E.04. Involvement of interleukin-1 receptors in sickness responses to lipopolysaccharide

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Background & Aim

Peripheral immune stimuli are signaled to the central nervous system to induce various kinds of sickness responses, such as fever, anorexia and stress hormone release. The proinflammatory cytokine interleukin-1β (IL-1β) has been suggested to play a major role in this signal transduction. However, there are some conflicting reports about the effect o inhibition/deletion of the transducing receptor for IL-1β, the IL-1 type I receptor (IL-1R1). In the present study, we first re-examined this issue by evaluating the effect of global gene deletion of IL-1R1 on lipopolysaccharide (LPS) induced anorexia, stress hormone release and fever. We then examined the contribution of tumor necrosis factor-α (TNFα) and IL-6 receptor signaling to the temperature response. Furthermore, by using animals with cell/tissue specific deletion of IL-1R1, we examined the role of IL-1R1s in brain endothelial cells, neural cells or peripheral nerves related to IL-1R1 dependent fever.

Materials & Methods

Animals

◇Global IL-1R1 knockout (KO) : B6.129S7-Il1r1tm1Imx/J

◇Mice with tissue-specific deletions of IL-1R1:

created by crossing mice possessing *loxP* sites flanking exon 5 of the *Il1r1* gene with mice expressing Cre recombinase under

-*Nestin* promoter (expressed in the central and peripheral nervous system)

-endogenous *Trpv1* locus (expressed by nociceptors in primary sensory ganglia)

-*human tissue plasminogen activator* (*HtPa) promoter* (expressed by neural crest derivates)

or mice expressing

-tamoxifen-inducible CreER^{T2} under the *Slco1c1* promoter (expressed in the cerebrovascular endothelium)

Temperature recording

Temperature data were sampled every minute for 9 hours by a transmitter (E-Mitter, Mini Mitter)

Injection of LPS or saline and taking samples

WT and KO mice were injected ip with LPS from *Escherichia coli* (serotype 0111:B4; 120 μg/kg, ip) or saline. One, 3 and 6 hours after injection, brains and blood were taken for analyses.

Immunohistochemistry

Brain sections were cut at 40 μm on a freezing microtome and incubated in primary antibody solution (anti-c-Fos antibody, Millipore, 1:10000; Millipore) for 60 hours at 4°C, treated with secondary antibody for 2 hours, and then with Avidin-Biotin complex solution for 2 hours at room temperature.

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Results

1. IL-1R1 deletion attenuates the febrile response to LPS

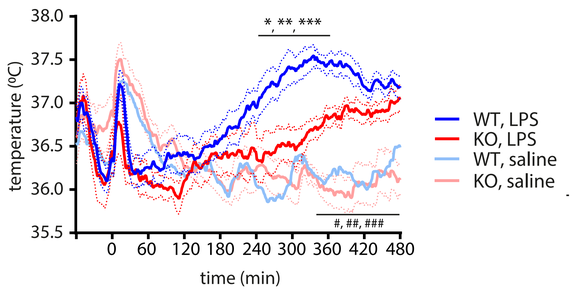

Figure 1. Effect of global IL-1R1 deletion on the febrile response to LPS.
LPS (120 μg/kg) or saline was injected intraperitoneally at 0h. *, **, and *** indicate $P < 0.05$, 0.01, and 0.001 between WT mice and IL-1R1 KO mice treated with LPS, and #, ##, and ### indicate $P < 0.05$, 0.01, and 0.001 between KO mice treated with LPS and saline, respectively. n = 7-9.

2. LPS induced stress hormone release and ano… unaffected in IL-1R1 KO mice

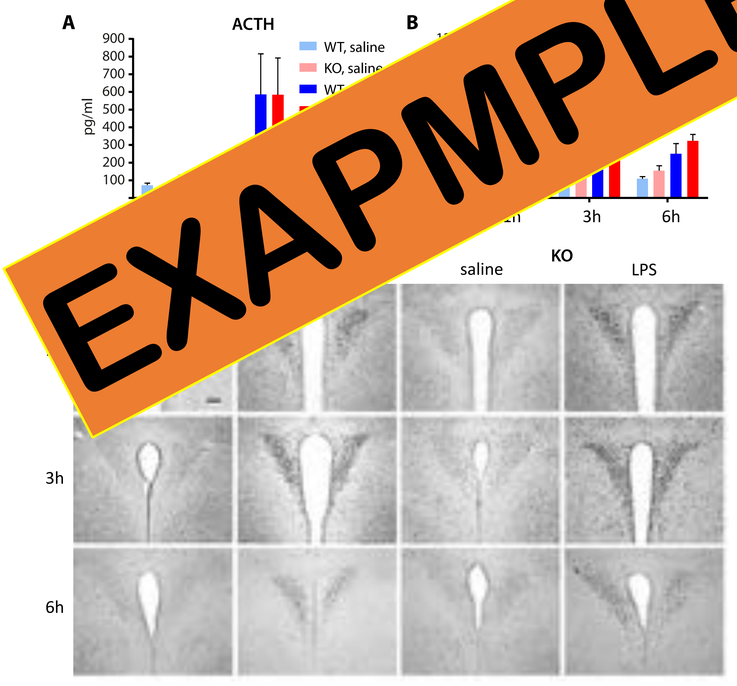

Figure 2. HPA-axis response to intraperitoneally injected LPS in WT and global IL-1R1 KO mice.
A: ACTH in plasma. B: Corticosterone in plasma. C: Expression of Fos protein in the paraventricular nucleus of the hypothalamus. Scale bar = 100 μm. In (A) and (B), n = 5-6 in the saline treated groups, and n = 7-9 in the LPS treated groups.

