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*Immunopathology and Infectious Disease*

# p150/95 (CD11c/CD18) Expression Is Required for the Development of Experimental Autoimmune Encephalomyelitis

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**p150/95 (CD11c/CD18, CR4) is a member of the $\beta_2$-integrin family of adhesion molecules and is considered an important phagocytic receptor. The role of p150/95 in the development of central nervous system demyelinating diseases, including multiple sclerosis, remains unexplored. To determine p150/95-mediated mechanisms in experimental autoimmune encephalomyelitis (EAE), we performed EAE using CD11c-deficient (CD11c$^{-/-}$) mice. EAE in CD11c$^{-/-}$ mice was significantly attenuated and characterized by markedly reduced spinal cord T-cell infiltration and interferon-$\gamma$ production by these cells. Adoptive transfer of antigen-restimulated T cells from wild-type to CD11c$^{-/-}$ mice produced significantly attenuated EAE, whereas transfer of CD11c$^{-/-}$ antigen-restimulated T cells to control mice induced a very mild, monophasic EAE. T cells from MOG$_{35–55}$ peptide-primed CD11c$^{-/-}$ mice displayed an unusual cytokine phenotype with elevated levels of interleukin (IL)-2, IL-4, and IL-12 but reduced levels of interferon-$\gamma$, tumor necrosis factor-$\alpha$, IL-10, IL-17, and transforming growth factor-$\beta$ compared with control mice. Overall, CD11c$^{-/-}$ T cells from primed mice proliferated comparably to that of control T cells on MOG$_{35-55}$ restimulation. Our results indicate that expression of p150/95 is critical on both T cells as well as other leukocytes for the development of demyelinating disease and may represent a novel therapeutic target for multiple sclerosis.** ***(Am J Pathol 2007, 170:2001–2008; DOI: 10.2353/ajpath.2007.061016)***

Multiple sclerosis (MS) is considered a T-cell-mediated autoimmune disease, with self-reactivity directed against numerous myelin-derived antigens, including myelin basic protein and myelin oligodendrocyte glycoprotein (MOG). A variety of other cell types, including macrophages, dendritic cells, glial cells, and $\gamma\delta$ T cells, as well as various blood-borne and membrane-anchored effector molecules (eg, cytokines, antibody, and complement), also contribute to MS pathogenesis and inflammation.[1–5] A classic feature of MS is the trafficking of antigen-specific T cells and macrophages into the central nervous system (CNS), where they initiate inflammation and destruction of oligodendrocytes and eventually neurons. The movement of these inflammatory cells into the CNS is regulated by a number of molecules including leukocyte/endothelial cell adhesion proteins and chemoattractant/activating molecules. CNS inflammatory model systems, including the MS model, experimental autoimmune encephalomyelitis (EAE), strongly suggest that the adhesion molecules VLA-4 and its ligand vascular cell adhesion molecule-1, as well as the $\beta_2$-integrin molecules LFA-1 and Mac-1 (CD11a and CD11b, respectively), play an integral part in this process.[6–8] The role of other $\beta_2$-integrin molecules, adhesion molecules, in particular CD11c/CD18 (CR4, p150/95), remains unexplored.

p150/95 is expressed by myeloid cells including macrophages, neutrophils, dendritic cells, and lymphocytes, and expression increases on treatment with a variety of chemoattractants, cytokines, phorbol esters, or on antigen-mediated activation.[9–17] In the CNS, microglia and infiltrating macrophages constitutively express this adhesion molecule, and p150/95 expression increases on ac-

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tivation of these phagocytic cells.[18–22] p150/95 binds to cells via a limited number of known ligands including iC3b, fibrinogen, intercellular adhesion molecule-1, and lipopolysaccharide (LPS)[10,15,23–26] and is important in the phagocytic clearance of bacteria and apoptotic cells.[14,27–31] Studies have also suggested a role for p150/95 in monocyte/endothelium interactions or conjugate formation between cytotoxic T cells and target cells of various types,[14,18,32–35] but the *in vivo* relevance of these findings remains unclear. These studies combined with our previous work demonstrating an important role for the other iC3b receptor, Mac-1, in the development and progression of EAE[6] prompted us to examine the role of p150/95 in this animal model of autoimmune demyelinating disease.

