multiple sclerosis, have produced conflicting findings. To clarify these results and to determine Mac-1-mediated mechanisms in EAE, we performed EAE using Mac-1-deficient mice. Mac-1 homozygous-deficient, but not Mac-1 heterozygous-deficient mice, had significantly delayed onset and attenuated EAE. Leukocyte infiltration was similar in both groups of mice in early disease but significantly reduced in spinal cords of receptor-deficient mice in late disease. Adoptive transfer of Ag-restimulated T cells from wild-type to Mac-1-deficient mice produced significantly attenuated EAE, whereas transfer of Mac-1-deficient Ag-restimulated T cells to control mice failed to induce EAE. T cells from myelin oligodendrocyte glycoprotein (MOG)35–55 peptide-primed Mac-1-deficient mice produced an altered array of cytokines compared with wild-type mice. Lymph nodes of myelin oligodendrocyte glycoprotein (MOG)35–55 peptide-primed Mac-1-deficient mice displayed an altered cytokine phenotype with elevated levels of TGF-β and IL-10, but reduced levels of IL-2, IFN-γ, TNF-α, IL-12, and IL-4 compared with control mice. Mac-1-deficient T cells from primed mice proliferated comparably to that of control T cells on MOG35–55 restimulation in vitro. However, the draining lymph nodes of MAC-1-deficient mice on day 10 after MOG35–55 immunization contained lower frequency of blast T cells than in control mice, suggesting poor priming. Our results indicate that Mac-1 expression is critical on both phagocytic cells and T cells for the development of demyelinating disease. The Journal of Immunology, 2005, 175: 6327–6333.

In multiple sclerosis (MS) and the corresponding animal model, experimental autoimmune encephalomyelitis (EAE), the development of disease requires the trafficking of effector cells, including macrophages and Ag-specific T cells, into the CNS. These cells initiate lesion formation and contribute to the inflammation and demyelination characteristic of the disease (1). Members of several families of adhesion molecules are crucial for the trafficking of effector cells into sites of inflammation, including CNS diseases (2–7). In MS, the α4β1-integrins, α1β1, and αβ2β2 in particular, appear important, because treatment of MS patients with Ab against α4-integrins reduces development of new lesions and results in fewer clinical relapses (8). Disease symptoms were, however, not completely ameliorated by this therapeutic approach, suggesting that other adhesion molecules, including the β2-integrins, may also contribute to disease development.

Members of the β2-integrin family of adhesion molecules have been implicated in the development of MS and EAE (reviewed in Refs. 5, 7). Previous in vitro studies using Abs to the β2-integrin, Mac-1 (itgam/itgb2, CD18/CD11b), suggested that the most likely function of this adhesion molecule in demyelinating disease is microglial or macrophage-mediated myelin phagocytosis (9–12). Mac-1-mediated phagocytosis of myelin is accompanied by production of the inflammatory mediators TNF-α and NO (13). However, studies on this adhesion molecule in the EAE model have produced conflicting results. Administration of anti-Mac-1 Ab in several cases delayed and attenuated adoptive-transferred EAE; however, the protective effect varied widely (10, 14, 15). Furthermore, these studies did not define whether anti-Mac-1 Ab treatment inhibited EAE development through reduced myelin phagocytosis, modulation of T cell and/or dendritic cell function, or a combination of these factors (16–19). Thus, the relative contribution of Mac-1 to the cellular infiltration, inflammation, and demyelination characteristic of EAE remains to be established.

To avoid the inherent limitations of Ab treatment, we examined the role of Mac-1 in the development and progression of EAE using Mac-1-deficient mice. We found that deletion of Mac-1 resulted in a profound protection in both active and adoptive-transferred EAE, suggesting an important role for Mac-1 on multiple cell types in EAE. Transfer of Mac-1-deficient Ag-specific T cells into wild-type mice did not lead to disease, thus implicating Mac-1 in T cell sensitization processes. We also observed that T cells from Mac-1-deficient mice proliferated well but produced an altered array of cytokines compared with wild-type mice. Lymph nodes of myelin oligodendrocyte glycoprotein (MOG)-immunized Mac-1-deficient mice had elevated numbers of CD4+ IFN-γ-producing T cells; however, substantially fewer of...
these Mac-1-deficient T cells were activated. Our results indicate that Mac-1 expression is critical not only on phagocytic cells but also on T cells for the development of demyelinating disease.

