IMMUNOREGULATORY CIRCUITS AMONG T-CELL SETS

I. T-Helper Cells Induce Other T-Cell Sets to Exert Feedback Inhibition*

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The T-lymphocyte population is divisible into several subclasses; each subclass possesses a distinctive genetic program which combines information for cell-surface phenotype and function (1). In the mouse, there is evidence that T cells which express the $Thy1^+Ly1^+Ly23^-$ surface phenotype ("Ly1 cells") are programmed for helper (T_H)[1] function. In contrast, T cells that express the $Thy1^+Ly1^-Ly23^+$ surface phenotype ("Ly23 cells") are programmed for suppressor (T_S) function (1). Isolation of these two T-cell subclasses in mice depleted of T cells ("B mice") has indicated that each belongs to an independent line or branch of thymus-dependent differentiation (2). A third major T-cell subclass, expressing the surface phenotype $Ly1^+2^+3^+$, can react to antigen and differentiate to $Ly23^+$ cytotoxic effector cells (3), suggesting that this subclass probably contains precursor cells that have acquired receptors for antigen but have not yet become committed to either T_H or $T_{C/S}$ function (3).

These findings, and others, are consistent with the view that functionally distinct T-cell sets carry cell surface components that are invariably associated with particular immunologic function. According to this idea, cells carrying the $Ly1^+Ly23^-$ surface phenotype are programmed for helper and not suppressive activity regardless of external conditions, such as the mode or type of antigen stimulation. To test this hypothesis we have stimulated purified populations of $Ly1^+2^-$ T cells with antigen in vitro, by using conditions devised to induce unselected T cells to express optimal levels of antigen-specific suppressive activity (4). We find that (*a*) stimulation of purified Ly1 cells under these conditions results in the generation of T_H but not T_S activity and (*b*) such hyperimmune Ly1 cells also induce a subset of nonimmune T cells to exert potent suppressive effects upon the antibody response. The surface phenotype of the T-cell set responsible for "feedback" inhibition is described in this study.

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[1] *Abbreviations used in this paper:* T-lymphocytes, thymus-derived lymphocytes; T_S, T-suppressor cells; T_H, T-helper cells; T_C, T-cytotoxic cells; SRBC, sheep erythrocytes; HRBC, horse erythrocytes; PFC, plaque-forming cell; TNP, trinitrophenyl.

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Materials and Methods

Mice. C57BL/6 (B6) mice 10–14 wk of age were obtained from The Jackson Laboratory, Bar Harbor, Maine. The congenic lines B6-Ly1^a and By-Ly2^{a}3^a (5), phenotypes Ly1.1,2.2,3.2 and Ly1.2,2.1,3.1, respectively, and B6-T1a^a (6, 7) were produced and supplied by Dr. E. A. Boyse, Memorial Sloan-Kettering Cancer Center, New York.

Production and Use of Antisera. Congenic anti-Thy 1.2, anti-Ly1.2, anti-Ly2.2, and anti-Ly3.2 was prepared as described previously (8). The antiserum (B6 × A-T1a^a) anti-A strain leukemia ASL1, heretofore termed "anti-TL" in reference to its reaction with thymocytes, and herein termed anti-Qa1 in reference to its reaction with peripheral T cells, is described elsewhere (6).

Ly1 CELLS. Highly purified Ly1 cells were obtained after incubation of 5×10^7 spleen cells/ml with anti-Ly2.2 (1:30 final dilution) + anti-Ly3.2 (1:30 final dilution) × ½ h at 4°C followed by ½ h incubation with selected rabbit C at 37°C as described previously (8). This treatment was repeated to obtain highly purified Ly1 populations. Such "twice-treated" cells were then passed over rabbit anti-Fab-coated Sephadex G-200 columns (9) or nylon wool columns (10). If contamination of the effluent population by Ig$^+$ cells was >3%, the cells were discarded. Controls for specificity of elimination by Ly antisera were performed as previously described (8, 9); controls for Qa1 specificity are shown (Table III).

