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A systems biology approach to the mutual interaction between yeast and the immune system

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ABSTRACT

Dendritic cells (DCs) are capable of sensing fungi and then to initiate an appropriate defense against the invading microbe. We studied interactions between host and microorganism by analyzing the transcriptional response of DCs stimulated by the harmless *Saccharomyces cerevisiae* and of this phagocytosed fungus. Pathway analyses provided insight into the mutual interactions. Of particular interest was the responses elicited by the DC in the fungus, including downregulation of the carbon-compound metabolism, and upregulation of lipid, fatty acid, glyoxylate and tricarboxylic acid cycles. This indicates that the yeast shifts to a starvation mode and induces morphogenetic and autophagic pathways as well as those associated with reshaping cell wall composition, to resist the immune clearance. This yeast response is independent of the presence of virulence traits as the same transcriptional cell reprogramming has also been observed in potentially pathogenic *C. albicans* hyphae phagocytosed by macrophages. When comparing our results with the previous findings, it appears that the yeast dimorphic switch is only one of the components of the evolutionarily conserved panels of survival strategies elicited by phagocytosis. In conclusion, a systems biology approach, which combines genomics and pathway analyses, provides a powerful strategy to further our understanding of complex host–pathogen interactions and may ultimately define the distinguishing features of pathogenicity and commensalism.

Introduction

Immune systems biology can be defined as the comprehensive, quantitative study of interactions between hosts and microbes over time, leading to the generation of models describing their dynamic behavior. The analysis of a biological system, its components and its properties benefits from harnessing as many types of data sets as possible, including analyses of genes, proteins, RNAs, small molecules, cells and their interactions.

Many studies have investigated immune cells which are particularly suited to functional genomics analyses because their responses to precise stimuli in a controlled environment can be clearly classified. Only in the past decade it has become clear that the innate immune system not only specifically recognizes various classes of microorganisms, but also initiates and modulates the subsequent adaptive responses that are delivered by T cells and B cells through their interactions with antigen-presenting cells, especially dendritic cells (DCs; Hoffmann, 2003; Akira, 2006; Beutler et al., 2007; Medzhitov, 2007). Innate immunity should not be considered as a set of discrete signaling pathways activated by a pathogen binding to a receptor; it is a complex network of interconnected pathways depending on many factors. The immune response is also not simply a function of the host; the pathogen itself with its variable virulence and complex interactions with the rest of the microbiome, can influence the innate immune response. Fungi are an important component of the system. *Candida albicans* and *Saccharomyces cerevisiae* are ubiquitous fungal organisms that often colonize the skin and the mucosal surfaces of normal individuals without causing disease. However, when normal host defense mechanisms are impaired (Muñoz et al., 2005; Sethi and Mandell, 1988), they can become pathogenic. Fatal opportunistic mycosis continues to be alarmingly frequent (Pfaller and Yu, 2001; Koch et al., 2004) as cure rates are limited by the reduced effectiveness and serious side effects of the few available drugs as well as increasing incidence of drug resistance (Sanglard, 2002; Kontoyiannis and

Abbreviations: ConA, Concavalanin A; DCs, dendritic cells; DEG, differentially expressed gene; FET, Fisher Exact test; FC, fold change; moDCs, monocyte derived dendritic cells; GM-CSF, granulocyte macrophage colony stimulating factor; GO, gene ontology; IQR, interquantile range; IL, interleukin; PAMP, pathogen associated molecular pattern; PBMCs, peripheral blood mononucleated cells; PBS, phosphate buffer saline; PRR, pattern recognition receptor; RMA, Robust Multichip Average; sBEF, signed binary enrichment factors; *SC*, *Saccharomyces cerevisiae*; TF, transcription factor; YPD, yeast peptone dextrose

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Lewis, 2002). Fungal infections are the result of a coordinated battle between the fungus and its host. Fungi are known to activate morphogenetic programs in response to host signals. The association between morphogenesis and virulence has long been known for pathogenic, dimorphic fungi, where one morphotype exists in the environment or during commensalism and another within the host during the disease process. Augmenting the ability of the immune system to eliminate a pathogen requires a sophisticated understanding of the molecular mechanisms that are involved in pathogen recognition and in the hosts' immune response.

The widespread use of microarrays has generated large amounts of data sets that are available in public microarray repositories. Genome-wide mRNA expression profiling by DNA microarrays has proven to be a powerful tool for characterizing the changes in immune response to pure and combined stimuli (Napolitani et al., 2005). In the context of fungal infections, global gene expression profiles have been generated to study DCs stimulated with *C. albicans* (Huang et al., 2001, Rizzetto et al., 2010) and the response of *C. albicans* to phagocytosis by macrophages or granulocytes (Lorenz et al., 2004; Rubin-Bejerano et al., 2003; Fradin et al., 2005). This is just an example of the potential of whole transcriptional analyses of host–pathogen interplay.

