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Research

Kinin B₂ receptor regulates chemokines CCL2 and CCL5 expression and modulates leukocyte recruitment and pathology in experimental autoimmune encephalomyelitis (EAE) in mice

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Abstract

Background: Kinins are important mediators of inflammation and act through stimulation of two receptor subtypes, B_1 and B_2. Leukocyte infiltration contributes to the pathogenesis of autoimmune inflammation in the central nervous system (CNS), occurring not only in multiple sclerosis (MS) but also in experimental autoimmune encephalomyelitis (EAE). We have previously shown that the chemokines CCL2 and CCL5 play an important role in the adhesion of leukocytes to the brain microcirculation in EAE. The aim of the present study was to evaluate the relevance of B_2 receptors to leukocyte-endothelium interactions in the cerebral microcirculation, and its participation in CNS inflammation in the experimental model of myelin-oligodendrocyte-glycoprotein $(MOG)_{35-55}$-induced EAE in mice.

Methods: In order to evaluate the role of B2 receptor in the cerebral microvasculature we used wild-type (WT) and kinin B2 receptor knockout $(B_2^{-/-})$ mice subjected to MOG_{35-55}-induced EAE. Intravital microscopy was used to investigate leukocyte recruitment on pial matter vessels in $B_2^{-/-}$ and WT EAE mice. Histological documentation of inflammatory infiltrates in brain and spinal cords was correlated with intravital findings. The expression of CCL5 and CCL2 in cerebral tissue was assessed by ELISA.

Results: Clinical parameters of disease were reduced in $B_2^{-/-}$ mice in comparison to wild type EAE mice. At day 14 after EAE induction, there was a significant decrease in the number of adherent leukocytes, a reduction of cerebral CCL5 and CCL2 expressions, and smaller inflammatory and degenerative changes in $B_2^{-/-}$ mice when compared to WT.

Conclusion: Our results suggest that B_2 receptors have two major effects in the control of EAE severity: (i) B_2 regulates the expression of chemokines, including CCL2 and CCL5, and (ii) B_2 modulates leukocyte recruitment and inflammatory lesions in the CNS.

Background

Bradykinin (BK) and its biologically active metabolites are the functional components of the kallikrein-kinin system. Kinins elicit a wide range of physiological effects including relaxation of vascular smooth muscle in arteries and arterioles, expression of adhesion molecules, leukocyte infiltration, formation of interendothelial gaps and protein extravasation from post-capillary venules, and pain transmission mechanisms [1,2]. The actions of kinins are mediated through stimulation of two subtypes of seven-transmembrane-domain G-protein-coupled receptors, namely B_1 and B_2. The B_2 receptor is constitutively expressed in various cell types, including endothelial cells, nerve fibers, leukocytes and mast cells [3,4]. The B_1 receptor is generally expressed at low levels under normal conditions but is up-regulated by cytokines in stressful situations, such as shock and inflammation [5-7]. Most of the physiological actions of kinins are believed to be mediated by stimulation of B2 receptors [8].

Experimental autoimmune encephalomyelitis (EAE) is an inflammatory disease of the CNS mediated by CD4+ Th1 cells that serves as experimental model of human multiple sclerosis (MS). A pathological hallmark of MS is infiltration of immune cells across the blood-brain barrier into the CNS causing myelin destruction and axonal injury [9]. It is thought that inappropriate leukocyte recruitment and activation in the brain results in disease symptoms and progression [10]. Thus, reduction of the migration of immune cells into the CNS is a relevant novel therapeutic strategy for the treatment of MS.

Although the potential role of the kinin system on leukocyte entry into the CNS in MS remains unclear, it has been shown that BK can interfere with the mechanism of leukocyte recruitment in various tissues [11]. BK may enhance the expression of adhesion molecules on endothelium cells [12]. BK antagonists reduce leukocyte-endothelium interactions after diverse inflammatory conditions, including global cerebral ischemia [4], leukocyte infiltration in murine mesenteric post-capillary venules [13] and lung inflammation in guinea pigs [14]. BK could potentially modify leukocyte recruitment by production of chemoattractant molecules, such as chemokines. For example, treatment with bradykinin receptor antagonists has been shown to reduce production of chemokines, including KC and MCP-1, after intestinal ischemia and reperfusion [15]. Several studies, have clearly demonstrated the relevance of chemokines for the recruitment of leukocytes into the brain of EAE mice [16-18].

Cross talk between cytokines and kinin receptors has been extensively investigated over the last several years [19-21]. Studies have demonstrated that pro-inflammatory cytokines regulate B_1 and B_2 receptor expression [11,22,23] and, conversely, blockade of kinins receptors modifies expression of cytokines and chemokines [24,25]. In the present work, we used B_2-deficient mice to assess the potential contribution of kinin receptors for the clinical course of disease, leukocyte recruitment, and modulation of chemokines expression in the CNS after EAE induction by MOG_{35-55}.

Methods

Animals

Mice B_2 knockout ($B_2^{-/-}$) mice were generated as previously described [24]. Knockout female C57BL/6 X sv129 mice (9–11 wks) and their wild-type (WT) littermate controls were housed under standard conditions and had free access to commercial chow and water. All procedures described in this study had prior approval from the local Ethics Committee that governs animal care and use in research.

EAE induction

$B_2^{-/-}$ and WT mice were immunized subcutaneously at the base of the tail with an emulsion containing 100 μg MOG_{35-55} peptide (MEVGWYRSPFSRVVHLYRNGK; (Dept Biophysics, Escola Paulista de Medicina, SP, Brazil) in Freund's complete adjuvant (CFA, Sigma) supplemented with 4 mg/mL *Mycobacterium tuberculosis* H37RA (Difco Laboratories). Pertussis toxin (Sigma), was injected (300 ng/animal, i.p.), on the day of immunization and 48 h later. Animals were monitored daily and neurological impairment was quantified on an arbitrary clinical scale and presented as mean clinical disease severity. Scores were: 0 = no clinical signs, 1 = tail paralysis (or loss of tail tone), 2 = tail paralysis and hind-limb weakness, 3 = hind-limb paralysis, 4 = complete hind-limb paralysis and front limb weakness [26]. The mice were weighed pre- and 14 days post-immunization, the peak of the disease [18]. "Sham animals" refers to WT animals without EAE induction.

