

CONTRASUPPRESSION

A Novel Immunoregulatory Activity*

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A reductionist approach is commonly used to gain insight into complex biological systems: individual components of the system are isolated so that the contribution of each individual element can be studied in detail. Dissection of the events that control blood clotting (1) or complement activation (2, 3) are examples of the successful use of this approach.

We have tried to use this approach to understand the highly complex system of immunoregulation. Our strategy has depended on the demonstration that the genetic program of many immunologically competent cells combines information for function (reflected by the synthesis of biologically active proteins by the cells) with the expression of an unique pattern of surface glycoproteins (4). These cell surface glycoproteins can serve as markers for fractionating lymphocytes into sets with the use of specific antisera and the contribution of each set to the regulation of immunity can then be determined.

This experimental approach has shown that cells which express the Ly-1⁺,2⁻ T cell set surface pattern (Ly-1 cells)¹ are programmed to act as inducer or initiator cells. All T-dependent immune responses so far studied require induction by Ly-1 cells for optimal activity (4, 5); Ly-1 cells induce B cells to make antibody; they induce macrophages and other nonspecific inflammatory cells to participate in delayed-type hypersensitivity reactions; they induce effector activity from killer cell precursors; and they induce suppressor T cells to express optimal suppression. Whether all these inducing functions of the Ly-1 cell set are invested in a single group of cells, or,

* Supported by grants CA-08593, AI-14216, AI-10497, CA-14349, and CA-18359 from the National Institutes of Health.

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¹ *Abbreviations used in this paper:* BSS, balanced salt solution; BRBC, burro erythrocytes; C', complement; HRBC, horse erythrocytes; FCS, fetal calf serum; Ly-1, Ly-1⁺,2⁻ T cell set; Ly-1,2, Ly-1⁺,2⁺ T cell set; Ly-2, Ly-1⁻,2⁺ T cell set; NMS, normal mouse serum; PFC, plaque-forming cells; SRBC, sheep erythrocytes; TsF, suppressor factor from T cells.

whether the Ly-1 set is a heterogeneous collection of cells with each inducer function being mediated by individual Ly-1 subsets is not fully resolved.

The use of antisera against a polymorphic gene product(s) controlled by the I-J subregion of the major histocompatibility complex (6) has given a partial answer to this question (7). Thus, Ly-1 cells treated with anti-I-J serum and complement induce B cells to secrete antibody but are deficient in their ability to induce suppressor cell activity, i.e., the I-J⁺ Ly-1 subset is specialized in inducing suppressive activity whereas cells in the I-J⁻; Ly-1² subset are specialized in inducing B cell activity (7). This finding indicates that not all inducer activities are equally invested in all Ly-1 cells.

Less information is available on the heterogeneity of regulatory T cells that express the Ly-1⁻, Ly-2⁺ phenotype (Ly-2 cells). Tada et al (8) have shown that an I-J⁺ fraction of Ly-2 cells is dependent upon an interaction with I-J⁺; Ly-1⁺; Ly-2⁺ (Ly-1,2) cells to manifest suppressive activity. This requirement for Ly-1,2 cells to make Ly-2 suppressor cell activity manifest has also been demonstrated by Germain and Benacerraf (9) and McDougal et al. (10). The precise cell surface phenotype of the final effector cell in these systems has not been determined. On the other hand, Kontiainen and Feldmann (11) have shown that Ly-2 cells can suppress the in vitro antibody response of cultured spleen cells that have been depleted of all Ly-2⁺ (Ly-2 and Ly-1,2) cells, suggesting that the Ly-2 cell set may also be heterogeneous.

These considerations led us to attempt to separate and characterize those Ly-2 T cells that act as suppressor cells in the presence of Ly-1,2 T cells from those acting as suppressor cells in their absence. In the course of these studies, we have found that regulatory Ly-2 T cells can be subdivided into I-J⁺ and I-J⁻ fractions. The I-J⁻ fraction contains effector cells that suppress Ly-1 helper T cell activity.³ Within the I-J⁺ fraction there is a subset of cells that has not been previously described. These cells interact with Ly-1,2 T cells to inhibit Ly-2-mediated suppressor cell activity. This report concerns the definition of this heretofore unrecognized immunoregulatory circuit which is composed of at least two T cell subsets that communicate via the use of a cell free product. We use the term "contrasuppression" to define the immunoregulatory activity that this cellular circuit produces.

Materials and Methods

Mice. C57BL/6J (B6), (C57BL/6 × B10.A)F₁ (B6AF₁), and B10.A/Sn mice, 8-12 wk of age, were obtained from The Jackson Laboratory, Bar Harbor, Maine. All other strains are maintained in our colony at Yale University School of Medicine, New Haven, Conn.

Antigens. Sheep erythrocytes (SRBC) were obtained from Colorado Serum Co., Denver, Colo.

