News & Views

OPEN ACCESS

EMBO reports

Fish TLR5 develops a taste for viral RNA

Alexander N R Weber[1,2,3,4,*]

These days not only humans but also freshwater fish battle with infections by RNA viruses (Zou & Nie, 2017). This observation prompted Liao *et al* to turn their attention to viral recognition in Grass carp (*Ctenopharyngodon idella*), the most important cultivated freshwater fish with 5.7 million tons and 13 billion USD in fishery exports per year (FAO, 2021). Grass carp and other freshwater fish, such as the model organism *Danio rerio* (zebrafish), have a sophisticated innate immune system that helps them to detect microbial and viral pathogens by employing a variety of pattern recognition receptors (PRRs; Zou & Nie, 2017). Toll-like receptors (TLRs) are one class of PRRs that detect microbe-associated molecular patterns (MAMPs), such as flagellin or viral double-stranded (ds)RNA. In mammals, TLR3 is specialized in sensing viral dsRNA (Liu *et al*, 2008), while TLR5 recognizes the MAMP flagellin (Yoon *et al*, 2012; Fig 1). The well-established notion of TLR5 as a purely "bacterial" flagellin TLR has now been challenged by Liao *et al* in this issue of EMBO Reports (Liao *et al*, 2022). The authors' intriguing and unexpected results indicate that fish TLR5 is involved in viral recognition, a function lost in mammals, and shed light on hitherto inexplicable links of mammalian TLR5 to antiviral immune signaling.

EMBO Reports (2022) 23: e55443
See also: **Z Liao *et al*** (August 2022)

Teleost fish have undergone whole genome duplication and grass carp encodes two tandem *TLR5* genes, *CiTLR5a* and *CiTLR5b*. Liao *et al* (2022) started by comparing the sensitivity of the two CiTLR5 paralogs to different MAMPs. Various flagellins or flagellated bacteria were sensed by CiTLR5a and CiTLR5b heterodimers for activation of NF-κB, a transcription factor regulating the expression of pro-inflammatory genes in both fish and mammals. Very surprisingly, they found that CiTLR5b can also function as a homodimer to signal to Interferon-responsive elements (ISRE) in response to a control MAMP, the dsRNA analog poly(I:C), or to viral infection of *Ctenopharyngodon idella* cells (Fig 1). Notably, the CiTLR5 ectodomains directly bound poly(I:C). Through a comprehensive series of domain swaps and mutagenesis, the authors showed that flagellin recognition requires canonical TLR5 recognition sites in both CiTLR5a and CiTLR5b. However, poly(I:C) binding sites differed between the TLR5 paralogs. These differences in ligand-receptor interactions correlated well with their surprising finding that *in vitro the* response of CiTLR5b to poly(I:C) was blocked by co-expression of CiTLR5a. Interestingly, in fish tissues CiTLR5a and 5b are co-expressed; however, 5b usually exceeds the mRNA expression of 5a by a factor of ~2–10 in most analyzed *C. idella* tissues, suggesting that, at steady-state levels, viral dsRNA sensing is operational. Indeed, viral challenge in live grass carp induced an antiviral transcriptional program that was dampened by RNA interference with CiTLR5b expression, confirming that CiTLR5b is involved in viral sensing *in vivo*. Although the structural framework for dsRNA vs. flagellin binding and NF-κB vs. ISRE signaling requires further exploration, Laio *et al*'s meticulous and comprehensive study does show that fish TLR5 is a *bona fide* viral sensor.

The study immediately extends its scope to another teleost, zebrafish, and also explores the intriguing possibility that mammalian TLR5 may respond to dsRNA. In fact, responsiveness to dsRNA appears a unique capacity of teleost TLR5: Whereas teleost tandem TLR5s can sense flagellin via TLR5a/b heterodimers and dsRNA via a TLR5b homodimer, mammalian TLR5 seems confined to sense only flagellin in a homodimeric manner (Fig 1). Nevertheless, the study of Liao *et al* has important implications for our understanding of mammalian TLR5 function.

Firstly, when it comes to the structural connotations of TLR5 sensing, most of what we know about flagellin-TLR5 recognition in mammals is based on the structure of a truncated zebrafish DrTLR5b homodimer in complex with *Salmonella* flagellin (Yoon *et al*, 2012). As insightful as this structure has been, this present study and another (Voogdt *et al*, 2018) remind us that in fish TLR5 homodimers cannot form an active flagellin-sensing complex, suggesting that the 2012 structure by Yoon *et al* is most likely that of an inactive flagellin-TLR5 complex. Therefore, conclusions drawn over the years about the human sensing of flagellin have to be treated with caution and require experimental confirmation by a human TLR5-flagellin complex. *En route*, the precise details of the supposedly active structures of a Ci (or Dr)TLR5a/b heterodimer in complex with flagellin and a Ci (or Dr)TLR5b homodimer in complex with poly (I:C) would be very informative and exciting to compare. In the meantime, it may prove insightful to compare the modeling studies offered by Liao *et al* with the first reported TLR structure of human TLR3 binding the same ligand (Liu *et al*, 2008). Furthermore, Liao's work raises the possibility that Ci/DrTLR5b may exist in two different "ON"