Intravital microscopy in mouse brain

Intravital microscopy of cerebral microvasculature was performed as previously described [18,27]. Briefly, the mice were anaesthetized by intraperitoneal injection of a mixture containing ketamine (150 mg/kg) and xylazine (10 mg/kg). The tail vein was cannulated for administration of fluorescent dyes. A craniotomy was performed using a high-speed drill (Dremel, USA) and the dura matter was removed to expose the underlying pial vasculature. Throughout the experiment, the mouse was maintained at 37°C with a heating pad (Fine Science Tools Inc., Canada) and the exposed brain was continuously superfused with artificial cerebrospinal fluid buffer at pH 7.4, containing in mmol/L: NaCl 132, KCl 2.95, $CaCl_2$ 1.71, $MgCl_2$ 0.64, $NaHCO_3$ 24.6, dextrose 3.71 and urea 6.7, at 37°C.

To assess leukocyte-endothelium interactions, leukocytes were fluorescently labeled by administration of rhodamine 6G (i.v., 0.5 mg/kg body weight) and observed using a microscope (Olympus B201, X20 objective lens, corresponding a 100 μm of area) outfitted with a fluorescent light source (epi-illumination at 510–560 nm, using a 590 nm emission filter). A silicon-intensified camera (Optronics Engineering DEI-470) mounted on the microscope projected the image onto a monitor (Olympus). Rolling leukocytes were defined as the number of white cells moving at a velocity lesser than that of erythrocytes cells. Leukocytes were considered adherent to the venular endothelium if they remained stationary for 30 seconds or longer.

Histopathology
Brains, cerebellum and the spinal cords were removed just after intravital microscopy. Transverse slices of brain, spinal cord and cerebellum were fixed by immersion in 10% formalin and processed for paraffin embedding. The sections (6.0 μm) were stained with H&E and examined in an Olympus BX51 microscope. Documentation was performed by a coupled digital camera and imaging capture software (Megacybernetics Pro-Express version 4.1 and cool-snap kit).

Measurement of chemokines
Brain tissue extracts were obtained from control and EAE mice (WT and $B_2^{-/-}$). Brains were removed after intravital microscopy, and the left and right hemispheres were stored on ice. Thereafter, using Ultra-Turrax, each hemisphere was homogenized in extraction solution (100 mg of tissue per 1 mL) containing 0.4 M NaCl, 0.05% Tween 20, 0.5% BSA, 0.1 mM phenylmetilsulfonil fluoride, 0.1 mM benzetonio chloride, 10 mM EDTA and 20 KIU aprotinin. The brain homogenate was spun at 10000 × g for 10 min at 4°C and the supernatants were stored at -70°C. The concentrations of CCL2, CCL3, CCL5, TNFα and IFNγ were determined in the supernatants of the brain extracts, at a 1:3 dilution in PBS containing 1% BSA, using an ELISA set-up commercially available according to the procedure supplied by the manufacturer (R&D Systems, Minneapolis, MN and Pharmingen, San Diego, CA).

Statistical analysis
Data are shown as mean ± SEM. Significance was assessed using an ANOVA parametric test with Bonferroni correction for multiple comparisons. Statistical significance was set at $P < 0.05$.

Results
Clinical assessment and histopathology
All WT and $B_2^{-/-}$ mice developed EAE after MOG_{35-55} administration. Clinical symptoms appeared on day 11 post MOG-injection and peaked around day 14 after

which were stable through day 18, the last day for animal evaluation. There were no deaths during the course of EAE development. However, $B_2^{-/-}$ mice showed a modest but statistically significant reduction (n = 7, mean clinical severity score = 2.4 ± 0.6) in neurological impairment at the peak of disease (day 14) and during the final plateau phase of disease when compared with their WT group counterparts (n = 7, mean clinical severity score = 3.6 ± 0.24) (Figure 1A). In addition, there was significant weight loss in EAE WT animals compared to the EAE $B_2^{-/-}$ group (Figure 1B).

Histopathological evaluation of the brain, cerebellum and spinal cord was performed in WT and $B_2^{-/-}$ mice at day 14 after EAE induction. Brains of EAE WT mice showed a marked perivascular infiltration of mononuclear cells around pial and cerebral cortex venules (Figure 2A). The inflammatory process extended from the meninges into cerebral cortex parenchyma and was characterized by
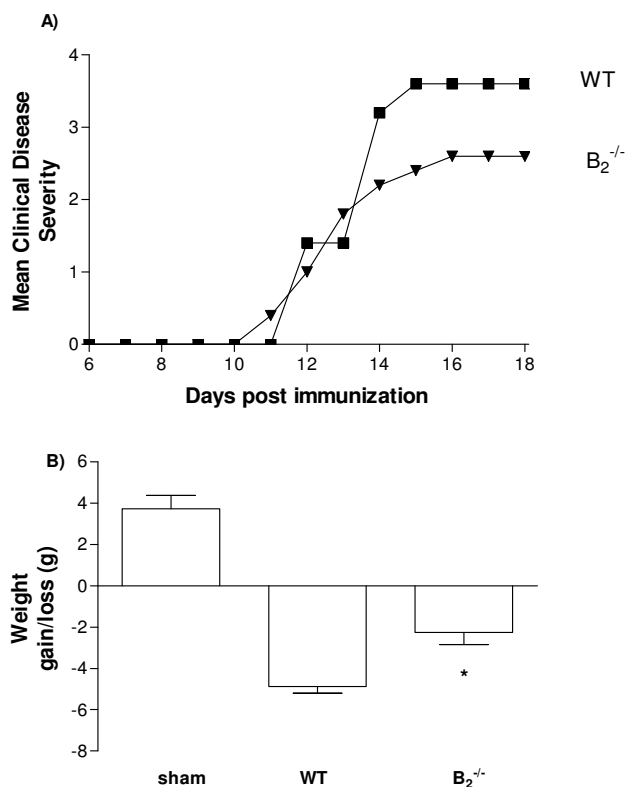