We report here the results of EAE studies using CD11c$^{-/-}$ mice. The absence of CD11c resulted in significantly attenuated disease severity with reduced cellular infiltration and demyelination compared with wild-type mice. In addition, adoptive transfer experiments demonstrated that p150/95 expression is required on T cells for the development of EAE; however, loss of p150/95 did not affect T-cell proliferation in *in vitro* restimulation assays. In fact at higher antigen concentration, T-cell proliferation was enhanced using CD11c-deficient T cells. Despite normal proliferation, CD11c$^{-/-}$ T cells isolated from either spleens or spinal cords of mice with EAE produced a profile of cytokines favoring an anti-inflammatory response. Our results suggest that p150/95 is important at multiple levels for the development of EAE, particularly at the level of effector T-cell functions, and may also contribute to both phagocytosis of myelin debris and leukocyte trafficking during the pathogenesis of EAE.

## Materials and Methods

### Mice

Mice containing a null mutation for CD11c were generated by gene targeting using 129/Sv-embryonic stem cells as previously described.[36] The CD11c mutation was then backcrossed onto the C57BL/6 strain for at least seven generations (The Jackson Laboratory, Bar Harbor, ME). 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. CD11c$^{-/-}$ mice have normal splenic and lymph node structure, architecture, and cellularity compared with control mice. In our hands, MOG-induced EAE in 129/Sv mice is essentially identical to that seen in C57BL/6 mice.

### Induction of Active and Transferred EAE

For active EAE, control and CD11c$^{-/-}$ mice were immunized with MOG peptide$_{35\text{-}55}$ as described,[37] except that the mice received only one MOG peptide injection. MOG peptide was synthesized by standard 9-fluorenyl-methoxycarbonyl chemistry and was >95% pure as determined by reversed phase-high performance liquid chromatography (Biosynthesis, Lewisville, TX). Onset and progression of EAE symptoms were 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; and 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 was calculated from the sum of the daily clinical scores observed between day 7 and day 30. For transferred EAE, spleens of control or CD11c$^{-/-}$ donors were removed 2 to 3 weeks after induction of active EAE and prepared as previously described.[37] Passive EAE was induced by injecting $\sim 5 \times 10^6$ purified T cells derived from wild-type mice into CD11c$^{-/-}$ mice or by injecting the same number of purified T cells derived from CD11c$^{-/-}$ mice into wild-type mice. In both cases, purified T cells derived from wild-type mice were injected into wild-type mice as a control to monitor disease development.

### Histopathology

Mice with actively induced EAE were sacrificed at 32 days after induction by $CO_2$ inhalation, and spinal columns were removed, fixed in 10% buffered formalin, and paraffin-embedded. Sections (5 $\mu$m thick) from the cervical, thoracic, and lumbar spinal cord were cut and either stained with hematoxylin and eosin for overall lesion evaluation and characterization of inflammatory responses or with Luxol fast blue for evaluation of demyelination. The extent of inflammation and demyelination was scored based on lesion size (0 to 4), and lesions were evaluated for lymphocyte accumulation, neutrophil infiltration, demyelination, axonal degeneration, and gliosis (0 to 4). Tissues were evaluated without identification as to experimental group. Severity scores were calculated as the mean overall segments of the products of the intensity scores multiplied by the extent scores for each lesion characteristic (inflammation, axonal degeneration, gliosis, and demyelination). The means of the individual lesion characteristic severity scores were summed to give the overall severity score.

### Isolation and Flow Cytometric Analysis of Leukocytes from Spinal Cords

Spinal cords were removed from control and CD11c$^{-/-}$ mice with active EAE (days 12 to 15) after perfusion with phosphate-buffered saline (PBS), 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 minutes), cells at the interface were removed and washed in PBS and stained as described. Cells obtained from spinal cords were incubated with anti-CD16/32 (24G2, FcR block) to prevent nonspecific staining. Spinal cord leukocytes were stained with anti-CD4-fluorescein isothiocyanate [GK1$_{(CR1)}$.5], anti-CD-8-phycoerythrin (53-6.7), anti-CD45-fluorescein isothiocyanate (30F11), anti-tumor necrosis factor-$\alpha$-phycoerythrin (MP6-XT22), and anti-interferon (IFN)-$\gamma$-fluorescein isothio-

cyanate (XMG1.2), all from eBiosciences, San Diego, CA. Stained cells and forward scatter were analyzed using a FACSCalibur and the data analyzed using CellQuest software (BD Biosciences, San Jose, CA).

