cellular components. In a system such as dry seeds, where repair mechanisms are probably inoperative, this damage could accumulate to such an extent that cell death and consequent loss of embryo viability occur. In hydrated systems, where membrane turnover (*1*), and thus presumably repair, does occur, membrane damage by free radical peroxidation probably does not accumulate (*14*). However, accumulation of this type of damage might well be important in the terminal deteriorative senescence changes in such systems if repair mechanisms fail with age.

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Serological Detection of Mixed Lymphocyte Culture Identity between Cells That Differ by One HL-A Haplotype

Abstract. *Antibody mediated cell dependent immune lympholysis (ABCIL), an extremely sensitive serological technique for detecting tissue sensitization, was used in a family segregation study. Two serums capable of demonstrating ABCIL were used to identify members of the family who differed by one HL-A haplotype but whose cells did not stimulate in mixed lymphocyte culture. Absorption study of one serum indicated that the ABCIL reaction of that serum was directed against a factor, independent of HL-A, that might be responsible for lymphocyte stimulation in mixed lymphocyte culture. Thus the ABCIL technique may be used to detect lymphocyte-defined gene products.*

Antibody mediated cell dependent immune lympholysis (ABCIL) is a sensitive technique for detecting evidence of tissue sensitization in humans. We have shown previously that the antibody in the ABCIL reaction is different from that reacting in the complement dependent cytotoxicity test (*1*), that it is frequently positive in transfused patients who are nonresponders by the cytotoxicity test (*2*), and that the ABCIL specificity is not defined by HL-A (*3*). We now have evidence from studies of one large family that ABCIL reactivity segregates with the HL-A antigens. Our data also show that ABCIL reactivity may be used to detect mixed lymphocyte culture (MLC) factors. These MLC factors or lymphocyte-defined (LD) determinants have been postulated to exert as much or perhaps more control than HL-A over the outcome of tissue grafting between unrelated individuals (*4*).

The cells of the grandparents of the family were first screened for ABCIL reactions with the use of several serums whose specificity in the microlymphocytotoxicity test was known. Of the 22 serums so screened, two gave positive reactions with cells of only one grandparent, a different grandparent for each serum. As judged by the complement dependent lymphocytotoxicity test both serums have antibody against HL-A12. However, there is no HL-A12 in this family, and these ABCIL reactions cannot be explained in terms of their known HL-A reactivity.

With serum 1, the cells of the grandfather were negative, but the cells of the grandmother were positive, whereas the converse is true with serum 2 (Fig. 1). These two serums were then used with the cells of some of their children, their grandchildren, and their daughter-in-law Ruth. As is shown in Fig. 1, the positive ABCIL reactions of serum 1 can be shown to segregate with haplotype *c* (and haplotype *x*) if we assign *a* and *b* as haplotypes 2, W15 and 2, 5 of the father; and *c* and *d* as haplotypes 1, W17 and 2, W5 of the mother;

and *x* and *y* as haplotypes 3, W5 and W32, W14 of the daughter-in-law. Similarly, positive ABCIL reactions of serum 2 segregate with haplotype *a*, although serum 2 is also weakly positive with the cells of grandchild Bruce, who carries haplotype *d* rather than the expected haplotype *a*. Apart from this weak reaction with serum 2, which we cannot explain, all other positive reactions are strong (*5*). Thus, in this family, these two serums contain ABCIL reactivities which segregate with two distinct HL-A haplotypes, *a* and *c*, one from each grandparent, and yet the complement dependent cytotoxic specificities are unrelated to the HL-A antigens of these haplotypes. These two haplotypes are both inherited by Eleanor and Don (Fig. 1).

By chance, each of these two ABCIL reactive serums also differentially mark Terry and Ruth, two parents in the second generation, although serum 1 is now marking haplotype *x* from outside the family (not haplotype *c*), and haplotypes *a* and *x* are inherited by Brian and Laurie in the third generation. That haplotypes *c* and *x* share characteristics is further established by absorption studies. These showed that ABCIL reactivity of serum 1 against haplotype *c* (Eleanor) could be removed by lymphocytes bearing haplotype *x* (Laurie), and vice versa. Terry's cells were used as controls in these absorption studies and did not remove ABCIL activity from serum 1. Results with platelet absorption were less conclusive, but suggested that ABCIL activity could not be absorbed by platelets at the dosage used (*6*). On the basis of these lymphoid cell absorptions, we believe that a factor linked to haplotype *c* may be identical to the factor linked to haplotype *x*, and that both are expressed on the lymphocyte membrane.

