# B-Cell Very Late Antigen-4 Deficiency Reduces Leukocyte Recruitment and Susceptibility to Central Nervous System Autoimmunity

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Natalizumab, which binds very late antigen-4 (VLA-4), is a potent therapy for multiple sclerosis (MS). Studies have focused primarily upon its capacity to interfere with T-cell migration into the central nervous system (CNS). B cells are important in MS pathogenesis and express high levels of VLA-4. Here, we report that the selective inhibition of VLA-4 expression on B cells impedes CNS accumulation of B cells, and recruitment of Th17 cells and macrophages, and reduces susceptibility to experimental autoimmune encephalomyelitis. These results underscore the importance of B-cell VLA-4 expression in the pathogenesis of CNS autoimmunity and provide insight regarding mechanisms that may contribute to the benefit of natalizumab in MS, as well as candidate therapeutics that selectively target B cells.

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Natalizumab, a monoclonal antibody (mAb) directed against the human α4 (CD49d) subunit of the integrin very late antigen-4 (VLA-4), is a potent treatment for relapsing–remitting multiple sclerosis (RRMS).[1] Studies of anti–VLA-4 treatment in experimental autoimmune encephalomyelitis (EAE), considered predominantly a T-cell–mediated disease, indicate that its effects on T cells,[2–4] in particular Th1 cells,[5] are responsible for the clinical benefit of natalizumab. The recent successful use of anti-CD20 B-cell–depleting agents in multiple sclerosis (MS) treatment trials[6] has renewed appreciation for the role of B cells in MS pathogenesis and interest in evaluating their response to MS therapeutics. Although VLA-4 is more highly expressed on the surface of mature B cells than on T cells,[7] less is known regarding the influence of anti–VLA-4 therapy on B cells than on T cells. One in vitro study suggested that engagement of VLA-4 on B cells with its endothelial ligand VCAM-1 is required for their migration across the blood–brain barrier (BBB).[8] In this regard, natalizumab treatment of MS has been associated with elevation of B cells in peripheral blood[9] and reduction in cerebrospinal fluid.[10] Thus, given these observations and the recent increased appreciation for the role of B cells in MS and EAE,[6,11–13] we questioned whether the clinical benefit of anti–VLA-4 therapy could also relate to its potential influence on B-cell trafficking into the CNS.

## Materials and Methods

### Mice

$\alpha4^{flox/flox}$ mice[14] (referred to as $\alpha4^{f/f}$ below) were kindly provided by Dr Thalia Papayannopoulou (University of Washington). CD19cre mice[15] and wild-type C57BL/6J mice were purchased from the Jackson Laboratory (Bar Harbor, ME). All studies have been approved by the University of California, San Francisco Institutional Animal Care and Use Committee and were in accordance with the US Public Health Service's Policy on Humane Care and Use of Laboratory Animals.

### Antigen

Recombinant human (rh) myelin oligodendrocyte glycoprotein (MOG) was provided by Dr C. C. A. Bernard and was synthesized, purified, and refolded as previously reported.[12]

### EAE Induction

EAE was induced in 8- to 12-week-old mice by immunization with 100μg rhMOG in complete Freund adjuvant containing 200μg *Mycobacterium tuberculosis* H37RA (DIFCO Laboratories, Detroit, MI) on day 0. Mice intraperitoneally (i.p.) received either 100ng (Fig 1) or 200ng (all other experiments) *Bordetella pertussis* toxin (List Biological Laboratories, Campbell, CA) on days 0 and 2. Mice were observed daily for clinical EAE.[12]

### In Vivo Blockade of α4

Mice received 200μg of rat anti-α4 antibody (PS/2) or rat IgG2b isotype control (LTF-2) (both Bio X Cell, West Lebanon, NH) i.p. on days 4, 7, and 10 after immunization.

