Tokai J Exp Clin Med., Vol. 10, No. 2, 3, pp. 239–244, 1985

Immunogenetic Composition of 94 Consecutive Danish Families Investigated with Respect to Bone Marrow Transplantation

Svend Erik CHRISTIANSEN, Fritz JØRGENSEN, Tom KRISTENSEN, Lars U.LAMM, Peter Wendelboe HANSEN and Flemming KISSMEYER-NIELSEN

Department of Clinical Immunology, The Tissue Typing Laboratory, Aarhus Kommunehospital, Denmark

The immunogenetic composition of 94 patients needing bone marrow transplantation and their core families primarily investigated to select family bone marrow donors have been further analysed to test for association between disease and HLA-region markers. From this material it is shown, that in the primary immunogenetic analysis of the family, inclusion of mixed lymphocyte culture analysis increases donor possibilities by approximately 14% when a reciprocal negative MLC response and phenotypic HLA-DR compatibility are accepted as criteria for transplantation. Further, the results indicate, that no association between HLA and leukemia seems to exist.

INTRODUCTION

In order to minimize the clinical complications in bone marrow transplantation (BMT) (especially graft versus host disease, GvHD) HLA genotypically identical siblings are generally selected as donors. Only a few centers have reported results from the use of unrelated donors phenotypically identical to the recipient or related donors genotypically haploidentical to the recipient (1, 2). In order to avoid induction of immunologic responses between recipient and donor, a certain degree of compatibility in the HLA-region is necessary; especially compatibility in the HLA-D region (viz. the class II antigens) seems to be a prerequisite, since disparity in this region induces immune reactivity against these or other alloantigens. At The IXth International Histocompatibility Workshop and Conference and the following WHO Nomenclature Meeting it was decided to divide the HLA-D region in to the HLA-DR, -DQ and -DP loci. However, scarcity of cellular and serological typing reagents make complete typings impossible in most centers. Alternatively, the Mixed Lymphocyte Culture (MLC) analysis/typing seems to be a functionally acceptable integral for (in-)compatibility testing for the above-mentioned HLA-D region determinants with respect to transplantation. Thus, from a theoretical point of view a reciprocal negative MLC response between donor and recipient is presently the best method for evaluation of the compatibility in the HLA-D region.

Apart from a monozygotic twin, HLA genotypically identical siblings have first priority as donors. Alternatively, haploidentical siblings or parents incompatible for the HLA-A, -B and -C region, but exhibiting HLA-DR phenotypical compatibility and reciprocally low- or negative-MLC responses, or a one-way negative response against the recipient may be accepted as donors. It must, however, be realized that GvHD in these phenotypically haploidentical combinations may be severe.

In this communication we demonstrate that inclusion of MLC analysis in the first immunogenetic evaluation of these families increases the frequency of donor possibilities to almost 44% as compared to approximately 31% when the first selection is based solely on

Svend Erik CHRISTIANSEN, Department of Clinical Immunology, The Tissue Typing Laboratory, Aarhus Kommunehospital, DK-8000 Aarhus C, Denmark

This Study was supported by grants from the Danish Medical Research Council

HLA-ABC typing, i.e. that generally suitable bone marrow donors will not be identified by applying only the latter approach for selection. Further, we have performed a genetic analysis in order to identify the genetic composition of 94 consecutive local families investigated with respect to BMT, due to an impression in this laboratory as well as in other centers, that there may exist an elevated frequency of homozygocity in the HLA-D region in patients needing BMT – possibly due to a non-random distribution of the HLA-antigens in their families.

MATERIAL AND METHODS

The present material comprises 94 consecutive Danish Caucasoid families selected by probands needing bone marrow transplantation and investigated in this laboratory. Diseases indicating investigation were leukemia, aplastic anemia and severe combined immunodeficiency disease (SCID). Family compositions are displayed in Table 1 and the number and subclassification of diseases in Table 3.

