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Activation of kinin receptor B1 limits encephalitogenic T lymphocyte recruitment to the central nervous system

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Abstract

Previous proteomic and transcriptional analyses of multiple sclerosis lesions[1, 2, 3] revealed modulation of the renin-angiotensin and the opposing kallikrein-kinin pathways. Here we identify kinin receptor B1 (Bdkrb1) as a specific modulator of immune cell entry into the central nervous system (CNS). We demonstrate that the Bdkrb1 agonist R838 (Sar-[D-Phe]des-Arg^9-bradykinin) markedly decreases the clinical symptoms of experimental autoimmune encephalomyelitis (EAE) in SJL mice[4, 5, 6], whereas the Bdkrb1 antagonist R715 (Ac-Lys-[D-βNal^7, Ile^8]des-Arg^9-bradykinin) resulted in earlier onset and greater severity of the disease. Bdkrb1-deficient (*Bdkrb1*$^{-/-}$) C57BL/6 mice[7] immunized with a myelin oligodendrocyte glycoprotein fragment, $MOG_{35–55}$, showed more severe disease with enhanced CNS-immune cell infiltration. The same held true for mixed bone marrow–chimeric mice reconstituted with *Bdkrb1*$^{-/-}$ T lymphocytes, which showed enhanced T helper type 17 (T_H17) cell invasion into the CNS. Pharmacological modulation of Bdkrb1 revealed that *in vitro* migration of human T_H17 lymphocytes across blood-

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Author Contributions

F.Z. and M.B. initiated the investigation of EAE in *Bdkrb1*$^{-/-}$ mice, previously characterized by I.S., M.A.M. and M.B. L.S., M.H.H. and A.P. contributed screens to the investigations. U.S.-T. performed EAE in *Bdkrb1*$^{-/-}$ mice including immunological read-outs under the supervision of O.A. T.P. and A.S. performed histological analysis. A.P., M.P. and U.S.-T. performed treatment of EAE with Bdkrb1 agonists and antagonists. U.S.-T. initiated EAE in *Bdkrb1*$^{-/-}$ bone marrow chimeras, and U.S.-T. together with V.S. and M.P. performed these investigations, including immunological analyses. U.S.-T., T.P., F.S. and I.B. investigated Bdkrb1 expression and small GTPase activity pattern in T cells. J.H., V.S. and U.S.-T. performed mouse T cell migration assays using multiphoton microscopy, and I.I. and A.P. performed human T_H1 and T_H17 cell migration assays. J.V.H. and T.P. performed immunohistochemical analysis of Bdkrb1 expression in tissue from individuals with multiple sclerosis. All authors analyzed the data; F.Z. and O.A. wrote the manuscript with U.S.-T.; F.Z., O.A., A.P., L.S. and M.B. edited the manuscript.

brain barrier endothelium is regulated by this receptor. Taken together, these results suggest that the kallikrein-kinin system is involved in the regulation of CNS inflammation, limiting encephalitogenic T lymphocyte infiltration into the CNS, and provide evidence that Bdkrb1 could be a new target for the treatment of chronic inflammatory diseases such as multiple sclerosis.

Introduction

Three independent mRNA and protein screens performed on inflammatory lesions of the central nervous system (CNS) (ref. 1,2) and on blood-brain barrier (BBB) membrane microdomains[3] revealed unexpected changes in both the renin-angiotensin and the kallikrein-kinin systems (KKS), two pathways that are known mainly for their role in blood pressure regulation and are proposed to counterbalance each other[8]. For example, in a large-scale analysis of mRNA transcripts from multiple sclerosis lesions, prokallikrein KLKB1 was more abundant in samples from all lesion types relative to control brain samples, whereas the kallikrein inhibitor kallistatin was less abundant[1]. In a proteomic study, neurolysin, which hydrolyzes bradykinin, was present in chronic active and chronic plaques, whereas lysosomal carboxypeptidase, which cleaves angiotensins as well as des-Arg^9-bradykinin, was found only in active plaques[2]. Kinins belong to a family of bioactive octa- to decapeptides generated from kininogens in a stepwise cleavage process[9]. Their biological activities are mediated via two pharmacologically distinct G protein–coupled receptors: kinin receptor B1 (Bdkrb1), which under physiological conditions is not found in immune cells, and the ubiquitously expressed kinin receptor B2 (Bdkrb2). In the mouse EAE model of multiple sclerosis, we found upregulation of Bdkrb1, bradykinin, des-Arg^9-bradykinin, kallikrein-1 and kallikrein-6 as well as low-molecular-weight kininogens (KNGL) in CNS tissue and the cerebrospinal fluid (Supplementary Fig. 1). Because kallikreins cleave the precursor kininogens to bradykinin, which mediates its effect primarily via Bdkrb2, and subsequent proteases generate des-Arg^9-bradykinin, acting via Bdkrb1, we assumed that the kallikrein-kinin system may contribute to chronic autoimmune neuroinflammation. Bdkrb1 expression has been found not only on brain endothelial cells[10] but also on T lymphocytes from individuals with multiple sclerosis[11, 12] and, as shown here, on parenchymal CD3$^+$ T cells within perivascular lesions (Fig. 1a).

In light of the known involvement of such T cells in the initiation of a myelin-specific immune attack, we induced EAE via adoptive transfer of activated proteolipid protein $(PLP)_{139–151}$–specific lymphocytes into naive SJL/J mice[5, 6]. We treated recipients with the Bdkrb1 agonist R838 (1 mg kg^{-1}), the Bdkrb1 antagonist R715 (1 mg kg^{-1}), or with vehicle, from day 0 to day 10 after transfer. Activation of Bdkrb1 resulted in a significantly lower maximum clinical disease severity and milder clinical deficits than occurred in vehicle-treated mice ($P < 0.01$), whereas blocking of Bdkrb1 led to accelerated disease onset ($P = 0.01$; Fig. 1b). To analyze the potential of Bdkrb1 activation for therapeutic use, we immunized SJL/J mice to produce relapsing-remitting EAE and treated them after disease onset with R838 (1 mg kg^{-1}) or vehicle. Bdkrb1 activation resulted in a significantly attenuated clinical disease course ($P < 0.05$; Fig. 1c).

