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**Interleukin-1 β induced activation of the hypothalamus-pituitary-adrenal axis is
dependent on interleukin-1 receptors on non-hematopoietic cells**

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Abstract

The proinflammatory cytokine interleukin-1 β (IL-1 β) plays a major role in the signal transduction of immune stimuli from the periphery to the central nervous system, and has been shown to be an important mediator of the immune-induced stress hormone release. The signaling pathway by which IL-1 β exerts this function involves the blood-brain-barrier and induced central prostaglandin synthesis, but the identity of the blood-brain-barrier cells responsible for this signal transduction has been unclear, with both endothelial cells and perivascular macrophages suggested as critical components. Here, using an irradiation and transplantation strategy, we generated mice expressing IL-1 type 1 receptors (IL-1R1) either in hematopoietic or non-hematopoietic cells and subjected these mice to peripheral immune challenge with IL-1 β . Following both intraperitoneal and intravenous administration of IL-1 β , mice lacking IL-1R1 in hematopoietic cells showed induced expression of the activity marker c-Fos in the paraventricular hypothalamic nucleus, and increased plasma levels of ACTH and corticosterone. In contrast, these responses were not observed in mice with IL-1R1 expression only in hematopoietic cells. Immunoreactivity for IL-1R1 was detected in brain vascular cells that displayed induced expression of the prostaglandin synthesizing enzyme cyclooxygenase-2 and that were immunoreactive for the endothelial cell marker CD31, but was not seen in cell positive for the brain macrophage marker CD206. These results imply that activation of the HPA-axis by IL-1 β is dependent on IL-1R1s on non-hematopoietic cells, such as brain endothelial cells, and that IL-1R1 on perivascular macrophages are not involved.

Key words: HPA-axis; corticosterone; ACTH; c-Fos; paraventricular nucleus; chimeric mice; bone marrow transplantation; brain endothelial cells; perivascular macrophages; cyclooxygenase-2

1. Introduction

The hypothalamic-pituitary-adrenal (HPA) axis plays a central role in the neuroendocrine responses to immune stimuli. Peripherally released pathogens induce massive secretion of corticotropin releasing hormone (CRH) from the hypothalamus, followed by secretion of adrenocorticotrophic hormone (ACTH) from the pituitary and glucocorticoids from the adrenal cortex. The proinflammatory cytokine interleukin-1 β (IL-1 β) has been shown to be an important mediator of the immune-induced glucocorticoid release (Berkenbosch et al., 1987; Besedovsky et al., 1986), but the signaling pathway by which IL-1 β exerts this function has not been determined. While there is a strong evidence that brain endothelial cells, by induced prostaglandin (PG) synthesis, are critical for the immune-elicited fever (Engström et al., 2012), it has been suggested that perivascular macrophages, located within the two sheets of the basal membrane of the cerebral blood vessels and hence on the abluminal side of the endothelial cells, mediate IL-1 β evoked HPA-axis activation, also by induced PG-synthesis (Schiltz and Sawchenko, 2002, 2003; Serrats et al., 2010). Deletion of the gene encoding microsomal prostaglandin E-synthase (Trebino et al., 2003), rendering the animals unable to elicit central PGE₂ synthesis upon immune stimulation (Engblom et al., 2003), results in attenuated corticosterone release to such stimuli (Elander et al., 2009), showing an important role for immune-induced PGE₂ in this response.

The evidence for a role of perivascular cells in the IL-1 β induced corticosterone release are mainly morphological demonstrations of cyclooxygenase (Cox)-2 induction in these cells in certain experimental paradigms (Schiltz and Sawchenko, 2002), as well as attenuated HPA-axis activation upon immune challenge (Serrats et al., 2010) in a

model of macrophage/monocyte depletion/degeneration (Serrats et al., 2010) induced by central injection of clodronate (Van Rooijen, 1989). However, these data are contradictory to the selective expression of interleukin-1 type 1 receptors (IL-1R1s) in brain endothelial cells, as shown by immunohistochemistry on sections from the rat brain, with no demonstrated labeling of perivascular cells (Konsman et al., 2004).

