Human TLR7 or TLR8 independently confer responsiveness to the antiviral compound R-848

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Toll-like receptors (TLRs) play an important role in the innate immune response to pathogens[1]. TLRs detect PAMPs (pathogen-associated molecular patterns) and stimulate immune cells *via* the MyD88-dependent interleukin 1 receptor (IL-1R)–TLR signaling pathway, which leads to activation of the transcription factor NF-κB[2]. In humans, ten members of this family (TLR1 to TLR10) have been reported to date. TLR2, TLR4 and TLR5 are crucial for the recognition of peptidoglycan, lipopolysaccharide and flagellin, respectively[3–5]. TLR6 associates with TLR2 and recognizes lipoproteins from mycoplasma[6]. TLR9 detects bacterial DNA containing unmethylated CpG motifs and TLR3 activates immune cells in response to double-stranded RNA[7–9]. The natural ligands for TLR1, TLR7, TLR8 and TLR10 are not known, although a synthetic compound with antiviral activity has now been described as a ligand for TLR7[10].

Hemmi *et al.* reported in *Nature Immunology* that, in experiments that used gene-deficient mice, the antiviral imidazoquinoline resiquimod (R-848) activates immune cells *via* the TLR7 MyD88-dependent signaling pathway[10]. They showed that macrophages from MyD88- and TLR7-deficient mice do not respond to R-848 stimulation, whereas macrophages from wild-type mice strongly induce the transcription factor NF-κB and the secretion of proinflammatory cytokines, such as tumor necrosis factor-α, as well as the regulatory cytokine IL-12. In addition, Hemmi *et al.* showed, by genetic complementation in HEK293 cells, that human TLR7 confers responsiveness to R-848[10].

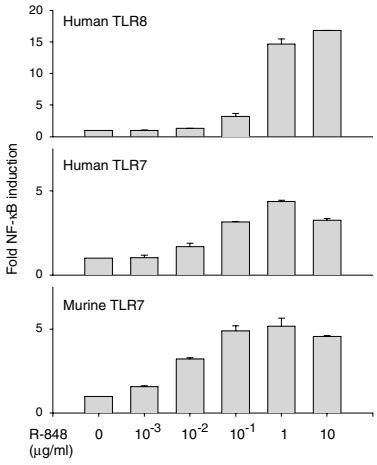

Figure 1. R-848 induces NF-κB activation *via* TLR8 or TLR7. HEK293 cells were transfected by electroporation with vectors expressing human TLR8, human TLR7 or murine TLR7 and a NF-kB luciferase reporter plasmid. Sixteen hours after transfection, cells were stimulated with R-848 (commercially synthesized by GLSynthesis, Worcester, MA) at the indicated concentrations for a further 7 h. TLR2-, TLR3- and TLR4-transfected cells did not induce NF-kB activation after stimulation with R-848; a negative result was also scored for murine TLR8 (data not shown[10]). Data are mean±s.d. from one representative of three independent experiments.

In the quest to identify ligands for TLRs, we also screened immunostimulatory synthetic compounds for their potential to activate HEK293 cells that were transiently transfected with TLR cDNAs and a NF-κB luciferase reporter plasmid. We found that R-848 induced NF-κB activation in HEK293 cells transfected with human TLR8 (GenBank accession number AF245703) in a dose-dependent manner (**Fig. 1**). In accordance with the findings of Hemmi *et al.*, human and murine TLR7 (GenBank accession numbers AF240467 and AY035889) also mediate R-848 recognition (**Fig. 1**)[10]. TLR7 showed a higher sensitivity to R-848, but TLR8 was able to induce NF-κB more effectively than TLR7 when higher concentrations of R-848 were used (**Fig. 1**). In contrast, HEK293 cells transiently transfected with murine TLR8 (GenBank accession number AY035890) did not activate NF-κB after stimulation with R-848 (data not shown), which suggests that TLR8 is nonfunctional in mice. This mute phenotype is in accordance with the observation that TLR7-deficient mice do not respond to R-848, even though TLR8 is present[10].

These results show that both human TLR7 and TLR8 can independently mediate recognition of the same antiviral compound, imidazoquinoline R-848, which suggests a possible redundancy among these receptors. The differential dose response observed between these receptors may allow fine-tuning of the immune response to high or low concentrations of this molecule as well as other ligands. Further studies will be necessary to identify the natural ligands for both receptors and clarify the status and function of murine TLR8.

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