To elucidate the role of Bdkrb1 in chronic CNS neuroinflammation, we immunized Bdkrb1-deficient (*Bdkrb1*$^{-/-}$) C57BL/6 mice[7] or wild-type (WT) C57BL/6 controls with myelin oligodendrocyte glycoprotein $(MOG)_{35–55}$. *Bdkrb1*$^{-/-}$ mice had both a significantly greater maximum clinical disease severity ($P < 0.05$) and greater clinical deficits (Fig. 1d). To rule out a possible compensatory role of the constitutively expressed Bdkrb2 in *Bdkrb1*$^{-/-}$ mice, we next immunized C57BL/6 mice lacking the genes encoding kinin receptors B1 and B2 (ref. 13) (*Bdkrb1*$^{-/-}$;*Bdkrb2*$^{-/-}$ mice) with $MOG_{35–55}$. Despite a recently reported potential proinflammatory role of Bdkrb2 (ref. 14), we found no difference between the EAE disease courses in *Bdkrb1*$^{-/-}$;*Bdkrb2*$^{-/-}$ and *Bdkrb1*$^{-/-}$ mice ($P > 0.05$), which indicates that Bdkrb1 has a dominant role in influencing the course of the disease (Fig. 1e).

Histological examination at day 23 after immunization (Fig. 1d) revealed large inflammatory infiltrates, including an elevated number of activated microglia and macrophages throughout the brain stem and spinal cord, in *Bdkrb1*$^{-/-}$ mice with EAE as compared to control WT mice with EAE (Fig. 2a,b). In parallel, the extent of demyelination and axonal damage, the main pathological feature of multiple sclerosis[15], was greater in the *Bdkrb1*$^{-/-}$ mice (Fig. 2c,d). In contrast to the strong effects of Bdkrb1 on disease severity and histological parameters, however, the myelin-specific T cell response as well as the activation status of T cells in the peripheral immune organs of the *Bdkrb1*$^{-/-}$ mice were unaltered as compared to those of the controls (Fig. 2e–h). Moreover, the proportion of $FoxP3^+$ $CD25^+$ regulatory T cells[16] within the $CD4^+$ lymphocyte population from draining lymph nodes was similar in the *Bdkrb1*$^{-/-}$ mice with EAE (13.1% ± 1.2) and the WT control mice with EAE (11.9% ± 0.8.; $P > 0.05$).

To further dissect which cell type is influenced by Bdkrb1 signaling during the disease, we generated bone marrow–chimeric mice[17] by injecting C57BL/6-CD45.2 bone marrow into lethally irradiated C57BL/6-CD45.1 recipient mice. In the first group, we reconstituted lethally irradiated congenic C57BL/6-CD45.1 mice with a mixed bone marrow consisting of a 5:1 ratio of T cell receptor (TCR) β-chain (*Tcrb*$^{-/-}$)-deficient and *Bdkrb1*-deficient bone marrow donors[18, 19]. The resulting *Bdkrb1*$^{-/-}$*Tcrb*$^{-/-}$ → WT mixed-bone-marrow chimera lacked Bdkrb1 on T cells. In a second group, we gave irradiated C57BL/6-CD45.1 mice *Bdkrb1*$^{-/-}$ bone marrow only, in which all reconstituted immune cells were devoid of Bdkrb1 (*Bdkrb1*$^{-/-}$ → WT). To rule out a bias through irradiation and BM reconstitution, we gave a third group of mice a 5:1 ratio of *Tcrb*$^{-/-}$ and C57BL/6 (WT) bone marrow (WT *Tcrb*$^{-/-}$ → WT). Two months after bone marrow grafting, we assessed reconstitution by FACS analysis of peripheral blood and PCR analysis of MACS-sorted $CD3^+$ and $CD11b^+$ cells (Supplementary Fig. 2a). Immunization with $MOG_{35–55}$ to produce active EAE revealed an earlier onset and markedly greater clinical deficits in *Bdkrb1*$^{-/-}$ → WT bone marrow chimeras as compared to WT *Tcrb*$^{-/-}$ → WT EAE controls ($P < 0.05$; Fig. 3a). This pattern was similar to the EAE disease courses in germline *Bdkrb1*$^{-/-}$ mice as compared to WT C57BL/6 controls (Fig. 1d). We found that mean EAE disease scores in *Bdkrb1*$^{-/-}$*Tcrb*$^{-/-}$ → WT were virtually indistinguishable from *Bdkrb1*$^{-/-}$ → WT EAE, pointing to the importance of T cell–expressed Bdkrb1 in CNS inflammation and to a somewhat accessory role for macrophage or endothelial kinin receptor B1 expression. The results of *ex vivo* proliferation assays of lymph node–derived T cells in response to stimulation with $MOG_{35–55}$ were the same for all three groups (Supplementary Fig. 2b),

indicating that antigen-specific immune responses against the encephalitogenic peptide were not compromised by kinin receptor B1 deficiency (Fig. 3a).

