

A SUPPRESSOR T CELL IN THE HUMAN MIXED LYMPHOCYTE REACTION*

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T lymphocytes are known to exert both positive and negative regulatory controls on effector cells in the immune response. The positive helper effects on antibody production were recognized first (1, 2). Later, suppressor functions were demonstrated in humoral immune responses (3-5) and more recently in delayed hypersensitivity (6) and the mixed lymphocyte reaction (MLR) (7).¹ It is now clear from the work of Cantor and Boyse (8) that there are two types of regulatory T lymphocytes, helper and suppressor cells, which belong to separate populations distinguishable by their Ly surface antigens.

Like helper T cells, suppressor T cells appear to be genetically controlled by the major histocompatibility complex (MHC). These regulatory genes map in the *I* region of the mouse MHC (*H-2*), which is serologically divisible into *A*, *B*, *J*, *E*, and *C* regions. Kapp et al. (9) showed that the immune response to the synthetic peptide L-glutamic acid⁶⁰-L-alanine³⁰-L-tyrosine¹⁰ in mice was regulated by an antigen-specific immune suppression gene. Tada et al. (10) have shown that immune T cells can release a supernatant factor which specifically suppresses the immune response of other mouse strains that share the same *I-J* region genotype. Rich and Rich (11) have also shown that the suppressor T cell in the MLR can release a factor that suppresses the response of responding cells sharing the same *I-C* region genotype. Murphy et al. (12) and Tada et al. (13) have shown that suppressor T cells express a unique *I* region determinant, *I-J*, on their surface.

Suppressor T cells are of interest in humans for two reasons. They may allow some insight into the controls on immune function exerted by the human MHC, the HLA system, and they may play roles in disease processes and organ transplantation. It has, for instance been suggested that they normally prevent the development of autoimmune disease processes (14). There has been one report of suppressor cells in a human disease. Waldman et al. (15) showed that T

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¹ Abbreviations used in this paper: FCS, fetal calf serum; HTC, homozygous-typing cell; MHC, major histocompatibility complex; MLC, mixed lymphocyte culture; MLR, mixed lymphocyte reaction; PBS, phosphate-buffered saline; Ts, suppressor T cells; W.H.', irradiated W.H.

lymphocytes from patients with common variable hypogammaglobulinaemia suppressed the production of immunoglobulin by normal lymphocytes in response to pokeweed mitogen.

In this study, a suppressor T cell was demonstrated in the human MLR. The cell was found in the mother of a large family who showed an unusual pattern of reactivity when tested in the MLR with her HLA homozygous husband and some other homozygous cells (16). Her T cells suppressed the response of cocultured HLA identical cells to the homozygous cells of the husband in a unidirectional MLR.

Materials and Methods

Blood Donors. The family H was contacted during a search for individuals homozygous for HLA-D (MLR stimulating) determinants. Other volunteers comprised one other HLA-D homozygote, T.I., and laboratory personnel. 60-ml venous blood was drawn into sterile bottles and defibrinated immediately.

Mixed Lymphocyte Cultures. Defibrinated blood was diluted twofold with phosphate-buffered saline (PBS) and layered onto 9% Ficoll-Hypaque. Each tube was centrifuged at 1,300 rpm for 35 min and the lymphocytes at the interface collected. These cells were washed once with RPMI 1640 medium (Grand Island Biological Co., Grand Island, N. Y.) containing 100 U/ml penicillin, 100 μ g/ml streptomycin, and 2 mM glutamine. They were then resuspended at 1×10^6 cells/ml in the same medium containing 10% heat inactivated pooled human A serum. Stimulating cells were irradiated in a cesium irradiator to a total dose of 6,000 rad, to abolish their capacity to proliferate, and to make the reaction unidirectional.

Cultures were set up in round-bottomed microtiter trays (Linbro Chemical Co., New Haven, Conn.). 50,000 responder cells were mixed with 50,000 irradiated stimulators in a vol of 0.15 or 0.20 ml. In three-way cultures, 50,000 cells from each donor were mixed in 0.15 ml. Cultures, set up in triplicate, were incubated in air/5% CO₂ for 6 days at 37°C. [³H]Thymidine (New England Nuclear, Boston, Mass.) was then added, 1 μ Ci per well, and the wells harvested in a MASH 2 harvester (Microbiological Associates, Bethesda, Md.) 18 h later.

Cell Separation. T and B cells were separated from peripheral blood lymphocytes by two methods. The first was based on the rosetting techniques described by Wilson et al. (17) and modified by W. F. Bodmer, Oxford University, Great Britain (personal communication). Lymphocytes were suspended in RPMI - 10% heat inactivated, sheep erythrocyte absorbed, fetal calf serum (FCS) at 5×10^6 cells/ml. An equal vol of 0.7% papain-treated sheep erythrocytes was added and the mixture allowed to stand for 15 min. The cells were then pelleted by centrifuging for 5 min at 800 rpm. The rosettes were then allowed to become more stable by incubating on ice. After 1 h the cells were layered onto 10% Ficoll-Isopaque and centrifuged at 1,200 rpm for 30 min. The B cells at the interface were collected and washed once in RPMI-10% normal human serum. The T-cell rosettes were incubated in 100% pooled heat-inactivated human A serum for 1 h at 37°C and then separated on 9% Ficoll-Isopaque. The efficiency of this method was checked by re-rosetting samples of the B- and T-cell preparations and counting the number of rosettes compared to nonrosetted lymphocytes.

