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CD43 Modulates Severity and Onset of Experimental Autoimmune Encephalomyelitis[1]

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Experimental autoimmune encephalomyelitis (EAE) is a mouse model of multiple sclerosis characterized by infiltration of activated $CD4^+$ T lymphocytes into tissues of the CNS. This study investigated the role of CD43 in the induction and progression of EAE. Results demonstrate that CD43-deficient mice have reduced and delayed clinical and histological disease severity relative to $CD43^{+/+}$ mice. This reduction was characterized by decreased $CD4^+$ T cell infiltration of the CNS of $CD43^{-/-}$ mice but similar numbers of Ag-specific T cells in the periphery, suggesting a defect in T cell trafficking to the CNS. The absence of CD43 also affected cytokine production, as myelin oligodendrocyte glycoprotein (MOG) 35–55-specific $CD43^{-/-}$ $CD4^+$ T cells exhibited reduced IFN-γ and increased IL-4 production. $CD43^{-/-}$ $CD4^+$ MOG-primed T cells exhibited reduced encephalitogenicity relative to $CD43^{+/+}$ cells upon adoptive transfer into naive recipients. These results suggest a role for CD43 in the differentiation and migration of MOG_{35-55}-specific T cells in EAE, and identify it as a potential target for therapeutic intervention. ***The Journal of Immunology,*** **2003, 171: 6527–6533.**

Experimental autoimmune encephalomyelitis (EAE)[3] is an autoimmune attack on myelin-producing cells of the CNS, which is initiated by $CD4^+$ T cells and characterized by an influx of activated lymphocytes and macrophages into the brain and spinal cord parenchyma (1, 2). The experimental condition results in localized inflammation and demyelination in the CNS that leads to limb weakness and eventual paralysis (1, 2), and mimics the human disease multiple sclerosis (MS) (3, 4). Myelin oligodendrocyte glycoprotein (MOG) 35–55 is a well-characterized target Ag of encephalitogenic T cells used to initiate EAE in the $H\text{-}2^b$ C57B/6 mouse model (5).

Studies have shown that T cell trafficking to the CNS is a critical factor in the ability of $CD4^+$ T cells to mediate disease (6). Adhesion molecules facilitate the infiltration of lymphocytes into the CNS of mice with EAE. Several reports have highlighted the importance of the expression of integrins such as very late Ag-4 on $CD4^+$ T cells that penetrate the blood brain barrier and mediate disease (7, 8). In these studies, only clones expressing very late Ag-4 could traffic to the CNS and thereby mediate EAE. Furthermore, up-regulation of ICAM-1, VCAM-1, and L-selectin on CNS vessels correlated with the immigration of $CD4^+$ T cells during the development of EAE (9). Thus, the ability of $CD4^+$ T cells to migrate into the CNS is a critical parameter of their encephalitogenicity. Determining factors that modulate T cell migration into the CNS could lead to the development of effective therapeutic intervention in MS.

CD43 (leukosialin, sialophorin) is a large sialoglycoprotein that is abundantly expressed by cells of hemopoietic origin, including both $CD4^+$ and $CD8^+$ T cells (10). Two distinct glycoforms of the molecule exist, and their expression is differentially regulated in $CD4^+$ and $CD8^+$ T cells (11, 12). CD43 has been implicated in the regulation of both T cell homing and activation (10), and has been shown to localize away from the immunological synapse by interaction with members of the ezrin-radixin-moesin family of cytoskeletal adaptor proteins (13–16). Early studies documented both positive and negative regulatory roles for CD43 in T cell activation (17–19), however these results may be reconciled by a recent report suggesting that CD43 plays a dynamic role in the progression of an immune response (12). Although earlier studies described a negative regulatory role for CD43 on T cell adhesion (18, 20), more recent studies have demonstrated a positive role for CD43 in T cell homing to secondary lymphoid organs and peripheral tissues (21–23). For example, anti-CD43 mAbs have been shown to prevent the migration of T cells to pancreatic islets and thereby prevent the development of diabetes in a nonobese diabetic model (23). A potential role for CD43 in T cell homing to the CNS was suggested in recent reports demonstrating delayed $CD8^+$ T cell migration into the CNS following intracranial infection of lymphocytic choriomeningitis virus (LCMV) virus in $CD43^{-/-}$ mice (12). In addition, increased expression of CD43 in the CNS was observed during EAE (9). Therefore, we sought to characterize EAE induction and progression in $CD43^{-/-}$ mice and to analyze the ability of $CD43^{-/-}$ T cells to adoptively transfer EAE. Our results indicate that disease is significantly attenuated in $CD43^{-/-}$ mice due to decreased lymphocyte trafficking to the CNS and cytokine dysregulation.

Materials and Methods

Mice

$CD43^{-/-}$ B6.129 mice were originally generated by Ardman and coworkers (18) and were purchased from The Jackson Laboratory (Bar Harbor, ME) along with the $CD43^{+/+}$ B6.129 wild-type control strain ($H\text{-}2^b$).

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[3] Abbreviations used in this paper: EAE, experimental autoimmune encephalomyelitis; MS, multiple sclerosis; MOG, myelin oligodendrocyte glycoprotein; LCMV, lymphocytic choriomeningitis virus.

