MAJOR ARTICLE

Early Stop Polymorphism in Human DECTIN-1 Is Associated with Increased *Candida* Colonization in Hematopoietic Stem Cell Transplant Recipients

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(See the editorial commentary by Bochud and Calandra on pages 733–5)

Background. Intensive treatment of hematological malignancies with hematopoietic stem cell transplantation (HSCT) is accompanied by a high incidence of opportunistic invasive fungal infection, but individual risk varies significantly. Dectin-1, a C-type lectin that recognizes 1,3-β-glucans from fungal pathogens, including *Candida* species, is involved in the initiation of the immune response against fungi.

Methods. Screening for the *DECTIN-1* Y238X polymorphism within a group of 142 patients undergoing HSCT was correlated with *Candida* colonization and candidemia. Furthermore, functional studies were performed on the consequences of the polymorphism.

Results. Patients bearing the Y238X polymorphism in the *DECTIN-1* gene were more likely to be colonized with *Candida* species, compared with patients bearing wild-type *DECTIN-1*, necessitating more frequent use of fluconazole in the prevention of systemic *Candida* infection. Functional assays demonstrated a loss-of-function phenotype of the polymorphism, as shown by the decreased cytokine production by immune cells bearing this polymorphism.

Conclusions. The Y238X polymorphism is associated with increased oral and gastrointestinal colonization with *Candida* species. This suggests a crucial role played by dectin-1 in the mucosal antifungal mechanisms in immunocompromised hosts. The finding that *DECTIN-1* polymorphisms rendered HSCT recipients at increased risk for fungal complications may contribute to the selection of high-risk patients who should be considered for antifungal prophylaxis to prevent systemic candidiasis.

The treatment of patients with hematological malignancies with a hematopoietic stem cell transplantation (HSCT) following myeloablative conditioning is accompanied by complications that include mucosal barrier injury, prolonged neutropenia, and graft-versus-host disease (GvHD), all of which contribute to fungal and other opportunistic infections [1, 2]. Traditional risk factors are predictive for incidence of invasive fungal disease in general, but the individual risk is more difficult to determine, although this is needed for a more guided use of antifungal prophylaxis and therapy.

Dectin-1 is a member of the C-type lectin receptor family that recognizes the β-1,3-glucan motif of the cell wall of pathogenic fungi [3]. Dectin-1 is mainly expressed by immune cells of the myeloid lineage (neutrophils, macrophages, and dendritic cells). Several studies have shown that dectin-1 belongs to the armamentarium of immune cells against fungal pathogens, including *Candida* species and *Aspergillus* species [4, 5]. Furthermore, dectin-1 synergizes with TLR2 and TLR4 signals and promotes Th1 and Th17 responses to activate antifungal host defense [6–8].

Recently, we have demonstrated that a polymorphism in *DECTIN-1* (Y238X, rs 16910526) is respon-

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sible for recurrent mucocutaneous fungal infections in a Dutch family (unpublished data). This polymorphism resulted in an early stop codon, which leads to the loss of the last 10 amino acids of the extracellular domain, and in a diminished capacity to bind β-glucans.

To test the hypothesis that *DECTIN-1* variants influence the susceptibility to fungal infection in general and to *Candida* infection in particular for HSCT recipients, we first assessed the frequency of *DECTIN-1* polymorphisms in a healthy Dutch population. We identified the Y238X mutation as an important polymorphism and the only one with significant functional consequences for the recognition of *Candida* species. Subsequently, we have investigated the impact of this *DECTIN-1* polymorphism on the incidence of mucosal *Candida* colonization and the occurrence of candidemia and other invasive fungal infections among 142 patients who received a sibling T cell–depleted allogeneic HSCT.

PATIENTS, MATERIALS, AND METHODS

Genetic screening for* DECTIN-1 *polymorphisms. DNA template of the *DECTIN-1* gene (also named *CLEC7A*) was taken from GenBank, chromosome position 12p13, NC_000012.10. Sequencing of the exonic and nearby intronic regions of the *DECTIN-1* gene in the 138 healthy volunteers was performed by applying the primers and conditions depicted in table 1. Genotyping for the presence of the Y238X polymorphism in the patient and donor groups was performed by applying the TaqMan single-nucleotide polymorphism (SNP) assay C_33748481_10 on the 7300 ABI Real-Time polymerase chain reaction system (Applied Biosystems). Patients, donors, and healthy volunteers had given informed consent to prospective collection of DNA samples for investigational use.

Flow cytometry. For staining of membrane-bound dectin-1, monocytes were incubated with 5 μg/mL murine anti–dectin-1 directed towards the stalk region (BD6) conjugated with biotin, or mouse immunoglobulin (Ig) G2b isotype control, followed by streptavidin–allophycocyanin conjugated goat anti-mouse antibody (Pharmingen). Dectin-1 expression was determined by flow cytometry (FACScalibur; BD Biosciences).

Confocal microscopy. Confocal laser scanning microscopy was performed as described by Meyer-Wentrup et al [9]. Cells were stained with 10 μg/mL mouse anti–dectin-1 (clone 259931; R&D Systems) or mouse IgG2b isotype control, followed by goat anti-mouse-Alexa647–conjugated secondary antibody (Molecular Probes). Samples were mounted in mowiol and analyzed by confocal laser scanning microscopy (Olympus FV1000).