IMMUNOGENETIC ANALYSIS OF PATIENTS AND CORE FAMILIES

The first immunogenetic investigation includes AB0-, Rhesus- and HLA-ABC typing by serologic techniques, GLO-typing and intrafamilial MLC-analysis. Further, the analysis includes tests for immunologic responses to mitogens and antigens, screen tests for lymphocytotoxic antibodies and intrafamilial serologically lymphocytotoxic cross-matches. If these investigations reveal any donor possibilities, a second confirmative analysis including intrafamilial MLC-analysis, mitogen responsiveness and lymphocytotoxic crossmatch is performed. In addition, other tests are performed – e.g. HLA-DR/Dw typing and PLT-testing. The above-mentioned tests are described in detail in Kissmeyer-Nielsen, F. and Kjerbye, K.E. (1967); Jørgensen, F. and Lamm, L.U. (1974); Madsen, M. *et al.* (1979); Lamm, L. U. *et al.* (1977) (4, 5, 6, 7).

SELECTION OF DONORS

According to our selection criteria, donor possibilities exist in the following situations (by decreasing priority) i) monozygotic twin, ii) HLA-genotypically identical siblings, iii) HLA-DR phenotypically identical parents or siblings with reciprocally low- or negative-MLC reactions, iv) HLA-DR heterozygous siblings or parents – to HLA-D region homozygous recipients indicated by a one-way low- or negative-MLC reaction of donor cells stimulated with recipient cells.

CALCULATIONS

Basic calculations and formulas are described by Mattiuz, P.L. *et al.* (1970). Further, conventional binomial and chi-square statistics are used.

RESULTS

Table 1 describes the distribution of offspring in the 94 families investigated. Further, the observed numbers of donor possibilities are compared to the expected values according to Mendelian inheritance within each group mentioned above. It is seen that no monozygotic twins (group 1) are present in the material. Genotypically HLA-identical siblings (group 2), are identified in frequencies in good accordance with expected values. Group 3 and 4 donors (MLC- negative but non-HLA-identical siblings or parents) are identified by inclusion of MLC family analysis. This inclusion increases the frequency of donor possibilities by approximately 14%. In total, acceptable donors can be identified in about 44% of the families investigated with respect to BMT.

The impression of non-random distribution of HLA-antigens among probands and their core families is analysed by i) investigation of the antigen distribution in the parental group and test for random matings. ii) Secondly, the HLA-antigen distribution of the probands is tested by comparison to normal material and to the Mendelian expected antigen frequencies calculated on the basis of gene frequencies in the parental group. iii) Finally, testing for abnormal frequency of homozygocity in the HLA-D region of the probands is performed in families with one or more sibs by comparing the actual number of homozygous probands to number of ditto among closest siblings.

The distribution of HLA-A and -B antigens in normal material from a Danish population ($n = 3164$) was compared to the distribution among the groups of (1) fathers, (2) mothers and (3) probands. The data are not shown, since no significant deviations from expected

frequencies were observed. Subdivision with respect to the different diseases does not alter this picture. (The HLA-A and -B antigens included in this comparison are shown in Table 2).

Table 2 illustrates the frequencies of sharing of HLA-A and -B antigens between parents. These calculations have been performed by extracting all the families in which the father possessed the antigen in question. Within these families the comparison between expected and observed numbers of matings sharing this antigen has been performed by chi-square statistics. The expected numbers of sharings are calculated by the formula:

I: $N \times p(X|\text{father}) \times p(X|\text{mother})$

where N indicates the number of families selected, X indicates the antigen in question and p the antigen frequency. Due to the selection of the families $P(X|\text{father}) = 1$. The corresponding antigen frequency among mothers equals the frequency in the normal population, i.e. the expected values equal the numbers of selected families multiplied by the population antigen frequency. Table 2 reveals some deviating chi-square values only in cases with small sample sizes ($N < 5$) indicating simple stochastic variations. The material is divided into the different diseases indicating investigation. Beside aplastic anemia (AA, $n = 5$) and severe combined immunodeficiency disease (SCID, $n = 1$) the leukemia group is subdivided into acute and chronic lymphoblast leukemia (ALL, $n = 58$, and CLL, $n = 0$) and acute and chronic myeloblast leukemia (AML, $n = 23$, and CML, $n = 7$). The results reveal no significant deviation from expected values, indicating antigenic sharing has not occurred between parents to the probands. This conclusion is valid only for the ALL- and AML-groups due to acceptable sample sizes.

