Cutting Edge: Loss of α4 Integrin Expression Differentially Affects the Homing of Th1 and Th17 Cells

Simon Glatigny, Rebekka Duhen, Mohamed Oukka and Estelle Bettelli

*J Immunol* 2011; 187:6176-6179; Prepublished online 14 November 2011;
doi: 10.4049/jimmunol.1102515

http://www.jimmunol.org/content/187/12/6176

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**Supplementary Material**

http://www.jimmunol.org/content/suppl/2011/11/14/jimmunol.1102515.DC1

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The neutralization of α4 integrin is currently used as treatment in several autoimmune diseases and is thought to prevent the entry of most immune cells in target tissues. In this study, we showed that selective deletion of α4 integrin in T cells did not prevent but delayed the development of experimental autoimmune encephalomyelitis. Whereas both Th1 and Th17 cells infiltrate the CNS of wild-type mice, T cells present in the CNS of mice lacking α4 integrin were mainly enriched in Th17 cells, suggesting that this T cell subset uses other integrins to access the CNS. In contrast, α4 integrin expression is important for Th1 cells to enter the CNS and for the stability of their Th1-associated genetic program. Therefore, our data suggest that anti-α4 integrin Ab treatment may be more efficient in the treatment of Th1- rather than Th17-mediated disease. *The Journal of Immunology*, 2011, 187: 6176–6179.

Experimental autoimmune encephalomyelitis (EAE) is an animal model of multiple sclerosis (MS) characterized by multifocal areas of leukocyte infiltration, demyelination, and axonal damage (1). Two subsets of myelin-specific CD4+ T cells have been implicated in the pathogenicity of MS and EAE: Th1 cells and Th17 cells (2–5).

Trafficking of autoreactive CD4+ T cells from the systemic compartment into the CNS are crucial early events in the development of MS and EAE lesions and involve specific adhesion molecules (1). α4 (Igα4) β1 (Igβ1) integrin or VLA-4 has been proposed as the major adhesion molecule allowing the entry of T cells in the CNS. Treatment of mice with an anti-α4 integrin Ab has been shown to dramatically reduce leukocyte adhesion and prevent the development of EAE in most animal models (6, 7), except in C57BL/6 mice (10). Therefore, it is important to determine the effect of Igα4 blockade on the migration of different subsets of immune cells in the CNS.

In this article, we show that mice with selective deletion of Igα4 on CD4+ T cells are still susceptible to EAE development. Whereas the number of CNS-infiltrating Th1 cells was significantly decreased, the number of CNS-infiltrating Th17 cells was not impaired in these mice. Using adoptive transfer experiments, we further show that the lack of Igα4 expression on Th1 cells impaired their phenotypic stability and their capacity to infiltrate the CNS. Together, our results suggest the efficacy of Igα4 neutralization for Th1-mediated EAE, but also distinct and new molecular requirements for Th17 cell entry into the CNS during EAE.

**Materials and Methods**

**Mice**

All mice are on the C57BL/6 background and used at 8–12 wk. Wild-type (WT), CD4Cre, TCRβ-deficient, and 2D2 transgenic mice were purchased from The Jackson Laboratory. Igα4+ mice were provided by Dr. Papayannopoulou (University of Washington) (11). All mice were bred and maintained under specific pathogen-free conditions at the Benaroya Research Institute (Seattle, WA), and all experiments were performed in accordance with the guidelines of the Benaroya Research Institute Animal Care and Use Committee.

**EAE induction**

Active EAE was induced as previously described (12). For passive transfer of EAE, 2D2 naive CD462+CD4+ T cells were cultured with irradiated splenocytes, anti-CD3 Ab (clone 145-2C11; 2.5 μg/ml), and either IL-12 (10 ng/ml) for Th1 cells or IL-6 (30 ng/ml), TGF-β (5 ng/ml), and anti–IFN-γ (10 μg/ml) for Th17 cells. After 3 d, 50 × 10⁶ cells were injected i.v. into TCRβ-deficient recipients with pertussis toxin at days 0 and 2. EAE was scored according to the following criteria: 0, no signs of disease; 1, loss of tail tone; 2, hind limb paraparesis; 3, hind limb paralysis; and 4, front and hind limb paralysis.

**Flow cytometry**

Cell suspensions from brain and spinal cord were prepared as previously described (12). Abs for CD4 (GK1.5), IFN-γ (XMG1.2), IL17A (TC11-18H11.1), and Igα4 (R1-2) were purchased from eBioscience and BioLegend. For surface cytokine staining, CD4+ T cells were stained with Miltenyi Biotec. Cells were acquired on an LSR II (BD Biosciences), and data were analyzed with FlowJo software (Tree Star).