Antisera. Anti-Ly-1.2 (C3H/An anti-C3H.CE-Lyt-1.2) and anti-Ly-2.2 (C3H/An × B6-Lyt-2.1 anti-ERLD) were prepared and tested for specificity as previously described (12). Monoclonal anti-Thy-1 reagents were generously provided by Dr. Phillip Lake, University College, London and Dr. Jonathan Sprent, University of Pennsylvania, Philadelphia, Pa. Anti-I-J^b serum was prepared by hyper-immunizing B10.A(5R) recipients with a mixture of B10.A(3R) spleen and lymph node cells (antiserum No. ASM-5). Anti-I-J^a sera were prepared by hyper-

² The designation "I-J⁻" is used to indicate that the cell type in question is not killed by complement-dependent treatment with our anti-I-J reagents. We cannot exclude the possibility that these I-J⁻ functional cell populations (a) express an I-J determinant not detected by our reagents or (b) express insufficient amounts of I-J antigen to be killed.

³ Although it is clear that I-J⁺; Ly-2 T cells are involved in the generation of suppressor activity, there is no previously published evidence that I-J-controlled determinants are expressed on Ly-2 effector cells that suppress responses made by Ly-1 T cells and B cells.

immunizing B10.A(3R) (antisera No. ASM-18 and No. AMS-19) and [B10.A(3R) × A.BY]F₁ (ASM-46) recipients with B10.A(5R) cells (6).

Cytotoxic Depletion of Cells. Depletion of cells bearing a given marker was achieved by incubating cells with antiserum diluted in balanced salt solution (BSS) for 30 min at 37°C (1 × 10⁷ cells/1 ml of 1:20-diluted anti-Ly sera/1 ml of 1:1,000-diluted anti-Thy-1 serum/1 ml of 1:5-diluted anti-I-J serum), washing, and incubating with rabbit complement for 45 min at 37°C (1 × 10⁷ cells/1 ml of 1:10-diluted complement). The cells were then washed twice in BSS and resuspended in tissue culture medium (13).

Preparation of Lymphocyte Subpopulations. T cells were prepared by adding unprimed spleen cells to plastic petri dishes coated with goat anti-mouse immunoglobulin, and harvesting the nonadherent fraction (14). B cells were prepared by treating unprimed spleen cells with anti-Thy-1.2 plus complement (13). Depletion of Ly-2-bearing T cells from unprimed spleen cell suspensions was achieved by treating spleen cells with anti-Ly-2.2 plus complement (Ly-1 T cells plus B cells) (13).

Antiserum Absorption. Absorption of the B10.A(3R) anti-B10.A(5R) serum (ASM-18) was performed by suspending 1 ml of 1:5-diluted serum with 3 × 10⁸ unprimed spleen plus lymph node cells for 1 h at room temperature.

Antigen Stimulation of Ly-2 T Cells. Ly-2 T cells were primed by culturing 1 ml of 10⁷ purified T cells with 0.025 ml of a 1% sheep erythrocytes (SRBC) suspension for 4 d in Falcon 3008 tissue culture dishes (Falcon Labware, Div. of Becton, Dickenson, & Co., Oxnard, Calif.) in a 5% CO₂-95% air incubator at 37°C. RPMI-1640 tissue culture media, fortified with 10% fetal calf serum (FCS), 100 mM glutamine, 25 mM Hepes, and 5 × 10⁻⁵ M 2-mercaptoethanol was used. After the 4-d culture period, the cells were harvested, washed, counted, treated with anti-Ly-1 plus complement, and added in graded numbers to appropriate assay cultures (13, 15).

Assay Cultures. Suppressor activity by the primed Ly-2 T cells was determined by adding these cells to cultures containing either unprimed spleen cells or anti-Ly-2 plus complement-treated unprimed spleen cells (Ly-1 T cells plus B cells) plus or minus additional, unfractionated unprimed T cells. Helper activity was determined by adding unprimed T cells to anti-Thy-1 plus complement-treated unprimed spleen cells (B cell source). All cells were suspended in RPMI-1640 tissue culture medium (see above), and 0.2 ml of the cells cultured with 0.025 ml of a 1% SRBC suspension in Falcon 3040 flat-bottomed microtiter trays (cell assays) or 1 ml of the cells cultured with 0.05 ml of a 1% SRBC suspension in Falcon 3008 plates (factor assays) in a 5% CO₂-95% air incubator at 37°C. At day 5, the anti-SRBC response was determined by enumerating the number of plaque-forming cells (PFC) per culture by the technique of Cunningham and Szenberg (16).

Purification of Ly-2 T Suppressor Factor. The detailed method for production of this factor has been described.⁴ Mice were immunized intraperitoneally with 0.2 ml of 20% SRBC twice at a 2-wk interval and killed 2 wk after the second immunization. Their spleen cells were treated with an anti-Ly-1 serum and rabbit complement (C') and then cultivated in vitro for 48 h at 1 × 10⁷/ml in RPMI-1640 supplemented with 10% FCS.

After incubation, the supernate was harvested, centrifuged at 2,500 rpm for 20 min and passed through a Millipore filter (Millipore Corp., Bedford, Mass.). (The role of antigen in helping [or suppressing] the production of these factors has not yet been fully determined).

