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Mannose-binding lectin gene polymorphisms are associated with major infection following allogeneic hemopoietic stem cell transplantation

Charles G. Mullighan, Sue Heatley, Kathleen Doherty, Ferenc Szabo, Andrew Grigg, Timothy P. Hughes, Anthony P. Schwarer, Jeff Szer, Brian D. Tait, L. Bik To, and Peter G. Bardy

Life-threatening complications such as graft versus host disease and infection remain major barriers to the success of allogeneic hemopoietic stem cell transplantation (SCT). While pretransplantation conditioning and posttransplantation immunosuppression are important risk factors for infection, the reasons that similarly immunosuppressed transplant recipients show marked variation in frequency of infection after allogeneic SCT are unclear. Mannose-binding lectin (MBL) deficiency is a risk factor for infection in other situations where immunity is compromised. We investigated associations between *MBL2* gene polymorphisms and risk of major infection following allogeneic SCT. Ninety-seven related allogeneic donor-recipient pairs were studied. Clinical data including survival, days of fever, graft versus host disease incidence and severity, and infection were collected by case note review. Five single-nucleotide polymorphisms in the *MBL2* gene were genotyped using the polymerase chain reaction and sequence-specific primers. *MBL2* coding mutations were associated with an increased risk of major infection following transplantation. This association was seen for donor ($P = .002$, odds ratio [OR] 4.1) and recipient ($P = .04$, OR 2.6) *MBL2* genotype. *MBL2* promoter variants were also associated with major infection. The high-producing haplotype *HYA* was associated with a markedly reduced risk of infection (recipient *HYA* $P = .0001$, OR 0.16; donor *HYA* $P = .001$, OR 0.23). Donor *MBL2* coding mutations and recipient *HYA* haplotype were independently associated with infection in multivariate analysis. These results suggest that *MBL2* genotype influences the risk of infection following allogeneic SCT and that both donor and recipient *MBL2* genotype are important. These findings raise the possibility that MBL replacement therapy may be useful following transplantation. (Blood. 2002;99:3524-3529)

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Introduction

Allogeneic hemopoietic stem cell transplantation (SCT) is the only curative therapy for a number of malignant and nonmalignant conditions. However, despite optimal donor-recipient HLA matching and supportive care, the success of this procedure continues to be compromised by life-threatening complications such as graft versus host (GVH) disease and infection.[1] Factors such as neutropenia, immunosuppressive agents given as GVH disease prophylaxis, GVH disease itself, and mucosal breaches from mucositis and instrumentation are established risk factors for infection.[2] However, the reasons that some allogeneic SCT recipients develop major infections and other similarly immunosuppressed allogeneic SCT recipients do not are unclear. The integrity of the recipient's innate immune response may be an important factor. Chemoradiotherapy given as pretransplantation conditioning ablates the recipient's adaptive immune response and cellular effectors of innate immune responses such as granulocytes, macrophages, and natural killer cells. Consequently, at a time of such profound immunosuppression, it is possible that noncellular innate host defenses less affected by conditioning will assume greater importance.

Mannose-binding lectin (MBL) is an important component of the innate immune response and is an attractive candidate for investigation in this setting. MBL is a member of the collectin family that binds to repeating carbohydrate moieties on a broad range of bacterial, viral, fungal, and protozoal pathogens independently of antibody[3] and directly or via complement activation opsonizes pathogens for phagocytosis.[4] Human MBL is encoded by the *MBL2* gene on chromosome 10 (*MBL1* is a pseudogene).[5] Five single-nucleotide polymorphisms influencing serum MBL levels have been identified.[6] Polymorphisms in exon 1 at codons 52 (Arg→Cys, allele "*D*"), 54 (Gly→Asp, allele "*B*"), and 57 (Gly→Glu, allele "*C*") result in disruption of assembly of MBL peptides into functional polymers and profound reduction in serum levels of functional MBL. An *MBL2* coding region containing any of the *B*, *C*, or *D* mutations is referred to as "*O*" and the wild-type "*A*." Thus, an individual heterozygous for a coding mutation is "*A/O*" and a homozygote or compound heterozygote "*O/O*." Two promoter polymorphisms, −550g/c (alleles named *H/L*) and −221c/g (*X/Y*) form 3 of 4 possible haplotypes: *HY*, *LX*, and *LY*. When these promoter variants lie on the same chromosome as a normal coding region ("*A*"), they form 3 haplotypes: *HYA*, *LYA*, and *LXA*, which are associated with high, intermediate, and low levels of MBL, respectively.[7,8] The *MBL2* coding mutations are in

