stretch of the Banning–Mission Creek fault has had no earthquakes above magnitude 4 since at least 1932 (*1*), it clearly has the potential for producing moderate earthquakes in the future.

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HL-A LD (Lymphocyte Defined) Typing: A Rapid Assay With Primed Lymphocytes

Abstract. *When human lymphocytes are cultured for 9 to 14 days with stimulating cells of a family member differing by a single HL-A haplotype they become "primed" to recognize specific HL-A LD (mixed lymphocyte culture) antigens. These primed lymphocytes respond specifically and rapidly when "restimulated" with cells of a person that contain the same LD antigens as those of the priming haplotype. Specific HL-A LD antigens can be detected within 24 hours by this primed LD typing.*

Rejection of a transplanted tissue or organ is initiated when the graft recipient's immune system recognizes genetically controlled "foreign" antigens on the grafted tissue. In humans a single genetic region, called HL-A or the major histocompatibility complex (MHC), appears to control the majority of strong antigens important in graft rejection (*1*). Minimizing antigenic disparity between donor and recipient (matching) for the MHC increases the probability that the transplant will survive.

Two methods are commonly used for detecting antigens associated with the major histocompatibility complex: (i) serological testing for HL-A SD (serologically defined) antigens, and (ii) mixed lymphocyte culture (MLC) tests that define disparity at an HL-A LD (lymphocyte defined) locus (or at several loci). In MLC tests, lymphocytes from one individual (the "responder") are cultured for 4 to 7 days with "stimulating" lymphocytes from another individual. To prevent their proliferation, stimulating cells are treated with mitomycin C or x-rays before they are mixed. When the stimulating cells are from unrelated persons or family members whose MHC is different from that of the responder, the untreated lymphocytes proliferate; this proliferation is assayed by incorporation of tritiated thymidine into the proliferating cells. All SD and LD loci are closely linked genetically, and within families they are inherited as a unit called a haplotype. However, since the SD and LD loci are genetically separable (*2*), both the serological and MLC tests are necessary in the evaluation of the MHC relationship between two individuals.

In transplants between SD matched persons who are not related, the frequency and severity of rejection generally have been much greater than in transplants between siblings with identical MHC's (*3*); moreover, most unrelated individuals who are SD identical are LD disparate when tested by the MLC assay. There is some evidence that MLC matching for HL-A LD antigens may be useful for predicting the success of a transplant (*4*).

Two major obstacles prevent the widespread use of MLC tests for transplant matching. (i) The result cannot be obtained in less than 4 to 5 days—a time that exceeds the limits for cadaver kidney preservation. (ii) Although MLC tests can identify individuals that are matched for their LD antigens, it does not indicate which specific LD antigens the two persons bear; therefore lymphocytes from all potential donors must be tested in MLC with lymphocytes from all potential recipients. This last problem would be alleviated by an "LD typing" method (analogous to serological typing that has been done for blood groups and HL-A SD antigens) that would identify specific LD antigens. Because LD typing would preclude the necessity of the recipient and potential donor being present in the same MLC-testing laboratory at the same time, the LD type of any potential tissue donor could be determined, and the donor organ or bone marrow could be sent to an LD matched recipient at any center in the world.

One approach to LD typing has been MLC testing with stimulating cells homozygous for an LD haplotype (*5*). Such cells should fail to stimulate cells of individuals bearing the LD antigens of the homozygous haplotype, since no foreign antigens are presented. LD antigens can be identified in this manner, but a homozygous cell donor must be found for every identifiable LD haplotype; rare LD haplotypes will be particularly difficult to obtain in homozygous form. Moreover, this test also requires several days. In other approaches to LD typing antiserums are used (*6*) in an attempt to define LD serologically. However, it is not clear whether these antisera actually detect the LD antigens.

We have developed an LD typing method, designated primed LD typing (PLT), that seems to have some advantages over these other methods. (i) PLT appears to recognize LD. (ii) Results are obtained in less than 2 days, usually within 24 hours. (iii) Even very rare LD haplotypes can be conveniently typed. This method is based on the finding (*7*) that lymphocytes stimulated in a primary MLC exhibit an accelerated secondary response when stimulated 14 days later.

PLT cells are prepared in MLC; cells of individual A are "primed" by stimulating

them with cells treated with mitomycin C (B_m cells) obtained from a person who differs from A by only a single MHC haplotype (for example, a parent or child of A). When the MLC proliferative response is essentially completed (after 9 to 14 days), the remaining cells are used as the PLT cells. These cells are "restimulated" with B_m cells or with cells from any other person who is to be LD typed, and their proliferation is assayed by uptake of tritiated thymidine. On restimulation with B_m cells, or with cells of family members having the same MHC that A recognized on B_m, a significant proliferative response is observed after as few as 24 hours. Stimulation by cells of unrelated persons ranges from none to that seen with B_m; those persons whose cells restimulate as well as B_m presumably bear LD antigens very similar to those recognized on B_m by A. Thus PLT cells should identify individuals bearing specific LD antigens. Many populations of PLT cells, each specific for antigens of a different LD haplotype, can easily be obtained via primary MLC's with cells from the appropriate members of different families.