Results

Definition of a T Cell Subset that Interferes with Ly-2 Suppressor T Cell Activity (Surface Phenotype Thy-1⁺; Ly-1⁺, 2⁺; I-J⁺) (Table I). Under appropriate experimental conditions, antigen-primed Ly-2 T cells will only suppress Ly-1 T cell helper activity in the absence of unprimed Ly-2⁺ T cells. Data from three experiments (Table I) demonstrate that the ability of 3 × 10⁴ SRBC-primed Ly-2 T cells to suppress responses by 2 × 10⁶ unprimed anti-Ly-2 plus C'-treated spleen cells (Ly-1 T and B cells) (Table I, line 1

⁴ Yamauchi, K., D. B. Murphy, F.-W. Shen, H. Cantor, and R. K. Gershon. Analysis of "I-J" MHC-restricted, cell-free products from "I-J"; Ly-2 T cell that suppress Ly-2-depleted spleen cells. Manuscript submitted for publication.

TABLE I
Definition of a T Cell Subset That Interferes with Ly-2 Suppressor T Cell Activity (Surface Phenotype: Thy-1⁺; Ly-1⁺, 2⁺; I-J⁺)

	3 × 10 ⁴ primed Ly-2 T cells*	2 × 10 ⁵ unprimed T cells treated with‡	10 ⁶ unprimed Ly-1 T and B cells§			Comments
			B10.A (Exp. I)	B10.A (Exp. II)	B6 (Exp. III)	
<i>PFC/culture</i>						
1.	—	No cells added	2,500	1,700	1,500	<i>Control.</i> Ly-1 T cells induce B cells to produce antibody.
2.	+	No cells added	500	100	500	<i>Suppression.</i> Primed Ly-2 T cells suppress helper activity.
3.	+	NMS + C'	2,600	1,600	1,500	<i>No suppression.</i> Unprimed T cells interfere with suppressor activity.
4.	+	Anti-I-J ^k (I) + C'	600	100		<i>Suppression.</i> anti-I-J ^k kills unprimed T cells that interfere with suppressor activity.
5.	+	Anti-Thy-1 (II) + C'	800			<i>Suppression.</i> Anti-Thy-1 kills unprimed T cells that interfere with suppressor activity.
6.	+	Mixture I + II	800			<i>Suppression.</i> I-J and Thy-1 determinants are both expressed on unprimed T cells that interfere with suppressor activity.
7.	+	Anti-Ly-2 + C'	700			<i>Suppression.</i> Anti-Ly-2 kills unprimed T cells that interfere with suppressor activity (suppression not overcome by additional Ly-1 T cells).
8.	+	Anti-Ly-1 + C'	400			<i>Suppression.</i> anti-Ly-1 kills unprimed T cells that interfere with suppressor activity.
9.	+	Anti-I-J ^k + C'			1,600	<i>No suppression.</i> Anti-I-J ^k fails to kill unprimed T cells that interfere with suppressor activity in I-J ^b strain (specificity control).
10.	+	Anti-I-J ^b + C'			600	<i>Suppression.</i> Demonstration that an unprimed I-J T cell interferes with suppressor activity in H-2 ^b mice.

* Ig-plate-nonadherent spleen cells cultured for 4 d with SRBC and treated with anti-Ly-1 plus C'.

‡ Ig-plate-nonadherent spleen cells. Anti-I-J^k, B10.A(3R) anti-B10.A(5R) (ASM-19); anti-I-J^b, B10.A(5R) anti-B10.A(3R) (ASM-5).

§ All cultures contain 10⁶ anti-Ly-2 plus C'-treated unprimed spleen cells (Ly-1 T cells and B cells). Mean PFC of triplicate cultures. B10.A (I-J^k), B6 (I-J^b).

|| NMS, normal mouse serum.

vs. line 2), can be blocked by the addition of 2 × 10⁵ unprimed T cells to the cultures (line 3). Identical results were obtained using cells from two different inbred strains, B10.A (experiment I and experiment II) and B6 (experiment III). Thus, cells present in the antigenically naive T cell preparation interfered with Ly-2 T cell-mediated suppression, i.e., displayed contrasuppressive activity. Treatment of the unprimed T cells from I-J^k mice (B10.A) with anti-I-J^k (line 4), anti-Thy-1 (line 5), anti-Ly-2 (line 7), or anti-Ly-1 (line 8) antisera plus C' ablated their contrasuppressive activity. In

addition, mixtures of independently treated anti-I-J^k and anti-Thy-1-treated cells (line 6), as well as mixtures of anti-I-J^k and anti-Ly-1- or anti-Ly-2-treated cells (data not shown), did not reconstitute contrasuppressive activity, suggesting that the cell responsible for the contrasuppressive activity expressed all four of these alloantigens. It also expresses the Qa-1 alloantigen (data not shown). Data from experiment III show that an I-J^b-bearing cell also interferes with Ly-2 suppressive activity in a second strain (B6) (line 10) and verifies the specificity of the anti-I-J^k antiserum (line 9). For the sake of simplicity, we will hereafter refer to the T cell that interferes with suppressive activity as an "Ly-1,2;I-J⁺ contrasuppressor cell."

