Host-microbe interactions in stem cell transplantation

Recognizing Candida in infection and inflammation

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Host-pathogen interactions at epithelial barriers play an important role in health and disease. This also applies to the clinical setting of stem cell transplantation (SCT) in which deregulated sensing of microbes and their cell wall components by pattern recognition receptors (PRRs) can contribute to inflammatory and infectious complications. The role of Candida species herein has recently been rediscovered since a ‘loss-of-function’ Y238X polymorphism in dectin-1, a C-type lectin receptor recognizing the β-1,3-glucan motif of Candida, resulted in diminished membrane expression and lower cytokine responses upon β-1,3-glucan recognition, and was associated with increased Candida colonization of SCT recipients, rendering them at risk for candidaemia. In addition, Candida colonization was associated with an increased incidence of acute graft-versus-host disease (GvHD), but only in those individuals with the wild-type dectin-1 allele. The Th17 mediated immune responses might provide a common link in these processes, as they have recently been implicated in anti-Candida immunity as well as the pathogenesis of GvHD. These new insights suggest that immunogenetics could contribute to a more individualized risk-based strategy for managing SCT recipients, for example concerning antifungal prophylaxis. In addition, modulating host-pathogen interactions by selectively modulating PRR activity could be exploited in SCT to achieve better outcomes.

Introduction

Intensive immune therapy, chemotherapy and stem cell transplantation (SCT) remain the only curative treatment options for most patients with a hematological malignancy. However, these treatments are still accompanied by complications caused by their devastating effects on epithelial barriers and the immune system leading to uncontrolled inflammation and infections. For many years the role of microorganisms in the pathogenesis of these inflammatory complications has been known. Besides their obvious role in causing sepsis, microorganisms and their microbe-associated molecular patterns (MAMPs) initiate and aggravate inflammation and immune responses in mucosal barrier injury (MBI), idiopathic pneumonia syndrome (IPS), and graft-versus-host disease (GvHD). These effects result from activation of pattern recognition receptors (PRRs), including Toll-like receptors (TLRs), Nod-like receptors (NLRs), and the family of C-type lectin receptors (CLRs), which are expressed predominantly on immune cells and epithelial cells. The final immune response resulting from host-pathogen interactions during infection and tissue damage depends on the complex interaction of multiple activated PRRs generating different cytokine profiles which direct adaptive immunity towards CD4+ T-lymphocyte helper 1 (Th1), Th2 and/or Th17.

Several non-synonymous single nucleotide polymorphisms (SNPs) in PRRs have consistently been associated with bacterial
and fungal infections, GvHD and IPS.6–8 These new insights could be used in the future to target antimicrobial prophylaxis, immunomodulation and the management of other complications. PRR-mediated immune responses can also be manipulated and exploited in the treatment of cancer and hematological diseases by induction of apoptosis or increasing graft-versus-leukemia (GvL) responses.9

Immunity to Candida: New Paradigms

Anti-Candida host defenses consist of epithelial and mucosal physical barriers, the resident commensal bacterial flora, antimicrobial peptides, and the innate and acquired immune cells. In particular, the phagocytic capacity of neutrophils, monocytes and macrophages is crucial in the phagocytic capacity of neutrophils, antimicrobial peptides, and the innate immune system. The resident commensal bacterial flora, anti-microbial peptides and chemokines.11 The role of Th17 responses in antifungal host defense. Th1 responses have an important role for host defense against systemic infections with Candida. However, several studies have shown recently that Th17 responses play a more dominant role in anti-Candida mucosal immunity.10 T-helper cells manifest the Th17 profile under the influence of pro-inflammatory cytokines IL-1β, IL-6, IL-23 and TGFβ, although the specific cytokines involved differ among species. These cytokines are released by dendritic cells, monocytes and macrophages, upon the activation of PRRs. Dectin-1, dectin-2 and the mannose receptor are very important for the induction of Th17 responses by Candida albicans. Th17 cells release several cytokines including IL-17 and IL-22, both of which are involved in enhancing epithelial barrier functions and in the production of anti-microbial peptides and chemokines.11 The contribution of Th17 responses to systemic anti-Candida immunity is less clear, but it is though to be less important. This is underscored by the reports on hereditary diseases affecting the Th17 signaling pathway, such as the hyper-IgE-syndrome and the recently discovered Q295X mutation in CARD9, as patients present with mucocutaneous, but not systemic, Candida infections.12 Normal functioning phagocytes in the presence of the pro-inflammatory cytokines are sufficient to control systemic infection.

