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MODULATION OF TREM1 SIGNALING IN MACROPHAGES INFECTED WITH *Aspergillus fumigatus*

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INTRODUCTION

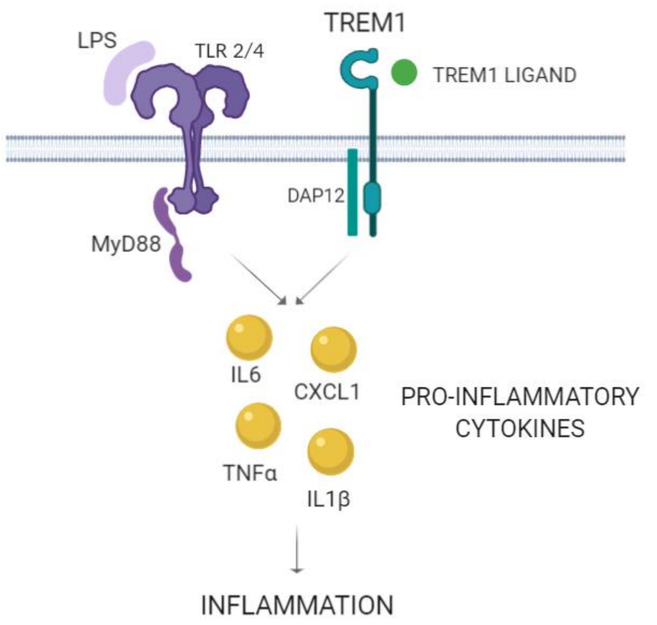


Fig 1. TREM1 signaling pathway. TREM1 amplifies the TLR signaling pathway to increase the production of pro-inflammatory cytokines and mediate inflammatory responses.

Pattern recognition receptors (PRR) play a central role recognizing *Aspergillus fumigatus* components and initiating immune responses in the lungs[1]. In the last few years, a new family of PRRs, called TREM, has been identified, of which TREM1 was the first to be characterized[2]. TREM1 is constitutively expressed in myeloid cells such as neutrophils and macrophages[3]. TREM1 potently synergizes with Toll-like receptors (TLRs) for a substantial amplification of the immune response and production of pro-inflammatory mediators such as TNFα, IL1β, IL6 and CXCL1[4,5]. TREM1 has both activator and inhibitory isoforms. For this reason the blockade of TREM1 with small molecules and peptides is being studied as a possible therapy target[6].

OBJECTIVES

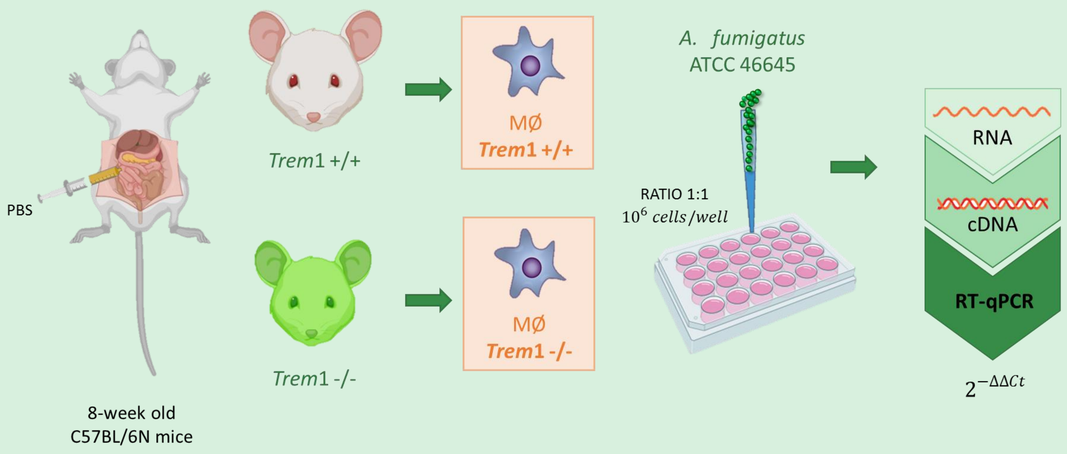
1. To investigate the functional **effect of TREM1 deletion** on the TLRs mediated signaling pathway against *A. fumigatus*.

2. To study the **immunomodulation** of the TREM1 response during **antifungal** therapy.

3. To study the **pharmacologycal inhibition** of TREM1 with **blocking peptides** and the effects on the immune response.

1 Expression analysis in *Trem*1 +/+ and *Trem*1 -/- macrophages infected with *A. fumigatus*

Peritoneal macrophages from *Trem*1 +/+ and *Trem*1 -/- mice were isolated and infected with *A. fumigatus.* After 6 hours RNA was extracted and expression levels of the membrane receptors TLR2, TLR4, MyD88 and the cytokines CXCL1, IL1β, IL6 and TNFα were analyzed by quantitative RealTime PCR (RT-qPCR). Fold changes in expression were calculated using the $2^{-\Delta\Delta Ct}$ method and normalized to the actine house keeping gene[7].



