ImmunoTools *special* Award 2014

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Comparative analysis of immune responses against cell wall carbohydrate constituents in various *Candida* spp.

The incidence of candidiasis has significantly increased over the past few decades as a result of rise of AIDS, aged population, immunosuppressive therapies and modern medical intervention. Candidiasis is an infection caused by yeasts in genus *Candida*, which affects several organs such as mouth, throat, gastrointestinal tract and vagina. Invasive candidiasis, occurring when *Candida* spreads throughout blood stream, is ranked as the fourth most important cause of hospital-acquired sepsis, and has emerged as a significant cause of morbidity and mortality. There are at least 17 different *Candida* spp. can cause candidiasis in human, and the six most common species are *C. albicans*, *C. glabrata*, *C. dubliniensis*, *C. krusei*, *C. parapsilosis* and *C. tropicalis*. *C. albicans* is the most common cause of candidiasis; however, the prevalence of non-albicans Candida (NAC) has dramatically increased recently. The second common causes of candidemia are *C. glabrata* and *C. tropicalis*, due to its resistance to fluconazole. *C. parapsilosis* is significantly found in neonates and transplant patients, while *C. krusei* transiently habitats as a commensal in man and has been frequently isolated only from mucosal surface. *C. dubliniensis* which shares diagnostic characteristics with *C. albicans*, has been associated with oral candidiasis in AIDS patients.

Innate immune sensing to invading fungi via the engagement of PAMPs (pathogen-associated molecular patterns) and PRRs (pattern-recognition receptors) importantly provides the foundation to develop an adaptive immunity. Carbohydrate constituents of fungal cell wall, which comprise mannan, glucan and chitin, are the

major sources of PAMPs that transduce signals to activate phagocytes and antigen presenting cells (APCs). Dendritic cell (DC) is the APC that bridges innate and adaptive immunity. The interaction between PAMPs from the pathogen and PRRs of DCs leads to the various expression of cytokines and surface molecules involving in T cell differentiation and functions. DCs express variety of PRRs which recognize polysaccharides of fungal cell wall. The structures and proportion of carbohydrate in cell wall are different in an individual fungus; consequently, these are critically influent in the type of immune responses. It has been reported that *C. albica*ns induced T_H1, T_H2 and T_H17 responses, while *A. fumigatus* induced T_H1 and T_H17, and *C. neoformans* preferentially stimulated T_H17. Similarly, cell walls of individual *Candida spp.* are different in polysaccharide structures and composition. However, there is still lack of a full understanding of how DCs act and regulate immune responses against the distinct *Candida spp..* Thus, we aim to investigate DC activation and its effects on T cell induction in response to six different *Candida spp.*, *C. albicans*, *C. glabrata*, *C. dubliniensis*, *C. krusei*, *C. parapsilosis* and *C. tropicalis*. Our study will provide the knowledge of candidiasis immuno-pathogenesis which is crucial for the development of effective anti-fungal therapies, especially in the context of the rising incidence of NAC infection.

To investigate *in vitro* DC responses, bone marrow-derived DCs (BM-DCs) will be stimulated with heat-killed *Candida* or their cell wall carbohydrate extracts. For DC maturation, the expression of MHC II, CD80, CD86 will be accessed by flow cytometry. For cytokine productions, ELISA will be performed to detect IL-12p70, IFN-γ (important for T_H1 differentiation), IL-4, IL-10 (critical for T_H2 generation), IL-6, IL-23, TGF-β (essential for T_H17 induction) and pro-inflammatory cytokines IL-1 and TNF-α. To examine the *in vivo* immune responses, the animal will be immunized with the cell wall carbohydrate extracts and the T cell population will be identified based on the marker CD3, CD4, CD8, CD25, Foxp3, CD44, and CD62L. Besides, other immune cells such as B cell, macrophage, NK cell and neutrophil will also be investigated. To determine T cell differentiation, splenocytes will be *ex vivo* re-stimulated, and IFN-γ, IL-4, IL-5, IL-17, and IL-21 will be assayed by ELISA. The interaction of *Candida spp*. with human monocyte-derived DCs will be also analyzed by using flow cytometry to investigate the level of CD1a, CD14, CD80, CD86 and HLA-DR expression, and using ELISA for the measurement of cytokines production. The reagents from ImmunoTools will majorly contribute to the investigation of all above immune responses.

References

1. Kauffman CA. Fungal infections in older adults. Clin Infect Dis. 2001 Aug 15;33(4):550-5.
2. Flevari A, Theodorakopoulou M, Velegraki A, Armaganidis A, Dimopoulos G. Treatment of invasive candidiasis in the elderly: a review. Clin Interv Aging.8:1199-208.
3. Arendrup MC. Candida and candidaemia. Susceptibility and epidemiology. Dan Med J. Nov;60(11):B4698.
4. Gutierrez J, Morales P, Gonzalez MA, Quindos G. Candida dubliniensis, a new fungal pathogen. J Basic Microbiol. 2002;42(3):207-27.
5. Silva S, Negri M, Henriques M, Oliveira R, Williams DW, Azeredo J. Candida glabrata, Candida parapsilosis and Candida tropicalis: biology, epidemiology, pathogenicity and antifungal resistance. FEMS Microbiol Rev. Mar;36(2):288-305.
6. Samaranayake YH, Samaranayake LP. Candida krusei: biology, epidemiology, pathogenicity and clinical manifestations of an emerging pathogen. J Med Microbiol. 1994 Nov;41(5):295-310.
7. Romani L. Immunity to fungal infections. Nat Rev Immunol. Apr;11(4):275-88.
8. Brown GD. Innate antifungal immunity: the key role of phagocytes. Annu Rev Immunol.29:1-21.
9. Free SJ. Fungal cell wall organization and biosynthesis. Adv Genet.81:33-82.