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SCID mice (B6.CB17-*Prkdc*^{scid}/SzJ) were purchased from The Jackson Laboratory and housed in a sterile environment. Mice were bred and housed in the Emory University Department of Animal Resources facility (Atlanta, GA) according to Institutional Animal Care and Use Committee protocols. For EAE experiments, mice were used at 8 wk of age.

EAE induction

EAE was induced according to the protocol of Mendel et al. (24). Briefly, female B6.129 or CD43$^{-/-}$ mice were immunized with 200 μg of $MOG_{35–55}$ (MEVGWYRSPFSRVVHLYRNGK) emulsified in CFA containing 5 mg/ml heat-inactivated *Mycobacterium tuberculosis* (H37 RA; Difco, Detroit, MI) on days 0 and 7 s.c. in the hind flank (24). On days 0 and 2, mice received 250–500 ng of pertussis toxin (Sigma-Aldrich, St. Louis, MO) i.p. Disease severity was monitored according to the following scale: 0, no disease; 1, flaccid tail; 2, hind limb weakness; 3, hind limb paralysis; 4, forelimb weakness; 5, moribund.

Histology

Spines were harvested and fixed in 10% formalin for ~1 wk. Spinal cords were then extracted, paraffin-embedded, and longitudinal sections were stained with H&E for the presence of inflammatory infiltrates or Luxol fast blue to detect demyelination. Inflammatory foci were scored in a blinded fashion.

Flow cytometric analysis of brain lymphocytes

For analysis of lymphocytic infiltrates, mice with the highest disease score in each group at the time point indicated were sacrificed. For day 25, CD43$^{+/+}$ mice ranged from 2.0 to 2.5 and CD43$^{-/-}$ mice ranged from 0.0 to 2.5. For day 150, the CD43$^{+/+}$ mice all had a score of 2.5 and the CD43$^{-/-}$ mice all had a score of 0.0. Brains were isolated and dissociated in 10% FBS. Cells were resuspended in 100% Percoll (Sigma-Aldrich), underlayed with 70% Percoll, and centrifuged at 2500 rpm for 20 min. The interface was collected and cells were stained with anti-CD4-allophycocyanin and anti-CD44-PE and analyzed by flow cytometry on a BD FACSCalibur (BD PharMingen, San Diego, CA). Data were analyzed using FlowJo software (Tree Star, San Carlos, CA).

Intracellular cytokine staining

Cultured splenocytes from $MOG_{35–55}$-primed CD43$^{+/+}$ or CD43$^{-/-}$ mice (5×10^5 per well) were incubated in a 96-well plate with 100 μM $MOG_{35–55}$ and 10 μg/ml brefeldin A. After 6 h in culture, cells were processed using an intracellular staining kit (Caltag Laboratories, San Diego, CA) and stained with anti-CD4-allophycocyanin and anti-IFN-γ-PE (BD PharMingen). Data are gated on CD4$^+$ T cells.

Proliferation assay

Cultured splenocytes from $MOG_{35–55}$-primed mice (5×10^5 per well) were incubated in a 96-well plate with the indicated concentration of $MOG_{35–55}$ peptide. After 48 h, cells were labeled with 0.4μCi/well [^{3}H]thymidine. Eighteen hours later, the plates were harvested on a FilterMate harvester (Packard Instrument, Meriden, CT) and analyzed on a Matrix 96 Direct β Counter (Packard Instrument).

IFN-γ and IL-4 ELISA

Splenocytes were incubated with 1 μM $MOG_{35–55}$ in a 24-well plate (5×10^6/well) and supernatants were harvested 48 h later. Microtiter plates were coated with 50 μl of purified anti-IL-4 (5 μg/ml, clone 11B11) or anti-IFN-γ (2 μg/ml, clone R4-6A2) overnight at 4°C. Captured cytokines were detected using biotinylated anti-IL-4 (100 μg/ml; BD PharMingen) or anti-IFN-γ (clone XMG1.2, 100 μg/ml; BD PharMingen) and detected using alkaline phosphatase-conjugated avidin (Sigma-Aldrich) and *p*-nitrophenylphoshphate (pNPP) substrate (Bio-Rad, Hercules, CA). Recombinant IL-4 or IFN-γ (BD PharMingen) was used as a standard.

Measurement of MOG-specific Ab

Mice were sacrificed at various time points after EAE induction and peripheral blood was harvested by cardiac puncture. Blood was allowed to clot at room temperature for 1 h and then overnight at 4°C. The clot was microcentrifuged at 13,000 rpm for 5 min and serum was isolated. Microtiter plates were coated with 50 μl of $MOG_{35–55}$ peptide (5 μg/ml) in $NaHCO_3$ coating buffer, pH 9.6, overnight at 4°C. Serum was titrated in blocking buffer, plated at 100 μl/well, and incubated overnight at 4°C (1/50 serum dilution is shown). Abs were detected using biotinylated anti-IgG1, anti-IgG2a, anti-IgG2b, anti-IgG3 (BD PharMingen), avidin-conjugated alkaline phosphatase (Sigma-Aldrich), and pNPP (Bio-Rad). Colorometric change was measured at 405 nm on a Microplate Autoreader (Bio-tek Instruments, Winooski, VT).

