

A SUPPRESSOR T CELL OF THE MIXED LYMPHOCYTE
REACTION IN MAN
SPECIFIC FOR THE STIMULATING ALLOANTIGEN
Evidence that Identity at HLA-D between Suppressor and Responder
is Required for Suppression*

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Recent investigations from this laboratory have documented the presence of suppressor T cells of the mixed lymphocyte reaction (MLR)¹ in two unrelated individuals (1).² That is, when T cells from these individuals were cocultured in an MLR with the responder cells of human leukocyte antigen (HLA) identical persons, the responses of such persons to allogeneic cells were inhibited. One of these suppressor cell donors, an HLA-Dw4 homozygous male, failed to respond in the MLR to almost all allogeneic cells and, therefore, his cells could be cocultured in MLR's with responder cells of varied haplotype to determine if they were suppressible.² Only those responders who were heterozygous or homozygous for the Dw4 antigen were inhibited, regardless of their associated HLA-A or B antigens, indicating that identity between suppressor and responder at *HLA-D* was required for suppression.

Another individual, J.H., failed to respond in an MLR to her husband, W.H., and when J.H.'s T cells were added to the responder lymphocytes of HLA identical persons, their responses to W.H. were suppressed (1). Unlike the other suppressor cell donor, J.H. responded vigorously when challenged by stimulator cells other than W.H. It was not possible, therefore, to map the restriction between the J.H. suppressor T cell and responder cells to a specific locus within HLA due to allogeneic stimulation of J.H.'s lymphocytes which occurred when responder cells other than those homozygous for HLA-Dw2 were tested.

The present studies are an effort to clarify the nature of determinants recognized by the antigen-specific MLR suppressor T cell from J.H. Advantage was taken of the observation that the subpopulation of J.H. suppressor lymphocytes was resistant to a dose of γ -irradiation which functionally eliminated MLR responder cells from J.H. After elimination of proliferative re-

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¹ *Abbreviations used in this paper:* HLA, human leukocyte antigen; MLR, mixed lymphocyte reaction.

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sponses on the part of J.H. with this technique, restriction between suppressor and responder was found to be localized to the *HLA-D* region.

Materials and Methods

Blood samples were obtained from a panel of healthy persons known to be homozygous or heterozygous for specific HLA antigens. All experiments were performed with lymphocytes from fresh venous blood.

Mixed Lymphocyte Cultures. As described previously (1),² lymphocytes from defibrinated blood were collected by Ficoll-Hypaque gradient centrifugation, washed, and then resuspended at 1×10^6 cells/ml in RPMI-1640 medium (Grand Island Biological Company, Grand Island, N.Y.) containing 100 U/ml penicillin, 100 μ g/ml streptomycin, 2 mM glutamine, and 10% pooled type A human serum. Stimulating cells were irradiated in a ¹³⁷Cesium irradiator (Mark I model 24 irradiator; J. L. Shepherd, & Associates, Glendale, Calif.) with a dose of 6,000 rads to abolish their capacity to proliferate and to make the reaction unidirectional. Mixed lymphocyte cultures were carried out in round bottom microtiter trays (Linbro Chemical Co., New Haven, Conn.) with 50,000 responder cells and 50,000 irradiated stimulators in a vol of 0.15 ml. In three-way cultures, 50,000 suppressor cells were cocultured with 50,000 responder cells and 50,000 irradiated stimulator cells in 0.15 ml. Cultures, prepared in sextuplicate, were incubated in air/5% CO₂ for 6 days at 37°C. [³H]thymidine (New England Nuclear Corp., Boston, Mass.) was then added, 1 μ Ci/well, and the plates harvested in a Multiple Sample Harvester (MASH II, Microbiological Associates, Bethesda, Md.) 18 h later.

Separation of T Cells and B Cells. Peripheral blood T cells and B cells were separated by a method dependent on B lymphocytes binding to a plastic flask coated with anti-immunoglobulin, as described previously (1).² Under these conditions, monocytes as well as B cells adhere to the flask. The nonadherent T cells were decanted and the B cells removed after 2 h incubation at 37°C with RPMI-50% human A serum containing 1.25 mM EDTA. The T cells were measured in the two fractions by rosetting with sheep erythrocytes (1). The B cells were measured by staining with fluorescein-conjugated anti-Ig.² Cell recovery was 75-90%, with T-cell fractions consisting of 90% rosetting cells and B-cell fractions consisting of 80-85% Ig-positive cells.