This work was supported by National Institutes of Health Grant R01 NS 059996 to E.B.

Received for publication September 1, 2011. Accepted for publication October 26, 2011.

This online version of this article contains supplemental material.

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www.jimmunol.org/cgi/doi/10.4049/jimmunol.1102515
Draining lymph nodes (dLN) were collected 8 d after immunization. Cells were cultured at 5 × 10⁶ cells/ml in the presence of different concentrations of MOG for 72 h. During the last 16 h, cells were pulsed with 1 μCi [³H]thymidine. [³H]thymidine incorporation was measured using a β-counter.

Results and Discussion
Itga4 is expressed at higher levels in Th1 cells compared with Th17 cells

Th cell subsets express distinct chemokine receptors and adhesion molecules conferring specific homing properties. Analysis of Itga4 expression has been previously performed on T cell clones or in vitro-polarized cells, but culture conditions could modulate Itga4 expression. In this study, we analyzed Itga4 expression on ex vivo effector Th1 and Th17 cell subsets isolated from dLN after MOG35–55 immunization using a secretion assay for IL-17A and IFN-γ (Fig. 1). Itga4 was expressed at higher levels on Th1 than Th17 cells (Fig. 1). In contrast, there was no difference in the expression of Itgb1 and Itga7 between Th1 and Th17 cells (Supplemental Fig. 1A, 1B). Therefore, the selective higher expression of Itga4 on Th1 cells raises the possibility that these cells might be more dependent on Itga4 for their entry in the CNS than Th17 cells.

Itga4 deletion on CD4+ T cells does not prevent the development of EAE

To determine whether the differential expression of Itga4 on Th cell subsets could affect the development of EAE, we used mice with specific deletion of Itga4 in T cells (CD4Cre Itga4fl/fl). Cre-mediated deletion of Itga4 in CD4+ T cells was efficient at the genomic level and resulted in >95% of CD4+ T cells lacking Itga4 in these animals (Supplemental Fig. 1C, 1D). The majority of WT control mice (95%) developed a monophasic disease with ascending paralysis between 10 and 14 d after immunization (Fig. 2A, Supplemental Table I). Although the mean clinical EAE score in CD4Cre Itga4fl/fl mice (2.5 ± 0.7) was similar to the one observed in WT control mice (2.7 ± 0.7), fewer CD4Cre Itga4fl/fl mice developed disease (incidence: 50 compared with 95% in WT mice; Fig. 2A, Supplemental Table I) and had a delay in disease onset (19.3 ± 4.3 d in CD4Cre Itga4fl/fl mice versus 12.3 ± 2.3 d in WT mice; Fig. 2A, Supplemental Table I). Thus, CD4Cre Itga4fl/fl mice developed EAE with delayed onset and less incidence than WT control mice. However, in agreement with a previous report showing blockade of leukocyte adhesion but limited effect of anti-Itga4 treatment in C57BL/6 mice MOG-induced EAE (8, 9), specific deletion of Itga4 on T cells did not abolish EAE susceptibility in our model. This suggests that pathological mechanisms including but not limited to the dominant type of CNS-infiltrating effector T cells may differ among mouse strains.

Itga4 deletion does not affect T cell priming and differentiation

VLA-4 has been proposed to play an important role in several immune functions such as lymphopoiesis, costimulation, and T cell migration (11, 13–17). Therefore, we compared these parameters between CD4Cre Itga4fl/fl and Itga4fl/fl mice. We did not detect any difference in the number, percentage, and phenotype of CD4+ and CD8+ T cells present in the lym-
pairs with Itgâ4 to form VLA-4, were not affected in CD4Cre Itgâ4â/â mice (Supplemental Fig. 1F). Next, we investigated whether the lack of Itgâ4 expression on T cells could interfere with the differentiation of naive T cells into pathogenic Th1 and Th17 cells. We determined that CD4+ T cells from CD4Cre Itgâ4â/â and Itgâ4â/â mice could differentiate equally well in Th1 and Th17 subsets (Fig. 2B) and that Itgâ4 deletion was equivalent in Th1 and Th17 cells (Supplemental Fig. 1F). MOG-specific T cell proliferation was also similar between CD4Cre Itgâ4â/â mice and Itgâ4â/â mice (Fig. 2C).