Materials and Methods

Mice

Mice containing a null mutation for CD11b were generated by gene targeting in 129/Sv-embryonic stem cells, as described previously (20). The CD11b mutation was then backcrossed onto the C57BL/6 strain (The Jackson Laboratory). Inbred C57BL/6 mice were used as controls for all experiments. All studies were performed with approval from the University of Alabama at Birmingham Institutional Animal Care and Use Committee.

Induction of active and transferred EAE

For active EAE control, Mac-1-deficient and mice heterozygous for the CD11b mutation were immunized with MOG peptide35–55, as described (21). MOG peptide was synthesized by standard 9-fluorenylmethoxycarbonyl chemistry and was >95% pure as determined by reversed-phase HPLC (Biosynthesis International). Onset and progression of EAE symptoms was monitored daily using a standard clinical scale ranging from 0 to 6 as follows: 0, asymptomatic; 1, loss of tail tone; 2, flaccid tail; 3, incomplete paralysis of one or two hind limbs; 4, complete hind limb paralysis; 5, moribund; 6, dead. Only mice with a score of at least 2 (flaccid tail) for more than 2 consecutive days were judged to have onset of EAE. For each animal, a cumulative disease index (CDI) was calculated from the daily clinical scores observed between days 7 and 30. For transferred EAE, spleens of control or Mac-1−/− donors were removed, 2 wk following induction of active EAE, and prepared as described previously (21). Passive EAE was induced by injecting 5 × 106 purified T cells derived from wild-type mice into Mac-1-deficient mice, or by injecting purified T cells derived from Mac-1-deficient mice into wild-type mice. In both cases, purified T cells derived from wild-type mice were injected in wild-type mice as a control to monitor disease development.

Histopathology

At day 30 postimmunization mice with actively induced EAE were sacrificed by CO₂ inhalation, and spinal columns were removed, fixed in 10% buffered formalin, and paraffin embedded. Sections (5-μm thick) from the cervical, thoracic, and lumbar spinal cord were cut and either stained with H&E for overall lesion evaluation and characterization of inflammatory responses or with Luxol fast blue (LFB) for evaluation of demyelination. The extent of inflammation and demyelination was scored based on lesion size (0–4), and lesions were evaluated for lymphocyte accumulation, neotrophy and neovascularization, axonal degeneration, and gliosis (0–4). Tissues were evaluated without identification as to experimental group. A lesion score for individual changes by the score for extent, and an overall score for each segment was calculated by multiplying the sum of the daily clinical scores observed between days 7 and 30. For transferred EAE, spleens of control or Mac-1−/− donors were removed, 2 wk following induction of active EAE, and prepared as described previously (21)

Isolation and flow cytometric analysis of leukocytes from spinal cords

Spinal cords were removed from control and Mac-1-deficient mice with active EAE (day 16) and treated with collagenase (2 mg/ml in PBS; Roche) and DNase I (Sigma-Aldrich; 5 μg/ml collagenase buffer) for 1 h at 37°C. Cords were ground through a cell strainer, washed in PBS, resuspended in 40% Percoll, and layered on 70% Percoll. After centrifugation at 2000 rpm (room temperature, 25 min), cells at the interface were removed, washed in PBS, and stained as described below.

T cell proliferation and cytokine and chemokine production

Ag-specific T cell proliferation assays were performed as described previously (21). Single-cell suspensions from spleens obtained 14 days after EAE induction were cultured in 96-well plates at 5 × 10³ cells/well with increasing concentrations of MOG35–55 peptide or 10 μg/ml anti-CD3, clodiomycin (BC1; National Cell Culture Center) in triplicate. After 48 h, cultures were pulsed with [³H]thymidine for an additional 18 h, and incorporation of thymidine was measured. The in vitro cytokine and chemokine analyses were performed essentially as described for the proliferation assay. Duplicate cultures were either left untreated or stimulated with MOG peptide alone (5 μg/ml). Culture supernatants were collected at 48 h for use in cytokine and chemokine ELISA kits. ELISA kits for murine cytokines (TNF-α, IFN-γ, IL-1β, IL-2, IL-12, IL-10) and chemokines (MIP-1α, RANTES, TGF-β) were purchased from R&D Systems. Each assay was performed according to the manufacturer’s instructions. Cytokine and chemokine production by cultures of wild-type and Mac-1-deficient cells is reported as the percentage compared with control. The data are pooled from two separate experiments.