Hence, in this family, two HL-A identical sib pairs, Eleanor and Don in the second generation and Brian and Laurie in the third generation, have cells that behave identically by the ABCIL test with these two selected serums, both directly and by absorption. These two pairs of HL-A identical siblings have one HL-A haplotype in common: HL-A2 and W15, derived from the grandfather, and marked by serum 2. The other HL-A haplotypes, however, are different and are derived from unrelated sources; nevertheless both are marked by serum 1.

It is generally accepted that the genetic determinants responsible for the stimulation in MLC are separate from, but closely linked to, the HL-A region (7). Recently certain MLC determinants in humans have been typed with the use of homozygous HL-A identical cells that do not stimulate in MLC. These cells are assumed to be homozygous for LD determinants, or homozygous for the gene products of the MLC locus. Such cells are then arbitrarily assigned an LD (or MLC) number, or letter, and can be used (after mitomycin treatment or x-irradiation) as stimulators in the MLC. They thereby are probes for one of the MLC gene products (or LD factors) on responder cells (8).

Our next question was whether the ABCIL reactivity of serum 1 was evidence that haplotypes c and x had identical capacity to stimulate or respond in MLC. This was tested by performing MLC studies within this family. In three separate experiments, lymphocytes from Laurie consistently failed to stimulate or respond to lymphocytes from Eleanor (9), although the Laurie cells were capable of a full response to and stimulation by cells from her parents and other related or unrelated persons except Brian whose cells were identical to hers with respect to HL-A (Table 1). Brian's cells, on the other hand, failed to respond to Eleanor's, but on one occasion they did stimulate hers to a stimulation index (S.I.) of 6.5, although this figure may
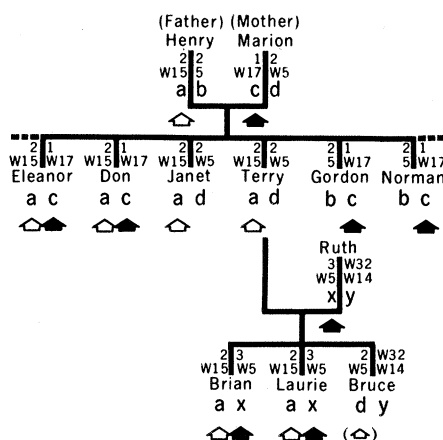

Fig. 1. The use of the ABCIL technique to show segregation of serums 1 and 2 in family F. Positive ABCIL reactions with serum 1 are indicated by closed arrows and with serum 2 by open arrows. (Other members in the second generation are not shown in this diagram.)

still represent nonstimulation when compared with other S.I. of the Eleanor cells responding to those of other individuals.

The data of the second of three experiments are shown in Table 1 and were analyzed for the stimulation of haplotype c on x, and vice versa, with other members of this family. These data showed that cells which differed by one serologically defined HL-A haplotype had a mean S.I. of 28.6 ± 14.9, unless this serologically defined difference was due only to haplotype x instead of c (or vice versa), where the mean S.I. was 3.0 ± 2.3. Similarly

when cells differed by two HL-A haplotypes, the mean S.I. was 38.0 ± 18.4, unless one of these two haplotype differences was due to haplotype x instead of c (or vice versa), where the mean S.I. was 21.1 ± 15.4. The latter is consistent for a single haplotype LD difference.