Figure 1
Clinical assessment of EAE. Clinical signs (n = 7 mice/group) were daily monitored, comparing $B_2^{-/-}$ (▼) and WT (■) mice during 18 days after EAE induction (A) and body weight gain or loss (B). Data is expressed as mean ± SEM. *P < 0.05 for $B_2^{-/-}$ versus WT on the peak of disease (day 14 post-induction).
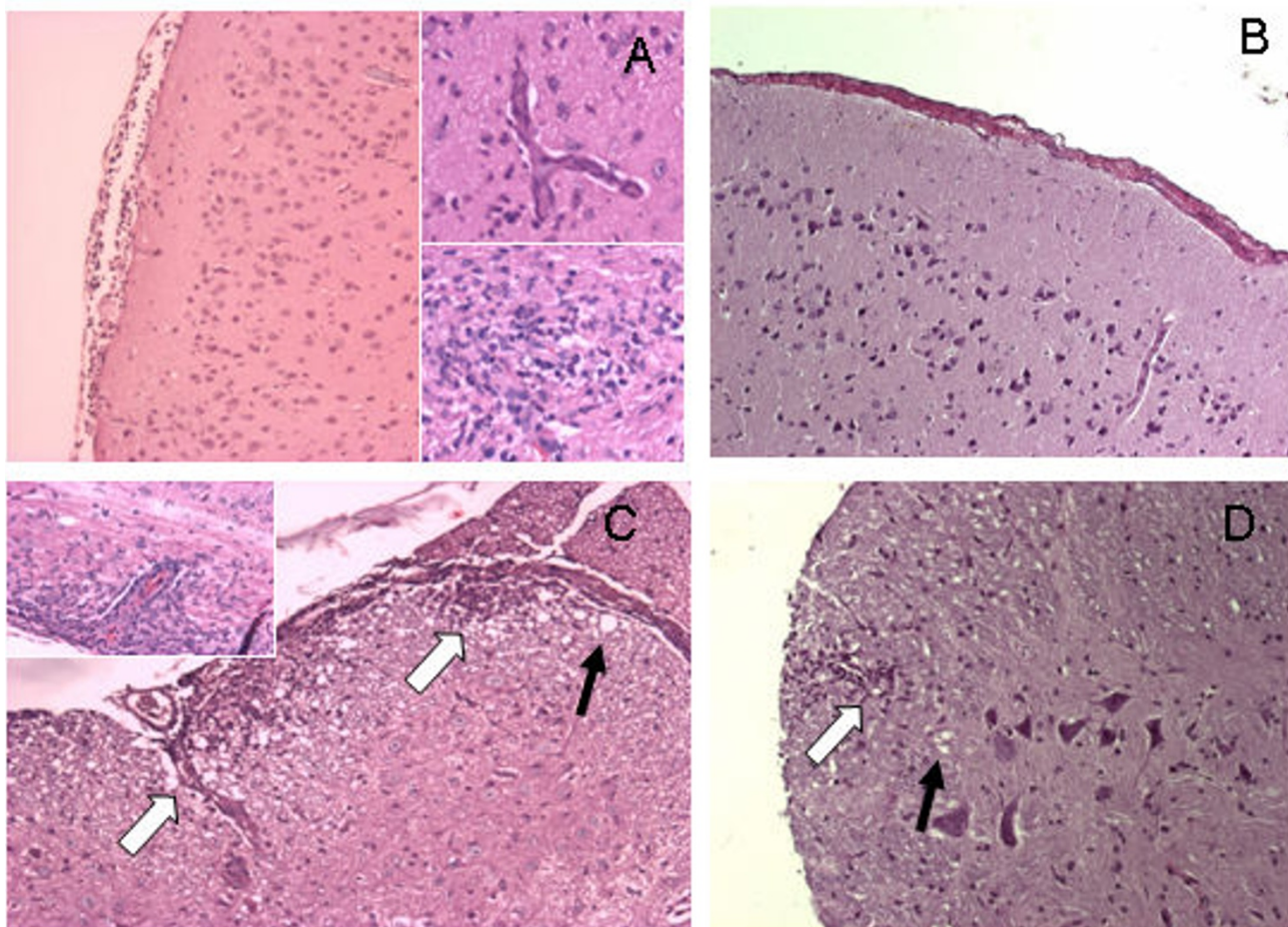
Figure 2
Histopathological analysis of EAE WT(A, C) and EAE B$_2$$^{-/-}$ mice (B, D). The analysis was performed on H&E-stained sections of brain (A, B) and spinal cord (C, D) at day 14. Observe the marked perivascular infiltration of mononuclear cells in WT brain meninges (A) and the perivascular and inflammatory infiltration of parenchyma (superior and inferior details in A) and spinal cord (C, white arrows). Degenerative changes of white matter are increased in the spinal cord of WT mice in comparison to B$_2$$^{-/-}$ mice (C, black arrows). Original magnification 150×.

focal intense mononuclear infiltration. The meninges and white matter of spinal cord of the EAE WT group presented an intense focal inflammatory response associated with white matter vacuolization (Figure 2C). However, the EAE B$_2$$^{-/-}$ group showed only discrete focal inflammatory infiltrates of mononuclear cells, predominantly in association with discrete white matter vacuolization (Figure 2B and 2D).

Rolling and adhesion of leukocytes in cerebral microvasculature on day 14

EAE pathogenesis requires migration of activated T cells from peripheral lymphoid tissue to the CNS, which presumably occurs in a multi-step manner. To migrate into sites of inflammation, leukocytes must first tether and roll along the vessel before they firmly adhere and emigrate out of the vasculature. Firm adhesion is triggered by the action of chemoattractant molecules, such as chemokines [26]. EAE induced an increase in leukocyte rolling which presented the same profile in all groups. The deletion of B$_2$ genes did not alter the rolling of leukocytes on pial vessel walls when compared to WT group (Figure 3A). EAE also induced an increase of leukocyte adhesion in WT mice (Figure 3B). On the other hand, leukocyte adhesion was diminished in B$_2$$^{-/-}$ in comparison with WT mice (Figure 3B). In summary, both leukocyte recruitment and histopathological alterations decreased in B$_2$$^{-/-}$ when compared to the EAE WT.

Chemokine release in the brain

In order to assess the involvement of kinin receptors in the cerebral chemokine release after EAE induction, brain tis-
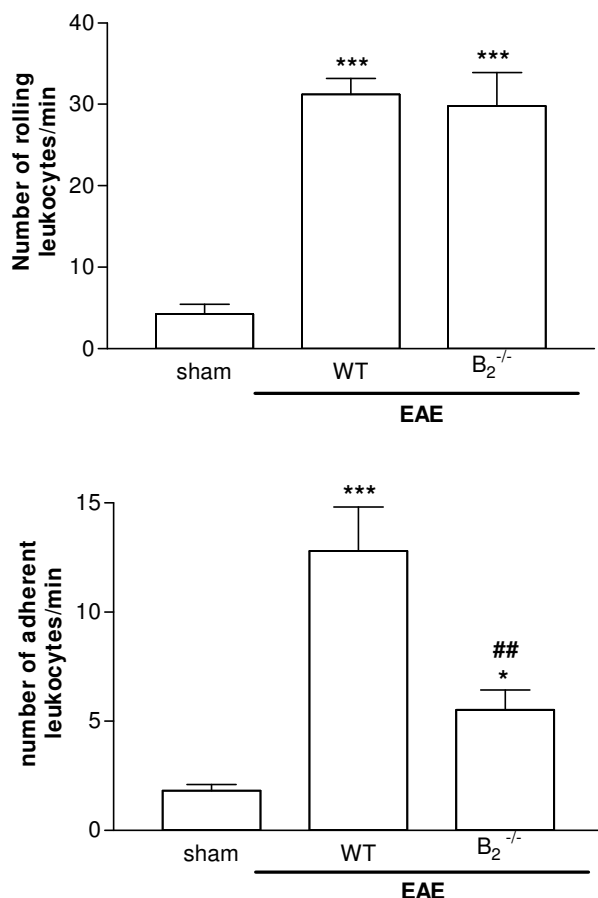
Figure 3
Leukocyte-endothelial interactions in pial vasculature. Intravital microscopy was used to assess leukocytes rolling (A) and adhesion (B) on day 14 post-immunization. The analysis was performed in 4–5 vessels per animal (n = 6 per group). Results are expressed as mean ± SEM. ***P < 0.001 when compared to control; ###P < 0.001 when compared to EAE WT mice.

