Journal of Bacteriology and Virology 2012. Vol. 42, No. 4 p.357 – 358
http://dx.doi.org/10.4167/jbv.2012.42.4.357

Letter to the Editor

Bacterial 23*S* Ribosomal RNA, a Ligand for Toll-like Receptor 13

Zahid Manzoor and Young-Sang Koh*

Department of Microbiology and Immunology, Jeju National University School of Medicine, Jeju, Korea

Toll-like receptors are required for detection of pathogen-associated molecular patterns and play critical roles in protection of host from infection. Murine TLR13 was recently reported to be involved in recognition of bacterial 23*S* ribosomal RNA sequence that is the binding site of different antibiotics.

Key Words: Toll-like receptor, Toll-like receptor 13, Bacterial ribosomal RNA, *Staphylococcus aureus*

In Science on August 31st, 2012 Oldenburg *et al* reported that TLR13 recognizes bacterial 23*S* ribosomal RNA (rRNA) sequence that is the binding site of macrolide, lincosamide, and streptogramin group antibiotics (1). Toll-like receptors (TLRs) are one family of pattern recognition receptors used by the mammalian innate immune system to detect pathogen-associated molecular patterns (2, 3). TLR11, -12 and -13 belong to TLR11 subfamily and are expressed in mice but represented in humans only by a pseudogene (4). TLR13 is primarily expressed in the spleen, particularly in dendritic cells and macrophages. TLR13 stimulation recruits MyD88 adaptor protein resulting in activation of TAK1, which subsequently induces translocation of NF-κB to the nucleus (5). NF-κB leads to the expression of genes which are responsible for production of the pro-inflammatory cytokines. Here we summarize the results regarding detection of the bacterial 23S rRNA sequence by TLR13 (1, 5).

Gram-positive bacteria are mainly sensed by TLR2 which is expressed on cell surface. Oldenburg *et al* found that mice lacking TLR2 are still responsive to *Staphylococcus aureus* or *Streptococcus pneumoniae*, which indicate that other TLRs play a role in the detection of these bacteria (1). They ruled out the possibility of other classes of pattern recognition receptors such as C-type lectins, RIG-I-like helicases, or nucleotide binding domain-and leucine-rich repeat-containing proteins in the detection of heat inactivated *S. aureus* or *S. pneumoniae* by using different knockout macrophages (1). They found that the production of cytokine was dependent on MyD88, which suggest that TLRs are mainly responsible for detection of these bacteria and there is no role of RIG-I-like helicases or nucleotide binding domain-and leucine-rich repeat-containing proteins (1). TLRs are involved in the recognition of viral infection and $Tlr13^{-/-}$ cells are highly susceptible to vesicular stomatitis virus infection (6).

Oldenburg *et al* focused on endosomal TLRs including TLR3, -7, -8, -9, -11, and -13 whether these have some role in cell activation. Pretreatment of bafilomycin in $Tlr2^{-/-}$ macrophages abrogated recognition of Gram-positive bacteria. Bacterial inoculums treated with ribonuclease A were unable to produce TNF-α in $Tlr2^{-/-}$ and $Tlr23479^{-/-}$ macrophages. From these results they concluded that an

Received: October 5, 2012/ Revised: October 10, 2012/ Accepted: October 15, 2012
*Corresponding author: Young-Sang Koh. Department of Microbiology and Immunology, Jeju National University School of Medicine, 102 Jejudaehakno, Jeju 690-756, Korea.
Phone: +82-64-754-3851, Fax: +82-64-702-2687, e-mail: yskoh7@jejunu.ac.kr
**This study was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology, Republic of Korea (2010-0023645).