Results

Evidence that J.H. Suppresses the MLR and that Suppression is Due to a T Cell. J.H. is an HLA-B7,Dw2 homozygous mother of 10, who responds normally in the MLR to most allogeneic cells but, surprisingly, not to her husband, W.H. This is an unexpected finding because W.H. (Bw35, Dw1 homozygous) has no HLA antigens in common with J.H., and W.H. behaves normally as a stimulator cell when tested with a random panel of responder cells (data not shown). Furthermore, although J.H. made a good anti-HLA-Bw35 antibody during her childbearing years, she has had no detectable HLA-A or B antibody since 1972, nor does she have detectable B-cell alloantibody at the time of this report (B. Colombe, and R. Payne, personal communication). The evidence that J.H. and W.H. are homozygous for HLA-B7,Dw2 and HLA-Bw35,Dw1, respectively, is presented elsewhere (1). New data from the VIIth International Histocompatibility Testing Workshop show that J.H.'s and W.H.'s B cells are lysed by different groups of B-cell alloantisera, which correlate with their previously assigned Dw types (B. Colombe and R. Payne, personal communication).

In a series of experiments reported by McMichael and Sasazuki (1), the failure of J.H. to respond to her husband, W.H., has been shown to be due to suppressor T cell. As indicated in Fig. 1, a one-way MLR between 50,000 J.H. lymphocytes and 50,000 irradiated W.H. stimulator cells results in little, if any

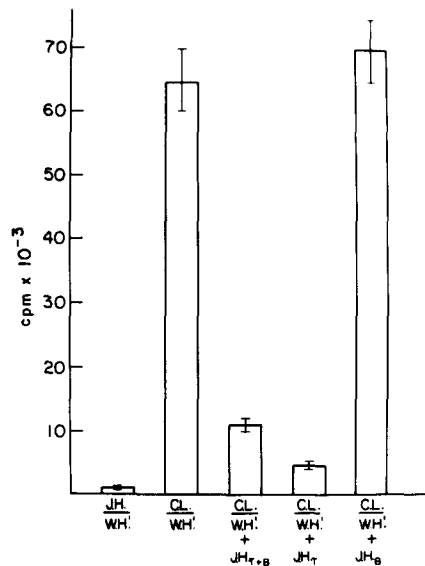


FIG. 1. The effect of J.H. lymphocytes on the one-way MLR between C.L. and W.H.'. J.H. and C.L. are both homozygous for HLA-B7,Dw2. W.H.', the irradiated stimulator cell, is homozygous for Bw35,Dw1. Each column height represents the MLR response in counts per minute, with bars showing the standard error of the mean of six experiments. The first column depicts J.H.'s response to W.H.'. The second column shows C.L.'s response to W.H.'. In the third column 50,000 peripheral blood lymphocytes from J.H. have been cocultured in an MLR between C.L. and W.H. In the fourth and fifth columns, 50,000 T-enriched cells or B-enriched cells have been cocultured in MLR's between C.L. and W.H. J.H. T cells were separated from B cells by a technique in which B cells were bound to immunoglobulin coated on a plastic surface (see Materials and Methods).

[³H]thymidine incorporation by J.H. (980 ± 92 cpm). In contrast, an MLR between 50,000 responder cells from an unrelated HLA-B7,Dw2 homozygous individual, C.L., and W.H. results in marked proliferation as measured by incorporation of [³H]thymidine ($64,927 \pm 5,120$ cpm). If 50,000 J.H. cells are cocultured with 50,000 C.L. cells and 50,000 irradiated W.H. stimulator cells, C.L.'s response to W.H. is markedly inhibited ($10,792 \pm 1,752$). As shown in Fig. 1, this inhibition is mediated by a T cell, confirming previous results (1).

