

## Typing for MLC (LD): I. Lymphocytes From Cousin-Marriage Offspring as Typing Cells

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**W**HEN it was observed that the majority of SD-identical unrelated individuals stimulated each other in the MLC test, it was postulated in agreement with earlier suggestions by others that MLC activation might be coded by a locus separate from the LA and Four loci.<sup>1-4</sup> The existence of such a MLC locus is now generally accepted.<sup>5,6</sup> About 10% of the MLC tests between SD-identical unrelated persons are negative, indicating that a linkage disequilibrium exists between the MLC locus and the LA and Four loci. It is furthermore likely that SD-identical unrelated individuals who are also LD-identical carry MLC determinants that occur rather frequently.

In this study we tried to recognize the MLC-locus determinants. To this end we used lymphocytes from double-homozygous offspring of cousin marriages. As shown in Table 1, it is possible with these cells to indicate whether another cell carries the same MLC determinant and if so whether the determinant occurs in a homozygous or heterozygous form. We will refer to the lymphocytes obtained from the double-homozygous cousin-marriage offspring as typing cells. Typing cells are for LD the equivalent of typing sera for SD.

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In this and the following paper we will describe a systematic approach to recognize the MLC (LD) determinants. This paper describes the definitions of five MLC determinants by MLC or LD testing. The basic principle of this approach is that if two individuals carry the same MLC determinants they will not simulate in the MLC test.

### MATERIALS AND METHOD

Test cells were selected from a file of more than 20,000 HL-A-typed individuals. Two suitable cell populations were used for this experiment: (1) cells of unrelated SD- and LD-identical individuals and (2) cells of children from cousin marriages that are homozygous at both HL-A loci. Both serotyping for SD and MLC testing for LD were done as described before<sup>7-10</sup> The combinatorial experiment was performed in a microtechnique<sup>11</sup>

### RESULTS

SD-identical individuals (137 persons) were first tested within their respective SD-identical groups in bidirectional and unidirectional MLC tests; 24 persons were repeatedly MLC-negative, with 1 or 2 SD-identical individuals. From 6 MLC-negative combinations with different HL-A phenotypes, we selected 8 individuals for the test (group A). From 52 cousin marriages we were able to select 11 children homozygous at both HL-A loci, with 6 different HL-A phenotypes. From each family that had homozygous children, the lymphocytes from one homozygous child were used for the experiment (group B). In the combinatorial experiment of group A and group B, 10 of these 14 cells were informative because they were involved in a MLC-negative combination. The other 4 cells were positive with all other cells in the test. Table 2 shows the results of the uni-

**Table 1. Typing for MLC (LD): Description and Interpretation of MLC Results Using Typing Cells Homozygous at LA, Four, and MLC (SD and LD)**

Pattern Number	Cell to Be Typed	Homozygous Typing Cell	MLC Reaction	MLC Type of X
1	X	a/a <sub>m</sub>	neg	a/a a/b or a/c etc.
2	X	a/a <sub>m</sub>	pos	b/c etc.
3	X <sub>m</sub>	a/a	neg	a/a
4	X <sub>m</sub>	a/a	pos	a/b or a/c or b/c etc.

Interpretation: 1 + 3, X = a/a; 2 + 3, should not occur; 1 + 4, X = a/b or a/c etc.; 2 + 4, X = b/c, d/e etc.

lateral MLC test between cells of these 10 informative individuals. The cells being MLC-negative between each other are surrounded by a solid line or a broken line when the cells of donor L are involved. The cells within each square share one or two MLC (LD) determinants. The determinant LD1 is shared by the cells G, H, O, and L, the weak stimulation between G and H on the one hand and O and L on the other (while O and L are mutually negative) suggests that O and L might share a determinant LD2. The determinant LD3 is shared by the cells L and K, while LD5 is present probably in a homozygous form in M and only on one chromosome in N (Table 1). The fourth LD determinant (LD4) is seen on the SD-identical cells I and F. The cells D and L might share a sixth LD determinant, but further confirmation is needed.