Adoptive transfer of CD4$^+$ T cells for induction of EAE

CD43$^{+/+}$ or CD43$^{-/-}$ mice were immunized with $MOG_{35–55}$ and lymph nodes and spleens were harvested as described above. Lymph node cells and splenocytes were cultured in vitro with 1 μM $MOG_{35–55}$ and IL-2 for 72 h. Cells were then Ficolled and CD4$^+$ T cells were positively selected by MACS separation using magnetic CD4$^+$ microbeads (Miltenyi Biotec, Auburn, CA) per manufacturer's instructions. In one experiment, 2.5×10^6 CD4$^+$ T cells, and in a second experiment, 5×10^6 CD4$^+$ T cells, were adoptively transferred by i.v. injection into SCID recipients on day 0. On day 2, mice received an i.p. injection of pertussis toxin (250 ng), and mice were then monitored for symptoms of disease. Because data were similar for both experiments, the results were pooled.

Statistical analyses

Statistical analyses were conducted using GraphPad Prism (Software for Science; GraphPad, San Diego, CA). Mean clinical scores were analyzed by the Mann-Whitney test, while disease incidence percentages were compared by Fisher's exact test. Mean high scores, mean day of onset, and mean day of peak disease were compared by Mann-Whitney test, and highest clinical scores were compared using the Student *t* test. Comparisons of IFN-γ ELISA data and Ab production were performed using the Student *t* test.

Results

CD43$^{-/-}$ mice exhibit reduced EAE severity and incidence

Previous studies by Onami et al. (12) demonstrated that CD43$^{-/-}$ mice exhibit delayed accumulation of CD8$^+$ infiltrates into the

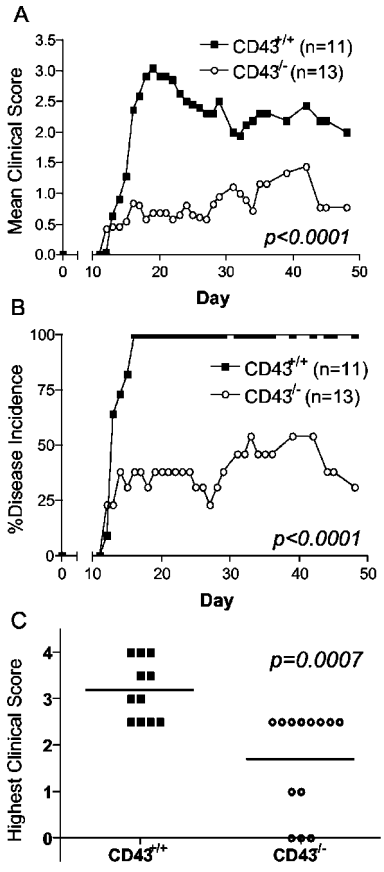

FIGURE 1. CD43$^{-/-}$ mice exhibit reduced EAE incidence and severity. CD43$^{+/+}$ or CD43$^{-/-}$ female mice were injected with $MOG_{35–55}$ and pertussis toxin and monitored for clinical signs of EAE. *A*, CD43$^{-/-}$ mice exhibit reduced disease severity during the course of disease. *B*, CD43$^{-/-}$ mice have reduced disease incidence relative to CD43$^{+/+}$ mice. Data shown represent results obtained from immunization of 13 wild-type and 11 knockout mice in three independent experiments.

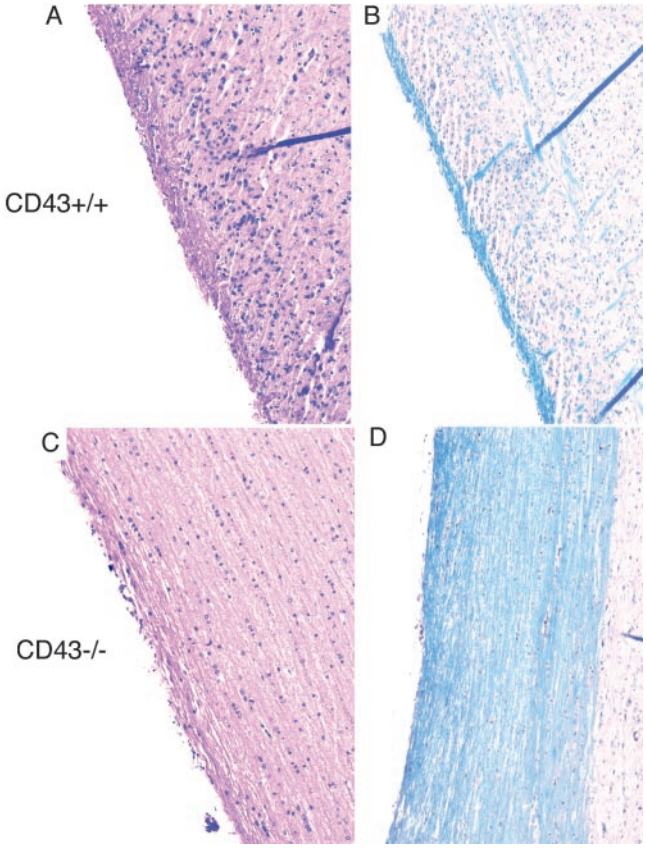

FIGURE 2. Reduced infiltration and demyelination in spinal cords of $CD43^{-/-}$ EAE mice. Spinal cords were harvested from $CD43^{+/+}$ or $CD43^{-/-}$ EAE mice 150 days after disease induction. Longitudinal sections were stained with H&E (*A* and *C*) or Luxol Fast Blue (*B* and *D*). Sections from $CD43^{-/-}$ mice exhibited less infiltration (*B*) and demyelination (*D*) relative to wild-type controls (*A* and *C*). Sections shown are representative of those taken from three mice per group.