Evidence that Identity at HLA-D between J.H. and Responder Lymphocytes is Required for Suppression. Only responders identical at both *HLA-B* and *HLA-D* loci to J.H. are uniformly inhibited by the J.H. suppressor cell (Table I). Responders heterozygous for the J.H. haplotype (HLA-B7,Dw2) are only weakly inhibited or not inhibited at all. The apparent lack of suppression of Dw2 heterozygous responders, however, may be due to proliferation of cells from J.H. responding to the non-B7,Dw2 haplotype in heterozygous cells, thereby masking suppression. It became possible to test this hypothesis after the discovery that the J.H. suppressor cell was resistant to a dose of γ -irradiation that functionally eliminated responder cells from the suppressor cell donor population. As illustrated in Fig. 2, all MLR responder activity in J.H. is lost at an irradiation dose of 1,000 rads. Suppressor activity, on the

TABLE I
The J.H. Suppressor Cell: Requirement for HLA-Dw2 in the Responder Cell

HLA	Responder			Response to W.H.	Response to W.H. + J.H.	Response to W.H. + J.H. _{x1,000}		
	A	B	D			%Δ cpm	%Δ cpm	
C.L.	2,3	7,7	2,2	64,927 ± 5,120	10,792 ± 1,752	-83.4	12,307 ± 1,929	-80.4
L.H.	3,3	7,7	2,2	86,935 ± 9,416	34,705 ± 4,451	-60.1	27,364 ± 2,671	-68.6
T.I.	1,3	7,7	2,2	48,633 ± 3,294	19,924 ± 1,334	-61.1	17,516 ± 1,683	-64.0
B.D.	2,2	7,27	2,-	86,263 ± 6,081	100,300 ± 7,713	+16.3	31,081 ± 4,456	-64.0
R.G.	2,1	7,8	2,3	122,433 ± 8,505	109,888 ± 8,398	-10.2	30,625 ± 3,664	-75.0
E.G.	3,30	7,15	2,4	72,180 ± 2,223	57,346 ± 4,454	-20.5	24,046 ± 1,792	-66.7
B.B.	2,2	7,17	2,-	39,424 ± 3,590	50,619 ± 6,212	+28.4	14,712 ± 1,230	-62.7
W.B.	3,29	7,12	2,-	57,188 ± 3,271	53,926 ± 4,284	-5.7	19,240 ± 3,460	-62.7
J.R.	10,1	18,8	2,3	48,672 ± 5,393	58,715 ± 4,598	+20.6	16,847 ± 862	-66.4
J.B.	1,1	8,8	3,3	52,155 ± 4,166	56,602 ± 7,365	+8.5	49,304 ± 3,491	-9.5
L.M.	2,11	12,12	4,4	33,462 ± 3,617	51,009 ± 6,909	+52.4	61,560 ± 4,183	+83.9
S.F.	2,24	13,27	4,6	45,714 ± 4,060	89,327 ± 6,600	+95.4	71,950 ± 5,388	+57.4
D.S.	2,3	14,40	5,-	53,116 ± 5,184	91,640 ± 8,005	+72.5	59,112 ± 2,427	+11.3
B.C.	1,29	17,35	6,-	29,641 ± 3,183	74,751 ± 6,146	+252.2	55,418 ± 6,352	+86.9
M.K.	28,28	7,14	-,-	47,211 ± 2,398	106,063 ± 10,962	+224.7	88,944 ± 5,507	+88.4
H.K.	1,2	8,7	3,-	20,575 ± 1,039	60,944 ± 5,258	+296.2	47,808 ± 4,814	+232.4
M.I.	2,32	12,35	107,-	41,929 ± 3,903	85,410 ± 6,075	+203.7	55,418 ± 3,027	+32.2
M.H.	2,29	12,27	1,107	32,566 ± 4,919	77,626 ± 5,822	+238.4	49,625 ± 5,589	+52.4

Responses in cpm represent the means of six experiments ± standard error.

other hand, remains intact after exposure to 1,000 rads, falls off partially after 2,000 rads, and is completely lost after 6,000 rads.