sues from WT and $B_2^{-/-}$ were examined. EAE induction enhanced cerebral production of the chemokines CCL2 and CCL5 in WT mice (Figure 4A, B). In $B_2^{-/-}$ mice, the levels of CCL2 were significantly lower than those found in WT mice (Figure 4A). In addition, the production of CCL5 was lower in $B_2^{-/-}$ mice when compared to their WT controls subjected to EAE (Figure 4B). There was no difference in cerebral CCL3 expression in WT mice before or after EAE induction (Figure 4C). Moreover, expression of CCL3 was similar in WT and gene-deficient mice subjected to EAE (Figure 4C).

Considering the important participation of IFNγ and TNFα in T-cell trafficking to CNS, we evaluated the role of kinin receptors on the cerebral production of these pro-

inflammatory cytokines. EAE WT and EAE $B_2^{-/-}$ mice showed increased TNFα and IFNγ in brain tissue, when compared to the control group (Figure 4D, E). However, no statistical difference was found in the cerebral cytokines levels between WT EAE and $B_2^{-/-}$ EAE mice.

Discussion
The kallikrein-kinin system is one of the first inflammatory pathways activated after tissue damage. Particularly because of its ability to increase vascular permeability, the kallikrein-kinin system has been investigated for its ability to promote brain edema and secondary brain damage in various models of central nervous system injuries, for example; bacterial meningitis [28], traumatic brain and spinal cord injury [29-32], and global cerebral ischemia [4]. Although, kinins are known to be released and have several effects in the CNS, their cerebral effects in EAE pathogenesis remain unclear.

It is known that pretreatment with B2 receptor antagonist markedly inhibits inflammatory responses in lungs and intestine [15,33] by suppressing leukocyte recruitment and activation in acute models of inflammation [14,34,35]. In addition, treatment with a B2 receptor antagonist decreases CD44, CD54, CD11a and CD11b expression in various cell types after arthritis induction in Lewis rats [36].

This study was designed to provide further insights into the molecular mechanisms of proinflammatory cell trafficking into the CNS in a murine model of EAE. As kinin receptors appear to mediate leukocyte-endothelium interaction induced by certain inflammatory stimuli [4,13], our aim was to determine the putative role of kallikrein-kinin system in the cerebral inflammatory response, via the kinin B2 receptor, after EAE development. For this, we used wild-type (WT) and kinin B2 receptor knockout ($B_2^{-/-}$) mice subjected to a MOG_{35-55}-induced EAE model of multiple sclerosis.

The MOG peptide can induce typical EAE disease in C57BL/6 mice and in other strains. MOG, which represents only ~0.05% of myelin proteins, elicits a major antibody response that has been correlated with disease severity and demyelination in both human and animal models of MS [37,38]. There is some evidence for activation of the kallikrein-kinin system during inflammation in this model [39]. In our hands, mice that lack the kinin B_2 receptor and are subjected to EAE had a statistically significant reduction in neurological impairment at the peak of disease (day 14) and during the final plateau phase of disease, when compared with the WT EAE group. Our results also reveal lesser degrees of weight loss in EAE $B_2^{-/-}$ mice when compared to WT EAE animals. These findings are consistent with a previous study that showed pro-
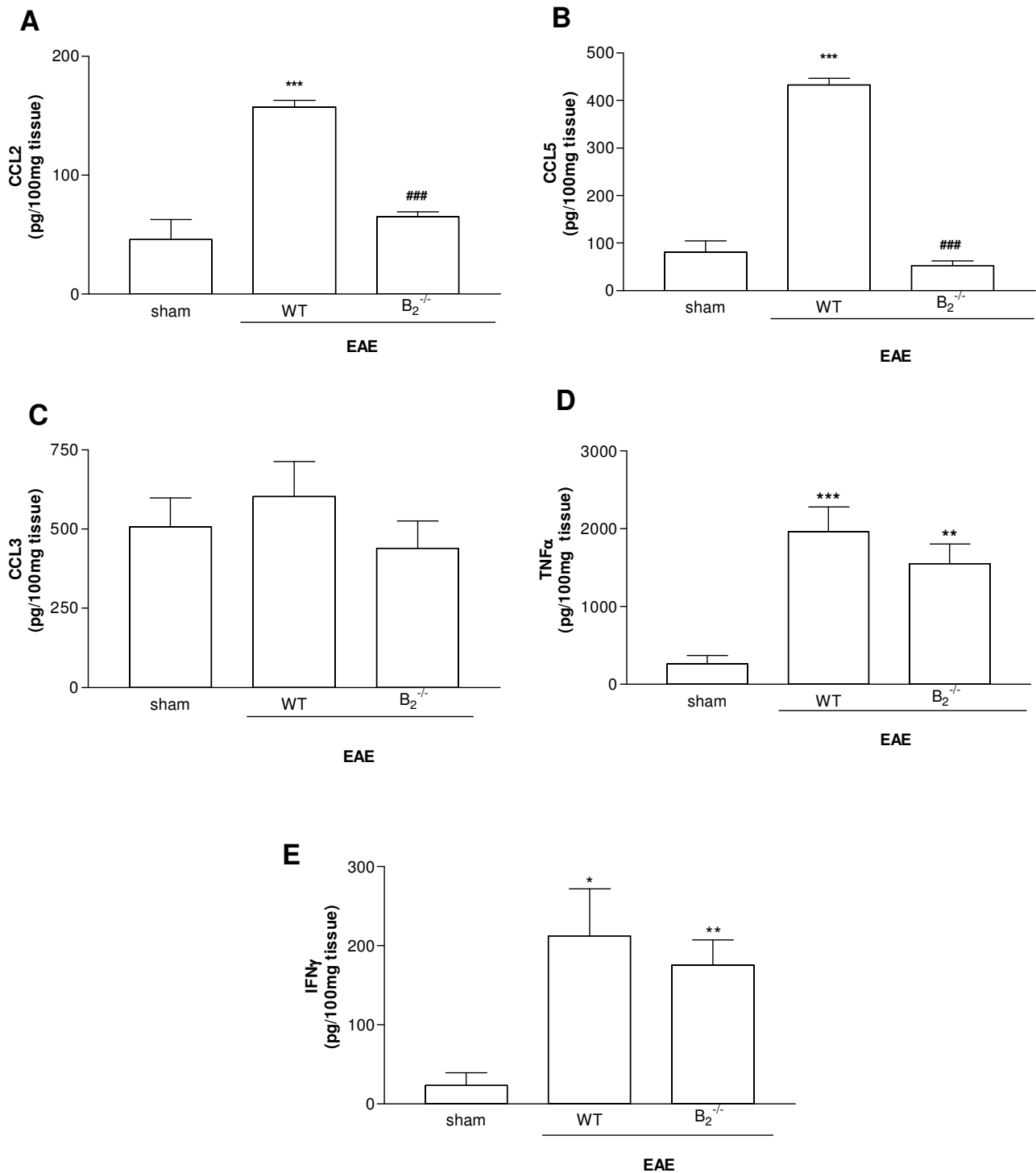
Figure 4
Kinetics of cytokine and chemokine production in the CNS of EAE mice. Cerebral levels of CCL2 (A), CCL5 (B), CCL3 (C), TNFα (D) and IFNγ (E) were measured by ELISA (n = 6). Statistically significant differences are indicated by: **P < 0.01 when compared with control; ##P < 0.01 when compared with WT.