endosomal RNA sensor is involved in detection of heat inactivated *S. aureus* (1). They generated two types of bone marrow-derived dendritic cells (DCs) called as conventional DCs (cDCs) and plasmacytoid DCs (pDCs). *Tlr23479*$^{-/-}$ CD8^{high} cDCs express TLR11, -12, -13 and signal regulatory protein α (Sirp)^{high} cDCs express TLR13 but lack TLR11, -12 while *Tlr23479*$^{-/-}$ pDCs express TLR12 and lack TLR11, -13. Using these cDCs and pDCs they showed that TLR13 acts as bacterial single-stranded RNA sensor. For identification of relevant RNA, they treated heat inactivated *S. aureus* with calf intestinal phosphatase which affect the integrity of 16*S* and 23*S* rRNA or double stranded RNA-specific RNase III or VI (1). They further extended their work and degraded large rRNAs, namely 16*S* and 23*S* rRNA and isolated high molecular weight rRNA. Low molecular weight portions from total RNA were unable to stimulate the cells but high molecular weight portions of bacterial RNA activated *Tlr23479*$^{-/-}$ cells. From these results they concluded that portion of large bacterial rRNA activates the cDCs in MyD88-dependant manner (1).

For characterization of a TLR responsible for detection of 23*S* rRNA, TLR13 was focused as they ruled out the involvement of TLR8. They identified the conserved bacterial 23*S* rRNA sequence "CGGAAAGACC" as a ligand for TLR13 (1). To verify the role of TLR13 in detection of bacterial RNA, TLR13 expression was targeted with small interfering RNA (1). Transfection of DCs with small interfering RNA against TLR13 resulted in significantly less production of IL-12 p40 mRNA upon treatment with bacterial RNA (5). To further explore the role of TLR13 in recognition of bacterial RNA, chinese hamster ovary cells were cotransfected with an NF-κB luciferase reporter and vectors expressing TLR13, -7, or -8, or empty vector, and then treated with bacterial RNA. They found that TLR13-transfected cells significantly induced NF-κB activation. Chinese hamster ovary cells expressing TLR13 induced NF-κB luciferase reporter activity in response to both live and heat killed *Streptococcus pyogenes* but failed to do so in the presence of ribonuclease A in the heat killed bacterial inoculums. This shows that TLR13 contributes in recognition of group A streptococci (5).

Oldenburg *et al* showed that bacteria can avoid TLR13 detection by modification in 23*S* rRNA (1). The erythromycin-resistance methylase family of methyltransferases confers resistance to the macrolide, lincosamide and streptogramin antibiotics by the methylation of 23*S* ribosomal RNA which leads to abolition of their ability to bind to the ribosome and exhibit their antibiotic activity (1, 7). They found that modification in 23*S* rRNA via methylation leads to antibiotic resistance and also eradicates immunostimulatory activity in clinical isolates of *S. aureus* (1).

They show that murine TLR13 is required for detection of bacterial conserved 23*S* ribosomal RNA sequence and bacteria can evade recognition via TLR13 by specific mechanisms of antibiotic resistance. Hence, they suggested that in humans there may be a related RNA-sensing pattern recognition receptor that can recognize the bacteria which escape TLR13 detection.

REFERENCES

1) Oldenburg M, Krüger A, Ferstl R, Kaufmann A, Nees G, Sigmund A, *et al*. TLR13 recognizes bacterial 23*S* rRNA devoid of erythromycin resistance-forming modification. Science 2012;337:1111-5.
2) Yuk JM, Jo EK. Toll-like receptors and innate immunity. J Bacteriol Virol 2011;41:225-35.
3) Koh YS. Nucleic acid recognition and signaling by Toll-like receptor 9: compartment-dependent regulation. J Bacteriol Virol 2011;41:131-2.
4) Roach JC, Glusman G, Rowen L, Kaur A, Purcell MK, Smith KD, *et al*. The evolution of vertebrate Toll-like receptors. Proc Natl Acad Sci U S A 2005;102:9577-82.
5) Hidmark A, von Saint Paul A, Dalpke AH. Cutting Edge: TLR13 is a receptor for bacterial RNA. J Immunol 2012;189:2717-21.
6) Shi Z, Cai Z, Sanchez A, Zhang T, Wen S, Wang J, *et al*. A novel Toll-like receptor that recognizes vesicular stomatitis virus. J Biol Chem 2011;286:4517-24.
7) Hajduk PJ, Dinges J, Schkeryantz JM, Janowick D, Kaminski M, Tufano M, *et al*. Novel inhibitors of Erm methyltransferases from NMR and parallel synthesis. J Med Chem 1999;42:3852-9.