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Infections in Patients with Innate Immunity, Primary Immunodeficiency and Neonates

1 Pathogenic Inflammation in Fungal Infections: the Contribution of the Th17 Pathway

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Background: The inflammatory response to fungi may serve to limit infection but an overzealous or heightened inflammatory response may contribute to pathogenicity, as documented by the occurrence of intractable fungal infections in patients with immunodeficiency disease or primary immunodeficiencies associated with heightened immune reactivity. IL-12, by initiating and maintaining Th1 responses, was thought to be responsible for overreacting immune and autoimmune disorders. The newly described Th17 pathway is now thought to contribute to immune pathogenesis previously attributed to the Th1 lineage.

Objective: This study examined the role of Th17 in inflammation and immunity to Candida albicans or Aspergillus fumigatus in experimental models of infections.

Methods: Mice with selected genetic deficiencies (i.e., IL-23, IL-12 or both or TLR-deficient) were exposed to C. albicans or A. fumigatus and assessed for susceptibility to infection, gut or airway inflammation, and parameters of innate and adaptive immunity.

Results: The Th17 pathway – and not the uncontrolled Th1 response – was associated with defective pathogen clearance, failure to resolve inflammation and initiate protective immune responses. Conditions of high-threat inflammation predisposed to Th17 activation, whereby the unrestricted fungal growth resulted from the activation of not only pathogenic Th17 cells but also Th2 cells, whose activation is strictly dependent on fungal burden. Blockade of IL-17/IL-23 prevented pathogenic inflammatory responses, ameliorated infections and restored protective Th1 antifungal resistance, thus causally linking pathogenic inflammation to Th17 development.

Conclusion and clinical implications: The above findings provide a molecular connection between the failure to resolve inflammation and lack of antifungal immune resistance and point to strategies for immune therapy of fungal infections that attempt to limit inflammation to stimulate an effective immune response. Inhibition of the Th17 pathway may potentially represent a novel strategy for the prevention of inflammatory immunity and allergy in fungal diseases.

2 Aspergillus Attenuates Distinct Host TLR2 & TLR4 Responses

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Background: Aspergillus fumigatus is the most commonly identified cause of invasive aspergillosis in immunocompromised hosts with significant attributable mortality and morbidity. To date, not much is known about the pathophysiology and host response to such mould infections.

Objective: We aimed to study how A. fumigatus may modulate the host innate immune system to its own advantage in the course of invasive disease.

Methods: Peripheral blood mononuclear cells (PBMCs), isolated from venous blood of healthy volunteers by density centrifugation on Ficoll-Hypaque, were preincubated with A. fumigatus conidia (107cfu/ml) as a priming stimulus in RPMI for 24h, after which cells were exposed to a secondary stimulus to detect the respective immunomodulatory effects. As secondary stimulus, TLR2 ligand Pam3Cys 10ug/ml or TLR4 ligand E. coli lipopolysaccharide 1ng/ml was used. Cytokine production (IL-6, TNF, IL-1b) was measured. Cytokine production (IL-6, TNF, IL-1b) was measured by ELISA. Quantitative PCR was performed for TLR2, TLR4 mRNA (24h after priming of PBMCs with Aspergillus); IL-1b and TNF mRNA (4h after secondary stimuli). Mean relative mRNA expression was measured. To analyse the involvement of phagocytic activity and dectin-1 receptor, cytochalasin B and laminarin were added respectively. Results were pooled from at least 3 sets of experiments.

Statistical significance was set at 0.05 (Wilcoxon Signed Rank).

Results: PBMCs primed with A. fumigatus conidia produced significantly less IL-6 and IL-1b in response to subsequent TLR2 and TLR4...
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Figure 1a. Asp conidia attenuates IL6 response in presence of TLR2/TLR4 ligands. Attenuated response only with TLR4 ligand in Asp hyphae.

Figure 1b. TNF production not attenuated by Asp conidia in contrast to IL6. Decreased TNF response with TLR4 ligand stimulation. TNF production remained unchanged (Figures 1a & b).

The observed TLR-2 modulation involved phagocytosis, as addition of cytochalasin B reversed the observed attenuation, while no influence was seen by signalling through TLR4. This effect was not mediated by dectin-1 receptor. IL-1β mRNA expression decreased in response to Aspergillus conidia. TNF, TLR2 and TLR4 mRNA expression was not significantly attenuated after exposure to Aspergillus.

Conclusions: We elicited a novel phenomenon by which A. fumigatus attenuates host immune response via distinct means. Monocytic phagocytosis of A. fumigatus conidia results in an attenuated proinflammatory IL-1β and IL-6 response via TLR2 likely secondary to depletion of surface TLR2. TLR4 modulation by conidia though, affects transcription processes upstream independent of phagocytosis. Induction of proinflammatory cytokines TNF and IL-1β/IL-6 by A. fumigatus purportedly involved distinct pathways.

3 TLR4 Receptor Polymorphisms are Associated with Gram-negative Bacteremia after Allogeneic Stem Cell Transplantation

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Background: Bloodstream infection (BSI) by Gram-negative organisms (GNR) occurs in approximately 20% of allogeneic hematopoietic stem cell transplant recipients (HSCT). Toll-like receptor 4 (TLR4), has been shown to be a major recognition receptor for Gram-negative pathogens and specifically lipopolysaccharide (LPS). The missense mutations TLR4Asp299Gly and co-segregating TLR4 Thr399Ile are found in approximately 6-10% of Caucasians. Heterozygosity for TLR4 Asp299Gly TLR4 is associated with hyporesponsiveness to LPS. We followed prospectively a cohort of 105 allogeneic HSCT to study the association of TLR4Asp299Gly and Thr399Ile in the donor or recipient with mutations with GNR BSI after HSCT.

Methods: The study was approved by the MSKCC Institutional Review Board. Patients were followed up to onset of BSI, death or end of study, whichever occurred first. DNA for donor and recipient was extracted from whole blood. TLR4 genotyping was performed by direct sequencing. Genotypes were tabulated as presence or absence of the minor allele at each locus, separately for the donors and recipients. “Risk genotype” was defined as heterozygous at TLR4 299AspGly and Thr399Ile. The cumulative incidence of infection was estimated by treating death prior to the onset of infection as a competing risk event, and compared between relevant groups using a modified chi-squared test.

Results: Ten of 95 (10.5%) donors and 8/88 (9.1%) recipients had the risk genotype. Twenty-one out of 105 (20%) patients developed BSI at 1-381 days post transplant. Twenty-seven percent (27%) of patients with BSI had donor risk genotype compared to 6.4% of patients without BSI (p<0.01). In contrast, examination of recipient genotypes showed similar frequencies of “risk genotype” among patients with or without BSI (5.5% versus 8.5%, p-value = 0.59) (Table 1). The cumulative incidence of infection was higher for patients with donor with risk genotype to patients without risk genotype. In contrast, when recipient genotypes were examined the cumulative incidence of BSI was similar between the two groups (Figure 1).

Table 1

<table>
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<tr>
<th>TLR4 Genotype</th>
<th>GNR BSI</th>
<th>P-value</th>
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<tr>
<td></td>
<td>Yes N (%)</td>
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<td>Donor Risk genotype</td>
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<tr>
<td>Total</td>
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<td>70 (100)</td>
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Figure 1. Cumulative incidence of Gram-negative BSI or mortality without BSI by donor type.

Conclusions: This is the first prospective study suggesting an association of TLR4 polymorphisms with Gram-negative bloodstream infection after allogeneic HSCT. The ability to predict genetic susceptibility to a common infection with substantial morbidity is of direct relevance to the design of strategies to protect most vulnerable patients.