The second method depended on B lymphocytes binding to solid phase anti-immunoglobulin. 2 ml of Cohn Fraction II (Mann Research Laboratories, Inc., New York) immunoglobulin at 2 mg/ml and water-soluble carbodiimide at 1 mg/ml in PBS were pipetted into a plastic tissue culture flask (Falcon Plastics, Division of BioQuest, Oxnard, Calif.). This flask was incubated on its side at room temperature for 1 h and then washed three times with PBS-5% FCS. Then 2 ml of a 1:20 dilution of rabbit anti-human immunoglobulin was added, incubated for 30 min at room temperature, and the flask washed out with PBS-5% FCS. 1.5×10^7 peripheral lymphocytes in RPMI-10% FCS were then added and allowed to stand for 30 min at room temperature. The nonadherent T cells were then decanted off and the B cells removed after 2 h incubation at 37°C with RPMI-50% human A serum containing 1.26 mM EDTA. T cells were counted for the two fractions by rosetting. Cell recovery was normally 75-90%.

Antibody-Mediated Lympholysis. The method described by Kovithavongs et al. (18) was used. Lymphocytes were prepared as described above and erythrocytes lysed by a 5-s hypotonic shock. 5

$\times 10^6$ target cells in 0.1 ml RPMI/10% FCS were labeled by incubating with 100 μ Ci chromium 51 (New England Nuclear) for 1 h at 37°C with shaking. To 2×10^6 effector cells (J.H.) in 1 ml RPMI/10% FCS was added 0.025 μ l of labeled targets in duplicate. 0.1 ml of normal human serum (antibody) was then added and the mixture incubated for 4 h at 37°C. The cells were centrifuged to a pellet and the chromium 51 released into the supernate was counted in a γ -counter. The fraction of chromium 51 released into the supernate was calculated as a percentage of the total radioactivity in each tube and the background release by target cells incubated alone was then subtracted.

Results

Mixed Lymphocyte Culture (MLC) in the Family H. The HLA types (determined by Dr. Rose Payne) and MLC results of family H are shown in Table I. J.H. is homozygous for HLA-B7 and W.H. is homozygous for BW35. In addition, both parents are homozygous at the *HLA-D* locus which controls stimulation in the MLR. In the human family studies in this laboratory, counts of <10,000 above background are considered to be nonstimulation, >20,000 to be stimulation, and 10–20,000 to be equivocal. On this basis J.H. stimulates no children in the MLR and has been shown by cross testing with reference homozygous typing cells (data not shown) to be homozygous for the specificity HLA-DW2. W.H. also appears to be *HLA-D* homozygous. Irradiated W.H. (W.H.' cells) stimulated two children, D.H. and K.H., out of four tested but the sibling pair K.H. (W28, W35/3, 7); D.H. (11, W35/2, 7) was mutually nonstimulating even though they inherited different paternal haplotypes. The responses of D.H. and K.H. to W.H.' may be due to back stimulation (19) and thereby anomalous. W.H. cells were submitted to the North American DW1 reference laboratory and his type was found to belong to the DW1 cluster. They failed to respond in the MLR to reference DW1 cells but stimulated some of them, suggesting that they carry a broader specificity [L. Marchuk and J. B. Dossseter (University of Alberta, Canada), personal communication]. A surprise finding, repeatedly confirmed in this family is that irradiated W.H.' cells do not stimulate J.H. lymphocytes. W.H.' has been found to behave normally as a stimulating cell when tested with a random panel. In addition, J.H. did not respond to cells from any of her children. J.H. has behaved normally as a responder when tested with unrelated individuals (Fig. 1). However, as reported by Sasazuki et al. (16) she gave some unexpected results when tested as a responder against 39 homozygous typing cells (HTC) in the 1975 Histocompatibility Testing Workshop. She failed to respond (relative response less than 20%) to three DW2 HTC, and also to six other cells; she responded normally to 30 other HTCs. The nonstimulating cells did not fall into any single DW cluster but four of them shared HLA antigens of the 5 complex. It has also been found that her cells do not respond to some HLA-D heterozygous cells (Table II).