### *T-Cell Proliferation and Cytokine and Chemokine Production*

Antigen-specific T-cell proliferation assays were performed as previously described.[37] Single cell suspensions from spleens obtained 14 days after EAE induction were cultured in 96-well plates at $5 \times 10^5$ cells/well with increasing concentrations of $MOG_{35-55}$ peptide in triplicate. After 48 hours, cultures were pulsed with [$^3$H]thymidine for an additional 18 hours, and incorporation of thymidine was measured. The *in vitro* cytokine assays were performed essentially as described for the proliferation assay. Duplicate cultures were either left untreated or stimulated with MOG peptide alone (5 $\mu$g/ml). Culture supernatants were collected at 48 hours for use in cytokine enzyme-linked immunosorbent assays. Enzyme-linked immunosorbent assay kits for murine cytokines [IFN-$\gamma$, tumor necrosis factor-$\alpha$, interleukin (IL)-2, IL-4, IL-10, IL-12, IL-17, and transforming growth factor-$\beta$] were purchased from R&D Systems (Minneapolis, MN). Each assay was performed according to the manufacturer's instructions. Cytokine production by cultures of wild-type and CD11c$^{-/-}$ cells is reported as the mean ± SEM of four mice per group. The data are pooled from two separate experiments.

### *Statistics*

Statistical significance between control and CD11c$^{-/-}$ mice for active and transferred EAE experiments was calculated using the Wilcoxon signed-rank test; for proliferation assays the Student's *t*-test was used. Results of evaluations for inflammation and demyelination were analyzed using analysis of variance for main effects and Tukey's test for pairwise mean comparisons.

## *Results*

### *Deletion of p150/95 Significantly Attenuates Active EAE*

To determine the role of p150/95 in EAE, we immunized wild-type and CD11c$^{-/-}$ mice using $MOG_{35-55}$ peptide and followed the course of disease for 30 days. CD11c$^{-/-}$ mice developed EAE slightly later than wild-type mice (19 days versus 16 days); however, the difference was not statistically significant (Figure 1, Table 1). Both groups of mice had a similar disease course during the acute phase of disease, but the chronic phase of disease was attenuated in CD11c$^{-/-}$ mice compared with wild type. The cumulative disease index for CD11c$^{-/-}$ mice was significantly lower than that of controls (32.1 versus 58, respectively; $P < 0.0001$, Wilcoxon signed-rank test) (Table 1).

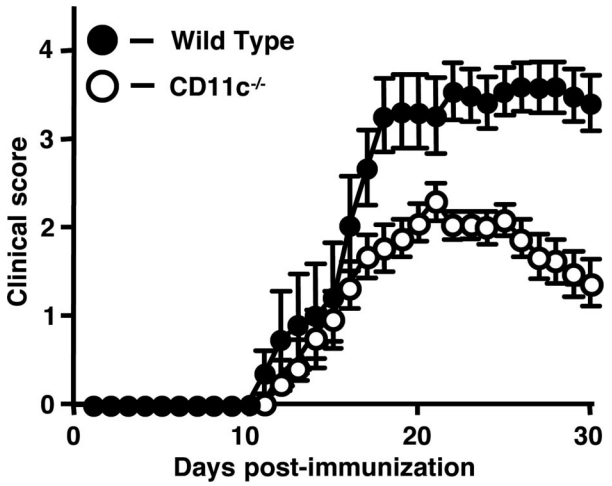


**Figure 1.** The clinical course of active EAE is attenuated in CD11c$^{-/-}$ mice. Active EAE was induced with $MOG_{35-55}$ peptide and symptoms scored for 30 days as described in Materials and Methods. Results shown are the daily mean clinical score for wild-type ($n = 11$) and CD11c$^{-/-}$ mice ($n = 17$) from three experiments.

### *Cellular Infiltration and Demyelination in CD11c$^{-/-}$ Mice with EAE*

We next performed histopathological analysis on spinal cords of wild-type and CD11c$^{-/-}$ 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. Representative spinal cord sections from wild-type mice obtained 32 days after disease induction had significant cellular infiltration in the meninges and white matter (Figure 2A) with corresponding demyelination (Figure 2B). Sections obtained from CD11c$^{-/-}$ mice had little cellular infiltration, inflammation, axonal degeneration, and demyelination throughout the spinal cord, compared with wild-type mice (Figure 2, C and D). The overall mean score for these parameters for all regions of the spinal cord in CD11c$^{-/-}$ mice was 0.82, whereas wild-type mice had a mean score of 3.1.

