

**OCCURRENCE OF RESTRICTED SUPPRESSOR
T-CELL ACTIVITY IN MAN***

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Dysfunction of suppressor cells has been implicated in the pathogenesis of immunodeficiencies (1), aplastic anemia (2), recurrent fungal infections (3), and autoimmunities such as systemic lupus erythematosus in humans (4), and its equivalent in the NZB mouse (5). In addition, evidence is accumulating that suppressor cells of lymphoid and/or macrophage origin may appear during the course of tumor growth both in rodents (6-8) and humans (9, 10).

Suppressor T cells can be induced by soluble (11, 12) and cell associated antigens (11, 13) and by concanavalin A (Con A) (14, 15). In some murine models it has been clearly demonstrated that the suppressor T-cell activity is genetically restricted in that the cell that is being suppressed, either directly or through suppressor factors, must share an *I* region component of the major histocompatibility complex with the suppressor cell (12, 13, 16). We describe here what we believe to be the first example of a genetically-restricted suppressor T-cell activity in man.

Materials and Methods

Venous blood mononuclear cells were prepared from donors by defibrination and centrifugation over a cushion of Ficoll Hypaque (F-H) followed by three washes before suspension in Eagle's minimal essential medium containing 10% heat-inactivated fresh normal male serum, 100 U of penicillin and 100 μ g streptomycin/ml, 1% nonessential amino acids, and 2 mM fresh glutamine. Lymphocytes to be used as stimulator cells in mixed leukocyte culture (MLC) were irradiated with a ^{60}Co source to 2,250 rads. Triplicate samples of 125,000 responder cells were incubated in a final vol of 0.2 ml with either 125,000 irradiated stimulator cells for 6 days or with a 1:800 dilution of PHA-P (Difco Laboratories, Detroit, Mich.) or concanavalin A (10 μ g/ml, Sigma Chemical Co., St. Louis, Mo.) for 3 days. The cultures were pulsed with 0.5 μ Ci of [^3H]TdR for the final 18 h of incubation before harvesting by the multiple sample harvester onto glass fiber filters for liquid scintillation counting (17).

For the fractionation of the mononuclear cells into T or non-T-cell subpopulations (18), the cells were resuspended in medium at 0.83×10^6 /ml final concentration containing 10 mM Hepes buffer and 32% fetal calf serum with a 50:1 ratio of sheep erythrocytes (SRBC) per lymphocyte and incubated at room temperature overnight in a 5% CO_2 atmosphere to form spontaneous SRBC rosettes. Cell pellets were gently resuspended, samples removed for enumeration of rosettes, and the remainder gently underlaid with F-H and centrifuged. The pellet and the interface were collected separately, and both were treated with 0.83% Tris- NH_4Cl to lyse the remaining SRBC. The unfractionated cells were treated in a similar fashion with the omission of SRBC in the medium. The fraction that remained at the interface after centrifugation over F-H was designated non-T (T-depleted), and the fraction that pelleted was designated T cells (T-enriched).

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TABLE I
Ability to Stimulate and Respond in One-Way MLC and Responsiveness to Mitogens of Blood Leukocytes from Patient C.H.

Responder lymphocytes	³ H-cpm at day 3 for mitogens and day 6 for MLC stimulated by						
	Patient C.H.	Normal C.D.	Normal P.K.	Normal T.D.	Normal S.C.	Mitogens	
						PHA	Con A
Patient C.H.	386	9,330	9,591	15,482	18,892	17,159	6,091
Normal C.D.	2,394	885	25,470	42,391	38,224	101,574	42,104
Normal P.K.	18,021	15,928	573	32,756	35,126	88,505	31,615
Normal T.D.	26,319	30,418	23,373	1,031	42,752	78,701	26,827
Normal S.C.	36,377	23,009	31,807	45,102	1,885	67,238	30,350

The numbers given are the arithmetic mean of three replicates of 125,000 responder and 125,000 irradiated stimulator cells or mitogens for each test combination. The standard deviations (data not shown) for all combinations shown in the Table averaged 12%.

