APPENDIX

Human TLR8 senses UR/URR-motifs in bacterial and mitochondrial RNA

A. Krüger[1]†, M. Oldenburg[1]†, C. Chebrolu[1]†, D. Beißer[2], J. Kolter[3], A. M. Sigmund[1], J. Steinmann[1], S. Schäfer[4], H. Hochrein[5], S. Rahmann[2], H. Wagner[6], P. Henneke[3], V. Hornung[7], J. Buer[1], C. J. Kirschning[1*]

[1]Institute of Medical Microbiology, University of Duisburg-Essen, 45147 Essen, Germany
[2]Institute of Human Genetics, University of Duisburg-Essen, 45147 Essen, Germany
[3]Centre of Chronic Immunodeficiency, University Medical Center Freiburg, 79106 Freiburg, Germany
[4]Clinic of Anesthesia, University of Duisburg-Essen, 45147 Essen, Germany
[5]Department of Research Immunology, Bavarian Nordic GmbH, 82152 Martinsried, Germany
[6]Institute for Medical Microbiology, Immunology and Hygiene, Technical University of Munich, 81675 Munich, Germany
[7]Institute of Clinical Chemistry and Clinical Pharmacology, University of Bonn, 53127 Bonn, Germany

†These authors contributed equally

[*]Correspondent

Appendix Supplemenentary Methods

Immunoblot analysis

Immunoblot analysis was performed as previously described [3] applying antibodies towards TLR7 and TLR8 (D7 and D3Z6J, respectively), cytoskeletal α-actinin-1, and MAP kinase ERK1/2 (all Cell Signaling). Positive controls were lysates of transiently specific TLR expression plasmid transfected HEK293 cells.

Mice

Wildtype (Wt) C57BL/6, *Tlr239*$^{-/-}$, and *Tlr2379*$^{-/-}$ mice the two latter of which were derived by cross breeding of single TLR knockout mice were used as sources of splenocytes and in vitro generated macrophages [5]. For in vivo analysis mice were sensitized with IFNγ (*i.v.*; 50 μg/kg; Peprotech*)* and 45 min later 4 mg/kg of ORN were injected *i. v.*. Blood was sampled by retrobulbar dotting. Animal experiments were approved by the State Office for Nature, Environment, and Consumerism of North Rhine-Westphalia, Recklinghausen, Germany (G1230/11).

Bacterial RNA preparation

Bacterial RNAs were prepared by acidic phenol extraction [5]. Briefly, bacteria were washed and solubilized in 50 nM sodium azide, raised in glass milk (Lysing Matrix B, Fastprep-24 Instrument, MPbio) upon which RNA was extracted and precipitated. RNA was separated using a 1% agarose gel and MOPS running buffer. Gel slices containing bacterial 16S or 23S rRNA were cut out and RNA was purified subsequently (Zymoclean gel RNA recovery kit, Zymoresearch). Gel slices of denaturing 8 M urea polyacrylamide (15%) gels containing bacterial 5S rRNA or tRNA were cut out and RNA was subsequently purified (Zymoclean small RNA PAGE clean up kit, Zymoresearch).

TLR7 mRNA knockdown

48 h upon differentiation THP-1 cells were transfected with siRNA (Qiagen) towards human TLR7 (ID SI02642402). The primers used for PCR were (MWG eurofins): human Tlr7, (sense) 5'-CCACAACCAACTGACCACTG-3', (antisense) 5'-CCACCAGACAAACCACACAG-3'.

MTLR13 and hTLR8 screening, alignment, and phylogenetic tree

The genome and protein sequences of the species illustrated were retrieved from Ensembl v68 and screened on the sequence level for the presence of the proteins TLR13 and TLR8 [35]. The genomic sequences were 6-frame translated and indexed for the protein similarity search tool RAPSearch v2.12. RAPsearch was used for a fast detection of possible local matches [36]. These regions were used subsequently to calculate optimal local alignments using the Smith-Waterman algorithm of the EMBOSS package v6.5.7 and the region with the best alignment score was kept. Since all TLRs possess a high sequence similarity, the best region was again globally aligned to all murine TLR protein sequences (TLR1-TLR13) using the global alignment algorithm Needleman-Wunsch of the EMBOSS package. The alignment with the highest score identifies the closest homolog to mTLR13 and hTLR8, respectively. Sequences identified in the genome represent possible genes or pseudogenes. In addition to the genomic screen, searches were performed on the protein level to identify possibly active genes in that they possess a protein product. Protein sequences of all species were aligned in the same way as the genomic sequences using RAPsearch, Smith-Waterman and Needleman-Wunsch algorithms. The pairwise-alignment between hTLR8 and mTLR13 was performed using Jalview and annotated with Uniprot features, e.g. domains or signal peptides. The NCBI taxonomy was used to generate a species tree including all analyzed species. The tree was annotated with the TLR8 and TLR13 homologs identified in the Ensembl genomes and protein sequences. For visualization FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/) was used.

References

35. Flicek P, Amode MR, Barrell D, Beal K, Billis K, Brent S, Carvalho-Silva D, Clapham P, Coates G, Fitzgerald S*, et al* (2014) Ensembl 2014. *Nucleic Acids Res* **42:** D749-755

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