We also analyzed leukocyte infiltration early in EAE development and observed that total CD45$^+$ leukocyte infiltration in the spinal cords of CD11c$^{-/-}$ mice was not different before disease onset (day 10, data not shown), but was substantially reduced during the acute phase of disease (day 15, Figure 3A). Of the leukocytes that did infiltrate the spinal cords of CD11c$^{-/-}$ mice, there were fewer CD4$^+$ and CD8$^+$ T cells compared with wild type

**Table 1.** EAE Symptoms in Wild-Type Mice and CD11c$^{-/-}$ Mice

| Mice | CDI* | Disease onset† | Disease incidence‡ |
|---|---|---|---|
| Wild type, $n = 11$ | 58 | 16 days | 100 |
| CD11c$^{-/-}$, $n = 17$ | 32.1 | 19 days | 94 |

*Cumulative disease index is the mean of the sum of daily clinical scores observed between days 7 and 30.

†Disease onset is defined as the 1st day of 2 consecutive days with a clinical score of two or more.

‡Disease incidence is defined as the percent of mice that displayed any clinical signs of disease

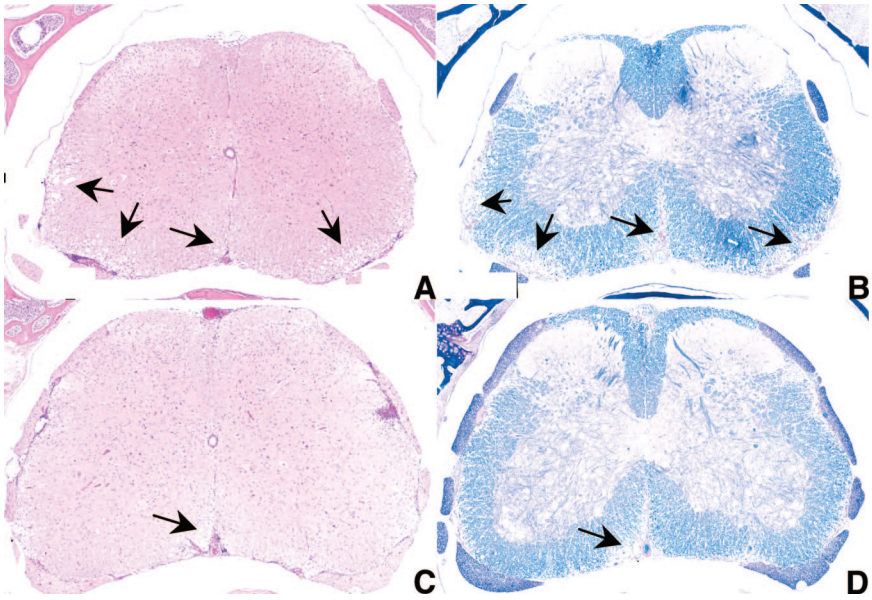


**Figure 2.** Leukocyte infiltration and demyelination are reduced in CD11c$^{-/-}$ mice in EAE. Spinal cords from wild-type and CD11c$^{-/-}$ mice ($n$ = 3 for each group) were obtained at 32 days after immunization, fixed in 10% buffered formalin, and paraffin-embedded. Sections from the cervical, thoracic, and lumbar regions (5 $\mu$m) were stained with H&E or Luxol fast blue (LFB) and scored as described in Materials and Methods. **A:** Representative section from a wild-type mouse stained with H&E. **Arrows** indicate widespread cellular infiltration and inflammation. **B:** Section from the same specimen as in **A** stained with LFB. **Arrows** indicates regions of significant demyelination. **C:** Representative section from an CD11c$^{-/-}$ mouse stained with H&E. Note the lack of cellular infiltration and inflammation. **D:** Section from the same specimen as in **C** stained with LFB. Little to no demyelination was observed throughout the white matter. Original magnifications, ×4.

(Figure 3B). CD4$^+$ and CD8$^+$ T-cell infiltration was reduced 39 and 53%, respectively, in CD11c$^{-/-}$ mice compared with controls. However, when normalized for the differences in overall CNS infiltration between the two groups, CD4$^+$ and CD8$^+$ T-cell infiltration was reduced 94.5 and 95.8%, respectively, in CD11c$^{-/-}$ compared with wild-type mice. These data demonstrate that the absence of p150/95 significantly reduces trafficking of leukocytes to the CNS. The reduced trafficking of CD11c$^{-/-}$ leukocytes was not attributable to changes in expression of the other $\beta_2$-integrins (data not shown).