Next, we reconstituted *Bdkrb1*-deficient mice with a mix of TCR-β-chain–deficient and C57BL/6-CD45.1 bone marrow (WT $Tcrb^{-/-} \rightarrow Bdkrb1^{-/-}$). After immunization with $MOG_{35–55}$, EAE course in these mice was compared to that in $Bdkrb1^{-/-} \rightarrow$ WT as well as $Bdkrb1^{-/-} \rightarrow Bdkrb1^{-/-}$ mice (Fig. 3b). Disease courses were comparable in mice deficient for *Bdkrb1* in the immune system; however, the disease was ameliorated in the mice reconstituted with WT T cells ($P < 0.05$). These data demonstrate that the protective effect of Bdkrb1 is mediated by its expression on T cells and exclude a possible contribution of Bdkrb1 expression in the CNS.

We therefore investigated whether Bdkrb1 would modulate CNS inflammation by affecting the migration of encephalitogenic T cells across the BBB (Fig. 3). To directly compare and quantify the homing capacity to the CNS *in vivo*, we reconstituted C57BL/6 mice with mixed bone marrow consisting of a 1:1 ratio of WT (C57BL/6-CD45.1) and $Bdkrb1^{-/-}$ (C57BL/6-CD45.2) bone marrow. Infiltration of $Bdkrb1^{-/-}$ T cells into the CNS, normalized to the reconstitution rate, was significantly higher than infiltration of WT T cells at disease peak (Fig. 3c). Moreover, we found more $CD4^+$ T cells in the CNS when encephalitogenic $Bdkrb1^{-/-}$ T cells, as opposed to WT T cells, were transferred to $Rag1^{-/-}$ recipients (Fig. 4a). In line with this, we observed an EAE incidence of 75% when transferring $Bdkrb1^{-/-}$ T cells but only of 33% with WT T cells (Supplementary Table 1).

Using an *in vitro* Transwell assay, we found that $PLP_{139–151}$- and $MOG_{35–55}$-specific $CD4^+$ T cells isolated from immunized SJL/J and $Bdkrb1^{-/-}$ mice were attracted to migrate through a monolayer of transformed mouse brain-derived endothelial cells (ref. 20) in a CXCL12 gradient–dependent manner. Although engagement of Bdkrb1 by R838 considerably reduced the number of migrated T cells, the additional application of the Bdkrb1 antagonist R715 restored migration. By contrast, the migration of $Bdkrb1^{-/-}$ cells was not altered (Fig. 3d). In PLP-specific T cells treated with R838, we did not find any alterations in adhesion molecule expression (LFA-1, VLA-4, ICAM, ALCAM, VCAM-1 and CD6; see Supplementary Table 2 for oligonucleotide primers). In contrast, we observed a markedly decreased polymerization of F-actin upon short-term Bdkrb1 activation (Fig. 3e). Moreover, in $CD4^+$ T cells the activity of the small GTPase RhoA—which controls T cell migration[21]—was also downregulated (Fig. 3f). Thus, activation of the G protein–coupled kinin receptor B1 directly regulates signaling events that are important for T lymphocyte motility.

Bdkrb1 seems to affect CNS inflammation and control the migration of proinflammatory T cells across the BBB into the CNS. Indeed, analysis of immune cells recovered from the CNS of mice with EAE revealed that after adoptive transfer of $Bdkrb1^{-/-}$ into $Rag1^{-/-}$ mice, the proportion of $CD4^+$ T cells within the CNS-infiltrating immune cells was significantly higher than that in mice that had received only WT T cells (Fig. 4a). Notably, within the same CNS-derived $CD4^+$ T cell population, the proportion of IL-17^+ $CD4^+$ T lymphocytes —regarded as crucial for the initiation and maintenance of autoimmune neuroinflammation[22]—was greater in $Bdkrb1^{-/-}$ mice with EAE, whereas the proportion of

IFN-γ–producing T cells was comparable, relative to those in WT control mice with EAE (Fig. 4b,c).

To investigate whether Bdkrb1 influences the development of IL-17–producing T helper type 17 (T_H17) cells (ref. 23) or the migration of T_H17 lymphocytes to the CNS, we used ovalbumin-transgenic C57BL/6 OT-2 mice and PLP-specific T cells isolated from EAE mice. The proportion of OT-2- and PLP-specific $CD4^+$ T_H17 lymphocytes generated with IL-23, IL-6, anti–IL-4, anti–IL-12 and TGF-β (ref. 24) did not differ in the presence or absence of the Bdkrb1 agonist R838 (Supplementary Fig. 3), demonstrating that Bdkrb1 has no impact on the generation of antigen-specific T_H17 lymphocytes. However, in the CNS of *Bdkrb1*$^{-/-}$*Tcrb*$^{-/-}$ → WT mice with EAE, in which the T cell population was deficient for Bdkrb1, the proportion of IL-17^+ subsets was markedly greater than among cells isolated from WT *Tcrb*$^{-/-}$ → WT mice with EAE. We did not observe any differences in the proportions of either IFN-γ–producing T cells or $FoxP3^+$ $CD25^+$ T cells (Fig. 4d–f).

Using *in vitro* migration assays, we next investigated the effect of R838 and R715 on the migration of mouse and human memory $CD45RO^+$ T_H17 and T_H1 lymphocytes (Supplementary Fig. 4)[25]. Addition of R838 resulted in a significant reduction in the migration of T_H17, but not T_H1, lymphocytes toward a CXCL12 chemokine gradient for mouse T cells and across human brain-derived microvascular endothelial cells for human T cells (Fig. 4g and Supplementary Fig. 5). These data also point to the important contribution of kinin receptor B1 as a modulator of the recruitment of pathogenic lymphocytes to the CNS. To find a possible explanation for the specific effect on T_H17 cells, we analyzed the expression of Bdkrb1 in T_H1 and T_H17 cells generated from different transgenic mouse strains (2d2, OT-2) as well as from human sources. In fact, T_H17 cells showed a markedly higher expression of Bdkrb1 than did T_H1 cells (Fig. 4h and Supplementary Fig. 5).