B CELLS. Highly purified B cells were obtained by treating spleen cells with anti-Ly1.2,2.2,3.2 and Thy1.2 × ½ h at 4°C, followed by exposure to rabbit complement at 37°C according to a previously described protocol (8). Again, this treatment was repeated to insure highly purified populations of B cells. Lack of any residual T-cell activity was determined by the presence or absence of a proliferative response to concanavalin A or a PFC response to SRBC.

T CELLS. Nonimmune T cells were obtained after anti-Fab column passage rather than nylon wool column passage was routinely used, since passage through nylon wool in some cases resulted in a significant decrease in feedback suppressive activity. In some experiments (Table IV), whole spleen cells were used as a source of nonimmune T cells + B cells.

Antigens. Sheep erythrocytes (SRBC) were obtained from Colorado Serum Co., Denver, Colo. Erythrocytes were conjugated to tri-nitrobenzenesulfonic acid according to the method of Rittenberg and Pratt (11).

In Vitro Stimulation of Lymphoid Populations by SRBC

IN VITRO STIMULATION OF SRBC-SPECIFIC T-SUPPRESSOR CELLS. 10^7 lymphoid cells (usually highly purified Ly1 cells; see above) were incubated along with 2×10^6 SRBC according to the method of Eardley and Gershon (4). At the end of 5 days, the remaining viable lymphoid cells were harvested, washed twice, and in some cases, treated again with anti-Ly2.2, anti-Ly3.2 + C, using the procedure described above for obtaining highly purified Ly1 populations. Various numbers of these stimulated cells were then added to fresh cell cultures containing spleen cells and SRBC (see below).

IN VITRO PRIMARY ANTI-SRBC RESPONSES. A modification of the cell culture technique described initially by Mishell and Dutton (12) was used to generate in vitro primary anti-SRBC PFC responses (13). PFC responses per culture were determined by the Cunningham modification of the Jerne plaque assay as described previously (13). The mean and standard error of PFC were calculated from triplicate cultures.

Results

A Comparison of the Suppressive Effects of Unselected T Cells and Ly1 T Cells after in Vitro Stimulation by SRBC (Table I). Purified Ly1 cells that have been stimulated in vitro with high concentrations of SRBC express no demonstrable suppressive activity when added to fresh cultures containing mixtures of purified nonimmune Ly1 cells, B cells, and sheep erythrocytes (Table I); the addition of similar numbers of SRBC-stimulated unselected T cells resulted in ≅70–95% reduction of the anti-SRBC response. Thus, even after in vitro stimulation by antigen under conditions devised to induce optimal suppressive

TABLE I

*Influence of SRBC-Stimulated Ly1 Cells on Primary in Vitro SRBC Responses**

	SRBC-stimulated T-cell Population (10^5)	Assay Culture: (4×10^6 purified B cells + Nonimmune T-cell population)	Anti-SRBC PFC/culture‡	Suppression (%)
Exp 1	None	Ly1 (10^6)	7,300 ± 100	–
	Ly1	Ly1 (10^6)	7,600 ± 660	0
	Unselected	Ly1 (10^6)	350 ± 25	95
Control	None	None	0	–
	Ly1	None	2,500 ± 360	–
	Unselected	None	240 ± 120	–
Exp 2	None	Ly1 (2×10^6)	4,600 ± 140	–
	Ly1	Ly1 (2×10^6)	6,900 ± 380	0
	Unselected	Ly1 (2×10^6)	1,380 ± 130	72
Exp 3	None	Ly1 (10^6) + 10^5 Unselected	2,900 ± 320	–
	Ly1	Ly1 (10^6) + 10^5 Unselected	600 ± 110	79
	Unselected	Ly1 (10^6) + 10^5 Unselected	420 ± 60	85
Exp 4	None	Ly1 (10^6) + 10^5 Unselected	13,600 ± 1,200	–
	Ly1	Ly1 (10^6) + 10^5 Unselected	2,270 ± 160	88
	Unselected	Ly1 (10^6) + 10^5 Unselected	270 ± 40	98
Exp 5	None	Unselected T (10^6)	10,230 ± 1,360	–
	Ly1	Unselected T (10^6)	2,240 ± 380	88
	Unselected	Unselected T (10^6)	530 ± 75	95
Exp 6	None	Unselected T (4×10^6)	1,900 ± 200	–
	Ly1	Unselected T (4×10^6)	500 ± 60	74