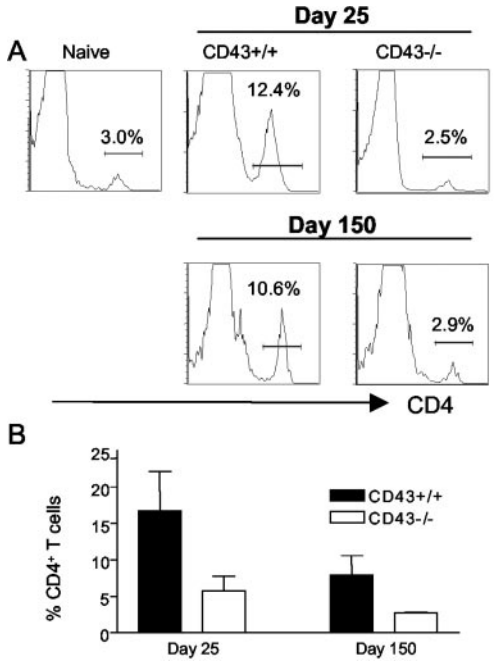

FIGURE 3. Reduced trafficking of $CD4^+$ T cells to the CNS in $CD43^{-/-}$ EAE mice. Brains from $CD43^{+/+}$ or $CD43^{-/-}$ EAE mice were removed at day 25 or day 150 postdisease induction, stained with anti-CD4, and analyzed by flow cytometry (*A*). Data were gated on live cells and are expressed as the percentage of $CD4^+$ T cells within this gate. Data from three mice per group from each time point are summarized in *B*. For day 25, $CD43^{+/+}$ mice ranged in disease score from 2.0 to 2.5 and $CD43^{-/-}$ mice ranged from 0.0 to 2.5. For day 150, the $CD43^{+/+}$ mice all had a score of 2.5 and the $CD43^{-/-}$ mice all had a score of 0.0.

CNS following intracranial infection with LCMV. Because CD43 is also expressed on activated $CD4^+$ T cells (11, 12), we hypothesized that CD43 might play a role in EAE progression. To test this hypothesis, B6.129 and B6.129 $CD43^{-/-}$ mice were immunized with $MOG_{35–55}$ emulsified in CFA, injected with pertussis toxin, and monitored for symptoms of disease (24–26). Results indicate that $CD43^{-/-}$ mice have reduced disease severity (Fig. 1*A*) and disease incidence (Fig. 1*B*) compared with $CD43^{+/+}$ mice in $MOG_{35–55}$-induced EAE. Whereas $CD43^{+/+}$ mice developed EAE with a mean high clinical score of 3.1, $CD43^{-/-}$ mice exhibited a mean high clinical score of only 1.6 (Fig. 1*C* and Table I). Of those $CD43^{-/-}$ mice that did exhibit symptoms of disease, none exhibited symptoms as severe as the majority of the $CD43^{+/+}$ mice (Fig. 1*C*), indicating that even among those mice that got sick, the disease course was lessened. $CD43^{-/-}$ mice also exhibited a delay in disease onset relative to $CD43^{+/+}$ mice, as the average day of onset was day 14 and 19 after induction, respectively (Table I). The day of peak disease in the $CD43^{-/-}$ mice was also delayed relative to wild-type controls (day 17 vs 24, respectively) (Table I). Several $CD43^{-/-}$ mice were observed up to 150 days postdisease induction and never exhibited the severe disease symptoms seen in the $CD43^{+/+}$ mice. In fact, by day 150, $CD43^{-/-}$ mice with a peak disease score of 2.0–2.5 had regressed to a score of 0 (data not shown).

To confirm the finding that the generation of EAE is less severe in $CD43^{-/-}$ mice, longitudinal sections of spinal cord from control or $CD43^{-/-}$ mice were examined in a blinded fashion for

Table I. *Clinical and histological features of EAE induced in $CD43^{-/-}$ mice*[a]

	Mean High Score[b]	Incidence[c]		Day of Onset[b]	Day of Peak Disease[b]
	(Mean ± SEM)	(Day 20)	(Day 40)	(Mean ± SEM)	(Mean ± SEM)
	$p = 0.009$	$p = 0.002$	$p = 0.016$	$p = 0.079$	$p = 0.011$
$CD43^{+/+}$ $n = 13$	3.1 ± 0.2	100%	100%	13.7 ± 0.4	17.2 ± 0.4
$CD43^{-/-}$ $n = 11$	1.6 ± 0.3	38%	54%	18.5 ± 2.7	24.5 ± 2.4

[a] Wild-type ($CD43^{+/+}$, $n = 13$) and CD43-deficient ($CD43^{-/-}$, $n = 11$) B6.129 mice were immunized for EAE with 200 μg $MOG_{35–55}$ emulsified in CFA and 250 ng of pertussis toxin. Results represent the cumulative data obtained from three independent experiments.

[b] Mean high scores and day of onset/day of peak disease data are expressed as the mean ± SEM. Statistical comparisons were comparisons done using the Mann-Whitney nonparametric test.