Cytokine stimulation assays. Isolation of mononuclear cells was performed as described previously [6]. Cells were incubated at 37°C for the indicated duration (4 h or 48 h) with either culture medium or the various stimuli: 10^5 heat-killed *Candida albicans* (heat-killed by incubation at 56°C for 30 min), the TLR2 agonist Pam3Cys (10 μg/mL) and β-glucan (10 μg/mL) or a combination of Pam3Cys and β-glucan. In a part of the experiments, after peripheral blood mononuclear cell (PBMC) isolation, monocytes were purified by $CD14^+$ MACS MicroBeads (Miltenyi Biotec) and stimulated as described above. Cytokine production was measured by enzyme-linked immunosorbent assay (R&D Systems).

Patients and donors. We performed a retrospective analysis involving 142 Dutch patients undergoing HSCT because of hematological malignancies. The patients and donors were consecutively admitted to our transplant unit from May 1996 through September 2007 for a human leukocyte antigen–identical sibling, partially T cell–depleted allogeneic HSCT. The characteristics of patients, donors, and HSCT procedures are given in table 2.

Treatment protocol. The treatment protocol has been described in detail elsewhere [10]. The conditioning regimen consisted of cyclophosphamide (60 mg/kg for 2 days) in combination with either total body irradiation (4.5 Gy for 2 days) or busulfan (4 mg/kg for 4 days). Idarubicin (42 mg/kg in 48 h) was often added to these conditioning regimen to reduce the risk of relapse in the setting of T cell–depleted HSCT [11]. On day 0, all patients were given an allogeneic HSCT containing 3.2×10^6 $CD34^+$ cells/kg (range, 0.6–11.6 $CD34^+$ cells/kg) and 0.5×10^6 $CD3^+$ cells/kg (range, 0.1–0.8 $CD3^+$ cells/kg).

Antimicrobial prophylaxis consisted of 500 mg of ciprofloxacin given twice daily and 500 mg of valaciclovir given 3 times daily. Surveillance cultures for *Candida* were collected twice weekly from hospital admission until hospital discharge, with the first culture samples obtained on the day of hospital admission, before the start of conditioning. Fluconazole (200 mg daily) was only prescribed to those who were colonized with *C. albicans, Candida tropicalis*, or *Candida parapsilosis* (not *Candida krusei* or *Candida glabrata*) when the yeast was present in both fecal cultures and mouth washes obtained on the same day, or when obtained from the same site on 2 consecutive occasions [12].

Invasive fungal infections were defined according to the European Organization for the Treatment of Cancer/Mycoses Study Group consensus guidelines, designating invasive fungal disease as possible, probable, or proven [13]. Oral mucositis was graded daily according to the validated Nijmegen Nursing Mucositis Scoring System (NNMSS) [14]. Acute GvHD was diagnosed by clinical signs or pathological examination of skin, gut, or liver biopsy samples and was graded according to the criteria of Glucksberg et al [15].

Statistical analysis. In multivariable logistic regression analyses, we investigated the association of the *DECTIN-1* status of the patient with *Candida* colonization, controlling for underlying hematological disease, age, and sex of the patient. In

Table 1. Primers and Polymerase Chain Reaction Conditions Applied to Amplify Every Exon and Proximal Intronic Regions of the *DECTIN-1* Gene to Perform Sequence Analysis

Exon, primer	Sequence, 5′–3′	$MgCl_2$, mM	Annealing temperature
1			
Forward	TTTCACCACGTTAGCCAAGCT	2.5	52°C
Reverse	CTGAAATAGTTTGCATCGGTT		
2			
Forward	CCCTTTATAAGTGAAATGGGC	1.75	60°C
Reverse	ACCGTGCAAGGCCAGATTTT		
3			
Forward 1	GCCAGTGATAAATCAGTTACT	3.5	56°C
Reverse 1	TTCTTCTTCTCCACCTTCTT		
Forward 2	TGGCAACATTTTCCCTTCTT	3.5	56°C
Reverse 2	GGCAAGGGCATAGTTAAAGG		
4			
Forward	TCATTACCTGGAATCTCCCTCT	2.5	56°C
Reverse	TGGCAACTAATTGGTTATTTCA		
5			
Forward	GCTGCTCGACAGAGGTTTTC	1.75	62°C
Reverse	GGATGGTCTCGATCTCCTGA		
6			
Forward	AATCACAGCCTCTCCCTTCA	2.5	60°C
Reverse	GATTTAAGCCTCCTTTTCCAA		

NOTE. For all amplicons, sequence analyses were carried out with the forward and the reverse primer. For the sequences of exon 3, technical difficulties were encountered. Therefore, we amplified exon 3 with 2 different primer pairs and aligned both forward assays after sequence analysis.

the analysis on the impact of early candidemia, the *DECTIN-1* status of only patients, but not of the donors, was considered of importance. Early candidemia was defined as that occurring on day 21 or earlier. In myeloablative stem cell transplantation, monocyte recovery usually occurs only 3–4 weeks after HSCT [16], and therefore, the genetic make-up of the donor is not considered to be relevant for the susceptibility to early candidemia. The association between the *DECTIN-1* status of the patient and the occurrence of early candidemia was studied using logistic regression models that accounted for confounding by including the age of patient, the underlying disease, the duration of neutropenia, the presence of colonization at hospital admission, and the presence of GvHD. In contrast, in the analysis of the impact of proven and probable invasive mold infections up to day 100, the *DECTIN-1* status of both patients and donors was included.