Here we re-examined this issue by first creating mice that selectively lacked IL-1R1s either in hematopoietically-derived cells, including perivascular macrophages, or in non-hematopoietic cells. This was obtained by subjecting wild type and IL-1R1 knockout mice to potentially lethal whole body gamma-irradiation, followed by transplantation of bone marrow cells from the opposite genotype. Through this strategy we generated mice on a wild-type background with hematopoietically derived IL-1R1 knockout cells, and mice on a IL-1R1 knockout background with hematopoietically derived wild-type cells, as well as wild type and IL-1R1 knockout mice transplanted with cells of the same genotype.

Our data show that HPA-axis activation and ACTH and corticosterone release elicited by IL-1 β injection intraperitoneally or intravenously are independent of IL-1R1s on hematopoietically derived cells. They also demonstrate that IL-1R1s are induced in the mouse brain by peripheral immune stimuli and expressed by brain endothelial cells but not by perivascular macrophages. These observations hence refute the idea that perivascular macrophages are critical for HPA-axis activation and stress hormone release. Instead they imply that, at least in mice, IL-1R1s on brain endothelial cells and other non-hematopoietic cells mediate these responses.

2. Materials and methods

2.1. Animals

Littermates of IL-1R1 knockout (KO) and wild-type (WT) mice were obtained by heterozygous breeding of animals derived from IL-1R1 KO mice originally obtained from the Jackson laboratories [B6.129S7-*Il1r1*^{tm1Imx}/J; (Glaccum et al., 1997)]. The latter were crossed once with wild-type C57BL/6 mice to generate heterozygotes. For transplantation experiments (donor bone marrow) IL-1R1 KO mice were crossed with a GFP-expressing strain [C57BL/6-Tg (CAG-EGFP)131Osb/LeySopJ; The Jackson Laboratory, Bar Harbor, ME]. The resulting heterozygous offspring were then crossed to generate GFP⁺IL-1R1^{-/-} and GFP⁺IL-1R1^{+/+} mice. The animals were housed one to four per cage on a 12-h light/dark cycle (lights on at 08.00 h) with water and food available *ad libitum*. All experimental procedures were approved by the Animal Care and Use Committee at Linköping University.

2.2. Irradiation and bone marrow transplantation

About 2-month-old KO and WT littermates were irradiated in a cage with two opposed fields, using a linear accelerator (Varian Clinac 600C; Varian, Palo Alto, CA, USA) to a total absorbed dose to water of 9 Gy, single fraction. Approximately 24 h after irradiation, the animals were injected i.v. with 2 x 10⁶ freshly prepared GFP⁺CD45⁺ bone marrow cells, as described in detail elsewhere (Engström et al., 2012). After the injection, mice were immediately transferred to an isolated room with autoclaved cages

(1-4 mice per cage) and received sterilized food and autoclaved water. During the first 3 weeks post transplantation, the water was supplemented with an antibiotic (Ciprofloxacin, 0.11 mg/ml; BMM Pharma, Stockholm, Sweden).

2.3. Flow cytometry

Blood from a tail vein was collected into heparinized saline. After separation using dextran and lysis of erythrocytes, leukocytes were re-suspended in phosphate-buffered saline (PBS) and analyzed for GFP expression by flow cytometry on a FACSCanto device (BD Biosciences, San Jose, CA, USA). Non-transplanted WT C57BL/6J mice and GFP+ mice were used as controls.

2.4. Injection of lipopolysaccharide (LPS), IL-1 β , or vehicle, and tissue collection

For determining HPA axis activity, IL1-R1 WT and KO mice were injected with IL-1 β (recombinant murine IL-1 β , 30 μ g/kg; Preprotech, Rocky Hill, NJ; catalog # 211-11B) intraperitoneally or intravenously via the tail vein. This dose has previously been shown to evoke the centrally elicited acute phase responses fever and anorexia in mice (Elander et al., 2007; Saha et al., 2005). Three hours later, the animals were killed by asphyxiation with CO₂ and decapitated. Brains were dissected out, fixed in 4% paraformaldehyde in PBS for 2 days, and then soaked in a solution of 30% sucrose in PBS and kept at 4°C until used for c-Fos immunostaining. At the same time, the blood

was collected into heparinized tubes. After centrifugation, plasma was taken and stored at -20°C until further use. For IL-1R1 immunohistochemistry, and to examine if the IL-1R1 expression was influenced by peripheral immune stimulation, WT animals were injected i.p. with LPS from *Escherichia coli* (Sigma-Aldrich, St. Louis, MO; serotype 0111:B4; 120 $\mu\text{g/kg}$, i.p.). Six hours later, the animals were killed with CO_2 and perfused with 4% paraformaldehyde. The brain were taken out, post-fixed overnight, and placed in a 30% sucrose solution at 4°C .