To finally demonstrate an influence of Bdkrb1 signaling on the migration pattern of T_H17 cells, we treated fluorescence-labeled mouse T_H17 lymphocytes with the Bdkrb1 modulators or vehicle before allowing them to infiltrate into syngeneic hippocampal slice cultures[26]. Two-photon microscopy analysis revealed a lower mean velocity (Supplementary Fig. 6) and reduced infiltrative behavior upon Bdkrb1 activation (Fig. 4i,j and Supplementary Movies 1, 2,3).

Altogether, our data suggest the existence of a hitherto unknown endogenous control mechanism that limits harmful antigen-specific immune responses targeting the CNS, and they define the kinin receptor B1 as an important regulator for the homing of encephalitogenic T lymphocytes into the CNS. Progress toward the development of new therapies for chronic inflammation is urgently needed[27, 28]. In principle, activation of the body's innate control mechanisms, such as those identified here, may offer certain advantages over previous strategies aimed at selectively blocking structures involved in inflammatory CNS infiltration[29]. Newly developed kinin receptor agonists with improved pharmacological properties in regard to half-life and receptor specificity may provide promising new tools for the therapeutic manipulation of the kallikrein-kinin system in immune-mediated diseases. For the related renin-angiotensin system, blockade by an angiotensin-converting enzyme inhibitor suppresses inflammation in the CNS (L.S.,

personal communication). Thus, modification of major systems known for their cardiovascular roles may open the way toward new therapies for chronic inflammatory diseases such as multiple sclerosis.

Online methods

EAE

We induced active EAE (in C57BL/6 and SJL/J) and adoptive transfer EAE (in SJL/J) as previously described[4, 5, 6]. *Bdkrb1*-deficient mice on the SV129 background were backcrossed to C57BL/6 to produce F_{10} offspring. For pharmacological modulation, we used the Bdkrb1 agonist R838 or antagonist R715 (Biosynthan). For adoptive transfer EAE in C57BL/6 $Rag1^{-/-}$ mice, we immunized C57BL/6 (WT) and $Bdkrb1^{-/-}$ mice using 200 μg $MOG_{35–55}$ and 200 ng pertussis toxin. Twelve days later, cells from draining lymph nodes and splenocytes were depleted of $CD8^+$ T cells (MACS, Miltenyi), re-stimulated for 72 h (15 μg ml^{-1} $MOG_{35–55}$), and intravenously injected into naive C57BL/6 $Rag1^{-/-}$ recipients (1×10^7 cells per mouse). All animal experiments were conducted according to protocols approved by the local Canadian and German animal welfare committees.

Generation of bone marrow chimeras

We generated conventional and mixed bone marrow chimeras as described previously[17, 18, 19]. Recipient mice were lethally irradiated with 1,100 cGy (split dose) and reconstituted with 12×10^6 donor bone marrow cells devoid of $CD90^+$ T cells (MACS). To track encephalitogenic $Bdkrb1^{-/-}$ and WT T cells into the CNS, cells were followed after disease induction and the proportion of CNS-derived T cells was analyzed by FACS. We quantified WT and $Bdkrb1^{-/-}$ T cells recovered from the CNS by using flow cytometry for CD45.1 and CD45.2. We checked reconstitution by analyzing donor-derived peripheral blood mononuclear cells for CD45.2 expression (95% purity). We determined the loss of Bdkrb1 in T cells from mixed bone marrow chimeras ($Bdkrb1^{-/-}Tcrb^{-/-} \rightarrow$ C57BL/6-CD45.1) by PCR (25 cycles) of magnetically sorted $CD3^+$ cells (95% purity Dynal CD3 Sort) from spleens. We analyzed Bdkrb1 expression on non-$CD3^+$ cells by magnetic enrichment of $CD11b^+$ cells (95% purity, Miltenyi).

Generation of T_H17 and T_H1 cells

Mouse cells—$CD4^+CD62L^+$ naive T cells were magnetically sorted from OT-2 mice and stimulated with ovalbumin (0.3 μM $OVA_{323–339}$; Pepceuticals) with irradiated antigen-presenting cells (APC) at a 1:5 ratio. T_H17 differentiation was achieved by addition of 3 ng ml^{-1} TGF-β, 20 ng ml^{-1} IL-23, 20 ng ml^{-1} IL-6, 5 μg ml^{-1} anti–IL-12 (C17.8) and 5 μg ml^{-1} anti–IL-4 (11B11). For T_H1 cells, 10 ng ml^{-1} IL-12 and 5 μg ml^{-1} anti–IL-4 (11B11) were added. Cells were kept in medium supplemented with rhIL-2 (Chiron) and re-stimulated every 7 d. R838 (500 nM) was added to the culture after 7 d and refreshed after medium exchange. After 14 d we checked cytokine production with PMA and ionomycin stimulation. PLP-specific T cells isolated from EAE mice were stimulated with $PLP_{139–151}$ (12.5 μg ml^{-1}; Pepceuticals) in the presence of irradiated APCs. T_H17 differentiation was achieved with IL-23 (20 ng ml^{-1}), 10 μg ml^{-1} anti–IL-12 (C17.8) and 10 μg ml^{-1} anti–IL-4

(11B11). R838 (500 nM) was added to the culture and refreshed after medium exchange. After 7 d, cytokine production was checked with PMA and ionomycin stimulation.

Human cells—$CD4^+$ and $CD4^+CD45RO^+$ T lymphocytes (purity > 97%) were magnetically isolated from the peripheral blood mononuclear cells of healthy human donors, who had given written informed consent (Centre Hospitalier de l'Université de Montréal, ethical approval experimental design number HD04.046) (ref. 25). T cells were cultured with autologous monocytes as APCs in a 2:1 ratio and stimulated with CD3-specific antibody (2.5 μg ml^{-1}; clone OKT3, eBioscience). For T_H1 polarization, we added recombinant hIL-12 (10 ng ml^{-1}) and anti-hIL-4 (5 μg ml^{-1}; clone 3007, R&D), whereas for T_H17 polarization, we cultured T cells with recombinant hIL-23 (10 ng ml^{-1}) and neutralizing antibodies against IFN-γ (5 μg ml^{-1}; clone K3.53, R&D) and IL-4 (R&D). We harvested cells on day 6 for cytokine determination using commercially available ELISA kits for IFN-γ (Becton Dickinson), IL-17 (Biosource) and IL-22 (R&D).