The results above indicate that the parental group is representative of a normal population with respect to the HLA-A and -B antigen distribution and secondly, that mating seems to be random. However, one still may expect disturbed ratios of observed and expected Mendelian HLA-phenotypes among patients, due to zygotic selection or secondary to association between HLA and diseases. As mentioned above, results from the HLA-AB antigen distribution showed that antigen distribution among probands fits well to normal antigen frequencies. However, this Hardy-Weinberg fit is relatively uncertain due to the small sample size. A more precise test for zygotic assortment is achieved by comparison of observed antigen frequencies to expected values estimated on the basis of gene frequencies among the fathers and mothers. These gene frequencies (q) are calculated by the formula of Bernstein:

II: $q = 1 - \sqrt{1 - P}$

where p indicates antigen frequency. Using the Hardy-Weinberg principle, the expected gene frequencies among probands are estimated and compared to observed frequencies. The expected frequencies are calculated according to formula II, where p now equals:

III: $p = 1 - [(1 - q(X|\text{father})) \times (1 - q(X|\text{mother}))]$

where X indicates the antigens and q the gene frequencies among the fathers and mothers, respectively. From Table 3 it appears that observed values fit very well to expected values—indicating that no disturbance in Mendelian segregation has occurred.

Our impression of many aberrant MLC-family patterns—especially a high frequency of homozygocity in the HLA-D region among probands—was tested by comparing frequency of homozygocity between proband and closest sibling (with respect to birth). Homozygocity was in this analysis defined as a one-way negative MLC-typing response against father and mother as responders. Results are illustrated in Table 4, which also contains sex ratios in the different disease groups. The material is in this analysis somewhat reduced, due to required number of informative families (minimum requirements: father, mother and one sib). This implies that only the disease groups ALL and AML could be investigated with a certain degree of security. It appears that no abnormalities are revealed among probands when compared to closest sib. This might indicate that the trait under investigation does not seem to be recessive or associated to the HLA-region. Incidentally, the sex ratio in the acute leukemias is very close to 2:1 in (dis-)favour to the males, which might indicate an inverse association to the X-chromosome.

CONCLUSION

The following conclusions can be drawn:

i) Inclusion of intra-familial MLC-analysis in the primary immunogenetic analysis of core-families to patients needing BMT increases the number of acceptable donor possibilities significantly.
ii) A normal distribution of the HLA-A, -B antigens exists in the parental population and that mating appears to be random.
iii) There exists a normal HLA-antigen distribution among patients and no zygotic assortment is established with respect to HLA.
iv) There appears a normal frequency of homozygocity in the HLA-D region among patients when compared to closest sibling.

On the basis of these conclusions it seems clear that no association between leukemia and HLA exists. No conclusions concerning severe combined immunodeficiency disease and aplastic anemia could be drawn due to small sample sizes.

REFERENCES

1) Clift RA, Hansen JA, Thomas ED, Buckner CD, Sanders JE, Mickelson EM, Storb R, Johnson FL, Singer JW and Goodell BW: Transplantation 28.3: 235–242, 1979
2) Duquesnoy RJ, Zeevi A, Marrari M, Hackbarth S and Camitta B: Transplantation 35.6: 566–571, 1983
3) Mattiuz PL, Ihde D, Piazza A, Ceppelini R and Bodmer WF: In: Histocompatibility Testing 1970 (ed., P.I. Terasaki) Munksgaard, Copenhagen, Denmark, pp. 193–205, 1970
4) Madsen M, Johnsen HE and Kissmeyer-Nielsen F: Tissue Antigens 13, 135–142, 1979
5) Lamm LU, Weitkamp LR, Jensson O, Bruun Pedersen G and Kissmeyer-Nielsen F: Tissue Antigens 11: 132–138, 1978
6) Jørgensen F and Lamm LU: Tissue Antigens 4: 482–494, 1974
7) Kissmeyer-Nielsen F and Kjerbye KE: In: Histocompatibility Testing 1967 (eds., E.S. Curtoni, P.L. Mattiuz and R.M. Tosi) Munksgaard, Copenhagen, Denmark, pp. 381–385, 1967

Table 1 Composition and donor frequencies in 94 consecutive Danish families investigated with respect to BMT