Evidence that the Low Response of J.H. to W.H.' is due to a Suppressor Cell. Because J.H. failed to respond to some cells that shared the BW35 specificity with her husband, it seemed possible that this might be due to specific suppression after multiple pregnancies. J.H. has 10 children, the youngest of whom was aged 9 yr at the time of these experiments. Because her husband is also HLA-B and D homozygous, she has therefore had repeated exposure to one HLA-B-D haplotype. She was originally investigated by Dr. Rose Payne because she made a good anti-HLA-BW35 antibody, during her childbearing years. At

TABLE I
HLA and MLR Data on Family H

Individual	HLA A-B-D type						Designation
Father W.H.	11, W35, DW1/W28, W35, DW1						A/B
Mother J.H.	3, 7, DW2/2, 7, DW2						C/D
Child D.H.	11, W35, DW1/2, 7, DW2						A/D
Child K.H.	W28, W35, DW1/3, 7, DW2						B/C
Child M.H.	11, W35, DW1/2, 7, DW2						A/D
Child E.H.	11, W35, DW1/2, 7, DW2						A/D
	Irradiated (stimulator) cells						
MLR	W.H.'	J.H.'	K.H.'	D.H.'	M.H.'	E.H.'	
Unirradiated (responder) cells							
W.H. A/B	1.7 1.5	53.8 18.3	35.0	15.0	27.5	16.6	
J.H. C/D	0.3 1.2	0.3 1.7	0.3	0.2	16.6	8.0	
K.H. B/C	18.1	0.8	0.5	5.7	—	—	
D.H. A/D	37.0	0.8	10.8	0.6	—	—	
M.H. A/D	1.3	11.2	—	—	0.6	2.2	
E.H. A/D	1.9	3.5	—	—	1.5	2.5	

The upper part of the table shows the HLA type of six members of family H. The lower part shows the family MLR, as $\text{cpm} \times 10^{-3}$. The MLR data from two separate experiments is shown: Experiment 1/Experiment 2.

the time of these studies however there was no detectable anti-HLA-A or B antibody in her serum. Even if it were present in very low amounts, the experiments were always carried out in pooled A serum so it seems unlikely that this antibody could suppress the MLC reaction. However, Barnstable et al. (20) have shown that a very high dilution of heterologous anti-human Ia antiserum would suppress the MLC so it is possible that if her serum contained anti-Ia antibody and washing of the cells was inadequate this might suppress the response. Alternatively, there might be a cellular suppression by either B cells, producing anti-HLA antibody on challenge with W.H., or by suppressor T cells. The experiments outlined below were designed to distinguish between these possibilities.

Advantage was taken of the fact that J.H. is DW2 homozygous. It was therefore possible to mix her cells with a second unrelated DW2 homozygote, T.I., and observe whether J.H. cells would suppress the response of T.I. to W.H.' There was some low level of stimulation between J.H. and T.I. as shown in Fig. 1, possibly due to minor MLC stimulating differences between the two cells, either HLA or non-HLA linked. Nevertheless, the experiments clearly and repeatedly showed that when 50,000 J.H. cells were mixed with 50,000 T.I. cells

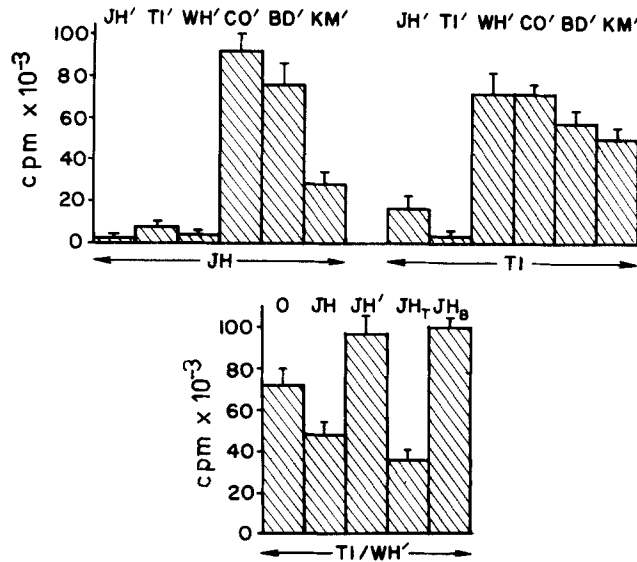


FIG. 1. The effect of J.H. cells on the MLR T.I./W.H.'. The upper histogram shows data from one experiment, performed in triplicate, on the response as counts per minute, of J.H. and T.I. to irradiated stimulating cells J.H.', T.I.', W.H.', C.O.', B.D.', and K.M.'. The lower histogram shows the effect of adding J.H. cell preparations to the mixture T.I./W.H.'. 0, no cells added, J.H., untreated J.H. (70% rosette-forming cells); J.H.', irradiated J.H., J.H._T, J.H. T-cell preparation (81% rosette-forming cells), J.H._B, J.H. B-cell preparation (11% rosette-forming cells). Each column height represents the mean counts per minute with bars showing the standard error.

TABLE II
HLA Type of Cells that Failed to Stimulate* J.H. in the MLR.

		Individual	
Family	W.H.	11, W34, DW1/W28, W35, DW1	
	K.H.	W28, W35, DW1/3, 7, DW2	
	D.H.	11, W35, DW1/2, 7, DW2	
	M.H.	11, W35, DW1/2, 7, DW2	
	E.H.	11, W35, DW1/27, DW2	
Homozygous type cells HLA-D identical	T.I.	3, 7, DW2/1, 7, DW2	
	C.L.	3, 7, DW2/2, 7, DW2	
	D.Ho.	3, 7, DW2/3, 7, DW2	
	20,049	3, W18, DW2/3, W18, DW2	
	12,003	7, DW2/7, DW2	
	14,001	7, DW2/7, DW2	
	14,006	12, DW2/12, DW2	
	HLA-D nonidentical	5,005	2, 5, -/W31, W15, -
		9,003	3, W35, DW5/3, W35, DW5
		3,003	2, 12, DW4/2, 12, DW4
5,004		2, W15, 108/2, W15, 108	
13,003		2, W35, 108/2, W35, 108	
Heterozygous unrelated	J.L.	3, 11, 18, 17 - -	
	D.B.	9, W32, 12, W14, DW4 -	

* Failure to stimulate indicated by a relative response of <20% (16).

TABLE III
Effect of Different J.H. Preparations on the Proliferative Response T.I./W.H.'

	J.H.	J.H.'	J.H. _T	J.H. _B
Preparation:	Untreated	Irradiated	T lymphocytes*	B lymphocytes‡
Number of experiments:	7	7	4	4
Percent suppression§ ± SE:	43.8 ± 6.1	-9.4 ± 18.1	59.6 ± 7.1	6.2 ± 17.9

* T lymphocytes prepared by rosetting in two experiments and by the anti-immunoglobulin method – two experiments; mean rosetting cell content 86%.

‡ B lymphocytes prepared by rosetting in two experiments and by the antiimmunoglobulin method in two experiments; mean nonrosetting cell content 83%.

§ Percent suppression was calculated from the mean of triplicate cpm data in each experiment by the formula percent suppression = $\left(1 - \frac{\text{J.H./T.I./W.H.' cpm}}{\text{T.I./W.H.' cpm}}\right) \times 100$. Mean of values ± SE was then calculated.

and challenged with 50,000 irradiated W.H.' cells, the response was below that obtained by mixing 50,000 T.I. cells alone with 50,000 W.H.' cells (Fig. 1, Table III). J.H. had therefore suppressed the response of T.I. to W.H.'. The control T.I.: W.H.' is rigorous because the interactions J.H. ↔ W.H.' and T. I. ↔ J.H. can still occur and even though at a low level would probably raise the base-line response. This contention is supported by the finding that when irradiated J.H.' cells were added the net response was usually enhanced (Fig. 1, Table III). This experiment also shows that the effect must be cellular because it is radiosensitive.

Shown in Fig. 2 is a comparison of the effect of the DW2 homozygous cells J.H. and T.I. on the response of a DW2 heterozygote (K.M.) to W.H.'. In the presence of J.H. cells the response of K.M. to W.H.' was reduced. Again the effect of adding unirradiated J.H. cells was impressive because J.H. cells could respond to the heterozygous K.M. and the expected response would be greater than that found for K.M. stimulated by W.H.' alone. When irradiated J.H.' cells were added the net response was consistently enhanced. In contrast to these results, the addition of unirradiated T.I. cells increased the response to the K.M.:W.H.' mixture. Irradiation of the T.I. cells reduced this effect. There was therefore a qualitative as well as quantitative difference between the effect of the DW2 homozygous cells J.H. and T.I. on the response of K.M. to W.H.'.

Time-Course of the Suppressive Effects of J.H. Cells. The previously described experiments were all harvested after 7-days culture which is normally the peak in the human MLR in this laboratory. If the three cell mixtures had accelerated kinetics it is possible that the apparent suppression at day 7 might, in fact, be the descending curve of an earlier peak. A time-course experiment was therefore carried out to exclude this possibility. Replicate cultures were set up on day 0 and harvested at days 3, 5, 7, and 9. These results (Figs. 3 and 4) show that the three cell mixtures also peaked at day 7 and that suppression was maximal at this time.

Evidence that the Suppressor Cell is a T Cell. Two methods of T- and B-cell separation were used in these experiments, one dependent on the ability of T cells to form rosettes with sheep erythrocytes and the other dependent on the failure of T cells to bind to anti-immunoglobulin. Both methods give T-cell

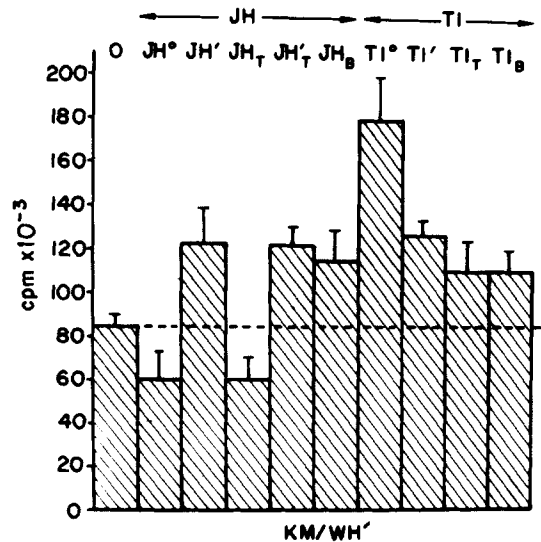


FIG. 2. The effect of adding J.H. cells or HLA B-D identical T.I. cells to the MLR K.M./W.H.' 0, no cells; J.H.⁰, T.I.⁰, untreated cells, J.H.', T.I.', irradiated cells; J.H._T, T.I._T (79% rosette-forming cells), T-cell preparations; J.H._B, T.I._B (12% rosette-forming cells), B-cell preparations. The figure represents an experiment with the height of each column representing the mean of triplicate cultures with the bars showing the standard error. The experiment was performed by using the same cell preparations as were used in Fig. 1.

preparations that were 81-93% pure as judged by (re)rosetting; the B-cell preparations contained 11-24% rosetting cells. These figures compared with a percentage of 55-70% rosetting cells in unseparated blood lymphocytes. Table III and Figs. 1 and 2 show the results of these experiments. T- and B-cell preparations were tested for their ability to suppress the response of T.I. to W.H.'. As 50,000 cells of each preparation were used it may be relevant that the relative numbers of T cells in the T-cell preparation were slightly increased, and of B cells in the B-cell preparation greatly increased. The experiment was thus biased towards finding a B-cell suppressor. The results showed that suppression was consistently enhanced by removing B cells and greatly reduced or abolished in the B-cell-rich preparations. The T-cell suppression was also reversible by high dose (6,000 rad) irradiation.

To eliminate the possibility of a nonspecific T-cell effect, the experiment was repeated by using separated T and B lymphocytes isolated from both J.H. and T.I. These cells were tested on K.M. Only T cells from J.H. suppressed the response to W.H.' (Fig. 2).

Evidence that J.H. T Cells Did Not Kill W.H.' Stimulator Cells. It is theoretically possible that if J.H. T lymphocytes killed W.H. stimulator cells, this might reduce stimulations in the MLR (21). J.H. cells were therefore mixed with chromium 51-labeled W.H. lymphocytes under conditions of the lymphocyte-mediated antibody-dependent cytotoxic assay. This assay does not require that these targets are blast cells, which is appropriate, as W.H. cells were irradiated in the MLR experiments. The experimental conditions for this assay were very close to those of the MLR culture, the antibody being 10% normal

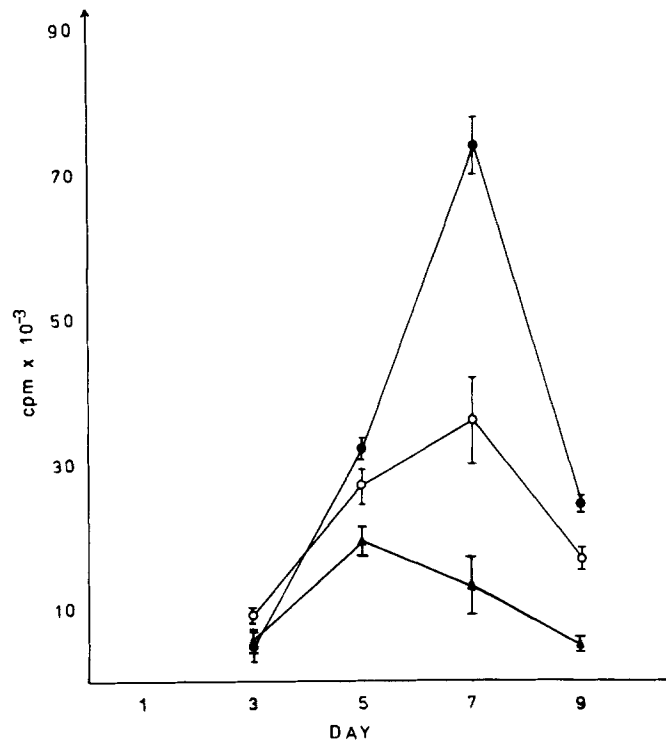


FIG. 3. Time-course of suppression by J.H. cells on the reaction T.I./W.H.'. —▲—▲—, J.H./W.H.'; —●—●—, T.I./W.H.' with no J.H. cells added; —○—○—, J.H. + T.I./W.H.'.

human serum. At a killer (J.H.) to target (W.H.) ratio of (20:1) and after 4 h incubation the percent chromium release from W.H. cells was 2.4 ± 0.8 (background release subtracted) compared to 3.3 ± 0.6 (background release subtracted) from a control target cell, C.O. A positive control serum gave 24% chromium release above background in the same experiment.

Specificity of the Suppressor T Cell. The effect of J.H. cells on the response of T.I. to nine stimulating cells is shown in Table IV. The proliferative response to four of these cells, including W.H., was suppressed. The response to the other five was greater than the control value T.I./X' as expected. J.H. lymphocytes therefore show antigen specificity in their suppressive effect. These findings are consistent with the finding that J.H. did not respond to certain stimulating cells (Table II). Thus J.H. did not respond to W.H., J.L., or D.B. and when added to T.I. cells the net response of T.I. to each of these cells was reduced. J.H. did respond to S.P. however and the response of T.I. to this cell was suppressed.

The J.H. suppressor cell therefore shows specificity when tested with a limited selection of cells and, with the exception of cell S.P., this specificity seems comparable to the pattern of low responsiveness seen when J.H. alone was tested as a responder with a panel of stimulating cells. Analysis of the HLA types of these stimulating cells (Tables II and IV) did not reveal an identifiable private specificity as the antigen recognized by the suppressor cell. It is most likely that this cell in fact recognizes one or more public specificities shared by MLR stimulating (*I* region) antigens of these cells.

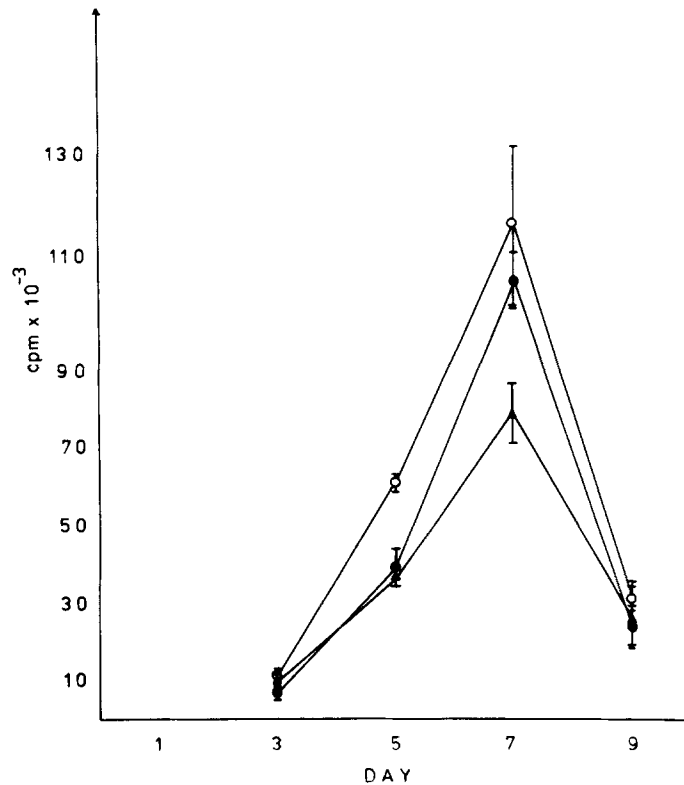


FIG. 4. Time-course of suppression by J.H. or T.I. cells on the reaction K.M./W.H.'. —●—●, K.M./W.H.' with no cells added; —○—○, K.M. + T.I./W.H.'; —▲—▲, K.M. + J.H./W.H.'.

TABLE IV
Specificity of the J.H. Suppressor Cell for the Stimulator:

Stimulator cell (X')	HLA type*	MLR TI ± J.H./X'	
		Suppression‡	Enhancement‡
		%	%
W.H.	11, W35, DW1/W28, W35, DW1	43.8	
J.L.	3, 11, W18, 27, —, —	33.8	
S.P.	3, W29, 12, W21, DW4	16.0	
D.B.	9, W32, 12, 14, DW4—	11.2	
C.O.	1, 2, 13, 27, —, —		7.9
K.M.	2, —, 7, W15, DW2, —		22.3
A.M.	2, W32, W40, 21, —, —		57.8
B.D.	1, 2, 27, 7, —, —		63.6
H.O.	9, —, W35, —, H0, H0		125.6

* HLA type shown as genotype s if known: A, B, D/A, B, D, or otherwise as a phenotype A, A, B, B, D, D.

‡ The percent suppression or enhancement was calculated by dividing the mean cpm of the mixture J.H. + T.I./X' by the mean cpm of the mixture T.I./X', subtracting from 1.0 and multiplying by 100. The results shown were collected from several different experiments in which different stimulating cells (X') were used; in each of the experiments J.H. cells suppressed the response of T.I. to W.H.'.

Discussion

The family H is special in several ways. Both parents are HLA-B and D homozygous which is a very rare situation. The mother J.H. fails to respond to the father in a one-way MLR. Specific low responsiveness is probably rare in the human MLR; if it was common it would greatly interfere with HLA-D typing using homozygous cells. Nonspecific low responsiveness however is relatively frequent.

These studies demonstrated that J.H. could reproducibly suppress the response of other HLA matched cells to W.H.'. That this was a cellular effect was shown by the effect of irradiation on this suppression. Although this effect was quantitatively rather variable, the net proliferation was always greater when irradiated J.H. cells were added to the T.I.-W.H.' mixture compared to when untreated J.H. cells were added. When the effect of the addition of J.H. cells was compared to the effect of the addition of the HLA identical T.I. cells on the mixture K.M./W.H.' there was a qualitative difference (Fig. 2). J.H. cells reduced the response; T.I. cells increased the response; irradiation of J.H. cells resulted in a higher proliferative response whereas irradiation of T.I. cells resulted in a lower proliferative response.