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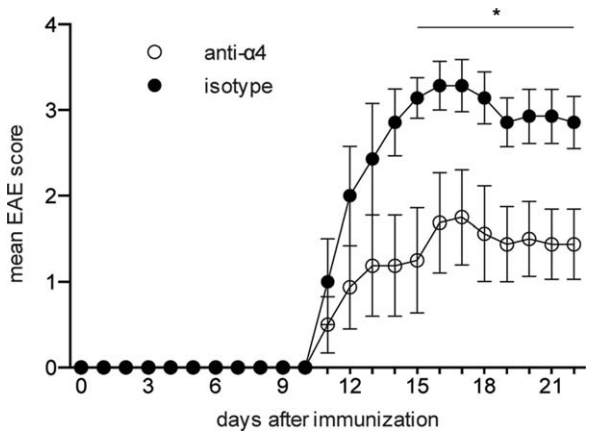


**FIGURE 1: α4-Blocking antibodies prevent recombinant human myelin oligodendrocyte glycoprotein (rhMOG)-induced experimental autoimmune encephalomyelitis (EAE) in C57BL/6 mice. EAE was induced in wild-type C57BL/6 mice by immunization with 100μg rhMOG. On days 4, 7, and 10 after immunization, 200μg of anti-α4 antibody (n = 8 mice) or IgG2b isotype control antibody (n = 7 mice) were injected intraperitoneally. Data shown are mean ± standard error of the mean. *$p \leq 0.05$ for days 15 to 22; Mann–Whitney *U* test.**

### *Cell Isolation*

Blood was collected via cardiac puncture. After erythrocyte lysis, leukocytes were washed. Spleen and CNS mononuclear cells were isolated after perfusion with phosphate-buffered saline (PBS).[12]

### *Flow Cytometric Analysis*

Antimouse FcRIIB/FcRIIIA mAb (2.4G2; BD, Franklin Lakes, NJ) was used to avoid nonspecific staining. Aqua dead cell stain kit was used for live/dead cell separation and CountBright counting beads (both Molecular Probes, Eugene, OR) for absolute cell number quantification. Antibodies to mouse CD19 PerCP-Cy5.5 (eBio1D3), B220 (CD45R) APC-Cy7 (RA3–6B2), MHC-II (I-A/I-E) PE-Cy7 (M5/114.15.2), CD80 (B7-1) APC (16-10A1), CD4 APC-Cy7 (RM4–5), and CD11b PE-Cy7 (M1/70) were purchased from eBioscience (San Diego, CA). Antibodies to B220 (CD45R) FITC (RA3–6B2), CD45 APC (30-F11), and CD49d PE (9C10) were purchased from BD. An isotype- and fluorochrome-matched control antibody (IgG2a kappa PE; R35–95; BD) was used to assess nonspecific staining for CD49d. Analysis was performed on a BD LRSFortessa flow cytometer using FACSDiva software (BD).

### *Intracellular Cytokine Staining*

Intracellular cytokine staining (ICS) was performed as described,[12] using aqua dead cell stain kit (Molecular Probes) and antibodies to CD4 PE-Cy7 (RM4–5), IL-17A PerCP-Cy5.5 (eBio17B7), and IFN-γ APC (XMG1.2; all eBioscience).

### *Detection of Anti-MOG Antibodies*

Anti-rhMOG immunoglobulin G (IgG) was measured with a noncommercial enzyme-linked immunosorbent assay as described,[12] using rhMOG and horseradish peroxidase–labeled goat antimouse IgG (Thermo Scientific, Waltham, MA).

### *Immunohistochemistry*

Mice were perfused with PBS followed by 10% formalin for fixation. Brains and spinal cords were dissected, paraffin-embedded, and sectioned. Immunohistochemical staining (CD3 and B220) and hematoxylin counterstaining were performed on representative sections.[12] Meningeal and parenchymal $B220^+$ B cells and $CD3^+$ T cells were counted by a blinded observer (R.A.S.).

### *Statistical Analysis*

Mann–Whitney *U* test was used for clinical scores. All other statistical analysis was performed using an unpaired *t* test. A value of $p \leq 0.05$ was considered significant.

## Results

In this investigation, EAE was induced by rhMOG, which requires the participation of both T and B cells.[12] Administration of anti-α4 mAb ameliorated EAE induced by rhMOG protein in C57BL/6 wild-type mice (see Fig 1). To investigate the role of VLA-4 expression on B cells in EAE pathogenesis, we created mice (CD19cre/$\alpha4^{f/f}$) that selectively lack B-cell α4 expression by crossing the CD19cre knockin, which selectively directs gene expression in B cells, but not in T cells or in other leukocytes,[15] (and data not shown) onto the $\alpha4^{f/f}$ background. VLA-4 was not detected on B cells from naive CD19cre/$\alpha4^{f/f}$ mice, whereas $CD4^+$ T cells expressed normal levels (Fig 2A). Similar results were observed in the blood and spleen of rhMOG-immunized mice (data not shown). In comparison to either CD19cre/$\alpha4^{f/WT}$ or CD19cre control mice, CD19cre/$\alpha4^{f/f}$ mice exhibited a reduction in EAE susceptibility, which was most pronounced at the peak of disease (see Fig 2B). Similar results were observed in an independent experiment using $\alpha4^{f/f}$ mice as control group (data not shown).