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absolute linkage disequilibrium with the promoter polymorphisms: 52Cys is always in *cis* with *HY*, and 54Asp and 57Glu with *LY*.[7] The nucleotide sequence of the *MBL2* gene and location of the polymorphisms can be found in GenBank sequence accession number Y16581. *MBL2* polymorphisms resulting in low serum levels are present in at least 30% of individuals.[8-10] MBL deficiency appears to be an important risk factor for infection in situations where the adaptive immune response is already compromised, for example, in early childhood,[11-13] primary immunodeficiency,[10,14] cystic fibrosis,[15] human immunodeficiency virus infection,[16] and following chemotherapy.[17,18] Because infection is also a frequent complication following allogeneic SCT, we hypothesized that *MBL2* genotype may influence risk of infection in this setting.

The aim of this retrospective study was to investigate the relationship between polymorphisms in the *MBL2* gene and the risk of major infection following allogeneic SCT. *MBL2* promoter and exonic polymorphisms were genotyped in 97 related donor-recipient allogeneic SCT pairs, for whom comprehensive clinical data were available.

Materials and methods

Patient and donors

Ninety-seven donor-recipient pairs undergoing allogeneic SCT from 1991 to 1998 in 3 Australian transplantation centers (Royal Adelaide Hospital, Adelaide, and Royal Melbourne and Alfred Hospitals, Melbourne) were studied as part of a large, multicenter study of genetic determinants of allogeneic SCT outcome. The study was approved by the Ethics Committee of the Australian Bone Marrow Donor Registry. Recipients were followed until July 1, 2001. Clinical data were obtained by review of case notes and results of microbiologic investigations. Fifty-seven recipients were male and 40 female. All donor-recipient pairs were of Caucasoid descent except one pair from India and one from Vietnam. Mean age at time of transplantation was 39.8 years (range 19-59). Donors were mostly sibling (91) and were other relatives in 6. Diagnoses were acute myeloid leukemia (33 recipients), chronic myeloid leukemia (21), non-Hodgkin lymphoma (13), acute lymphoblastic leukemia (10), aplastic anaemia (3), myelodysplasia (5), myelomatosis (4), myelofibrosis (4), and neuroblastoma, Hodgkin disease, biphenotypic acute leukemia, and Ewing sarcoma (1 each). Seventy recipients were at high risk for relapse, and 27 were standard risk (defined as chronic myeloid leukemia in first chronic phase or acute myeloid leukemia in first complete remission). Forty-nine received unmanipulated bone marrow grafts, and 48 granulocyte colony-stimulating factor–mobilized peripheral blood stem cells, 9 of which were T-cell depleted. Conditioning regimens were intravenous cyclophosphamide (120 mg/kg) and total body irradiation (12-13.2 Gy) for 46 recipients; cyclophosphamide (120 mg/kg) and oral busulfan (16 mg/kg) for 34 recipients; busulfan (16 mg/kg), cyclophosphamide (120 mg/kg), and etoposide (30 mg/kg) for 11 recipients; and other non–total body irradiation–based chemotherapeutic regimens in the remaining 6 patients. GVH disease prophylaxis was cyclosporine alone in 47 recipients and cyclosporine with short-course methotrexate (days 1, 3, 6, and 11) in 50 recipients.[19] All recipients received standard supportive care, including isolation in high-energy particulate-air–filtered rooms, insertion of central venous or Hickman catheters, and administration of prophylactic antimicrobials. These included ganciclovir, if donor or recipient was cytomegalovirus (CMV) immunoglobulin G–positive, at 5 mg/kg 3 times per week from the time of count recovery (neutrophils $> 1.5 \times 10^9$/L and platelets $> 50 \times 10^9$/L) until day 84 after transplantation; oral cotrimoxazole or inhaled pentamidine from count recovery until 12 months after transplantation; acyclovir (500 mg orally 3 times per day or 125 mg intravenously twice daily) or valaciclovir (500 mg orally twice daily) until at least 3 months following cessation of immunosuppressive therapy; fluconazole 200 mg daily orally or intravenously until count recovery or commencement of intravenous amphotericin; oral norfloxacin 400 mg orally twice daily for 38 recipients from time of transplantation until engraftment or commencement of systemic antibiotics; mouth care with topical antiseptics (Amosan) and nystatin; and intravenous immunoglobulin (Intragam, CSL, Parkville, Australia) 500 mg/kg weekly from time of transplantation until day 84.

Mannose-binding lectin genotyping

The −550 (*H/L*), −221 (*X/Y*), and codon 52Cys, 54Asp, and 57Glu *MBL2* polymorphisms were genotyped in 93 recipients and 90 donors using the polymerase chain reaction and sequence-specific primers as previously described.[10] In this technique, combinations of −550 and −221 alleles, and −221 alleles with each of the coding polymorphisms are directly amplified using forward and reverse allele-specific primers. Not all donors and recipients of each transplantation pair could be genotyped due to lack of DNA. Genotyping was successfully performed for both donor and recipient of 87 transplantation pairs. Genotyping was performed independently of clinical data collection.

Statistical analysis

Data were managed in Filemaker Pro (FileMaker, Santa Clara, CA). Univariate association analyses between categoric variables were performed using contingency tables and the Fisher exact test. Associations between categoric and continuous variables were analyzed using the Student *t* test. Multivariate analysis was performed using logistic regression analysis (StatView, SAS, Cary, NC).

Results

Outcome measures

Median duration of follow-up was 469 days (range, 9-3742). Overall 1-year survival was 56%, with no significant differences between the transplantation centers. Median time to neutrophil count recovery (defined as $> 0.5 \times 10^9$/L [500×10^6/L] for 2 consecutive days) was 16 days (range, 7-35). Mean days of fever (defined as temperature above 38°C) was 11 (range, 0-66). Fifty-five recipients experienced a total of 98 episodes of major infection, with a median time to onset of first major infection of 20 days (range, 1-300). A major infection was defined as a microbiologically confirmed systemic, disseminated, invasive, or rapidly progressive infection. A diagnosis of pneumonia required compatible clinical or radiologic findings and identification of a causative organism from sputum, nasopharyngeal aspirates, bronchoscopy, blood, or open/transbronchial lung biopsy. Episodes of infection caused by CMV were included if positive CMV antigen or culture results were deemed clinically significant and treatment was administered. The following were not included as major infective episodes: commonly encountered skin contaminant bacteria (eg, coagulase-negative staphylococci) detected in a single blood culture bottle only when multiple were taken, *Clostridium difficile* diarrhea, dermatomal varicella zoster reactivation, ocular or labial herpes simplex, nonpneumonic respiratory infections, culture-negative interstitial pneumonitis, and culture-negative fever. Twenty-eight recipients experienced a major infective episode while neutropenic and 36 following neutrophil recovery. Twenty-six recipients experienced multiple infective episodes. Causative agents were bacterial in 52 patients, viral in 21 patients, and fungal (not including *Pneumocystis carinii*) in 7. A breakdown of causative agents and number of infective episodes is provided in Table 1. There was no association between age at or date of transplantation and risk of infection.

MBL2 genotypes

Thirty-eight (40.9%) of 93 recipients and 38 (42.2%) of 90 donors carried an *MBL2* coding mutation. Promoter and coding haplotype and allele frequencies are listed in Table 2. Observed frequencies did not

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Table 1. Major infective episodes occurring in 97 allogeneic SCT recipients

Type of infection	No. of episodes: Prior to neutrophil recovery	No. of episodes: After neutrophil recovery	No. of episodes: Total	No. of patients
Bacteremia	32	30	62	47
CMV viremia	0	6	6	6
CMV enteritis	0	2	2	2
CMV pneumonitis	0	3	3	3
Pneumonia, bacterial	2	6	8	7
Pneumonia, viral (influenza A, RSV, VZV)	0	7	7	7
Pneumonia, *Pneumocystis*	0	1	1	1
Fungal infection	1	6	7	7
Toxoplasma	0	1	1	1
Disseminated VZV	0	1	1	1
Total	35	63	98	

Occurrence of major infection following SCT is shown both as the number of infective episodes (stratified into neutropenic and nonneutropenic episodes) and the number of patients experiencing each type of infection. Because some patients had multiple episodes, the total number of patients is greater than the overall number of patients experiencing an episode of major infection (n = 55). RSV indicates respiratory syncytial virus; VZV, varicella zoster virus; CMV, cytomegalovirus.

differ significantly from those predicted by Hardy-Weinberg equilibrium analysis and were in accordance with previously published frequencies.[9,10]

Mannose-binding lectin polymorphisms and risk of major infection

Both *MBL2* coding and promoter polymorphisms were associated with risk of infection following transplantation. The presence of a coding mutation (52Cys, 54Asp, or 57Glu) was associated with an increased frequency of major infection. This association was seen when the analysis was performed for donor coding mutations (infection in 76% recipients when donor coding mutation is present vs 44% when no mutation, $P = .002$, odds ratio [OR] 4.1), recipient coding mutations (68% vs 45%, $P = .04$, OR 2.6), coding mutations present in either donor or recipient (69% vs 41%, $P = .007$, OR 3.1), and coding mutations present in both donor and recipient (79% vs 50%, $P = 0.01$, OR 3.7). These data are summarized in Table 3. The observation of associations between infection and both donor and recipient genotype is not explained by genetic matching: Of the 87 transplantation pairs for whom both donor and recipient genotypes were available, only 38 (43.7%) were *MBL2*-identical. Furthermore, donor *MBL2* coding mutations were associated with infection in both recipients with and without coding mutations, although due to low sample numbers following stratification, *P* values were of borderline significance. Ten (76.9%) of 13 *A/A* recipients developed infection when their donor carried a mutation (*A/O* or *O/O*), compared with 17 (43.6%) of 39 *A/A* recipients whose donors were also of *A/A* genotype ($P = .06$, OR 3.9, 95% confidence interval [CI] 0.98-16.2). Similarly, examining the subgroup of recipients carrying an *MBL2* coding mutation (*A/O* or *O/O*), 19 (79.2%) of 24 experienced an episode of major infection when their donor carried a mutation, compared with 6 (50%) of 12 whose donor was of wild-type (*A/A*) genotype ($P = .10$, OR 3.5, 95% CI 0.86-15.9). While few recipients were homozygous or compound heterozygotes for *MBL2* coding mutations, 4 of 5 such recipients experienced a major infective episode, compared with 45% of those without an *MBL2* mutation ($P = .05$, OR 8.4, 95% CI 0.87-76.5).

Data were also analyzed following stratification of infective episodes as occurring before or after neutrophil count recovery. The presence of donor *MBL2* coding mutations was associated with infections occurring after neutrophil recovery but not infections prior to neutrophil recovery. Infections occurred after neutrophil recovery in 20 (53%) of 38 recipients whose donor carried a coding mutations, compared with 13 (25%) of 52 recipients whose donor did not carry a coding mutation ($P = .007$, OR 3.3, 95% CI 1.4-8.2). In contrast, neutropenic infections occurred in 13 (34%) of 38 recipients whose donor carried a mutation, compared with 12 (23%) of 52 without ($P = .35$, OR 1.7, 95% CI 0.7-4.4).

Associations were also observed between *MBL2* promoter polymorphisms and risk of infection. The *HYA* haplotype was associated with a significantly lower frequency of major infections when present in recipients or donors (Table 4). For example, major infection occurred in 41% of 56 recipients carrying the *HYA* haplotype, compared with 81% of 37 recipients lacking this haplotype ($P = .0001$, OR 0.16). Similar associations were seen with donor *HYA* (44% vs 83%, $P = .001$, OR 0.23). The associations between recipient *HYA* and infection were observed both in

Table 2. *MBL2* allele and haplotype frequencies in allogeneic SCT recipients and donors

Allele/haplotype	No. of recipients (%) n = 93	No. of donors (%) n = 90
Coding genotype		
A/A	55 (59.1)	52 (57.8)
A/D	12 (12.9)	12 (13.3)
A/B	21 (22.6)	25 (27.7)
A/C	0	0
Total *A/O*	33 (35.5)	37 (41.1)
B/C	1 (1.1)	0
B/D	2 (2.2)	0
D/D	2 (2.2)	1 (1.1)
Total *O/O*	5 (5.5)	1 (1.1)
Total with coding mutation (*A/O* or *O/O*)	38 (40.9)	38 (42.2)
Promoter genotypes		
− 550 alleles (*H/L*)		
HH	13 (14.0)	13 (14.1)
HL	52 (55.9)	49 (54.4)
LL	28 (31.1)	28 (31.1)
− 221 alleles (*X/Y*)		
XX	4 (4.3)	1 (1.1)
XY	30 (32.3)	34 (37.8)
YY	59 (63.4)	55 (61.1)
Promoter haplotypes		
HYA/A	40 (43.0)	40 (44.4)
HYA/O	16 (17.2)	14 (15.6)
Total *HYA*	56 (60.2)	54 (60.0)
LYA/A	28 (30.1)	30 (33.3)
LYA/O	11 (11.8)	12 (13.3)
Total *LYA*	39 (41.9)	42 (46.7)
LXA/A	28 (30.1)	24 (26.7)
LXA/O	6 (6.5)	11 (12.2)
Total *LXA*	34 (36.6)	35 (38.9)
Sufficient (*A/A*, *LYA/O*, and *HYA/O*)	78 (83.9)	77 (85.6)
Insufficient (*O/O*, *LXA/O*, and *LXA/LXA*)	15 (16.1)	13 (14.4)

MBL2 gene allele, genotype, and haplotype frequencies are shown as numbers and percentages of recipients and donors carrying each variant. For coding region alleles, "*A*" refers to a wild-type region, "*D*" to the codon 52Cys mutation, "*B*" to the 54Asp mutation, and "*C*" to the 57Glu mutation. "*O*" refers to the presence of any of the *MBL2* coding mutations. "*A/O*" is a coding mutation heterozygote and "*O/O*" a coding mutation homozygote or compound heterozygote. "*H*" and "*L*" refer to alleles at the − 550 *MBL2* promoter polymorphism and "*X*" and "*Y*" to the − 221 alleles. For example, "*HYA/O*" refers to the − 550 *H* allele, the − 221 *Y* allele, and a normal coding region all lying on the same chromosome ("*HYA*") and a coding mutation on the other chromosome ("O"). *MBL2*-"insufficient" genotypes denote those shown in previous studies to be associated with very low levels of circulating MBL.

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Table 3. Associations between *MBL2* coding mutations and major infection after transplantation

Sample group	No. with coding mutation	No. without coding mutation	Major infection when mutation is present, no. (%)	Major infection when mutation is absent, no. (%)	*P*	OR (95% CI)
Donor, n = 90	38	52	29 (76)	23 (44)	.002	4.1 (1.6-10.3)
Recipient, n = 93	38	55	26 (68)	25 (45)	.04	2.6 (1.1-6.3)
Donor or recipient, n = 87	52	35	36 (69)	18 (41)	.007	3.1 (1.3-7.3)
Donor and recipient, n = 87	24	63	19 (79)	32 (50)	.01	3.7 (1.2-11.1)

This table details the analyses showing significant positive associations between the presence of *MBL2* coding mutations and risk of major infection after SCT. Infection frequencies refer to number of recipients experiencing an episode of major infection—not the number of episodes of infection. The codon 52, 54, and 57 mutations have been grouped together for this analysis because they have similar effects on MBL levels. The associations between *MBL2* mutations and infection were seen with donor and recipient *MBL2* genotype.

recipients carrying a coding mutation (ie, *HYA/O*, $P = .04$, OR 0.23) and recipients with no coding mutations (*HYA/A*, $P = .001$, OR 0.09) (Table 5). Associations between donor *HYA* and infection were also examined following stratification according to the presence or absence of donor *MBL2* mutations (Table 5). Identical trends to those seen with recipient *HYA* were observed but did not reach significance for *HYA/A* donors. Furthermore, none of the 5 recipient *HYA/HYA* homozygotes and only 2 of the 8 donor *HYA/HYA* homozygotes experienced a major infection. The *LYA* and *LXA* haplotypes were not individually associated with major infection in either donors or recipients. *MBL2* genotypes were also stratified as "insufficient" (associated with very low levels of circulating MBL[7]) or "sufficient" (Table 2). The presence of *MBL2*-insufficient recipient genotypes was significantly associated with major infection. Thirteen (86.7%) of 15 recipients with an *MBL2*-insufficient haplotype experienced an episode of major infection, compared with 40 (51.3%) of 78 recipients with *MBL2*-sufficient haplotypes ($P = .01$, OR 6.2, 95% CI 1.3-29.2). A similar but not significant trend was seen for donor *MBL2*-insufficient genotypes (10 [76.9%] of 13 developed infection vs 42 [54.5%] of 77, $P = .13$, OR 2.8, 95% CI 0.7-10.1).