J.H. lymphocytes were, therefore, exposed to 1,000 or 6,000 rads and tested for their ability to suppress the response of a variety of responder cells to W.H. As shown in the second half of Table I, J.H. lymphocytes exposed to 1,000 rads (J.H._{x1,000}) inhibit the responses of all persons heterozygous or homozygous for HLA-Dw2, regardless of the associated HLA-A or B antigens. Cells lacking Dw2 are not suppressed. Thus, J.R., who possesses the genotype (A10,B18,Dw2/A1, B8,Dw3) is suppressed by J.H._{x1,000}, but neither M.K. (A28,B7,D-/A28,B14,D-) nor H.K. (A2,B7,D-/A1,B8,Dw3) is suppressed.

When J.H. lymphocytes are irradiated with 6,000 rads (J.H._{x6,000}) and tested on the same panel of responder cells, there is no suppression. Furthermore, the MLR responses of non-Dw2 responder cells to W.H. are no greater in the presence of J.H._{x6,000} than J.H._{x1,000}, confirming that non-Dw2 cells are not suppressed by J.H._{x1,000} (data not shown).

Specificity of the J.H. Suppressor Cell for Determinants in the Irradiated Stimulator Cell, W.H. Only when W.H. or a few other cells are present as the irradiated stimulator, is J.H. suppression of the MLR detectable (2). In general, these are cells to which J.H. fails to respond in a one-way MLR. Previous surveys (1, 2) indicated that such cells often, but not always, share the HLA-Bw35 specificity with W.H. Thus, the J.H. suppressor cell appeared to recognize determinants in the irradiated stimulator cell as well as D locus specificities in the responder. This dual specificity is illustrated in Fig. 3, in which J.H. inhibits the response of an HLA-B7,Dw2 homozygous responder, C.L., to W.H., but not to three other allogeneic cells (C.O., S.P., and B.C.).

We considered the possibility that a strong proliferative response by J.H. to C.O. might be masking a simultaneous but weaker suppressive response. If this were so, elimination of J.H.'s ability to proliferate by exposure to 1,000

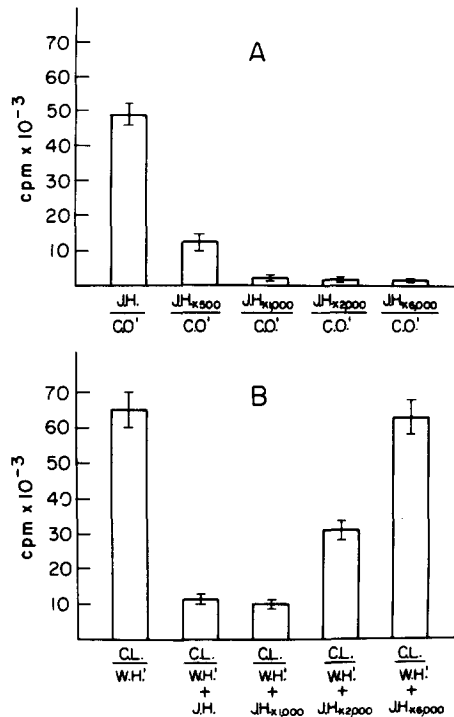


FIG. 2. The sensitivity of J.H. MLR responder cells and suppressor cells to γ -irradiation. The upper panel (A) demonstrates the sensitivity of J.H. responder cells to increasing doses of γ -irradiation as measured in one-way MLR's between 50,000 J.H. cells and 50,000 irradiated (6,000 rads) cells from an allogeneic donor, C.O. The responses represent the mean of six experiments at each radiation level. The lower panel (B) shows the sensitivity of J.H. suppressor cells to increasing doses of γ -irradiation as measured by the ability of 50,000 J.H. cells to inhibit the MLR between C.L. and W.H. '.

rads irradiation should reveal suppression of the response to C.O. As shown in Table II, however, in spite of the fact that J.H._{x1,000} can no longer proliferate in response to C.O., these cells cannot suppress the responses of other Dw2-positive individuals to C.O. Non-Dw2 responders are similarly unaffected by J.H._{x1,000}.

Although the determinants on W.H. recognized by the J.H. suppressor T cell do not correlate with a known private HLA specificity in all cases such determinants are clearly distinct from those recognized by the suppressor cell on Dw2-positive responder cells. Thus, the response of W.H. to J.H. is not diminished when neither cell is irradiated in a two-way MLR, nor is the response of W.H. to other cells inhibited by the addition of J.H. (data not shown).