Although VLA-4 is recognized primarily for its role in endothelial transmigration of leukocytes, the interaction of VLA-4 with its receptor, VCAM-1, can also mediate costimulation.[16] Therefore, selective elimination of B-cell VLA-4 expression could conceivably alter EAE susceptibility via its influence on peripheral immune responses and/or CNS B-cell migration. First, in comparison between control and CD19cre/$\alpha4^{f/f}$ mice with EAE, there were no evident differences in peripheral B-cell activation as measured by expression of CD80 or MHC II (see Fig 2C). Second, there were no significant differences in peripheral numbers of Th1 and Th17 cells, indicating that selective elimination of B-cell VLA-4 expression did not alter in vivo antigen presentation by B cells or other antigen-presenting cells (see Fig 2D). Lastly, as

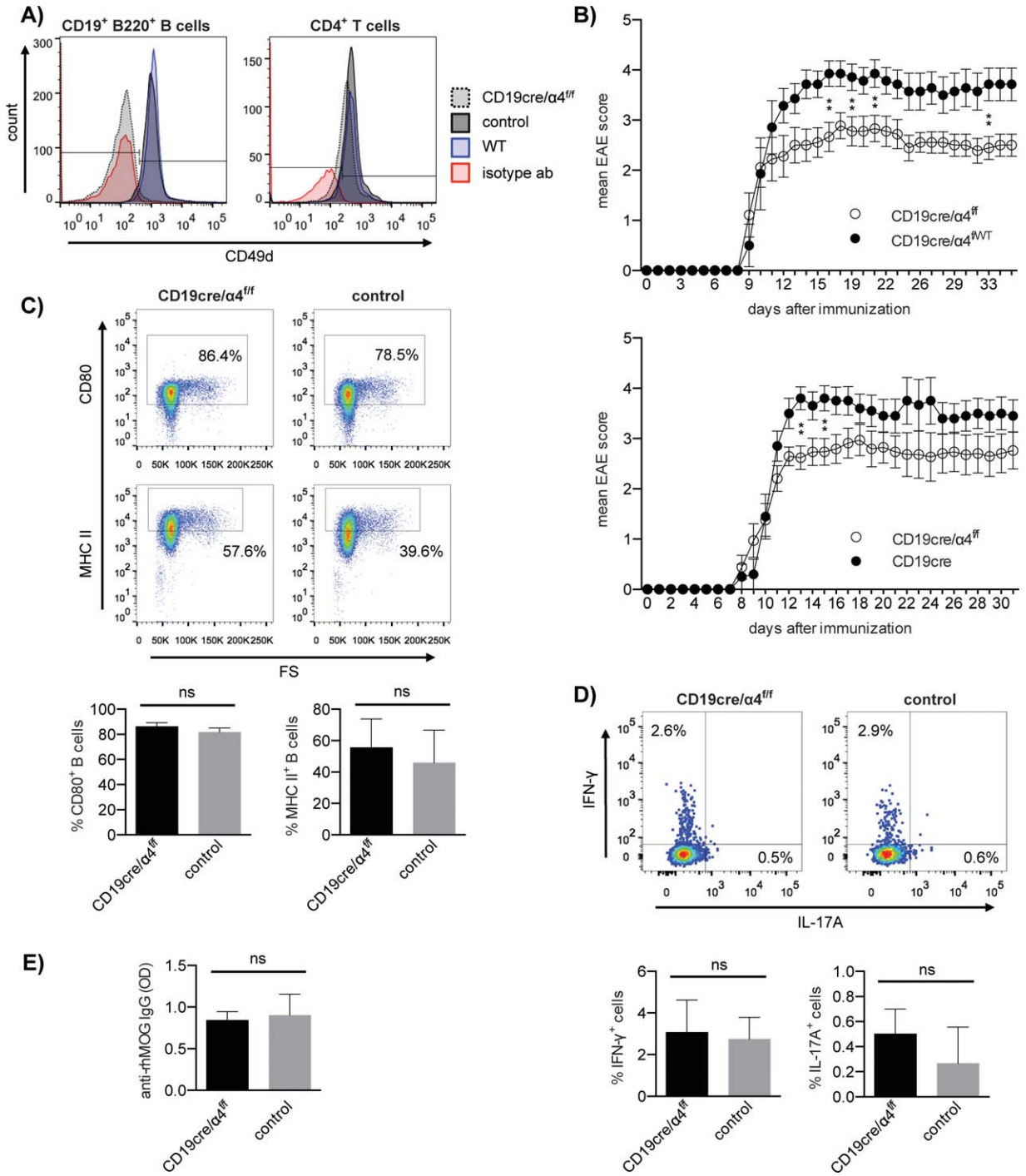


FIGURE 2: Selective α4-deficiency on B cells reduces disease severity in recombinant human myelin oligodendrocyte glycoprotein (rhMOG)-induced experimental autoimmune encephalomyelitis (EAE), whereas peripheral B-cell properties appear to be unchanged. (A) α4 (CD49d) surface expression on $CD19^+B220^+$ B cells and $CD4^+$ T cells in the peripheral blood of naive $CD19cre/\alpha4^{f/f}$ or control mice assessed by flow cytometry. An isotype control antibody (ab) was used to define the negative population, and a healthy wild-type (WT) mouse served as a positive control. Histograms show 1 representative mouse per group (n = 4 mice/group). (B) $CD19cre/\alpha4^{f/f}$ (n = 9 mice) and $CD19cre/\alpha4^{f/WT}$ control mice (n = 7 mice) were immunized with 100μg rhMOG (upper panel). $CD19cre/\alpha4^{f/f}$ (n = 17 mice) and CD19cre control mice (n = 10 mice) were immunized similarly (lower panel). In the lower panel, cumulative data from 2 independent experiments are shown. Similar results were obtained in another independent experiment with an $\alpha4^{f/f}$ control group (not shown). Data shown represent mean disease score ± standard error of the