I-J and Ly-2 Markers Distinguish Contrasuppressor T Cells from Helper T Cells (Table II). The observation that Ly-1 T cells fail to overcome Ly-2 T cell-mediated suppressive activity (Table I, line 7) suggested that the observed inhibition of suppression could not be accounted for by a simple excess of helper cell activity. Two other lines of evidence support this conclusion. First, aliquots of the same Ly-1 T cells that could not overcome suppression (see Table I, line 7) exhibited strong helper activity when added to purified B cells (Table II, line 3). Second, anti-I-J plus C' treatment had no effect on helper activity (Table II, line 4), although this treatment eliminated the contrasuppressor cell (Table I, line 4). Thus, Ly-1,2;I-J⁺ T cells mediate contrasuppressive activity, whereas the Ly-1⁺,2⁻;I-J⁻ T cells that induce B cells to make antibody do not.

Further Evidence for a T Cell Subset That Interferes with Ly-2 Suppressor T Cell Activity (Studies with a Cell-free Product) (Table III). Confirmation of the above observations comes from experiments conducted with biologically active cell-free products obtained from antigen-stimulated Ly-2 T cells (Materials and Methods). Addition of a supernate from cultured-primed Ly-2 cells to fresh cultures containing unprimed unfractionated T cells plus B cells did not suppress helper activity and thus appeared to lack suppressive activity (Table III, lines 1 and 2). However, addition of the same amount

TABLE II
I-J and Ly-2 Markers Distinguish Contrasuppressor Cells From Helper Cells

	2 × 10 ⁵ unprimed T cells treated with*	5 × 10 ⁵ unprimed B cells‡	Comments
		PFC/culture	
1.	No cells added	100	<i>Negative control.</i>
2.	NMS + C'	2,200	<i>Positive control.</i> Helper T cells induce B cells to produce antibody.
3.	Anti-Ly-2 + C'	2,100	<i>Help.</i> Helper T cells are not killed by anti-Ly-2; the same treatment eliminated contrasuppressive activity (Table I, line 7).
4.	Anti-I-J ^k + C'	2,100	<i>Help.</i> Helper T cells are not killed by anti-I-J; the same treatment eliminated contrasuppressive activity (Table I, line 4).
5.	Anti-Thy-1 + C'	400	<i>Marginal help.</i> Helper T cells are killed by anti-Thy-1.
6.	Anti-Thy-1 + C'	500	<i>Marginal help.</i> Helper T cells are killed by anti-Ly-1.

* Aliquots of Ig-plate-nonadherent B10.A spleen cells utilized in Table I, experiment I. Anti-I-J^k, B10.A(3R) anti-B10.A(5R) (ASM-19).

‡ All cultures contain unprimed anti-Thy-1 plus C'-treated spleen cells (B cell source). Mean PFC of triplicate cultures.

TABLE III
 Further Evidence for a T Cell Subset That Interferes with Ly-2 Suppressor T Cell Activity (Studies with Cell-free Material (Factor[s]))

	Factor(s) from primed Ly-2 T cells added to cultures*	5 × 10 ⁶ unprimed spleen cells treated with‡	PFC/culture§		Comments
			Exp. I	Exp. II	
1.	—	NMS + C'	1,700	1,300	No suppression.
2.	+	NMS + C'	1,700	1,200	
3.	—	Anti-Ly-2 + C'	1,700	900	Suppression. Removal of an Ly-2 ⁺ acceptor cell reveals latent suppressive activity in factor preparation.
4.	+	Anti-Ly-2 + C'	300	300	
5.	—	Anti-I-J ^b + C	ND	800	Suppression. Removal of an I-J ⁺ acceptor cells reveals latent suppressive activity in factor preparation
6.	+	Anti-I-J ^b + C	ND	200	

* Culture supernate from in vivo primed anti-Ly-1 plus C' treated B6 spleen cells (experiment I) or B6AF₁ spleen cells (experiment II).

‡ B10 spleen cells (experiment I) or B6AF₁ spleen cells (experiment II).

§ Mean PFC of triplicate cultures.

of this supernate to unprimed and Ly-1 T cells plus B cells resulted in a substantial suppression of the PFC response (lines 3 and 4). A similar suppression was revealed when the unprimed T cells were treated with an anti-I-J serum (lines 5 and 6). Because anti-Ly-2 or anti-I-J plus C' treatment of the unprimed spleen cells was required for the expression of the suppressive activity, it can be concluded that I-J⁺;Ly-2⁺ contrasuppressor cells in the unprimed T cell preparation interfered with the suppressive activity mediated by the cell-free products of Ly-2 suppressor T cells. These results provide evidence that suppressive activity can be inhibited at a stage distal to the generation of Ly-2 suppressor effector cells and the release of their biologically active mediators.

In sum, the data presented show that (a) Thy-1⁺;Ly-1,2;I-J⁺ contrasuppressor cells interfere with Ly-2 suppressor cell activity; (b) this contrasuppressive activity is not produced by Ly-1⁺,2⁻;I-J⁻ helper T cells; and (c) contrasuppression can occur after the generation of suppressor effector cells. Although the precise mechanism by which contrasuppression is brought about remains to be resolved, it is clear that the activity which we have described is distinguishable from activities previously associated with either helper or suppressor systems.