Stratification according to type of infection showed that *MBL2* coding mutations and the absence of the *HYA* haplotype were associated with bacterial infection (Table 6). These associations were independent of the presence of neutropenia. It was not possible to determine if *MBL2* polymorphisms were also independently associated with viral infections, because all recipients who experienced an episode of major viral infection had also experienced an antecedent bacterial infection. The number of observed fungal infections was inadequate for meaningful statistical analysis to be performed.

Multivariate analysis was performed by logistic regression to assess the independence of associations between donor and recipient *MBL2* variants and infection. Four independent variables (donor and recipient *HYA*, donor and recipient *MBL2* coding mutations) and one outcome variable (major infection) were analyzed. The *HYA* haplotype in recipients ($P = .002$, likelihood ratio 0.17, 95% CI 0.06-0.54) and donor *MBL2* coding mutation ($P = .03$, likelihood ratio 2.8, 95% CI 1.2-7.9) were independent risk factors for the development of major infection. Neither donor *HYA* nor recipient *MBL2* coding mutations were significantly associated with major infection in multivariate analysis. Pearson *P* value for goodness of fit for this logistic regression model was .66.

Associations of *MBL2* polymorphisms with other outcome measures

While the duration of neutropenic fever appeared longer in patients with recipient or donor *MBL2* mutations, this trend did not reach significance (11.1 vs 8.5 days for recipient mutations, $P = .09$). No association between any of the *MBL2* alleles and haplotypes and duration of inpatient stay or early death (occurring in the first 30 days) was observed.

Associations between *MBL2* variants and GVH disease were also examined. Acute GVH disease was graded by standard criteria.[20] Eighty-two patients survived to be evaluable for acute GVH disease. Twenty-two (27%) did not develop acute GVH disease, 20 (24%) developed grade I disease, 22 (27%) grade II, 11 (13%) grade III, and 7 (9%) grade IV. The occurrence of multiple major infective episodes was associated with higher grades of acute GVH disease: 17 (42.5%) of 40 recipients who developed grades II-IV acute GVH disease experienced multiple major infections, compared with 5 (11.9%) of 42 patients with no or grade I acute GVH disease ($P = .001$, OR 5.5, 95% CI 1.8-16.8). There were no associations between any of the *MBL2* polymorphisms and acute GVH disease overall, treatment requiring GVH disease (grades II-IV), or severe GVH disease (III-IV). Sixty-nine patients survived beyond 100 days and thus were evaluable for chronic GVH disease by standard criteria.[21] Thirty-two did not develop chronic GVH disease, 7 developed limited, and 30 extensive chronic GVH disease. Multiple infections were associated with chronic GVH disease (13 of 37 patients with chronic GVH disease experienced multiple infections vs 4 of 29 without chronic GVH disease, $P = .04$, OR 3.4, 95% CI 1.0-11.9). In the 65 recipients graded for chronic GVH disease and genotyped for *MBL2*, the *HYA* haplotype was associated with chronic GVH disease in univariate analysis: 18 (44%) of 41 *HYA*-positive recipients developed chronic GVH disease, compared with 17 (71%) of 24 *HYA*-negative recipients ($P = .03$, OR 0.32, 95% CI 0.11-0.94). However, only the occurrence of multiple episodes of major infection was independently associated with chronic GVH disease in multivariate analysis ($P = .04$).

Discussion

This retrospective study is the first report of a genetic risk factor for major infection following allogeneic hemopoietic SCT. The presence of *MBL2* coding mutations was associated with increased risk of major infection, and the *HYA* haplotype, previously reported to be associated

Table 4. Associations between *MBL2 HYA* haplotype and major infection after transplantation

Sample group	No. HYA^+	No. HYA^-	Major infection when HYA^+, no. (%)	Major infection when HYA^-, no. (%)	*P*	OR (95% CI)
Donor, n = 90	54	36	24 (44)	30 (83)	.001	0.23 (0.29-0.59)
Recipient, n = 93	56	37	23 (41)	30 (81)	.0001	0.16 (0.06-0.43)
Donor and recipient, n = 87	43	44	17 (40)	34 (77)	.0003	0.19 (0.08-0.49)

This table details the negative association between the HYA haplotype and risk of infection. Again, this association is seen for donor and recipient genotype.

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Table 5. Associations between *MBL2 HYA* infection stratified according to presence or absence of coding mutations

Sample group	No. *HYA*$^+$	No. *HYA*$^-$	Major infection when *HYA*$^+$, no. (%)	Major infection when *HYA*$^-$, no. (%)	*P*	OR (95% CI)
Recipients without coding mutation (HYA/A), n = 55	40	15	16 (40)	13 (87)	.001	0.09 (0.02-0.47)
Recipients with coding mutation (HYA/O), n = 38	16	22	7 (45)	17 (77)	.04	0.23 (0.06-0.93)
Donors without coding mutation (HYA/A), n = 52	40	12	15 (37)	8 (67)	.07	0.30 (0.08-1.17)
Donors with coding mutation (HYA/O), n = 38	14	24	9 (64)	22 (92)	.03	0.16 (0.03-1.0)

Data regarding *HYA* and infection has been stratified according to the presence or absence of *MBL2* coding mutations. *HYA* is associated with infection in recipients with and without coding mutations. Similar trends are seen for donor genotype.

with high MBL levels,[7,22] was associated with reduced risk of infection. MBL is known to be an important innate immune defense against a broad array of bacterial, viral, fungal, and protozoal pathogens.[3] Consequently, analyses were performed to examine associations between bacterial, viral, and fungal infections in this patient group. There were significant associations between *MBL2* coding mutations and the *HYA* haplotype and risk of bacterial infection. It was not possible to examine viral infections in a discrete analysis because all patients experiencing viral infections had previously had a bacterial infection. The number of patients experiencing invasive fungal infections was too small for a meaningful statistical analysis to be performed.

This study extends recent reports of associations of *MBL2* coding mutations and low MBL levels with duration of fever and burden of infection following conventional-dose chemotherapy.[17,18] There are several important differences between the present study and those of Peterslund and Neth.[17,18] Both groups examined the relationship between MBL and infection in patients with a variety of malignancies following a number of different chemotherapeutic regimens. The unifying risk factor for infection in these studies was chemotherapy-induced neutropenia. Patients undergoing allogeneic SCT are at considerably greater risk for life-threatening infection than those receiving conventional-dose chemotherapy. Allogeneic SCT recipients receive myeloablative chemotherapy with its attendant nonhemopoietic toxicities to the liver, lungs, skin, and gut. Allogeneic SCT recipients also experience major breaches in physical defenses by indwelling central venous catheters and mucosal injury from mucositis and GVH disease. Furthermore, these patients also have profound and prolonged defects in cellular and humoral immunity and are reliant on the transplanted donor cells to restore innate and adaptive immunity.[23] The study of Neth et al[18] showed an increase in MBL levels in patients without coding mutations experiencing infection. There was, however, no analysis of the well-characterized promoter polymorphisms in this study, and coding mutations did not fully explain the variation in MBL levels observed. It is known that *MBL2* coding region mutations have a greater effect on basal MBL levels than the promoter variants.[7,8] This may not be the case following high-dose chemoradiotherapy, when promoter variants such as *HYA*, which allow high levels of MBL transcription, may result in high MBL levels and afford relative protection from infection. MBL is known to be synthesized by the liver as an acute phase reactant, and the promoter region of the *MBL2* gene contains response elements to several of the key mediators released during high-dose chemoradiotherapy, such as interferon-γ and interleukin-2.[24-27] Thus, the ability to substantially increase MBL levels at times of stress after SCT may determine risk of infection. This might explain the striking protective effect of the recipient *HYA* haplotype against infection observed in our study.