2.5. Hormone assays

For determination of plasma corticosterone levels, an enzyme immunoassay kit (COTEIA corticosterone kit; Immunodiagnostic systems, Boldon, UK) was used. The minimum detection concentration was 0.55 ng/ml. By using a 4-PL curve fit, a standard curve with an R value of 1.000 was obtained [for details, see (Elander et al., 2009)]. The concentrations of ACTH in plasma following intraperitoneal injection of IL-1 β were determined with a bead-based analysis kit (#MBN1A-41K; Millipore, Billerica, MA), using the Luminex-100 system, as described in detail previously (Ruud and Blomqvist, 2007). The minimum detectable concentration was 1.8 pg/ml. For samples obtained after intravenous IL-1 β injection, an EIA kit (EKE-001-21; Phoenix Pharmaceuticals, Burlingame, CA) was used, since the bead-based kit was no longer available. The assay range was 80-2000 pn/ml and there was no cross-reactivity with rat β -endorphin, α -MSH or LH-RH.

2.6. Immunohistochemistry

Brain sections were cut at 30 μ m on a freezing microtome, collected in cold cryoprotectant buffer (0.1 M phosphate buffer, 30 % ethylene glycol, 20 % glycerol), and stored at -20°C until further use. Immunohistochemical staining was performed according to protocols described in detail elsewhere (Engström et al., 2012). For single staining of c-Fos protein or IL-1R1, free-floating sections were pretreated with 0.3% H_2O_2 in PBS for 30 min, followed by 1% bovine serum albumin in PBS for 2 h, and then incubated in goat anti-IL-1R1 antibody (AF771; 1:1000; R&D Systems, Minneapolis, MN) or rabbit anti-c-Fos antibody (PC38 (Ab-5); 1:10,000; Millipore) in 0.3% Triton X-100 in PBS (PBST) at 4°C for 60 h, washed three times with 0.03% PBST, and processed using a Vectastain ABC kit (Vector Labs, Burlingame, CA). Peroxidase activity was detected by incubation for 1-3 min in 0.5 mg/ml of 3, 3'-diaminobenzidine tetrahydrochloride (Sigma-Aldrich) dissolved in 0.1 M Tris-HCl, with 0.01% hydrogen peroxide and 0.25% nickel ammonium sulfate. Counts of the number of c-Fos-ir cells were done under bright-field illumination from one section per animal through the paraventricular hypothalamic nucleus.

For dual- or triple-labeling immunofluorescence, free-floating sections were treated with rat anti-CD31 antibody (MCA2388GA; 1:1000; Serotec, Düsseldorf, Germany) as a marker for endothelial cells (Williams et al., 1996) or rat anti-CD206 antibody (MCA2235; 1:1000; Serotec) as a marker for perivascular macrophages (Galea et al., 2005), together with the antibodies against GFP (ab13970, made in chicken; 1:1000; Abcam, Cambridge, UK) or IL-1R1 and Cox-2 (sc-1747-R, made in rabbit; 1:1000; Santa Cruz Biotechnology, Santa Cruz, CA). Bound primary antibody was then

detected with Alexa Fluor Dyes-conjugated secondary antibodies (405 donkey anti-rat IgG, 488 donkey anti-goat IgG, and 555 donkey anti-rabbit IgG; 1:1000; Invitrogen, Carlsbad, CA). For dual-labeling immunofluorescence, the sections were treated with the anti-CD206 antibody and the chicken anti-GFP antibody and then the proper secondary antibodies (568 donkey anti-rat IgG and 488 donkey anti-chicken IgG, 1:1000; Invitrogen). Sections were analyzed on a Nikon 80i microscope equipped with epi-fluorescence, and a Zeiss Axio Observer Z1 fluorescence microscope connected to a Zeiss LSM 700 confocal unit with 405, 488, 555 and 639 nm diode lasers.