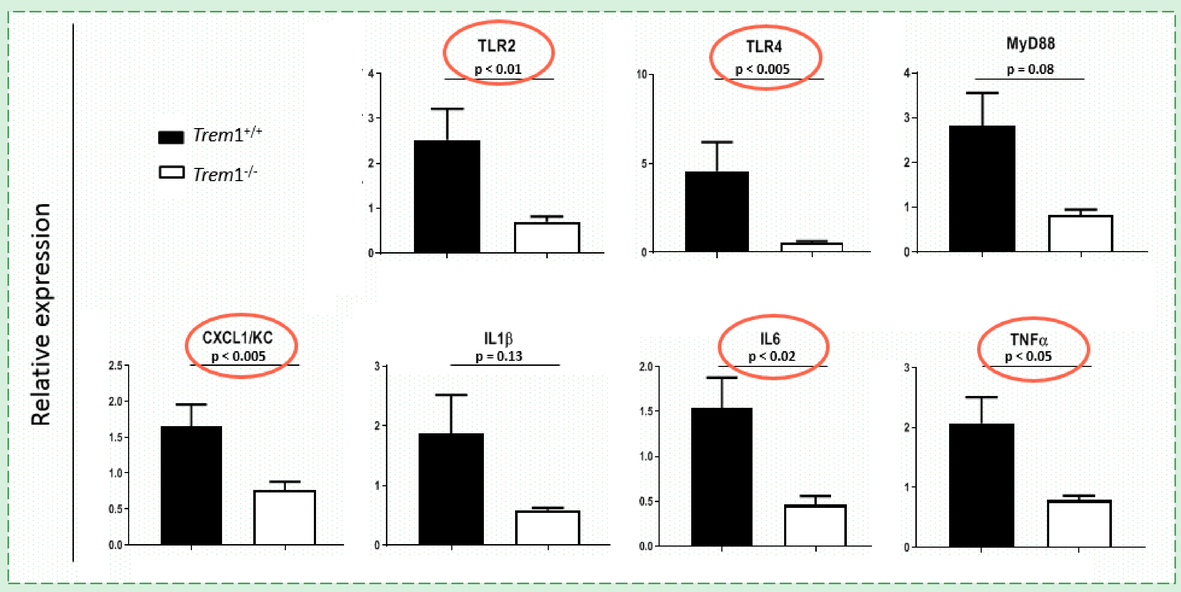
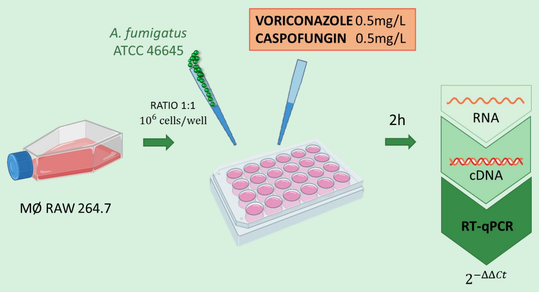


Fig 2. mRNA expression levels by RT-qPCR of TLR2, TLR4, MyD88, CXCL1, IL1β, IL6 and TNFα in murine macrophages isolated from the peritoneal cavity of *Trem*1$^{-/-}$ and *Trem*1$^{+/+}$ mice. P-values for statistical significance were calculated using the Mann Whitney U test (p<0.05).

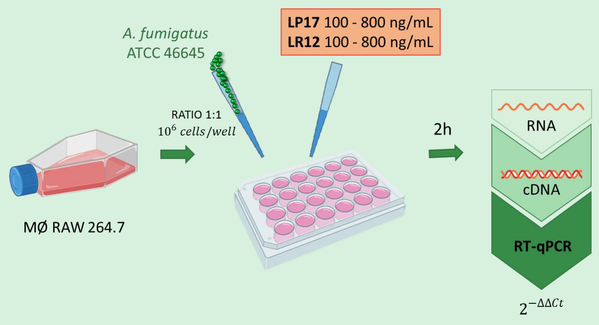
2 Immunomodulation of the TREM1 response during antifungal therapy

In order to evaluate the effect of antifungal agents on myeloid cells during infection with *A. fumigatus,* RAW 264.7 macrophages were infected with *A. fumigatus* and treated with Caspofungin (CAS) and Voriconazole (VCZ). After 2 hours of infection, RNA was extracted and expression levels of TREM1 and the cytokines CXCL1, IL1β, IL6 and TNFα were analyzed by quantitative RealTime PCR.
Fold changes were calculated as described previously described.



3 Pharmacologycal inhibition of TREM1 with blocking peptides in macrophages infected with *A. fumigatus*

Expression levels of TREM1 and the cytokines CXCL1, IL1β, IL6 and TNFα were quantified after infection with *A. fumigatus* of RAW 264.7 macrophages that were treated with two blocking peptides LP17 and LR12 at two different concentrations, 100 and 800 ng. Fold changes were calculated as described previously described.



