Brain, Behavior, and Immunity 33 (2013) 123–130

Contents lists available at SciVerse ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Interleukin-6 primarily produced by non-hematopoietic cells mediates the lipopolysaccharide-induced febrile response

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ARTICLE INFO

Article history:
Received 31 January 2013
Received in revised form 15 June 2013
Accepted 25 June 2013
Available online 1 July 2013

Keywords:
Interleukin-6
Hematopoietic cells
Bone marrow transplantation
Fever

ABSTRACT

Interleukin-6 (IL-6) is critical for the lipopolysaccharide (LPS)-induced febrile response. However, the exact source(s) of IL-6 involved in regulating the LPS-elicited fever is still to be identified. One known source of IL-6 is hematopoietic cells, such as monocytes. To clarify the contribution of hematopoietically derived IL-6 to fever, we created chimeric mice expressing IL-6 selectively either in cells of hematopoietic or, conversely, in cells of non-hematopoietic origin. This was performed by extinguishing hematopoietic cells in wild-type (WT) or IL-6 knockout (IL-6 KO) mice by whole-body irradiation and transplanting them with new stem cells. Mice on a WT background but lacking IL-6 in hematopoietic cells displayed normal fever to LPS and were found to have similar levels of IL-6 protein in the cerebrospinal fluid (CSF) and in plasma and of IL-6 mRNA in the brain as WT mice. In contrast, mice on an IL-6 KO background, but with intact IL-6 production in cells of hematopoietic origin, only showed a minor elevation of the body temperature after peripheral LPS injection. While they displayed significantly elevated levels of IL-6 both in plasma and CSF compared with control mice, the increase was modest compared with that seen in LPS injected mice on a WT background, the latter being approximately 20 times larger in magnitude. These results suggest that IL-6 of non-hematopoietic origin is the main source of IL-6 in LPS-induced fever, and that IL-6 produced by hematopoietic cells only plays a minor role.

1. Introduction

Fever is an adaptive defense that is necessary for the protection and survival of the host in the presence of pathogenic agents (Kluger et al., 1975). The signaling pathways regulating fever are complex and still remain unclear. It is however generally accepted that prostaglandin E_2 (PGE_2), acting on prostaglandin E receptor type 3 (EP_3) expressing neurons in the preoptic area of the hypothalamus, is the principal mediator of fever (Engblom et al., 2003; Lazarus et al., 2007). In experimental animals, lipopolysaccharide (LPS), a component of the cell wall envelope of Gram-negative bacteria, is commonly used to provoke a febrile response (Oka et al., 2003; Romanovsky et al., 1998; Rudaya et al., 2005). A large part of the LPS-elicited production of PGE_2 and concomitant fever response is thought to involve cytokines, such as interleukin-6 (IL-6) and interleukin-1β (IL-1β) (Conti et al., 2004; Nadjar et al., 2005; Rummel et al., 2011).

IL-6 is a critical proinflammatory cytokine for the febrile response, because neither IL-6 knock-out (IL-6 KO) mice, nor animals treated with IL-6 antiserum develop fever upon peripheral immune stimulation (Cartmell et al., 2000; Chai et al., 1996; Kozak et al., 1998). However, depending on species, IL-6 *per se* has been reported to induce no or only weak to moderate fever (Blatteis, 1990; Cartmell et al., 2000; Dinarello et al., 1991; Harre et al., 2002; Nilsberth et al., 2009a; Rummel et al., 2006; Sakata et al., 1991; Wang et al., 1997). Several explanations for these contradictory observations have been proposed. For example, it has been suggested that IL-6 needs to reach a threshold level to activate the transcription factor STAT3 and initiate fever (Rummel et al., 2004). Another hypothesis is that IL-6 and IL-1β act in a synergistic way, based on the finding that IL-6 induces fever if administered together with a subfebrile dose of IL-1β (Cartmell et al., 2000; Harden et al., 2008). Thus, although the presence of IL-6 has been shown to be necessary for fever, its role in the regulation of the febrile response is not clear.