2.7. Statistical analyses

Data were analyzed by a one-way (i.v. injections) or two-way (i.p. injections) ANOVA, followed by Sidak's post hoc test. The differences were considered statistically significant at $P < 0.05$.

3. Results

3.1. Extensive replacement of hematopoietic cells after irradiation and bone marrow transplantation

Using the irradiation and transplantation protocol employed here, a high level of replacement in blood and brain of native hematopoietic cells with transplanted cells has been shown by us previously (Engström et al., 2012; Hamzic et al., 2013; Ruud et al., 2013). In the present study, the proportion of GFP⁺ cells among the white blood cells in the different groups of chimeras was in average 84% (SEM 1%), as determined by flow

cytometry (Fig. 1A). Microscopic examination (Fig. 1B) of 12 randomly selected fields from the brain of each of four mice showed that 80.3% (SEM 3.4%) of the CD206 positive cells (perivascular macrophages) also expressed GFP and hence derived from the transplanted cells.

In addition to the replacement in the brain of perivascular cells, there was also infiltration of bone marrow derived microglia in the brain parenchyma (not shown), as reported by us in some detail in previous studies using the same irradiation and transplantation protocol (Engström et al., 2012; Hamzic et al., 2013). These cells, which similar to the perivascular macrophages are Iba1 positive (Engström et al., 2012; Imai et al., 1996) but that do not display the mannose receptor (CD206) which specifically characterizes perivascular macrophages, constitute however only a small portion of the microglial cells (see Engström et al., 2012; Hamzic et al., 2013).

3.2. IL-1R1 in non-hematopoietic cells plays an indispensable role for the release of ACTH and corticosterone induced by intraperitoneal or intravenous injection of IL-1 β

We first performed functional analyses of the role of IL-1R1 in hematopoietic and non-hematopoietic cells, respectively, for ACTH and corticosterone release induced by intraperitoneally injected IL-1 β , by comparing the hormone response between the different groups of mice chimeric for the IL-1R1. As expected, IL-1 β enhanced plasma corticosterone and ACTH in mice with intact IL-1R1 expression (WT $\rightarrow$ WT) but not in mice lacking IL-1R1 (KO $\rightarrow$ KO). WT mice transplanted with hematopoietic cells from

IL-1R1 knockout animals (KO→WT) showed normal IL-1 β -induced increase of plasma ACTH and corticosterone (Fig. 2A). In contrast, these responses were not observed in mice with IL-1R1 expression only in hematopoietic cells (WT→KO) (Fig. 2A), suggesting that it is non-hematopoietic cells that plays a critical role for the IL-1 β /IL-1R1 signaling that enhances plasma levels of stress hormones. Similar results were obtained following intravenous injection of IL-1 β (Fig. 3A).

3.3. Intraperitoneal injection of IL-1 β activates neurons in the paraventricular hypothalamic nucleus via IL-1R1 on non-hematopoietic cells

Next we performed immunostaining for c-Fos in the paraventricular hypothalamic nucleus (PVH) of chimeric mice to examine the role of IL-1R1 in hematopoietic and non-hematopoietic cells, respectively, for the IL-1 β -induced neuronal activation in this nucleus. Mice with intact IL-1R1 expression (WT→WT) and WT mice transplanted with hematopoietic cells from IL-1R1 KO mice (KO→WT) showed large numbers of c-Fos-ir cells in the PVH, while mice lacking IL-1R1 either globally (KO→KO) or selectively in non-hematopoietic cells (WT→KO) showed no induced c-Fos expression, irrespective of whether IL-1 β was injected intraperitoneally (Fig. 2B, C) or intravenously (Fig. 3B, C). These results demonstrate that hematopoietic cells are not necessary for the neuronal activation of the PVH induced by intraperitoneal injection of IL-1 β .

3.4. LPS induces IL-1R1 expression in the brain vessels

To detect IL-1R1 in the brain, we performed immunohistochemical staining on brain sections from WT mice treated with intraperitoneal injection of saline or LPS, with sections from KO mice treated with LPS as a negative control. Brains were taken out 6 h after the injection. In saline-treated WT mice, there were almost no IL-1R1-ir cells seen in the brain (Fig. 4A). In contrast, many IL-1R1-ir cells, located in the brain vessels, were seen in LPS-treated WT animals, but not in the LPS-treated KO mice, demonstrating the specificity of the antibody (Fig. 4A). Little or no staining was seen in the brain parenchyma. The findings show that IL-1R1 is induced in brain vessels upon peripheral immune challenge.