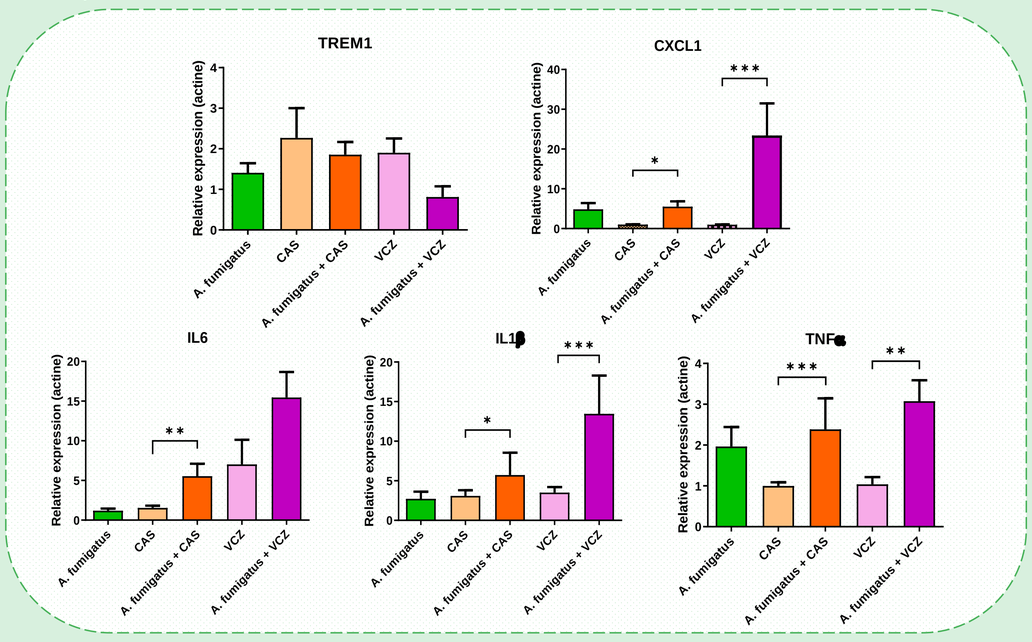


Fig 3. TREM1 and cytokines gene expression levels in RAW264.7 macrophages infected with *A. fumigatus* and treated with caspofungin and voriconazole. The cytokines and TREM1 levels were determined using RT-qPCR. P-values for statistical significance were calculated using the Mann Whitney U test (p<0.05). *<0.05; **<0.005; ***<0.0005.

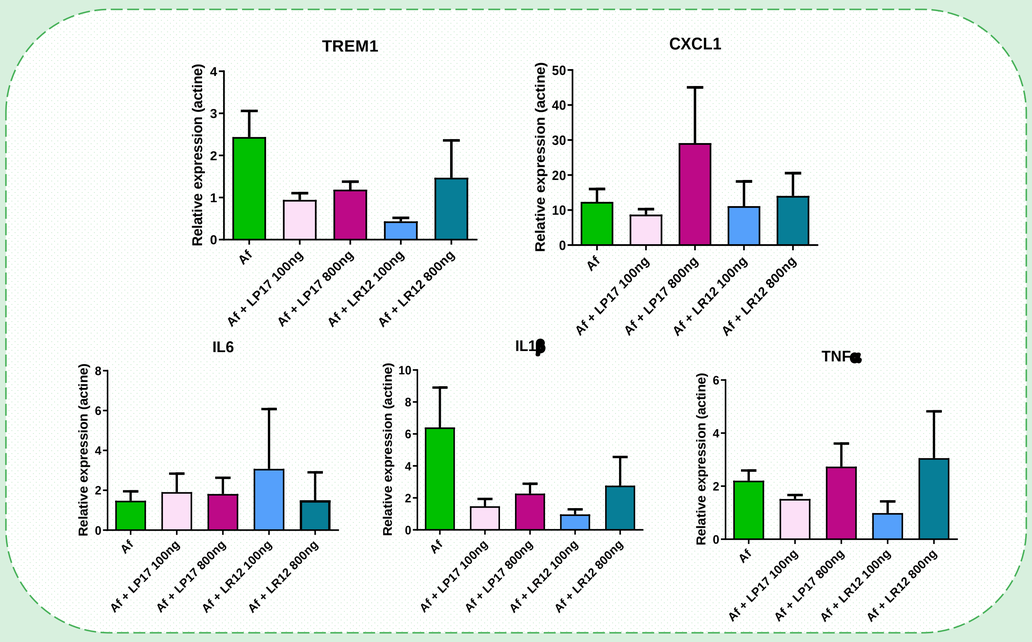


Fig 4. TREM1 and cytokines gene expression levels in RAW264.7 macrophages infected with *A. fumigatus* and treated with LP17 and LR12. The cytokines and TREM1 levels were determined using RT-qPCR.

CONCLUSIONS

1. TREM1 modulates the TLR signaling in macrophages by altering the expression of both receptor and effector proteins that are critical to the response against *A. fumigatus*.

2. The possibility of modulating the inflammatory response in a favorable direction through the TREM1 pathway may represent a new approach for the development of novel immunotherapeutic antifungals to treat patients with Invasive Aspergillosis.

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