Bone Marrow Failure

The frequency of HLA class I alleles in Japanese patients with bone marrow failure

The frequencies of the HLA-B*4002 and HLA-A*0206 alleles in patients with aplastic anemia (AA) (n=32; 21.9%) and paroxysmal nocturanl hemoglobinuria (PNH) (n=24; 22.9%), respectively, were significantly different from those in controls (n=371; 8.6%, p<0.002 and 7.7%, p<0.001, respectively), suggesting that each specific allele in AA or PNH may be related to the immunologic pathophysiology of these disorders.

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Autoreactive T lymphocytes are implicated in the immune mechanisms involved in the bone marrow failure (BMF) syndrome, including aplastic anemia (AA), paroxysmal nocturnal hemoglobinuria (PNH), and myelodysplastic syndrome (MDS).[1] In order to clarify the frequency and some clinical significance of the human leukocyte antigen (HLA) class I alleles in the BMF syndrome, we investigated these alleles using a high-resolution method of genotyping[2,3] in 78 Japanese patients with BMF, including 32 with AA, 24 with PNH, and 22 with MDS. The diagnosis and grading of the severity of AA were based on the criteria of the International Agranulocytosis and Aplastic Anemia Study Group[4] and that of Frickhofen *et al.*,[5] respectively. The diagnosis of PNH and MDS was made according to the International PNH Interest Group criteria[6] and French-American-British criteria,[7] respectively.

As shown in Table 1, the reticulocyte counts, values of lactate dehydrogenase, and proportions of complement-sensitive erythrocytes in PNH patients were significantly higher than those in patients with AA (p<0.0005, p<0.0005, and p<0.0005, respectively) or MDS (p<0.0005, p<0.0005, and p<0.0005, respectively). Also, the duration of illness in PNH patients was longer than that in AA patients (p<0.002) or in MDS patients (p<0.0005). Moreover, differences in hemoglobin concentrations were found between AA and MDS patients (p<0.02). Chromosomal analysis showed a high frequency of abnormal karyotypes only in MDS patients, but there were no differences between them in the other parameters.

The frequencies of the HLA-A and -B alleles in 78 patients with BMF are summarized in Table 2. The frequencies of the HLA-B*4002 allele in AA patients and the HLA-A*0206 allele in PNH patients were significantly different from those in controls[8] (p<0.002 and p<0.001, respectively). In addition, the frequency of the HLA-A*0206 allele in PNH patients was significantly different from that in AA patients (p<0.01). The frequencies of all the other alleles were not significantly different between the patients and healthy individuals or between the patients. Twenty-one (65.6%) of 32 AA patients, nine (37.5%) of 24 PNH patients, and nine (40.9%) of 22 MDS patients had HLA-B40 haplotype. However, the frequencies of all the haplotypes were not significantly different between the patients or between the patients and healthy individuals. Ten (58.8%) of 17 patients with severe or very severe AA and three (20%) of 15 patients with non-severe AA had the HLA-B*4002 allele. The proportion of patients with severe or very severe AA with the HLA-B*4002 allele was significantly higher than that of non-severe AA patients with the allele (p<0.05). In addition, of the 13 AA patients with the HLA-B*4002 allele, 10 (76.9%) had severe or very severe disease, whereas of the 19 AA patients without the alleles, only 7 (36.8%) had severe or very severe AA (p<0.05). Subsequently, statistical analyses showed that the reticulocyte counts (138±73×10^9/L) and values of lactate dehydrogenase (2399±235 IU/L) in PNH patients (n=10) with the HLA-A*0206 allele were significantly higher than those in PNH patients (n=14) without the allele (78±34×10^9/L, p<0.02; and 972±770 IU/L, p<0.05, respectively). All the other parameters were not significantly different between the two groups. Although differences in the proportions of complement-sensitive erythrocytes were not found between the two groups, the frequency of PNH patients with over 30% of complement-sensitive erythrocytes was significantly higher in PNH patients with the HLA-A*0206 allele than in PNH patients without the allele (80% versus 28.6%, p<0.05). This is the

Table 1. Summary of clinical, hematologic, and laboratory findings at the time of examination of HLA class I genotype in patients with BMF.