[c] Comparison of disease incidence analyzed by Fisher's exact test.

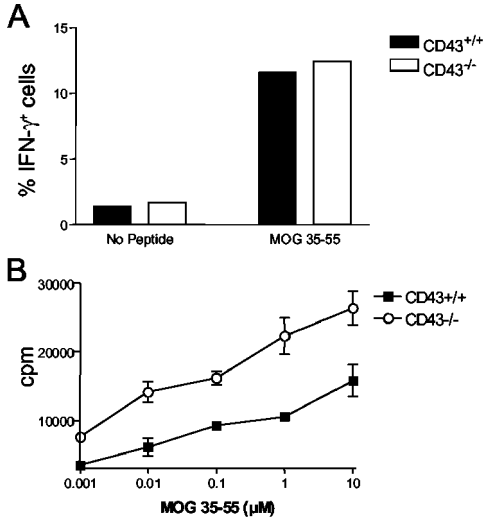

FIGURE 4. Hyperproliferation and equivalent IFN-γ production by CD43$^{-/-}$ MOG-primed splenocytes. Splenocytes from CD43$^{+/+}$ or CD43$^{-/-}$ EAE mice were harvested and restimulated with 10 μM MOG_{35-55} for measurement of intracellular IFN-γ (*A*) or various concentrations of MOG_{35-55} in a proliferation assay (*B*). Data shown are representative of three independent experiments using pooled splenocytes from two to four mice per group.

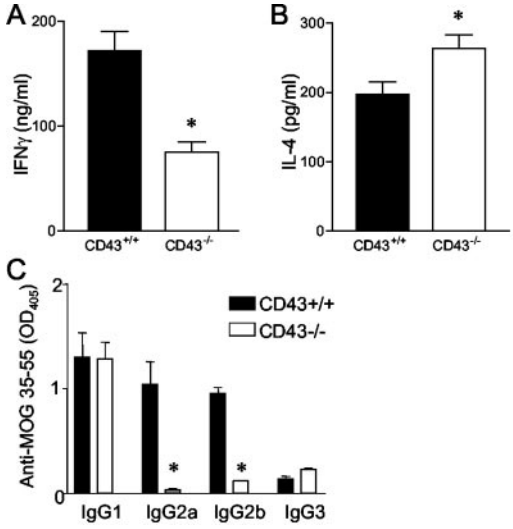

FIGURE 5. Splenocytes from CD43$^{-/-}$ EAE mice exhibit reduced IFN-γ and increased IL-4 in response to MOG_{35-55}. CD43$^{+/+}$ or CD43$^{-/-}$ MOG-primed splenocytes were restimulated in vitro with 10 μM MOG_{35-55} for 48 h, and supernatants were analyzed for the presence of IFN-γ (*A*) or IL-4 (*B*) by ELISA. Serum was also collected from CD43$^{-/-}$ EAE mice and analyzed for the presence of anti-MOG_{35-55} IgG by ELISA. Data shown are representative of three independent experiments using pooled splenocytes from two to four mice per group. $*$, $p > 0.01$.

inflammatory infiltrates and demyelination. Significantly less inflammatory infiltration and demyelination was identified in spinal cord sections from CD43$^{-/-}$ mice (Fig. 2, *B* and *D*) relative to those obtained from wild-type controls (Fig. 2, *A* and *C*).

Reduced CD4$^+$ infiltrates into brains of CD43$^{-/-}$ EAE mice

To determine whether the reduced disease severity seen in CD43$^{-/-}$ mice was due to decreased T cell trafficking to the brain, CNS mononuclear cells from brain tissue of CD43$^{+/+}$ or CD43$^{-/-}$ mice were examined for the presence of CD4$^+$ infiltrates by flow cytometry following EAE induction. During both the acute (day 25) and chronic (day 150) phases of disease, an increased number of CD4$^+$ T cells were observed in CD43$^{+/+}$ mice relative to CD43$^{-/-}$ mice at both time points (Fig. 3. The CD4$^+$ cells in the CNS were all CD44$^+$, consistent with an activated phenotype that could mediate disease (data not shown). These results show that CD43$^{-/-}$ mice lacked appreciable infiltration of CD4$^+$ T cells in the CNS even 150 days post-EAE induction.

One interpretation of these data is that CD43$^{-/-}$ MOG-specific CD4$^+$ T cells have a defect in trafficking to the CNS, while an alternative interpretation is that fewer CD4$^+$ T cells are activated in response to immunization with MOG in CD43$^{-/-}$ mice. To determine the number of Ag-specific CD4$^+$ T cells in the periphery of CD43$^{-/-}$ EAE mice, splenocytes were analyzed by intracellular IFN-γ staining after activation with MOG_{35-55}. Results indicated that splenocytes from CD43$^{-/-}$ EAE mice contained similar numbers of MOG_{35-55}-specific T cells (Fig. 4*A*). Paradoxically, we observed increased proliferation (Fig. 4*B*) and IL-2 production (data not shown) in response to MOG_{35-55} peptide in CD43$^{-/-}$ splenocytes relative to wild type. This result is consistent with previous data suggesting that activated CD43$^{-/-}$ T cells exhibit decreased apoptosis in vitro (12). Thus, CD43$^{-/-}$ mice generate similar numbers of MOG_{35-55}-specific CD4$^+$ T cells, but exhibit defects in CD4$^+$ T cell trafficking to the CNS.