Yet in order to understand pathogenicity, one needs to define the baseline of a healthy-normal response. Therefore, this study was designed to use the non-pathogenic yeast strain *S. cerevisiae* to uncover the mechanisms governing the interaction between microbes and the human organism. We recently described the immune response of DCs exposed to *S. cerevisiae* (Rizzetto et al., 2010). Here we investigated the transcriptional response of the DCs as well as of the yeast cells internalized by those DCs. The results obtained were compared with the published analyses of the paradigmatic pathogenic fungus, *C. albicans*. By dissecting the transcriptome at the pathway level, we investigated the specific cellular networks that determine how the fungus "sees" the immune system, and the immune signaling pathways induced by this harmless eukaryotic microorganism.

Material and methods

Yeast culture and preparation

SK1 yeast strain (MATa/α HO gal2 cupS can1R BIO) was cultured at different growth phases in complete medium (YPD, 2% yeast extract, 1% peptone, 2% glucose). Exponentially growing yeasts were cultured for 18 h, collected, washed twice with sterile water and resuspended at 10^8 cells/ml. Stationary phase cells were grown for 5 days, collected and treated as above.

DC preparation and stimulation

Peripheral blood mononucleated cells (PBMCs) were isolated from buffy coat blood samples from healthy volunteers by Ficoll-Hypaque density gradient centrifugation (Biochrom AG, Berlin, Germany). Monocytes were isolated from low density PBMCs by magnetic enrichment with anti-CD14 beads (Miltenyi Biotec, Bergisch Gladbach, Germany). Cells were cultured in the presence of granulocyte macrophage colony stimulating factor (GM-CSF 800 U/ml, Gentaur, Belgium) and recombinant interleukin (IL)-4 (1000 U/ml, Gentaur, Belgium) for 6 days to allow DC differentiation (Sallusto and Lanzavecchia, 1994). DC activation was induced by yeast in the different life stages. Serial dilution of yeast preparations was added to the monocyte-derived dendritic cells (moDCs) at different stimuli:DC ratios.

Internalization assay

S. cerevisiae cells were biotinylated using 10 mg/ml sulfo-NHS-LC-biotin (Sigma Aldrich, St. Louis, MO) 50 mM $NaHCO_3$ pH 8.5 for 2 h at 4 °C. The remaining reactive biotin molecules were inactivated by incubation in 100 mM Tris–HCl pH 8.0 for 40 min at 4 °C. DCs were allowed to ingest biotinylated yeast on sterile glass coverslips for 2 h using a stimuli:DC ratio of 4:1. Cells were then fixed in 4% paraformaldehyde for 30 min and DCs labeled with Concavalanin A (ConA, Sigma Aldrich, St. Louis, MO) for 30 min at 37 °C. To distinguish between internalized and attached/non-ingested yeast, *S. cerevisiae* cells were conjugated with streptavidin (Sigma Aldrich, St. Louis, MO) and counter-stained with Calcofluor white M2R (Sigma Aldrich, St. Louis, MO) (2.5 M) for 15 min in the dark. Calcofluor white is a fungus-specific stain that binds specifically to the yeast cell wall chitin and is not taken up by DCs. After several washes, the ability of DCs to ingest yeast cells was observed using confocal microscopy.

Whole genome transcriptome analyses of DC

3×10^6 DCs were cultivated either alone, or with spores, exponentially growing yeast, or stationary phase yeast in a stimuli:DC ratio of 4:1. After 4 h, cells were collected. RNA preparation, labeling, hybridization on a GeneChip® HG-U133A array (Affymetrix) and scanning were performed according to the Affymetrix reference protocols. The analysis was performed on three different donors. Array data files (CEL files) were pre-processed and normalized using the Robust Multichip Average procedure (RMA; Irizarry et al., 2003). Annotations were updated following a procedure devised by Dai et al. (2005). Computation was performed with the RMAExpress program (http://rmaexpress.bmbolstad.com). Microarray data have been submitted to the Array Express repository (experiment ID E-MEXP-1745, www.ebi.ac.uk/microarray-as/ae).

Raw data, in the form of $\log_2$-transformed values, were subjected to interquantile range (IQR) filtering to reduce noise. Differential gene expression analysis was carried out using the Rank Product algorithm (Hong et al., 2006), taking the differences between donors into account.

p-Values estimating differential expression were corrected for multiple testing (Fold Discovery Rate) and genes with a corrected *p*-value ≤ 0.05 were selected. Differential gene expression between stimulated and unstimulated cells was expressed as fold change (FC) in natural scale.

Expression profiling of ingested S. cerevisiae

S. cerevisiae was cultured in the presence or absence of DCs (stimuli DCs:ratio, 4:1) for 4 h. DC cultures were then treated with zymoliase (2 mg/ml, Sigma-Aldrich, St. Louis, MO) to eliminate ungested yeast cells, and DCs were lysated with a hypotonic solution. RNA was isolated from ingested yeast cells by the hot acid phenol extraction protocol. cDNA was synthesized from 20 to 30 μg of total RNA (control and ingested yeast) by reverse transcription using the Agilent kit (Quick Amp Labeling, Agilent), incorporating Cy3-dCTP or Cy5-dCTP (Quick Amp Labeling, Agilent) into the cDNA corresponding to each sample to be compared. Microarrays were provided by Agilent (G2519F Yeast V1 Oligo Microarray kit). For each condition tested, the total RNA from three different experiments was analyzed. Expression ratios were obtained from the average of three independent microarray