Fungal recognition: dectin-1. Cells of the innate and acquired immune system elicit antifungal immune responses after the activation of a panel of PRRs.13 PRRs that are involved in yeast recognition belong to the classes of TLRs and CLRs, but recently also the Nod-like receptor NALP3 has been implicated in yeast sensing.14 Several yeast cell wall components are recognized by innate immune cells, of which mannann is recognized by the mannose receptor, dectin-2 and TLR4, phospholipomannan by TLR2 and β-glucan by dectin-1.14 Dectin-1 is mainly expressed by immune cells of the myeloid lineage, though keratinocytes, enteroctyes and γδ-T-cells also do so. Dectin-1 signals through Syk-CARD9 and Raf-1 and synergizes with TLR2 and other PRRs for the induction of anti-Candida responses.15 Activation of dectin-1 increases phagocytosis and intracellular killing by monocytes and macrophages and induces the production of pro-inflammatory cytokines. The Syk-CARD9 pathway is of particular importance in eliciting Th17 responses through the generation of IL-6, TNFα and IL-23 in dendritic cells.16 In studies with dectin-1 knock-out mice, the importance of dectin-1 in both mucosal and systemic Candida infection has been established, but little is known about its role in Candida infections of humans.17 Recently, we have confirmed the importance of dectin-1 in the sensing of Candida in human infections.18 In a family with multiple subjects suffering from recurrent mucocutaneous fungal infections the early stop codon polymorphism Y238X was discovered in dectin-1. This polymorphism leads to the loss of the last ten amino acids of the extracellular carbohydrate recognition domain involved in the binding of fungal derived β-glucan. Defective surface expression of dectin-1 due to the presence of the Y238X polymorphism results in lack of β-glucan recognition and impaired cytokine responses (IL-6, TNFα and IL-17) by monocytes and macrophages (Fig. 1). Moreover, cytokine production was decreased to a lesser degree in heterozygous individuals. In contrast, monocytes/macrophages and neutrophils of affected patients exhibited phagocytosis and normal killing of Candida albicans. This underlines the redundant nature of dectin-1 in the phagocytosis and killing of yeast pathogens, which explains the absence of invasive candidiasis in these patients. The defective function of myeloid cells with regard to cytokine release in the patients bearing the Y238X dectin-1 polymorphism is the most likely cause of the clinical phenotype. However, defective

Figure 1. Dectin-1 polymorphism Y238X leads to functional defects. IL-17 production capacity of peripheral blood mononuclear cells from healthy volunteers (dectin-1 wt, five individuals), patients heterozygous (dectin-1 het, two individuals) or patients homozygous for the mutation (dectin-1 hom, three individuals) stimulated for 5 days with heat-killed Candida albicans. Adapted from Ferwerda et al. NEJM 2009.18
dectin-1 signaling in epithelial cells and intra-epithelial γδ-T-cells can conceivably have contributed to the clinical picture, especially since these cells express dectin-1 and produce cytokines and anti-microbial peptides upon activation.25

Candida in SCT

Colonization, infection and immunogenetics. Fungal infections including those due to Candida species pose a great threat in the treatment of hematology patients, especially in the setting of SCT where they contribute to significant morbidity and mortality. This has resulted in the use of antifungal prophylaxis resulting in both reduced infection and mortality.22 Anti-Candida prophylaxis particularly with fluconazole is now widely adopted for it decreases the incidence of candidaemia from 9–10% to 1–2%.24 Nevertheless, the issue of prophylaxis is far from settled, because there is considerable over-treatment resulting in increased selective pressure leading to resistance, potential drug interactions, side effects and unnecessary costs. Therefore, a more individual risk-based approach would be welcome, targeting only those at high risk of infection and mortality.24

Candida colonization is the major risk factor for candidaemia among patients with neutropenia and mucosal barrier injury following intensive chemotherapy, and occurs in 28–57% of patients. During admission the proportion increases further under the pressure of antibacterial drugs, tissue damage and corticosteroids.25 However, a substantial number of patients remain free from colonization. Predicting Candida colonization or establishing it at an early time point is necessary if prophylaxis is to be targeted, but this remains a challenge. We have used a culture-guided approach, treating only those patients deemed to be colonized. This approach reduced the risk of over-treatment, but the incidence of candidaemia still remained high in colonized patients. Therefore, we investigated the impact of the newly discovered dectin-1 polymorphism Y238X on our SCT recipients and found a significantly increased risk for Candida colonization in patients bearing the dectin-1 polymorphism on admission; 84.6% vs. 31.5% in wild-type patients and on the day of SCT; 92.3 vs. 45.1%, (p < 0.001).25 The polymorphism results in decreased Th17 responses suggesting that dectin-1 contributes to the mucosal anti-Candida defenses in SCT recipients.18 Although the dectin-1 polymorphism does not increase the risk for invasive candidiasis in otherwise healthy persons, it is a reasonable hypothesis that it would influence the susceptibility to candidaemia in SCT recipients during neutropenia and MBL. However, we were unable to confirm this assumption as the prevalence of invasive candidiasis was low, probably as a result of prescribing fluconazole prophylaxis. The clear impact of the dectin-1 polymorphism on Candida colonization suggests that immunogenetics helps in achieving a more individualized approach to antifungal prophylaxis and treatment. However, confirmation of these findings in an independent cohort is needed before any clinical implications should be considered.