* The indicated T-cell populations (Ly1 or unselected) were incubated in vitro with 2×10^6 SRBC $\times 5$ days: 10^5 cells were added to assay cultures containing 4×10^6 B cells and the indicated nonimmune T-cell populations (see Materials and Methods for details of procedure).

‡ Mean PFC ± standard error of triplicate SRBC-stimulated assay cultures.

activity by unselected T cells, purified Ly1 cells do not suppress the interaction between nonimmune Ly1 cells and B lymphocytes.

Moreover, addition of SRBC-stimulated Ly1 cells to purified B cells resulted in the induction of a substantial anti-SRBC response; addition of SRBC-stimulated unselected T cells did not (Exp 1: control). Taken together, the above findings show that purified Ly1 cells do not have the capacity (or, more precisely, have lost the differentiative option) of directly suppressing the T_H-B interaction: Ly1 cells are programmed to induce B cells to secrete antibody.

SRBC-Activated Ly1 Cells can Induce Ly2$^+$ Cells to Exert Feedback Suppressive Effects (Table I). One conclusion drawn from the above experiments was that antigen-stimulated Ly1 cells induce B cells to differentiate to antibody-forming cells (but do not directly suppress Ly1/B-cell interactions). We then asked whether antigen-stimulated Ly1 cells might induce *other* sets of T lymphocytes, including Ly2$^+$ cells, to develop immunoregulatory activity. We observed that the addition of small numbers of unselected nonimmune T cells to fresh cultures containing SRBC-immune Ly1 cells + nonimmune Ly1 cells

(identical to the culture populations of Exp 1 and 2) resulted in ≅80–90% reduction of the SRBC response (Table I, Exp 3 and 4). This reduction was noted when as few as 10^5 unselected nonimmune T cells were deliberately added to assay cultures containing SRBC-stimulated Ly1 cells + B cells (Exp 3, 4). In four separate experiments, addition of unselected nonimmune T cells to cultures containing small numbers of SRBC stimulated Ly1 cells resulted in ~75–90% inhibition of the anti-SRBC response (Exp 1, 2 vs. 3–6).

These findings suggest that signals from in vitro activated Ly1 cells can induce nonimmune T-cell populations containing $Ly2^+$ cells (i.e., cells sensitive to anti-Ly2 + C) to exert potent feedback suppressive effects. Additional experiments, in which graded doses of SRBC-stimulated Ly1 cells were added to cultures containing a fixed number of nonimmune $Ly2^+$ cells showed that the level of suppression was directly proportional to the numbers of SRBC-stimulated Ly1 cells added to the cultures (Fig. 1); i.e., the degree of feedback suppressive activity exerted by a fixed number of nonimmune $Ly2^+$ cells increased in direct proportion to the level of SRBC-activated T_H activity added to the assay cultures.

After in Vivo Immunization by SRBC, Ly1 Cells Induce Feedback Inhibition by Nonimmune Ly123$^+$ Cells (Table II; Fig. 2). The data above indicate that Ly1 cells that have been exposed in vitro to high concentrations of SRBC (*a*) induce purified B cells to secrete anti-SRBC antibody, and (*b*) induce nonimmune $Ly2^+$ cells to express substantial suppressive effects. To test whether the activities of in vitro stimulated Ly1 cells reflect cellular mechanisms governing the magnitude of the antibody response in vivo, we examined the influence of Ly1 cells obtained from mice immunized 1–4 wk previously with high concentrations (10^8) of SRBC upon in vitro primary responses to SRBC (Table II). Such isolated Ly1 cells, combined with purified B cells, produced substantial SRBC responses (group A). Addition of nonimmune T cells to these cultures resulted in a substantial reduction of the anti-SRBC PFC response (group B). No inhibition was seen when the nonimmune T-cell population was depleted of cells of the $Ly123^+$ subclass (group C).

These findings indicate that (*a*) in vivo immunization procedures also lead to the formation of Ly1 cells that can induce nonimmune T cells to exert feedback suppressive effects, and (*b*) the surface phenotype of cells responsible for feedback suppressive activity in the nonimmune T-cell population is $Ly1^+2^+3^+$.

Finally, feedback inhibitory effects exerted by a fixed number of $Ly123^+$ nonimmune T cells could not be overcome by increasing the number of SRBC-immune Ly1 cells over a 10-fold range (Fig. 2), again indicating the potency of $Ly123^+$ feedback inhibition (Table I) and confirming the conclusion drawn from analysis of the effects of in vitro stimulated Ly1 cells: the level of $Ly2^+$ feedback inhibition increases in proportion to the amount of Ly1 T_H signal in the cultures (see Fig. 1).

Cells Mediating Feedback Suppression also Express Surface Qa1 (Table III). To further delineate the surface phenotype of nonimmune cells responsible for feedback suppression, we asked whether such cells express Qa1 locus products. Qa1 surface components are coded for by gene(s) mapping between H2-D and T1a; these components are expressed selectively on a subset of T cells (6). We examined the effects of anti-Qa1 + C upon feedback suppressive activity

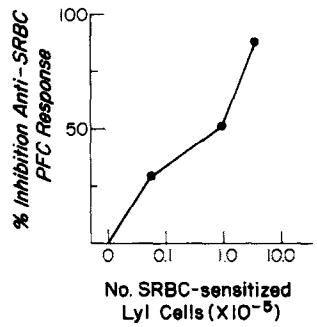

FIG. 1. Increasing numbers of SRBC-sensitized Lyl cells induce increasing amounts of feedback inhibition by nonimmune T-cells. Increasing numbers of Lyl cells that had been incubated in vitro 5 days with 2×10^6 SRBC were added to the following SRBC-stimulated assay cultures: population A: 5×10^6 B-cells + 10^6 nonimmune Lyl cells + 3×10^6 SRBC, or population B: population A + 2×10^5 nonimmune T-cells. The percent inhibition of the anti-SRBC response was determined by the following calculation:

$$\frac{\alpha\text{-SRBC PFC response (A)} - \alpha\text{-SRBC PFC response (B)}}{\alpha\text{-SRBC PFC response (A)}}.$$

Each point represents the mean percent inhibition of two to four separate experiments.

TABLE II

*After in Vivo Immunization Lyl Cells can Induce Feedback Inhibition by Nonimmune Lyl23⁺ T-Cells**

	Group	Assay population: SRBC-immune Lyl cells (1×10^5)	Assay population: B cells (4×10^6)	Nonimmune T-cell Population Added (10^6)	Anti-SRBC PFC Response: Exp 1	Anti-SRBC PFC Response: Exp 2	Anti-SRBC PFC Response: Exp 3	% Inhibition PFC Response: Exp 1	% Inhibition PFC Response: Exp 2	% Inhibition PFC Response: Exp 3
Experimental cultures	A	+	+	None	9,400	970	1,200	—	—	—
	B	+	+	Unselected	970	225	385	89	83	83
	C	+	+	(Lyl + Ly23)	9,100	1,925	1,320	2	0	0
Control cultures	D	−	+	Unselected	4,100	750	900			
	E	−	+	None	0	50	0			
	F	+	−	None	0	0	0			