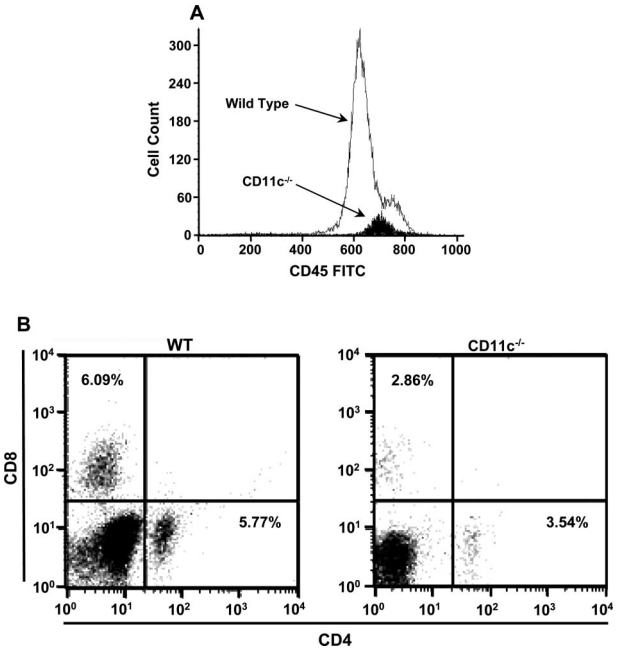


**Figure 3.** Leukocyte subset infiltration in the spinal cord of CD11c$^{-/-}$ mice with EAE is reduced compared with control mice. **A:** Leukocytes isolated from spinal cords of control ($n$ = 5) and CD11c$^{-/-}$ mice ($n$ = 5) as described in Materials and Methods were immunostained for CD45. The infiltration of CD45$^+$ leukocytes at day 15 after immunization was markedly reduced in CD11c$^{-/-}$ mice compared with controls. **B:** Leukocytes isolated from spinal cords of control ($n$ = 5) and CD11c$^{-/-}$ mice ($n$ = 5) as described in Materials and Methods were immunostained for CD4 and CD8. The results shown are from cells pooled within each group of mice.

## *Transfer of Wild-Type MOG-Sensitized T Cells to CD11c$^{-/-}$ Mice Modestly Attenuates Transferred EAE, Whereas CD11c$^{-/-}$ MOG-Sensitized T Cells Induce Mild, Monophasic EAE*

We also induced EAE by adoptively transferring MOG-sensitized T cells from wild-type mice to CD11c$^{-/-}$ mice. Onset of EAE in CD11c$^{-/-}$ recipient mice was identical to control transfers and similar to the course of EAE observed during active EAE (Figure 4A, Table 2). The overall severity of disease as assessed by cumulative disease index was significantly lower in CD11c$^{-/-}$ recipient mice (48.1 versus 28.4; $P < 0.0001$, Wilcoxon signed-rank test). To determine whether p150/95 deficiency on anti-

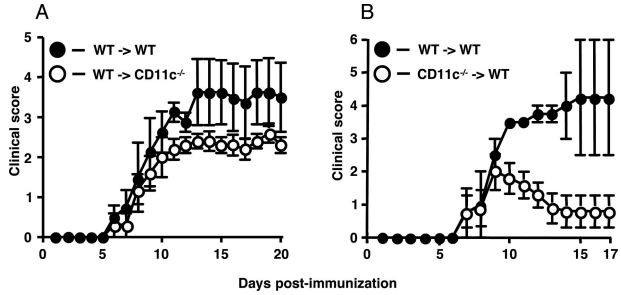


**Figure 4.** The clinical course of adoptively transferred EAE is attenuated in CD11c$^{-/-}$ mice. **A:** Transferred EAE was induced in wild-type ($n$ = 5) and CD11c$^{-/-}$ mice ($n$ = 5) mice by injecting encephalitogenic T cells (~5 × 10$^6$) derived from wild-type mice with active EAE. Results shown are the daily mean clinical score from two separate experiments. **B:** Transferred EAE was induced in wild-type ($n$ = 5) mice by injecting encephalitogenic T cells (~5 × 10$^6$) derived from CD11c$^{-/-}$ mice with active EAE. As a control, transferred EAE was induced in wild-type mice ($n$ = 2) 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.