longed survival, improved motor function, and smaller cerebral infarcts in $B_2^{-/-}$ after experimental stroke [40]. However, leukocyte recruitment was not investigated in this study. On the other hand, our findings of improvement of neurological clinical score in $B_2^{-/-}$ mice associated with impaired leukocyte recruitment parameters are in contrast to the findings of Xia et al. [41], which provide evidence for a protective role of the kinin B_2 receptor against ischemic stroke. In the latter study, the authors also found increased neutrophil accumulation in infarcted brain areas in $B_2^{-/-}$ mice. Recently these results have been consistently contested, leading to the conclusion that the deficiency of bradykinin receptor B2 is not detrimental in experimental stroke [42], and may be beneficial in other pathological conditions.

EAE pathogenesis requires the infiltration of leukocytes into brain parenchyma. In a previous study undertaken by our group, we showed that there are increased leukocyte-endothelial cell interactions (rolling and adhesion) in brains of EAE mice, as assessed by cerebral intravital microscopy [18]. In the present series of experiments, leukocyte adherence, but not rolling, was suppressed in $B_2^{-/-}$ mice submitted to the EAE protocol.

The latter findings were corroborated by histopathological examination of brain sections, which showed inhibition of mononuclear cell influx and inflammatory lesions on $B_2^{-/-}$ mice after EAE induction. In addition, histopathological analysis of spinal cord for EAE WT mice showed a marked inflammatory response with intense vacuolization in white matter, contrasting with very discrete infiltrates and degenerative changes in $B_2^{-/-}$ mice after EAE induction. Thus, the present study clearly suggests an important role for B_2 in mediating leukocyte adherence to vessels in brain, and extensively in spinal cord, as suggested by histology of both sites in the same animal.

These suggestions draw support from described patterns of kinin receptor distribution in the CNS. High-density kinin binding sites have been identified in the CNS, mainly in cerebral blood vessels [2]. Previous studies have demonstrated the presence of B_1 receptor in the brain and spinal cord in different species [43]. In addition, B_2 receptors have been localized in experimental brain tumors [44]. Chen et al have also shown that the B_2 receptor is widely expressed within rat brain [45]. In contrast to available knowledge on bradykinin B_2 receptors in brain, the distribution of these receceptors in spinal cord and the potential functional roles in spinal cord remain unclear. RT-PCR studies reveal the presence of B_1 and B_2 receptor mRNA in neurons of dorsal root ganglia in mice [46]. Murone et al. have demonstrated the distribution of B_2 receptors in the brain and spinal cord of guinea pig and sheep by autoradiography [47,48]. Further studies using

intravital microscopy of spinal cord vessels are necessary in order to define functional roles of these receptors in pathological conditions.

Activation of kinin receptors may underlie production of chemokines in several models of inflammation, including response to the cytokine IL-1β in mesenteric venules [49], inflammatory pain [50,51], and allergic rhinitis [21]. Considering previous data, it may be possible that kinin receptors could be regulating the expression of chemokines and, consequently, leukocyte trafficking after EAE induction. Our previous studies showed that the chemokines CCL2 and CCL5, but not CCL3, are involved in the adherence, but not rolling, of leukocytes in the microvasculature of EAE mice [18]. In this study we demonstrate that CCL5 expression is greatly suppressed in EAE $B_2^{-/-}$ mice, when compared to EAE WT. These findings could partially explain the inhibition of leukocyte adherence observed in $B_2^{-/-}$ mice, as a probable consequence of decreased levels of CCL5.

The chemokine CCL2 and its receptors CCR2 are thought to play an important role in the pathogenesis of EAE in several conditions. For example, both CCL2 and CCR2 are expressed in brains of patients with MS [52,53] and deletion of CCR2 leads to an almost complete inhibition of MOG_{35-55}-induced EAE in mice [16]. Blocking of the CCL2-CCR2 axis with neutralizing antibodies limits the development of subsequent relapses [54]. Our previous studies have also shown a role for CCL2 in leukocyte adherence during murine EAE [18]. In the present experiments, CCL2 levels were lower in $B_2^{-/-}$ mice than in WT mice after EAE induction. Previous studies have shown that CCL2 may be critical not only for leukocyte recruitment but also for the activation of T cells during inflammatory and immune responses [53,55]. Thus, it is possible that the inhibition of CCL2 production observed in $B_2^{-/-}$ mice would lead to lesser activation of infiltrated leukocytes and, hence, stabilization of the severity of clinical signs.

There are many studies supporting the clinical involvement of TNF-α in EAE. Elevated expression of TNF-α can be found in the CNS during acute episodes of disease, and blockage of TNF-α with neutralizing antibodies and soluble receptors will ameliorate signs of EAE [56,57]. In order to evaluate the role of the B_2 receptor in cerebral TNF-α expression after EAE induction, brain tissue extracts were obtained from control, WT EAE and EAE $B_2^{-/-}$ mice. We found a significant increase in TNF-α levels at day 14 (peak of disease) in both WT and $B_2^{-/-}$ compared to control mice. Absence of B_2 receptor did not modulate TNF-α expression after EAE induction. These results are consistent with those of Cunha et al. [58] who demonstrated that B_2 kinin receptor antagonist, at a dose that inhibits carra-

geenin-induced hypernociception, does not inhibit release of TNF-α [54]. Direct, bradykinin-independent, release of cytokines was also suggested in rats after administration of large doses of LPS [59].