To compare percentages between 2 independent groups, we used the χ^2 test or Fisher's exact test when appropriate. Differences in the cytokine production capacity were tested by the Student's *t* test. $P < .05$ was considered to represent a statistically significant difference.

RESULTS

DECTIN-1 ***polymorphism screening in healthy individuals.*** Genetic variation in the *DECTIN-1* gene was investigated for 138 Dutch healthy volunteers and revealed the polymorphisms shown in figure 1. A small number of polymorphisms were identified, with the Y238X (rs16910526) in exon 6 being the only exonic polymorphism. This polymorphism was present in 19 (13.8%) of 138 individuals, all of whom were heterozygous, resulting in an allele frequency of 6.9%. This polymorphism was identified earlier in a family that had previously been analyzed for mucocutaneous *Candida* infection (unpublished data); 3 members of this family were homozygous for the Y238X polymorphism.

Dectin-1 protein expression. We investigated the consequence of the polymorphism on dectin-1 expression and localization at the protein level. Monocytes isolated from individuals who were homozygous for the wild-type *DECTIN-1* allele and from individuals heterozygous or homozygous for the Y238X polymorphism were analyzed for dectin-1 expression by flow cytometry and confocal microscopy. Monocytes from the individuals homozygous for the Y238X polymorphism

Table 2. Clinical Characteristics of the Study Group

Variable	Homozygous wild-type for *DECTIN-1*	Heterozygous for *DECTIN-1* Y238X	*P*
No. of recipients	126	15	
No. of donors	116	22	
Male sex, %			
Recipients	65	60	.78
Donors	56	64	.64
Age, mean years (range)			
Recipients	47.5 (18.5–64.4)	42.8 (19.2–59.8)	.11
Donors	47.4 (14–75.6)	43.3 (23.8–67.6)	.24
Diagnosis			.79
AML/ALL	60 (47.6)	8 (53.3)	
CML/MPS	26 (20.6)	3 (20)	
MDS	23 (18.3)	…	
Lymphoma/CLL	17 (13.5)	4 (26.7)	
Conditioning regimen			.25
Ida-Cyclo-TBI	79 (62.7)	10 (66.7)	
Ida-Cyclo-Bus	15 (11.9)	1 (6.7)	
Cyclo-TBI	27 (21.4)	1 (6.7)	
Cyclo-Bus	5 (4.0)	3 (20)	
TBI	106 (84.1)	11 (73.3)	
Stem cell source			>.99
Peripheral blood	72 (57.1)	9 (60.0)	
Bone marrow	54 (42.9)	6 (40.0)	
T cell depletion			.43
CD34 selection	61 (48.5)	9 (60.0)	
Counterflow elutriation	41 (32.5)	4 (26.7)	
CD3/CD19 selection	24 (19.0)	2 (13.3)	
Duration of neutropenia,[a] mean days (range)	11.9 (6–20)	11.0 (6–15)	.23
Acute GvHD			.77
Grade 0–I	81 (64.3)	11 (73.3)	
Grade II-IV	39 (31.0)	4 (26.7)	
Grade III-IV	10 (7.9)	1 (6.7)	
NA	6 (4.7)	…	

NOTE. Data are no. (%) of patients, unless otherwise indicated. All patients received a human leukocyte antigen–matched sibling partially T cell–depleted hematopoietic stem cell transplant. Graft-versus-host disease (GvHD) prophylaxis consisted of only cyclosporine for all patients. Differences between the study groups were compared using the Pearson χ^2 test or Fisher's exact test and with the use of the independent Student's *t* test where appropriate. No statistically significant differences were obtained. AML/ALL, acute myeloid and lymphatic leukemia; Bus, busulphan; CLL, chronic lymphatic leukemia; CML/MPS, chronic myeloid leukemia/myeloproliferative syndrome; Cyclo, cyclophosphamide; Ida, idarubicin; MDS, myelodysplastic syndrome; NA, not applicable; TBI, total body irradiation.

[a] Neutropenia was defined as $\leq 0.1 \times 10^9$ cells/L.

exhibited no dectin-1 expression on the cell surface, whereas cells from individuals heterozygous for the Y238X polymorphism had intermediate cell surface expression, compared with cells from individuals with the wild-type allele (figure 2*A*). In line with this, no cell surface expression of dectin-1 could be detected on monocytes isolated from individuals who were homozygous for the Y238X polymorphism, in contrast with wild-type cells, when monocytes were analyzed by confocal microscopy (figure 2*B*). However, dectin-1 mRNA expression was demonstrated to be equal between the genotypes (data not shown). Thus, these findings demonstrate that dectin-1 protein expression is absent from the cell membrane of monocytes from individuals who are homozygous and intermediate on monocytes from individuals who are heterozygous for the Y238X polymorphism.

Cytokine production. Functional consequences of the

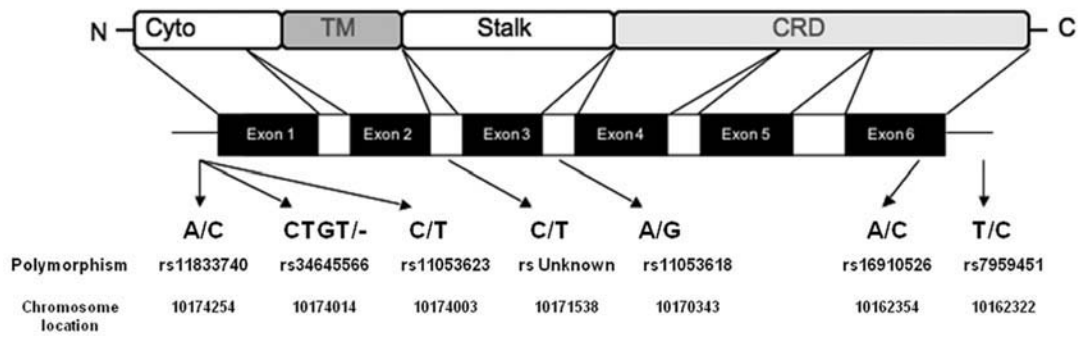

Figure 1. Schematic drawing of the *DECTIN-1* gene (also known as *CLEC7A*), consisting of an N-terminal cytoplasmatic tail (Cyto), encoded by exon 1, a transmembrane region (TM; exon 2), a stalk region (exon 3), and the carbohydrate recognition domain (CRD; exon 4–6). Intronic polymorphisms were detected in 5′UTR, intron 2, intron 3, exon 6, and 3′UTR. All variations are depicted together with their corresponding chromosome location and rs number, if available.

Y238X polymorphism were investigated in monocytes and PBMCs isolated from individuals bearing only the wild-type *DECTIN-1* allele and individuals who were heterozygous or homozygous for the Y238X polymorphism. Interleukin (IL)–1β induction by *C. albicans* was lower in cells from individuals bearing the Y238X polymorphism (figure 3*A*; $P < .05$). Dectin-1 has been previously demonstrated to amplify TLR2 signaling [6]; this effect was absent in cells isolated from individuals homozygous for the Y238X allele (figure 3*B*). In contrast, IL-18 and interferon (IFN)–γ production was not defective in cells isolated from these individuals (figure 3*C*; $P =$ not significant). Although a tendency toward a lower IL-18 production capacity has been observed in cells from individuals heterozygous for Y238X, compared with cells bearing only the wild-type *DECTIN-1* allele, this did not reach statistical significance. IL-18 production in cells from individuals homozygous for the Y238X mutation was similar to production in cells from individuals with the wild-type allele. Furthermore, IFN-γ production capacity is practically equal between the genotypes.

DECTIN-1 ***Y238X polymorphism in patients and donors.*** The *DECTIN-1* genetic status could be determined for 141 patients and 138 donors. Insufficient amounts of DNA precluded the determination of genetic status in 1 patient and 4 donors. Fifteen (10.6%) of 141 patients and 22 (15.9%) of 138 donors had the *DECTIN-1* Y238X polymorphism; all individuals were heterozygous. In 9 patient-donor pairs, both individuals were heterozygous for the polymorphism. There were no statistically significant differences between the clinical characteristics of patients with and patients without the *DECTIN-1* Y238X polymorphism (table 2). No difference was detected in the severity of mucositis between patients with and patients without the *DECTIN-1* Y238X polymorphism, with a mean NNMSS score of 3.8 vs 3.9 on day 0 and 7.2 vs 7.6 on day 7 ($P =$ not significant.).

Candida ***species colonization.*** Seven patients who received secondary antifungal prophylaxis at hospital admission were excluded from the study, as were 11 patients with missing colonization data, leaving 124 patients eligible for analysis of *Candida* colonization. At hospital admission, 46 (37.1%) of 124 patients were colonized with *Candida* species. Patients who were heterozygous for the Y238X polymorphism were statistically significantly more often colonized than were patients with *DECTIN-1* wild-type alleles (11 [84.6%] of 13 vs 35 [31.5%] of 111; $P < .001$) (odds ratio [OR], 11.9; 95% confidence interval [CI], 2.5–56.8). After adjusting for diagnosis, the OR was 12.2 (95% CI, 2.5–59.7); after adjusting for patient age and sex, the OR was 12.0 (95% CI, 2.5–57.1). On the day of HSCT (day 0), this difference persisted: 12 (92.3%) of 13 patients vs 50 (45.1%) of 111 patients ($P = .001$). The unadjusted OR was 14.6 (95% CI, 1.8–116.5) and the OR adjusted for diagnosis, patient age, and sex was 15.5 (95% CI, 1.9–125.6).

Patients with a *DECTIN-1* polymorphism were more likely than other patients to receive fluconazole (9 [69.2%] of 13 vs 42 [37.8%] of 111; $P = .03$) (table 3). Among those patients who received fluconazole, eradication was achieved in 1 (11.1%) of 9 patients who were heterozygous for the Y238X polymorphism, compared with 14 (33.3%) of 42 patients who bore only the wild-type *DECTIN-1* allele ($P = .25$). Colonizing species were *C. albicans* (87% of patients), *C. glabrata* (8.1%), and sporadically, *C. krusei, Candida kefyr, Candida parapsilosis,* and *Candida dubliniesis* (1.6% each). No difference in the frequency of colonization with particular *Candida* species was observed between patients who were heterozygous for Y238X and patients who bore only the wild-type *DECTIN-1* allele.