Heterozygosity for the W.H. phenotype is apparently sufficient for recognition by J.H. suppressor cells. Thus, the responses of other Dw2-positive individuals to the H children, each of whom carries the J.H. and W.H. haplotypes, are suppressed by J.H. (Table III). The potency of the suppression by J.H. on these responses (as tested on an unrelated Dw2 homozygous responder cell, C.L.) tends to be about half of that seen when W.H. is the stimulator, possibly

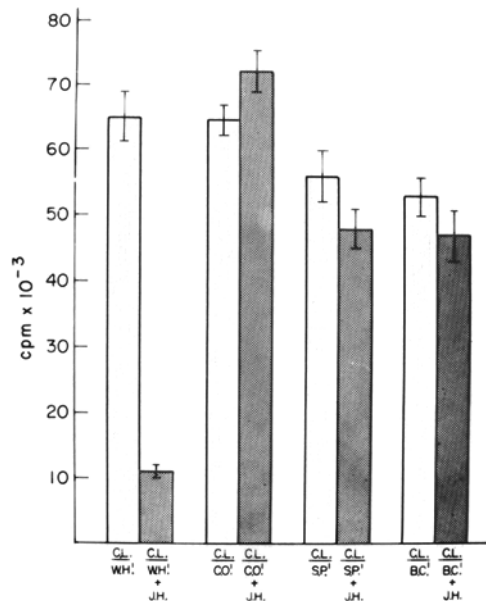


FIG. 3. Specificity of the J.H. suppressor cell for the MLR stimulating cell. Unshaded columns represent one-way MLR's between C.L. (B7,Dw2 homozygous) and four different irradiated stimulator cells. Shaded columns represent the same MLR's in the presence of 50,000 J.H. cells. Each result represents the mean of six experiments.

TABLE II
Failure of the J.H. Suppressor Cell to Inhibit MLR Response to C.O.

Responder		Response to C.O.	Response to C.O. + J.H.	Response to C.O. + J.H. _{x1,000}
J.H.	2,2	49,892 ± 3,406	57,419 ± 9,417	48,386 ± 4,468
T.I.	2,2	84,152 ± 5,812	79,803 ± 6,751	76,442 ± 6,564
C.L.	2,2	64,977 ± 6,297	72,516 ± 8,327	69,548 ± 7,057
L.H.	2,2	75,170 ± 6,466	71,460 ± 6,550	65,691 ± 5,333
B.D.	2,-	71,630 ± 9,562	99,512 ± 5,863	82,400 ± 8,110
R.G.	2,3	84,583 ± 7,369	121,290 ± 8,864	91,322 ± 6,714
J.B.	3,3	44,240 ± 4,710	88,918 ± 4,721	67,716 ± 4,800
L.M.	4,4	87,509 ± 6,726	116,392 ± 9,677	101,712 ± 8,399
D.S.	5,-	78,782 ± 5,995	109,025 ± 9,840	92,246 ± 9,474
B.C.	6,-	65,340 ± 3,889	78,207 ± 7,118	75,418 ± 6,120
M.H.	1,107	40,096 ± 3,565	89,040 ± 9,140	46,190 ± 1,899

Responses in cpm represent the means of six experiments ± standard error.

indicating a gene-dose effect. Nonetheless, the fact that J.H. fails to respond in a one-way MLR to any of her children (1) is evidence that such suppression can be effective.

Search for an MLR Suppressor Cell in other Multiparous Women. Because J.H. had been exposed repeatedly to the same foreign haplotype (W.H.) in her 10 pregnancies, and because her MLR suppressor T cell is specific for W.H., 6 other multiparous women were investigated for possible MLR suppressor cells. Although each subject had at least six pregnancies by a single partner, no suppression was observed when the subject's cells were cocultured in MLR's

TABLE III
Suppression by J.H. of the Responses of an HLA Matched Donor (C.L.) to H Children

HLA	Stimulator cell			Response by:		Suppression by J.H. %
	A	B	D	C.L.	C.L. + J.H.	
W.H.	11,28	35,35	1,1	90,593 ± 5,208	8,512 ± 655	90.6
K.H.	2,28	7,35	2,1	56,828 ± 3,839	32,884 ± 1,446	42.2
M.H.	2,11	7,35	2,1	34,683 ± 4,501	18,376 ± 1,670	47.0
D.H.	2,11	7,35	2,1	79,654 ± 9,411	41,102 ± 2,697	48.4
R.H.	3,11	7,35	2,1	38,590 ± 3,796	22,085 ± 3,378	42.8
E.H.	2,11	7,35	2,1	86,308 ± 6,350	40,610 ± 5,439	53.1

Responses in cpm represent the means of six experiments ± standard error.