Flow cytometry and apoptosis assay

Cells obtained from spinal cords or draining lymph nodes were incubated with anti-CD16/32 (24G2, FcR block) to prevent nonspecific staining. Spinal cord leukocytes were stained with anti-CD-FITC (GK1.5), anti-CD8-PE (53-6.7), and anti-CD45 (30F11) (all from eBioscience). Single-cell suspensions of draining lymph node cells, obtained at day 10 after immunization, were stained with anti-CD4-FITC (GK1.5), eBioscience, anti-IFN-γ (BD Pharmingen), or anti-IL-10 (BD Pharmingen). Frequency of apoptotic T cells was determined by staining with annexin V and 7-aminoactinomycin D (AAD; BD Pharmingen) after gating out B220+ cells (anti-B220-biotin, RA3-6B2). Streptavidin-allophycocyanin (BD Pharmingen) was used to detect biotinylated Abs. To assay for early apoptotic events in T cells, we measured loss of mitochondrial membrane potential by incubating with dihexyloxacarbocyanine (DiOC6; Molecular Probes) at a final concentration of 20 nM for 15 min at 37°C (22). Spleen and lymph node mononuclear cells were also stained with anti-CD4, anti-CD25 (PC61.5; eBioscience), and anti-CD69 (H1.2F3; eBioscience) at various time points after induction of EAE to enumerate changes in activation status and frequency of lymphocyte subsets. Stained cells and forward scatter were analyzed using a FACS-Calibur (BD Biosciences).

Statistics

Statistical significance between control and Mac-1-deficient mice for active and transferred EAE experiments was calculated using the Mann-Whitney U test, and for proliferation assays Student’s t test was used. Results of evaluations for inflammation and demyelination were analyzed using ANOVA for main effects and Tukey’s test for pairwise mean comparisons.

Results

Deletion of Mac-1 significantly attenuates active EAE

To determine the role of Mac-1 in EAE, we performed active EAE using MOG35–55 Peptide. We induced EAE in wild-type and Mac-1-deficient mice and followed the course of disease for 30 days.

We found that Mac-1-deficient mice developed very mild EAE, with a substantial delay in the onset of disease (27 days vs 17 days; p < 0.05) compared with wild-type mice (Fig. 1A; Table I). The CDI for Mac-1-deficient mice was markedly lower than that of control mice (24.3 vs 68.6; Table I), and the overall course of disease was significantly reduced (p = 0.01, Mann-Whitney U test). The attenuated EAE observed using Mac-1-deficient mice was not seen in mice heterozygous for the mutation (Fig. 1B). The onset of disease in Mac-1-heterozygotes was not delayed (15 vs 14 days) nor was disease severity significantly different from that of wild-type mice (Table I; p > 0.05).

![FIGURE 1.](http://www.jimmunol.org/) The clinical course of active EAE is markedly attenuated in Mac-1-deficient mice, but not mice heterozygous for CD11b. A. Active EAE was induced with MOG peptide35–55, and symptoms were scored for 30 days as described in Materials and Methods. Results shown are the daily mean clinical score for wild-type (n = 13) and Mac-1-deficient mice (n = 8) from two experiments. B. Active EAE was induced in wild-type (n = 5) and mice heterozygous for CD11b (n = 5) mice. The results shown are the daily mean clinical score from one experiment.
Cellular infiltration and demyelination in Mac-1-deficient mice with EAE

We performed histopathological analysis on spinal cords of wild-type and Mac-1-deficient mice with active EAE to determine the extent and nature of the cellular infiltrate and the amount of demyelination between the two groups of mice. Sections from Mac-1-deficient mice overall had significantly less cellular infiltration, gliosis, axonal degeneration, and demyelination throughout the spinal cord, particularly in the cervical and thoracic segments, compared with wild-type mice (p < 0.05, Tukey’s test). Representative sections from a Mac-1-deficient mouse showed limited infiltration and inflammation (Fig. 2A) and demyelination (Fig. 2C). As expected, wild-type mice had extensive cellular infiltration in the meninges and white matter (Fig. 2B). The cellular infiltrate in lesions in Mac-1-deficient mice was largely devoid of CD3+ T cells compared with wild-type mice (data not shown).