All cells used in these studies were human peripheral blood leukocytes purified by the Ficoll-Hypaque technique. In the primary MLC, 1×10^7 responding cells were cultured in Falcon flasks with 1×10^7 stimulating cells from a family member differing from the responder by a single MHC haplotype. After 9 to 14 days, these cultures contain PLT cells, which are either restimulated or preserved frozen (liquid nitrogen temperature). In the secondary or restimulation phase, 5×10^4 PLT cells and 1×10^5 x-irradiated stimulating cells were cultured together in a volume of 0.15 ml. Proliferation was assayed by incorporation of tritiated thymidine ending at 24 or 48 hours (*8*).

Lymphocytes from members of a single family were cultured in four different primary MLC's (Table 1). After 13 days, cells from these cultures were restimulated with x-irradiated cells from each of the two parents and six children. The four MHC haplotypes in the family, determined by SD typing, were designated with letters "a" through "d". For each PLT cell (that is, each column of the table), maximum restimulation is caused only by those family members having the haplotype recognized in the primary cultures; cells of other family members restimulate to varying lesser degrees.

That even this lesser stimulation is caused by the MHC is demonstrated in Table 1, most clearly in column 7. Although MHC identical to C6, siblings C4 and C5 would be expected to differ from C6 for many of their other loci. Any antigens potentially recognizable by C6 on cells of C4 and C5 must be present on cells of one of the parents. However, lymphocytes primed simultaneously with cells of both parents did not respond to MHC-identical siblings. This, with corroborating results from two other families, suggests that non-MHC antigens do not by themselves cause restimulation.

In other experiments we have shown that MHC-controlled restimulation is not caused primarily by products of the two best known SD loci. First, unrelated persons having the same SD antigens presented by B_m cells in the primary culture have often restimulated very little compared with B_m cells; thus shared SD antigens are not sufficient to cause a good secondary response. Second, persons whose SD antigens do not cross-react with those of B_m have restimulated as well as B_m; thus shared SD antigens are not necessary to cause a good secondary response. Because we lack the necessary serums, we have not been able to examine the influence of the third SD locus; however, we have no reason to expect this SD locus to behave differently from the other two (*9*).

If our method is to be used for LD typing, it is essential to demonstrate that it does identify LD antigens on unrelated individuals. Siblings sharing a single MHC haplotype stimulate each other less on the average in a standard MLC than siblings differing for both MHC haplotypes (*10*). Thus unrelated persons postulated to share LD antigens by PLT testing should stimulate each other less on the average in a standard MLC than persons who do not share LD.

Results from experiments testing this hypothesis are presented in Table 2. Lymphocytes from a family were used to prepare PLT cells for all four of the family's MHC haplotypes. For example, PLT cells from a primary culture with the mother as responder (haplotypes y and z) and a son Fred as stimulator (haplotypes w and y) were used to define the LD antigens of haplotype w. PLT cells prepared by using Fred as responder and the mother as stimulator defined LD antigens of haplotype z. Cells from 11 unrelated persons were tested as stimulators in the PLT test; restimulation by these cells was 3 to 104 percent of that elicited by cells from the specific family member who was the stimu-

Table 1. Results of 1- and 2-day restimulation (counts per minute) of PLT cells from a single family, by stimulating cells from members of that family. Numbers are mean count per minute from triplicate cultures.

Primary MLC	24-hour PLT			51-hour PLT			
Responder	C6=bd*	C5=bd	F=ab	C6=bd	C5=bd	F=ab	C6=bd
Stimulator	F=ab	C3=bc	C6=bd	F=ab	C3=bc	C6=bd	F + M = ab + cd
Haplotype recognized	a	c	d	a	c	d	a + c
PLT							
Stimulators for PLT:							
F=ab	2185	239	0	30875	11523	0	26133
M=cd	172	1863	361	6356	26190	7335	19779
C1=ac	1941	1963	33	28910	26744	1225	30868
C2=ad	2326	281	550	29757	8427	7303	24228
C3=bc	299	1807	–64	6604	24999	859	24599
C4=bd	36	–8	455	–50	429	6634	–85
C5=bd	–26	0	437	–265	0	5354	–223
C6=bd	0†	39	565	0	349	7390	0

*Family members are designated by capital letters, and their MHC haplotypes by lowercase letters. For example, child C6 has MHC haplotypes b and d. †For this column, restimulation (counts per minute) by C6, who was the responder in the primary culture, was subtracted from all numbers in the column. These "background" counts, ranging from 307 to 733 for the seven columns, were subtracted from all numbers in each column.