An intriguing finding from this study concerns the role of donor as well as recipient *MBL2* genotype. The associations of both donor and recipient genotype with risk of infection raises questions regarding the relative importance of MBL synthesis by donor and recipient following allogeneic SCT. Several observations suggest that both donor and recipient genotype are important. Only 43.7% of donors and recipients shared identical *MBL2* genotypes, indicating that the observed associations with both donor and recipient genotype are not solely due to genetic matching. The *MBL2 HYA* haplotype was associated with infection in recipients with and without coding mutations, showing that the association of *HYA* was not secondary to linkage disequilibrium between the coding mutations and the promoter variants. Furthermore, donor *MBL2* mutations were associated with infection in both recipients with and without coding mutations and also with infection following neutrophil count recovery but not prior to this time. Finally, donor *MBL2* coding and recipient *HYA* genotype were independently associated with infection in multivariate analysis. The association with recipient *MBL2* genotype was expected given the current understanding that the predominant site of MBL synthesis during the acute phase response is the liver.[24-27] However, functional studies examining nonhepatic sites of MBL synthesis in humans are very limited. The association between donor genotype and infection following neutrophil count recovery suggests that lymphocytes, macrophages, dendritic cells, or the progeny of hemopoietic stem cells in the donor graft may synthesize MBL in amounts sufficient to influence susceptibility to infection. While functional data in humans are lacking, recent evidence has shown that MBL-C, the murine homolog of human *MBL2*, is expressed by lymphocytes.[28] It is also possible that the association between donor genotype and infection is secondary to linkage disequilibrium with other as-yet-unidentified immunoregulatory genes or that donor MBL levels prior to stem cell harvest influence the donor immune repertoire. However, our results and these preliminary murine functional data suggest that nonhepatic sites of MBL synthesis may be important in

Table 6. *MBL2* polymorphism associations with bacterial infections

MBL variant	Sample group, total no. in group	Total no. with variant	Bacterial infection when variant is present, no. (%)	Bacterial infection when variant is absent, no. (%)	*P*	OR (95% CI)
Coding mutation	Donor, n = 90	38	29 (76)	23 (44)	.002	4.1 (1.6-10.3)
	Recipient, n = 93	38	27 (71)	27 (49)	.04	2.4 (1.1-6.3)
HYA haplotype	Donor, n = 90	54	25 (46)	30 (81)	.0008	0.2 (0.08-0.54)
	Recipient, n = 93	56	26 (46)	30 (79)	.001	0.23 (0.09-0.59)

MBL2 coding mutations and the HYA haplotype, when present in either donor or recipient, are associated with the occurrence of bacterial infection after SCT.

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vivo. Further studies of *MBL2* genotype, synthesis, and kinetics after transplantation are warranted.

Acute GVH disease is another frequently lethal complication of allogeneic SCT and has been described as an exaggerated response to infection.[29] Furthermore, other triggers of the innate immune response, such as lipopolysaccharide, have emerged as key mediators and targets for intervention in GVH disease.[29,30] Consequently, it was of interest to examine associations between *MBL2* polymorphisms and incidence of GVH disease. While the presence of multiple major infective episodes was significantly associated with higher grades of acute GVH disease, no associations or trends between *MBL2* coding or promoter polymorphisms and acute GVH disease were observed. Recipient *HYA* was associated with chronic GVH disease in univariate analysis but was not independently associated in multivariate analysis. Thus, while the innate immune response has been implicated in GVH disease pathogenesis in other studies,[29,30] there is no evidence from our data that MBL has a role in the pathogenesis of this complication.

There is considerable interest in the role of purified or recombinant MBL as a potential therapeutic agent.[31-33] Early data suggest that administration of purified MBL is safe and may be effective in ameliorating infection frequency in MBL-deficient individuals.[31] Intensive antimicrobial treatment for infection after SCT is often toxic or unsuccessful, and existing strategies to prevent infection such as prophylactic antimicrobials and intravenous immunoglobulin (which contains no MBL) are incompletely effective. Furthermore, the increased susceptibility to infection after allogeneic SCT extends well beyond the initial period of neutropenia, and host immune competence may never be fully regained.[34] Thus, if MBL deficiency is confirmed by future genetic and functional studies to be a major risk factor for infection after SCT, this clinical setting would be an ideal scenario for a clinical trial of MBL replacement therapy.

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