**Table 2.** Transferred EAE Symptoms in Wild-Type Mice and CD11c$^{-/-}$ Mice

| Mice | CDI* | Disease onset† | Disease incidence‡ |
|---|---|---|---|
| WT > WT, $n = 4$ | 41.8 | 9.3 days | 100 |
| WT > CD11c$^{-/-}$, $n = 5$ | 28.1 | 9.4 days | 100 |
| WT > WT, $n = 2$ | 35.5 | 8.5 days | 100 |
| CD11c$^{-/-}$ > WT, $n = 5$ | 12.5 | 8 days | 80 |

*Cumulative disease index is the mean of the sum of daily clinical scores observed between days 0 and 20 (WT > CD11c$^{-/-}$ experiments) or 0 and 17 (CD11c$^{-/-}$ > WT experiments).

†Disease onset is defined as the 1st day of 2 consecutive days with a clinical score of two or more.

‡Disease incidence is defined as the percent of mice that displayed any clinical signs of disease.

gen-specific T cells would result in attenuated disease, we performed transferred EAE using MOG-sensitized T cells from CD11c$^{-/-}$ mice (Figure 4B, Table 2). Wild-type mice receiving CD11c$^{-/-}$ T cells developed EAE at the same time as control transfers; however, the disease peaked 3 days after onset of symptoms and remitted to a significantly milder form of disease (cumulative disease index: 35.5 versus 12.5; $P = 0.002$, Wilcoxon signed-rank test).

## *CD11c$^{-/-}$ T Cells Proliferate Comparably but Have an Altered Cytokine Profile Compared with Wild-Type T Cells*

To test the possibility that attenuated active and transferred EAE in CD11c$^{-/-}$ mice could be attributable to impaired T-cell proliferation, we performed *in vitro* proliferation assays as previously described.[37] Stimulation of MOG-sensitized T cells from wild-type and CD11c$^{-/-}$ mice with various concentrations of MOG revealed no overall significant difference in proliferation (Figure 5; $P = 0.64$, unpaired *t*-test). Interestingly, at the highest MOG peptide concentrations (2 and 4 $\mu$g/ml), T cells from CD11$^{-/-}$ mice proliferated significantly more than those from control mice ($P = 0.009$, unpaired *t*-test). The increased T-cell proliferation seen in CD11c$^{-/-}$ mice, using the higher concentrations of MOG peptide, is consistent with a nearly twofold increase in IL-2 production observed on *in vitro* restimulation of splenic T cells from CD11c$^{-/-}$ mice compared with control (Figure 6A). The levels of several proinflammatory cytokines produced by CD11c$^{-/-}$ T cells (including IFN-$\gamma$, tumor necrosis factor-$\alpha$, and IL-17) were markedly lower, although IL-12 levels were elevated more than 1.5-fold relative to control mice (Figure 6A). The production of anti-inflammatory cytokines such as IL-4, IL-10, and transforming growth factor-$\beta$ by CD11c$^{-/-}$ T cells was comparable or only modestly reduced compared with control mice. We also examined for the production of IFN-$\gamma$ by CD4$^+$ and CD8$^+$ T cells in the spinal cord of wild-type and CD11c$^{-/-}$ mice during the acute phase of EAE development (15 days after infection) (Figure 6B). Surprisingly, we observed essentially no IFN-$\gamma$-producing CD4$^+$ and CD8$^+$ T cells that infiltrated the spinal cords of CD11c$^{-/-}$ mice compared with controls. In contrast, CD11c$^{-/-}$ splenic T cells readily produced IFN-$\gamma$ at the same time period after induction (Figure 6A).

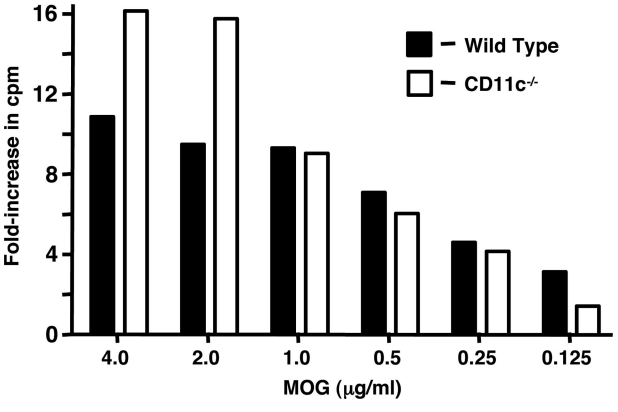


**Figure 5.** CD11c$^{-/-}$ T cells proliferate comparably to wild-type T cells. Encephalitogenic T cells enriched by nylon-wool adherence from the spleens of wild-type ($n = 4$) or CD11c$^{-/-}$ mice ($n = 4$) undergoing active EAE, or T cells from healthy controls (naïve cells), were co-cultured with irradiated splenic APCs plus MOG peptide (0.125 to 4 $\mu$g/ml). The cells were pulsed with [$^3$H]thymidine and harvested at 18 hours for determination of radioisotope incorporation. The results shown are expressed as the mean + SEM of fold induction of wild-type or CD11c$^{-/-}$ T-cell proliferation relative to background proliferation.