An I-J⁺;Ly-2 T Cell Subset Is Required for the Induction of Contrasuppressive Activity (Table IV). The biological activity of Ly-1,2 T cells, which is the phenotype of the cell we have found to be responsible for contrasuppression, depends, to a large degree, on the nature of the inducing signal they receive (5).⁵ Therefore, we looked to see if (a) Ly-2 T cells act to induce Ly-1,2 T cell-dependent contrasuppression, and if so, (b) if contrasuppressor-inducing activity and suppressor activity are mediated by separate

⁵ Yamauchi, K., D. B. Murphy, H. Cantor, and R. K. Gershon. Analysis of antigen specific, Ig restricted cell-free material made by I-I⁺;Ly-1 cells (Ly-1;TsiF) that induces Ly-2⁺ cells to express suppressive activity. Manuscript submitted for publication.

subsets of Ly-2 T cells. Because the expression of I-J-controlled markers had been successfully used to separate different types of Ly-1 inducer T cells (Introduction), we tried to determine if the cellular expression of I-J could also be used to identify these potentially different Ly-2 T cell subsets. We found that 3×10^4 antigen-stimulated Ly-2 T cells could suppress the response of unprimed Ly-1 T and B cells (Table IV, line 2), but that this suppressive activity was abrogated by the addition of 2×10^5 unprimed T cells to the assay cultures (line 3). However, if the antigen-stimulated Ly-2 T cells were treated with anti-I-J plus C', they could act as potent suppressor cells even in the presence of 2×10^5 unprimed T cells (lines 4 and 5). Thus, the activity of the Ly-1,2;I-J⁺ contrasuppressor T cells described above is not autonomous. An inducing signal from antigen-stimulated Ly-2 T cells is required to make their activity manifest. This inducing signal does not come from the Ly-2 suppressor T cell itself (which is I-J⁻) but rather from a second Ly-2 cell subset (contrasuppressor inducer) that expresses an I-J marker. Whether Ly-2 suppressor T cells lack detectable amounts of all I-J products or bear an I-J-subregion product not detected by our antisera remains to be determined.

Cells with the Contrasuppressor-inducer Phenotype (I-J⁺;Ly-1⁻,2⁺) Produce a Cell-free Product That Has the Same Activity as Contrasuppressor-inducer Cells (Table V). To further clarify the role of I-J⁺;Ly-2 T cells in the contrasuppression system, we asked if cell-free

TABLE IV
An I-J⁺;Ly-2 T Cell Subset Is Required for the Induction of Contrasuppressive Activity

	3 × 10 ⁴ primed Ly-2 T cells treated with*	2 × 10 ⁵ unprimed T cells‡	10 ⁶ un-primed Ly-1 T and B cells	Comments
			PFC/culture	
1.	No cells added	—	1,800	Control. Ly-1 T cells induce B cells to produce antibody.
2.	NMS + C'	—	100	Suppression. Primed Ly-2 T cells suppress helper activity.
3.	Anti-I-J ^k abs. 5R + C'¶	+	1,300	Marginal suppression. Primed Ly-2 T cells induce contrasuppressor T cells to inhibit suppression.
4.	Anti-I-J ^k + C'	+	100	Suppression. Anti-I-J treatment kills primed Ly-2 T cells which induce contrasuppressor cells, but does not kill primed Ly-2 suppressor T cells.
5.	Anti-I-J ^k abs. 3R + C'¶¶	+	100	Suppression. I-J ^b strain fails to absorb antibody reactive with I-J ^k primed Ly-2 T cells which induce contrasuppressor cells (specificity control).

* Ig-plate-nonadherent B10.A spleen cells cultured for 4 d with SRBC, and treated with anti-Ly-1 plus complement.

‡ (—) without or (+) with Ig-plate-nonadherent spleen cells.

§ All cultures contain 10⁶ anti-Ly-2 plus C'-treated unstimulated spleen cells (Ly-1 T cells and B cells). Mean PFC of triplicate cultures.

¶ B10.A(3R) anti-B10.A(5R) (ASM-18) absorbed with B10.A(5R) (I-J^k) cells (NMS equivalent). This serum should not contain anti-I-J activity.

¶¶ B10.A(3R) anti-B10.A(5R) (ASM-18) absorbed with B10.A(3R) (I-J^b) cells. This serum should contain anti-I-J activity.

material from antigen-primed Ly-2 T cells could induce the same contrasuppressive activity as the cells. We found that like the producer cells (Table III) the addition of some factor(s) from antigen-stimulated Ly-2 T cells did not suppress the response of unprimed unfractionated spleen cells (Table V, line 4), whereas factor(s) derived from an aliquot of the same cells that had been treated with anti-I-J plus C' did suppress the response (Table V, line 5). Thus, an I-J⁻;Ly-2 T cell produces material that suppresses the expression of helper activity but does not induce contrasuppression, whereas the product of I-J⁺;Ly-2 T cells induces Ly-1,2;I-J⁺ T cells to display contrasuppressive activity.

Further Evidence That the Ly-2 T Cell Responsible for Delivering a Suppressive Signal Can Be Separated from the Cell That Activates Contrasuppression by Virtue of Differential Expression of I-J Subregion-controlled Products (Table VI). In the experiments depicted in Table VI, biologically active cell-free products were obtained from antigen-stimulated, non-anti-I-J-treated Ly-2 T cells (see Materials and Methods and Table III). However, the products were passed through an anti-I-J immunoabsorbent. This maneuver turned a moderately suppressive factor(s) to a much more potent suppressive factor(s). Thus an I-J⁺;Ly-2⁺ T cell (Table V), produces an I-J⁺ product that can activate contrasuppressor cells. These activated cells can obscure the suppressive activity of I-J⁻;Ly-2 T cells and their products.