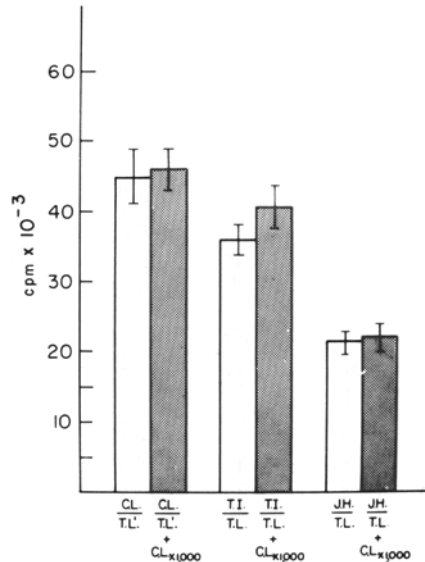


FIG. 4. Failure of C.L. to suppress the responses of HLA matched donors to her husband, T.L. Unshaded columns represent one-way MLR's between three unrelated HLA-B7,Dw2 homozygous responders (including C.L.) and T.L.'. T.L.', the irradiated stimulator, is HLA-A1,2 B8,7 Dw3,2. Shaded columns represent the same MLR's in the presence of 50,000 C.L. cells exposed to 1,000 rads (C.L._{x1,000}). Each result represents the mean of six experiments.

between an HLA identical cell and the appropriate paternal cell. The data from one such study are summarized in Fig. 4. C.L., an HLA-B7,Dw2 homozygous mother of nine, has, like J.H., been exposed repeatedly in pregnancy to a single allogeneic cell. Unlike J.H., however, C.L. responds in the MLR to her husband, T.L. Also, neither addition of C.L. nor addition of C.L. irradiated with 1,000 rads to MLR's between other Dw2 homozygous individuals and her husband's cell are suppressive.

Discussion

These investigations demonstrate the presence in J.H., an HLA-Dw2 homozygous mother of 10, of a T lymphocyte which suppresses the responses of other

Dw2-positive persons to W.H. The possibility that J.H. is cytotoxic to W.H. rather than inhibitory of MLR responses to W.H. seems unlikely because (a) J.H. does not suppress the responses to W.H. of persons who are not HLA-Dw2 positive; (b) the MLR responses of W.H. are not suppressed by the addition of J.H.; (c) no cytotoxicity of W.H. by J.H. could be detected with an antibody-mediated cytotoxicity assay (2). On the other hand, the possibility that J.H. is cytotoxic only for that subset of Dw2 responder cells which recognizes the W.H. haplotype, cannot be excluded. Such a mechanism of MLR suppression can only be excluded if it is shown that the inhibitory effects of J.H. are mediated by a soluble factor or factors. The existence of antigen-specific and nonspecific suppressor factors has been documented in other immunologic systems (3-6), and experiments are in progress to evaluate this possibility in J.H.

Localizing the restriction between MLR suppressor and responder cells to HLA-Dw2 was made possible by the fact that J.H. suppressor cells were resistant to a dose of γ -irradiation that functionally eliminated J.H. responder cells. After addition of J.H. cells exposed to 1,000 rads, the responses of Dw2-positive responder cells to W.H. were inhibited regardless of their associated HLA-A or B antigens. We considered the possibility that J.H. inhibits the responses of non-Dw2 cells in the MLR, but that suppression is masked by the response of these cells to J.H. If this were so, the responses of non-Dw2 cells to allogeneic cells would have been greater in the presence of J.H. _{$\times 6,000$} than J.H. _{$\times 1,000$} . This was not found, however. Thus, the J.H. suppressor T cell appears to be specific for Dw2-positive responder cells.

