How does a cell “know” the difference between an inflammatory stimulus and a phagocytic stimulus?

The far-sighted macrophage

Long distance surveillance

Soluble microbial components

Secretion of cytokines and chemokines to attract and activate other immune cells

The near-sighted macrophage

- Phagocytosis
- Respiratory Burst
- Killing & Digestion
- Antigen Processing

Secretion of cytokines and chemokines to attract and activate other immune cells
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A far-sighted receptor mechanism

Lipopeptide

TLR2

TLR6

MyD88

Mal

IRAKs

Transcriptional regulation

NF-kB, AP-1, etc.

A near-sighted receptor mechanism

Dectin-1

Yeast

Src

Syk

-P

Phagocytosis

Respiratory Burst

NFAT, SRF

NF-kB

But there’s this inconvenient observation:

- For 30 years or more folks have been using “soluble” β-glucans to block yeast recognition by macrophages…

"Laminarin" a soluble glucan (storage polysaccharide) prepared from brown algae

So, it must be too small, right?
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β-glucan
β-(1,3)-glucan
β-(1,6)-glucan branch
MW = 1500 Da

Dectin-1
β-glucan
MMDB ID: 64601
β-glucan
Jones & Coworkers, Protein Science (2007)
MW = 1500 Da

MW = 32 KDa
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MW = 130 KDa

MW = 400 KDa

β-glucans have many structures, there will be many binding sites available

MW = 400 KDa

So we got some β-glucans...

Laminarin avg. 10 kDa From Seaweed
Low MW avg. 16 kDa
Med. MW avg. 150 kDa
Med. High MW avg. 220 kDa
High MW avg. 400 kDa
Whole Glucan Partides >95% pure From S. cerevisiae

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Soluble β-glucans bind to Dectin-1

Wild type

Dectin-1+/+

Dectin-1-/-

DTAF-MMW (220 kDa)

Bright field

13

Soluble glucans do not work – regardless of their size or the assay

A

B

C

D

E

F

G

14

How does Dectin-1 discriminate between soluble and particulate β-glucans?

15
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Im mobilized β-glucans trigger Dectin-1 signaling

Immobilized β-glucans trigger Dectin-1 signaling

0 1 2 3 4 5
ng/ml

unstim. MHMW HMW cMHMW cHMW

Soluble

Soluble glucans coated onto bottoms of tissue culture plates

wild type Dectin-1 

β-glucans

Immunological synapse

Immunological synapse

Resting T cell Plasma Membrane
Abundant Phosphatase (CD45) Primes Signaling Kinases, but Generally Prevents Spurious TCR Signaling

Antigen Presenting Cell

ICAM1

MHC/peptide

Signaling

APC

CD45

TCR

Immunological synapse (2)
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CD45 and CD148

- Membrane tyrosine phosphatases
- Dual role in ITAM signaling
  1. Activating – remove inhibitory phosphorylation from Src-family kinases
  2. Inhibitory – limit downstream Tyr kinase-based signaling

19

CD45ABC

\[ \approx 50 \text{ nm} \]
\[ \approx 20-30 \text{ nm} \]

phosphatase domains (active)

CD45RO

\[ \approx 13 \text{ nm} \]

20

Zap70

CD45

CD4

\( \text{CD4} \) alternatively spliced

- CD45 is found on the surface of all nucleated hematopoietic cells
- On T and B cells it comprises up to 10% of the cell surface area
- The cytoplasmic tail contains two phosphatase domains
- Although there is low sequence identity between extracellular regions in different species, the cytoplasmic region is highly conserved

21

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Do CD45 and CD148 regulate Dectin-1 signaling?

Reactive Oxygen Production

- CD148-/-
- CD45-/-
- CD45-/- CD148-/-

Do Dectin-1 & CD45 form a synapse-like structure during phagocytosis?

Dectin-1 phagocytic synapse

Do CD45 and CD148 regulate Dectin-1 signaling?

Reactive Oxygen Production

- CD148-/-
- CD45-/-
- CD45-/- CD148-/-

Do Dectin-1 & CD45 form a synapse-like structure during phagocytosis?

Dectin-1 phagocytic synapse

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Dectin-1 phagocytic synapse (2)

Dectin-1 (Green)
CD45 (Red)
Zymosan (Blue)

Dectin-1 phagocytic synapse (3)

Dectin-1-expressing RAW264.7 macrophages

Dectin-1 phagocytic synapse – Aspergillus fumigatus

Dectin-1/CD45-expressing RAW264.7 macrophages

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in Distinguishing Particulate and Soluble Stimuli
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Soluble glucans bind & cluster Dectin-1, but do not sufficiently exclude the membrane phosphatases to allow signaling to progress.

Soluble glucans immobilized on a plastic surface activate signaling.
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Dectin-1 phagocytic synapse
– plate-coated β-glucan

Uncoated

HMM β-glucan-coated

Dectin-1 CD45

What happens if you also coat the plastic surface with anti-CD45 antibodies as well to prevent movement of the proteins?

CD45 co-immobilization

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Summary

- “Phagocytic synapse” formation allows a cell to distinguish between microbes in the immediate vicinity and components shed from microbes at a distance
- Prevents inappropriate initiation of phagocytosis and direct anti-microbial responses

On the term “phagocytic synapse”

- The term “phagocytic synapse” or “engulfment synapse” has previously been proposed in the literature to describe the re-organization of macrophage cell surface proteins upon engagement of apoptotic cells
- In this context, the term has been applied simply to denote the “points of contact” between phagocytic cells and the receptors & ligands found there
- It is not clear yet whether this contact site bears any functional similarity to the “Immunological Synapse” that forms between an APC and a T cell
- It is this similarity that has lead to the use of the term “Phagocytic Synapse” to denote the mechanism by which cells discriminate soluble from particulate ligands

Do other receptors require a “phagocytic synapse”?

Myosin II-dependent exclusion of CD45 from the site of Fcγ receptor activation during phagocytosis

Myosin II contributes to FcγR activation and subsequent F-actin assembly at the nascent phagocytic cup; while FcγRs cluster beneath an IgG-opsonized particle without myosin II activity, myosin II is required to exclude CD45 and permit full activation of signaling and phagocytosis
Further reading: