IMMUNOREGULATORY CIRCUITS AMONG T-CELL SETS

Identification of a Subpopulation of T-Helper Cells that Induces Feedback Inhibition*

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Antigen-stimulated Ly1 cells induce B cells to secrete antibody and induce a nonimmune set of T cells (surface phenotype $Ly123^{+}Qa1^{+}$) to participate in specific suppressor activity (1, 2). We have referred to this suppressive T-T interaction as feedback inhibition because (*a*) the level of suppression exerted by a fixed number of nonimmune T cells increase in direct proportion to the numbers of antigen-stimulated Ly1 cells ($0.5-5 \times 10^{5}$) in cell culture and (*b*) one consequence of Ly123-associated suppression is a reduction in the delivery of T-helper activity to B cells.

These observations indicate that cells of the Ly1 set play a central role in regulating antibody formation. It is therefore important to determine whether all Ly1 cells are programmed to induce nonimmune T cells to exert feedback inhibition or whether this immunologic function is invested in a specialized set of Ly1 cells. If the latter explanation were the case, then the intensity and duration of the immune response after stimulation by antigen would depend in part upon the degree of activation of Ly1 cells that induce feedback inhibition.

A direct approach to this question comes from the finding that a portion of $Ly1^{+}$ cells also express Qa1 surface components.[1] We find that (*a*) $Ly1:Qa1^{+}$ cells are responsible for induction of feedback inhibition and (*b*) signals from both $Ly1:Qa1^{+}$ and $Ly1:Qa1^{-}$ cells are required for optimal formation of antibody by B cells.

Materials and Methods

Mice. C57BL/6 (B6) mice 10- to 14-wk of age were obtained from the Jackson Laboratory, Bar Harbor, Maine. The congenic lines B6-Ly1ᵃ and B6-Ly1ᵃ3ᵃ, phenotypes Ly1.1,2.2,3.2 and Ly1.2,2.1,3.1, respectively, and B6-T1aᵃ were produced and supplied by Dr. E. A. Boyse, Memorial Sloan-Kettering Cancer Center, New York.

Production and Use of Antisera. Congenic anti-Thy1.2, anti-Ly1.2, anti-Ly2.2, and anti-Ly3.2 were prepared as described previously (3). The antiserum (B6 × A-T1aᵃ) 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 (4).

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[1] *Abbreviations used in this paper:* C, complement; NMS, normal mouse serum; PFC, plaque-forming cells; TNP, trinitrophenyl; SRBC, sheep erythrocytes.

The proportion of Ly1 cells expressing surface Qa1 was estimated from the lytic effects after sequential exposure of cells to Ly and Qa1 antisera with selected rabbit sera as a source of complement (C),[2] according to a protocol detailed previously (5).

Preparation of Lymphocyte Subpopulations

Ly1 Cells.   Highly purified Ly1 cells were obtained as described previously (1). Controls for specificity of elimination by Ly antisera were performed as previously (3, 5); controls for Qa1 specificity are shown.

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 (1). This treatment was repeated to insure highly purified populations of B cells. Lack of any residual B-cell activity was determined by the presence or absence of a proliferative response to concanavalin A or a plaque-forming cell (PFC) response to sheep erythrocytes (SRBC).

T Cells.   Nonimmune T cells were obtained after passage through RαMFab-coated Sephadex G-200 rather than nylon wool columns since passage through nylon wool in some cases results in a significant decrease in feedback suppressive activity.

Antigens.   SRBC were obtained from Colorado Serum Co., Denver, Colo. Erythrocytes were conjugated to tri-nitrobenzene-sulfonic acid according to the method of Rittenberg and Pratt (6).

In Vitro Stimulation of Lymphoid Populations by SRBC.   In vitro stimulation of Ly1 cells by SRBC. 10^7 highly purified Ly1 cells (see above) were incubated with 2×10^6 SRBC according to the method of Eardley and Gershon (7). At the end of 5 days, the remaining viable lymphoid cells were harvested, washed twice, and treated again with anti-Ly2.2, anti-Ly3.2 + C. 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 (8) of the cell culture technique described initially by Mishell and Dutton (9) was used to generate in vitro primary anti-SRBC PFC responses. PFC responses per culture were determined by the Cunningham modification of the Jerne plaque assay as described previously (1). The mean and standard error of PFC were calculated from triplicate cultures.

Results

Approximately ⅔ of Ly1 Cells also Express Surface Qa1 (Table I).   To determine the proportion of Ly1 cells that also express the Qa1 surface phenotype, highly enriched Ly1 cells were treated with anti-Qa1 + C. This analysis indicates that approximately 60% of Ly1 cells also express Qa1.