Migration assay

Mouse cells—We performed chemotaxis experiments with $PLP_{139–151}$- and $MOG_{35–55}$-specific T cells using laminin-coated, 6.5 mm Transwells (Costar) with a confluent monolayer of bEnd3 cells[20]. Except for *in vitro*–differentiated T_H1 and T_H17 cells, T cells were pretreated for 24 h with 70 U ml^{-1} TNF-α and IFN-γ to induce Bdkrb1 expression, and challenged with either R838, R715 or both (500 nM of each) for an additional 3 h. We induced migration by adding 200 ng ml^{-1} recombinant CXCL12 (R&D) to the lower chamber.

Human cells—BBB endothelial cells were isolated from CNS tissue specimens obtained from temporal lobe resections from young adults undergoing surgery for the treatment of intractable epilepsy, as described previously[10, 25]. Informed consent and ethical approval were obtained before surgery (Centre Hospitalier de l'Université de Montréal, approval HD04.046). Human T cell migration was assessed using a 24-well-plate modified Boyden chamber[3, 25]. T_H1 and T_H17 lymphocytes were challenged with R838 or R715 (500 nM each) for 3 h. One million cells per condition were loaded in the upper chamber, and the absolute number of cells that transmigrated to the lower chamber was counted after 18 h.

Statistics

Data were analyzed with SPSS and presented with Prism 4 (GraphPad).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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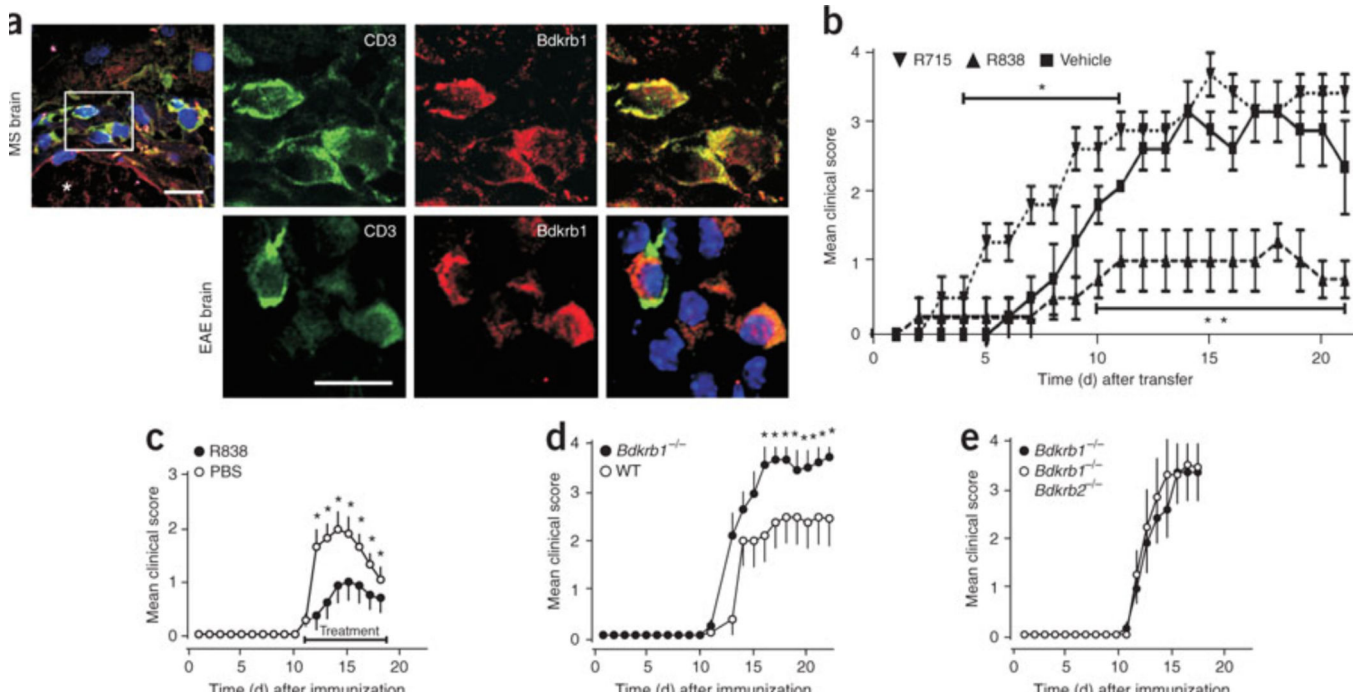

Figure 1. Morphological and functional evidence for the involvement of the kallikrein-kinin system in autoimmune CNS inflammation

