Stem Cell Transplantation

Recipient interferon-γ 3/3 genotype contributes to the development of chronic graft-versus-host disease after allogeneic hematopoietic stem cell transplantation

Microsatellite polymorphism (CA)n within the first intron of the interferon-γ gene was assessed in 160 recipients of an allogeneic hematopoietic stem cell transplant (HSCT). IFN-γ 3/3 was found to be associated with an increased risk of chronic graft-versus-host disease (GvHD) (11/27 vs 26/133, *p*=0.02). Forward logistic regression analysis confirmed the role of IFN-γ 3/3 genotype as one of the risk factors for manifestation of chronic GvHD (OR=3.180, *p*=0.018) together with previous acute GvHD (OR=2.752, *p*=0.024), cyclosporine A monotherapy (OR=2.607, *p*=0.029) and malignant disorders (OR=4.371, *p*=0.032).

haematologica 2005; 90:425-426
(*http://www.haematologica.org/journal/2005/03/425.html*)

Chronic graft-versus-host disease (GvHD) is the most common non-relapse complication affecting long-term survivors of allogeneic hematopoietic stem cell transplantation (HSCT). Over 60% of the recipients of either sibling or alternative HSC transplants who survive over 100 days develop this complication, resulting in dysfunction of numerous organ systems and an often profound state of immunodeficiency. It is the most frequent cause of poor long-term outcome and quality of life after allogeneic HSCT.[1,2] Many factors have been reported to contribute to the development of chronic GvHD with the most important one being the occurrence of significant acute GvHD. Other risk factors include older recipient age, female to male (F-M) transplantation, peripheral blood progenitor cells (PBPC) instead of bone marrow (BM) as the source of hematopoietic stem cells for the transplant, mismatched or unrelated donors, donor lymphocyte infusion and lack of T-cell depletion.[2] After cord blood transplantation the rate of chronic GvHD is relatively low, even in patients with significant acute GvHD.[3] Recent reports have described associations between the polymorphic features of cytokine encoding genes and the outcome of allogeneic HSCT, including some correlations with chronic GvHD.[4-6] In the present study, 160 recipients of allogeneic HSCT were studied for the incidence of chronic GvHD in relation to the interferon (IFN)-γ gene microsatellite $(CA)_n$ polymorphism (Table 1).

The previous manifestation of significant acute GvHD is the most important factor affecting the development of chronic GvHD.[2] Also in our present study more patients with previous acute GvHD had chronic GvHD symptoms than did those with no history of acute disease (0.36 vs 0.14, *p*=0.002). Among the other factors found to contribute to the risk of chronic GvHD, malignant disorders, F-M transplantation, conditioning regimen, GvHD prophylaxis, and PBPC as the source of hematopoietic stem cells were identified in our group of patients (in univariate analysis). All these factors had been previously reported in many studies from various centers.[1,2,4-6] In our present work, patients carrying the IFN-γ 3/3 genotype more frequently developed acute (9/34 vs 18/126, *p*=0.08) and chronic GvHD (11/17 vs 26/133, *p*=0.020) as compared to patients with other IFN-γ alleles. The first

Table 1. Patients' characteristics. Acute GvHD was graded according to the consensus conference on acute GvHD grading.[10] Chronic GvHD staging was performed according to the *limited/extensive* classification, proposed by Seattle group in 1980 and recently revised.[2]

Number of patients	*160*
Date of transplant	1993 -2003
Age (median, range), yrs	23 (0.3 - 55)
Adults > 16 yrs	111
Children ≤ 16 yrs	49
Donor/recipient gender	
Female-Female (F-F)	26
Male-Male (M-M)	59
Female-Male (F-M)	45
Male-Female (M-F)	30
Transplant material	
Bone marrow (BM)	74
Peripheral blood progenitor cells (PBPC)	86
Diagnosis	
Hematologic malignancies	127
Chronic myeloid leukemia (CML)	52
Acute lymphocytic leukemia (ALL)	29
Acute myeloid leukemia (AML)	25
Hodgkin's disease (HD)	6
Anemia's	23
Fanconi's anemia (FA)	9
Severe aplastic anemia (SAA)	14
Immunodeficiences	10
Conditioning regimen	
Myeloablative*	75
Reduced intensity conditioning (RIC)°	85
GvHD prophylaxis	
Cyclosporine A (CsA)	101
CsA + methotrexate (MTX)/CsA + mycophenolate mofetil (MMF)	49/10
Acute GvHD, grades	
0	96
I	30
II	25
III	6
IV	3
Chronic GvHD	
Limited/extensive	18/19

**standard myeloablative: busulphan (16 mg/kg b.w.) + cyclophosphamide + (vepesid or thio-Tepa or anti-thymocyte globulin); aggressive myeloablative: busulphan (16 mg/kg b.w.) or total body irradiation + cyclophosphamide; °RIC: busulphan (8 mg/kg b.w.) or melphalan (140 mg/m^2) + fludarabine + anti-thymocyte globulin.*

relation of IFN-γ 3/3 genotype and acute GVHD had already been found by Cavet *et al.*[5] and was confirmed by our previous study[7,8] while the latter one is a novel observation not formerly described. The association of IFN-γ 3/3 was confronted with other, previously identified, risk factors for chronic GvHD in multivariate forward logistic regression analysis. The following factors were found to contribute to the risk of chronic GvHD in this multivariate analysis: malignant disorders (OR = 4.371, 95%CI: 1.133-16.87, *p*=0.032), IFN-γ 3/3 (OR = 3.180, 95%CI: 1.222-8.271, *p*=0.018), acute GvHD (OR = 2.752, 95%CI: 1.142-6.629, *p*=0.024), and cyclosporine A monotherapy

Table 2. Univariate association of recipient IFN-γ 3/3 genotype with chronic GvHD.