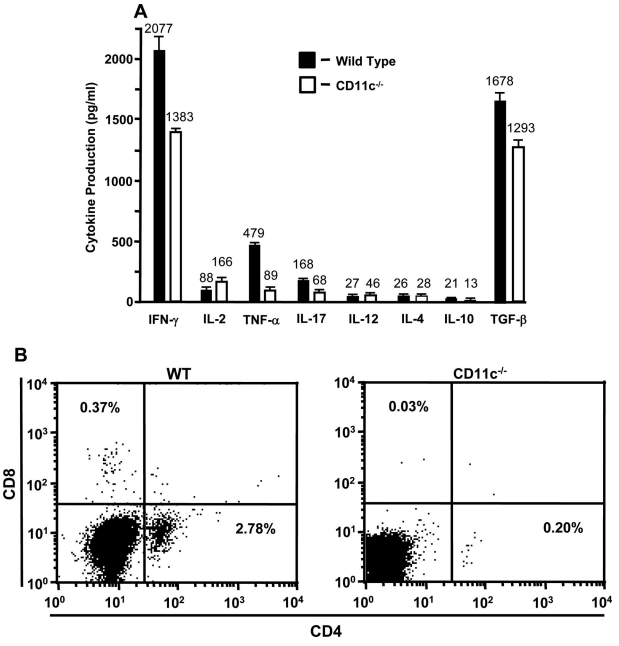


**Figure 6.** Splenic CD11c$^{-/-}$ T cells produce a unique repertoire of cytokines during EAE. **A:** Encephalitogenic T cells enriched by nylon-wool adherence from the spleens of wild-type ($n = 4$) or CD11c$^{-/-}$ mice ($n = 4$) undergoing active EAE (day 15) were co-cultured with irradiated splenic APCs from naïve donors and stimulated with MOG peptide (1 $\mu$g/well). Supernatants were collected 48 hours after stimulation and assayed by enzyme-linked immunosorbent assay to quantitate production of each cytokine. Cytokine production by cultures of wild-type and CD11c$^{-/-}$ cells is reported as the mean ± SEM in picograms per milliliter. The mean value for each cytokine is shown above the bar. **B:** Production of IFN-$\gamma$ in CD4$^+$ and CD8$^+$ T cells isolated from the spinal cords of control and CD11c$^{-/-}$ mice with active EAE. Leukocytes isolated from spinal cords of control ($n = 5$) and CD11c$^{-/-}$ mice ($n = 5$) as described in Materials and Methods were immunostained for CD4, CD8, and IFN-$\gamma$. The data shown are derived from gating on IFN-$\gamma$-producing cells. The results shown are from cells pooled within each group of mice.

## Discussion

The results we report here demonstrate that p150/95 plays an important role in the progression of the inflammatory events leading to demyelination and paralysis during EAE. The mechanisms underlying CD11c-mediated protection in EAE are difficult to readily decipher attributable, in part, to poor understanding of the major role(s) of p150/95 in the host immune response. Functionally, p150/95 is best known for its contribution to phagocytosis of bacteria (particularly *Mycobacterium*) and apoptotic cells.[14,27–31] Resting and activated microglia as well as infiltrating macrophages all express p150/95, and expression increases on activation of these phagocytic cells.[18–22] This expression pattern of p150/95 on macrophages and microglia, combined with the reduced demyelination observed in the spinal cords of CD11c$^{-/-}$ mice (Figure 2), supports a role for p150/95 in promoting myelin damage and subsequent neuronal injury during EAE. However, to date, no studies using antibodies to block p150/95-mediated phagocytosis in the CNS have been performed, unlike for Mac-1 in which both *in vitro* and *in vivo* studies have clearly established a role for this adhesion molecule in this process.[38–42] Studies directly addressing the role of p150/95 in phagocytosis in demyelinating disease and other CNS inflammatory diseases are required to enhance our understanding of this potentially important aspect of p150/95 biology.