mean (SEM). $^{**}p \leq 0.01$; Mann–Whitney *U* test; differences were significant ($p \leq 0.05$) for days 14 to 35, except day 28, in the upper panel and for days 12 to 16 in the lower panel. (C) Surface activation markers CD80 (B7-1) and MHC II were assessed on B cells (gated on viable $CD19^+B220^+$ cells) by flow cytometry in the spleen at peak of disease (day 14 post-immunization with 100μg rhMOG) in $CD19cre/\alpha4^{f/f}$ or control mice. Similar results were obtained in the blood, at a later time point (31 days postimmunization), and for CD86 (B7-2; not shown). Flow cytometry plots show 1 representative mouse per group. Bar graphs represent mean ± SEM of n = 3 mice/group. ns = not significant; *t* test. (D) Th1 (IFN-γ) and Th17 (IL-17A) T-cell differentiation is measured by intracellular cytokine staining in the spleen 31 days postimmunization. Flow cytometry plots show 1 representative mouse per group (gated on viable $CD4^+$ T cells). Bar graphs represent mean ± SEM of n = 3 mice/group. ns = not significant; *t* test. Similar results were obtained at peak of disease (14 days postimmunization; not shown). (E) Serum immunoglobulin G (IgG) antibodies against rhMOG were detected by enzyme-linked immunosorbent assay. The optical density (OD) at 450nm is indicated. Serum was obtained on day 31 postimmunization with 100μg rhMOG in $CD19cre/\alpha4^{f/f}$ or control mice (n = 3 mice/group) and diluted 1:9,000 before analysis. ns = not significant; *t* test. Control mice were CD19cre in all experiments, except in B, upper panel, where they were $CD19cre/\alpha4^{f/WT}$. FS, forward scatter.

immunization with rhMOG protein can elicit secretion of pathogenic (demyelinating) antibodies,[17] we examined MOG-specific antibodies in control and CD19cre/$\alpha4^{f/f}$ mice with rhMOG-induced EAE. No significant differences in anti-rhMOG IgG titers were observed (see Fig 2E). By these measures, selective elimination of VLA-4 expression did not significantly influence proinflammatory peripheral B-cell function.