Studies on the Specificity of the I-J⁺ Factor That Activates Contrasuppressor Cells (Table VII). Previous studies have shown that the suppressor factor(s) made by I-J⁻;Ly-2

TABLE V
Cells with the Contrasuppressor-inducer Phenotype (I-J⁺;Ly-1⁻,2⁺) Produce Cell-free Material That Has the Same Activity as Contrasuppressor-inducer Cells

	Factor(s) from primed Ly-2 T cells treated with*	5 × 10 ⁶ unprimed spleen cells treated with‡	PFC/culture§		Comments
			Exp. I	Exp. II	
1.	No factor(s) added	NMS + C'	1,300	1,700	<i>Control.</i> Unprimed T cell induce B cells to produce antibody.
2.	No factor(s) added	Anti-Ly-2 + C'	1,300	1,700	<i>Control.</i> Anti-Ly-2 treatment has no effect on helper activity.
3.	NMS + C'	Anti-Ly-2 + C'	350	300	<i>Suppression.</i> Primed Ly-2 T cells produce a factor(s) that suppresses helper activity.
4.	NMS + C'	NMS + C'	800	1,700	<i>Marginal or no suppression.</i> Factor(s) from primed Ly-2 T cells activate contrasuppressor T cells to inhibit suppressor activity.
5.	Anti-I-J + C'	NMS + C'	400	350	<i>Suppression.</i> Anti-I-J treatment kills primed Ly-2 T cells which produce factor(s) that activates contrasuppressor cells, but does not kill primed Ly-2 T cells which produce factor(s) that suppresses helper activity.

* Culture supernate from in vivo primed anti-Ly-1 plus C'-treated B6AF₁ spleen cells (experiment I) or B6 spleen cells (experiment II). Anti-I-J^k, B10.A(3R) anti-B10.A(5R) (ASM-18) used in experiment I. Anti-I-J^b, B10.A(5R) anti-B10.A(3R) (ASM-5) used in experiment II.

‡ B10 spleen cells used in experiment I, (B6A)F₁ spleen cells used in experiment II.

§ Mean PFC of triplicate cultures.

TABLE VI
Contrasuppressor-inducer Cells Produce I-J⁺ Cell-free Material That Has the Same Activity as Do The Cells

Factor(s) from primed Ly-2 T cells treated with*	PFC/culture‡	Comments
No factor(s) added	1,900	<i>Control.</i>
NMS column filtrate§	900	<i>Suppression.</i> Primed Ly-2 T cells produce a factor that is moderately suppressive.
Anti-I-J ^b column filtrate	200	<i>Increased suppression.</i> Removal of I-J-binding material changes moderate suppression to severe suppression, i.e., it removes contrasuppression.

* Culture supernate from primed anti-Ly-1 plus C'-treated B6 spleen cells.

‡ Mean PFC of triplicate cultures of unfractionated B6 spleen cells.

§ NMS conjugated to Sepharose beads.

|| Anti-I-J^b (ASM-5).

TABLE VII
Antigen Specificity of Contrasuppressor-inducer Factor Is More Cross-reactive Than Is the Ly-2 Suppressor Factor(s) Specificity

Factor(s) from primed Ly-2 T cells treated with*	PFC/culture‡	Comments
No factor	2,600	<i>Control.</i>
No treatment of factor	4,000	<i>No suppression.</i>
Abs. (HRBC)§	750	<i>Suppression.</i> Absorption with cross reacting erythrocytes removes material that blocks "latent" suppression in Ly-2 factor preparations
Abs. (BRBC)§	700	
Abs. (SRBC)§	2,800	<i>No suppression.</i> Expected result since suppressive material is SRBC specific.

* Culture supernatant from primed anti-Ly-1 plus complement treated B6 spleen cells.

‡ Mean PFC of triplicate cultures of unfractionated B6 spleen cells.

§ Factor preparation absorbed with cells in parentheses.

cells can be removed by absorption with specific antigen, but not with other heterologous erythrocytes.⁴ Also, other studies have suggested that suppressive interactions between regulatory cells can be inhibited by cross-reactive antigens (17, 18). If the previously noted interference with suppression and/or inhibition of tolerance by cross-reacting antigen was related to contrasuppression, one might expect that the cells in the contrasuppressor circuit would be more cross-reactive than the cells in the suppressor circuit. Thus, we attempted to determine if the contrasuppressor-inducing factor made by the I-J⁺;Ly-2 T cells could be absorbed by heterologous erythrocytes other than the ones used to induce production of a suppressor factor from T cells (TsF).

The results presented in Table VII demonstrate quite clearly that such is the case. In this experiment, both horse erythrocytes (HRBC) and burro erythrocytes (BRBC) removed the contrasuppressor activity and left the SRBC-specific suppressive activity intact. This is not a dose effect because TsF cannot be absorbed with inappropriate erythrocytes, even when the dose of TsF is limiting. We can therefore conclude that contrasuppressor-inducer material has a broader specificity than does the suppressor

factor(s). Thus it is possible in those circumstances where suppressed immunological reactivity was rescued by cross-reacting or modified antigens, cells in the contrasuppressor circuit may have been involved.