No. of probands with			≥ one HLA-identical sibling donor			≥ one non-HLA-ABC-identical among siblings		Donor possibility among parents		No donor possibility	
			abs.	rel.	expt.	abs.	rel.	abs.	rel.	abs.	rel.
0	siblings	7	0	0,0%	0%	0	0,0%	1	14,3%	6	85,7%
1	-	42	10	23,8%	25%	1	2,4%	5	11,9%	26	61,9%
2	-	31	11	35,5%	44%	0	0,0%	2	6,5%	18	58,1%
3	-	7	2	28,6%	58%	2	28,6%	1	14,3%	2	28,6%
4	-	2	1	50,0%	68%	1	50,0%	0	0,0%	0	0,0%
5	-	3	3	100,0%	76%	0	0,0%	0	0,0%	0	0,0%
6	-	2	2	100,0%	82%	0	0,0%	0	0,0%	0	0,0%
total		94	29	30,9%	36%	4	4,3%	9	9,6%	52	55,3%

Selection of donors primarily by HLA-ABC typing reveals 30,9% donor possibilities. Inclusion of MLC analysis in the initial investigation increases this frequency to 44,8% (30,9 + 4,3 + 9,6).

Table 2 Antigen sharing between parents in different disease groups

HLA-	p[2] %	ALL, 57(58)[1] numbers[4] of sharing[5]				AML, 15(23) numbers of sharing				CML, 3(7) numbers of sharing				A.A, 4(5) numbers of sharing				SCID, 1(1) numbers of sharing			
		N[3]	exp.	obs.	χ^2	N	exp.	obs.	χ^2	N	exp.	obs.	χ^2	N	exp.	obs.	χ^2	N	exp.	obs.	χ^2
A1	31,38	23	7.22	7	0,01	3	0,94	2	1,20	1	0,31	0	0,31	2	0,63	0	0,63	1	0,31	0	0,31
A2	53,41	30	16,02	17	0,06	9	4,81	5	0,01	2	1,07	1	0,00	1	0,53	1	0,42	1	0,53	1	0,42
A3	27,69	14	3,88	5	0,32	3	0,83	2	1,65	0	-	-	-	2	0,55	0	0,55	0	-	-	-
A9	17,51	13	2,28	2	0,03	4	0,70	1	0,13	0	-	-	-	1	0,18	0	0,18	0	-	-	-
A10	9,01	3	0,27	0	0,27	1	0,09	0	0,09	0	-	-	-	0	-	-	-	0	-	-	-
A11	10,46	2	0,21	0	0,21	0	-	-	-	0	-	-	-	1	0,10	1	8,10	0	-	-	-
A19	18,81	8	1,50	0	1,50	4	0,75	1	0,08	2	0,38	0	-	0	-	-	-	0	-	-	-
A28	8,79	7	0,62	0	0,62	2	0,18	1	3,74	0	-	-	-	0	-	-	-	0	-	-	-
A b1	-	-				-				-				-				-	-	-	-
B5	10,81	3	0,32	1	1,45	4	0,43	0	0,43	1	0,11	0	0,11	1	0,11	1	7,20	0	-	-	-
B7	28,79	15	4,32	5	0,11	6	1,73	4	2,98	1	0,29	0	0,29	0	-	-	-	1	0,29	1	1,74
B8	23,10	16	3,70	2	0,78	5	1,16	1	0,02	1	0,23	0	0,23	1	0,23	0	0,23	1	0,23	0	0,23
B12	25,09	11	2,76	1	1,12	4	1,00	1	0,00	1	0,25	1	2,25	1	0,25	0	0,25	0	-	-	-
B13	4,02	1	0,04	0	0,40	0	-	-	-	0	-	-	-	0	-	-	-	0	-	-	-
B14	3,89	1	0,04	0	0,04	1	0,04	0	0,04	1	0,04	0		0	-	-	-	0	-	-	-
B15	17,89	9	1,61	10	1,61	1	0,18	0	0,18	0	-	-	-	1	0,18	0	0,18	0	-	-	-
B16	5,22	4	0,21	1	2,97	1	0,05	1	18,05	0	-	-	-	0	-	-	-	0	-	-	-
B17	8,19	9	0,74	0	0,74	0	-	-	-	0	-	-	-	0	-	-	-	0	-	-	-
B18	7,40	5	0,37	0	0,37	0	-	-	-	0	-	-	-	0	-	-	-	0	-	-	-
B21	3,29	1	0,03	0	0,03	1	0,03	0	0,03	0	-	-	-	0	-	-	-	0	-	-	-
B22	3,51	1	0,04	0	0,04	0	-	-	-	0	-	-	-	0	-	-	-	0	-	-	-
B27	8,41	5	0,42	0	0,42	0	-	-	-	0	-	-	-	0	-	-	-	0	-	-	-
B35	13,08	9	1,18	0	1,18	1	0,13	1	5,82	0	-	-	-	2	0,26	2	11,64	0	-	-	-
B40	18,30	10	1,83	2	0,02	0	-	-	-	1	0,18	1	3,74	1	0,18	1	3,74	0	-	-	-
B b1	-	-																			