The contribution of p150/95-mediated phagocytosis to demyelination may be minor given that leukocyte infiltration into the CNS of CD11c$^{-/-}$ mice is substantially lower than that seen in control mice (Figure 3). There is little experimental support for p150/95 as an adhesion molecule important in leukocyte migration. Numerous studies have documented a role for p150/95 in both monocyte/endothelium interactions using static *in vitro* adhesion assays and in conjugate formation between cytotoxic T cells and target cells of various types.[14,18,32–35] However, *in vivo* adhesion studies investigating the contribution of p150/95 in mediating leukocyte/endothelial interactions under normal or inflammatory conditions are lacking. Thus the importance of p150/95 in leukocyte trafficking remains primarily unexplored. Nevertheless, our results provide strong indirect evidence suggesting that p150/95 may be important for cellular trafficking into the CNS.

p150/95 expression increases on activated B and T cells, particularly cytotoxic T cells, the latter of which suggests a role in adhesive events leading to target cell killing[15–17,32,35] These studies also raise the possibility that p150/95 contributes to the development and or stability of the immunological synapse along with LFA-1. The absence of p150/95 on either T cells or antigen-presenting cells (APCs) could result in reduced T-cell activation and an altered pattern of cytokine production, a finding we obtained with CD11c$^{-/-}$ mice in EAE (Figure 6). Despite the unusual pattern of cytokines produced by CD11c$^{-/-}$ splenic T cells during EAE, there was no significant reduction in the antigen-specific proliferative capacity of T cells derived from these mice as determined by *in vitro* assays (Figure 5). Nevertheless, the markedly attenuated EAE observed when CD11c$^{-/-}$ T cells were transferred to control mice (Figure 4) indicates that loss of this $\beta_2$-integrin on T cells is more critical to disease development than loss of expression on APCs and other leukocyte subsets. This finding coupled with the development of attenuated EAE when control T cells were transferred to CD11c$^{-/-}$ mice, argues that the expression of p150/95 on dendritic cells is not critical for the development of EAE. p150/95 is the common cell surface marker for the identification of dendritic cells, although not all dendritic cells appear to express p150/95.[43] More importantly, little information is available for a functional role for p150/95 on dendritic cells. Nevertheless, the absence of p150/95 on either APCs or T cells may alter the extent and rate of activation events for both cell types leading to the attenuated disease phenotype we report here.

Our data combined with previous studies show that three of the four $\beta_2$-integrins play critical, nonredundant roles in demyelinating disease. Numerous reports have shown that treatment of mice with anti-CD11a (LFA-1) and CD11b (Mac-1) antibodies attenuates or prevents the development of EAE.[44–47] In support of these antibody studies, we have observed markedly attenuated MOG-induced EAE using CD11b$^{-/-}$ and CD11a$^{-/-}$ mice[6] (K. Dugger, J. Hu, D. Bullard, and S.R. Barnum, unpublished observations). In contrast, the deletion of CD11d (namely $\alpha$-D) had no effect of the development or progression of EAE,[48] although antibodies to CD11d were protective in animal models of spinal cord injury.[49–51] Taken together, it is clear that LFA-1, MAC-1, and p150/95 contribute uniquely to demyelinating disease, despite their overlapping ligand specificity and expression on leukocytes.

Therapeutic approaches targeting integrin function in neurodegenerative diseases, including demyelinating disease, have been investigated for more than a decade. Antibody-mediated inhibition of several members of both the $\alpha$4 and $\beta$2 integrin families attenuates or prevents EAE.[6,38–40,44–46,52–54] Most importantly, anti-$\alpha$4 integrin antibody has become a major component of the treatment arsenal for relapsing-remitting MS, despite the potentially serious side effects for a small subset of patients.[55–58] The data we present here for p150/95 suggest that members of the $\beta$2-integrin family of adhesion molecules, particularly Mac-1 and p150/95, represent a viable therapeutic option for MS. Unlike LFA-1, which is important in trafficking of numerous cell types as well as T-cell activation events, inhibition of Mac-1 and p150/95 may affect a repertoire of functions in demyelinating disease without leaving the host significantly immunocompromised. Given the overlapping functions of Mac-1 and p150/95, deletion of both receptors may result in a complete inhibition of disease similar to that seen when their common ligand intercellular adhesion molecule-1 is deleted.[59] Thus, the $\beta$2-integrin family members, including p150/95, may offer new therapeutic approaches for demyelinating disease.

## *Acknowledgment*

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