Results

In our studies of the immune function of cancer patients (17), the pattern of response illustrated in Table I has been not infrequently observed. Bladder cancer patient C.H. had a decreased response to both mitogens and allogeneic cells when compared to the level of response of normal individuals. In addition, his leukocytes stimulated normal donor C.D. only weakly while adequately stimulating three other normal donors, which is generally interpreted as a one-way MLC (19) typing match. However, in these experiments, the dose of stimulator cells was also titrated. Unexpectedly, leukocytes from patient C.H. produced a reciprocal pattern of stimulation distinct from that usually seen in MLC, i.e., as the number of stimulator cells was increased, the weak response of normal C.D. decreased (Table II). Yet, on the same day, when the patient's cells were used to stimulate lymphocytes of other normal individuals, they induced perfectly normal dose-response patterns (cf. normal stimulator cells S.D. and B.D. Table II). Since patient C.H. stimulated lymphocytes from some normal individuals well and yet apparently suppressed other normals, we suspected that his leukocytes might contain both competent stimulator cells as well as a population of suppressor cells which were acting in a restricted fashion.

To test this hypothesis, the patient's leukocytes were depleted of T cells by removing SRBC rosetting cells (18) to determine whether the T-depleted lymphoid cells would stimulate lymphocytes from a normal individual who did not respond to the unfractionated cells (Table III). The unfractionated cells and the T-enriched cells failed to stimulate significant blastogenesis in the suppressible¹ normal responder lymphocytes, but rather exhibited the reciprocal dose-response pattern, i.e., decreasing stimulation with increasing numbers of stimulating cells. However, the patient's T-depleted (non-T) cells were as effective in stimulating that suppressible normal donor as were comparable cells from an unrelated normal individual.

Since the patient's non-T-cell fraction was capable of stimulating both suppressible and nonsuppressible responders, we next examined whether the patient's T cells were actively interfering with the ability of lymphocytes from the suppressible normal to respond to allogeneic stimuli (Table IV). For this

¹ For clarity of presentation, normal donors whose leukocytes responded only weakly and with a reciprocal dose-response pattern to this patient's stimulator leukocytes in one-way MLC were designated as suppressible normals, whereas those normal donors responding to his stimulator cells in a normal fashion are designated nonsuppressible.

TABLE II
Pattern of Decreasing MLC Responsiveness of a Suppressible Normal Donor When Incubated with Increasing Numbers of Irradiated Stimulator Cells from Patient C.H.

Number of Responder lymphocytes	Number of Stimulator lymphocytes	³ H-cpm at day 6 with stimulator cells from			
		Normal C.D.	Normal S.D.	Normal B.D.	Patient C.H.
Normal C.D. 125,000	250,000	—	62,420	42,432	1,860
	125,000	554	46,038	24,555	5,874
	62,500	—	26,856	12,099	9,175

Lymphocytes were prepared and used in MLC as described in Materials and Methods.

TABLE III
MLC Response of a Suppressible Normal Donor to Subpopulations of Lymphocytes from Patient C.H. and a Normal Donor

Responder lymphocytes	Number of stimulator lymphocytes	³ H-cpm at day 6 of MLC stimulated by cells from					
		Normal J.C.			Patient C.H.		
		Unfractionated	T	Non-T	Unfractionated	T	Non-T
Normal L.L. 125,000	125,000	45,872	28,586	50,116	866	778	45,208
	62,500	42,189	27,545	37,828	2,313	3,393	36,174

Normal \times normal_r = 546 cpm. Percent E rosettes for C.H., 46%; J.C., 62%.

TABLE IV
Ability of T Cells from Patient C.H. to Suppress the MLC Response of Lymphocytes of Some Normals but Not Others

Responder lymphocytes	Non-T stimulator lymphocytes	³ H-cpm at 6 days of incubation with 62,500 non-T stimulator cells and	
		Nothing	125,000 Patient C.H. T lymphocytes
Suppressible normal L.L. 125,000	Normal L.L.	6,034	810
	Normal P.K.	32,249	1,215
	Normal R.F.	29,996	1,257
	Normal J.G.	44,144	3,410
	Patient C.H.	20,886	1,573
Nonsuppressible normal P.K. 125,000	Normal L.L.	24,502	24,876
	Normal P.K.	2,572	21,797
	Normal R.F.	13,481	31,883
	Normal J.G.	37,962	51,800
	Patient C.H.	15,219	31,777

Unfractionated responder lymphocytes were incubated with irradiated non-T stimulator lymphocytes with or without 125,000 irradiated patient T lymphocytes as modulator cells.

experiment, unfractionated lymphocytes from a suppressible and from a non-suppressible individual were used as responding cells. These cells were stimulated by the irradiated non-T-cell fraction from several allogeneic normal individuals in one-way MLC with or without the patient's irradiated T cells added as modulator cells. Clearly, the patient's cells abrogated the responsiveness of the suppressible normal to this battery of allogeneic stimulator cells, but did not interfere with the response of nonsuppressible normal donors. Similar experiments (data not shown) showed that this patient's lymphocytes

also depressed the response of the same suppressible normals to mitogenic stimulation.