Ly1 Cells that Induce Feedback Inhibition Express Qa1 (Table II).   As noted previously (1), addition of SRBC-stimulated Ly1 cells (10^5) to cultures containing nonimmune spleen cells resulted in substantial inhibition of anti-SRBC PFC formation (Table II, group A vs. group B). In contrast, addition of Ly1:Qa1⁻ cells (10^5) (group C) to such cultures did not result in significant inhibition.

Quantitative Analysis of the Contribution of Ly1:Qa1⁺ Cells to Induction of Feedback Inhibition by Resting T Cells and Antibody Formation by B Cells (Fig. 1; Tables II and III).   The above findings indicate that the ability to induce feedback inhibition is dependent on Ly1 cells that also express the Qa1 surface phenotype (Ly1:Qa1⁺ cells). A second function invested in cells of the Ly1 set is the passage of the helper signal to the B cell. We therefore analyzed the relative contributions of Ly1:Qa1⁺ and Ly1:Qa1⁻ cells to induction of antibody formation of B lymphocytes: a fixed number (10^5) of Ly1 cells containing increasing proportions of Ly1:Qa1⁺ cells were added to SRBC-stimulated

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[2] C. E. Stanton et al. The Qa-1 antigenic system: relation of Qa-1 phenotypes to lymphocyte sets, mitogen responses, and immune functions. Manuscript submitted for publication.

TABLE I

Analysis of the Proportion of Ly1 Cells that also Express Qa1

Group	Highly enriched Ly1 T cells treated with the following (+ C):	Lysis	
		B6-T1[a] Donor	B6 Donor (specificity control)
		%	
A	NMS	12	9
B	Anti-Thy-1.2	87	84
C	Anti-Ly-1.2	83	80
D	Anti-Ly-2.2 + anti-Ly-3.2	8	6
E	Anti-Qa1	56	6
F	Group C treated with anti-Qa1	17	10

Spleen cells from B6 T1a or B6 donors were passed through anti-Fab coated columns before treatment with anti-Ly2.2 + anti-Ly3.2 in the presence of selected rabbit complement. This procedure resulted in a cell population that contains approximately 83% Thy1.2$^+$ cells (group B vs. group A), and 80% Ly1$^+$ cells. This population lacked Ly2$^+$3$^+$ cells (group D vs. group A). Treatment of this population with anti-Qa1 + C resulted in approximately 55% lysis of the B6-T1a population but no detectable lysis of B6 (Qa1$^-$) cells. Since approximately 10-15% of highly enriched Ly1 cells consisted of Thy1$^-$Ly$^-$ cells (groups B and C vs. group A), and because previous studies have suggested that a small proportion of non-T cells express surface Qa1, we determined the proportion of cells remaining after lysis with anti-Ly1.2 that was sensitive to anti-Qa1$^+$ (group F vs. group A). The proportion of Ly1:Qa1$^+$ cells determined from the specific lysis obtained in group E less the specific lysis obtained in group F is approximately 61%.

TABLE II

Role of Ly1:Qa1$^+$ Cells in Induction of Feedback Inhibition

Group	SRBC-Stim-ulated Ly1 cells (10^5)	Control SRBC-stimulated lymphocyte cultures*	Anti-SRBC/PFC/culture			Inhibition (% standard)
			Exp. 1	Exp. 2	Exp. 3	
A	—	+	6,133	1,150	3,320 (2610)‡	Standard
B	Ly1	+	1,173	475	650 (570)‡	79
C	Ly1:Qa1$^-$	+	5,173	3,650	9,690 (660)‡	0 (+180)
D	Ly1	—	ND	0	0 (0)	—
E	Ly1:Qa1$^-$		ND	0	0 (0)	

* 5 × 10^6 spleen cells from B6 T1a[a] donors were incubated with 3 × 10^6 SRBC under conditions described in Materials and Methods.