Our observation that selective B-cell VLA-4 deficiency reduced EAE susceptibility, but did not impact peripheral immune responses, indicates its primary effect may be attributed to its influence on B-cell migration. Flow cytometric analysis revealed a significant reduction in absolute numbers of CNS-infiltrating $CD19^+B220^+$ B cells in CD19cre/$\alpha4^{f/f}$ mice (Fig 3A), findings that were confirmed by immunohistological staining. Reduction of $B220^+$ ($CD45R^+$) B cells was most pronounced in the meninges, which contained the majority of CNS-infiltrating B cells in control mice with EAE (see Fig 3B). There were no clearly detectable differences in the histological appearances of microglia or astrocytes in lesions in CD19cre/$\alpha4^{f/f}$ and control mice with EAE.

The paucity of CNS B cells in CD19cre/$\alpha4^{f/f}$ mice was also associated with a decrease in the total number of CNS-infiltrating hematopoietic cells ($CD45^{hi}$), $CD45^{hi}CD11b^+$ macrophages, and $CD45^{hi}CD11b^-CD4^+$ T cells (see Fig 3C–E). Similarly, meningeal $CD3^+$ T cells were reduced in CD19cre/$\alpha4^{f/f}$ mice, as measured by immunohistochemistry (see Fig 3B). Moreover, the frequency of Th17 but not Th1 cells was significantly reduced in the CNS of CD19cre/$\alpha4^{f/f}$ mice with EAE (see Fig 3F). In summary, our results indicate that conditional B-cell VLA-4 deficiency inhibited CNS B-cell accumulation, reduced recruitment of other effector leukocytes, and suppressed development of EAE.

## Discussion

Since 1992, when it was reported that anti–VLA-4 antibody treatment could prevent EAE,[4] providing the foundation for advancing development of natalizumab in MS,[1] mechanistic studies of anti–VLA-4 therapy have focused primarily on its influence on T cells. However, B-cell VLA-4 expression promotes B-cell trafficking across the BBB,[8,18] and clinical studies suggest this process may be inhibited by natalizumab.[9,10] Clinical investigations demonstrating pronounced beneficial effects of anti-CD20 B-cell–depleting mAbs in the treatment of RRMS[6] have renewed interest in understanding the cellular and humoral contribution of B cells in MS pathogenesis and response to therapy.[18] To assess the influence of VLA-4 expression on B cells in CNS autoimmune disease, we created mice that were deficient in VLA-4 on B cells only and studied these mice in an EAE model that requires the participation of B cells.[12] Mice containing VLA-4–deficient B cells demonstrated a modest but significant and reproducible reduction in EAE susceptibility, which was associated with a marked decrease in CNS B cells as well as significantly fewer CNS proinflammatory Th17 cells and macrophages. In contrast, selective B-cell VLA-4 deficiency did not alter peripheral B-cell activation, modulate peripheral T-cell function, or reduce anti-MOG antibodies. Thus, our results indicate that the reduction in both clinical EAE and other CNS leukocytes was due to the selective block in accumulation of B cells within the CNS, and not an effect on peripheral immunity. Similarly, reduction in VLA-4–dependent CNS B-cell recruitment may contribute to the benefit of natalizumab therapy in MS.

The proinflammatory Th17 subset was described in 2006, 2 years after the initial approval of natalizumab. Of interest, 1 recent study demonstrated that anti–VLA-4 prevented EAE induced by myelin-specific Th1, but not Th17 cells.[5] VLA-4–deficient Th17 cells induced an atypical form of EAE, which was attributed to their ability to enter the brain through use of the integrin LFA-1 ($\alpha L\beta2$ integrin) instead of VLA-4. Our results, demonstrating that selective B-cell VLA-4 deficiency was associated with a reduction in CNS Th17 cells, are complementary to the study demonstrating that anti–VLA-4 directly inhibited CNS accumulation of Th1 cells.[5] Anti–VLA-4 mAb may also indirectly block accumulation of proinflammatory Th17 cells through binding of VLA-4 on B cells that may enter the CNS in a VLA-4–dependent manner.

Although effective in MS, natalizumab has exacerbated neuromyelitis optica (NMO).[19] There may be several contributing factors. First, NMO is a humoral autoimmune disease that is associated with aquaporin-4 (AQP4)-specific antibodies, which are generated primarily outside of the CNS. We observed that B-cell VLA-4 deficiency did not influence peripheral humoral responses. Second, neutrophils are abundant in NMO lesions[20] and are thought to contribute to NMO pathogenesis. Th17 cells can promote CNS neutrophil accumulation,[21] and AQP4-specific T cells in NMO exhibit Th17 polarization.[22] Third, as resting human neutrophils do not express VLA-4,[23] CNS migration of this leukocyte subset may be unopposed by natalizumab. Thus, one can speculate that natalizumab treatment, which may be less effective in Th17-mediated CNS disease,[5] could facilitate CNS neutrophil accumulation in NMO.