Table 2. Average stimulation (counts per minute) in standard MLC's of family responders by unrelated persons cross-reacting highly with both or one of the responder's LD haplotypes or showing no cross-reaction. For each responder, the 11 unrelated stimulators were each placed in one of the three categories indicated. The first column of figures, for example, shows the average response of the father to unrelated persons "highly cross-reactive" in PLT tests defining antigens of (i) both haplotypes w and x, (ii) haplotype w or x but not both, and (iii) neither w or x. Numbers in parentheses are numbers of stimulators included in the calculation of each average.

Number of responder's LD haplotypes, with which stimulator cross-reacts highly	Stimulation (count/min) of family responders, and responder's LD haplotypes			
	Father wx	Fred wy	Bill wz	Mother yz
Both	3311(1)	8857(1)	2139(1)	
One	9390(4)	13136(3)	4879(3)	4783(4)
None	13529(6)	15653(7)	9607(7)	10771(7)

lator in the primary culture. Unrelated persons were arbitrarily considered highly cross-reactive for a given LD haplotype if they restimulated at least 80 percent as much as the average for family members known to have the specific haplotype.

Table 2 shows the average response, in a standard 5-day MLC, of each family member to those unrelated persons cross-reacting highly in PLT tests with both, one, or neither of the LD haplotypes of that family member. For each family member the trend of MLC response magnitudes is in the predicted direction: Those persons that are highly cross-reactive by PLT for both LD haplotypes of a particular responder stimulate that responder the least. Similarly those that are highly cross-reactive with one haplotype stimulate less than those which do not cross-react highly with either haplotype. The probability of finding this consistent trend by chance alone is less than .0025. It is important to note that the observed low stimulation in the standard primary MLC by certain unrelated individuals "highly cross-reactive" for one or two LD haplotypes was predicted from the high stimulation by those same individuals in specific secondary MLC combinations (PLT tests).

As a further test of the ability to do LD typing by our method, two groups of three unrelated persons were chosen by PLT criteria. One group consisted of three persons that were all "highly cross-reactive" for one LD haplotype, and the other three persons were "highly cross-reactive" for a different LD haplotype. Standard MLC tests were done between all possible pairs from these six persons. The average MLC (in counts per minute) when responder and stimulator were from opposite groups was 7550; the average when responder and stimulator were from the same group was only 5154, about two-thirds as great. This difference was highly significant ($P < .001$), demonstrating that, on the average, individuals shared more LD antigens with persons in their own group than with persons in the other group. These results, and those in Table 2, show that our method does identify cell components that cause MLC stimulation—that is, that it does type for LD antigens.

Because of the rapidity of the method, and the ease of obtaining PLT cells, the PLT test can easily be applied in an international LD typing program. PLT cells for any LD antigen can be obtained, with the use of cells from appropriate members of any family in which that antigen is found. Since we have found that fresh PLT cells and those preserved by freezing give quantitatively similar results, the freezing methods of Netzel *et al.* (*11*) could be used to prepare typing trays that contain frozen PLT cells defining many different LD antigens. The LD type of any person could be rapidly determined by stimulation of the different PLT cells in this panel.

The ability to identify LD antigens may be important in other ways besides its obvious application to transplant matching. LD antigens are controlled by the same region of the MHC (*12*) that controls the magnitude of immune responses to certain specific antigens (*13*). Moreover, the LD region has been associated with susceptibility to oncogenic viral infections in the mouse (*14*), and certain LD antigens have been associated with disease of immune etiology in man (*15*). Thus PLT may be an important criterion for diagnosing human disease, as well as a specific probe allowing greater understanding of the function and genetic fine structure of the MHC in man and in other species.

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Lactosyl Ceramidosis: Normal Activity for Two Lactosyl Ceramide β-Galactosidases

Abstract. *Lactosyl ceramide β-galactosidase activities in the fibroblasts from the previously described patient with so-called "lactosyl ceramidosis" were reexamined with the two recently developed assay methods which appear to measure two genetically distinct enzymes that can degrade this substrate. No deficiency of either of the lactosyl ceramide–cleaving enzymes was observed. In addition, sphingomyelinase activity was only one-sixth of normal, while all other enzymes examined were within the normal ranges.*

In 1970 Dawson and Stein (*1*) reported on a child with a severe neurovisceral disease which has come to be called "lactosyl ceramidosis." This child had symptoms which included mental regression, spasticity, cerebellar ataxia, increased deep tendon reflex, and increasing redness of the maculae. Biopsy of liver demonstrated storage of glycolipids, especially glucosyl ceramide, lactosyl ceramide (lac-cer), and ganglioside GM_{M3}. Lactosyl ceramide was also increased in red cells, plasma, bone marrow, urine sediment, and brain (*1*). The concentration of sphingomyelin in these tissues was within normal limits according to the initial and subsequent report (*1, 2*).