‡ Qa1 specificity control: in the same experiment B6 (T1a$^-$)Ly1 cells treated with anti-Qa1 + C were added to B6 SRBC-stimulated lymphocytes.

cultures containing nonimmune spleen cells or purified B cells (Fig. 1). Addition of these Ly1 populations (containing increasing proportions of Ly1:Qa1$^+$ cells) to SRBC-stimulated spleen cell cultures shows that (*a*) 10^5 Ly1:Qa1$^-$ cells do not induce feedback inhibitory activity and (*b*) 10^5 Ly1 cells containing increasing proportions of Ly1:Qa1$^+$ cells induced progressively greater feedback inhibition of the anti-SRBC response. The addition of these same Ly1 populations (containing increasing proportions of Ly1:Qa1$^+$ cells) to cultures containing purified B cells + SRBC indicated
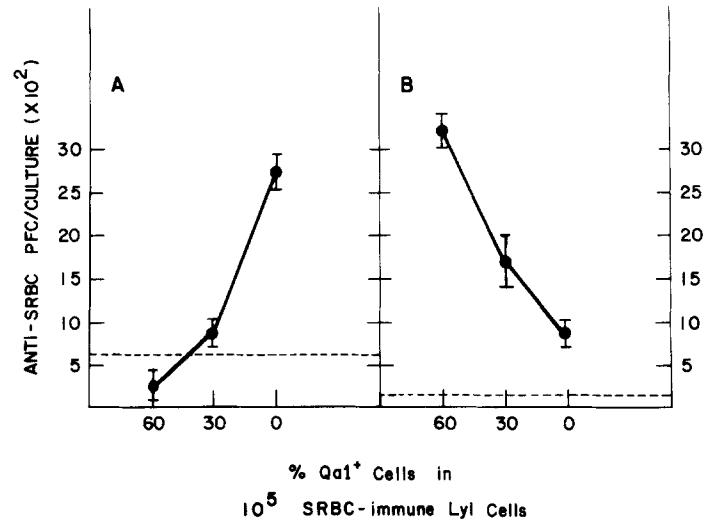
FIG. 1. Contribution of Ly1:Qa1⁺ cells to the induction of feedback suppression and antibody formation. Ly1 cells obtained from donors immunized with 5×10^8 SRBC 5 days previously were treated twice with anti-Qa1 + C (Ly1:Qa1⁻ cells). Ly1 cells containing $\approx 30\%$ Qa1 cells were produced by mixing 5.5×10^4 Ly1:Qa1⁻ cells with 4.5×10^4 NMS + C treated Ly1 cells; 10^5 Ly1 cells containing $\approx 60\%$ Ly1:Qa1⁺ cells were produced by mixing 0.9×10^5 NMS + C-treated Ly1 cells with 0.1×10^5 anti-Qa1-treated Ly1 cells. The ability of a fixed number (10^5) of Ly1 cells containing graded proportions of Ly1:Qa1⁺ cells to induce feedback suppression in cultures containing normal spleen cells and SRBC is shown in panel A. The ability of these Ly1 populations to induce B cells to secrete antibody in cultures containing 4×10^6 highly purified B cells + SRBC is shown in panel B.

TABLE III

Role of Carrier-Primed Ly1:Qa1⁺ Cells in Induction of B Cells to Produce Anti-Hapten Antibody

SRBC-Immune Ly1 cells (10^5)		B Cells (5×10^6)	Anti-TNP PFC/culture*
Qa1⁺	Qa1⁻		
%	%		
None		+	0
60	40	+	890 ± 40
40	60	+	560 ± 110
0	100	+	210 ± 35

* 10^5 SRBC-stimulated Ly1 cells containing increasing proportions of Ly1:Qa1⁺ cells (obtained as described in legend to Fig. 1) were added to cultures containing purified B cells and 3×10^6 TNP SRBC. The numbers of anti-TNP PFC per culture were determined 5 days later.

that (a) Ly1:Qa1⁻ cells were sufficient to induce B cells to form antibody and (b) Ly1:Qa1⁺ cells also contribute to the induction of antibody formation by B cells, since optimal PFC responses were obtained in cultures containing 60% Ly1:Qa1⁺ cells and 40% Ly1:Qa1⁻ cells.

The development of anti-trinitrophenyl (TNP) PFC in lymphocyte cultures stimulated with TNP SRBC depends upon the presence of SRBC-immune (carrier-primed) T-helper cells. We therefore also examined the ability of a fixed (and limiting)

