Under the Radar Screen:
How Bugs Trick Our Immune Defenses

Session 3: Toll-like receptors (TLRs)

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Introduction to Toll-like Receptors (TLRs)

Historical Background

TLRs as the link between innate and adaptive immunity

What kind of Pathogen Associated Molecular Patterns do TLRs recognize

What's known about TLR structure and activity

The detailed signaling pathways which TLRs

Introduction to Toll-like Receptors (TLRs)

The discovery of the Toll-like receptors

1985: Christiane Nüsslein-Volhard identified Toll genes (*Toll = cool, weird in German*) in *Drosophila melanogaster* as gene important in embryogenesis in establishing the dorsal-ventral axis.

1989: Janeway predicts that our immune system uses PAMPs (pathogen-associated molecular patterns) as tools to identify molecules that would always be associated with infection. The concept that immune systems decide when to initiate a response based on the microbial nature of the molecules encountered became known as the pattern recognition hypothesis

1994: Nomura et al. described the first reported human Toll-like receptor

1996: Jules Hoffmann et al. find that Toll has a role in the fly's immunity to fungal infection

1997: Janeway and Medzhitov discovered a human protein with structural similarity to drosophila Toll that could activate immune response genes in human cells (TLR4).

1998: Beutler discovered that a mouse strain with an altered response to bacterial lipopolysaccharide (called LPS or endotoxin) was due to a mutation in the TLR4 gene.

There are 11 TLR family members in human and 12 in mice. Each responds to a distinct set of microbial products. Because the specificity of Toll-like receptors (and other innate immune receptors) cannot easily be changed in the course of evolution, these receptors recognize molecules that are constantly associated with threats (i.e. pathogen or cell stress), that are not subject to mutation, and are highly specific to these threats (i.e. cannot be mistaken for self molecules).

Overview

Article 1: A46R ……

- Inhibits multiple IL-1–induced signals
- Interacts with MyD88 and antagonizes MyD88-dependent signaling
- Targets the TIR domain-rich TLR4 signaling
- Antagonizes TRIF-dependent pathways (TLR3 signaling)
- Is expressed early during infection and contributes to VV virulence
- A46R and A52R are not functionally redundant (but cooperate in interfering with TLR3 signaling)

Article 2:

- Imidazoquinoline-induced response is MyD88-dependent
- $TLR7^{-/-}$ cells respond to CpG DNA = the TLR signaling is not compromised
- $TLR7^{-/-}$ cells do not respond to imidazoquinoline (but TLR signaling is OK)
- Imidazoquinoline-induced signaling is TLR7-dependent
- In vivo Imidazoquinolines do not trigger an immune response in $TLR7^{-/-}$ mice

Paper 1

Vaccinia virus protein A46R targets multiple Toll-like–interleukin-1 receptor adaptors and contributes to virulence

Julianne Stack, Ismar R. Haga,Martina Schröder,Nathan W. Bartlett,Geraldine Maloney,Patrick C. Reading,Katherine A. Fitzgerald,Geoffrey L. Smith,and Andrew G. Bowie

Questions

A. In Figure 2e in the paper, the authors change from HEK 293 cells to RAW 264.7 cells. What might have motivated their choice?

B. Why is studying TLR4 signaling using the chimeric protein CD4-TLR4 such a good / simple model system?

C. If the authors already show by IP that TLR4, MAL and TRAM pull down A46R (Fig. 3b-d), why do they do an GST-pulldown in Fig. 3e?

D. Do you consider the action of the A46R protein from Vaccina Virus to be a good example of immune evasion? (Which TIR-domain containing proteins does A46R interact with?)

Paper 2

Small anti-viral compounds activate immune cells via the TLR7 MyD88−dependent signaling pathway

Hiroaki Hemmi, Tsuneyasu Kaisho, Osamu Takeuchi, Shintaro Sato, Hideki Sanjo, Katsuaki Hoshino, Takao Horiuchi, Hideyuki Tomizawa, Kiyoshi

Takeda & Shizuo Akira

Questions

A. What might have motivated the authors to study the pathways by which drugs like Imiquimod stimulate the immune system? (do they perhaps have unique tools?)

B. Have the authors properly motivated their choice to make an TLR7$^{-/-}$ mouse?

C. Why might the authors have chosen to perform three different types of assays in Figure 3a,b and c and what does it tell you about the cell-types that they study?

D. What is the function of the CpG control? Has the control been consistently used throughout the manuscript?