This finding was confirmed in the third experiment in which the cells of 12 members of this family and an unrelated individual were reacted in MLC giving 137 combinations, each tested in triplicate, and all done at one time. Cells that differed by two HL-A haplotypes gave a mean S.I. of 31.2 ± 28.3 (N = 47), except for the group where one of these two HL-A haplotype differences was due to haplotype x instead of c (or vice versa), where the mean S.I. was 15.0 ± 12.5 (N = 24). This difference is highly significant (P < .005). There is no significant difference between this mean S.I. of 15.0 ± 12.5 and the mean S.I. of 20.4 ± 18.1 (N = 52) for cells differing by one HL-A haplotype (exclusive of single differences due to haplotype c or x). When data from these latter two groups are pooled, the pooled S.I. is 18.7 ± 16.6 (N = 76). This pooled S.I. is significantly different (P < .01) from the mean S.I. of 31.2 ± 28.3 for cells that differ by two HL-A haplotypes exclusive of x and c differences. Thus, experiment 3 confirms the results of the 72 MLC combinations of experiment 2. We conclude from these studies that haplotype c and x were

Table 1. Mixed lymphocyte culture done within family F, plus an unrelated individual (experiment 2). Responding cells were mixed with stimulating cells treated with mitomycin C and cultured for 5 days; radioactive thymidine was then added. The results are given as counts per minute. The stimulation index given in parentheses.

Responding cells	[³H]Thymidine incorporation (count/min) after the addition of stimulating cells from								
	Eleanor (ac)	Terry (ad)	Norman (bc)	Gordon (bc)	Bruce (dy)	Laurie (ax)	Brian (ax)	Ruth (xy)	Unrelated (9, 10) (12, W10)
Eleanor (ac)	204	6529 (32)	6871 (33.7)	9461 (46.4)	12216 (59.6)	399 (1.95)	1323 (6.5)	9592 (47)	13452 (65.9)
Terry (ad)	8505 (49.7)	171	7884 (46.1)	9614 (36.2)	4572 (26.7)	9517 (55.7)	8576 (50.2)	15308 (89.5)	15273 (89.3)
Norman (bc)	5381 (24.9)	8984 (41.5)	216	251 (1.2)	6342 (29.4)	3102 (14.4)	6613 (30.6)	11171 (51.7)	11054 (51.2)
Gordon (bc)	7232 (25.8)	8145 (29.1)	201 (0.7)	280	9990 (35.7)	4306 (15.4)	6387 (22.8)	10912 (38.9)	6673 (23.8)
Bruce (dy)	11624 (38.4)	3245 (10.7)	9176 (30.3)	12256 (40.4)	303	11407 (37.6)	11976 (39.5)	9722 (32.1)	12612 (41.6)
Laurie (ax)	1132 (2.3)	5532 (11.3)	3734 (7.4)	3644 (7.4)	10756 (21.9)	491	381 (0.8)	9889 (20.1)	7683 (15.6)
Brian (ax)	3444 (1.4)	10683 (4.4)	12689 (5.2)	9348 (3.9)	18046 (7.4)	1187 (0.5)	2423	17897 (7.4)	19466 (8.0)
Ruth (xy)	13079 (18.5)	16042 (22.6)	9627 (13.6)	12986 (18.3)	16756 (23.7)	21481 (30.3)	21608 (30.5)	708	19729 (27.8)
Unrelated (9, 10) (12, W10)	10278 (32.6)	10510 (33.4)	9627 (30.5)	11124 (35.3)	9682 (30.7)	10704 (34.0)	14910 (47.3)	13384 (42.5)	315

identical in their capacity to stimulate or respond in MLC.

Thus serum 1 in these two families marks a common MLC gene product, or LD factor, though this is associated with HL-A1 and W17 in family F, and HL-A3 and W5 in Ruth's family. It is reasonable also to assume that serum 2 is marking the MLC region of the haplotype HL-A2, W15, although our data only show that it is not marking the HL-A antigens that this serum was known to detect by the cytotoxicity test, but a factor that segregates with HL-A. The ABCIL procedure as reported in our study may provide a rapid serological approach to the detection of LD factors and is now being used to investigate the correlation between ABCIL reactivity of certain selected serums and stimulation in unidirectional MLC in unrelated individuals.

