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TLR Polymorphisms and the Risk of Invasive Fungal Infections

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Allogeneic hematopoietic stem-cell transplantation is a potentially lifesaving cancer therapy that, at least temporarily, renders patients highly immunocompromised and vulnerable to infection. *Aspergillus fumigatus*, a common environmental fungus that causes invasive infections in immune-compromised persons, is particularly problematic in patients who have undergone this treatment.¹ Although the risk of the development of aspergillosis correlates with the degree of immune-suppression and the intensity of exposure to fungal spores, these factors alone do not explain why this infection develops in approximately 5 to 10% of patients who have received these transplants, whereas it does not develop in the remaining 90 to 95% of patients. A study reported by Bochud and colleagues in this issue of the *Journal*² begins to shed light on additional risk factors by correlating innate immune-receptor polymorphisms with the risk of the development of invasive aspergillosis after allogeneic hematopoietic stem-cell transplantation.

Innate immune receptors are expressed on or within mammalian cells and, on binding to microbial molecules, induce the expression of factors that restrict microbial tissue invasion and enhance microbial killing.³ The most extensively investigated innate immune receptors are the toll-like receptors (TLRs). Toll, a protein first described in *Drosophila melanogaster* as a regulator of development in flies,⁴ was subsequently discovered to mediate an innate immune defense against fungal infection in fruit flies by inducing production of the antimicrobial peptide drosomycin.⁵ A long hunt for innate immune receptors in mammals led to the discovery of TLR4,^{6,7} the receptor that detects lipopolysaccharide, a component of gram-negative bacteria that causes septic shock. Humans harbor 10 genes encoding TLRs, each with distinct specificities that extend from microbial glycolipids and lipoproteins to nucleic acids and bacterial flagellins.³

Studies in mice show increased susceptibility to infection when TLR signaling is impaired, and mutations in genes encoding TLRs or downstream signaling proteins increase the risk of infection among humans.⁸ For example, a common mutation resulting in a deficiency of TLR5, a receptor that responds to bacterial flagellin, is associated with increased susceptibility to *Legionella pneumophila* infection.⁹ Point mutations in TLR2, which responds to microbial glycolipids and lipoproteins, have been associated with a higher risk of the development of lepromatous than of tuberculoid leprosy. Associations between *TLR1* and *TLR6* polymorphisms and the development of invasive aspergillosis in patients who have received allogeneic hematopoietic stem-cell transplants have also been reported.¹⁰

TLR4 variants have been described in humans, and two mutations within the coding region of the *TLR4* gene decrease responsiveness to lipopolysaccharides.¹¹ The study by Bochud et al. involving patients who received allogeneic hematopoietic stem-cell transplants shows that stem-cell transplants from donors expressing the hyporesponsive TLR4 variant render recipients more susceptible to invasive aspergillosis.

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The authors first investigated 336 patients who underwent allogeneic hematopoietic stem-cell transplantation from unrelated donors between 1995 and 2003, a period that extends from the “old era” (when amphotericin B was the major therapeutic option for invasive mold infections) to the “new era” of less toxic antifungal azoles and echinocandins. Proven or probable invasive aspergillosis was diagnosed in 33 patients.

Single-nucleotide polymorphisms (SNPs) in four *TLR* genes (*TLR2*, *TLR3*, *TLR4*, and *TLR9*) were characterized in transplant recipients and donors; one haplotype, referred to as S4 by the authors, was found to be associated with an increased risk of invasive aspergillosis. The S4 haplotype is defined by four SNPs within or near the *TLR4* gene, two of which change the amino acid sequence of TLR4 to the lipopolysaccharide-hyporesponsive form. The association of the S4 haplotype with invasive aspergillosis was confirmed in a validation study that compared 103 case patients who had invasive aspergillosis with 263 control patients.

The association of the S4 haplotype with invasive aspergillosis was significant only in recipients of unrelated allografts, who presumably required greater immunosuppressive therapy to prevent graft-versus-host disease; this suggests that the “susceptibility phenotype” may be apparent only in patients with more profound degrees of general immunosuppression. Furthermore, the S4 haplotype of the donor, but not the recipient, was associated with invasive aspergillosis, indicating that TLR function in bone marrow–derived cells — perhaps in neutrophils, monocytes, macrophages, or dendritic cells — is critical. The authors found that cytomegalovirus (CMV) infection independently increases the risk of invasive aspergillosis, and they found that transplant recipients receiving non-S4 haplotype grafts in the absence of CMV infection have a very low risk of the development of invasive aspergillosis — a finding that may allow for more focused use of prophylactic antifungal agents after allogeneic hematopoietic stem-cell transplantation.

The finding that *TLR4* mutations affect susceptibility to *A. fumigatus* infection might be considered surprising, since this receptor is involved principally in the response to bacterial lipopolysaccharides. Since *A. fumigatus* does not produce lipopolysaccharides, TLR4 may bind other, nonlipopolysaccharide molecules produced by this fungus. Experiments in mice showing that inflammatory responses to *A. fumigatus* are, in part, mediated by TLR4 provide support for this finding.¹² An *A. fumigatus*–derived ligand for TLR4 has not been identified, however.

TLR-mediated activation of innate immune effector cells (e.g., macrophages, granulocytes, or dendritic cells) provides a direct mechanism to inactivate pathogenic microbes.³ An alternative indirect mechanism for a TLR-mediated defense against invasive infections has been suggested by recent studies of innate immune responses to microbial colonization of mucosal surfaces. Commensal bacteria inhabiting the intestine, for example, stimulate TLRs, including TLR4, and induce the expression of antimicrobial molecules by epithelial cells.^{13,14} Thus, even in the absence of overt infection, the innate immune system in mammals actively responds to colonizing bacteria and establishes an “innate immune tone” that fortifies mucosal barriers and restricts microbial invasion.

Can differences in the sensitivity of TLRs for their respective ligands affect the innate immune tone? Circulating levels of acute-phase reactants in persons expressing TLR4 variants suggest that the basal innate immune tone correlates with TLR sensitivity to lipopolysaccharides.¹⁵ So, an alternative explanation for the finding of Bochud et al. is that persons receiving stem cells that express the high-affinity TLR4 variant have an elevated innate immune tone that, more generally, increases resistance to infection. Determining how *TLR* polymorphisms influence a defense against pathogens will make for an exciting scientific journey that may, in time, result in new strategies to treat or prevent microbial infections.

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