When J.H. peripheral blood cells (normally 10-20% B cells) were enriched for T cells the suppressive effect was enhanced. When J.H. cells were enriched for B cells the suppressive effect was always reduced and was reversed in two out of four experiments. These results strongly suggest that the effect is due to a T cell. The low degree of suppression seen with two of the B-cell preparations was probably due to contamination by T cells, which was as high as 20%. Suppression by a T-cell-dependent antibody producing B cell is very unlikely because of the above results. Also, the number of B cells present in 50,000 purified T cells was only around 2,500. In an active secondary response, only 1-2 of 2,500 B cells might be expected to secrete antibody specific of the antigen (W.H.) so that, in the unlikely event that culture conditions were appropriate, given a Poisson distribution of these B cells the effect would be extremely variable from well to well. Standard errors of the triplicates were usually within 10% of the mean (Figs. 1-4).

The possibility that J.H. T lymphocytes include a cytotoxic cell that killed the target either directly or by means of very small amounts of antibody carried over by the J.H. cells, was considered. A cytotoxic assay was performed under conditions very similar to the MLR culture, except that the killer:target (J.H.:W.H.) ratio was increased from 1:1 to 20:1. No increase of chromium release over the low levels seen with a control cell was seen and this mechanism is therefore unlikely. It is concluded that the suppression was due to a suppressor T (Ts) cell.

Experiments with different stimulating cells showed that the suppressor T cell showed antigen specificity. In a survey of the effect of J.H. on suppression of the response of T.I. to a limited series of stimulating cells, three cells were found that shared specificity with W.H. Two of these also failed to stimulate J.H. directly indicating that the two basic observations of a low response of J.H. cells to W.H.' and suppression of a third party response to W.H.' may share a common mechanism, the same Ts cell. The specificity this cell recognized did not

fall into any recognized type and may represent a public specificity shared by the cells that fail to stimulate J.H.

The origin of the Ts cell is not known. It might be related to the repeated exposure of J.H. to the W.H. *HLA* haplotype during 10 pregnancies. This seems to be the most likely explanation. However, it might be that the DW2 type of J.H. is broader and that the nonresponsiveness to W.H.' is genetically determined. Supporting this is the finding of Sasazuki et al. (16) that a second DW2 homozygous responder cell (20049) failed to respond to the same homozygous typing cells as J.H. This cell was not available for study in these experiments and this finding needs confirmation. It does raise the point that self tolerance with regard to the MLC reaction might be due to suppressor T cells. J.H., when used as a stimulating cell in the MLR behaved as expected and failed to stimulate either DW2 homozygous or heterozygous cells. These points may be resolved when J.H. and 20049 are typed both serologically and by a further series of homozygous typing cells in the forthcoming VIIth Histocompatibility Testing Workshop.

Ts cells have been described in a number of immune responses (3, 5, 10). They can, in the mouse, be clearly distinguished by their Ly antigens (4). Ts cells have been described in the MLR by Rich and Rich (7) and these results are in many ways similar to theirs in the mouse. However there are apparent differences. The suppressor cell described by Rich and Rich was mitomycin resistant whereas the J.H. cell was radio sensitive. The dose of irradiation used was high and recent data (A. J. McMichael and T. Sasazuki, unpublished observation) show that a lower dose of 1,500 rad left suppressor activity intact. The Ts cell of Rich and Rich was not antigen specific but this may reflect the relatively small numbers of antigenic differences tested in inbred mice compared to those between outbred humans. The J.H. Ts cell showed specificity for antigen though it did not fit in with any recognized pattern of a private HLA specificity. It is possible that the specificity is for a public specificity shared by the BW35-DW1 haplotype and certain other HLA antigens, but not 3, 2, 7, or DW2.

This demonstration of antigen-specific Ts cells in humans raises questions about their relationship with the HLA system. In similar situations in mice Ts cells produce a suppressor factor which is active in suppressing T cells that share Ia determinants with the suppressor cells (10, 11). Because of the allogeneic stimulation that would occur, it was not possible to test whether J.H. Ts cells would suppress the response of cells that did not carry HLA B7-DW2 to W.H.'. Experiments are in progress to generate a suppressor factor to answer this question. If antigen-specific suppression is shown to be controlled by the HLA complex and can be shown to be restricted to a particular subregion, this will have important bearing on understanding the mechanisms that underly HLA and disease associations.

Summary

Lymphocytes from an HLA-B7 DW2 homozygous multiparous woman, J.H., failed to respond in the mixed lymphocyte reaction to lymphocytes from her DW1 homozygous husband, W.H., and certain other homozygous typing cells. J.H. lymphocytes could suppress the response of HLA matched responders to

W.H. This effect was shown to be radiosensitive and due to a T cell. The suppressor cell showed antigen specificity.