1) numbers of families, - reduced due to requirement of both a father and a mother. 2) antigen frequencies (%) in the normal population. 3) observed numbers of families with fathers phenotypically positive antigens. 4) expected numbers of antigen sharings. 5) observed numbers of ditto.

Table 3 Test for zygotic assortment

Antigen	PARENT		PROBANDS			
	gene freq. father	gene freq. mother	exp. antigen freq.	exp. gene freq.	obs. antigen freq.	χ^2
A1	0.209	0.163	0.338	0.186	0.2903	0.008
A2	0.329	0.297	0.528	0.313	0.6022	0.009
A3	0.127	0.170	0.275	0.149	0.2283	0.010
A9	0.127	0.103	0.217	0.115	0.2283	0.001
A10	0.025	0.023	0.047	0.024	0.0323	0.007
A11	0.019	0.059	0.077	0.039	0.0538	0.010
A19	0.094	0.100	0.185	0.097	0.2088	0.003
A28	0.058	0.041	0.097	0.050	0.1290	0.008
A bl	Σ: 0.989	Σ: 0.956		Σ: 0.973		Σ: 0.054
B5	0.058	0.023	0.080	0.041	0.0968	0.003
B7	0.156	0.176	0.305	0.166	0.2581	0.009
B8	0.163	0.129	0.271	0.146	0.2366	0.005
B12	0.120	0.129	0.234	0.125	0.2258	0.000
B13	0.006	0.023	0.029	0.015	0.0323	0.000
B14	0.019	0.017	0.036	0.018	0.0215	0.010
B15	0.071	0.072	0.138	0.072	0.1290	0.025
B16	0.032	0.029	0.060	0.031	0.0538	0.001
B17	0.058	0.029	0.085	0.044	0.1183	0.009
B18	0.032	0.023	0.054	0.028	0.0538	0.000
B21	0.013	0.035	0.048	0.024	0.0323	0.008
B22	0.013	0.029	0.036	0.018	0.0430	0.001
B27	0.032	0.047	0.077	0.040	0.1075	0.009
B35	0.078	0.041	0.116	0.060	0.1196	0.000
B40	0.078	0.136	0.203	0.107	0.2796	0.021
B bl .	Σ: 0.928	Σ: 0.934		Σ: 0.932		Σ: 0.101

Test for zygotic assortment based on Hardy-Weinberg principle, but using the actual gene frequencies among brothers and fathers. Expected and observed values among probands are compared by chi-square statistics.

Table 4 Test for homozygocity in the HLA-D region among probands by comparison to closest sib

MLC-pattern		ALL	AML	CML	SCID	AA
pt.	c.s.	n = 50	n = 13	n = 2	n = 1	n = 3
+	–	1	2	0	0	1
–	+	2	2	0	0	0
+	+	1	0	0	0	0
–	–	46	9	2	1	2
♂		40	16	7	0	2
♀		18	7	0	1	3

\+ indicates HLA-D region homozygocity.
No deviation between proband and healthy closest sibling with respect to frequency of homozygocity in the HLA-D region was observed. Sex ratio (♂/♀) in leukemia group equals 2:1.
pt. = patient.
c.s. = closest sibling.