The specificity of the nonimmune contrasuppressor cell that is activated by the primed inducer cell has been more difficult to determine. Clonal deletion type experiments must be done to ascertain the specificity of this type. We are presently attempting to address this important question.

It is important to emphasize that our results do not indicate that Ly-2 T cells cannot suppress the response of unfractionated spleen cells. It is just that it is an easier task to accomplish when the cells in the contrasuppressor circuit are removed. The data we have chosen to present best illustrate this point.

Discussion

The data presented show that antigen-stimulated $I-J^+$;Ly-2 T cells produce a biologically active cell-free product (factor) that induces unprimed Ly-1,2; $I-J^+$ T cells to inhibit the suppressive activity mediated by antigen-stimulated $I-J^-$;Ly-2 T cells. Because (a) the net effect of this cellular interaction is inhibition of suppressive activity, and (b) excess helper activity cannot account for this inhibition, the phenomenon can best be described by the term "contrasuppression." Thus, Ly-2; $I-J^+$ inducer T cells, the cell-free product that they produce, and Ly-1,2; $I-J^+$ T cells are components of a contrasuppression system or circuit. (The effector cell in this circuit is probably an Ly-1; $I-J^+$ T cell.⁶) The ability of contrasuppressor cells to block the activity of a suppressive cell-free product, produced by $I-J^-$;Ly-2 suppressor T cells, shows that contrasuppression can be effected even after the generation of suppressor effector cells and release of their biologically active products.

The key differentiation marker utilized to distinguish components of the contrasuppression circuit from those of helper or suppressor circuits is an I-J-subregion-controlled determinant. Thus, both the Ly-2 inducer cell and the Ly-1,2 acceptor T cells in the contrasuppressor circuit, but not Ly-2 suppressor effector T cells or helper T cells,⁷ bear an I-J determinant detected by our reagents in our systems. In addition, preliminary data (D. B. Murphy, unpublished observations) show that an anti- $I-J^k$ reagent (ASM-19), which kills Ly-1,2 contrasuppressor T cells, does not kill the $I-J^+$;Ly-1,2 T cells in the feedback suppression circuit (7). These studies therefore provide further evidence for genetic and serologic complexity of the I-J subregion, and suggest that different I-J subregion determinants are expressed in T cell subsets in the contrasuppression and suppression circuits.

Besides documenting a heretofore uncharacterized immunoregulatory interaction, the results we have presented make several other interesting points. For example, they

⁶Green, D. R., D. D. Eardley, A. Kimura, D. B. Murphy, K. Yamauchi, and R. K. Gershon. Immunoregulatory circuits which modulate responsiveness to suppressor cell signals: characterization of an effector cell in the contrasuppressor circuit. Manuscript submitted for publication.

⁷The reason why we cannot demonstrate $I-J^+$;Ly-1 helper T cells (19) or helper factors (20) is not clear, but may have something to do with the nature of the antigens studied as suggested by Howie et al. (20). Alternatively, the apparent helper function these authors have described is in reality not a result of helper function. We have shown that I-J antisera can remove apparent helper activity in some instances. However in those instances, if suppressor cells were removed from the system, the helper cell signal was not affected by the I-J antisera. Thus, the actual functional helper cell was $I-J^-$ and $I-J^+$ contrasuppressor cells were required for $I-J^-$ helper activity to be come manifest (G. M. Iverson, D. R. Green, W. Ptak, and R. K. Gershon, manuscript in preparation).

show an elegant symmetry in the immunoregulatory apparatus. As previous studies have shown (4, 5), the majority of cells that express the $Ly-1^+, 2^-$ phenotype are programmed to induce immunological effector cells that make a positive contribution in the immune response, e.g., they induce cells to make antibody, effect delayed-type hypersensitivity responses, and express cytotoxic functions. However, the $Ly-1^+, 2^-$ set also contains a small subset of $I-J^+$ cells whose function is to activate suppressor cells that tend to counteract the positive signals produced by the majority of cells in the $Ly-1$ cell set (7).

We have now shown a similar type of functional split in the cells that express the $Ly-1^-, 2^+$ phenotype. Although the predominant function of immunoregulatory $Ly-1^-, 2^+$ cells is to suppress immune responses, they contain an identifiable and separable subset that has the job of inducing other cells to counteract the suppressive signal. As with the small subset of $Ly-1^+, 2^-$ cells that is involved in inducing an activity that opposes the predominant activity of the other cells in the set (e.g., suppression), the $Ly-1^-, 2^+$ cells, which induce the alternative opposing activity (e.g., interference with suppression or contrasuppression) also express a gene product(s) controlled by the I-J subregion. As discussed above, the I-J markers expressed on the cells in suppressor circuit and those expressed on the cells involved in contrasuppression are probably controlled by different loci.