Possibly the most important observation in our study is that selective B-cell VLA-4 deficiency was associated with reduced CNS recruitment of other effector cells. This finding may be particularly relevant to therapies that selectively target B cells. In this regard, despite knowledge that B cells constitute a minority of

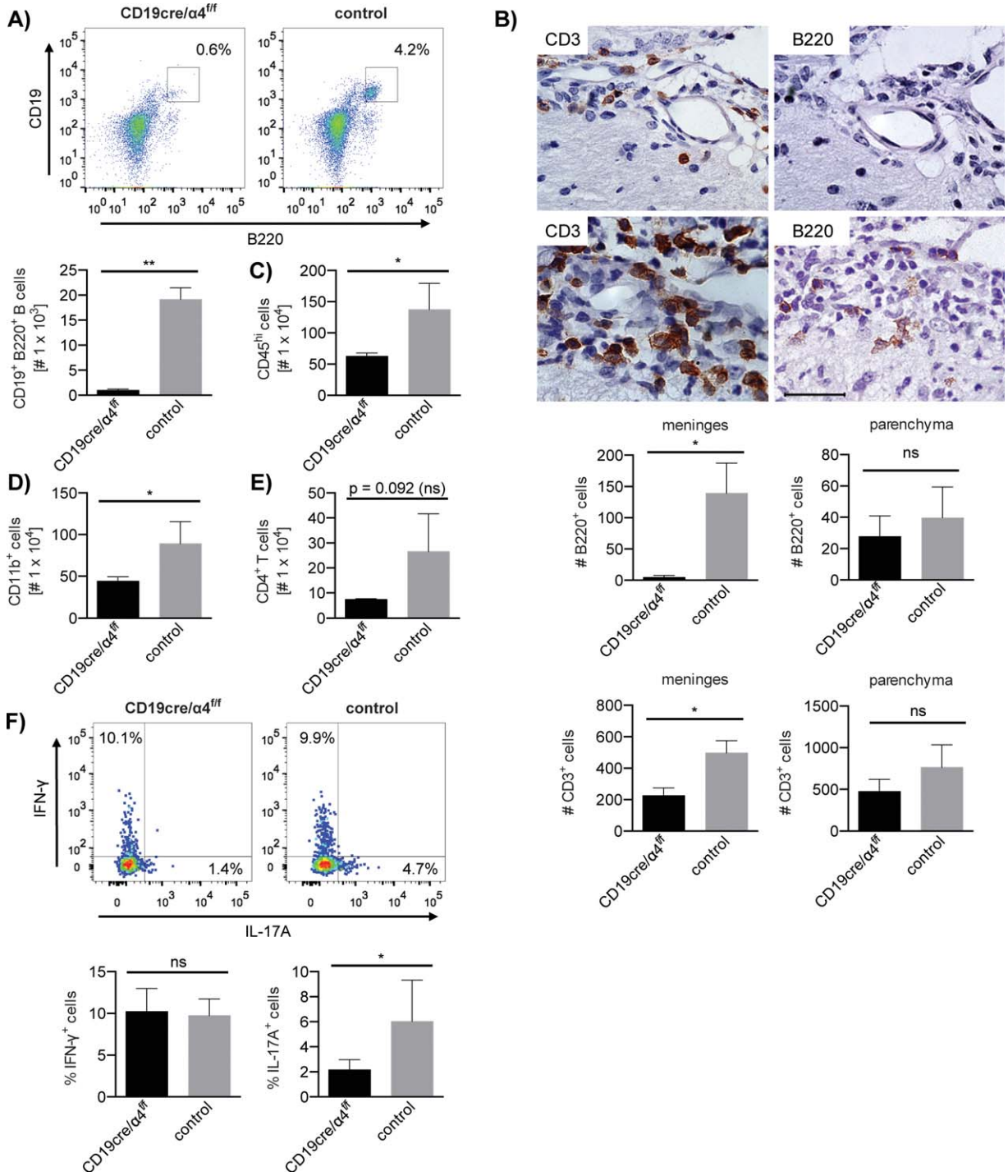


FIGURE 3: Selective B-cell α4 deficiency impairs B-cell migration into the central nervous system (CNS) in recombinant human myelin oligodendrocyte glycoprotein (rhMOG)-induced experimental autoimmune encephalomyelitis (EAE) and reduces CNS recruitment of $CD11b^+$ macrophages and T cells. CD19cre/$\alpha4^{f/f}$ or control mice (CD19cre) were immunized with 100μg rhMOG. (A) Frequency and absolute numbers of CNS-infiltrating $CD19^+B220^+$ B cells at peak of disease (day 14) in CD19cre/$\alpha4^{f/f}$ or control mice. Flow cytometry plots show 1 representative mouse (gated on viable $CD45^{hi}CD11b^-$ lymphocytes). Similar results were obtained at a later time point (31 days postimmunization; not shown). n = 3 mice/group. (B) Leptomeningeal infiltrates in the brains of CD19cre/$\alpha4^{f/f}$ (upper picture panels) and control (lower picture panels) mice with EAE 31 days postimmunization. CD19cre/$\alpha4^{f/f}$ and control mice have abundant $CD3^+$ T cells (left panels); $B220^+$ B cells are present in the control mouse but not the CD19cre/$\alpha4^{f/f}$ mouse (right panels). Immunohistochemistry was done with hematoxylin counterstain. Scale bar = 20μm. Bar graphs show mean number of $B220^+$ B cells (upper graph panels) and $CD3^+$ T cells (lower graph panels) in the meninges (left panels) or parenchyma (right panels) of n = 3–4 mice/group. (C) Nonresident $CD45^{hi}$ cells (gated on viable cells), (D) $CD11b^+$ macrophages (gated on viable $CD45^{hi}$ cells), and (E) $CD4^+$ T cells (gated on viable $CD45^{hi}CD11b^-$ cells) in the CNS at peak of disease (day 14 