These results prompted us to determine whether leukocyte infiltration was significantly lower during early disease development. For these studies, we induced active EAE in control and Mac-1-deficient mice and isolated spinal cords 16 days later. Leukocytes were isolated from the spinal cords as described in Materials and Methods and analyzed by flow cytometry for T cell subsets and monocytes. We observed almost identical levels of infiltration of CD4− cells in the spinal cords of control and Mac-1-deficient mice (48.7 vs 49.7, respectively) (Fig. 3A, upper right quadrant). CD8− T cells also readily infiltrated the spinal cords of Mac-1-deficient mice compared with control mice during EAE (14.3 vs 8.4, respectively; data not shown). To determine the extent of myeloid/monocytic cell infiltration between the two groups of mice, we first gated on the CD11b+, nonlymphoid cells and then analyzed by forward and side scatter. Myeloid cell infiltration was similar between control and Mac-1-deficient mice. The results shown are from cells pooled within each group of mice.

FIGURE 2. Leukocyte infiltration and demyelination are reduced in Mac-1-deficient mice in EAE. Spinal cords from wild-type and Mac-1-deficient mice were obtained at 30 days postimmunization, fixed in 10% buffered formalin, and paraffin embedded. Sections from the cervical, thoracic, and lumbar regions (5 μm) were stained with H&E or LFB and scored as described in Materials and Methods. A, Representative section from a Mac-1-deficient mouse stained with H&E. Arrow indicates single region of cellular infiltration and inflammation. B, Section from the same specimen as in A stained with LFB. Arrow indicates single region of demyelination. C, Representative section from a wild-type mouse stained with H&E. Arrows indicate widespread cellular infiltration and inflammation. D, Section from the same specimen as in C stained with LFB. Arrows indicate regions of extensive demyelination throughout the white matter. Original magnification, ×4.
data demonstrate that the absence of Mac-1 does not prevent trafficking of leukocytes to the CNS.

Transfer of wild-type MOG-sensitized T cells to Mac-1-deficient mice attenuates transferred EAE; in contrast, Mac-1-deficient MOG-sensitized T cells do not induce EAE

We also induced EAE by adoptively transferring MOG-sensitized T cells from wild-type mice to Mac-1-deficient mice. In these experiments, we observed delayed onset of disease and significantly less overall disease severity in Mac-1-deficient recipient mice compared with control mice (Fig. 4A; Table II; p = 0.0007, Mann-Whitney U test). To determine whether Mac-1 deficiency on Ag-specific T cells would also result in attenuated disease, we performed transferred EAE using MOG-sensitized T cells from Mac-1-deficient mice. Wild-type mice receiving these cells did not develop any clinical signs of EAE, whereas wild-type mice receiving MOG-sensitized, Mac-1-sufficient cells developed EAE (Fig. 4B; Table II).

Mac-1-deficient T cells proliferate comparably to wild-type T cells but have an altered cytokine profile