Shift in cytokine profile in MOG_{35-55}-specific CD43$^{-/-}$ T cells

Despite the similar numbers of IFN-γ-positive T cells in wild-type and CD43$^{-/-}$ splenocytes, a difference in the mean fluorescence intensity of the IFN-γ cells was observed ($\sim$30%, data not shown). To examine whether this decreased mean fluorescence intensity indicated a reduction in IFN-γ production, cytokine levels were examined by ELISA analysis of supernatants of cultured MOG-specific T cells from CD43$^{+/+}$ or CD43$^{-/-}$ mice. We observed a decrease in the amount of IFN-γ produced by CD43$^{-/-}$ splenocytes in response to MOG_{35-55} and a concomitant increase in IL-4 production (Figs. 5, *A* and *B*). These results suggested that CD43$^{-/-}$ MOG-specific T cells exhibited a less inflammatory cytokine profile.

To confirm this finding and to further characterize the biological impact of cytokine profile skewing, we examined the IgG subtype profile of serum anti-MOG Abs in CD43$^{+/+}$ or CD43$^{-/-}$ mice. The presence of IFN-γ in the extracellular milieu induces B cell class switching to IgG2a, while the presence of IL-4 induces class switching to IgG1 (27). A dramatic decrease in serum levels of IgG2a and IgG2b was observed in CD43$^{-/-}$ mice (Fig. 5*C*), consistent with reduced IFN-γ needed to drive Ab class-switching to IgG2a. Therefore, the decrease in MOG-specific IFN-γ observed in CD43$^{-/-}$ mice is biologically significant. No change in the levels of IgG1 or IgG3 was observed (Fig. 5*C*). Overall, these data suggest that in addition to a defect in CD4$^+$ T cell trafficking to the CNS, CD43$^{-/-}$ CD4$^+$ T cells also exhibit a less inflammatory response consistent with decreased disease in an EAE model.

Delayed onset of adoptive transfer EAE in recipients of CD43$^{-/-}$ CD4$^+$ T cells

Because CD43 is expressed on many cell types of hemopoietic origin, the effect of the loss of CD43 on CD4$^+$ T cells was specifically investigated. To accomplish this, CD43$^{+/+}$ or CD43$^{-/-}$ mice were primed with MOG_{35-55}, and their lymph nodes were harvested 10 days later and restimulated in vitro with specific peptide. CD4$^+$ T cells were then purified and adoptively transferred into B6 SCID recipients. Staining with CD44 revealed a similar number of CD44^{high} cells in both the CD43$^{+/+}$ and CD43$^{-/-}$

Table II. *Clinical features of EAE induced by adoptive transfer of CD43$^{-/-}$ T cells*[a]

Cells		Mean High Score[b] (Mean ± SEM)	Incidence[c] (Day 8)	Incidence[c] (Day 18)	Day of Onset[b] (Mean ± SEM)
		$p = 0.038$	$p = 0.023$		$p = 0.01$
CD43$^{+/+}$	$n = 7$	2.2 ± 0.1	6/7 (86%)	6/7 (86%)	5.5 ± 0.5
CD43$^{-/-}$	$n = 5$	1.5 ± 0.3	1/5 (20%)	4/5 (80%)	11.3 ± 1.7

[a] Wild-type (CD43$^{+/+}$) and CD43-deficient (CD43$^{-/-}$) CD4$^+$ MOG-primed T cells were adoptively transferred into SCID mice (2–5 × 10^6 T cells/mouse) on day 0. Mice received an injection of pertussis toxin (250 ng) on day 2. Results represent cumulative data obtained from two independent experiments.

[b] Results are expressed as the mean (±SEM). Comparisons analyzed by Mann-Whitney nonparametric test; only those mice that developed disease were included in the analysis.

[c] Comparison of disease incidence analyzed χ^2 test.

cultures (~40%, data not shown), suggesting a similar number of Ag-specific cells were transferred in both groups.

Results demonstrate a striking difference in the day of disease onset, with a mean day of onset of 5.5 in the recipients of CD43$^{+/+}$ CD4$^+$ cells compared with 11.3 in the recipients of CD43$^{-/-}$ CD4$^+$ T cells (Table II). Although the disease severity in adoptive transfer recipients was less robust overall than that observed in immunized mice, results indicate a moderate decrease in the mean high score in recipients of CD43$^{-/-}$ CD4$^+$ T cells as compared with CD43$^{+/+}$ T cells (2.2 ± 0.1 vs 1.4 ± 0.2) (Fig. 6*A*, Table II). A significant difference in disease incidence was also detected over the course of the disease (Fig. 6*B*, Table II). These results indicate that CD43$^{-/-}$ CD4$^+$ T cells have a reduced and delayed ability to induce EAE as compared with wild-type CD4$^+$ T cells, and suggest that the observed decrease in disease severity in CD43$^{-/-}$ mice is due to the absence of CD43 on CD4$^+$ T cells.