postimmunization). n = 3 mice/group. (F) Th1 (IFN-γ secreting) and Th17 (IL-17A secreting) T-cell differentiation is measured by intracellular cytokine staining in the CNS 31 days postimmunization. Flow cytometry plots show 1 representative mouse per group (gated on viable $CD4^+$ T cells). n = 6 mice/group (pooled from 2 independent experiments). Similar results were obtained at peak of disease (14 days postimmunization; not shown). All data shown in bar graphs are mean frequency (%) or total number (#) ± standard error of the mean. *$p \le 0.05$, **$p \le 0.01$; ns = not significant; *t* test.

infiltrating cells in characteristic lesions of RRMS,[24] B-cell depletion in RRMS results in a marked reduction in gadolinium-enhancing CNS lesions.[6] Anti-CD20 B-cell depletion in EAE significantly reduces meningeal B cells.[11] Similarly, the observation that selective B-cell VLA-4 deficiency not only inhibited accumulation of meningeal B cells, but also reduced CNS recruitment of other leukocytes, may provide an additional mechanism for reduction in CNS inflammatory lesions by therapies that eliminate B cells. Our study, which highlights the importance of VLA-4 on B cells in the pathogenesis of CNS autoimmunity, should promote investigations to further understand how CNS B cells influence cellular immune responses in situ.

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## Authorship

K.L.-H. designed research, performed the experiments, analyzed data, and wrote the paper. S.A.S. performed the experiments, analyzed data, and discussed the results at all stages. C.C.A.B. provided reagents. R.A.S. performed the neuropathologic analysis. S.S.Z. designed research, analyzed data, wrote the paper, and supervised the study.

## Potential Conflicts of Interest

S.S.Z.: consultancy, honoraria, Biogen Idec, EMD Serono, Genzyme, Novartis, Questcor, Roche, Teva Pharmaceuticals; data safety monitoring board, Lilly, BioMS, Teva, Opexa Therapeutics.

