ImmunoTools *special* Award 2014

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Candida colonization in *Clostridium difficile* infected patients: the pathogenic role of Th17

Clostridia are motile, gram-positive bacteria, ubiquitous in nature and especially prevalent in soil. Human gut hosts many bacteria including *Clostridium difficile (CD).* When antibiotics are administered they can kill commensal microorganisms, causing *CD* proliferation, which can create illness. *Clostridium difficile infection (CDI)* is recognized as the main cause of infectious diarrhoea that develops in high-risk patients: i.e. previous antibacterial and antacid therapy, older age, severe underlying illness and immune compromisation. It is currently estimated that there are ~500,000 cases of CDI per year in US hospitals and long-term care facilities, based on annual data from the state of Ohio in 2006. CDI severity is increasing, as 1% to 5% of affected patients require intensive care unit (ICU) admission and their disease leads to colectomy or death.

Candida is a genus of yeasts and is the most common cause of fungal infections worldwide. Many species are harmless commensals or endosymbionts of hosts including humans; however, when mucosal barriers are disrupted or the immune system is compromised they can invade and cause disease. *Candida albicans* is the most commonly isolated species, and can cause infections in humans and other animals. *Candida* species are the fourth leading cause of nosocomial bloodstream infection (BSI) in the United States. Wenzel and colleagues have estimated the number of cases of nosocomial candidemia and it ranges from 2.5% to 10% of all patients admitted to U.S. hospitals. In particular BSIs represent 10% of all nosocomial infections, and 8% of nosocomial BSIs are caused by *Candida* species. Recent observations hypothesized a possible link between CDI, Candida colonization and candidemia. The morbidity and mortality of candidemia are substantial and it is clear that fungal diseases have emerged as important public health problem. Our study conducted in 1300-bed teaching hospital Policlinico "Umberto I" in Rome showed that *Clostridium difficile* infection (CDI) was significantly associated with Candida colonization (83% CDI positive vs 67% CDI negative; $\chi 2=3.91$; $P<0.05$) and that *Candida albicans* was the species more often implicated. Furthermore two studies highlighted a correlation between CDI and candidemia showing that the risk of candidemia in subjects with CDI is higher than for those without CDI (OR 3.02; 95% CI 1.47-6.17; p=0.001) while the risk of bloodstream infection for any

aetiology other than Candida is similar for subjects with or without CDI (OR 1.16; 95% CI 0.70-1.92; p=0.57). However, further studies are needed to assess the pathogenic mechanism underling the correlation between candidemia and CDI. Candidemia mortality rates correlates to the timing of antifungal administration and the importance of early treatment of candidemia has been clearly demonstrated. An adequate antifungal administration within 48 h from the first positive blood culture was achieved in 7.3%-25.5% of treated patients. Therefore, the knowledge of the factors predisposing to the onset of candidemia needs to be addressed. For these prone patients, optimized treatments should be considered, such as prophylactic antimicotic therapy after CDI. The discovery of Th17 cells as a distinct T helper cell population set the stage for discoveries revealing a key role for this new T cell subset in antifungal immunity. Th17 are abundant at steady state in gut-associated tissues, particularly the small intestinal lamina propria, but they accumulate only in the presence of luminal commensal microbiota.

The pathogenic mechanism of increased *Candida* colonization in CDI patients needs to be addressed since it might represent the possible reservoir for the onset of candidemia. In this study we will be investigate Th17 as one of the possible susceptibility factor of the onset of candidemia in CDI.

Aim of our study is to investigate the role of Th17 CD4 T cells subsets for candidemia onset in CDI patients. Phenothypical assays will be performed on local and peripheral Th17. For this purpose anti-CD3, anti-CD4, anti CD8, anti-Tbet, anti-RORC, anti CCR6, anti CXCR3, anti CCR4 and anti-CD161 fluorochrome conjugate antibodies will be used. Immunophenotype assays will be performed with flow cytometry. *C*ytokine production will be assessed by flow cytometer and ELISA quantification of CXCL8, IL-17A, IL-17F, GM-CSF, IFNγ and IL10. rh IFNgamma, rh IL-2, rh, rh IL-17A, rh IL-17B, rh IL-17F will be used to assess functional effeciency of Th17 after in vitro candida specific T cells expansion.