Association Between Donor MBL Promoter Haplotype and Graft Survival and the Development of BOS After Lung Transplantation

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Background. Lung transplantation is a well accepted therapy for end-stage lung disease, despite high mortality rates. Mortality after transplantation is mainly caused by allograft failure in the first years after transplantation. Mannose binding lectin (MBL), a recognition molecule of innate immunity, has been associated with transplant outcome in other solid organ transplantation. In this study, the effect of donor- and recipient-MBL genotype on lung transplant outcome was investigated.

Materials and Methods. All lung transplantations performed in our center, except from retransplantations and combined lung–liver or heart–lung transplantations, were included. Genotyping of the MBL2 variants (promoter: L/H, Y/X, and P/Q allele and exon 1: A/D, A/B, and A/C allele) was performed in donor and recipient DNA. Analyses on graft survival and the development of bronchiolitis obliterans syndrome were performed with Kaplan-Meier (log rank) survival analysis.

Results. Of the 277 included cases, DNA was available from 189 donors and 200 recipients and genotyping of the promoter single nucleotide polymorphisms was successful in 184 donors and 198 recipients and of the exon 1 single nucleotide polymorphisms in 181 donors and 193 recipients. Patients who received a graft from a donor with an X-allele had better graft survival ($P=0.007$) and bronchiolitis obliterans syndrome free survival ($P=0.007$). Recipient MBL genotype was not associated with transplant outcome.

Conclusion. The donor X-allele, which corresponds to the LXPA haplotype is associated with superior lung transplant outcome. Our findings might prove to be important in finding ways to optimize outcome after lung transplantation.

Keywords: Lung transplantation, Mannose binding lectin, Genotype, Donor, Outcome.

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Lung transplantation is nowadays a well accepted therapy for end-stage lung disease. The International Society for Heart and Lung Transplantation reports an increasing number of procedures every year, up to 2169 transplantations performed in 2005. Because of advances in surgical management and immunosuppression, 1-year patient survival rate has increased relatively more impressively compared with 5- and 10-year survival rates. However, mortality rate remains highest in the first year after transplantation (*1*).

In lung transplantation, there are several causes of respiratory failure. Technical problems, ischemia–reperfusion (I/R) injury, acute rejection, and bacterial infection are the most common in the early perioperative phase, whereas on the other hand bronchiolitis obliterans syndrome (BOS) or chronic rejection as well as fungal, viral, and mycobacterial infections are becoming more prevalent after 3 months (*1*, *2*).

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The innate immune system has been shown to play a role in acute allograft rejection, BOS, infection and long-term outcome after lung transplantation (*3–6*). One of the key players in innate immunity is the complement system, which can be activated by the classical, alternative, and lectin pathway. The major recognition molecule of the lectin pathway is mannose binding lectin (MBL), a serum protein that belongs to the collectin family (*7*).

A single gene on chromosome 10 encodes the protein (*8*). MBL deficiency, which in many populations is the most common immunodeficiency (*9*), is strongly associated with gene polymorphisms in exon 1 (*10*) and in promoter regions (*11*). For exon 1, three independent alleles and their polymorphisms have been described (allele D, B, and C with allele A being the wild type at all three exon positions). In the promoter and 5′-UTR region, three polymorphic positions with alleles L/H, Y/X, and P/Q, respectively, have been identified (*12*, *13*). Furthermore, seven different haplotypes have been described (HYPA, LYQA, LYPA, LXPA, HYPD, LYPB, and LYQC) (*12*, *14*). Those with an exon polymorphism or an LX promoter variant are associated with low levels of the protein, whereas the HY promoter haplotype has been related to high MBL serum levels (*11*, *12*).

In several clinical studies, MBL deficiency has been associated with disease susceptibility and worse prognosis of disease with respect to morbidity and mortality (*15–18*). In liver and heart transplantation, (haplotypes encoding for) low-serum MBL levels have been reported to have a negative influence on transplant outcome (a major risk for severe infection and the development of transplant-

associated coronary artery disease and acute rejection, respectively) (*19*, *20*).

In contrast to the findings for heart and liver transplantation, there is evidence that low serum MBL might be favorable after pancreas–kidney and kidney transplantation. In these studies, low serum MBL level was associated with superior graft survival (*21*, *22*).

With respect to lung transplantation, in all the considerations outlined above, MBL donor genotype might be of importance, because there are indications that MBL is, besides largely produced in the liver, partly produced in the alveolar cells of the lungs (*23*). But, also the recipient MBL status might play a role.

In this study, the effect of MBL genotype on lung transplantation outcome was investigated.

MATERIALS AND METHODS

Patients and Clinical Data

In this single center study, all transplant procedures from November 1990 till October 2006 were included. Exclusion criteria were combined transplantations (lung–liver or lung–heart) and retransplantations. The primary endpoint was overall graft loss, defined as the need to retransplant or patient mortality. Furthermore, the development of BOS (*24*) in relation to MBL genotype was analyzed.

The immunosuppressive regimen was a combination of induction therapy and maintenance with a calcineurin-inhibitor, a nucleoside-antagonist and steroids. Before 2001 antithymocyte globulin induction therapy was given, followed by maintenance therapy with cyclosporin A, azathioprine, and prednisolone. Since 2001, the regimen included anti-CD25 induction, tacrolimus, azathioprine, and prednisolone.

DNA Isolating and Geno- and Haplotyping of MBL Polymorphisms

DNA from the donors and recipients was isolated from −20°C stored leukocytes suspension using QiAamp-colomns (QiAamp Blood Kit, cat no. 51106 from Qiagen, Westburg bv, Leusden, The Netherlands). The six known MBL2 functional variants were analyzed: promoter and 5′-UTR variants on position −619 (rs11003125, allele L/H), −290 (rs7096206, allele Y/X), and −66 (rs7095891, allele P/Q) and exon variants on position +154 (rs5030737, allele A/D), +161 (rs1800450, allele A/B), and +170 (rs1800451, allele A/C) (*13*).

Genotyping was performed on an ABI7900HT platform following the manufacturers' procedures. TaqMan assays for rs1800451, rs7096206, and rs5030737 were readily ordered (Applied Biosystems Assay-on-Demand). Assays for rs11003125 and rs7095891 were obtained through the Assay-by-Design service and finally, the assay for rs1800450 was a reorder of a previously published synthesis (*25*). Primer and probe details of the latter three single nucleotide polymorphisms (SNPs) are available on request.

Haplotype reconstruction was performed using the PHASE algorithm (*26*).

Statistical Analysis

A power analysis was performed to detect the number of transplant procedures needed to be included to detect a difference in 1-year graft survival of 10% between donors or recipients with and without MBL polymorphisms. Based on literature about a lung transplantation cohort transplanted between 1990 and 2005, 1-year graft survival was estimated between 70% and 80% (*27*). With a significance of 95% and a power of 90%, we needed 58 transplant procedures per group. Estimating that 33% of the population would have a polymorphism, a study group of 174 procedures was necessary.

Statistical analyses were performed with SPSS software, version 14.0 (SPSS Inc., Chicago, IL). Kaplan-Meier (log rank) univariate survival analysis was used to test the difference between the groups. Multivariate corrections were performed with Cox regression analysis. Results were considered statistically significant at P less than 0.05.

RESULTS

Clinical Features

Three hundred five lung transplantations were performed between November 1990 and October 2006. Twenty-eight procedures concerned retransplantations or combined transplantations and were excluded. Of the remaining 277 cases, DNA could be isolated from 189 donors and 200 recipients and genotyping of all three promoter SNPs was successful in 184 donors and 198 recipients and of the three exon SNPs in 181 donors and 193 recipients. Ninety-nine percent of the reconstructed haplotypes (368 of 372 donor haplotypes and 396 of 400 recipient haplotypes) could be assigned to one of the seven known haplotypes. In the other four cases, failure to determine all six allele variants resulted in unknown haplotypes; those cases were excluded from further haplotype analysis. All six SNPs were in Hardy-Weinberg equilibrium. Failure to isolate DNA was mainly because in earlier years storage of leukocyte suspension was not a standard procedure. Baseline characteristics of the 277 included cases are shown in Table 1.

MBL Allele Variants—Genotyping and Haplotyping

Table 2 shows the frequencies of the six allele variants in our study population. Our findings resemble the frequencies previously described in the European population, except from the H-promoter allele in donors (34.2% vs. 43.5%; in other populations frequencies range from 6.9% to 96.5%) and the X-promoter allele in both donors and recipients (25.6 and 20.4% vs. 14.7%; in other populations 4.9% to 18.6%) (*13*). Haplotype frequencies are shown in Table 3.

MBL Genotypes and Haplotypes—Graft Survival

The main causes of graft loss in this cohort were patient died with a functioning graft (36%), BOS (35%), primary graft failure (15%), and graft infection (11%). The mean time to graft loss was 2.7±3.3 years, with 44% graft loss in the first year after transplantation.

With Kaplan-Meier survival analysis, patients who received a graft from a donor with a Y/X or X/X polymorphism (which means hetero- or homozygous LX promoter haplotype) had a better overall graft survival than the Y/Y donor group (P=0.007, Fig. 1a). In addition to the donor X-allele, recipient female sex was significantly associated with better

TABLE 1. Baseline characteristics

Donors	
Male/female	152/125
Age (yr)	36±14
Type	
HBD	270
DCD	7
Recipients	
Male/female	150/127
Age (yr)	43±13
Diagnosis	
CF	59
COPD	128
Pulmonary fibrosis	41
Pulmonary hypertension	26
Other	23
Transplantation	
Time on waiting list (mo)	15±13
Type	
Left	16
Right	45
Bilateral	216
HLA mismatches	
A 0/1/2	22/146/109
B 0/1/2	6/90/181
DR 0/1/2	20/125/132
Immunosuppression	
ATG, CsA, Aza, pred	161
antiCD25, FK506, Aza, pred	116

HBD, heart beating donor; DCD, donor after cardiac death; CF, cystic fibrosis; COPD, chronic obstructive pulmonary disease; ATG, anti-thymocyte globulin; CsA, cyclosporin A; Aza, azathioprine; pred, prednisolone; FK506, tacrolimus.

TABLE 2. Allele and genotype frequencies of the MBL2 promoter and exon 1 variants

SNP	Allele	Allele name		Frequency (%) Donors	Recipients
−619	C/G	L/H	LL	45.7	38.0
			LH	40.2	43.5
			HH	14.1	18.5
			H-allele	34.2	40.3
−290	G/C	Y/X	YY	56.0	63.7
			YX	37.0	31.9
			XX	7.1	4.4
			X-allele	25.6	20.4
−66	C/T	P/Q	PP	59.9	59.8
			PQ	37.4	35.1
			QQ	2.7	5.2
			Q-allele	21.4	22.8
+154	C/T	A/D	AA	83.1	81.7
			AD	16.4	17.8
			DD	0.5	0.6
			D-allele	8.7	9.5
+161	G/A	A/B	AA	73.8	75.1
			AB	23.5	23.2
			BB	2.7	1.7
			B-allele	14.5	13.3
+170	G/A	A/C	AA	97.2	96.1
			AC	2.8	3.9
			CC	0	0
			C-allele	1.4	2.0

SNP, single nucleotide polymorphism.

TABLE 3. Frequencies of the reconstructed haplotypes

Allele combination	Haplotype	Frequency (%) Donors	Recipients
GGCCGG	HYPA	25.8	31.3
CGTCGG	LYQA	20.4	20.5
CGCCGG	LYPA	4.1	3.8
CCCCGG	LXPA	25.8	20.5
GGCTGG	HYPD	8.4	9.1
CGCCAG	LYPB	14.1	13.1
CGTCGA	LYQC	1.4	1.8

graft survival in a univariate Cox regression analysis (hazard ratio 0.61, 95% confidence interval [CI] 0.39–0.98). The effect of the donor X-allele corrected with recipient sex changed the hazard ratio from 0.49 (95% CI 0.29–0.84, $P=0.009$) to 0.51 (95% CI 0.30–0.87, $P=0.013$).

MBL exon variants of the donor did not significantly influence graft survival ($P=0.274$, Fig. 1b), neither did recipient MBL genotype (Fig. 1c,d).

Kaplan-Meier analysis of the donor LXPA haplotype compared with not having the LXPA haplotype confirmed the beneficial effect of the donor X-allele on graft survival ($P=0.006$). Further haplotype analysis did not give new insights.

Furthermore, analyses on combined genotypes of donors and recipients and their effect on graft survival were performed. The results were, however, not statistically significant.

HLA Mismatch

The combined effect of HLA mismatches and MBL gene polymorphisms was assessed. In donor–recipient combinations with less than two human leukocyte antigen (HLA)-A or HLA-DR mismatches, the result of a donor X-allele on enhanced graft survival was evident. However, with two mismatches on HLA-A or DR, the favorable effect of an X-allele was no longer detectable (Fig. 2a,b for HLA-DR, HLA-A is not displayed). For the recipient genotype, separate analysis for high and low HLA mismatch and the Y/X allele in relation to graft survival did not give new insights (HLA-DR mismatch <2 $P=0.18$, HLA-DR 2 $P=0.61$).

Secondary Endpoints

Data on the development of BOS was available from 154 recipients. After introducing the new immunosuppres-

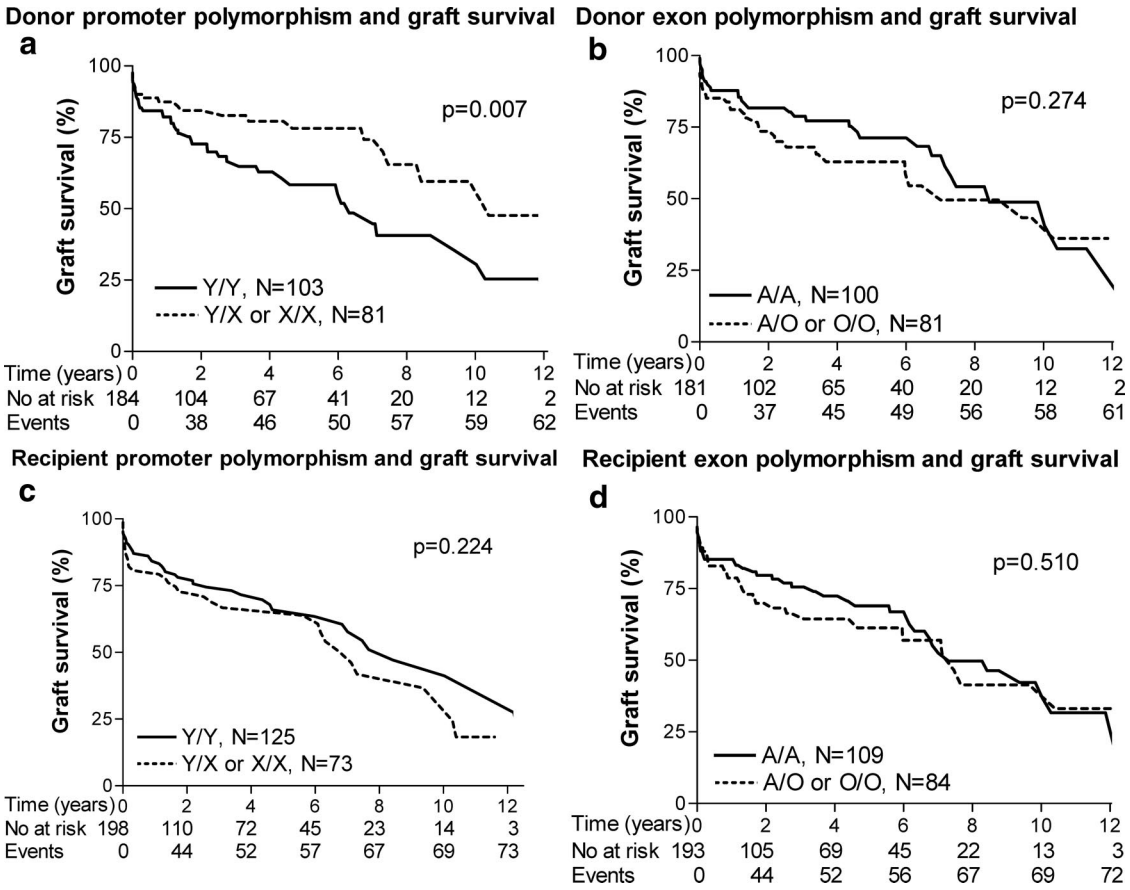

FIGURE 1. (a–d) Kaplan-Meier survival curves including censored cases. Y/Y, homozygous Y-allele; Y/X, heterozygous Y- and X-allele; X/X, homozygous X-allele; A/A, no exon polymorphisms; A/O or O/O, hetero- or homozygous exon polymorphism, respectively (d, b or c). No at risk = number at risk.

sive regimen in 2001 1-year BOS free survival increased dramatically from 72% to 96% ($P<0.000$). Therefore, a separate analysis was performed for patients transplanted before and after 2001. BOS free survival was significantly better for recipients who received a graft from a donor with an Y/X or X/X genotype compared with the Y/Y group ($P=0.007$) transplanted before 2001 (Fig. 3a). In a univariate Cox regression analysis also the number of HLA-DR mismatches was significantly associated with BOS in this subgroup (hazard ratio 2.98, 95% CI 1.41–6.29). However, after correcting the effect of the donor X-allele with the number of HLA-DR mismatches, the effect of the donor X-allele was still present (hazard ratio changed from 0.32 [95% CI 0.13–0.77, $P=0.011$] to 0.35 [95% CI 0.14–0.86, $P=0.021$]).

After introducing the new immunosuppressive regimen, the disadvantage of the Y/Y group disappeared ($P=0.487$) (Fig. 3b). This finding could be confirmed with LXPA haplotype analysis. Furthermore, we observed a negative effect of the donor HYPA haplotype on the development of BOS; patients who received a graft from a donor with a HYPA haplotype (homozygous or heterozygous) during the old immunosuppressive regimen, showed an inferior BOS free survival compared with the rest of the group ($P=0.005$, graph similar to Fig. 3a). Again, the disadvantage disappeared during the new immunosuppressive treatment protocol ($P=0.401$).

There were no differences in the development of BOS in relation to the recipient X/Y allele (during the old immunosuppressive regimen $P=0.672$, after introducing the new immunosuppressive regimen $P=0.340$).

DISCUSSION

Despite advancement in surgical management and immunosuppressive therapy, allograft survival after lung transplantation is still much lower compared with other solid organ transplants. Because, innate immunity plays an important role in several causes of respiratory failure after lung transplantation, such as, I/R injury (*28*), infection (*3*), and BOS (*6*), we investigated the role of MBL, a major player in innate immunity, in lung transplantation. Although the MBL protein is mainly synthesized in the liver, local production by alveolar cells in the lung has been reported (*23*). We hypothesized that local production of MBL in lung tissue could play a role in transplant outcome.

The results of this study showed an association between donor MBL genotype and lung transplant outcome. A superior graft and BOS-free survival was found in patients who received a graft from a donor with an X-allele, which corresponds to an LXPA haplotype. This association seemed to disappear with a high number of HLA-A or HLA-DR mismatches for graft survival and with better immunosup-

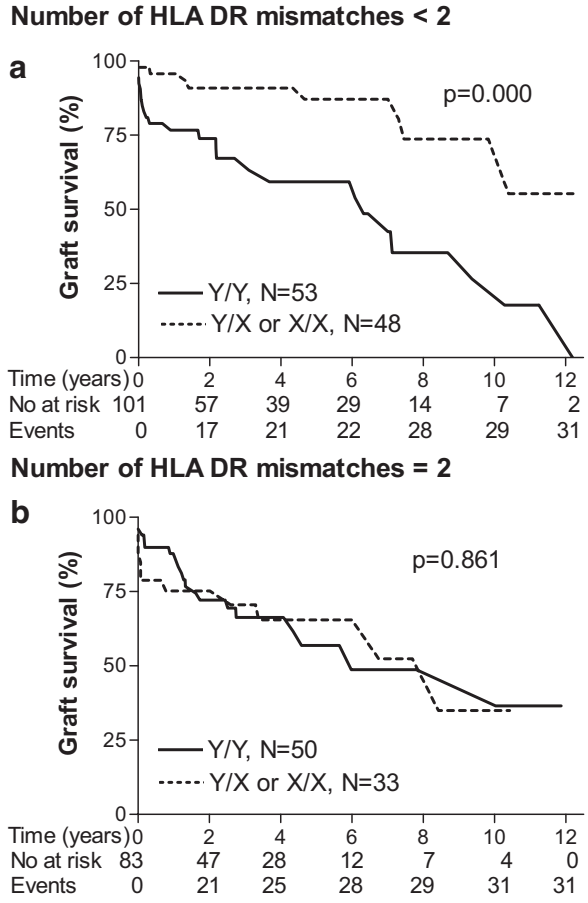

FIGURE 2. (a and b) Kaplan-Meier survival curves including censored cases. Donor genotype: Y/Y, homozygous Y-allele; Y/X, heterozygous Y- and Xallele; X/X, homozygous X-allele; No at risk, number at risk.

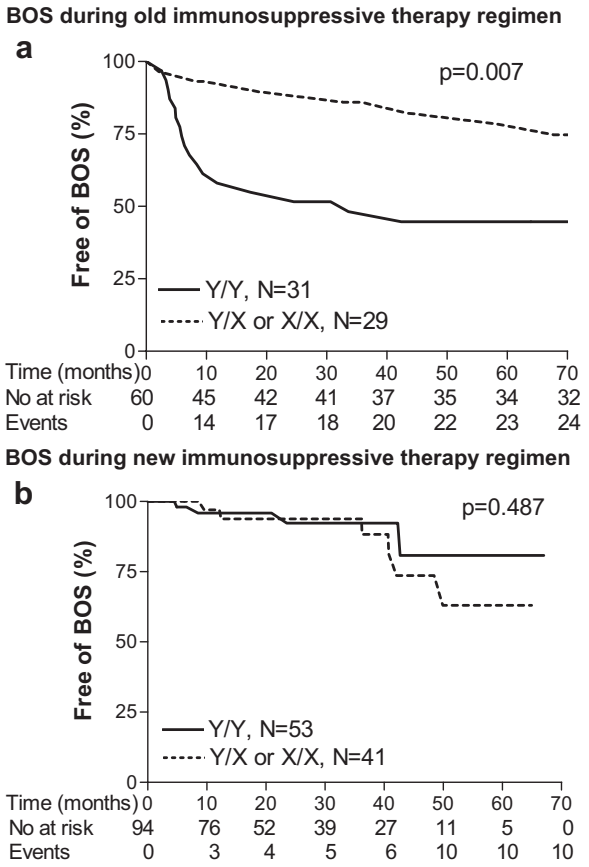

FIGURE 3. (a and b) Kaplan-Meier survival curves including censored cases. Donor genotype: Y/Y, homozygous Y-allele; Y/X, heterozygous Y- and Xallele; X/X, homozygous X-allele; BOS, bronchiolitis obliterans syndrome; No at risk, number at risk.

pressive therapy for BOS. Furthermore, a negative effect of the donor HYPA haplotype on the development of BOS was observed.

Allele frequency in the population studied was different compared with previous studies. The frequency of the H-allele in the donor population studied was lower and the frequency of the X-allele in both donor and recipient population was higher. However, all six SNPs were in Hardy-Weinberg equilibrium and haplotyping did not give notable unknown haplotypes. Therefore, the differences seem to be based on coincidence or due to differences in composition of our population compared with the European population, which has been previously described.

It is known that the LX haplotype is associated with low-MBL serum levels, while on the other hand the HY haplotype encodes a high production of serum MBL (*11*, *12*, *14*). With the results from this study, it could be concluded that locally produced MBL in the lung seems to be a factor in peri- and posttransplant injury. So, low levels of MBL produced in the transplanted organ could be beneficial for allograft survival. In the setting of lung transplantation, peripheral MBL deficiency in the graft may imply less tissue damage and inflammation and diminished antigen presentation. It seems to be likely that patients with less peripheral MBL activity might be protected against two of the main causes of graft loss in the study cohort, namely BOS (which pathogenesis is characterized by both alloantigen independent factors, like infections (*29*), and antigen dependent factors, like acute rejection (*30*)) and primary graft failure because of I/R injury.

A similar hypothesis for the importance of locally produced MBL was given by Jakab et al. (*31*). A strong association between X-allele homozygosity and juvenile onset of systemic lupus erythematosus was found. The authors hypothesized that homozygosity for the X-allele leads to a low degree of hepatic MBL expression and an absolute absent peripheral (extrahepatic) MBL expression. This will lead to peripheral MBL deficiency and therefore interference with local antigen sequestration and immune regulation in tissues.

The major improvement of allograft survival in the Groningen cohort was mainly caused by reduction in the incidence of BOS. After change of immune suppression, the frequency of 1-year posttransplant BOS dropped from 28% to 4%. Therefore, to analyze the potential role of MBL in the pathogenesis of BOS, the transplant cohort before 2001 was studied. A statistically significant association was found between low-producing MBL haplotype and the occurrence of BOS. We hypothesize that with inferior immune suppression (pre-2001 protocol) the pathophysiological mechanism of

BOS is partially determined by local MBL activity. This effect is, however, overruled by better immunosuppressive therapy.

Unfortunately, we are not informed in detail about the infectious complications in lung transplant patients. Therefore, it can not be ruled out that change in infection frequency might explain the difference in BOS before and after 2001. However, with more advanced immune suppressive regimens infectious complications are generally more frequently found.

A high number of HLA mismatches, especially HLA-DR, has previously been shown to have negative effects on transplant outcome by multivariate analysis (*32*, *33*). In the cohort used for this study, the DR matchings effect was not significant. However, we observed an overruling effect of the number of HLA-A and -DR mismatches on the effect of the promoter polymorphism of the donor; the superiority of survival of grafts derived from donors with an X-allele disappeared with a high number of HLA-A or HLA-DR mismatch.

In this study, we demonstrated that the donor promoter genotype which is previously related to low-serum MBL levels is related to superior transplant outcome in the setting of lung transplantation. We hypothesize that inflammatory damage, caused by I/R and a potential (chronic) response from the recipient against the allograft, is partially triggered by locally produced MBL. With functional MBL levels, damage of the lung tissue might therefore be enhanced. A comparable mechanism has been suggested for kidney and pancreas kidney transplantation. Low-serum MBL levels have, in kidney and pancreas–kidney transplantation, been associated with better transplant outcome (*21*, *22*).

Further research on MBL genotyping in relation to serum levels and locally produced MBL in donors, and their effects on I/R and chronic injury is needed to confirm this.

In a rat model for postischemic myocardial reperfusion injury, MBL inhibition resulted in reduced tissue damage (*34*). In future, MBL inhibition, during preparation of donors with an unfavorable genotype might become an option to optimize outcome after lung transplantation. Furthermore, if the donor has an unfavorable genotype, optimal HLA matching and immunosuppressive therapy might be of greater importance.

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