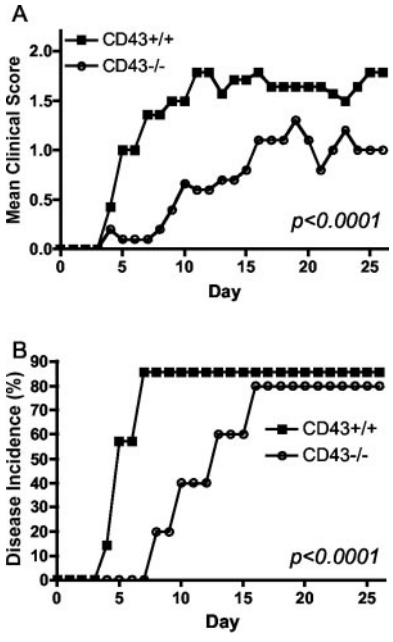

FIGURE 6. Adoptively transferred CD43$^{-/-}$ CD4$^+$ MOG-primed T cells are less encephalitogenic than their wild-type counterparts. MOG-primed T cells from CD43$^{+/+}$ or CD43$^{-/-}$ donors were stimulated in vitro for 72 h, MACS-sorted for CD4$^+$ T cells (>85% purity was achieved), and transferred i.v. into SCID recipients (2 × 10^6 CD4$^+$ cells/mouse in the first experiment and 5 × 10^6 cells/mouse in the second). Recipients also received an injection of pertussis toxin on day 2 posttransfer. Mice were then monitored for the symptoms of EAE. Because data obtained was similar in both experiments, results were pooled. Recipients of CD43$^{-/-}$ MOG-primed CD4$^+$ T cells exhibited reduced disease severity (*A*) and disease incidence (*B*) relative to recipients of CD43$^{+/+}$ cells. Values of p were calculated by Mann-Whitney test comparing disease scores during days 4–26 (those after disease onset in recipients of wild-type cells).

Adoptive transfer recipients of CD43$^{-/-}$ T cells exhibit skewed cytokine profile

To determine whether the CD43$^{-/-}$ adoptively transferred T cells exhibited a skewed cytokine profile relative to recipients of CD43$^{+/+}$ CD4$^+$ MOG-primed T cells, splenocytes were isolated from adoptive transfer recipients on day 26 posttransfer. After restimulation with MOG$_{35–55}$ in vitro for 72 h, supernatants were analyzed by ELISA. Results indicate that splenocytes from recipients of CD43$^{-/-}$ mice exhibited reduced IFN-γ secretion and increased IL-4 secretion (Fig. 7). These data corroborate the results of in vivo immunization experiments in CD43$^{-/-}$ mice that suggest a role for CD43 in determining Th cell phenotype. Interestingly, T cells isolated from adoptive transfer experiments exhibited a much more dramatic shift in cytokine phenotype than T cells isolated from immunized mice (Fig. 4).

Discussion

In this study, we addressed the induction and progression of MOG$_{35–55}$-induced EAE in CD43-deficient mice. A dramatic reduction in disease severity was observed in CD43$^{-/-}$ mice as

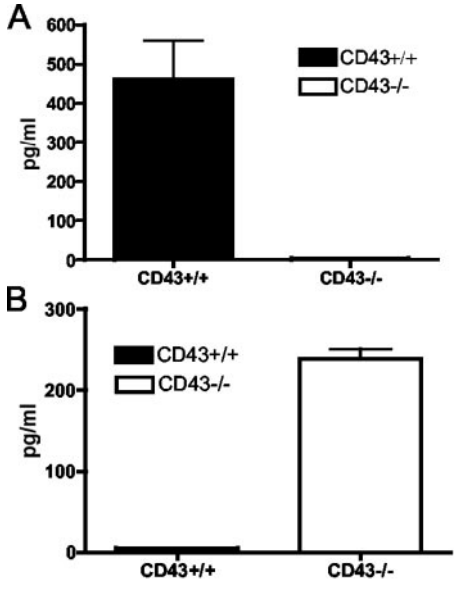

FIGURE 7. Splenocytes from recipients of CD43$^{-/-}$ MOG-primed T cells exhibit reduced IFN-γ and increased IL-4 secretion. Splenocytes from adoptive transfer recipients at day 26 posttransfer and restimulated in vitro with 10 μM MOG$_{35–55}$. Forty-eight hours later, supernatants were harvested and assayed for IFN-γ and IL-4 by ELISA. Data are representative of three individual mice from two independent experiments.

compared with their wild-type counterparts (Fig. 1). This reduction in disease severity was not due to a global defect in T cell activation, as knockout and wild-type mice exhibited similar numbers of Ag-specific T cells in the spleen, and $CD43^{-/-}$ MOG-specific T cells proliferated very well in response to MOG peptide (Fig. 4). Instead, our results demonstrate that in the absence of CD43, MOG-specific T cells exhibited significantly reduced homing into brain and spinal cord tissue and produced less IFN-γ and more IL-4 (Figs. 2, 3, and 5). This defect in CNS trafficking could be directly due to the absence of CD43, which has been reported to be an adhesion molecule important in the migration of cells to peripheral tissue. This has been suggested in other model systems; for example, anti-CD43 mAbs have been shown to prevent the migration of T cells to pancreatic islets and thereby prevent the development of diabetes in nonobese diabetic mice (23). Our data suggest the loss of CD43 ameliorates disease in an EAE model, and therefore further support an important role for CD43 in lymphocyte migration. Importantly, these results identify CD43 as a potential target for therapeutic intervention in EAE and MS.