Another surprising result is the amount of functional heterogeneity that can be found in the $Ly-1^-, 2^+$ T cell set. Although this T cell set represents <10% of the T cell pool, it contains at least four subsets with distinct functions; (a) killer cells; (b) suppressor cells; (c) cells that amplify suppressor activity (8-10); and (d) the subset we have described in this report, which acts to induce other cells to countermand suppressor signals. Two of these cell subsets (e.g., the cells involved in contrasuppression and those that amplify suppressor activity) have not yet been clearly separated by criteria other than function, although preliminary data indicates that Qa-1 is present on the former but not the latter cell subset (10). These two functionally distinct $Ly-1^-, 2^+$ subsets can be distinguished from the other two $Ly-1^-, 2^+$ cells (e.g., killer and suppressor cells) by the I-J subregion-encoded antigens that are expressed.

The question of the biological significance of the contrasuppressive activity we have described must be addressed. The results indicate that there are at least two separate modes by which suppressor cell activity can be regulated. Previous results have shown that the activation of suppressor T cells is under control of an $Ly-1$ inducer cell (7, 17, 21). The results we have presented show that even after the $Ly-1^-, 2^+$ suppressor cell is activated, its ability to perform suppressive functions can be controlled by a closely related cell which induces contrasuppressive activity.

This being the case, the question of why $Ly-1^-, 2^+$ suppressor cell activity needs more than one level of regulation arises. One could hypothesize that because the $Ly-1^-, 2^+$ suppressor cell needs an $Ly-1$ partner inducer cell, it could be regulated by suppressing its own inducer cell that at least in theory should lead to a fall off in suppressor activity. Almost certainly, this series of regulatory interactions does operate during the immune response (22). Still, another level of regulation that might act more quickly and efficiently would be one that interferes with the message of the suppressor cells, without waiting for the inactivation of the inducer cell and the subsequent return to homeostasis. The contrasuppressor cell could perform this role.

This type of rapid counteraction of suppression could give the system increased

flexibility. In addition, it is a mechanism by which microenvironmental immune regulation could be brought about. Thus, if there was a special anatomical site where high levels of immune activity were needed (for example, in the gut) the local release of an activator of the contrasuppressor circuit could allow for this, while keeping systemic immunity relatively suppressed. In the example chosen for illustration (regulation of immune responses in the lymphoid tissue of the gut), this latter point would be of some importance, as suppressed systemic immunity could act to help prevent anaphylactic or immune complex reactions caused by nontoxic antigens entering the blood from the intestines. In line with this notion, we have obtained evidence that Peyer's patches contain particularly high numbers of contrasuppressor inducer cells (J. Gold, D. R. Green, and R. K. Gershon, manuscript in preparation).

Another important situation where microenvironmental immune regulation such as the type we envision for contrasuppression might be important would be at sites where pathogenic viruses are multiplying and interferon is being released. We are presently testing the possible role of interferon as an inducer of contrasuppression. Thus, the level of systemic immunity could be set at a certain mode by suppressor mechanisms, and yet allow effector cells to escape from this suppression or regulation in certain microenvironments where high levels of immune activity are required.

Another possible role for the cellular interaction we have described stems from the need of the immune system to be able to respond, in a secondary fashion, even when suppressive elements that are sufficient to interfere with a primary response may be present. Thus, the cell interaction that we have described, which interferes with suppression, may be crucial for the expression of at least some secondary immune responses.

The antigenic specificity of the cells in the contrasuppressor circuit remains to be fully characterized. The increased cross-reactivity (of the inducer factor(s) (vis a vis suppressor-inducer or suppressor factors) is intriguing. This observation makes it worthwhile to reexamine the old experiments showing that cross-reacting or modified antigens are inimical to tolerance production or maintenance (18, 19, 23) to see if these phenomena were produced by the activation of the contrasuppressor circuit.

Considerably more data is needed before the importance of the suppression-interfering activity (contrasuppression) that we have described can be assessed. However, at least in theory, it offers a potentially highly effective way by which several important immunological attributes could be controlled.

Summary

We have described an interaction between two T cell subsets that results in interference with the expression of Ly-1⁻,2⁺ (Ly-2) T cell-mediated suppression. We refer to this novel immunoregulatory activity as contrasuppression. The T cell responsible for the induction of contrasuppression (inducer cell) expresses the phenotype Ly-1⁻,2⁺;I-J⁺;Qa-1⁺. This phenotype distinguishes it from suppressor effector cells which we find to be I-J⁻.^{2,3} An I-J⁺ soluble mediator from the contrasuppressor inducer cell acts on another cell (acceptor cell) that expresses the phenotype Ly-1⁺,2⁺;I-J⁺;Qa-1⁺. This phenotype distinguishes it from T helper cells. Both the inducer cell (or its biologically active mediator) and its acceptor cell are required for the expression of contrasuppression. Because contrasuppressor cells can block the suppressive activity of cell-free mediators released by Ly-2 suppressor T cells, the

mechanism of contrasuppression is either separate from or in addition to the inactivation of suppressor cells themselves. The potential importance of contrasuppressor activity in the regulation of suppressor T cell activity in allowing immunologic memory to be expressed and in permitting microenvironmental immune regulation is discussed.

We thank Astrid Swanson for her expert secretarial skills in the preparation of this manuscript.

Received for publication 10 December 1980 and in revised form 19 February 1981.

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