* 10^5 Lyl cells from donors immunized with 10^9 SRBC i.v. 5–17 days earlier were combined with 4×10^6 purified B-cells (obtained after incubation of nonimmune spleen cells with anti-Thyl + anti-Ly + C; see Materials and Methods). The suppressive effects of different nonimmune T-cell sets upon the generation of anti-SRBC PFC are indicated. Percent inhibition was determined by the following calculation:

$$\frac{\text{PFC group A} - \text{PFC group B (or group C)}}{\text{PFC group A}}$$

of nonimmune spleen cells from a congenic pair of B6 mouse strains differing only at the Qa1 locus (B6-T1^a and B6[T1a$^-$]). Again, addition of SRBC-immune Lyl cells to nonimmune cell populations lacking Ly123 cells induced substantial SRBC PFC responses; addition of Lyl cells to unselected spleen cells (containing Ly123 cells) did not (groups A and B). Addition of Lyl cells to spleen cells treated with anti-Qa1 + C also produced substantial responses (group C). Removal of feedback suppression after treatment with anti-Qa1 + C reflected specific elimination of Qa1 + C cells since treatment of nonimmune cells from

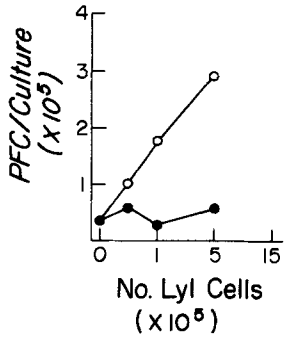

FIG. 2. PFC responses of SRBC-stimulated cell cultures containing graded numbers of SRBC-immune Ly1 cells (abscissa) and either 6×10^6 normal (NMS + C treated) spleen cells (●) or 6×10^6 spleen cells depleted of Ly123 cells (○) (i.e., mixtures of 3×10^6 α-Ly2 + C-treated spleen cells + 3×10^6 α-Ly1 + C-treated spleen cells). Each point represents the mean PFC of cultures from three separate experiments; in no case was the standard error of the mean greater than 43%.

TABLE III

Feedback Suppression is Mediated by Qa1$^+$Ly123$^+$ T Cells

	Group	SRBC-Immune Ly1 Cells (2×10^5)	Nonimmune population		Anti-SRBC PFC/culture	
			T Cells (2×10^6)	B Cells (4×10^6)	Exp 1	Exp 2
Experimental cultures	A	+	Ly1 + Ly23	+	2,835	ND
	B	+	Unselected	+	1,065	250
	C	+	Qa1$^-$	+	2,510	1,150
	D	+	Qa1 specificity control*	+	910	280
	E	+	Qa1$^-$ + (Ly1 + Ly23)	+	2,950	ND
Control cultures	F	+	–	–	0	0
	G	–	Unselected	+	915	600
	H	–	–	+	50	0

* B6(Tla$^-$) Ly1 cells added to B6(Tla$^-$) cells that had been treated with anti-Qa1 + C.

B6-T1a$^+$ donors but not B6(T1a$^-$) donors effectively decreased feedback suppressive activity (group D). The formal possibility that feedback suppression reflected an obligatory interaction between two separate cells, one Ly123$^+$Qa1$^-$ and the other Qa1$^+$Ly123$^-$, is unlikely since mixtures of anti-Ly + C treated cells (containing the putative Qa1$^+$Ly$^-$ population) and anti-Qa1 + C treated cells (containing the putative subset of Ly123$^+$Qa1$^-$ cells) did not exert detectable feedback suppressive effects (group E).