1 Department of Immunology, Interfaculty Institute of Cell Biology, Tübingen, Germany
2 iFIT – Cluster of Excellence (EXC 2180) "Image-Guided and Functionally Instructed Tumor Therapies", University of Tübingen, Tübingen, Germany
3 German Cancer Consortium (DKTK) and German Cancer Research Center (DKFZ) Partner Site Tübingen, Tübingen, Germany
4 CMFI – Cluster of Excellence (EXC 2124) "Controlling Microbes to Fight Infection", University of Tübingen, Tübingen, Germany
*Corresponding author. Tel: +49 7071 29 87623; Fax: +49 7071 29 4579; E-mail: alexander.weber@uni-tuebingen.de
DOI 10.15252/embr.202255443 | EMBO Reports (2022) 23: e55443 | Published online 28 June 2022

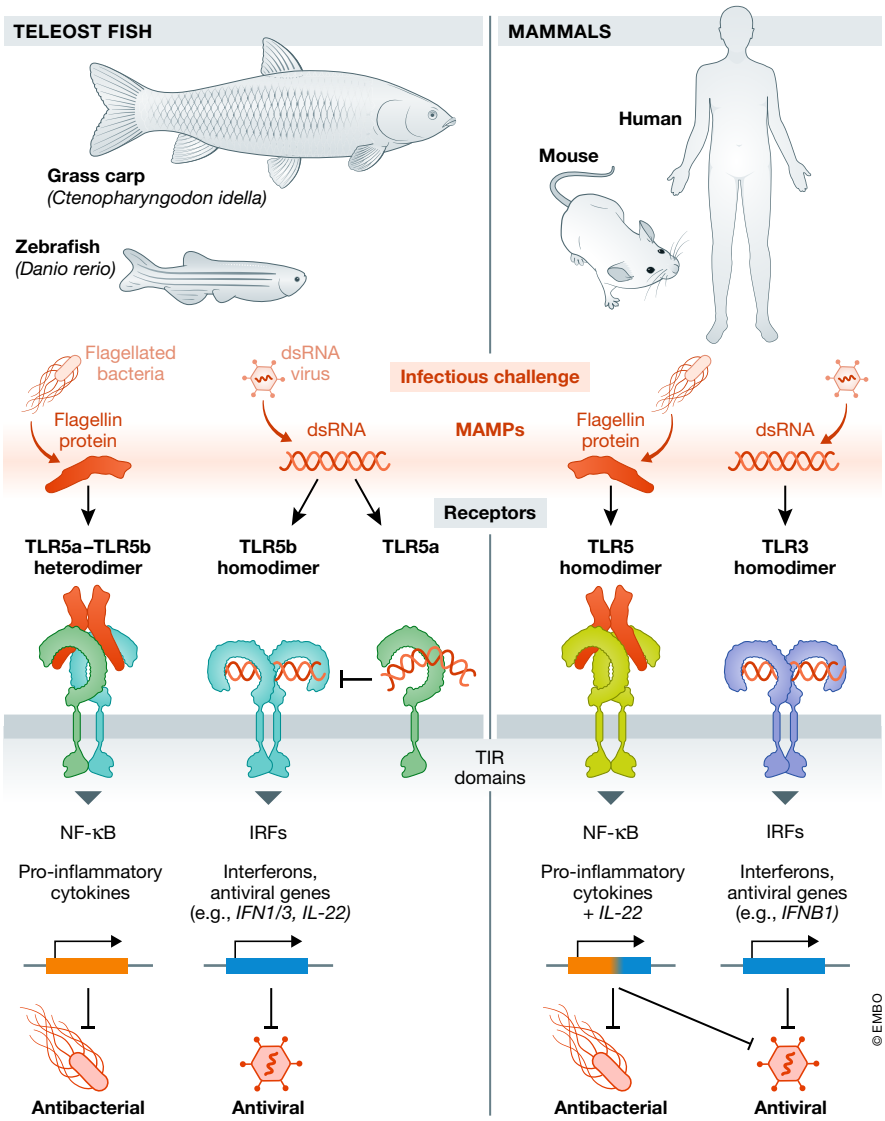

Figure 1. Viral sensing is a unique function of fish TLR5.

In teleosts, TLR5 proteins can be involved in both flagellin and dsRNA sensing. Specifically, TLR5a-TLR5b heterodimers recognize flagellin and induce NF-κB-dependent cytokine genes. However, TLR5b homodimers are able to respond to dsRNA to trigger antiviral transcriptional programs. Conversely, in mammals bacterial flagellin and viral dsRNA sensing are sensed separately by TLR5 and TLR3, respectively, and trigger largely distinct transcriptional profiles. A possible reminiscence of an antiviral role for mammalian TLR5 is the regulation of IL-22 upon flagellin engagement. The structural basis for these similarities and differences both at the ligand engagement and intracellular TIR domain remains elusive.

states, one signaling toward NF-κB together with Ci/DrTLR5a upon flagellin engagement and another signaling toward ISRE in response to poly(I:C). Further comparative studies of intracellular Ci/Dr vs. human TLR5(a/b) and TLR3 Toll/Interleukin-1 receptor(TIR) signal transduction domains may thus prove insightful.