Invasive fungal disease. Patients who received early antifungal prophylaxis with either itraconazole, voriconazole, or posaconazole, starting from day 0 or earlier and until day 21, were excluded (18 patients, 7 of whom had received secondary prophylaxis and 11 of whom had participated in antimicrobial studies). The overall incidence of candidemia until day 21 was

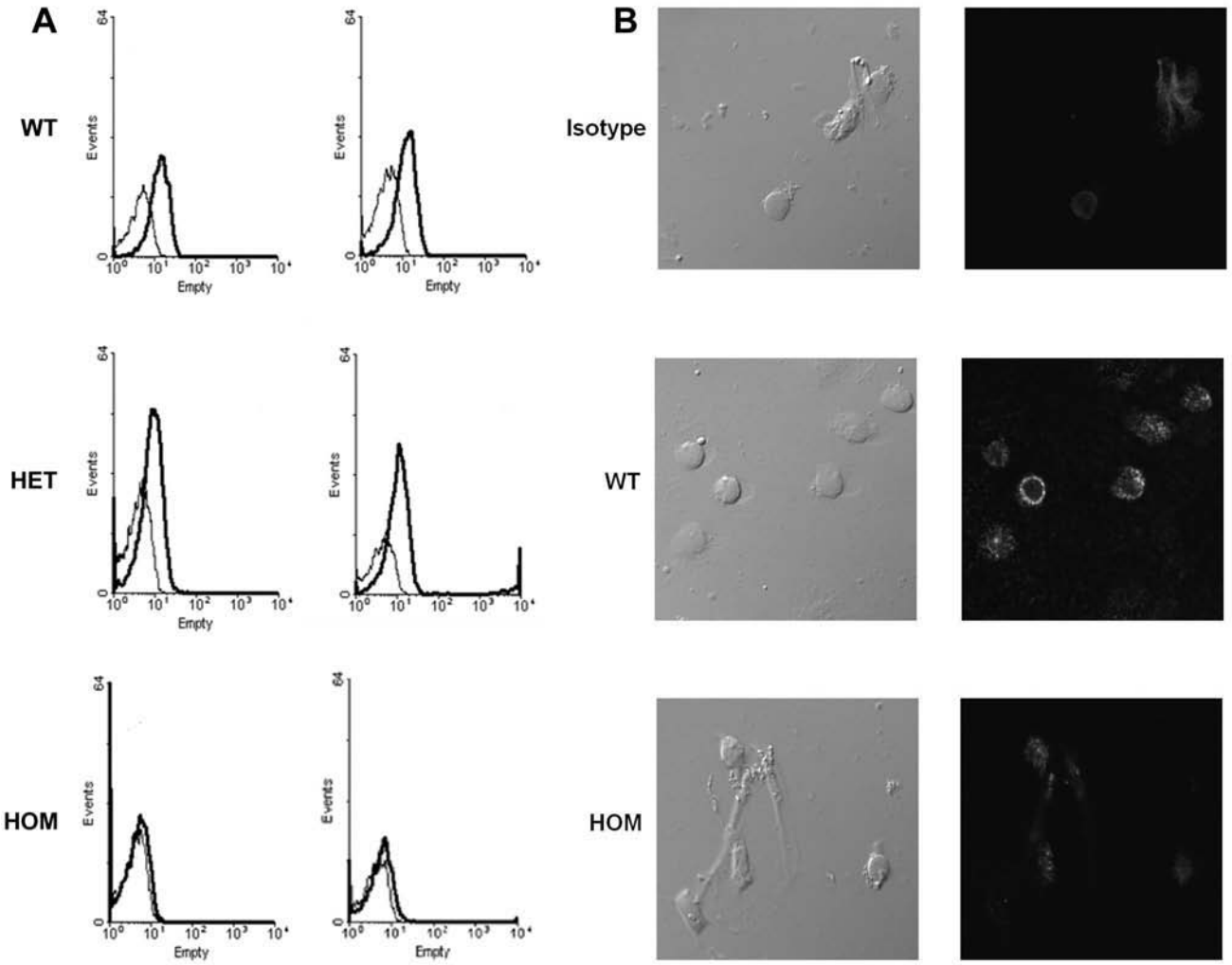

Figure 2. Flow cytometry graphs of extracellular dectin-1 staining *(A)* and *(B)* fluorescent confocal staining of dectin-1 *(right panel)* and light microscopic image *(left panel)* on unstimulated monocytes derived from individuals homozygous for the wild-type *DECTIN-1* allele (WT) and heterozygous (HET) or homozygous (HOM) for the *DECTIN-1* Y238X polymorphism.

8.9% (11 of 123 patients). There was no statistically significant difference in the incidence of early candidemia between patients with and patients without the *DECTIN-1* Y238X polymorphism (2 [18.2%] of 11 vs 9 [8.0%] of 112; $P = .26$) (table 3); the OR was 2.5 (95% CI, 0.5–13.6). However, this study was not designed to detect differences in the risk of developing invasive candidiasis, because patients who were colonized were prescribed fluconazole to prevent systemic *Candida* infection, and the study was underpowered to find a difference.

Candidemia was caused by *C. albicans* in 7 of 11 cases, with the remainder of the cases being due to *C. glabrata* (1 case), *C. parapsilosis* (2), and *C. dubliniesis* (1). Eight (15.7%) of 51 patients who were colonized and received fluconazole experienced candidemia. Two (3.6%) of 55 patients who were not colonized and who therefore received no fluconazole developed candidemia.