Carrier-Reactive T_H Cells are Targets of Feedback Suppression (Table IV). Feedback suppression by Ly123 cells might reflect a direct inhibition of induction of T_H cells, delivery of the T_H message, or direct suppression of B-cell differentiation to antibody-forming cells. To address this question, we exploited the observation of Kettman (14) that the development of anti-TNP PFC in this system depends upon the presence of SRBC-immune (carrier-primed) T-helper cells. Therefore, SRBC-immune Ly1 cells were added to cultures stimulated

TABLE IV
Feedback Inhibition in Cultures Stimulated with TNP-SRBC

SRBC-Immune Ly1 cells (1×10^5)	Nonimmune T cells (2×10^6)	B cells (5×10^6)	Anti-TNP-PFC	Anti-SRBC-PFC
–	Unselected	+	75 ± 30	1,000 ± 75
+	Unselected	+	250 ± 65	700 ± 90
+	(Ly1 + Ly23)	+	1,925 ± 410	7,100 ± 440
–	–	+	0	50 ± 8
+	–	–	0	0

The indicated lymphoid cell populations were incubated 5 days in 1-ml cultures containing 3×10^6 TNP-SRBC. PFC responses/culture were determined against SRBC and TNP-HRBC. Background PFC to HRBC were invariably <100 PFC/culture.

with TNP-SRBC conjugates. The presence of Ly123 cells in the nonimmune T-cell population resulted in a marked inhibition of *both* the anti-TNP PFC responses as well as the anti-SRBC response in this system. This finding suggests that feedback suppression reflects, at least in part, inhibition of T_H activity.

Discussion

These experiments were initially designed to determine whether cells carrying the $Ly1^+2^-$ surface phenotype are programmed for helper activity regardless of external conditions, such as the mode or type of antigen stimulation. We have found that in vitro stimulation of purified populations of Ly1 cells with sheep erythrocytes, using culture conditions devised to induce optimal T-suppressive (T_S) activity (4), invariably results in the generation of SRBC-specific T helper (T_H) but not T_S activity. These findings indicate that the $Ly1.2^+2.2/3.2^-$ surface phenotype is a stable, invariant marker of T cells that are programmed to express only helper activity and have lost the capacity to directly suppress the antibody response. In general, they support the view that the genetic program for a single differentiated set of cells combines information for surface phenotype and function.

In the course of these studies, we found that highly purified, antigen-stimulated Ly1 cells, in addition to inducing B cells to secrete antibody, can induce or activate other sets of T cells to express substantial suppressive effects. The surface phenotype of the set of cells exerting such feedback suppression in these experiments is: $Ly1^+2^+3^+Qa1^+$ (referred to below as Ly123 cells for ease of discussion). It is important to note that the Ly123 cell population responsible for feedback suppression was surprisingly resistant to a single treatment with anti-Thy1 + C (data not shown). We are presently testing two explanations for this observation: (*a*) Treatment with most anti-Thy1 antisera + C eliminates the large majority, but not all, T lymphocytes, or (*b*) Feedback regulatory cells expressing the $Ly123^+Qa1^+$ phenotype express relatively low surface concentrations of Thy1 and are thus relatively insensitive to lysis by Thy1 antiserum + C.

Perhaps the most striking characteristic of Ly123 feedback suppression is the

ability of extremely small numbers of these cells to exert potent feedback inhibitory effects in the fact of relatively large numbers of SRBC-immune Ly1 cells: fewer than 10^5 nonimmune Ly123 cells were capable of inhibiting ~80–90% of the PFC response induced by 10^6 Ly1 cells (Table I). This inhibition was not overcome by increasing numbers of SRBC-immune Ly1 cells; in fact, the degree of Ly123-mediated inhibition increased in direct proportion to the level of T_H activity in the system (Fig. 1).

The potency of extremely small numbers of $Ly2^+$ cells in this system is reminiscent of the interaction between Ly1 and $Ly2^+$ cells observed during the in vitro generation of alloreactive cytotoxic effector activity (15). In these experiments, extremely small numbers of Ly23 cells generated substantial levels of alloreactive cytotoxic effector activity only if co-cultured with relatively large numbers of Ly1 cells. Similarly, the present experiments indicate that extremely small numbers of $Ly2^+$ cells, in this case cells expressing the $Ly123^+Qa1^+$ phenotype, can be induced to express substantial levels of T-suppressive activity in the presence of relatively large numbers of SRBC-immune Ly1 cells.