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References

1. Claman, H. N., E. A. Chaperon, and R. F. Triplett. 1966. Thymus-marrow cell combinations. Synergism in antibody production. *Proc. Soc. Exp. Biol. Med.* 122:1167.
2. Miller, J. F. A. P., and G. F. Mitchell. 1969. Thymus and antigen-reactive cells. *Transplant. Rev.* 1:13.
3. Gershon, R. K., and K. Kondo. 1971. Infectious immunological tolerance. *Immunology.* 21:903.
4. Herzenberg, L. A., K. Okumura, and C. M. Metzler. 1975. Regulation of immunoglobulin and antibody production by allotype suppressor T cells in mice. *Transplant. Rev.* 27:57.
5. Baker, P. J., P. W. Stashak, D. F. Amsbaugh, and B. Prescott. 1974. Regulation of the antibody response to type III pneumococcal polysaccharide IV. Role of suppressor T cells in the development of low dose paralysis. *J. Immunol.* 112:2020.
6. Zembala, M., and G. L. Asherson. 1973. Depression of the T cell phenomenon of contact sensitivity by T cells from unresponsive mice. *Nature (Lond.).* 244:227.
7. Rich, S. S., and R. R. Rich. 1974. Regulatory mechanisms in cell-mediated immune responses. I. Regulation of mixed lymphocyte reactions by alloantigen-activated thymus-derived lymphocytes. *J. Exp. Med.* 140:1588.
8. Cantor, H., and E. Boyse. 1975. Functional subclasses of T lymphocytes bearing different Ly antigens I. The generation of functionally distinct T-cell subclasses is a differentiative process independent of antigen. *J. Exp. Med.* 141:1376.
9. Kapp, J. A., C. W. Pierce, S. Schlossman, and B. Benacerraf. 1974. Genetic control of immune response in vitro. V. Stimulation of suppressor T cells in nonresponder mice by the terpolymer L-glutamic acid ⁶⁰-L-alanine ³⁰-L-tyrosine¹⁰ (GAT). *J. Exp. Med.* 140:648.
10. Tada, T., M. Taniguchi, and T. Takemari. 1975. Properties of primed suppressor T cells and their products. *Transplant. Rev.* 26:106.
11. Rich, S. S., and R. R. Rich. 1975. Regulatory Mechanism in cell-mediated immune responses. II. A genetically restricted suppressor of mixed lymphocyte reactions released by alloantigen-activated spleen cells. *J. Exp. Med.* 142:1391.
12. Murphy, D. B., L. A. Herzenberg, K. Okumura, L. A. Herzenberg, and H. O. McDevitt. 1976. A new subregion (*I-J*) Marked by a locus (*Ia-4*) controlling surface determinants on suppressor T lymphocytes. *J. Exp. Med.* 144:699.
13. Tada, T., M. Taniguchi, and C. S. David. 1976. Properties of the antigen-specific suppressor T-cell factor in the regulation of the antibody response of the mouse. IV. Specific subregion assignment of the gene(s) that codes for the suppressive T-cell factor in the *H-2* histocompatibility complex. *J. Exp. Med.* 144:713.
14. Allison, A. C., A. M. Denman, and R. D. Barnes. 1971. Cooperating and controlling functions of thymus derived lymphocytes in relation to autoimmunity. *Lancet.* 2:135.

15. Waldman, T. A., M. Durm, S. Broder, M. Blackman, R. M. Blaese, and W. Strober. 1974. Role of suppressor T cells in pathogenesis of common variable hypogammaglobulinaemia. *Lancet*. 2:609.
16. Sasazuki, T., A. McMichael, R. Payne, F. C. Grumet, R. Radvany, B. Colombe, H. McDevitt, P. Stastny, L. Marchuk, and J. Dosseter. 1975. New and previously recognized MLC alleles. In *Histocompatibility Testing*. F. Kismeyer-Nielsen, editor. Munksgaard, Copenhagen, Denmark. 464.
17. Wilson, A. B., D. G. Haegert, and R. A. Coombs. 1975. Increased sensitivity of the rosette forming reaction of human T lymphocytes with sheep erythrocytes afforded by papain treatment of the sheep cells. *Clin. Exp. Immunol.* 22:171.
18. Kovithavongs, T., P. R. McConnachie, and J. B. Dosseter. 1974. Immunity to tissue sensitization, HLA and non-HLA as detected by the ABCIL system. *Transplantation (Baltimore)*. 17:453.
19. Sasazuki, T., A. McMichael, R. Radany, R. Payne, and H. McDevitt. 1976. Use of high dose irradiation to block back stimulation in the MLC reaction. *Tissue Antigens*. 1:91.
20. Barnstable, C. J., E. A. Jones, W. F. Bodmer, J. G. Bodmer, B. Arce-Gomez, D. Snary, and M. Crumpton. 1977. Genetics and serology of the HLA linked human Ia antigens. *Cold Spring Harbor Symp. Quant. Biol.* In press.
21. Schellekens, P., and V. P. Eijsvogel. 1972. Lymphocyte transformation in vitro. III. Mechanism of stimulation in the mixed leucocyte culture. *Clin. Exp. Immunol.* 7:229.