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Interleukin-1β 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-1β (IL-1β) 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-1β 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-1β. Following both intraperitoneal and intravenous administration of IL-1β, 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 cells positive for the brain macrophage marker CD206. These results imply that activation of the HPA-axis by IL-1β is dependent on IL-1R1s on non-hematopoietic cells, such as brain endothelial cells, and that IL-1R1 on perivascular macrophages are not involved.

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 adrenocorticotropic hormone (ACTH) from the pituitary and glucocorticoids from the adrenal cortex. The proinflammatory cytokine interleukin-1β (IL-1β) 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-1β 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-1β 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), rending the animals unable to elicit central PGE_2 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 PGE_2 in this response.

The evidence for a role of perivascular cells in the IL-1β 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 in a model of macrophage/monocyte depletion/degeneration (Serrats et al., 2010) induced by central injection of clodronate (Van Rooijen,

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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-1β 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 2×10^6 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-1β, or vehicle, and tissue collection

For determining HPA axis activity, IL1-R1 WT and KO mice were injected with IL-1β (recombinant murine IL-1β, 30 μg/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 CO_2 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 μg/kg). Six hours later, the animals were killed with CO_2 and perfused with 4% paraformaldehyde. The brain was 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-1β 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-1β 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 μm 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, rinsed 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