The incidence of proven and probable invasive mold infection up to day 100 was 3.3% (4 of 120 patients), with 4 probable and no proven mold infections. Three probable cases were due to *Aspergillus* species, and 1 probable case was due to *Rhizomucor* species. There was no statistically significant difference between pairs with and without a *DECTIN-1* Y238X polymorphism; 1 (5.0%) of 20 patients with the Y238X polymorphism had a probable case, compared with 3 (3.0%) of 100 patients with the *DECTIN-1* wild-type allele ($P = .52$) (table 3). However, the study was underpowered to detect a difference, especially because of the very low incidence of mold infection.

DISCUSSION

In this study, we demonstrate that a newly characterized polymorphism in *DECTIN-1* is associated with increased suscep-

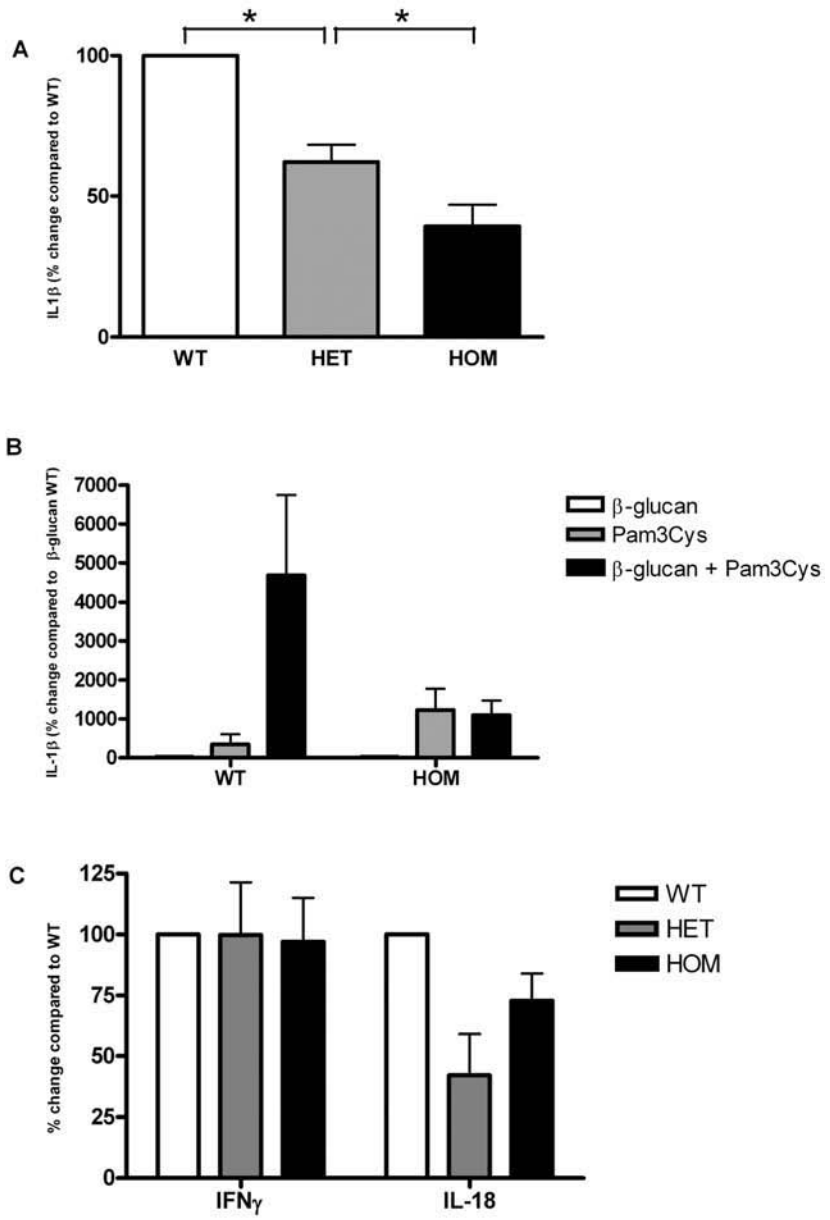

Figure 3. Cytokine production capacity of interleukin (IL)–1β, IL-18, and interferon (IFN)–γ after stimulation of monocytes during 4 h *(A)* or peripheral blood mononuclear cells (PBMCs) during 48 h with β-glucan, Pam3Cys, or β-glucan/Pam3Cys *(B)* or with heat-killed *Candida albicans (C)*. Cells were obtained from individuals with the wild-type allele (WT; $n = 5$) or individuals heterozygous (HET; $n = 4$) or homozygous (HOM; $n = 3$) for the *DECTIN-1* Y238X polymorphism. Data are percentages compared with WT ($\pm$ standard deviation). *$P < .05$.

tibility to fungal colonization among HSCT recipients. As a consequence, among patients who were immunocompromised as a result of HSCT, the need to prescribe fluconazole to prevent systemic *Candida* infection was, in part, defined by the presence of this polymorphism.

Dectin-1 is one of the most important pattern recognition receptors for fungal pathogens in general and *Candida* species, in particular. Polymorphisms in pattern recognition receptors are known to be associated with an increased susceptibility to fungal infection [17, 18]. We hypothesized that genetic variants of *DECTIN-1* could influence susceptibility to *Candida* colonization and infection in HSCT recipients.

Screening of all 6 exons of the *DECTIN-1* gene in a healthy Dutch population revealed 1 exonic polymorphism and several intronic SNPs. Because the intronic SNPs are not likely to affect dectin-1 function, we considered the Y238X polymorphism to be the only polymorphism that could alter dectin-1 function and could influence susceptibility to fungal infection.