The inability of Mac-1-deficient T cells to induce transferred EAE in wild-type mice and the markedly attenuated active EAE in Mac-1-deficient mice could be due to a poor proliferative capacity of Mac-1-deficient T cells. To test this possibility, we performed in vitro proliferation assays as described previously (21). Stimulation of MOG-sensitized T cells from wild-type and Mac-1-deficient mice with various concentrations of MOG revealed no significant differences in proliferation (p > 0.05, Student’s t test), although there was modest decline in proliferation at higher concentrations of MOG peptide (Fig. 5A). This may indicate the development of anergy or the inability of these cells to persist. Mac-1-deficient T cells also proliferated well in response to anti-CD3 Ab compared with wild-type T cells (Fig. 5B). To determine whether the cytokine repertoire produced by Mac-1-deficient T cells could account, at least in part, for the disease phenotype, we examined cytokine production of MOG-sensitized wild-type and Mac-1-deficient T cells. Fig. 6 shows that Mac-1-deficient T cells produced an altered cytokine profile compared with wild-type mice, with elevated levels of TGF-β, IL-10, and MCP-1 and reduced levels of IFN-γ, IL-2, TNF-α, IL-12, and MIP-1α and RANTES compared with wild-type T cells. IL-4 production by Mac-1-deficient T cells was ~50% lower than that of wild-type T cells.

Table II. Transferred EAE symptoms in wild-type mice and Mac-1-deficient mice

<table>
<thead>
<tr>
<th>Group</th>
<th>CD10</th>
<th>Disease Onset (Days)</th>
<th>Disease Incidence(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT &gt; WT (n = 5)</td>
<td>70.2</td>
<td>6</td>
<td>100</td>
</tr>
<tr>
<td>WT &gt; Mac-1−/− (n = 5)</td>
<td>30</td>
<td>12</td>
<td>100</td>
</tr>
<tr>
<td>WT &gt; WT (n = 6)</td>
<td>35</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>Mac-1−/− &gt; WT (n = 6)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

a CD10 is the mean of the sum of daily clinical scores observed between days 7 and 30. WT, Wild-type.

Mac-1-deficient T cells are functional, but not activated during EAE

The absence of Mac-1 on T cells may be associated with decreased survival as a result of enhanced activation-induced cell death (AICD), as observed in mice deficient for CDS (23). If this was the case, we might expect Mac-1-deficient mice to contain lower numbers of effector cells. However, on day 16 after MOG immunization, draining lymph nodes of Mac-1-deficient mice had significantly elevated numbers of CD4+/IFN-γ-expressing effector T cells and a small increase in the frequency of CD4+/IL-10-expressing T cells, consistent with the phenotype of T regulatory cells (Fig. 7A). We did not, however, observe an increase in CD4+/CD25+ T cells in Mac-1-deficient mice (data not shown). In addition, the frequency of apoptotic annexin V+, AAD CD4+ T cells was similar in MOG-induced wild-type and Mac-1-deficient mice (data not shown). Taken together, these data indicate that the primary mechanism of resistance of Mac-1-deficient mice to EAE is not due to T cell unresponsiveness, enhanced AICD, or frequency of regulatory T cells.

To assess the activation state of T cells in the draining lymph nodes, we compared the forward scatter of lymph node-derived cells from unimmunized mice and MOG-immunized wild-type and Mac-1-deficient mice 10 days after MOG immunization (Fig. 7B). We observed fewer cells undergoing blast in Mac-1-deficient mice compared with control mice (25.6 ± 9.7 vs 38.8 ± 3.8, respectively). As expected, unimmunized mice (wild-type or Mac-1-deficient) had few blast T cells (Fig. 7B). We also examined the

FIGURE 4. The clinical course of adoptive-transferred EAE is markedly attenuated in Mac-1-deficient mice. A, Transferred EAE was induced in wild-type (n = 5) and Mac-1-deficient (n = 5) mice by injecting encephalitogenic T cells (5 × 10^6) derived from wild-type mice with active EAE. Results shown are the daily mean clinical score from three separate experiments. B, Transferred EAE was induced in wild-type (n = 6) mice by injecting encephalitogenic T cells (5 × 10^6) derived from Mac-1-deficient mice with active EAE. As a control, transferred EAE was induced in wild-type mice (n = 5) by injecting encephalitogenic T cells (5 × 10^6) derived from wild-type mice with active EAE. Results shown are the daily mean clinical score from three separate experiments.