B_2 is partially necessary for B_1 receptor induction as supported by the consistent finding of B_2 participation in B_1 mRNA and protein induction in several models of inflammation [60]. The expressed B_1 is relevant to the control of CCL5 production and to ensuing leukocyte adherence and tissue inflammation. In addition to these B_1-dependent effects, B_2 receptor activation is necessary for the production of CCL2 in brains of EAE mice. Previous studies using rodent models of traumatic brain injury have corroborated the finding that B_2 receptor is present and involved in cerebral alterations [61,62]. Our study clearly demonstrates that blockade of B_2 receptors is beneficial in EAE mice in reducing inflammatory events such as leukocyte adhesion and activation. We should note, however, that various studies have demonstrated an important role for adhesion molecules in both EAE and MS pathogenesis [63-66] and, at this time, we can not yet exclude the possibility that there is altered expression of adhesion molecules such as ICAM-1 and VCAM-1 on brain endothelial cells of B_2 deficient animals.

Conclusion
Our study proposes an important and previously unappreciated involvement of the kallikrein-kinin system in the pathogenesis of EAE. In summary, our results clearly show a definite role for B_2 receptors in mediating leukocyte adherence, chemokine production and clinical disease outcome. Altogether, our data indicate that blockade of kinin receptors, especially B_2, may represent an additional and novel therapeutic strategy for the treatment of MS.

List of abbreviations
ANOVA: analysis of variance; BK: bradykinin; BSA: bovine serum albumine; CFA: complete Freund adjuvant; CNS: central nervous system; CCL: chemokine ligand; CCR: chemokine receptor; ELISA: enzyme linked immunosorbent assay; ICAM: intercellular adhesion molecule; IFNγ: interferon gama; IL-1β: interleukin-1β; MCAO: middle cerebral artery occlusion; MOG: myelin oligodendrocyte glycoprotein; MS: multiple sclerosis; PBS: phosphate buffer sodium; SEM: standard error mean; TNFα: tumor necrosis factor alpha; VCAM: vascular cell adhesion molecule; WT: wild type.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
ACS, MMT and JCT conceived the study, and wrote the manuscript. ACS carried out the experiments and analyzed the results, with the following exceptions: LJ synthetize the peptide MOG. ER extracted RNA samples and prepared cDNA from the brain. RMEA helped in the histological studies. JBP, JLP and MB provided B_2-/- animals. All authors read and approved the final manuscript.

References
1. Couture R, Lindsey CJ: **Brain kallikrein-kinin system: from receptors to neuronal pathways and physiological functions.** *Handbook of chemical anatomy* 2000, **16**:241-298.
2. Couture R, Harrison M, Vianna RM, Cloutier F: **Kinin receptors in pain and inflammation.** *Eur J Pharmacol* 2001, **429**:161-176.
3. Ahluwalia A, Perretti M: **B1 receptors as a new target. Could this B the 1?** *TiPS* 1999, **20**:100-103.
4. Lehmberg J, Beck J, Baethmann A, Uhl E: **Bradykinin antagonists reduce leukocyte endothelium interactions after global cerebral ischemia.** *J Cerebral Blood Flow & Metab* 2003, **23**:441-448.
5. Prat A, Weinrib L, Becher B, Poirier J, Duquette P, Couture R, Antel JP: **Bradykinin B1 receptor expression and function on T lymphocytes in acute multiple sclerosis.** *Neurology* 1999, **53**:2087-2092.
6. Ferreira J, Campos MM, Araújo R, Bader M, Pesquero JB, Calixto JB: **The use of kinin B1 and B2 receptor knockout mice and selective antagonists to characterize the nociceptive responses caused by kinins at spinal level.** *Neurophamacol* 2002, **43**:1188-1197.
7. Noda M, Kariura Y, Amano T, Manago Y, Nishikawa K, Aoki S, Wada K: **Kinin receptors in cultured rat microglia.** *Neurochem International* 2004, **45**:437-442.
8. Marceau F, Hess JF, Bachvarov DR: **The B1 receptors for kinins.** *Pharmacol Rev* 1998, **50**:357-386.
9. Bradl M, Hohlfeld R: **Molecular pathogenesis of neuroinflammation.** *J Neurol Neurosurg Psychiatry* 2003, **74**:1364-1370.
10. Antel JP, Owens T: **Immune regulation and CNS autoimmune disease.** *J Neuroimmunol* 1999, **100**:181-189.
11. Calixto JB, Cabrini DA, Ferreira J, Campos MM: **Kinins in pain and inflammation.** *Pain* 2000, **87**:1-5.
12. Tayeh MA, Scicli AG: **Angiotensin II and bradykinin regulate the expression of P-selectin on the surface of endothelial cells in culture.** *Proc Assoc Am Physicians* 1998, **110**:412-421.
13. McLean PG, Ahluwalia A, Perreti M: **Association between kinin B(1) receptor expression and leukocyte trafficking across mouse mesenteric postcapillary venules.** *J Exp Med* 2000, **192**:367-380.
14. Perron MS, Gobeil F Jr, Pelletier S, Regoli D, Sirois P: **Involvement of bradykinin B1 and B2 receptors in pulmonary leykocyte accumulation induced by Sephadex beads in guinea pigs.** *Eur J Pharmacol* 1999, **376**:83-89.
15. Souza DG, Lomez ES, Pinho V, Pesquero JB, Bader M, Pesquero JL, Teixeira MM: **Role of bradykinin B2 and B1 receptors in the local, remote, and systemic inflammatory responses that follow intestinal ischemia and reperfusion injury.** *J Immunol* 2004, **172**:2542-2548.
16. Izikson L, Klein RS, Charo IF, Weiner HL, Luster AD: **Resistance to experimental autoimmune encephalomyelitis in mice lacking the CC chemokine receptor (CCR)2.** *J Exp Med* 2000, **192**:1075-1080.

17. Glass WG, Hickey MJ, Hardison JL, Liu MT, Manning JE, Lane TE: **Antibody targeting of the CC chemokine ligand 5 results in diminished leukocyte infiltration into the central nervous system and reduced neurologic disease in a viral model of multiple sclerosis.** *J Immunol* 2004, **172:**4018-4025.

18. dos Santos AC, Barsante MM, Arantes RME, Bernard CCA, Teixeira MM, Carvalho-Tavares J: **CCL2 and CCL5 mediate leukocyte adhesion in experimental autoimmune encephalomyelitis – an intravital microscopy study.** *J Neuroimmunol* 2005, **162:**122-129.