Previous experiments have demonstrated that Ly1 cells can (*a*) induce B-cells to produce antibody, (*b*) induce pre-killer cells to differentiate to killer-effector cells, and (*c*) induce monocytes and macrophages to participate in inflammatory reactions (1). As demonstrated in the present experiments, Ly1 cells also induce other sets of T cells to develop potent suppressive activity. These findings, taken together, indicate that cells of the Ly1 set are programmed to signal other sets of cells to fulfill their respective genetic programs. The present experiments also imply that, like the formation of antibody, the generation of immunologic suppression after stimulation by antigen is not an autonomous function: both require induction by Ly1 cells.

The specificity of the feedback effects described here have not been studied, although it is likely that at least one target of suppression is the T_H cell or delivery of the T_H product (Table IV). The problem of specificity can be summed up as follows: although induction of the Ly123-mediated suppression may reflect signals from specifically activated immune Ly1 cells, we do not know whether the target of feedback suppression is solely the relevant antigen-reactive T-helper cell (or its product). If so, is such specificity due to Ly123 cells bearing receptors for the antigen or receptors specific for idiotypic determinants carried on the immune Ly1 population? Resolution of this question is now in progress using more well-defined antigens that elicit antibodies which carry identifiable idiotypic markers (16).

The magnitude and duration of an antibody response is governed by a complex series of inductive and suppressive interactions among subsets of lymphocytes and macrophages. To delineate these interactions, it is essential to establish whether expression of a particular surface phenotype is a reliable indicator of a cell population's helper-suppressor potential. Demonstration that cells expressing the $Ly1^+23^-$ surface phenotype carry help but are unable to directly suppress, even after antigen stimulation under "suppressive" conditions, allowed dissection of the cellular basis of feedback inhibition by $Ly123^+$ T-cells.

These experiments also bear on current strategies used to define and characterize T-suppressor cells. The observation that addition of cell population X to a complex population of lymphoid cells results in a reduction of the response does not imply that population X contains suppressor cells; it indicates only that the cell population in question can induce a suppressive effect. An apt analogy is that the production of antibody by T-depleted mice after the addition of thymocytes does not necessarily imply that thymocytes produce antibody.

In summary, our results are consistent with the current immunological paradigm that $Ly1^+23^-$ T-cells act as obligatory helper (T_H) cells and cannot be induced to act directly as suppressor cells, even by modes of immunization which induce high levels of T_S activity. In addition, the data indicate that, after immunization, Ly1 T_H cells can induce cells expressing the $Ly123^+Qa1^+$ phenotype to exert potent inhibitory effects. These findings suggest that activation of resting Ly123 cells by immune Ly1 T_H cells may represent an important homeostatic immunoregulatory mechanism in vivo. We test this proposition in the following paper (17).

Summary

These experiments test the hypothesis that cells carrying the $Ly1^+23^-$ surface phenotype are programmed exclusively for helper and not suppressive activity regardless of external conditions such as the mode or type of antigen stimulation. To this end, we have stimulated purified populations of Ly1 cells with antigen in vitro using conditions devised to induce unselected T cells to express optimal levels of antigen specific T-suppressor activity. We find that after such stimulation, Ly1 cells generate SRBC-specific T-helper activity but *not* T-suppressive activity. These findings establish that the *Ly1.2*$^+$*,2.2/3.2*$^-$ surface phenotype is a stable, and probably invariant, marker of T cells that are programmed to express only helper activity and have lost the capacity to directly suppress the antibody response. These findings support the concept that the genetic program for a single differentiated set of cells combines information for cell surface phenotype and function.

We also demonstrate that antigen-stimulated Ly1 cells, in addition to inducing B cells to secrete antibody, can induce or activate other sets of resting T cells to develop profound suppressive effects. The surface phenotype of this feedback suppressive T-cell set is shown to be: $Ly1^+2^+3^+Qa1^+$. These findings, taken together, indicate that activation of resting Ly123 cells by immune Ly1 T_H cells may represent an important homeostatic immunoregulatory mechanism.

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