(a) Histopathology for CD3 (green), kinin receptor B1 (Bdkrb1; red) and cell nuclei (Hoechst; blue), including overlay analysis in the CNS from a human with multiple sclerosis (top) or from a mouse with EAE (bottom). Asterisk marks the lumen of a blood vessel. Scale bars, 10 μm. (**b**) Pharmacological modulation of Bdkrb1 in adoptive transfer EAE in SJL mice. One of two experiments is shown. Mice were distributed into three groups and received intraperitoneal injections of either the Bdkrb1 agonist R838, the Bdkrb1 antagonist R715 or vehicle daily for days 0–10 ($n = 6$ per group). (**c**) Therapeutic treatment effect for the Bdkrb1 agonist R838, given after disease onset twice daily, is demonstrated in SJL mice immunized with $PLP_{139–151}$ ($n = 6$ per group). Representative results from two independent experiments are shown. (**d**) Bdkrb1 deficiency enhances autoimmune neuroinflammation. Clinical scores for wild-type (WT) EAE ($n = 9$) and *Bdkrb1*$^{-/-}$ EAE ($n = 9$) mice after immunization with $MOG_{35–55}$. Representative results from three independent EAE experiments are shown. (**e**) Combined disruption of *Bdkrb1* and *Bdkrb2* results in a disease course similar to that seen with *Bdkrb1* disruption alone. *Bdkrb1*$^{-/-}$;*Bdkrb2*$^{-/-}$ ($n = 5$) and *Bdkrb1*$^{-/-}$ mice ($n = 6$) were immunized with $MOG_{35–55}$. Representative results from two independent EAE experiments are shown. For all EAE courses, mean disease scores ± s.e.m. are displayed; $^{**}P < 0.01$, $^{*}P < 0.05$, repeated-measures ANOVA.

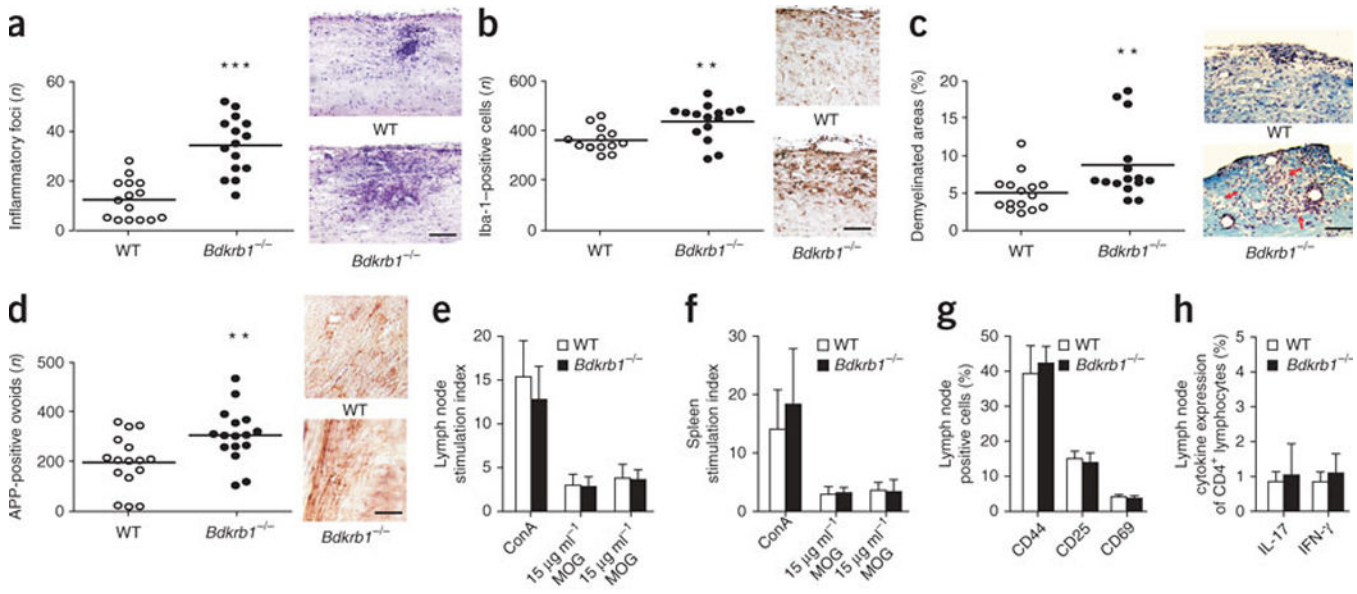

Figure 2. Bdkrb1 deficiency leads to enhanced EAE pathology

(**a–d**) Histopathological analysis of three sections per mouse, comprising the assessment of inflammation by H&E staining (**a**), microglia and macrophage infiltration by immunohistochemistry for Iba-1 (**b**), demyelination by luxol fast blue staining (arrows indicate demyelinated areas) (**c**) and axonal damage by immunohistochemistry for APP (**d**). Representative images from spinal cord longitudinal sections and corresponding quantifications are shown. Scale bars, 50 µm. (**e–h**) Deficiency of Bdkrb1 has no impact on myelin-specific inflammatory responses in the periphery. Proliferation in response to $MOG_{35–55}$ in cells from draining lymph nodes (**e,g,h**) or spleens (**f**) from *Bdkrb1*$^{-/-}$ or wild-type (WT) mice with EAE killed after immunization with $MOG_{35–55}$ but before the onset of disease. Shown are the results of [^{3}H]thymidine incorporation assays in response to $MOG_{35–55}$ (**e,f**) and flow cytometric assessment of the expression of surface activation markers and cytokines (**g,h**). Values are means ± s.d.; $^{**}P < 0.01$, $^{***}P < 0.001$, Mann-Whitney *U*-test.

