



CCR5 deletion mutation and its association with the risk of developing acute graft-versus-host disease after allogeneic hematopoietic stem cell transplantation

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Background and Objectives. Recently published data have suggested a potential role of CC chemokine receptor-5 (CCR5) in a mouse model of graft-versus-host disease (GvHD). It has also been described that a 32-nucleotide deletion within the CCR5 gene (CCR5 Δ 32) leads to complete loss of functional CCR5 in subjects homozygous for this mutation and decreased expression in heterozygous individuals. We analyzed CCR5 genotypes and their relationship to transplant outcome.

Design and Methods. A total of 349 individuals, comprising 186 recipients and 163 donors of allogeneic hematopoietic stem cell transplants, were typed for CCR5 polymorphisms.

Results. Recipients carrying the CCR5 Δ 32 allele developed acute GvHD (grades I-IV) less frequently than did patients lacking the CCR5 deletion mutation (11/35 vs. 76/151, $p=0.033$). This association was still valid after correcting for other known variables (recipient age, donor-recipient gender relation, type of donor, conditioning regimen, diagnosis, stem cell source and GvHD prophylaxis) by logistic regression (OD=0.391, $p=0.023$). Transplantation from a donor other than a matched sibling (OD=2.007, $p=0.028$), recipient age (OD=2.117, $p=0.041$) and myeloablative conditioning regimen (OD=2.235, $p=0.014$) were found to be factors associated with an increased risk of GvHD. Moreover, acute GvHD symptoms were not observed in any of the recipients carrying the CCR5 Δ 32 allele transplanted from donors with this deletion mutation (0/11 vs. 70/151, $p=0.002$).

Interpretation and Conclusion. The presence of the CCR5 Δ 32 allele in recipients constituted an independent and protective factor associated with a decreased risk of GvHD. This protective effect of the CCR5 deletion mutation was particularly marked in patients transplanted from donors also carrying the CCR5 Δ 32 allele.

Key words: CCR5 deletion mutation, acute GvHD, HSCT.

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Despite improvements in allogeneic hematopoietic stem cell transplantation (HSCT), acute graft-versus-host disease (GvHD) remains a significant problem after transplantation, and is still a major cause of post-transplant mortality. Disease progression is characterized by the differentiation of alloreactive T cells to effector cells leading to tissue damage, recruitment of additional inflammatory cell populations and further cytokine dysregulation.^{1,2} A characteristic feature of all inflammatory reactions is extensive recruitment of leukocytes to the site of inflammation. This process involves several families of proteins, including pro-inflammatory cytokines, adhesion molecules and chemokines.^{3,4} Chemokines constitute a superfamily of polypeptide mediators, a key component of the leukocyte recruitment process, which together with their receptors play a major role in the inflammatory and immune responses that mediate the outcome of allografts. The coordinated expression of chemokines and their receptors may, therefore, be important

in the directed migration of alloreactive T cells during GvHD.

Studies using murine models of acute GvHD have demonstrated the critical role of several chemokines and their receptors [e.g. macrophage inflammatory protein (MIP)-1 α , MIP-2, monokine induced by interferon- γ (Mig), monocyte chemotactic protein (MCP)-1, MCP-3 and CC chemokine receptor 5 (CCR5)] which direct T-cell infiltration into target tissues during acute GvHD.⁵⁻⁷ It has been shown that CCR5-expressing T lymphocytes are recruited to the liver during acute GvHD in mice⁵ while disrupting the gene encoding for CCR5 interrupts recruitment of T cells into Peyer's patches in the gut and as a result prevents acute GvHD in non-irradiated haploidentical recipients.⁸ In contrast, transferring CCR5-deficient (CCR5^{-/-}) T cells to lethally irradiated mice was associated with earlier onset and worsening of GvHD.⁹ It has also been documented that polarized human Th1 cells, known to be implicated in the pathomechanism of acute GvHD, preferentially express CXCR3

and CCR5.¹⁰ In recent human studies, Jaksch *et al.*,¹¹ who monitored the expression of four chemokine receptors, CCR1, CCR2, CCR5, and CXCR3, by quantitative real time polymerase chain reaction (PCR), observed increasing gene-expression levels for CCR5, CXCR3, CCR1, and CCR2 chemokine receptors a few days before acute GvHD was clinically diagnosed. CCR5 was identified as a co-receptor for the human immunodeficiency virus-1 (HIV-1) by Deng *et al.*¹² and Dragic *et al.*¹³ CCR5 and CXCR4 facilitate the fusion of HIV-1 with the plasma membrane of CD4⁺ cells. The human CCR5 gene maps to the short arm of chromosome 3p21.31. It encodes a 352-amino acid protein with a calculated molecular mass of 40,600 Da and a potential N-linked glycosylation site.^{14,15} Several mutations have been detected within the CCR5 gene and its promoter, including point mutations (such as single nucleotide polymorphisms within the promoter region CCR5-58755-A/G, CCR5-59029-A/G, CCR5-59653-C/T, CCR5m303), a deletion mutation, or microsatellites (IRI3.1, D3S4579 and IRI3.2, D3S4580) located respectively 11 kb upstream and 68 kb downstream of the CCR5 gene deletion. The 32-base pair (bp) deletion mutation (CCR5Δ32) causes a shift in the reading frame, which results in a severely truncated protein that is unable to reach the cell surface. This 32 bp deletion in the encoding region of CCR5 leads to complete loss of the functional CCR5 receptor in subjects homozygous for the mutation and decreased expression in heterozygous patients. It has been documented that CD3⁺ positive cells derived from CCR5Δ32 heterozygotes have reduced surface expression of CCR5 and a weaker response to its ligands.¹⁶

The CCR5 deletion mutation is estimated to have occurred 700-2000 years ago.^{17,18} Since then its frequency has increased to 13% in some northern European populations.^{17,19} CCR5Δ32 is absent in Africans and most Asian populations, and its frequency in African Americans ($f=0.02$) can be explained by admixture. CCR5Δ32 does not produce a functional protein, explaining the near-complete protection against HIV-1 infection in individuals homozygous for the allele.²⁰ Individuals with the heterozygous CCR5Δ32/CCR5 genotype express lower levels of CCR5 correlating with relatively low viral loads²¹ and slower progression to autoimmune deficiency syndrome (AIDS).²²⁻²⁴

In the present study we investigated the relationship between the incidence and severity of acute or chronic GvHD, associated with the presence of defective CCR5 expression, caused by the 32-bp deletion mutation in the recipients and donors of allogeneic hematopoietic stem cell transplants. To our knowledge this is the first study in humans describing the association between the CCR5 gene polymorphism and outcome of HSCT.

Design and Methods

Characteristics of the HSCT patient group

One hundred and eighty-six patients (132 adults over 16 years of age and 54 children) who underwent allogeneic HSCT in our Transplantation Unit and 163 stem cell donors were studied for the CCR5 gene polymorphism in relation to transplant outcome. This work was approved by the local ethics committee.

One hundred and three patients were transplanted from matched sibling donors while 68 and 15 patients were grafted from matched unrelated (MUD) or family haploidentical donors, respectively. For HLA matched sibling transplants HLA typing had been performed either serologically or by low-resolution molecular typing for HLA-A and B and by high-resolution DNA typing for DRB1. Family haploidentical and unrelated patient-donor pairs were matched on the basis of DNA high resolution typing for HLA-A, B, C, DRB1, and DQB1.

As shown in Table 1, there were differences in the conditioning regimen [myeloablative or reduced intensity conditioning (RIC)], source of stem cells [bone marrow (BM) or peripheral blood stem cells (PBSC)], GvHD prophylaxis [cyclosporine (CsA) or CsA with methotrexate (MTX) or CsA with mycophenolate mofetil (MMF)] and donor-recipient gender relation (sex matched or mismatched) among the group of patients.

Acute GvHD was graded according to the criteria of the consensus conference on acute GvHD grading.²⁵ Among 186 patients, 99 did not develop acute GvHD; of the 87 patients who developed acute GvHD, 48 had grades II-IV disease (Table 1). Sixty patients developed chronic GvHD. Sixty-four patients died after transplantation (including 28 who died before +100 day post-transplant).

CCR5 genotyping

DNA was extracted from peripheral blood mixed with EDTA using silica membranes (QiAmp Blood kit and RNeasy Mini kit, Qiagen, Hilden, Germany) following the recommendations of the manufacturer. The 32-nucleotide deletion within the CCR5 encoding gene (CCR5Δ32 polymorphism) was analyzed using a previously described PCR technique²⁶ with some modifications. In brief, a pair of primers (5'-CTT CAT TAC ACC TGC AGC TCT-3'; 5'-CAC AGC CCT GTG CCT CTT CTT C-3') flanking the region of the 32-nucleotide deletion in the CCR5 gene was used to generate wild-type and deleted DNA fragments of 182 bp and 150 bp, respectively. PCR amplification was conducted at 94°C for 3 min, followed by two cycles of 94°C for 40s; 68°C for 40s; 72°C for 40s then two cycles with annealing temperatures of 64°C and 62°C; and 30 cycles of 94°C for 40s; 58°C for 40s and 72°C for 40s with a final elongation step at 72°C for 5 min.

Table 1. Patients' characteristics.

Patients (female/male)	186 (69/117)
Age, median (range) years	24 (0.3-55)
Diagnosis	
Hematologic malignancies	151
Anemias or immunodeficiencies	35
Donor	
Sibling	103
Alternative (haploidentical/matched unrelated)	15/68
Conditioning regimen*	
Myeloablative (standard/aggressive)	101
Non-myeloablative	83
No conditioning	2
Source of hematopoietic stem cells	
Peripheral blood	107
Bone marrow	79
GvHD prophylaxis	
CsA alone	137
Multiagent (CsA + MMF or MTX)	49
Acute GvHD, n (%)	
Grades I-IV	87 (47%)
Grades II-IV	48 (26%)
No acute GvHD	99 (53%)
Chronic GvHD, n (%)°	
Present	60 (38%)
Absent	98 (62%)
Survival status, n (%)	
Dead	64 (25%)
Alive	122 (75%)

*Therapy: standard myeloablative: busulfan + (cyclophosphamide or total body irradiation) + antithymocyte globulin; aggressive myeloablative: busulfan + cyclophosphamide + (vepesid or thiotepa); non-myeloablative: (busulfan or melphalan) + fludarabine + antithymocyte globulin. CsA: cyclosporine A; MMF: mycophenolate mofetil; MTX: methotrexate). °28 patients who died before day +100 after HSCT were excluded from the analysis of chronic GvHD.

Statistical analysis

Univariate analyses of the distribution of CCR5 genotypes in patients with and without post-transplant complications were performed using Fisher's exact test or χ^2 test as appropriate. The Statistical Package for Social Scientists (SPSS, SYSTAT 10) was used for multivariate complete logistic regression analysis. Probability values <0.05 were considered statistically significant, and those between 0.05 and 0.1 as indicative of a trend.

Results

Distribution of CCR5 alleles and genotypes

The distribution of CCR5 genotypes was similar in the recipients and donors (Table 2). The frequencies of the CCR5 Δ 32 and wild type allele were 0.108 and 0.892, respectively, in the recipients and 0.113 and 0.887, respectively, in the donors. These frequencies are similar to those

Table 2. Distribution of CCR5 genotypes in HSCT recipients and donors.

CCR5 genotype	Recipients (n=186)	Donors (n=163)
	n (%)	n (%)
CCR5 Δ 32 homozygous	5 (2.7%)	4 (2.4%)
CCR5 Δ 32 heterozygous	30 (16.2%)	29 (17.8%)
CCR5 wild type homozygous	151 (81.1%)	130 (79.8%)

reported for other central European populations.^{17,19,27} As the numbers of individuals (recipients or donors) homozygous for the mutated allele were very low, in the further analyses the presence of the CCR5 Δ 32 allele (associated with no or low expression of functional CCR5) was considered as one of the factors affecting the risk of post-transplant complications.

Recipient CCR5 Δ 32 deletion mutation as an independent risk factor for acute GvHD

The presence of the CCR5 Δ 32 deletion mutation was found to be associated with a lower susceptibility to acute GvHD. Recipients carrying the CCR5 Δ 32 allele (associated with defective CCR5 expression) had acute GvHD (grades I-IV) less frequently than did patients lacking the CCR5 deletion mutation (11/35 vs. 76/151, $p=0.033$). This relationship was also seen for grades II-IV acute GvHD (5/35 vs. 43/151, $p=0.060$). The highest grades of GvHD were observed in recipients lacking the CCR5 Δ 32 allele. Sixteen out of 17 recipients with grades III-IV acute GvHD were homozygous for the wild type allele.

The relationship of the CCR5 Δ 32 allele with a lower risk of acute GvHD was observed in recipients' groups stratified with respect to age, diagnosis, conditioning regimen, GvHD prophylaxis, donor-recipient gender relation, type of donor or source of stem cells for transplantation (*individual data not shown*). For all these comparisons the frequency of acute GvHD was lower in the groups of recipients carrying the CCR5 Δ 32 mutation than in CCR5 Δ 32-negative individuals.

This protective role of the recipient CCR5 Δ 32 allele was confirmed by multivariate logistic regression analyses for acute GvHD (grades I-IV and grades II-IV). The following factors were considered: donor and recipient sex, recipient age, donor-recipient gender, intensity of conditioning regimen (myeloablative vs non-myeloablative), diagnosis (hematologic malignancies vs others), source of stem cells (bone marrow vs peripheral blood) GvHD prophylaxis (CsA vs multiagent), and type of donor (haploidentical/matched unrelated vs sibling donor). These results are presented in Table 3.

Following logistic regression analysis, three variables appeared to be significantly associated with the risk of

Table 3. Multivariate analyses of risk factors for acute GvHD.

Factor*	Acute GvHD grades I-IV			Acute GvHD grades II-IV		
	OD	95%CI	p	OD	95% CI	p
Type of donor	2.007	1.078-3.736	0.028	2.020	1.001-4.075	0.050
Recipient age	2.117	1.032-4.345	0.041	2.380	1.075-5.272	0.030
Conditioning regimen	2.235	1.175-4.252	0.014	2.331	1.108-4.902	0.026
Recipient CCR5Δ32	0.391	0.174-0.880	0.023	0.336	0.130-1.031	0.047

*type of donor (haploidentical/matched unrelated vs. sibling donor); recipient age (over vs. below or equal 35 years of age); conditioning regimen (myeloablative vs. reduced intensity conditioning or no conditioning), CCR5Δ32 (the presence of the CCR5Δ32 deletion mutation in the recipients vs. lack of the CCR5Δ32 allele).

developing acute GvHD. The presence of CCR5 deletion mutation was found to be associated with a lower risk of developing acute GvHD (OD=0.391, $p=0.023$). Transplantation from a haploidentical/matched unrelated donor (OD=2.007, $p=0.028$), recipient age (over 35 years) (OD=2.117, $p=0.041$) and myeloablative conditioning regimen (OD=2.235, $p=0.014$) were found to be factors associated with an increased risk of acute GvHD (grades I-IV). Similar correlations were observed with multivariate analysis for grades II-IV acute GvHD (Table 3).

This analysis confirmed the contribution of recipient CCR5 polymorphism to the risk of developing acute GvHD. Thus the presence of CCR5Δ32 allele was found to be an independent risk factor associated with a lower risk of developing acute GvHD (grades I-IV and II-IV). A similar, although not statistically significant, relationship was observed for more severe acute GvHD. More patients with the wild type homozygous genotype developed grades III and IV GvHD than those carrying the CCR5Δ32 allele. Among the 35 recipients carrying the CCR5 deletion mutation, only one developed grade III acute GVHD and none developed grade IV disease. In contrast, 16 out of 151 patients homozygous for the wild type allele had severe acute GVHD (11 with grade III and five with grade IV).

Organ damage in CCR5Δ32 allele-positive recipients with acute GvHD

The skin, gastrointestinal tract and liver are the major targets of GvHD. Previous studies in murine models suggested that transfer of CCR5^{-/-} T cells resulted in reduced donor cell infiltration in liver and lymphoid tissues.^{8,28} We, therefore, also looked to see whether there was any difference in the distribution of the CCR5Δ32 allele in patients with acute GvHD involving different organs. No difference was found between the organ manifestations of acute GvHD according to either recipient or donor CCR5 genotype.

Recipient CCR5 gene polymorphism and the incidence of chronic GvHD, relapse or death

As expected, the incidence of chronic GvHD was more frequent among patients with acute GvHD (37/72 vs. 23/86, $p<0.002$ and 23/37 vs. 37/121, $p<0.001$, for grades I-IV and II-IV acute GvHD, respectively). However, the presence of the CCR5Δ32 allele in recipients was not found to be associated with chronic GvHD. The incidence of chronic GvHD was similar in patients with or without the CCR5Δ32 allele (10/25 vs. 50/128; $p=ns$).

No significant difference was observed in the incidence of relapse in recipients with or without the CCR5Δ32 allele (4/35 vs. 14/151; $p=ns$). The CCR5 polymorphism was not found to affect the patients' survival (*data not shown*).

Donor CCR5 genotype and HSCT outcome

Although some direct associations of donor genotype with the risk of GvHD could be expected from previous animal studies,^{8,9,28} in the present study no relationships were found between the presence of the CCR5Δ32 allele in donors and transplant outcome: development of acute GvHD - independently of the severity of the disease and stratification of transplant recipients with respect to age, diagnosis, conditioning regimen, type of donor or source of stem cells for transplantation; manifestation of chronic GvHD independently of the *limited/extensive* classification; the incidence of relapse or death (*individual data not shown*).

Analysis of different combinations of donor-recipient CCR5 genotypes demonstrated that the presence of the CCR5 deletion mutation in both the recipients and their donors was the most protective combination. None of 11 recipients carrying the CCR5Δ32 allele transplanted with a graft from a donor also carrying the CCR5Δ32 allele developed acute GvHD (grades I-IV) whereas 70 out of 151 donor-recipient pairs carrying the other combinations of CCR5 alleles (0.00 vs 0.46, $p=0.002$) did so. Thus the protective effect of the CCR5Δ32 deletion mutation was especially seen in recipients transplanted with donors carrying the CCR5Δ32 allele.

Discussion

Acute GvHD is a major complication following allogeneic stem cell transplantation. The recent literature has suggested a potential role for CCR5 in a mouse model of GvHD.⁸ In this model, acute GvHD was prevented when recruitment of T cells into Peyer's patches in the gut was interrupted by disrupting the gene encoding for CCR5. Moreover, the presence of the CCR5 deletion mutation has been documented to lead to either complete loss of the functional CCR5 receptor or its decreased expression in subjects homozygous or heterozygous for this mutation, respectively.¹⁶ In the present study we investigated the relationship between the

incidence and severity of acute GvHD and the CCR5 gene polymorphism associated with defective CCR5 expression in humans.¹⁶

We found that the presence of the CCR5 Δ 32 allele in the recipient was associated with a significantly lower incidence of developing acute GvHD after allogeneic HSCT. After adjusting for other variables known to be associated with the development of acute GvHD by logistic regression analysis, the independent contribution of the CCR5 gene polymorphism to the risk of acute GvHD was confirmed. Interestingly, the protective effect of the CCR5 deletion mutation was particularly evident in mutation-positive recipients transplanted with grafts from donors carrying the CCR5 Δ 32 allele as none of those recipients developed acute GvHD (grades I-IV). Furthermore severe acute GvHD (grades III or IV) was seen only in recipients transplanted with donors homozygous for the wild type genotype (lacking the CCR5 deletion mutation). To our knowledge this is the first study in humans describing the association between the CCR5 gene deletion mutation polymorphism and the outcome of allogeneic HSCT, although several studies have analyzed the role of this CCR5 gene polymorphism in relation to the outcome of organ transplantation (i.e. kidney,^{29,30} heart³¹ or liver transplantation^{32,33}).

Interestingly, Jaksch *et al.* reported increasing gene-expression levels for CCR5, CXCR3, CCR1, and CCR2 chemokine receptors a few days before acute GvHD was clinically diagnosed.¹¹ In their study, the expression of functional chemokine receptors, including CCR5, was found to contribute to the risk of developing acute GVHD in patients after allogeneic HSCT. Thus, two independent studies, that of Jaksch *et al.*¹¹ and the present one, suggest that the presence of functional CCR5 (assessed by CCR5 gene expression or polymorphism) is associated with the development of acute GvHD in humans. Increased expression of the CCR5 gene associated with lack of the CCR5 deletion mutation¹⁶ were found in these two independent studies to be factors contributing to the risk of acute GvHD in patients undergoing allogeneic HSCT. These results suggest that chemokine receptors may not only act as a potential target for modulation of acute GvHD but also as a diagnostic marker for early detection of the disease.

We found that recipient CCR5 Δ 32 allele carriership was associated with a lower incidence and severity of acute GvHD but not with the other post-transplant complications (chronic GvHD, relapse, survival). No direct relationship with donor genotype was found, although a significantly lower incidence of acute GvHD was observed among recipients carrying the CCR5 Δ 32 allele grafted from donors with the same deletion mutation as compared to among the other donor-recipient pair combinations. This association of recipient CCR5 Δ 32 allele and lowered incidence of acute GvHD was an unexpected observation considering the recent data from murine

models of GvHD,^{8,9,28} suggesting that associations would be found with donor CCR5 genotype rather than recipient genotypes. However, these studies also indicated that the role of CCR5 in allogeneic bone marrow transplants and GvHD is quite complex as the absence of donor expression of CCR5 on T cells ameliorates GvHD in models using no conditioning of the recipient⁸ while, on the other hand, in a murine transplant model administered intensive conditioning, the overall effect of absent CCR5 expression on donor cells was a greater incidence/severity of GvHD^{9,28} and donor T-cell expansion.²⁸ Interestingly, in the present study, a relationship, albeit not statistically significant, was seen between the incidence of GvHD and pre-transplant conditioning regimen. Grafts from donors having one wild type allele induced more GvHD in recipients who had received myeloablative conditioning regimen than in recipients who had had non-myeloablative conditioning and who did not receive any conditioning (0.29 vs. 0.07; p =ns). This observation concurs with the results of Wysocki *et al.*,⁹ who showed that transferring CCR5-deficient (CCR5^{-/-}) T cells to lethally irradiated mice was associated with earlier onset and a worsening of GvHD. Welniak *et al.*²⁸ suggested that transplantation of full MHC-mismatched donor bone marrow and splenocytes from CCR5-knockout mice might result in higher interferon- γ and tumor necrosis factor- α production by recovered T cells and better proliferation in response to a T-cell mitogen with a lower expansion of CD8⁺ T cells, indicating that CCR5 plays a role in down-regulating donor alloreactive CD8⁺ T-cell expansion.

It is difficult to explain why the lack of functional chemokine receptor on recipient cells protects against GvHD. However, our study suggests that also the expression of CCR5 on recipient cells, especially early after transplantation, plays a role in the alloresponse leading to GvHD. Interestingly, recent studies in solid organ transplant recipients have suggested that the expression of CCR5 may play an important role in directing T cells into allografts to mediate acute rejection. A lack of functional CCR5 on recipient cells has been found to be associated with a better outcome of solid organ transplants. For example, prolonged survival of cardiac allografts was reported in recipients either lacking CCR5 or treated with CCR5 neutralizing monoclonal antibody.³⁴ In clinical renal transplantation, the presence of a homozygous mutation encoding non-functional CCR5 in recipients was associated with decreases in acute rejection and better graft outcomes as compared to recipients expressing functional receptor.³⁰ Data from the present study would suggest that a lack of functional CCR5 on recipient cells may play a protective role also in HSCT. It could be speculated that enhanced migration of recipient T cells lacking functional CCR5 to lymphoid tissue in response to pro-inflammatory chemokines induced by a conditioning regimen (irradiation) may

limit T-cell engraftment and expansion. Indeed the study of Wysocki *et al.*⁹ showed that, in irradiated recipients, T cells lacking CCR5 had enhanced migration to CXCL10 (the interferon-inducible CXCR3 ligand). In addition, it has been documented that CD4⁺CD25⁺ T regulatory cells (T regs), whose presence is associated with a lower risk of developing acute GvHD, need CCR5 to function.³⁵ All these results, from the studies in mice and the present one in humans, indicate that the role of CCR5 in allogeneic HSCT and GvHD is more complex than initially thought. Moreover, a direct association between levels of CCR5 and differentiation of monocytes to macrophages has been documented.³⁶ It has also been found that cross-linking of CCR5 on monocytes leads to activation and differentiation of monocytes into dendritic cells while following the cross-linking of CCR5, monocytes synthesize high levels of M-CSF, RANTES, MIP-1 α , and MIP-1 β associated with a readily detectable down modulation of CD14, CD4, CCR5, and CXCR4 expression.³⁷ It would, therefore, be of interest to look at changes in CCR5 expression at different times after transplantation, during acute GvHD and in different cell populations including not only lymphocytes, but also those that might function as professional antigen-presenting cells such as monocytes/macrophages. However, these aspects remain to be investigated.

There also has been some intriguing data suggesting that the CCR5 gene polymorphism may have a role in the perpetuation of viral infections. Initial indications on this came from studies in HIV-1 infected patients. Homozygosity for the CCR5 Δ 32 allele or lower expression of CCR5 in these patients was found to be associated with lack of clinical symptoms and heterozygous individuals had slower progression to AIDS.²⁰⁻²⁴

Furthermore, very recent data showed that the absence of CCR5 Δ 32 allele or increased expression of CCR5 in peripheral blood cells was associated with increased Epstein-Barr virus load in patients after allogeneic HSCT (*preliminary data reported in*^{38,39}).

These data support the theory that CCR5 genotype may be of prognostic value for transplant outcome. These results add significantly to those of studies highlighting the potential importance of non-HLA polymorphisms in HSCT^{40,41} and indicate that non-HLA genotyping may be useful for identifying patients at the highest risk of complications. Thus, CCR5 and probably also its ligands might be novel target molecules for therapeutic interventions in patients undergoing allogeneic HSCT. However, the results of the effect of the CCR5 gene polymorphism need to be confirmed in other patient-donor pairs to assess whether analysis of this polymorphism could be included in standard patient-donor genotyping to complement traditional histocompatibility testing and increase the ability to predict the risk of transplant-related complications.

AL worked out the concept of the study on an association between CCR5 and HSCT outcome. He supervised the laboratory and clinical work and proposed points for the discussion. He revised the manuscript prior to submission. KBK was primarily responsible for the CCR5 genotyping (performed all technical work) and statistical analysis, drafting the manuscript and creating all the tables in the paper. She contributed to the conception and design of the study and critically revised the manuscript for content. DD and KS were responsible for the collection and analysis of clinical data. All investigators participated in the interpretation of the results and the preparation of the manuscript. The authors declare that they have no potential conflict of interest.