Parameters	*AA (n=32)*	*PNH (n=24)*	*MDS (n=22)*
Male-to-female ratio	16:16	17:7	16:6
Mean age (years)	53±20 (17-82)	52±16 (23-82)	59±18 (19-87)
White blood cell count (×10^9/L)	3.6±1.6 (0.7-6.5)	4.3±3.0 (1.7-13.8)	4.1±2.1 (0.6-8.9)
Neutrophil count (x10^9/L)	1.869±1.069 (0.035-4.260)	2.828±2.840 (0.572-12.696)	2.272±1.892 (0.048-6.764)
Hemoglobin (g/dL)	11.2±2.8 (5.8-16.0)	10.5±2.9 (5.9-16.3)	9.0±2.7 (5.8-16.6)
Reticulocyte count (×10^9/L)	53±29 (4.0-120)	104±61 (12-269)	48±32 (8.3-119)
Platelet count (×10^9/L)	97±78 (3.0-260)	120±64 (21-239)	124±133 (7.0-469)
Lactate dehydrogenase* (IU/L)	249±87 (129-525)	1567±1736 (197-7203)	277±156 (145-744)
Duration of illness (months)	63±76 (2-343)	150±120 (0-456)	43±32 (3-130)
Chromosomal analysis (abnormal/normal karyotypes)	0/32	0/24	10/12
Proportion of CD55$^-$ and CD59$^-$ erythrocytes (%)	0.40±0.44 (0.07-2.17)	43.2±34.9 (1.3-100)	0.37±0.29 (0.04-1.31)

*Data are presented as a mean value±standard deviation or a ratio, with the range of values for each parameter shown in brackets. *:normal range in this study: 119 to 229 IU/L.*

Table 2. **Frequencies of the HLA-A and -B alleles in 78 Japanese patients with BMF and in healthy Japanese individuals.**

Gene allele*	Frequency of each allele (%) AA (n=32)	PNH (n=24)	MDS (n=22)	Healthy controls (8) (n=371)
A				
02011	17.2	10.4	11.4	11.5
0206	4.7	22.9	11.4	7.7
1101	10.9	6.3	(2.3)†	8.2
2402101	35.9	29.2	34.1	37.9
2601	6.3	10.4	13.6	8.1
2602	4.7	(2.1)†	(4.5)†	2.3
31012	6.3	6.3	6.8	7.1
3303	6.3	8.3	6.8	9.7
B				
15011	6.3	8.3	6.8	8.7
3501	7.8	10.4	(4.5)†	7.6
3901	(1.6)†	6.3	(4.5)†	4.4
4001	7.8	6.3	(4.5)†	4.2
4002	21.9	12.5	13.6	8.6
4006	9.4	–	(4.5)†	3.9
44031	6.3	6.3	6.8	8.7
51011	(1.6)†	8.3	6.8	7.7
52011	14.1	16.7	18.2	10.7
5401	7.8	10.4	9.1	7.7

**: The alleles shown here were found in at least three of the patients with each disease. †: The frequencies (%) found in fewer than two of the patients with each disorder are given in brackets.*

first report on the frequency of HLA class I alleles in patients with BMF in Asian countries. The frequencies of HLA-A and -B alleles or haplotypes in healthy individuals differ very greatly between the USA population, with its mixture of races, and the Japanese population, a single race.[8,9] Maciejewski *et al*.[9] using a low-resolution method, but not a high-resolution method, of HLA genotyping found that the frequencies of HLA-B14 alleles in 212 AA patients and of HLA-Cw06 alleles in 112 MDS patients were significantly higher than those in USA controls, whereas they did not find a high frequency of any HLA class I alleles in 46 hemolytic PNH patients. Among the cases of AA, MDS, and pure hemolytic PNH, they included, respectively, 24, 4 and 1 patients of Asian origin, explaining one reason for the differences between the two results. Subsequently, as far as we know, this is the first report indicating that the HLA-B*4002 allele may be related to the grade of severity of AA, and that the HLA-A*0206 allele may contribute to selection of PNH clones through some immune mechanism of $CD8^+$ T lymphocytes.

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*Key words: bone marrow failure (BMF), aplastic anemia (AA), paroxysmal nocturnal hemoglobinuria (PNH), HLA-B*4002 allele, HLA-A*0206 allele.*

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