FIGURE 5. Mac-1-deficient T cells proliferate comparably to wild-type T cells. Encephalitogenic T cells enriched by nylon wool adherence from the spleens of wild-type (n = 4) or Mac-1-deficient mice (n = 4) undergoing active EAE, or T cells from healthy controls (naive cells), were cocultured with irradiated splenic APCs plus MOG peptide (0.06–2 μg/ml). The cells were pulsed with [3H]thymidine and harvested at 18 h for determination of radioisotope incorporation. The results shown are from two experiments and are expressed as the mean ± SEM of fold induction of wild-type or Mac-1-deficient T cell proliferation relative to background proliferation.
activation state of T cells derived from wild-type and Mac-1-deficient lymph nodes by staining with DiOC₆, a fluorometric dye that assesses changes in mitochondrial membrane potential (22, 24). Activated T cells have elevated DiOC₆ staining due to increased metabolic activation during the expansion phase of the immune response, and cells poised to undergo apoptosis exhibit loss of mitochondrial potential and lower DiOC₆ staining (25). On day 10 after MOG immunization, we observed a much greater frequency of DiOC₆₉ T cells in draining lymph nodes of wild-type mice than in Mac-1-deficient mice (231 ± 6.4 vs 83.5 ± 23.5; Fig. 7C). Although the frequency of T cells undergoing blast was higher in wild-type mice, the frequency of CD4⁺ cells that up-regulated CD69 was similar in both groups of mice. The frequency of DiOC₆₈ (apoptotic) T cells in lymph node-derived CD4⁺ T cells was comparable between both kinds of mice (Fig. 7C).

Discussion

The results we report in this study demonstrate an important role for Mac-1 in the development and progression of demyelinating disease. Mac-1 is a well-established contributor to the trafficking of monocyte/macrophages into sites of inflammation and to phagocytosis of opsonized pathogens and apoptotic cells (2–4, 6, 7). Our findings indicate, however, that Mac-1 is not required for trafficking of T cells and monocyte/macrophages to the spinal cord early in EAE progression (Fig. 4). In contrast, we observed few infiltrating leukocytes in the spinal cords of Mac-1-deficient mice during the chronic phase of active EAE (Fig. 2). The differences in cellular infiltration in acute vs chronic EAE remain unclear, but may be due, in part, to poor priming or an increased predisposition to tolerance development (26).

The markedly limited demyelination we observed in the spinal cords of Mac-1-deficient mice reiterates the importance for Mac-1 in microglial and macrophage-mediated myelin phagocytosis. Although previous studies have implicated Mac-1 in phagocytosis of myelin, the use of Mac-1-deficient mice overcomes the technical limitations of in vitro culture systems and Ab treatment (9–12). Myelin phagocytosis may also be indirectly affected by the altered T cell cytokine profile we observed in Mac-1-deficient mice (Fig. 6). Decreased T cell production of IFN-γ, TNF-α, and IL-12, combined with elevated TGF-β and IL-10, may lead to poor activation of resident microglia and infiltrating perivascular macrophages and dendritic cells. As a result, these phagocytes may secrete lower levels of inflammatory mediators including complement, cytokines, and reactive-oxygen species further limiting disease development. In addition, it is possible that the absence of Mac-1 on phagocytes may prevent full activation of these cells with regard to production of cytokines and/or reactive-oxygen species. Failure to appropriately activate the phagocytic cell population may be particularly important given the recent studies demonstrating a critical role for these cells in disease development (27, 28).

Although the contribution of phagocytic cells to demyelination may be blunted in the absence of Mac-1, it is clear that T cells derived from Mac-1-deficient mice with EAE are unable to induce disease on transfer to wild-type mice. Is the failure to develop EAE in these transfer experiments due wholly, or in part, to the lack of Mac-1 expression on T cells? We think that cytokine production by Mac-1-deficient T cells maybe a critical factor in the failure to develop disease in this set of transfer experiments. Mac-1-deficient T cells produced an altered repertoire of cytokines consistent with this suggestion. The level of Th1 cytokines such as IFN-γ, IL-2,
TNF-α, and IL-12 were lower than in control mice, although IFN-γ levels were only marginally decreased. The markedly lower IL-4 levels raises the possibility of a Th2 deficiency in this experimental setting. This result was unexpected given that this cytokine is considered critically important in modulating the severity of EAE (29, 30). Interestingly, T cells from Mac-1-deficient mice produced elevated levels of TGF-β and IL-10, compared with control mice. IL-10 is well established as a regulator of the autoreactive T cell repertoire in EAE and other autoimmune disease models (31–33). Although we noted a small increase in CD4+/IL-10-producing T cells (Fig. 6) and elevated levels of IL-10 and TGF-β, we did not observe the presence of CD25+ T cells suggesting little role for regulatory T cells in the attenuated disease phenotype seen in Mac-1-deficient mice. This observation might indicate differences in functional activity of T regulatory cells between MAC-1-deficient and wild-type mice. At this time, it is unknown whether the pattern of cytokine production by Mac-1-deficient T cells in EAE is due to altered congenital T cell development (18).