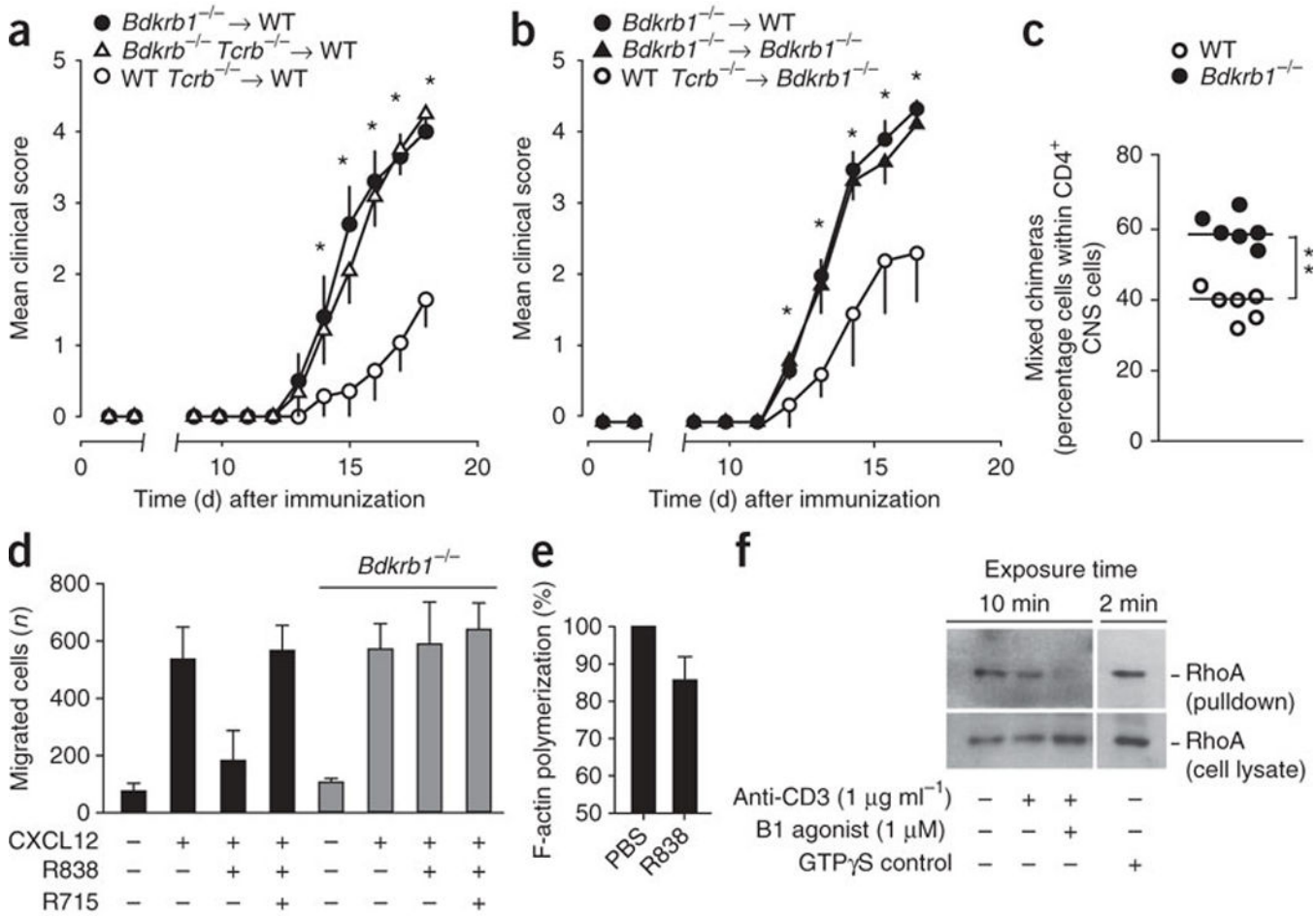

Figure 3. Bdkrb1 controls the migratory capacities of T cells targeting the CNS

(a) Induction of EAE by $MOG_{35–55}$ immunization of lethally irradiated C57BL/6-CD45.1 recipients reconstituted with Bdkrb1-deficient (*Bdkrb1*$^{-/-}$ → WT; $n = 5$) bone marrow or a mix of TCR-β-chain– and Bdkrb1-deficient bone marrow (*Bdkrb1*$^{-/-}$*Tcrb*$^{-/-}$ → WT; $n = 6$). To control for the impact of the transplantation procedure, control mice received a mix of TCR-β-chain–deficient and C57BL/6 WT bone marrow (WT *Tcrb*$^{-/-}$ → WT, $n = 7$). (**b**) EAE course in Bdkrb1-deficient mice that received a mix of TCR-β-chain–deficient and C57BL/6-CD45.1 bone marrow (WT *Tcrb*$^{-/-}$ → *Bdkrb1*$^{-/-}$, $n = 5$), as compared to that in *Bdkrb1*$^{-/-}$ → WT mice ($n = 4$) and *Bdkrb1*$^{-/-}$ → *Bdkrb1*$^{-/-}$ ($n = 5$) chimeras. Mean clinical disease scores ± s.e.m. are given for all three groups. $^{*}P < 0.05$, repeated-measures ANOVA. Representative results from three (**a**) and two (**b**) independent EAE experiments are shown. (**c**) C57BL/6 recipients were reconstituted with mixed *Bdkrb1*$^{-/-}$ (C57BL/6-CD45.2) and WT (C57BL/6-CD45.1) bone marrow (1:1 ratio; $n = 6$), and $CD4^+$ T cells were isolated from the CNS at disease peak. $^{**}P < 0.01$, Mann-Whitney *U*-test. (**d**) Migratory capacity, toward a CXCL12 chemokine gradient in a Transwell system, of $PLP_{139–151}$-specific T cells (from immunized SJL mice) and $MOG_{35–55}$-specific $CD4^+$ T cells (from immunized *Bdkrb1*$^{-/-}$ mice) that were seeded on a mouse bEnd3 brain–derived endothelial cell monolayer. Bdkrb1 was modulated by incubating T cells with the Bdkrb1 agonist R838 or the Bdkrb1 antagonist R715 before application to the endothelial monolayer. (**e**) Decreased

F-actin polymerization of $CD4^+$ T cells upon Bdkrb1 activation. (**f**) Downregulation of the small GTPase RhoA in $CD4^+$ T cells by activation of Bdkrb1. Shown is an immunoblot of a cell extract prepared from T cells after RhoA pulldown. GTPγS-loaded controls were used as a positive control for RhoA pulldown.