It would be interesting to investigate polymorphisms in other immune genes with respect to Candida infection in SCT recipients.26 Candidate genes include those involved in mucosal antifungal immunity, e.g., human β-defensin-1 (hBD1), TLR2, TLR4, mannose-binding lectin (MBL) and NLRP3/CARD8, and those involved in the IL-23/Th17 signaling pathway such as the IL-23 receptor (IL-23R) and CARD9. Polymorphisms in MBL have been associated with increased mucocutaneous candidiasis, and in SCT these polymorphism were associated with major infections, though not candidiasis.26 TLR4 polymorphisms (Asp299Gly/Thr399Ile) contribute to host susceptibility to fungal infections including those due to Candida spp. However, only the association with aspergillosis has been established in SCT patients.6

Candida in graft-versus-host disease. We extended our first study to explore retrospectively the impact of the Y238X polymorphism and Candida colonization on major SCT outcome endpoints, including disease-free and overall survival, relapse and the occurrence of GVHD (unpublished data). We found no significant impact of the dectin-1 polymorphism Y238X on any of these outcomes, but interestingly, Candida colonization was associated with an increased risk of acute GVHD (Fig. 2). This however seemed to depend on the dectin-1 genetic status. The incidence of acute GVHD was increased among patients from wild-type dectin-1 pairs who where colonized with Candida species compared to non-colonized patients (41.9 vs. 20.4%, OR = 2.6, 95%CI: 1.02–6.58, p = 0.04). This effect on acute GVHD was however not observed in patients from pairs bearing at least one dectin-1 Y238X allele (23.5 colonized vs. 20.4% not colonized, OR = 1.2, ns), although this group was too small to draw any firm conclusions.

Microorganisms residing on the mucosal surfaces have long been given a putative role in the pathogenesis of acute GVHD and the use of selective gut decontamination has been shown to decrease the incidence of acute GVHD.27 Although the focus so far has been on gut bacteria, our data underscore the importance of yeast. Moreover, in a study by Marr et al. a decreased incidence of gut acute GVHD was observed when using early fluconazole prophylaxis,23 supporting the association between Candida colonization and acute GVHD.

The link between Candida colonization and acute GVHD might prove to be the Th17/IL-23 axis. Candida elicits powerful Th17 responses and these responses confer anti-Candida immunity.28 However, Th17 responses have also been implicated in fungal-induced tissue damage29 and overproduction of IL-17 can also lead to autoimmune diseases.30 Moreover, recent studies have suggested a role for Th17/IL-23 in the pathogenesis of recent studies.31,32 Although most studies have been performed in mice, the association of the IL-23R polymorphism in donors and the occurrence of acute GVHD suggests an important role in humans as well.33 The exact role of Th17 responses in the pathogenesis of GVHD has to be defined in humans, but host-pathogen interactions at the mucosal surfaces seem to play a regulatory role on immune responses through the modulation of Th17 responses. Hence, Candida may have a greater impact on SCT than we have considered hitherto (Fig. 3).

Candida in graft-versus-leukemia? Activation of PRRs by microbial components can modify allo-reactive immune

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generate Th17 responses, could contribute to novel anti-cancer strategies.38

Future Directions

How humans coexist and cope with their microbial flora and external pathogens remains an exciting field of investigation. The regulatory immune network involved in maintaining homeostasis is extremely complex. Host-pathogen interactions are of utmost importance both in health and disease, and even apparently small defects can have serious consequences as in the clinical setting of SCT in which
chemotherapy and radiotherapy induce profound barrier injury and immune deficits in the host.

Studies on the impact of polymorphisms in innate immune genes have increased our understanding of antimicrobial host defenses and the pathogenesis of SCT-related inflammatory complications. Thus far, these new insights have not led to improved outcomes but it is conceivable that in the future immunogenetics could contribute to more individualized risk-based strategies for using prophylaxis and therapy. Polymorphisms in dectin-1 (Y238X) and other PRRs involved in fungal sensing may be employed to direct anti-fungal prophylaxis to keep over-treatment to a minimum. However, more studies are needed in larger and more homogenous SCT cohorts to define consistent disease associations with SNPs and assess interventions in prospective trials.

Conclusion

Recent studies have renewed the interest for Candida and fungal recognition by PRRs in SCT. This interest extends beyond colonization and infection, due to an apparent link between Candida, Th17 and acute GvHD. This hypothesis, and in particular the cooperative activation of adoptively transferred donor T cells infused after allogeneic bone marrow transplantation in the mouse, Blood 2007; 109:4564-74.

Conclusions of the literature review (1-10) lead to the hypothesis that GvHD and GvL should be further investigated. Modulating host-pathogen interactions by selectively modulating PRR activity could be exploited in SCT to achieve better outcomes.

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