19. Larrivée JF, Bachvarov DR, Houle F, Landry J, Huot J, Marceau F: **Role of mitogen-activated protein kinases in the expression of the B1 kinin receptors induced by tissue injury.** *J Immunol* 1998, **160:**1419-1426.

20. Koyama S, Sato E, Nomura H, Kubo K, Miura M, Yamashita T, Nagai S, Izumi T: **Bradykinin stimulates type II alveolar cells to release neutrophil and monocyte chemotactic activity and inflammatory cytokines.** *Am J Pathol* 1998, **153:**1885-1993.

21. Eddleston J, Christiansen SC, Jenkins GR, Koziol JA, Zuraw BL: **Bradykinin increases the in vivo expression of CXC chemokine receptors CXCR1 and CXCR2 in patients with allergic rhinitis.** *J Allergy Clin Immunol* 2003, **111:**106-112.

22. Campos MM, Souza GEP, Calixto JB: **In vivo B1 kinin-receptor up-regulation. Evidence for involvement of protein kinases and nuclear factor kappa B pathways.** *Br J Pharmacol* 1999, **127:**1851-1859.

23. Phagoo SB, Reddi K, Anderson KD, Leeb-Lundberg IM, Warburton D: **Bradykinin B1 receptor up-regulation by interleukin-1β and B1 agonist occurs through independent and synergistic intracellular signaling mechanisms in human lung fibroblasts.** *J Pharmacol Exp Ther* 2001, **298:**77-85.

24. Pesquero JB, Araujo RC, Heppenstall PA, Stucky CI, Silva JA, Walther T Jr, Oliveira SM, Pesquero JL, Paiva AC, Calixto JB: **Hypoalgesia and altered inflammatory responses in mice lacking kinin B1 receptors.** *Proc Natl Acad Sci* 2000, **97:**8140-8145.

25. Stewart JM: **Bradykinin antagonists: discovery and development.** *Peptides* 2004, **25:**527-532.

26. Kerfoot SM, Kubes P: **Overlapping roles of P-selectin and alpha 4-integrin to recruit leukocytes to the central nervous system in experimental autoimmune encephalomyelitis.** *J Immunol* 2002, **169:**1000-1006.

27. Carvalho-Tavares J, Hickey MJ, Hutchison J, Michaud J, Sutcliffe IT, Kubes P: **A role for platelets and endothelial selectin in tumor necrosis factor-induced leukocyte recruitment in the brain microvasculature.** *Circ Res* 2000, **87:**1141-1148.

28. Lorenzl S, Ködel U, Frei K, Pfister HW: **Effect of the bradykinin B2 receptor antagonist Hoe140 in experimental pneumococcal meningitis in the rat.** *Eur J Pharmacol* 1996, **308:**335-341.

29. Stover JF, Dohse NK, Unterberg AW: **Significant reduction in brain swelling by administration of nonpeptide kinin B2 receptor antagonisy LF 160687Ms after controlled impact injury in rats.** *J Neurosurg* 2000, **92:**853-859.

30. Gorlach C, Hortobagyi T, Hortobagyi S, Benyo Z, Relton J, Whalley ET, Wahl M: **Bradykinin B2, but not B1, receptor antagonist has a neuroprotective effect after brain injury.** *Neurotrauma* 2003, **18:**833-888.

31. Kaplanski J, Pruneau D, Asa I, Artru AA, Azez A, Ivashkova Y, Rudich Z, Shapira Y: **LF 16-0687 Ms, a bradykinin B2 receptor antagonist, reduces brain edema and improves long-term neurological function recovery after closed head trauma in rats.** *J Neurotrauma* 2002, **19:**953-964.

32. Hellal F, Pruneau D, Palmier B, Faye P, Croci N, Plotkine M, Marchand-Verrechia C: **Detrimental role of bradykinin B2 receptor in a murine model of diffuse brain injury.** *J Neurotrauma* 2003, **20:**841-851.

33. Souza DG, Pinho V, Pesquero JL, Lomez ES, Poole S, Juliano L, Correa A Jr, de A Castro MS, Teixeira MM: **Role of the bradykinin B2 receptor for the local and systemic inflammatory response that follows severe reperfusion injury.** *Br J Pharmacol* 2003, **139:**129-139.

34. Abraham WM, Burch RM, Farmer SG, Sielczak MW, Ahmed A, Cortes A: **A bradykinin antagonist modifies allergen-induced mediator release and late bronchial responses in sheep.** *Am Rev Respir Dis* 1991, **143:**787-796.

35. Bandeira-Melo C, Calheiros AS, Silva PM, Cordeiro RS, Teixeira MM, Martins MA: **Suppressive effect of distinct bradykinin B2 receptor antagonist on allergen-evoked exudation and leukocyte infiltration in sensitized rats.** *Br J Pharmacol* 1999, **127:**315-320.

36. Sainz IM, Uknis AB, Isordia-Salas I, Dela Cadena RA, Pixley RA, Colman RW: **Interactions between bradykinin (BK) and cell adhesion molecule (CAM) expression in peptidoglycan-polysaccharide (PG-PS)-induced arthritis.** *FASEB J* 2004, **18:**887-889.

37. Bernard CCA, Johns TG, Slavin A, Ichikawa M, Ewing C, Liu J, Bettdapura J: **Myelin oligodendrocyte glycoprotein: a novel candidate autoantigen in multiple sclerosis.** *J Mol Med* 1997, **75:**77-88.

38. Genain CP, Cannella B, Hauser SL, Raine CS: **Identification of autoantibodies associated with myelin damage in multiple sclerosis.** *Nat Med* 1999, **5:**170-175.

39. Germain L, Barabe J, Galeano C: **Increased blood concentration of des-Arg9-bradykinin in experimental allergic encephalomyelitis.** *J Neurol Sci* 1988, **83:**211-217.

40. Groger M, Lebesgue D, Pruneau D, Relton J, Kim SW, Nussberger J, Plesnila N: **Release of bradykinin and expression of kinin B2 receptor in the brain: Role for cell death and brain edema formation after local cerebral ischemia in mice.** *J Cereb Blood Flow Metab* 2005, **25:**978-989.

41. Xia CF, Smith RS Jr, Shen B, Yang ZR, Borlongan CV, Chao L, Chao J: **Postischemic brain injry is exacerbated in mice lacking the kinin B2 receptor.** *Hypertension* 2006, **47:**752-761.

42. Kleinschnitz C, Austinat M, Bader M, Renné T, Stoll G: **Deficiency of bradykinin receptor B2 is not detrimental in experimental stroke.** *Hypertension* 2008, **51:**e41.