3.5. Endothelial cells are the major source of IL-1R1

To determine which type of cells that expresses IL-1R1, we performed triple-immunostaining for IL-1R1 and Cox-2, together with cell-specific markers for brain vascular cells, on brain sections from mice killed 6 h after peripheral LPS injection. IL-1R1-ir and Cox-2-ir were mostly co-localized, and also co-localized with CD31, demonstrating that these antigen were located on brain endothelial cells (Fig. 4B). In contrast, IL-1R1-ir, as well as Cox-2-ir never co-localized with CD206, as marker for perivascular macrophages (Fig. 4C). Hence, these findings imply that the immune stimulus induced the expression of IL-1R1 and Cox-2 in brain endothelial cells, but not in perivascular macrophages.

4. Discussion

The present study demonstrates that IL-1R1s on non-hematopoietic cells are both necessary and sufficient for eliciting stress hormone release in response to peripheral immune challenge with IL-1 β . They also show that IL-1R1s on hematopoietically derived cells, including perivascular cells, neither are sufficient nor necessary for this response. These functional data are well in line with the present morphological demonstration that IL-1R1s are expressed by brain endothelial cells, likely to mediate at least part of the IL-1 β elicited response, but not by perivascular cells. Our data also show that IL-1 β -induced neuronal activation of the paraventricular nucleus, which contains hypophysiotropic CRH-expressing neurons, also is dependent on IL-1R1 on non-hematopoietic cells but not on IL-1R1 on hematopoietic cells.

The present morphological data are consistent with previous immunohistochemical work in rats, which has demonstrated that IL-1R1 protein in the brain is exclusively expressed on endothelial cells (Konsman et al., 2004), which in turn is largely consistent with findings from *in situ* hybridization studies in that species (Cunningham et al., 1992; Engblom et al., 2002; Ericsson et al., 1995). It should be noted, however, that whereas IL-1R1-ir was readily detected in brain endothelial cells in naïve rats (Konsman et al., 2004), its full demonstration in the mouse in the present study required prior peripheral immune challenge. Immune-elicited induction of IL-1R1 mRNA has previously been reported in peripheral cells and organ (Saccani et al., 1998), and the present results are consistent with such an induction also in the brain. While it previously have been reported that IL-1 receptors are down-regulated in brain after

immune challenge with LPS (Ban et al., 1993), those findings were obtained using radiolabeled IL-1 α ligands, and not by immunohistochemical detection of the IL-1R1.

No IL-1R1-ir was seen in perivascular macrophages, neither in naïve animals nor after peripheral immune challenge. This observation is similar to that in rats, in which perivascular macrophages did not display any IL-1R1-ir or IL-1R1 mRNA (Engblom et al., 2002; Konsman et al., 2004). While this does not exclude that perivascular macrophages express IL-1R1, but at low level that does not permit detection by immunohistochemistry or in situ hybridization, our functional observations in the hybrid mice imply that any IL-1R1 mediated signaling in perivascular macrophages is neither sufficient nor necessary for eliciting HPA-axis activation. Thus, whereas WT mice transplanted with KO bone marrow displayed the same strong IL-1 β induced neuronal activation in the PVH (as determined by their c-Fos expression) and similar ACTH and corticosterone release as WT $\rightarrow$ WT mice, IL-1R1 KO mice transplanted with WT bone marrow were completely devoid of these responses. With the qualification that the transplanted cells are able to perform the full spectrum of normal function (see below), the present data are clearly contradictory to the idea that perivascular macrophages, and not endothelial cells, mediate IL-1 β induced HPA-axis activation (Serrats et al., 2010). While that work, which was based on a model using toxin-induced elimination of macrophages (Van Rooijen, 1989), was done in rats, and the divergent results hence could be explained by species differences, the apparent absence of IL-1R1 on perivascular macrophages also in that species (Engblom et al., 2002; Konsman et al., 2004), seems to contradict any IL-1 β mediated receptor dependent signaling by these cells in the rat as well.