Blood leukocytes from this particular patient have been used as stimulator cells in the experimental protocol shown in Table I with leukocytes from 27 different unselected normal individuals as responder cells. Deficient stimulation of these normal leukocytes by the patient's stimulator cells similar to that shown in Table I was seen in only three cases. In 11 of these 27 normal combinations, the patient's leukocytes as stimulator cells were titrated to determine the dose-response curve. Only in MLC with those same three normals did his stimulator cells produce the reciprocal pattern of a further reduction in stimulation with increasing numbers of stimulator cells identical to that shown in Table II. In 12 experiments similar to that shown in Table IV we used the patient's irradiated cells as modulator cells in one-way MLC with normal donor lymphocytes as responder cells. In each of the 12 experiments, his cells suppressed only the reactivity of leukocytes obtained from those three normals. In five of these experiments, enrichment of E-rosetting cells was performed and these cells were suppressive, whereas the T-depleted cells were not.

In an attempt to clarify whether this restriction in suppressor activity was under genetic control, as reported in the animal models, HLA typing of the patient and the three suppressible normals was performed. The serological typing data are at present inconclusive as the patient did not type for the HLA-B locus² and reagents for human Ia typing were not available.

As another approach to establishing whether this T-cell suppression was genetically restricted, we reasoned that if there were a genetic restriction, the lymphocytes of one or both parents of each of the suppressible normals should be suppressed by the patient's lymphocytes. We were able to test the natural parents of two of the suppressible normal donors in an experimental design similar to that shown in Table IV. Unfractionated lymphocytes from both the mother and father of each of those two donors were stimulated with several different allogeneic leukocytes to induce a MLC response. The patient's and unrelated normal donor's irradiated leukocytes were then added as modulator cells. The results showed that only one parent of each of the suppressible normals was suppressed by the patient's leukocytes. Thus, there appeared to be a probable genetic restriction to the expression of this suppressor activity, although at present the gene or linkage group with which the suppression is associated remains to be established.

Discussion

The present results suggest that genetic restriction on T-cell suppression may exist in humans as well as in mice. At present, it is not possible to estimate the frequency of this phenomenon in cancer patient populations or speculate whether it is disease-related. The data reported here are from the study of a male patient who had not had transfusions or chemotherapy and who continues to have recurrent bladder carcinomas. We have subsequently found a second patient who has allergic asthma who exhibits this type of

² Typing for HLA-A and HLA-B courtesy of the Fred Hutchinson Cancer Research Center.

restricted suppressor activity intermittently. Addition of his leukocytes as third party cells to MLC reactions depressed the ability of leukocytes from one normal donor but not others to respond to unrelated allogeneic stimulator cells. When his leukocytes were used as stimulator cells in MLC, they produced the reciprocal pattern of stimulation against that one normal donor but not others.

This finding of a genetically-restricted T-suppressor activity in man supports the generality of similar recent findings in animal models (12, 13, 16). The present experiments also emphasize the difficulties in detecting suppressor cells in man when the activity may be restricted and, thus, detectable only with normal indicator (responder) leukocytes from a small percentage of randomly selected individuals, except in the rare event that the subject being tested for suppressor cells has an identical twin or both parents living, healthy, and geographically available. Obviously, more studies are required to establish whether this apparently genetically-restricted suppression is linked to the HLA complex and related to the incidence and course of disease.

Summary

Blood leukocytes from an immunologically hyporesponsive patient with urinary bladder carcinoma were found to be deficient in their ability to stimulate 3 of 27 responder leukocyte preparations from normal individuals in one-way mixed leukocyte culture (MLC). The patient's T-depleted leukocytes, however, functioned adequately as stimulator cells. T-enriched lymphocytes from this patient suppressed the MLC responsiveness of those three normals but not the responsiveness of other normals. The patient's cells suppressed the MLC responsiveness of only one of each of the parents of two of the normals who could be suppressed by the patient's leukocytes suggesting a possible genetic restriction to this suppressor cell activity.

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