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5. We define the strength of ABCIL reactions by dividing the experimental ⁵¹Cr releases of each individual with his spontaneous ⁵¹Cr release (*1–3*). The figure thus obtained from Bruce is 2.0-fold and for others 3.0- to 11.0-fold.
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9. Eleanor's cells responding to Laurie's mitomycin C–treated cells (EL$_m$) gave an S.I. of 1.95 to 2.4. The S.I. values for LE$_m$, EB$_m$, BE$_m$, BL$_m$, and LB$_m$ were 1.9 to 2.3, 1.9 to 6.5, 1.4 to 2.6, 0.5 to 0.9, and 0.8 to 1.0, respectively.
10. We thank the family under study for their interest and participation, National Institutes of Health for supplying many of the serums, and J. Kijewski for technical assistance.

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Dendritic Spine "Dysgenesis" and Mental Retardation

Abstract. *Golgi studies reveal abnormally long, thin spines and the absence of short, thick spines on dendrites of cortical neurons in retarded children with normal karyotypes. The degree of dendritic spine loss and abnormality appears to be related to age and the severity of developmental retardation. Dendritic spine "dysgenesis" is a common feature of the microstructural pathology that occurs in profound mental retardation of unknown etiology.*

Dendritic spines originally described by Cajal in Golgi preparations are postsynaptic targets for a major proportion of the synaptic inputs to pyramidal and spiny stellate neurons of the cerebral cortex (*1*). The significance of the relation between dendritic spines and synapses has not been fully exploited in studies of human neuropathological processes, particularly in conditions that yield few specific findings in routine microscopic studies despite clinical evidence of severe cognitive and behavioral deficits. Such conditions are frequently encountered in infants and children with profound mental retardation.

Marin-Padilla (*2*) first described dendritic spine abnormalities in two infants with trisomic chromosomal anomalies known to be associated with mental retardation. In a recent study I also observed unusual dendritic spines in an infant with seizures and mental retardation of unknown etiology (*3*). This observation called into, question the specificity of dendritic spine abnormalities in chromosomal disorders and prompted the Golgi study reported below of cortical dendritic spines in retarded children or infants with normal karyotypes.

The rapid Golgi method was applied to small blocks of unfixed or fixed (with formalin or glutaraldehyde) cerebral cortex tissues from 30 pediatric autopsy cases. These included six infants and children between 3 months and 12 years of age with profound mental or motor retardation (or both) in whom no etiological factors could be defined as a basis for their severe developmental deficits (*4*). A small block of cortical tissue was also obtained from a 10-month-old retarded child (who is still alive) during brain biopsy performed for the purpose of diagnosis and family counseling. Mild to moderate microcephaly was noted in four other subjects and clinical seizures in three, but there was no correlation between microcephaly and seizures (*4*). Cortical tissue was obtained postmortem from 24 pediatric deceased patients who

had normal developmental and neurological histories and these tissues were used for evaluating presumably normal characteristics of cortical dendritic spines and spine distribution at different ages.

Peters and Kaiserman-Abramof (*5*) identified three basic types of dendritic spines on pyramidal neurons in Golgi and electron microscope studies of the adult rat parietal cortex: stubby (ST), mushroom-shaped (MS), and thin (TH) spines. Similar types are also identifiable in different proportions on different dendritic segments of neurons in human cerebral cortex. As shown in Fig. 1, A1 and A2, and Fig. 2, A1, proximal apical dendritic segments from motor cortex pyramidal neurons in a neurologically normal 6-month-old infant exhibit a preponderance of ST and MS spines. Basilar dendrites and distal apical dendritic segments have a high proportion of TH spines (Fig. 1, A3), in agreement with Peters and Kaiserman-Abramof (*5*).

Dramatically different spines are observed on proximal apical dendritic segments in cortical tissue from the brain biopsy of a 10-month-old retarded child (Fig. 1B). In addition to an absence of ST and MS spines a predominance of unusually long (4 to 8 μm), very thin spines with prominent terminal heads is observed. Electron microscope studies of the tissues of this patient confirm the presence of long, thin spines in synaptic relation with morphologically normal presynaptic axonal terminals. The contrasting features of typical apical dendritic segments from the normal 6-month-old infant and the 10-month-old retarded infant are illustrated in camera lucida drawings (Fig. 2A). The entanglement of long, thin spine pedicles on large dendritic processes is particularly striking in the preparations from the retarded child. The finding of abnormal dendritic spines in Golgi preparations of glutaraldehyde-fixed fresh tissue removed at cortical biopsy rules out a contribution of postmortem artifact.