Association of IFN-γ genotype with chronic GvHD	*Incidence of chronic GvHD in patients having and lacking IFN-γ 3/3*		
Variable N. of	*Fractions patients*	*p of cases*	
Previous acute GvHD (grades II-IV)	**6/9 vs 8/25**	**0.66 vs 0.32**	**0.079**
No or mild acute GvHD (grades 0-I)	5/18 vs 18/108	0.28 vs 0.16	0.206
Previous acute GvHD (grades I-IV)	**8/14 vs 15/50**	**0.57 vs 0.30**	**0.062**
De novo (grades 0)	3/13 vs 11/83	0.23 vs 0.13	0.286
Sibling donor	**8/19 vs 20/91**	**0.42 vs 0.22**	**0.065**
Alternative donor	3/8 vs 6/42	0.37 vs 0.14	0.144
Children (< 16 yrs.)	2/8 vs 7/41	0.25 vs 0.17	0.457
Adults	**9/19 vs 19/92**	**0.47 vs 0.21**	**0.019**
CsA monotherapy	**7/19 vs 13/82**	**0.37 vs 0.16**	**0.045**
CsA with (MTX or MMF)	4/8 vs 13/51	0.50 vs 0.25	0.157
Peripheral blood progenitor cells	5/14 vs 18/72	0.36 vs 0.25	0.300
Bone marrow	**6/13 vs 8/61**	**0.46 vs 0.13**	**0.013**
Female donor-male recipient	5/10 vs 10/35	0.50 vs 0.25	0.186
others	**6/17 vs 16/98**	**0.35 vs 0.16**	**0.072**
Myeloablative conditioning	**8/15 vs 15/60**	**0.53 vs 0.25**	**0.038**
RIC	3/12 vs 11/73	0.25 vs 0.15	0.311
Hematologic malignancies	**11/21 vs 23/106**	**0.52 vs 0.22**	**0.006**
Non-malignant disorders	0/6 vs 3/27	0.00 vs 0.11	0.536
Overall	**11/27 vs 26/133**	**0.41 vs 0.19**	**0.020**

IFN-γ microsatellite polymorphism (CA)n within the first intron was analyzed by a PCR-STR technique as previously described.[8] Note that the IFN-γ 3 allele corresponds to 13 CA repeats. p values were assessed by Fisher's exact test. Probability values <0.05 were considered statistically significant, and those between 0.05 and 0.1 as indicative of a trend. Significant associations and tendencies are given in bold.

(OR=2.607, 95%CI: 1.104-6.152, p=0.029). The use of PBPC for transplantation (OR=2.094, 95%CI: 0.894-4.904, p=0.089) appeared to be a less significant factor. In addition, no significant association was observed between IFN-γ 3/3 homozygosity and overall patients' survival (assessed employing Kaplan-Meier analysis). However, in the group of fatal cases, IFN-γ 3/3 genotype was more frequently detected in patients who died as a result of chronic GvHD (4 out of 5 cases) than in patients who died from other complications (2 out of 31 cases) (0.90 vs 0.07, p=0.001).

Cytokine production is under genetic control and the presence of particular genotypes associates with cytokine gene expression and protein production. This was also shown for IFN-γ.[9] After mitogen stimulation peripheral blood mononuclear cells obtained from IFN-γ 2/2 homozygous healthy individuals produced more IFNγ than did cells taken from IFN-γ 3/3 homozygotes (Pravica *et al.*[9] *and own unpublished observation*). Our study documents that IFN-γ 3/3 genotype, associated with decreased IFNγ production, constitutes an independent risk factor for chronic GvHD. It is well known that production of Th1 type cytokines (including IFN-γ) play a crucial role during the initiation and perpetuation of acute GvHD, while Th2 type cytokines are more frequently secreted during the chronic phase of the disease. The presence of IFN-γ low-producing genotype in patients with chronic GvHD may reflect this switch from Th1 into Th2 cytokines in the chronic phase of GvHD. Therefore, the relationship of IFN-γ 3/3 with chronic GvHD may mirror an association of this genotype with acute GvHD.[5,7,8] In fact, IFN-γ 3/3 genotype is associated with chronic GvHD following acute GvHD and not that seen *de novo* (Table 2). This would suggest that the presence of IFN-γ 3/3 genotype affects acute GvHD while alloreactivity associated with the acute phase of the disease promotes chronic GvHD.

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Key words: IFN-γ gene polymorphism, (CA)n microsatellite, IFNγ production, chronic GvHD, allogeneic hematopoietic stem cell transplantation.

Funding: this work was supported by the FP6 AlloStem grant (LSHB-CT-2004-503319).

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