## References

1. Polman CH, O'Connor PW, Havrdova E, et al. A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. N Engl J Med 2006;354:899–910.
2. Baron JL, Madri JA, Ruddle NH, et al. Surface expression of alpha 4 integrin by CD4 T cells is required for their entry into brain parenchyma. J Exp Med 1993;177:57–68.
3. Theien BE, Vanderlugt CL, Eagar TN, et al. Discordant effects of anti-VLA-4 treatment before and after onset of relapsing experimental autoimmune encephalomyelitis. J Clin Invest 2001;107:995–1006.
4. Yednock TA, Cannon C, Fritz LC, et al. Prevention of experimental autoimmune encephalomyelitis by antibodies against alpha 4 beta 1 integrin. Nature 1992;356:63–66.
5. Rothhammer V, Heink S, Petermann F, et al. Th17 lymphocytes traffic to the central nervous system independently of alpha4 integrin expression during EAE. J Exp Med 2011;208:2465–2476.
6. Hauser SL, Waubant E, Arnold DL, et al. B-cell depletion with rituximab in relapsing-remitting multiple sclerosis. N Engl J Med 2008;358:676–688.
7. Niino M, Bodner C, Simard ML, et al. Natalizumab effects on immune cell responses in multiple sclerosis. Ann Neurol 2006;59:748–754.
8. Alter A, Duddy M, Hebert S, et al. Determinants of human B cell migration across brain endothelial cells. J Immunol 2003;170:4497–4505.
9. Krumbholz M, Meinl I, Kumpfel T, et al. Natalizumab disproportionately increases circulating pre-B and B cells in multiple sclerosis. Neurology 2008;71:1350–1354.
10. Stuve O, Marra CM, Jerome KR, et al. Immune surveillance in multiple sclerosis patients treated with natalizumab. Ann Neurol 2006;59:743–747.
11. Weber MS, Prod'homme T, Patarroyo JC, et al. B-cell activation influences T-cell polarization and outcome of anti-CD20 B-cell depletion in central nervous system autoimmunity. Ann Neurol 2010;68:369–383.
12. Molnarfi N, Schulze-Topphoff U, Weber MS, et al. MHC class II-dependent B cell APC function is required for induction of CNS autoimmunity independent of myelin-specific antibodies. J Exp Med 2013;210:2921–2937.
13. Pierson ER, Stromnes IM, Goverman JM. B cells promote induction of experimental autoimmune encephalomyelitis by facilitating reactivation of T cells in the central nervous system. J Immunol 2014;192:929–939.
14. Scott LM, Priestley GV, Papayannopoulou T. Deletion of alpha4 integrins from adult hematopoietic cells reveals roles in homeostasis, regeneration, and homing. Mol Cell Biol 2003;23:9349–9360.
15. Rickert RC, Roes J, Rajewsky K. B lymphocyte-specific, Cre-mediated mutagenesis in mice. Nucleic Acids Res 1997;25:1317–1318.
16. Udagawa T, Woodside DG, McIntyre BW. Alpha 4 beta 1 (CD49d/CD29) integrin costimulation of human T cells enhances transcription factor and cytokine induction in the absence of altered sensitivity to anti-CD3 stimulation. J Immunol 1996;157:1965–1972.
17. Marta CB, Oliver AR, Sweet RA, et al. Pathogenic myelin oligodendrocyte glycoprotein antibodies recognize glycosylated epitopes and perturb oligodendrocyte physiology. Proc Natl Acad Sci U S A 2005;102:13992–13997.
18. Lee-Chang C, Zephir H, Top I, et al. B-cell subsets up-regulate alpha4 integrin and accumulate in the cerebrospinal fluid in clinically isolated syndrome suggestive of multiple sclerosis onset. Neurosci Lett 2011;487:273–277.
19. Barnett MH, Prineas JW, Buckland ME, et al. Massive astrocyte destruction in neuromyelitis optica despite natalizumab therapy. Mult Scler 2012;18:108–112.
20. Lucchinetti CF, Mandler RN, McGavern D, et al. A role for humoral mechanisms in the pathogenesis of Devic's neuromyelitis optica. Brain 2002;125(pt 7):1450–1461.

21. Herges K, de Jong BA, Kolkowitz I, et al. Protective effect of an elastase inhibitor in a neuromyelitis optica-like disease driven by a peptide of myelin oligodendroglial glycoprotein. Mult Scler 2012; 18:398–408.

22. Varrin-Doyer M, Spencer CM, Schulze-Topphoff U, et al. Aquaporin 4-specific T cells in neuromyelitis optica exhibit a Th17 bias and recognize Clostridium ABC transporter. Ann Neurol 2012;72:53–64.

23. Bochner BS, Luscinskas FW, Gimbrone MA Jr, et al. Adhesion of human basophils, eosinophils, and neutrophils to interleukin 1-activated human vascular endothelial cells: contributions of endothelial cell adhesion molecules. J Exp Med 1991;173:1553–1557.

24. Kutzelnigg A, Lassmann H. Pathology of multiple sclerosis and related inflammatory demyelinating diseases. Handb Clin Neurol 2014;122:15–58.