Chemokines are central to the trafficking of encephalitogenic T cells and macrophages into the CNS during EAE (34, 35). The expression of chemokines by Mac-1-deficient T cells was also atypical for the attenuated EAE phenotype seen in Mac-1-deficient mice. For example, the expression of MCP-1 was higher in Mac-1-deficient mice despite markedly reduced cellular infiltration compared with control mice. MCP-1 plays an important role in recruitment of T cells and macrophages to the CNS in EAE, and mice deficient in MCP-1 are resistant to EAE (36). In contrast, the expression of MIP-1α, a chemokine not required for the development of EAE (37), was sharply lower compared with controls. In addition, the expression of RANTES, a chemokine important in T cell function as well as trafficking (38, 39), was also lower compared with control mice. Taken together, our data suggest that subtle shifts in cytokine and chemokine production occur in Mac-1-deficient mice that have a profound effect on disease severity.

It can be argued that Mac-1 deficiency in the mouse could lead to an intrinsic inability of T cells to differentiate into effector cells. This seems unlikely because T cells from Mac-1-deficient mice do respond, although less efficiently than wild-type T cells, to several staphylococcal superantigens (18) and to MOG peptide in in vitro restimulation assays (Fig. 5A). Furthermore, when stimulated by PMA and ionomycin (bypassing the TCR) (18) or anti-CD3 (Fig. 4B), Mac-1-deficient T cells proliferate normally. Thus, Mac-1 expression does not appear to be critical for T cell activation or costimulatory events in EAE. In addition, our results indicate that attenuated disease in Mac-1-deficient mice was not due to increased cell death through apoptosis or AICD. Surprisingly, our results indicate that T cells from Mac-1-deficient mice display a phenotype characteristic of unstimulated cells with respect to blast formation though the frequency of CD4+CD69+ T cells were similar in draining lymph nodes of Mac-1-deficient and wild-type mice. This may reflect lower threshold for Mac-1-deficient T cells to develop anergy. This prediction is supported by the lower levels of proliferation observed in Mac-1-deficient T cells at higher concentrations of MOG restimulation (Fig. 5). Until recently, few studies have focused on the role of Mac-1 in T cell-mediated processes. The combined observations that Mac-1-deficient T cells respond to Ag in vitro, and that Mac-1-deficient mice develop at least mild active EAE suggests that Mac-1-deficient T cells receive Ag-specific stimulation from APCs. It is also possible that Mac-1-deficient T cells are not capable of receiving all the in vivo signals required to fully develop into effector cells, perhaps due to altered cytokine receptor expression.

Inhibition of integrin function as a therapeutic approach in demyelinating disease has been investigated for over a decade (5). Ab-mediated inhibition of α4-integrins and LFA-1 can attenuate EAE (14, 40–43). In MS, anti-α4-integrin Ab immunotherapy has successfully reduced both new lesion development and clinical relapses (8), despite the recent setbacks in clinical trials. In EAE, anti-Mac-1 Abs delayed onset and severity of disease when administered immediately after transfer of encephalitogenic T cells or at the first signs of clinical disease (10, 14). The protective effect was attributed, in large part, to inhibition of phagocytosis, not modulation of the immune response. Our results support these earlier Ab-based therapeutic approaches. However, they also suggest that Mac-1 expression on T cells is critical to the development of the autoimmune response and that it’s role in phagocytosis of myelin debris is a downstream event. Taken together, these results suggest that Mac-1 may be an important integrin target for MS immunotherapy.

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Disclosures
The authors have no financial conflict of interest.

References