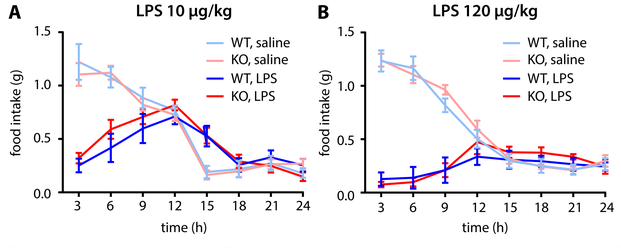

Figure 3. Food intake during 24 h in WT and global IL-1R1 KO mice injected intraperitoneally with a low (A) and a moderate dose (B) of LPS or saline. n = 7-10.

3. Neither TNFα inhibition nor IL-6 receptor blockade abolish the LPS induced febrile response in IL-1R1 KO mice

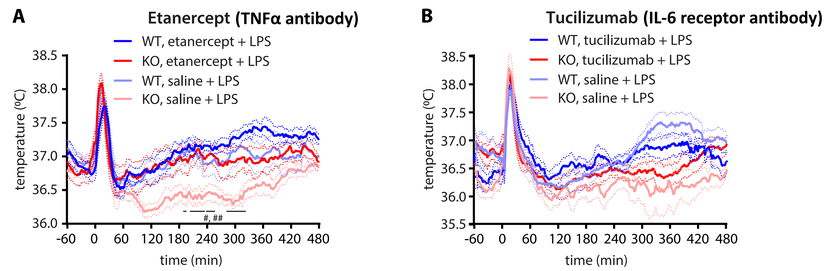

Figure 4. Effects of the TNFα antibody etanercept (A) and the IL-6 receptor antibody tocilizumab (B) on the febrile response to LPS.
In (A), #, and ## indicate $P < 0.05$, and 0.01, between LPS injected global IL-1R1 KO mice treated with etanercept and saline, respectively. n = 8-12 in (A), and 6-8 in (B). Solid lines and dotted lines indicate the mean SEM.

4. Endothelial cell deletion of IL-1R1 attenuates LPS induced fever

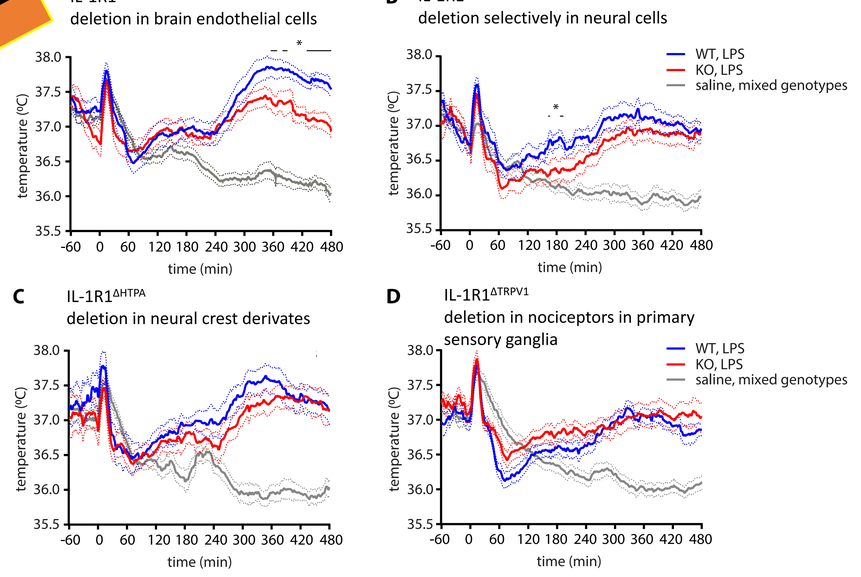

Figure 6. Febrile response to LPS in mice with IL-1R1 deletion selectively in (A) brain endothelial cells, (B) neural cells, (C) neural crest derivates, and (D) nociceptive C-bers.
WT mice are IL-1R1$^{fl/fl}$ littermates. * indicates $P < 0.05$ between WT mice and IL-1R1 KO mice. n=7-16 for LPS treated animals; n=12-30 for saline treated animals.

Summary

-IL-1R1 deletion reduces LPS-induced fever but not anorexia or HPA-axis activation
-Neither TNFα nor IL-6 receptors account for the febrile response in IL-1R1 KO mice
-IL-1R1s on brain endothelial cells are involved in the febrile response to LPS
-IL-1R1s on neural cells or peripheral nerves are not critical for LPS induced fever