Table 3. Observed *Candida* Species Colonization and Invasive Fungal Disease among Hematopoietic Stem Cell Transplant Recipients

Clinical outcome	Percentage (ratio) of recipients		*P*	OR
	Homozygous wild-type for *DECTIN-1*	Heterozygous for *DECTIN-1* Y238X		
Candida species colonization at hospital admission	31.5 (35/111)	84.6 (11/13)	<.001	11.9
Candida species colonization on day of HSCT (day 0)	45.1 (50/111)	92.3 (12/13)	.001	14.6
Surveillance-culture guided fluconazole therapy	37.8 (42/111)	69.2 (9/13)	.03	3.7
Early candidemia (day 21 or earlier)	8.0 (9/112)	18.2 (2/11)	.26	2.5
Invasive mold disease (day 100 or earlier)	3.0 (3/100)	5.0 (1/20)	.52	1.7

NOTE. The genetic *DECTIN-1* status of the patient-donor couples (either patient, donor, or both heterozygous for the Y238X polymorphism) was included in this analysis of the impact of the polymorphism on the occurrence of invasive mold disease on day 100 or earlier. HSCT, hematopoietic stem cell transplantation; OR, odds ratio.

To characterize the functional consequences of the Y238X polymorphism in more detail, flow cytometry and confocal microscopy were performed. Expression of dectin-1 was absent on the cell membrane of cells isolated from individuals who were homozygous for the Y238X allele, which suggested a defective transport of the mutated form of dectin-1 to the cell membrane. Accordingly, dectin-1 expression was intermediate on cells from individuals who were heterozygous for the polymorphism.

After stimulation with heat-killed *C. albicans* or β-glucan, IL-1β secretion was intermediate in cells isolated from individuals who were heterozygous for the Y238X polymorphism and low in cells from individuals who were homozygous for this polymorphism, compared with secretion in cells from individuals who were homozygous for the wild-type allele. Moreover, the previously described synergism between TLR2 and dectin-1 signals [6] was completely absent in individuals homozygous for the Y238X polymorphism. These data demonstrate the loss-of-function effect of the Y238X polymorphism for dectin-1 activity. However, no difference in the production of IL-18 and IFN-γ could be observed between the different *DECTIN-1* genotypes. This could well be because of a certain redundancy of dectin-1 signaling in production of these cytokines, and this is accompanied by a normal *Candida*-killing activity by neutrophils from individuals bearing the Y238X polymorphism (data not shown), resulting in an adequate host defense in systemic infections.

In the follow-up of these genetic and immunological studies, we demonstrate that this polymorphism has a significant impact on oral and gastrointestinal mucosal colonization with *Candida* species in HSCT recipients, which defined the need for the use of fluconazole in our approach. This is in line with the finding of recurrent mucocutaneous candidiasis in a family with individuals who were homozygous for the *DECTIN-1* polymorphism (unpublished data). The overall rate of fungal colonization at hospital admission was comparable to that in earlier studies, with colonization rates of 28%–57% [19, 20]. These data strongly support a role of dectin-1 mediated mechanisms for mucosal anti-*Candida* defense.

The mechanisms of the increased susceptibility to mucosal colonization with *Candida* in individuals bearing the *DECTIN-1* polymorphism cannot be definitively pinpointed, although the defective cytokine responses are probably involved. Although monocytes are important for mucosal defenses, epithelial cells may also contribute to these effects. Interestingly, the expression of dectin-1 on epithelial cells has been recently demonstrated, and the interaction of these cells with fungal pathogens leads to chemokine release [21]. One has to consider, however, that in the setting of HSCT, both monocytes and epithelial cells of the mucosa are damaged for a prolonged period, although they are largely intact at hospital admission and during the first days of conditioning. On the other hand, the residential macrophages of the mucosa are less affected, and it is most likely that the impact of the *DECTIN-1* polymorphism is exerted at their level.

The presence of the *DECTIN-1* polymorphism did not result in a significantly higher incidence of candidemia, although that might have been expected, because mucosal colonization is associated with systemic candidiasis in HSCT recipients who experience mucosal barrier injury [22, 23]. Importantly, in this cohort, patients were prescribed oral fluconazole when colonized, which is known to reduce the incidence of candidemia [20, 24]. Therefore, fluconazole was most likely a confounding factor, precluding a definitive conclusion regarding the role of the *DECTIN-1* Y238X polymorphism for susceptibility to early candidemia, although our data may suggest an increased risk (OR, 2.5; 95% CI, 0.5–13.6). In addition, our study was underpowered to study the impact of the polymorphism on the occurrence of invasive mold infections because of the low incidence of these infections.

In our culture-guided approach, fluconazole could safely be withheld for those individuals who were not colonized with

Candida species, preventing overtreatment and accompanied adverse effects and costs. However, those individuals who were colonized remained at significant risk for candidemia, necessitating better ways to predict, determine, and treat colonization at an early stage. Determining the *DECTIN-1* status before HSCT might be a factor contributing to a more risk-adapted prophylactic approach. Because *Candida* colonization is associated with invasive disease among HSCT recipients, we propose that patients bearing the polymorphism should be considered for antifungal prophylaxis to prevent systemic candidiasis. Nevertheless, future studies in prospective trials are necessary to further confirm the impact of this newly characterized *DECTIN-1* polymorphism and to define its role in selecting adequate prophylaxis or early treatment for HSCT recipients.