Collectively, these results indicate that specific deletion of Itgâ4 on T cells does not interfere with T cell priming, but instead may affect the homing of pathogenic T cells in the CNS.

Itgâ4 deletion decreases Th1 cells but not Th17 homing into the CNS

To evaluate this hypothesis, we determined the numbers of CD4+ T cells infiltrating the CNS of CD4Cre Itgâ4â/â mice during EAE. At the peak of the disease, there was a decrease in the number of CNS-infiltrating CD4+ T cells in CD4Cre Itgâ4â/â (52.9 ± 8.4 × 10^3) compared with control mice (126.5 ± 17.2 × 10^3). Before EAE onset and at the peak of the disease, there was a similar proportion of IL-17A+ IFN-γ+ and IL-17A+CD4+ T cells in the brain of CD4Cre Itgâ4â/â and WT control mice (Fig. 3, Supplemental Fig. 1G). However, we detected a significant decrease in the percentage and absolute number of CD4+ T cells that produced only IFN-γ in the CNS of CD4Cre Itgâ4â/â mice compared with control mice (Fig. 3, Supplemental Fig. 1G). Importantly, deletion of Itgâ4 was equivalent in Th1 and Th17 cells (Supplemental Fig. 1F), and the residual proportion of CD4+ T cells, which remained Itgâ4+ (4%), did not increase in CNS-infiltrating CD4+ T cells (Supplemental Fig. 1C). Of note, because a recent report suggests that IFN-γ+ T cells present in the CNS during EAE represent ex-Th17 cells (18), the tracking of T cell subsets by cytokine secretion might underestimate the number of CNS-infiltrating Th17 cells in our model. Despite this possibility, there was still a drastic decreased Th1/Th17 ratio in the CNS of CD4Cre Itgâ4â/â mice compared with WT mice (Fig. 3C). This suggests two important mechanisms of Itgâ4 action: 1) Itgâ4...
costimulation of T cells could promote Th1 cells switching to a Th17 phenotype in the CNS; or 2) fewer Th1 cells entered the CNS because their trafficking is dependent on Itga4.

*Loss of Itga4 delays Th1-induced EAE*

To address some of these possibilities, we differentiated CD4+ T cells from 2D2 CD4Cre Itga4fl/fl and 2D2 mice into either Th17 or Th1 cells and transferred them into T cell-deficient recipient mice (Fig. 4A, 4B). Th17 cells derived from either 2D2 or 2D2 CD4Cre Itga4fl/fl mice induced a severe disease (Fig. 4B), and a significant percentage of Th17-transgenic cells recovered from the CNS had maintained IL-17A expression (Fig. 4C, top panel). The transfer of Th1 cells lacking Itga4, in contrast, resulted in delayed disease onset compared with the disease induced by Itga4-sufficient Th1 cells (Fig. 4B). Surprisingly, cells recovered from the CNS of animals transferred with Itga4-deficient Th1 cells were mainly IL-17A-producing cells (Fig. 4C, bottom panel), indicating that in the absence of Itga4 on CD4+ T cells, the disease was mainly Th17 but not Th1 mediated. These results suggest the intriguing possibility that in the absence of Itga4, Th1 cells become unstable and revert to a Th17-like phenotype. The other possibility is that uncommitted cells from the pool of naïve donor cells may have differentiated into Th17 cells and have overgrown Th1 cells in vivo, causing EAE independently of Itga4 and in the absence of Th1 cell entry in the CNS (Fig. 4B, 4C, bottom panel).

**Conclusions**

In this study, we demonstrate that elimination of Itga4 on CD4+ T cells limited the access of Th1 cells in the CNS and affected their phenotypic characteristics, but had less of an effect on the trafficking of Th17 cells. In accordance with these observations, the selective deletion of Itga4 in CD4+ T cells did not prevent the development of Th17-induced EAE but delayed Th1-induced EAE. These findings suggest that anti-Itga4 might be more efficient at treating Th1- rather than Th17-mediated MS. A differential effect of immunomodulatory drugs on effector T cell subsets and disease development has been reported recently in another study (19). These observations together with ours suggest that the Th1 cells-induced CNS autoimmunity might be fairly well controlled by current available drugs but that more specific drugs are required for Th17-induced and dominated CNS pathologies.

**Acknowledgments**

We thank Dr. Papayannopoulou for providing the Itga4fl/fl mice and Alice Yuan for technical assistance.

**Disclosures**

The authors have no financial conflicts of interest.

**References**