The results of this study also revealed, however, that MOG-specific $CD43^{-/-}$ exhibit a less inflammatory cytokine profile, suggesting that the absence of CD43 during T cell activation has functional consequences for the differentiation of Th cells. Similar findings have been identified, concluding that $CD43^{-/-}$ T cells with a variety of Ag-specificities (including MOG_{35-55}) exhibit a Th2 profile (A. I. Sperling, unpublished data). Although the observed increase in IL-4 production by $CD43^{-/-}$ T cells was statistically significant (Fig. 5*B*), the lack of concomitant increase in IgG1 levels (Fig. 5*C*) and the absence of eosinophilic infiltration in the spinal cord (data not shown) suggest that it may not be biologically significant. In contrast, a dramatic decrease in serum levels of IgG2a was observed (Fig. 5*C*). Several reports suggest that anti-myelin IgG2a may be better able to fix complement than IgG1 (28–30), and anti-myelin IgG2a has been shown to exacerbate disease in vivo (30). Furthermore, the secretion of IFN-γ by autoreactive T cells is known to result in the activation of resident microglia and later cause the influx of activated peripheral macrophages that then release inflammatory mediators that damage oligodendrocytes and inhibit the production of myelin (1, 2). Therefore, the reduction in IFN-γ may be more responsible for the observed decreased disease severity observed in $CD43^{-/-}$ mice.

Although it is possible that reduced T cell trafficking and altered cytokine profile independently contribute to the amelioration of disease, it is also possible that a causative relationship exists between the two. Evidence exists that suggests a shift from Th1 to Th2 phenotype could be directly responsible for deficits in T cell homing. For example, Th1 cells have been shown to express chemokine receptors CCR5 and CXCR3, whereas Th2 cells primarily express CCR4 and CCR8 (31–33). Encephalitogenic T cells and those isolated from lesions in MS patients express CCR5 and CXCR3 and migrate toward RANTES and macrophage inflammatory protein-1α (34). In addition, Austrup et al. (35) demonstrated that only Th1 cells could bind to P- and E-selectin and thereby efficiently migrate to sites of inflammation. Therefore, it is possible that the phenotype shift itself could contribute to the observed decrease in lymphocyte trafficking to the CNS. Preliminary results indicate that there is no difference in the level of CCR5 expression on $CD43^{-/-}$ MOG_{35-55}-primed T cells as compared with wild-type controls (M. L. Ford and B. D. Evavold, unpublished observations). Experiments to examine patterns of expression of other chemokine receptors are ongoing.

CD43 is highly expressed on many hemopoetic cell types, including T cells, B cells, granulocytes, and monocytes. Our adoptive transfer studies using $CD43^{+/+}$ or $CD43^{-/-}$ MOG-primed donor T cells demonstrate that the absence of CD43 on T cells negatively affects their ability to induce EAE in SCID recipients. SCID recipients were used in our studies to minimize potential minor histocompatibility-specific responses among B6.129 F2 hybrid mice. Several groups have documented enhanced EAE induction in SCID mice after adoptive transfer of myelin-specific murine T cells (36). Both $CD43^{+/+}$ and $CD43^{-/-}$ T cells undergo homeostatic proliferation following transfer into lymphopenic hosts (data not shown), leading to an expanded population of encephalitogenic T cells. A delay in disease onset and lower disease severity was observed in the recipients of $CD43^{-/-}$ T cells (Fig. 6), indicating that at least a portion of the reduced disease severity seen in the $CD43^{-/-}$ mice is due to the effect on T cells. Analysis of adoptive transfer of $CD43^{+/+}$ $CD4^{+}$ T cells into $CD43^{-/-}$ recipients may reveal an additional role for CD43 on other cell types during the induction and progression of EAE.

The results of our experiments both in the $CD43^{-/-}$ immunized mice (Fig. 1, Table I) and the recipients of adoptively transferred $CD43^{-/-}$ cells (Fig. 6, Table II) reveal a delay in disease kinetics relative to wild-type counterparts, suggesting $CD43^{-/-}$ T cells may be delayed in trafficking to the CNS. This finding is substantiated by the observation that $CD8^{+}$ T cells exhibited a delay in trafficking to the CNS following intracranial infection (12). However, in the viral model, the short delay in T cell trafficking did not protect the mice from the effects of an inflammatory immune response in the brain, and all died a few days after their wild-type counterparts (12). In contrast, the apparent delayed kinetics of $CD43^{-/-}$ migration in the EAE model impacted the overall severity of disease, in that $CD43^{-/-}$ mice never exhibited severe disease (Fig. 1, Table I). The fact that delayed kinetics did not affect the eventual outcome during acute viral infection but does seem to impact the immune response to a self-Ag may be explained by the robustness of the immune response in the two instances. Although viral infections such as LCMV present high doses of Ag and induce a strong and sustained immune reaction, the immune response to low dose, self-Ags is much weaker and therefore may be more susceptible to the absence of auxiliary factors such as CD43. The results from both the immunization and adoptive transfer experiments indicate that $CD43^{-/-}$ T cells are capable of mediating a less severe and delayed disease, suggesting that CD43 is just one element capable of modulating a complex and multifactorial disease process. It is possible that effective therapy for the treatment of MS will be combinatorial in nature, and require targeting several different facets of the autoreactive immune response.

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