The irradiation and transplantation procedure used here has been employed by us in three previous studies (Engström et al., 2012; Hamzic et al., 2013; Ruud et al., 2013). In these studies transplanted cells from WT mice were found to be able to respond to an immune challenge with production of Cox-2 [resulting in elevated levels of PGE₂ in plasma (Engström et al., 2012)] and of IL-6 (Hamzic et al., 2013), and they were demonstrated to largely rescue the WT phenotype in experimental paradigm involving LPS induced anorexia (Ruud et al., 2013). Furthermore, as seen both in the present study and in previous reports (Engström et al., 2012; Hamzic et al., 2013), the transplanted perivascular cells in the brain express the scavenger receptor CD206, similar to the resident cells, suggesting a normal function of these cells.

However, as we have discussed in some detail previously (Hamzic et al., 2013), the irradiation has been reported to compromise the immune response in the brain, and in addition to the replacement of perivascular macrophages by transplanted cells, there is also infiltration in the brain parenchyma of bone-marrow derived microglia (Engström et al., 2012; Hamzic et al., 2013; Simard et al., 2006; present study). Little such infiltration was seen in non-irradiated mice given bone marrow transplantation (Vallieres and Sawchenko, 2003), indicating that the irradiation might compromise the integrity of the blood-brain barrier (Diserbo et al., 2002) and hence raising the concern that it might change the immune response in the brain. Addressing this issue, Turrin et al. compared the brain expression of innate immune markers between normal and chimeric mice following LPS administration. They found no differences and concluded that the innate immune response of chimeric mice is similar to that of WT mice. In the same vein, we previously demonstrated that the LPS induced release of IL-1 β in plasma was similar in irradiated mice as in non-irradiated mice, and we also showed that the

febrile response to LPS was unaffected by the irradiation, suggesting a normal innate immune response (Engström et al., 2012).

We found here that the IL-1R1 was co-localized with induced Cox-2 expression in cells that also stained for the endothelial cell marker CD31. This observation is consistent with findings in rats, showing IL-1R1 and Cox-2 mRNA in von Willebrand-positive endothelial cells (Engblom et al., 2002), and in line with our previous demonstration in mice that peripheral administration of IL-1 β , similar to LPS, evokes Cox-2 expression in brain endothelial cells, but not in other blood-brain barrier cells (Engström et al., 2012). Studies using IL-1R1 knockdown in endothelial cells have shown that the IL-1 β -induced Cox-2 expression in the endothelial cells is dependent on intact IL-1R1 on these cells (Ching et al., 2007), which together with morphological data showing co-localization of the IL-1R1, Cox-2 and mPGES-1 in the endothelial cells (Ek et al., 2001; Engblom et al., 2002) hence suggests a functional link between ligand binding on the endothelial cells and induced PGE₂ synthesis by these cells (Bazan, 2001).

Observations in mPGES-1 KO mice, which cannot evoke PGE₂ synthesis upon immune challenge (Engblom et al., 2003; Engström et al., 2012), have shown that the induced PGE₂ synthesis is involved in the stress hormone release in response to immune insults (Elander et al., 2009), and the present data are consistent with the idea that this PGE₂ synthesis occurs in brain endothelial cells. It should be noted however, that this involvement may apply only to the later phases of the stress hormone response, because the immediately occurring corticosterone release, at least when LPS is used as immune stimulus, is unaffected by mPGES-1 deletion (Elander et al., 2009) and seems to be

driven by a Cox-1 and not a Cox-2 dependent mechanism (Elander et al., 2010). These observations have bearing on the interpretation of the present data on induced c-Fos expression in the PVH, suggestive of activation of hypophysiotropic cells in this nucleus. While we here found that the IL-1 β induced c-Fos expression in the PVH was dependent on IL-1R1s on non-hematopoietic cells, this observation does not necessarily imply that the IL-1 β signaling that give rise to this response occurs via endothelial cells and induced PGE₂ synthesis, because c-Fos expression in PVH upon immune challenge with intraperitoneally injected LPS has been shown to be unaffected by genetic deletion of the prostaglandin synthesizing enzymes Cox-1, Cox-2 and mPGES-1 (Elander et al., 2009). In the work with endothelial specific IL-1R1 knockdown it was shown that such knockdown abolished c-Fos expression in the PVH after intravenous but not after intraperitoneal injection of IL-1 β , suggesting a PGE₂ dependent pathway for the immune signaling after the former but not after the latter administration route (Ching et al., 2007).

