INNA TE IMMUNITY

Layers of fungal complexity

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URLs

Entrez Genome Project:

<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genome>

Candida albicans

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genome&prj6cmd=Retrieve&dopt=Overview&list_uids=9526

A recent paper in the *Journal of Clinical Investigation* reports the results of an elegant analysis of the contribution made by the different layers of the *Candida albicans* cell wall to innate immune stimulation.

The *C. albicans* cell wall comprises an outer mannoprotein layer containing short chains of O-linked mannosyl residues and long, branched chains of N-linked mannosyl residues, and an inner layer containing chitin and β -glucans. Innate immune cells recognize pathogen-associated molecular patterns (PAMPs) using pattern-recognition receptors (PRRs), such as the Toll-like receptors (TLRs) and C-type lectins. Previous work had indicated that components of the mannan layer interact with TLR2, TLR4 and the mannose receptor (MR; a classical C-type lectin) and that β -glucans are detected by TLR2 and the non-classical C-type lectin dectin 1.

In a systematic genetic approach, Mihai Netea, Neil Gow and colleagues used a combination of *C. albicans* isogenic mutants, receptor blockade and knockout mice to determine the precise PAMP–PRR interactions involved in *C. albicans* recognition, and the relative contribution of each interaction. Each of the *C. albicans* mutants was depleted in a specific cell-wall component: the *mnn4* mutant is defective in phosphomannan, the *och1* mutant has a defect in N-linked mannosylation, the *mnt1 mnt2* mutant is defective in O-linked mannosylation, and the *pmr1* mutant is defective in both N-linked and O-linked mannosylation. The ability of each mutant strain to stimulate the release of proinflammatory cytokines from human mononuclear cells (MNCs) was analysed. The fact that the *pmr1* mutant showed greatly reduced stimulation compared with wild-type *C. albicans* initially established the importance of mannosylation. The relative importance of the N-linked and O-linked residues was then assessed by comparing the effects of the *och1* and *mnt1 mnt2* mutants — the absence of N-linked mannose residues reduced cytokine production by 70% whereas the absence of O-linked residues reduced it by 15–30%. The loss of phosphomannan had no effect.

The PAMPs were then matched up with their relevant PRRs by assessing the stimulation of cytokine release in the presence of specific receptor-blocking antibodies. Cytokine release by the *och1* mutant was inhibited by an anti-TLR4 antibody and not an anti-MR antibody

and the situation was reversed for the *mnt1 mnt2* strain. Furthermore, cytokine production in TLR4-deficient mice decreased after stimulation with the *och1* mutant strain but not the *mnt1 mnt2* strain. Taken together, these results indicate that TLR4 recognizes O-linked mannosyl residues and MR recognizes N-linked residues.

Finally, the contribution of β -glucan to immune stimulation was examined by looking at two different scenarios, the stimulation of cytokine release by the *och1* mutant in TLR4-deficient mice and the stimulation of cytokine release from human MNCs by the *mnt1 mnt2* mutant in the presence of an anti-MR antibody. In both situations, there should be no immune signal from either N-linked or O-linked mannosyl residues. Cytokine production was reduced by ~80% and this residual activity could be removed by the presence of laminarin, a dectin 1 inhibitor.

So, this multilayered approach to a multilayered organism has revealed the presence of three innate immune recognition systems and their differential importance. The results suggest that the N-linked mannose–MR interaction is most important, followed by the O-linked mannose–TLR4 interaction and then the β -glucan–dectin 1 interaction, which is consistent with the idea that *C. albicans* is recognized from the outside in.

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ORIGINAL RESEARCH PAPER Netea, M. G. et al. Immune sensing of *Candida albicans* requires cooperative recognition of mannans and glucans by lectin and Toll-like receptors. *J. Clin. Invest.* **116**, 1642–1650 (2006)