Secondly, the study begs the intriguing question whether mammalian TLR5 still has a connection to antiviral immune responses, that is, is there a "reminiscence of antiviral function" in human TLR5? CiTLR5b stimulation with dsRNA or viral infection triggered the transcription of *interferon (IFN) 1*, *IFN3* and/or *interleukin 22 (IL22) in vitro* as well as *in vivo* (Fig 1). The antiviral properties of IFNs in humans are textbook knowledge, but human IL-22 was also shown to have antiviral properties (Das *et al*, 2020) and to display similarity to fish IL-22 (Siupka *et al*, 2014). Liao *et al* now help us to make more sense of reports linking TLR5 activity with antiviral properties in humans: for example, hTLR5-induced IL-22 restricted rotavirus infection in the intestine (Zhang *et al*, 2020), and flagellin reduced influenza A virus replication independently of type I IFN and IL-2, thereby improving the efficacy of the antiviral drug, oseltamivir (Georgel *et al*, 2019).

Collectively, this study uncovers fascinating and completely unexpected facets of TLR5 sensing that have relevance beyond fish. Importantly, it also re-opens many questions that appeared settled and warrants exciting structural and biochemical work that could provide interesting new insights into the human immune system.

Acknowledgements

I thank Libera Lo Presti for editorial support. I apologize to colleagues whose work was not mentioned due to space restrictions. ANRW was supported by the University of Tübingen Medical Faculty, Deutsches Konsortium für Translationale Krebsforschungs (German Cancer Consortium, DKTK), the Deutsche Forschungsgemeinschaft (German Research Foundation, DFG) Research Grant We-4195/18-1, Clusters of Excellence "iFIT – Image-Guided and Functionally Instructed Tumor Therapies" (EXC 2180) and "CMFI – Controlling Microbes to Fight Infection (EXC 2124)." Gefördert durch die Deutsche Forschungsgemeinschaft (DFG) im Rahmen der Exzellenzstrategie des Bundes und der Länder—EXC 2180 und EXC 2124. Open Access funding enabled and organized by Projekt DEAL.

Disclosure and competing interests statement

The author declare that they have no conflict of interest.

References

Das S, St Croix C, Good M, Chen J, Zhao J, Hu S, Ross M, Myerburg MM, Pilewski JM, Williams J *et al* (2020) Interleukin-22 inhibits respiratory syncytial virus production by blocking virus-mediated subversion of cellular autophagy. *iScience* 23: 101256

FAO (2021) FAO yearbook. Fishery and Aquaculture Statistics 2019/FAO annuaire. Statistiques des pêches et de l'aquaculture 2019/FAO anuario. Estadísticas de pesca y acuicultura 2019. Rome/Roma. Food and Agriculture Organization (FAO) of the United Nations

Georgel AF, Cayet D, Pizzorno A, Rosa-Calatrava M, Paget C, Sencio V, Dubuisson J, Trottein F, Sirard JC, Carnoy C (2019) Toll-like receptor 5 agonist flagellin reduces influenza a virus replication independently of type I interferon and interleukin 22 and improves antiviral efficacy of oseltamivir. *Antiviral Res* 168: 28 – 35

Liao Z, Yang C, Jiang R, Zhu W, Zhang Y, Su J (2022) Cyprinid-specific duplicated membrane TLR5 senses dsRNA as functional homodimeric receptors. *EMBO Rep* 23: e54281

Liu L, Botos I, Wang Y, Leonard JN, Shiloach J, Segal DM, Davies DR (2008) Structural basis of toll-like receptor 3 signaling with double-stranded RNA. *Science* 320: 379 – 381

Siupka P, Hamming OJ, Fretaud M, Luftalla G, Levraud JP, Hartmann R (2014) The crystal structure of zebrafish IL-22 reveals an evolutionary, conserved structure highly similar to that of human IL-22. *Genes Immun* 15: 293 – 302

Voogdt CGP, Wagenaar JA, van Putten JPM (2018) Duplicated TLR5 of zebrafish functions as a heterodimeric receptor. *Proc Natl Acad Sci USA* 115: E3221 – E3229

Yoon SI, Kurnasov O, Natarajan V, Hong M, Gudkov AV, Osterman AL, Wilson IA (2012) Structural basis of TLR5-flagellin recognition and signaling. *Science* 335: 859 – 864

Zhang Z, Zou J, Shi Z, Zhang B, Etienne-Mesmin L, Wang Y, Shi X, Shao F, Chassaing B, Gewirtz AT (2020) IL-22-induced cell extrusion and IL-18-induced cell death prevent and cure rotavirus infection. *Sci Immunol* 5: eabd2876

Zou PF, Nie P (2017) Zebrafish as a model for the study of host-virus interactions. *Methods Mol Biol* 1656: 57 – 78