A possible alternative signaling route that elicits c-Fos expression in the PVH following intraperitoneal injection of IL-1 β could, in addition to immune-induced synthesis and transfer into the brain of IL-1 β by the endothelial cells themselves (Verma et al., 2006), be via IL-1R1 expressed on peripheral nerves (Dantzer, 2001). Thus, it has been reported that vagal afferents express IL-1R1 (Ek et al., 1998) and that they mediate behavioral responses to immune challenge (Maier et al., 1998). Moreover, blockage of neural signaling in somatic nerves by local anesthesia has been shown to abolish centrally elicited responses to peripheral immune challenge (Belevych et al., 2010; Roth and De Souza, 2001). Considering the findings in previous studies (Ching et al., 2007; Elander et al., 2009), it is hence conceivable that the c-Fos expression in the

PVH seen in the present study following intraperitoneal injection of IL-1 β is mediated by such a direct neuronal route, whereas the activation that was seen after intravenous injection of IL-1 β may be dependent on signaling across the blood-brain barrier.

In conclusion, the present study shows that activation of the HPA-axis by IL-1 β in mice is dependent on IL-1R1s on non-hematopoietic cells such as brain endothelial cells and peripheral nerves, but that IL-1R1 on hematopoietic cells, including perivascular macrophages, are not involved.

Conflict of interest statement

No conflict of interest is declared by the authors.

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Figures

Figure 1. Reconstitution of transplanted bone marrow cells. (A) Percentage of GFP labeled white blood cells in recipient mice. WT→WT: WT mice transplanted with WT bone marrow; KO→KO: KO mice transplanted with KO bone marrow; WT→KO: KO mice transplanted with WT bone marrow; KO→WT: WT mice transplanted with KO bone marrow. Squares, triangles, circles, and diamonds represent individual values in the respective group. Horizontal lines show the mean. (B) Confocal micrographs showing dual-immunostaining for GFP (green) and CD206 (red), a marker for perivascular macrophages. Arrowheads point at dual-labeled cells and arrows at single-labeled cells. Scale bar: 25 μ m.

Figure 2. Activation of the HPA axis 3 h after intraperitoneal injection of IL-1 β (30 μ g/kg) or vehicle. (A) Plasma concentration of corticosterone and ACTH. $**P < 0.01$, and $***P < 0.001$ between treatments ($n = 9-11$). Error bars = SEM. (B) Representative images of immunostaining for c-Fos in the PVH. Scale bar: 200 μ m. 3v, 3rd ventricle. (C) Counts of the number of c-Fos-ir cells in the PVH in the different chimeras. $***P < 0.001$ between treatments ($n = 5$). Error bars = SEM.

Figure 3. Activation of HPA axis 3 h after intravenous injection of IL-1 β (30 mg/kg) or vehicle. (A) Plasma concentration of corticosterone and ACTH. * $P < 0.05$, and ** $P < 0.01$ vs. vehicle treated WT→WT mice ($n = 4-9$, except for KO→KO where $n = 1$). Error bar = SEM. (B) Representative images of immunostaining for c-Fos in the PVH. Scale bar: 200 μ m. 3v, 3rd ventricle. (C) Counts of the number of c-Fos-ir cells in the PVH in the different chimeras. *** $P < 0.001$ vs WT→KO mice ($n = 3$, except for KO→KO where $n = 1$). Error bars = SEM.

Figure 4. Immunostaining for IL-1R1s. (A) IL-1R1 expression in the cerebral cortex and hypothalamus 6 h after injection of saline or LPS in wild-type and KO mice. (B-C) Confocal micrographs showing immunostaining for IL-1R1 (green), Cox-2 (red) and the cell specific markers CD31 identifying endothelial cells (B), and CD206 identifying perivascular macrophages (C) in brains of WT mice 6 hours after LPS injection. Scale bar in A is 50 μ m, and in B-C 20 μ m.