Table IV

Comparison of Ly1:Qa1⁻ Cells and Ly1 Cells Containing Similar T_H Activity

SRBC-Stimulated cell cultures containing			Purified B cells (5 × 10⁶)	Anti-SRBC PFC/culture
SRBC-Primed Ly1:Qa1⁻ cells (× 10⁵)	SRBC-Primed Ly1 cells (× 10⁵)	Nonimmune T + B cells (5 × 10⁶)		
None		+		290 ± 30
4.0		+		2,850 ± 380
	1.0	+		150 ± 60
4.0			+	1,475 ± 480
	1.0		+	1,890 ± 220

number of SRBC-stimulated Ly1 cells (10⁵) containing increasing proportions of Ly1:Qa1⁺ cells to exert carrier-specific helper function. Ly1:Qa1⁻ cells were sufficient to induce B cells to form anti-TNP PFC; optimal anti-TNP PFC responses were produced in cultures approximately 60% Ly1:Qa1⁺ and 40% Ly1:Qa1⁻ cells (Table III). These experiments show that, in addition to inducing T cells to exert feedback inhibition, Ly1:Qa1⁺ cells in concert with Ly1:Qa1⁻ cells signal B cells to produce antibody. Whether isolated Ly1:Qa1⁺ cells are sufficient to induce B cells to produce antibody is not yet established.

The inability of isolated Ly1:Qa1⁻ cells to induce feedback inhibition could be explained as follows: 10⁵ Ly1:Qa1⁻ cells lack significant T-helper activity (compared with 10⁵ Ly1 cells) and therefore do not induce nonimmune T cells to exert suppressive effects. To test this possibility, the ability of larger numbers of Ly1:Qa1⁻ cells to induce feedback inhibition was examined (Table IV). Although 4 × 10⁵ Ly1:Qa1⁻ cells induced B cells to generate virtually the same number of anti-SRBC PFC as did 10⁵ Ly1 cells, addition of these cells to SRBC-stimulated cultures containing nonimmune T + B cells did not inhibit the response (in fact, a substantial enhancement was noted).

Discussion

Members of the Ly1 set are divisible into two populations according to differential expression of the Qa1 surface phenotype. Approximately ⅔ of Ly1 cells from adult mice are Qa1⁺ (Ly1:Qa1⁺) and ⅓ are Qa1⁻ (Ly1:Qa1⁻). These studies emphasize again that the genetic program of differentiated T-lymphocyte sets combines information for cell surface phenotype and immunologic function. Cells of the Ly1:Qa1⁺ set are required for induction of feedback inhibition; cells of the Ly1:Qa1⁻ set are unable to induce nonimmune T cells to exert significant inhibitory effects.

It is of special interest that both sets of Ly1 populations are required for optimal induction of antibody formation by B cells. These experiments do not allow a choice to be made between the two most obvious explanations for this effect: (*a*) Optimal generation of the helper signal requires an interaction between Ly1:Qa1⁻ and Ly1:Qa1⁺ populations, or (*b*) Ly1:Qa1⁺ and Ly1:Qa1⁻ populations each deliver a separate signal to the B cell. If the former hypothesis is correct, supernates of antigen-stimulated Ly1 cell cultures containing both Qa1⁺ and Qa1⁻ members should contain substantially greater helper activity (as tested with isolated B cells) than those of recombined supernates obtained from isolated antigen-stimulated Ly1:Qa1⁻ and

Ly1:Qa1$^+$ cell cultures. In addition, it will be of interest to determine the role of Ly1:Qa1$^-$ and Ly1:Qa1$^+$ T cells in systems that have implied a requirement for more than one T-helper cell (10–15). The latter experiments (15) demonstrate that a subset of Ly1 inducer cells recognizes V_H gene products; whether Ly1:Qa1$^+$ cells specifically interact with resting T cells via V_H-associated cell surface determinants is currently being examined.

These findings, taken together with previous reports (1, 2), favor the proposal that the ability of an antigenic determinant to induce a detectable antibody response may depend largely on the ratio of Ly1:Qa1$^+$ and Ly1:Qa1$^-$ T-cell clones that bear receptors for that antigen. According to this view, antigenic determinants that are unable to elicit immune responses in certain genetically inbred strains may preferentially stimulate Ly1:Qa1$^+$ cells which in turn activate the T-suppressor system. The regulatory interactions between Ly1:Qa1$^+$ and Ly123:Qa1$^+$ T cells may also be particularly important in governing the duration and intensity of certain inflammatory reactions such as delayed type hypersensitivity and IgE-mediated hypersensitivity. These reactions are especially susceptible to Ly1-induced feedback inhibition (16, 17). Definitive analysis of these reactions now depends upon studies of isolated, antigen-specific Ly1:Qa1$^+$ cell populations.

Analysis of the cell-free products of homogeneous populations of Ly1:Qa1$^+$ cells is also of particular interest, since the experiments reported here indicate that at least some products of this cell set are potent activators of the Ly2$^+$ suppressive system and thus may prove useful in strategies designed to selectively suppress the immune response to defined antigens.