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References

1. Antin JH, Ferrara JL. Cytokine dysregulation and acute graft versus host disease. *Blood* 1992;80:2964-8.
2. Lichtman AH, Krenger W, Ferrara JLM. Cytokine Networks. In: Ferrara JLM, Deeg HJ, Burakoff SJ, eds. *Graft versus host disease*. New York: M. Dekker, Inc., 1997:179-218.
3. Gouwy M, Struyf S, Proost P, Van Damme J. Synergy in cytokine and chemokine networks amplifies the inflammatory response. *Cytokine Growth Factor Rev* 2005;16:561-80.
4. Coelho AL, Hogaboam CM, Kunkel SL. Chemokines provide the sustained inflammatory bridge between innate and acquired immunity. *Cytokine Growth Factor Rev* 2005;16:553-60.
5. Murai M, Yoneyama H, Harada A, Yi Z, Vestergaard C, Guo B, et al. Active participation of CCR5(+)/CD8(+) T lymphocytes in the pathogenesis of liver injury in graft-versus-host disease. *J Clin Invest* 1999;104:49-57.
6. Serody JS, Burkett SE, Panoskaltsis-Mortari A, Ng-Cashin J, McMahon E, Matsushima GK, et al. T-lymphocyte production of macrophage inflammatory protein-1 α is critical to the recruitment of CD8(+) T cells to the liver, lung, and spleen during graft-versus-host disease. *Blood* 2000;96:2973-80.
7. New JY, Li B, Koh WP, Ng HK, Tan SY, Yap EH, et al. T cell infiltration and chemokine expression: relevance to the disease localization in murine graft-versus-host disease. *Bone Marrow Transplant* 2002;29:979-86.
8. Murai M, Yoneyama H, Ezaki T, Suematsu M, Terashima Y, Harada A, et al. Peyer's patch is the essential site in initiating murine acute and lethal graft-versus-host reaction. *Nat Immunol* 2003;4:154-60.
9. Wysocki CA, Burkett SB, Panoskaltsis-Mortari A, Kirby SL, Luster AD, McKinnon K, et al. Differential roles for CCR5 expression on donor T cells during graft-versus-host disease based on pretransplant conditioning. *J Immunol* 2004;173:845-54.
10. Bonecchi R, Bianchi G, Bordignon PP, D'Ambrosio D, Lang R, Borsatti A, et al. Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. *J Exp Med* 1998;187:129-34.
11. Jaksch M, Remberger M, Mattsson J. Increased gene expression of chemokine receptors is correlated with acute graft-versus-host disease after allogeneic stem cell transplantation. *Biol Blood Marrow Transplant* 2005;11:280-7.
12. Deng H, Liu R, Ellmeier W, Choe S, Unutmaz D, Burkhart M, et al. Identification of a major co-receptor for primary isolates of HIV-1. *Nature* 1996;381:661-6.
13. Dragic T, Litwin V, Allaway GP, Martin SR, Huang Y, Nagashima KA, et al. HIV-1 entry into CD4⁺ cells is mediated by the chemokine receptor CC-CCR-5. *Nature* 1996;381:667-73.
14. Mummidi S, Ahuja SS, McDaniel BL, Ahuja SK. The human CC chemokine receptor 5 (CCR5) gene: multiple transcripts with 5-prime-end heterogeneity, dual promoter usage, and evidence for polymorphisms within the regulatory regions and noncoding exons. *J Biol Chem* 1997;272:30662-71.
15. Germana Paterlini M. Structure modeling of the chemokine receptor CCR5:

- implications for ligand binding and selectivity. *Biophys J* 2002; 3: 3012-31
16. Venkatesan S, Petrovic A, Van Ryk DI, Locati M, Weissman D, Murphy PM. Reduced cell surface expression of CCR5 in CCR5 Δ 32 heterozygotes is mediated by gene dosage, rather than by receptor sequestration. *J Biol Chem* 2002;277:2287-301.
 17. Stephens JC, Reich DE, Goldstein DB, Shin HD, Smith MW, Carrington M, et al. Dating the origin of the CCR5- Δ 32 AIDS-resistance allele by the coalescence of haplotypes. *Am J Hum Genet* 1998;62:1507-15.
 18. Libert F, Cochaux P, Beckman G, Samson M, Aksenova M, Cao A, et al. The Δ CCR5 mutation conferring protection against HIV-1 in Caucasian populations has a single and recent origin in Northeastern Europe. *Hum Mol Genet* 1998;7:399-406.
 19. Martinson JJ, Chapman NH, Rees DC, Liu YT, Clegg JB. Global distribution of the CCR5 gene 32-basepair deletion. *Nat Genet* 1997;16:100-3.
 20. Liu R, Paxton WA, Choe S, Ceradini D, Martin SR, Horuk R, et al. Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. *Cell* 1996;86:367-77.
 21. Michael NL, Chang G, Louie LG, Mascola JR, Dondero D, Birx DL, et al. The role of viral phenotype and CCR-5 gene defects in HIV-1 transmission and disease progression. *Nat Med* 1997; 3:338-40.
 22. Dean M, Carrington M, Winkler C, Huttley GA, Smith MW, Allikmets R, et al. Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CKR5 structural gene. *Science* 1996;273:1856-62.
 23. Zimmerman PA, Buckler-White A, Alkhatib G, Spalding T, Kubofcik J, Combadiere C, et al. Inherited resistance to HIV-1 conferred by an inactivating mutation in CC chemokine receptor 5: studies in populations with contrasting clinical phenotypes, defined racial background, and quantified risk. *Mol Med* 1997;3:23-36.
 24. de Roda Husman AM, Koot M, Cornelissen M, Keet IP, Brouwer M, Broersen SM, et al. Association between CCR5 genotype and the clinical course of HIV-1 infection. *Ann Intern Med* 1997;27:882-90.
 25. Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J, et al. 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant* 1995;15:825-8.
 26. Smith KM, Crandall KA, Kneissl ML, Navia BA. PCR detection of host and HIV-1 sequences from archival brain tissue. *J Neurovirol* 2000;6:164-71.
 27. Gonzalez E, Bamshad M, Sato N, Mummidi S, Dhanda R, Catano G, et al. Race-specific HIV-1 disease-modifying effects associated with CCR5 haplotypes. *Proc Natl Acad Sci USA* 1999; 96:12004-9.
 28. Welniak LA, Wang Z, Sun K, Kuziel W, Anver MR, Blazar BR, et al. An absence of CCR5 on donor cells results in acceleration of acute graft-vs-host disease. *Exp Hematol* 2004;32:318-24.
 29. Abdi R, Tran TB, Sahagun-Ruiz A, Murphy PM, Brenner BM, Milford EL, et al. Chemokine receptor polymorphism and risk of acute rejection in human renal transplantation. *J Am Soc Nephrol* 2002;13:754-8.
 30. Fischereder M, Luckow B, Hocher B, Wuthrich RP, Rothenpieler U, Schneeberger H, et al. CC chemokine receptor 5 and renal-transplant survival. *Lancet* 2001;357:1758-61.
 31. Fildes JE, Walker AH, Howlett R, Bittar MN, Hutchinson IV, Leonard CT, et al. Donor CCR5 Δ 32 polymorphism and outcome following cardiac transplantation. *Transplant Proc* 2005; 37:2247-9.
 32. Moench C, Uhrig A, Lohse AW, Otto G. CC chemokine receptor 5 Δ 32 polymorphism-a risk factor for ischemic-type biliary lesions following orthotopic liver transplantation. *Liver Transpl* 2004;10:434-9.
 33. Schroppel B, Fischereder M, Ashkar R, Lin M, Kramer BK, Mardera B, et al. The impact of polymorphisms in chemokine and chemokine receptors on outcomes in liver transplantation. *Am J Transplant* 2002;2:640-5.
 34. Gao W, Faia KL, Csizmadia V, Smiley ST, Soler D, King JA, et al. Beneficial effects of targeting CCR5 in allograft recipients. *Transplantation* 2001; 72: 1199-205.
 35. Wysocki CA, Jiang Q, Panoskaltis-Mortari A, Taylor PA, McKinnon KP, Su L, et al. Critical role for CCR5 in the function of donor CD4⁺CD25⁺ regulatory T cells during acute graft-versus-host disease. *Blood* 2005;106:3300-7.
 36. Tuttle DL, Harrison JK, Anders C, Sleasman JW, Goodenow MM. Expression of CCR5 increases during monocyte differentiation and directly mediates macrophage susceptibility to infection by human immunodeficiency virus type 1. *J Virol* 1998; 72:4962-9.
 37. Nimura F, Zhang LF, Okuma K, Tanaka R, Sunakawa H, Yamamoto N, et al. Cross-linking cell surface chemokine receptors leads to isolation, activation, and differentiation of monocytes into potent dendritic cells. *Exp Biol Med* 2006;231:431-43.
 38. Bogunia-Kubik K, Jaskula E, Lange A. The presence of CCR5 Δ 32 deletion mutation associates with a lower incidence of acute GvHD while enhanced CCR5 expression increases the risk of EBV infection. *Haematologica* 2005; 90 Suppl 2:226[abstract 564].
 39. Jaskula E, Bogunia-Kubik K, Lange A. CCR5 gene expression may affect the risk of EBV infection: preliminary report. *Bone Marrow Transplant* 2005; 35 Suppl 2:S169[abstract P628].
 40. Dickinson AM, Charron D. Non-HLA immunogenetics in hematopoietic stem cell transplantation. *Curr Opin Immunol* 2005;17:517-25.
 41. Bogunia-Kubik K, Wysoczanska B, Lange A. Non-HLA gene polymorphisms and the outcome of allogeneic hematopoietic stem cell transplantation. *Curr Stem Cell Res Ther* 2006; 1:239-53.