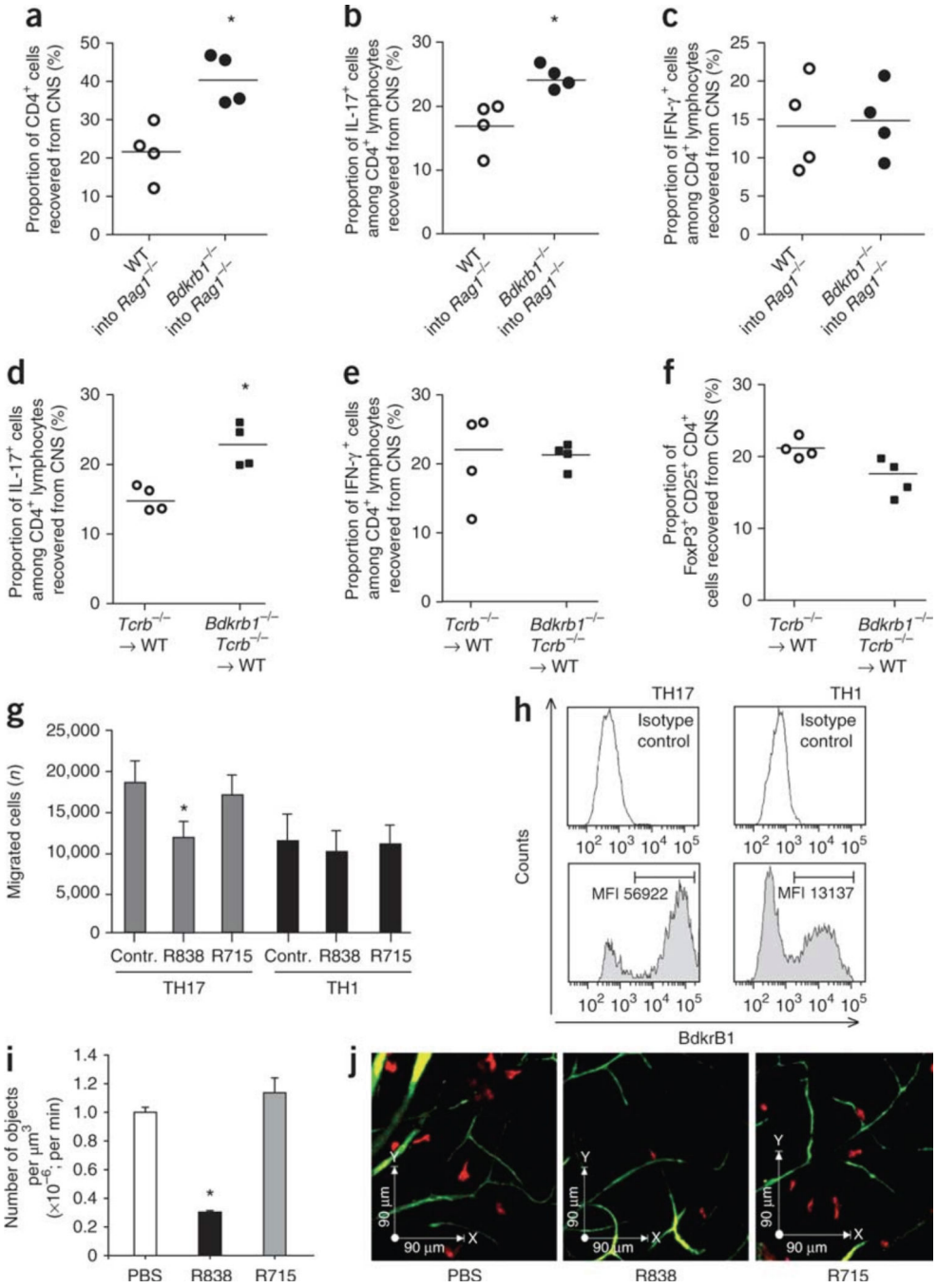

Figure 4. Bdkrb1 activation primarily targets the invasion of T_H17 cells

(**a–c**) Proportions of $CD4^+$ cells in the CNS (**a**) and of $IL\text{-}17^+$ and $IFN\text{-}\gamma^+$ cells within the $CD4^+$ T cell population (**b,c**) in C57BL/6 *Rag1*$^{-/-}$ mice with adoptive transfer EAE induced by injection of *Bdkrb1*$^{-/-}$ or WT T cells. Immune cells were isolated from the CNS of four mice per group at day 13 after cell transfer (which corresponds to the time of disease onset). (**d–f**) Proportions of $IL\text{-}17^+$ cells (**d**), $IFN\text{-}\gamma^+$ cells (**e**) and $FoxP3^+$ $CD25^+$ cells (**f**) within the $CD4^+$ T cell population from CNS-invading immune cells recovered from bone marrow chimeric mice with T cells lacking Bdkrb1 or from control mice (see Fig. 3a). (**g**) Bdkrb1

activation primarily targets the migration of human memory CD45RO$^+$ T_H17 rather than T_H1 cells across human brain-derived microvascular endothelium; $^*P < 0.05$, Mann-Whitney *U*-test. (**h**) Increased expression of Bdkrb1 in human T_H17 cells analyzed by FACS. (**i,j**) Activation of Bdkrb1 decreased the average number of Celltracker Orange–labeled T_H17 cells after application to hippocampal slice cultures for multiphoton microscopy. The average number of T cells per minute and per defined volume (between 60–120 m depth) over time is shown, including quantification (**i**) (see also Supplementary Movies 1,2,3) and representative overviews (**j**). Data shown are means ± s.e.m.; $^*P < 0.05$, Mann-Whitney *U*-test.