#### DISCUSSION

This study is based on the assumption that if two normal (unrelated) individuals do not stimulate each other in the MLC test, this implies that they share MLC determinants. In this manner the first three individuals were found who shared one or more MLC determinants.<sup>3,12</sup> The same approach was used by Mempel and Albert,<sup>13</sup> who not only confirmed our findings but extended them by using cells from SD and

LD double-homozygous offspring of unrelated parents. We have done the same, but found that these cells were often not homozygous for LD.

For this reason we collected lymphocytes from cousin-marriage offspring which were homozygous not only for SD but also (barring crossover) for LD, as the two haplotypes came from the same great-grandparent. With this approach we were able to recognize four of the five MLC determinants of Table 2.

The reaction with the typing cell L should be considered as preliminary. Even if one assumes that a crossover in the parent or grandparents of L has occurred, it is difficult to interpret the data obtained with L without allowing for the possibility that one chromosome can carry several (even more than two) LD determinants. There is a priori nothing against this possibility, which would be in agreement with the findings for the Ir genes, but because of the basic importance of such an observation, we feel that further confirmation is needed.<sup>14</sup> Another explanation could be that one of the two 1,8 haplotypes was not inherited from a great-grandparent but was introduced by one of the grandparents. Especially with frequently occurring haplotypes such as 1,8, this is a possibility one should consider. Lebrun et al.<sup>15</sup> have reported unexpected positive unilateral MLC tests between SD-homozygous children as stimulating cells and their parents as responder cells. The mechanism by which this is brought about is as yet unclear, but the use of typing cells is only possible if this phenomenon does not interfere. Our findings so far indicate that when typing cells are used that are SD- and LD-homozygous because they are obtained from cousin marriages, this is at least in some instances not a problem. We would like to submit that just as there exist in serology poor and good sera, in LD typing we will find poor and good typing cells. Typing cells from cousin marriages seem to be better than those from

Table 2. Typing for MLC (LD)

	G <sub>m</sub>	H <sub>m</sub>	O <sub>m</sub>	L <sub>m</sub>	K <sub>m</sub>	M <sub>m</sub>	N <sub>m</sub>	I <sub>m</sub>	F <sub>m</sub>	D <sub>m</sub>	
G	0	0	+	+	+++	+++	+++	+++	+++	+++	1, 2, 8, W10
H	0	0	+	+	++	++	+++	+++	+++	++	1, 2, 8, W15
O	0	+	0	0	++	++	+++	++	++	++	1, 2, 8, 12
L	+	(+)	(+)	0	+	++	++	(+)	+	+	1, 8/1, 8
K	+++	++	++	0	0	(+)	++	++	++	+++	2, W15/2, W15
M	++	++	+++	++	++	0	++	++++	++++	+++	3, 7/3, 7
N	++	+	++	+++	++	0	0	++	+++	++	2, 3, 7, W10
I	+++	+++	+++	+	+++	+++	+++	0	0	+++	1, 2, 8, W10
F	+++	+++	+++	++	+++	+++	+++	0	0	+++	1, 2, 8, W10
D	++	++	+++	+	+++	+++	+++	+++	+++	0	1, W30/W31, 8, W10

It is assumed that the cells G, H, O, and L carry MLC determinant LD1 and O and L MLC determinant LD2, which is in this panel included in LD1. It is possible that L and K share determinant LD3. M carries a determinant LD5 in a homozygous form that is also present on N in a heterozygous form. The SD-identical cells I and F share at least one determinant called LD4.

0 = 0-2,000 c.p.m.; (+) = 2,000-3,000 c.p.m.; + = 3,000-7,500 c.p.m.; ++ = 7,500-15,000 c.p.m.; +++ = > 15,000 c.p.m.

unrelated couples. The above approach provides us also with cell combinations that are LD-identical but different for SD (e.g., cells G, H, O, and L).

Although the procedure used in this experiment is probably the most reliable one for recognizing MLC determinants, it is a cumbersome and time-consuming method

In the next paper we will report on a quicker serologic approach. Final proof that we are recognizing MLC determinants will be obtained if cells that show an identical pattern with typing cells can be shown not to stimulate each other in the MLC test.

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