IL-6 is a pleiotropic cytokine with widespread expression. IL-6 is synthesized and secreted by monocytes/macrophages (Bauer et al., 1988; Callery et al., 1992; May et al., 1988; Northoff et al., 1987), fibroblasts (Helfgott et al., 1987; May et al., 1988), brain endothelial cells (Kakumu et al., 1992; Reyes et al., 1999; Verma et al., 2006), muscle cells (Andreasen et al., 2011), and hepatocytes (Panesar et al., 1999; Saad et al., 1995) following challenge with bacterial endotoxin. IL-6 has also been shown to be produced by

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http://dx.doi.org/10.1016/j.bbi.2013.06.006

astrocytes (Benveniste et al., 1990; Beurel and Jope, 2009), microglial cells (Sawada et al., 1992; Woodroofe et al., 1991), adipocytes (Flower et al., 2003), and neurons (Ringheim et al., 1995; Vallieres and Rivest, 1997) following different stimuli. IL-6 is thus synthesized by several different cells, however it has been difficult to determine the contribution of a specific cell type *in vivo*. For instance, the role of IL-6 produced by hematopoietic cells versus that produced by cells of non-hematopoietic lineage in LPS-induced fever remains to be clarified.

Here we examined the role of IL-6 derived from hematopoietic and non-hematopoeitic cells, respectively, in LPS-induced fever in mice chimeric for IL-6, meaning that they expressed IL-6 selectively either in cells of hematopoietic or non-hematopoietic origin. Chimeric mice were produced by exposing IL-6 KO and wild-type mice (WT) to whole body irradiation followed by transplantation with bone marrow cells from the opposite genotype. Thus, two chimeras were generated: WT mice with KO bone marrow (expressing IL-6 derived from non-hematopoietic cells) and KO mice with WT bone marrow (expressing IL-6 from cells of hematopoietic origin). This experimental set-up made it possible to distinguish between the febrile response mediated by IL-6 produced by non-hematopoietic cells and IL-6 produced by hematopoietically derived cells. The results show that IL-6 produced by cells of non-hematopoietic origin primarily is responsible for eliciting the febrile response following peripheral challenge with LPS, whereas IL-6 from hematopoietically derived cells only plays a minor but significant and time dependent role in the LPS-induced fever.

2. Materials and methods

2.1. Animals

C57 BL/6 mice deficient in IL-6 (IL-6 KO) and their WT littermates were used (Jackson Laboratory, Bar Harbor, ME). For transplantation experiments (donor bone marrow), the IL-6 KO mice were crossed with a C57BL/6 strain expressing green fluorescent protein (GFP) [C57BL/6-Tg(CAG-EGFP)C14-Y01-FM131Osb; kindly provided by Dr. Masaru Okabe, Osaka University, Japan (Okabe et al., 1997)] and the resulting IL-6 heterozygous mice were then crossed to generate GFP^+ IL-6 KO and GFP^+ WT mice, used as bone marrow donors. All mice were housed one to three per cage in a pathogen-free facility at an ambient temperature of 20–21 °C and with humidity between 30% and 50%, with food and water available *ad libitum* on a 12:12-h light–dark cycle (lights on at 07.00 h). The donor mice were 8–10 weeks old at the time of experiment. The mice receiving bone marrow transplantation were 2–5 months old at the time of irradiation and transplantation and 7–10 months old when sacrificed for experiments. Body weight (±SD) at the time of the experiment was for WT → WT: 32.4 g ± 5.1; for WT → KO: 30.6 g ± 3.8; for KO → WT: 31.3 g ± 5.9; and for KO → KO: 32.4 g ± 5.9 [there were no significant differences between the groups, as analyzed by a one-way ANOVA ($P = 0.46$)]. All experimental procedures were approved by the Animal Care and Use Committee at the Linköping University.