Summary

Purified Ly1 cells induce other T-cell sets to exert potent feedback inhibitory activity and this T-T interaction has been shown to play an important role in regulating in vivo immune responses. Approximately ⅔ of Ly1 cells also express the Qa1 surface phenotype (Ly1:Qa1$^+$ cells). The experiments reported here indicate that Ly1:Qa1$^+$ cells are responsible for induction of feedback inhibition and that signals from both Ly1:Qa1$^+$ cells and Ly1:Qa1$^-$ cells are required for optimal formation of antibody by B cells.

References

1. Eardley, D. D., J. Hugenberger, L. McVay-Boudreau, F. W. Shen, R. K. Gershon, and H. Cantor. 1978. Immunoregulatory circuits among T-cell sets. I. T-helper cells induce other T-cell sets to exert feedback inhibition. *J. Exp. Med.* **147:**1106.

2. Cantor, H., L. McVay-Boudreau, J. Hugenberger, K. Naidorf, F. W. Shen, and R. K. Gershon. 1978. Immunoregulatory circuits among T-cell sets. II. Physiologic role of feedback inhibition in vivo: absence in NZB mice. *J. Exp. Med.* **147:**1116.

3. Shen, F. W., E. A. Boyse, and H. Cantor. 1975. Preparation and use of Ly antisera. *Immunogenetics.* **2:**591.

4. Stanton, T. H., and E. A. Boyse. 1976. A new serologically defined locus, Qa1, in the Tla region of the mouse. *Immunogenetics.* **3:**525.

5. Cantor, H., and E. A. Boyse. 1975. Functional subclasses of T lymphocytes bearing different Ly antigens. I. The generation of functionally distinct T cell subclasses as a differentiative process independent of antigen. *J. Exp. Med.* **141:**1376.

6. Rittenberg, M., and K. Pratt. 1969. Anti-trinitrophenyl (TNP) plaque assay. *Proc. Soc. Exp. Biol. Med.* **32:**575.

7. Eardley, D. D., and R. K. Gershon. 1976. Induction of specific suppressor cells in vitro. *J. Immunol.* **117:**313.

8. Cantor, H., F. W. Shen, and E. A. Boyse. 1976. Separation of helper T cells from suppressor T cells expressing different Ly components. II. Activation of antigen: after immunization, antigen-specific suppressor and helper activities are mediated by distinct T cell subclasses. *J. Exp. Med.* **143:**1391.

9. Mishell, R. I., and R. W. Dutton. 1967. Immunization of dissociated spleen cell cultures from normal mice. *J. Exp. Med.* **126:**423.

10. Marrack (Hunter), P. C., and J. W. Kappler. 1975. Antigen specific and non-specific mediators of T cell/B cell cooperation. I. Evidence for their production by different cells. *J. Immunol.* **114:**1116.

11. Janeway, C. A., Jr. 1975. Cellular cooperation during in vivo antibody responses. I. The effect of cell number on the response. *J. Immunol.* **114:**1394.

12. Herzenberg, L., K. Okumura, H. Cantor, V. Sato, F. W. Shen, E. A. Boyse, and L. Herzenberg. T cell regulation of antibody responses: demonstration of allotype-specific helper T cells and their specific removal by suppressor T cells. *J. Exp. Med.* **144:**330.

13. Ward, K., H. Cantor, and E. A. Boyse. 1977. Clonally-restricted interactions among T and B cell subclasses. *In* ICN-UCLA Symposia on Molecular and Cellular Biology. C. F. Fox, editor. Academic Press, Inc., New York. Volume 8.

14. Tada, T., T. Toshitada, K. Okumura, M. Nonaka, and T. Tokuhisa. 1978. Two distinct types of helper T cells involved in the secondary antibody response: independent and synergistic effects of Ia$^-$ and Ia$^+$ helper T cells. *J. Exp. Med.* **147:**446.

15. Woodland, R. T., and H. Cantor. 1978. Idiotype-specific T-helper cells are required to induce B-memory cells to secrete antibody. *Eur. J. Immunol.* In press.

16. Askenase, P. W., B. J. Hayden, and R. K. Geshon. 1975. Augmentation of delayed-type hypersensitivity by doses of cyclophosphamide which do not affect antibody responses. *J. Exp. Med.* **141:**697.

17. Watanabe, N., S. Kojima, F. W. Shen, and Z. Ovary. 1977. Suppression of IgE antibody production in SJL mice. II. Expression of Ly-1 antigen on helper and non-specific suppressor T cells. *J. Immunol.* **118:**485.