43. Shughrue PJ, Ky B, Austin CP: **Localization of B1 bradykinin receptor mRNA in the primate brain and spinal cord: an in situ hybridization study.** *J Comp Neurol* 2003, **465:**372-384.

44. Liu Y, Hashizume K, Chen K, Samoto K, Ningaraj N, Asotra K, Black KL: **Correlation between bradykinin-induced blood-tumor barrier permeability and B2 receptor expresión in experimental braintumors.** *Neurol Res* 2001, **23:**379-387.

45. Chen EY, Emerich DF, Bartus RT, Kordower JH: **B2 bradykinin receptor immunoreactivity in rat brain.** *J Comp Neurol* 2000, **427:**1-18.

46. Seabrook GR, Easter A, Dawson GR, Bowery BJ: **Modulation of long-term potentiation in CA1 region of mouse hippocampal brain slices by GABAA receptor benzodiazepine site ligands.** *Neuropharmacology* 1997, **36:**823-830.

47. Murone C, Perich RB, Schlawe I, Chai SY, Casley D, Macgregor DP, Muller-Esterl W, Mendelsohn FA: **Characterization and localization of bradykinin b2 receptors in the guinea-pig using a radioiodinated Hoe140 analogue.** *Eur J Pharmacol* 1996, **306:**237-247.

48. Murone C, Paxinos G, McKinley MJ, Oldfield BJ, Muller-Esterl W, Mendelsohn FA, Chai SY: **Distribution of bradykinin b2 receptors in sheep brain and spinal cords visualized by in vitro autoradiograph.** *J Comp Neurol* 1997, **381:**203-218.

49. Duchene J, Lecomte F, Ahmed S, Cayla C, Pesquero J, Bader M, Perretti M, Ahluwalia A: **A novel inflammatory pathway involved in leukocyte recruitment: role for the kinin B1 receptor and the chemokine CXCL5.** *J Immunol* 2007, **179:**4849-56.

50. Poole S, Lorenzetti BB, Cunha JM, Cunha FQ, Ferreira SH: **Bradykinin B1 and B2 receptors, tumour necrosis factor alpha and inflammatory hyperalgesia.** *Br J Pharmacol* 1999, **126:**649-56.

51. Cunha TM, Verri WA Jr, Silva JS, Poole S, Cunha FQ, Ferreira SH: **A cascade of cytokines mediates mechanical inflammatory hypernociception in mice.** *Proc Natl Acad Sci* 2005, **102:**1755-1760.

52. Sorensen TL, Tani M, Jensen J, Pierce V, Luchinetti C, Folcik VA, Qin S, Rottman J, Sellebjerg F, Strieter RM: **Expression of specific chemokines and chemokine receptors in the central nervous system of multiple sclerosis patients.** *J Clin Invest* 1999, **103:**807-815.

53. Mahad DJ, Ransohoff RM: **The role of MCP-1 (CCL2) and CCR2 in multiple sclerosis and experimental autoimmune encephalomyelitis (EAE).** *Semin Immunol* 2003, **15:**23-32.

54. Kennedy KJ, Strieter RM, Luckacs NW, Karpus WJ: **Acute and relapsing experimental autoimmune encephalomyelitis are regulated by differential expression of the CC chemokines macrophage inflammatory protein-1 alpha and monocyte chemotactic protein-1.** *J Neuroimmunol* 1998, **92:**98-108.

55. Gu L, Tseng S, Horner RM, Tam C, Loda M, Rollins BJ: **Control of Th2 polarization by chemokine monocyte chemoattractant protein-1.** *Nature* 2000, **404**:407-411.
56. Ruddle NH, Bergman C, McGrath KM, Lingenheld EG, Grunnet ML, Padula SJ, Clark RB: **An antibody to lymphotoxin and tumor necrosis factor prevents transfer of experimental allergic encephalomyelitis.** *J Exp Med* 1990, **172**:1193-1200.
57. Körner H, Lemckert FA, Chaudhri G, Etteldorf S, Sedgwick JD: **Tumor necrosis factor blockade in actively induced experimental autoimmune encephalomyelitis prevents clinical disease despite activated T cell infiltration to the central nervous system.** *Eur J Immunol* 1997, **27**:1973-1981.
58. Cunha TM, Verri WA Jr, Fukada SY, Guerrero AT, Santodomingo-Garzón T, Poole S, Parada CA, Ferreira SH, Cunha FQ: **TNF-alpha and IL-1beta mediate inflammatory hypernociception in mice triggered by B1 but not B2 kinin receptor.** *Eur J Pharmacol* 2007, **573**:221-229.
59. Ferreira SH, Lorenzetti BB, Poole S: **Bradykinin initiates cytokine mediated inflammatory hyperalgesia.** *Br J Pharmacol* 1993, **110**:1227-1231.
60. Calixto JB, Medeiros R, Fernandes ES, Ferreira J, Cabrini DA, Campos MM: **Kinin B1 receptors: key G-protein-coupled receptors and their role in inflammatory and painful processes.** *Br J Pharmacol* 2004, **143**:803-818.
61. Pruneau D, Paquet JL, Luccarini JM, Defrêne E, Fouchet C, Franck RM, Loillier B, Robert C, Bélichard P, Duclos H, Cremers B, Dodey P: **Pharmacological profile of LF 16-0687 Ms, a new potent non-peptide bradykinin B2 receptor antagonist.** *Immunopharmacology* 1999, **43**:187-94.
62. Ding-Zhou L, Margaill I, Palmier B, Pruneau D, Plotkine M, Marchand-Verrecchia C: **LF 16-0687 Ms, a bradykinin B2 receptor antagonist, reduces ischemic brain injury in a murine model of transient focal cerebral ischemia.** *Br J Pharmacol* 2003, **139**:1539-1547.
63. Ukkonen M, Wu K, Reipert B, Dastidar P, Elovaara I: **Cell surface adhesion molecules and cytokine profiles in primary progressive multiple sclerosis.** *Mult Scler* 2007, **13**:701-707.
64. Correale J, Bassani Molinas Mde L: **Temporal variations of adhesion molecules and matrix metalloproteinases in the course of MS.** *J Neuroimmunol* 2003, **140**:198-209.
65. Bullard DC, Hu X, Schoeb TR, Collins RG, Beaudet AL, Barnum SR: **Intercellular adhesion molecule-1 expression is required on multiple cell types for the development of experimental autoimmune encephalomyelitis.** *J Immunol* 2007, **178**:851-857.
66. Chaudhary P, Marracci GH, Bourdette DN: **Lipoic acid inhibits expression of ICAM-1 and VCAM-1 by CNS endothelial cells and T cell migration into the spinal cord in experimental autoimmune encephalomyelitis.** *J Neuroimmunol* 2006, **175**:87-96.