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References

1. Barnes PD, Marr KA. Risks, diagnosis and outcomes of invasive fungal infections in haematopoietic stem cell transplant recipients. Br J Haematol **2007**; 139:519–31.
2. Gratwohl A, Brand R, Frassoni F, et al. Cause of death after allogeneic haematopoietic stem cell transplantation (HSCT) in early leukaemias: an EBMT analysis of lethal infectious complications and changes over calendar time. Bone Marrow Transplant **2005**; 36:757–69.
3. Yokota K, Takashima A, Bergstresser PR, Ariizumi K. Identification of a human homologue of the dendritic cell-associated C-type lectin-1, dectin-1. Gene **2001**; 272:51–60.
4. Gow NA, Netea MG, Munro CA, et al. Immune recognition of *Candida albicans* beta-glucan by dectin-1. J Infect Dis **2007**; 196:1565–71.
5. Steele C, Rapaka RR, Metz A, et al. The beta-glucan receptor dectin-1 recognizes specific morphologies of *Aspergillus fumigatus.* PLoS Pathog **2005**; 1:e42.
6. Ferwerda G, Meyer-Wentrup F, Kullberg BJ, Netea MG, Adema GJ. Dectin-1 synergizes with TLR2 and TLR4 for cytokine production in human primary monocytes and macrophages. Cell Microbiol **2008**; 10: 2058–66.
7. Brown GD, Herre J, Williams DL, Willment JA, Marshall AS, Gordon S. Dectin-1 mediates the biological effects of beta-glucans. J Exp Med **2003**; 197:1119–24.
8. Leibundgut-Landmann S, Gross O, Robinson MJ, et al. Syk- and CARD9-dependent coupling of innate immunity to the induction of T helper cells that produce interleukin 17. Nat Immunol **2007**; 8:630–8.
9. Meyer-Wentrup F, Figdor CG, Ansems M, et al. Dectin-1 interaction with tetraspanin CD37 inhibits IL-6 production. J Immunol **2007**; 178: 154–62.
10. van der Velden WJ, Blijlevens NM, Maas FM, et al. NOD2 polymorphisms predict severe acute graft-versus-host and treatment-related mortality in T-cell-depleted haematopoietic stem cell transplantation. Bone Marrow Transplant **2009** (epub ahead of print).
11. Schaap N, Schattenberg A, Bar B, et al. Outcome of transplantation for standard-risk leukaemia with grafts depleted of lymphocytes after conditioning with an intensified regimen. Br J Haematol **1997**; 98: 750–9.
12. Prentice HG, Kibbler CC, Prentice AG. Towards a targeted, risk-based, antifungal strategy in neutropenic patients. Br J Haematol **2000**; 110: 273–84.
13. Ascioglu S, Rex JH, de PB, et al. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. Clin Infect Dis **2002**; 34:7–14.
14. Potting CM, Blijlevens NA, Donnelly JP, Feuth T, van Achterberg T. A scoring system for the assessment of oral mucositis in daily nursing practice. Eur J Cancer Care (Engl) **2006**; 15:228–34.
15. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. Transplantation **1974**; 18:295–304.
16. Storek J, Dawson MA, Storer B, et al. Immune reconstitution after allogeneic marrow transplantation compared with blood stem cell transplantation. Blood **2001**; 97:3380–9.
17. Carvalho A, Pasqualotto AC, Pitzurra L, Romani L, Denning DW, Rodrigues F. Polymorphisms in toll-like receptor genes and susceptibility to pulmonary aspergillosis. J Infect Dis **2008**; 197:618–21.
18. van der Graaf CA, Netea MG, Morre SA, et al. Toll-like receptor 4 Asp299Gly/Thr399Ile polymorphisms are a risk factor for *Candida* bloodstream infection. Eur Cytokine Netw **2006**; 17:29–34.
19. Glasmacher A, Hahn C, Molitor E, Sauerbruch T, Schmidt-Wolf IG, Marklein G. Fungal surveillance cultures during antifungal prophylaxis with itraconazole in neutropenic patients with acute leukaemia. Mycoses **1999**; 42:395–402.
20. Marr KA, Seidel K, White TC, Bowden RA. Candidemia in allogeneic blood and marrow transplant recipients: evolution of risk factors after the adoption of prophylactic fluconazole. J Infect Dis **2000**; 181:309–16.
21. Saegusa S, Totsuka M, Kaminogawa S, Hosoi T. *Candida albicans* and *Saccharomyces cerevisiae* induce interleukin-8 production from intestinal epithelial-like Caco-2 cells in the presence of butyric acid. FEMS Immunol Med Microbiol **2004**; 41:227–35.
22. Martino P, Girmenia C, Micozzi A, De BF, Boccanera M, Cassone A. Prospective study of *Candida* colonization, use of empiric amphotericin B and development of invasive mycosis in neutropenic patients. Eur J Clin Microbiol Infect Dis **1994**; 13:797–804.
23. Blijlevens NM, Donnelly JP, de Pauw BE. Impaired gut function as risk factor for invasive candidiasis in neutropenic patients. Br J Haematol **2002**; 117:259–64.
24. Slavin MA, Osborne B, Adams R, et al. Efficacy and safety of fluconazole prophylaxis for fungal infections after marrow transplantation: a prospective, randomized, double-blind study. J Infect Dis **1995**; 171: 1545–52.