Protein unmasks pathogenic fungi to activate immune response

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In this week's issue of the journal Proceedings of the National Academies of Sciences (PNAS), Whitehead Institute scientists describe a mechanism by which immune cells can distinguish between pathogenic and non-pathogenic fungi and modulate the immune response accordingly.

The work builds on earlier research that identified how certain immune cells, called macrophages, determine whether a foreign cell is a fungus.

The protein dectin-1, which resides in the macrophage cell membrane, recognizes beta-glucan, a sugar molecule that supports the cell walls of fungi. Once dectin-1 detects beta-glucan, indicating the presence of a fungal threat, it can trigger many responses in its macrophage, including engulfment of the fungal cell; production of reactive oxygen species (ROS) that are toxic to the fungal cell; and secretion of inflammatory cytokines and chemokines that recruit other immune cells to the fight.

To observe dectin-1's activity in living cells, researchers in the labs of Whitehead Founding Member Gerald Fink and Member Hidde Ploegh collaborated to tag the protein while integrated into the cell membranes of macrophages. Maximilian Popp, a former graduate student in the Ploegh lab, had refined this tagging method, which does not interfere with normal cell functions. Earlier tagging methods had relied on attaching a bulky green fluorescent protein (GFP) to the protein of interest. Although GFP tagging is a valuable research method, it can induce unwanted changes in protein and cellular behavior.

"For a lot of proteins, GFP tagging is incompatible with their function because the GFP is so big, about 25 kilodaltons," says Popp. "We were trying to avoid this by attaching a very, very small, about 2 kilodalton tag that is very bright. They're so small that they don't have the negative effects that GFP does. And the tags attach to dectin at the exclusion of all of the other cell surface molecules of the same cell."

Using Popp's method, called sortagging, Alexandre Esteban, a postdoctoral researcher in the Fink lab and first author of the PNAS paper, determined that dectin-1 associates with the protein galectin-3 in macrophages. Although known to recognize pathogenic fungi, galectin-3's specific role in the immune response to fungi had yet to be identified.

According to Esteban's work on established cell lines and mouse

macrophages, galectin-3 supplements a major weakness of dectin-1. The dectin-1-dependent immune response hinges on dectin-1's ability to recognize beta-glucan. But over the millennia of human/fungal interactions, pathogenic fungi have evolved to mask their beta-glucan with something of a "shag carpet" of proteins and other sugar molecules on their cell surfaces.

Galectin-3 recognizes and then binds to specific sugar molecules from this outer layer that are only present in the pathogen Candida albicans. Such recognition flags the fungal cells as pathogens and modulates dectin-1's immune responses.

"Until now, we didn't know that dectin-1 requires a partner to modulate its response, depending on if the fungi are pathogenic or non-pathogenic," says Esteban. "With this work, we determined the way that immune cells discriminate between pathogenic and non-pathogenic fungi."

Esteban notes that research studying galectin-3 and its role in the immune system should enhance our understanding of invasive fungal infections and may one day lead to discovery of novel targets for antifungal drugs.

More information: "Fungal recognition is mediated by the association of dectin-1 and galectin-3 in macrophages," *PNAS*, the week of August 8-12, 2011

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