2.2. Irradiation and bone marrow transplantation

WT and IL-6 KO littermates of mixed gender were used. Groups were balanced to contain about equal proportions of male and female mice. They were irradiated in a cage with two opposed fields, using a linear accelerator (Varian Clinac 600C) with a 6 MV photon spectra to a total absorbed dose in water of 9 Gy, single fraction. The irradiation procedure followed the same experimental set-up as described earlier (Eliasson et al., 2010). Mice were injected intravenously within 6–18 h post-irradiation with 2×10^6 freshly prepared GFP^+CD45^+ bone marrow cells from either IL-6 KO or WT mice, in a total volume of 0.2 ml. Cells to be transplanted were collected from femurs and tibias after crushing the bones in ice-cold Dulbecco's phosphate buffered saline (PBS) (PAA Laboratories, Pasching, Austria) supplemented with 5% fetal calf serum (FCS) using a mortar and pestle. The bone marrow mixture was repeatedly passed through a 14 gauge needle and the resulting cell suspension, also containing small bone fragments, was transferred to a Falcon tube in which the bone fragments were allowed to sediment. The medium was carefully decanted and centrifuged. The obtained pellet was dissolved in ice-cold Dulbecco's PBS supplemented with 5% FCS and subsequently filtered through a 70 μm nylon mesh (BD Biosciences). Cells were then transferred to ice-cold Dulbecco's PBS supplemented with 5% FCS containing anti-mouse CD45 magnetic microbeads (1:10; Miltenyi Biotec, Bergisch Gladbach, Germany) and incubated for 20 min at 4 °C.

$CD45^+$ cells were enriched according to the manufacturer's instructions using the quadroMACS separation system with LS columns (Miltenyi Biotec). After an additional filtration, the cells were counted in a Bürker chamber and diluted to a final concentration of 10^7 cells/ml in Dulbecco's PBS. Aliquots of 0.2 ml of the final cell solution, one for each recipient mouse, were kept on ice until the moment of injection. After the injection, mice were immediately transferred to an isolated room with autoclaved cages (1–3 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, 0.1 mg/ml; BMM Pharma, Stockholm, Sweden).

2.3. Flow cytometry

Peripheral blood was collected by lateral tail vein bleeding into heparin treated tubes (5000 IE/ml; Leo Pharma, Malmö, Sweden) 5 months post transplantation. Equal volumes of PBS supplemented with 1% FCS and 2% Dextran (Sigma, St. Louis, MO) were added to each sample followed by incubation at 37 °C for 20 min. The upper phase was transferred to a new tube and centrifuged at 3000 rpm for 4 min. The supernatant was carefully decanted, and the pellet dissolved with 0.2 ml ammonium chloride (StemCell Technologies, Grenoble, France) and incubated for 2 min to lyse residual erythrocytes. The cells were resuspended in PBS supplemented with 1% FCS and analyzed for GFP expression by flow cytometry on a FACSCanto device (BD Biosciences, Franklin Lakes, NJ) using FACSDiva software (BD Biosciences). Non-transplanted WT C57BL/6 mice and GFP^+ mice were used as controls.

2.4. Immunohistochemistry

Mice were asphyxiated with CO_2 and perfused with 0.9% saline, followed by 4% ice-cold paraformaldehyde in phosphate buffer (0.1 M, pH 7.4). The brains were dissected and post-fixed for 3 h in the same fixative at 4 °C and subsequently transferred to ice-cold PBS containing 30% sucrose overnight. Sections were cut transversely at 30 μm on a freezing microtome, collected in sterile bins containing cold cryoprotectant (0.05 M sodium phosphate buffer, 30% ethylene glycol, 20% glycerol), and stored at −20 °C until use. For antibody detection under bright field illumination, the brain sections were stained for GFP immunoreactivity using a chicken anti-GFP antibody (1:1000; Abcam, Cambridge, UK) overnight at room temperature, and, following rinses with PBS, incubated with biotinylated goat anti-chicken antibody (1:1000; Vector Laboratories, Peterborough, UK) and avidin–biotin complex (1:1000; Vector) for 2 h each at room temperature. Color was developed using 3,3′-diaminobenzidine tetrahydrochloride (Sigma) containing 0.01% H_2O_2 and 2.25% nickel ammonium sulfate