These data are interpreted as indicating that genes in or near the HLA-Dw2 locus code for suppressibility, possibly via receptor molecules on suppressed cells which bind to the suppressor cell or to a product of the suppressor cell. A similar interpretation was made on the basis of analogous findings in studies of a Dw4 MLR suppressor T cell.² In neither example has a family been available for study with a crossover between HLA-B and the appropriate HLA-D antigen. Localization of restriction to the *D* locus must, therefore, remain tentative until such families are studied. Nonetheless, on the basis of these current data, it is inferred that genes in the *HLA-D* region code for structures, presumably on T cells, which are recognized by MLR suppressor cells, as well as for determinants primarily on B cells (the so-called Ia antigens) which are recognized by MLR responder cells (7, 8).

The specificity of the J.H. suppressor T cell for both responder and stimulator cells is qualitatively different than the other human MLR suppressor T cell studied in this laboratory,² or the MLR suppressor factor of Rich and Rich (5, 6) in the mouse, which lack specificity for the stimulator cell in the MLR. Several possible explanations for the apparent dual specificity of the J.H. suppressor cell might be considered in light of the current data. First, it is possible that the J.H. suppressor cell recognizes all allogeneic cells but is only detectable when J.H. responds weakly to the stimulatory alloantigen. Such an explanation seems unlikely because under conditions in which J.H. responder cells are irradiated, suppression remains specific for the stimulator cell. That is, only certain cells (such as W.H.) stimulate suppression, and cells that induce a proliferative response by J.H. do not induce suppression even after J.H. responder cells are functionally eliminated by γ -irradiation.

A second explanation of the dual specificity of J.H. suppression, that the suppressor cell is induced by a limited number of allogeneic cells, but once induced it suppresses the responses of Dw2 cells to all stimulator cells in the MLR is also unlikely, because under conditions in which the suppressor is present, J.H. retains the ability to respond to other allogeneic cells. Thus, for example, in the three-cell experiment in which J.H. is cocultured with a cell heterozygous for Dw2 and W.H. as the irradiated stimulator, proliferation of J.H. responder cells occurs to the non-Dw2 haplotype. That this response is not suppressed by J.H. suppressor cells, presumably present in the culture, suggests that the suppressor cell itself is specific for determinants in the irradiated stimulator W.H., as well as for the Dw2 antigen in the responder. Apparently, only those clones of Dw2 cells capable of responding in the MLR to W.H. are affected by the J.H. suppressor T cell.

Additional data indicate that determinants on the W.H. stimulator cell recognized by the J.H. suppressor T cell are distinct from determinants on Dw2 responder cells also recognized by J.H. Cells from W.H., the husband of J.H. and father of her 10 children, are recognized by the suppressor, but the responses of W.H. in the MLR are not inhibited by J.H. All of the H children carry their father's haplotype and are, therefore, recognized by the J.H. suppressor cell. Such results are consistent with the hypothesis that the J.H. suppressor cell arose in vivo as a result of sensitization to repeated fetal grafts with the same paternal haplotype. In this regard, the possibility that other multiparous women might have a similar suppressor cell was explored in studies of six additional subjects, but no MLR suppressor cell was found. Studies are in progress of other conditions in which the existence of an MLR suppressor cell might explain an apparent weakness of cell-mediated immune responses, such as thymic or lymph node irradiation and successful organ transplantation. If MLR suppressor cells can be induced in other persons, possible beneficial effects on patients with autoimmune disease and recipients of organ transplants might be anticipated.

Summary

It has previously been shown that J.H., a human leukocyte antigen (HLA)-Dw2 homozygous multiparous woman, fails to respond in a mixed lymphocyte reaction (MLR) to her Dw1 homozygous husband W.H., and that her T cells suppress the responses of HLA matched responders to W.H. The present studies take advantage of the observation that J.H. suppressor cells resist a dose of γ -irradiation which functionally eliminates her MLR responder cells. J.H. cells, depleted of alloreactive cells, suppress the responses of Dw2 heterozygous or homozygous cells to W.H., regardless of their associated HLA-A or B antigens. Only when W.H. or a few other cells are present as the irradiated stimulator is J.H. suppression of Dw2 responses detected. Thus, the J.H. suppressor T cell recognizes determinants in the irradiated stimulator cells as well as *D* locus products in the responder.

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