

SEPARATION OF HELPER T CELLS FROM SUPPRESSOR T CELLS EXPRESSING DIFFERENT Ly COMPONENTS

II. Activation by Antigen: after Immunization, Antigen-Specific Suppressor and Helper Activities are Mediated by Distinct T-Cell Subclasses*

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There is increasing evidence that after appropriate activation by antigen, T cells can specifically suppress an antibody response (reviewed in reference 1). The cellular basis of this is not understood. Possibly a single T-cell subclass can suppress or help, depending on the circumstances of immunization. Or exposure to antigen may generate suppressor and helper activities by different T-cell subclasses. This latter alternative is supported by the finding that help and suppression are properties of different T-cell subclasses belonging to T-cell populations activated polyclonally by concanavalin A (2).

In this report we examine the question whether T cells mediating specific helper and suppressor activities, after immunization with sheep erythrocytes (SRBC), belong to different subclasses.

Materials and Methods

Animals. B6 mice and Ly-congenic stock were obtained as described previously (2).

Antisera. The preparation and use of Ly-1.2, Ly-2.2, and Thy-1.2 antisera have been described (2, 3).

Controls for Ly Specificity. All experiments were controlled for Ly serological specificity by the use of Ly-congenic mouse strains according to the stipulations prescribed by Shen et al. (3).

Generation of Anti(α)¹-SRBC plaque-forming cells (PFC) In Vitro. A modification of the technique described initially by Mishell and Dutton was used (2).

T-Cell Purification. Rabbit antimouse Fab (R α MFab) columns and nylon wool columns were used as described (4). The proportion of Ig⁺ cells in these cell populations ranged from 1-6%.

Adoptive Primary Antibody Responses. Thymectomized, lethally irradiated (800 R) C57BL/6 (B6) mice, reconstituted with 10⁷ bone marrow (BM) cells treated with α Thy-1, were used as "B" mice. 4 wk after BM reconstitution, column-purified T cells treated with different Ly antisera were administered intravenously to these hosts along with 1-5 \times 10⁸ SRBC.

The Use of Immunoabsorbent Columns for Positive Selection of Ly Subclasses. Sephadex G200

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¹ Abbreviations used in this paper: α , anti; BM, bone marrow; B6, C57BL/6; HRBC, horse erythrocytes; NMS, normal mouse (B6) serum; PFC, plaque-forming cells; R α MFab, rabbit antimouse Fab.

columns coated with R α MFab which had been purified over a Sepharose 4B immunoabsorbent column, were conjugated to cyanogen bromide-activated Sephadex G200 beads according to Schlossman and Hudson (5). These columns specifically bind Ig⁺ lymphocytes (4, 5). For Ly subclass separation, approximately 100×10^6 lymph node and spleen T cells, highly purified by passage through a nylon wool column were incubated with α Ly-2 (dilution 1:40) at 4°C for 1/2 h, washed twice, and passed through a 10 ml R α MFab-coated column. Cells adherent to the R α MFab column, representing some 40% of the starting population were eluted with 10 ml of normal mouse sera diluted 1:10 in phosphate-buffered balanced salt solution (BSS). (The Ly phenotypes of the effluent and eluted cell populations are shown in Table III.)

Results

I. Conventions. The following notations are used in this section: T cells, spleen and lymph node cells from B6 mice passed through R α MFab-coated columns or nylon wool columns (see Materials and Methods). B cells: B6 spleen cells treated twice with α Thy-1.2 plus complement (C).

Ly subclasses notation: Because there is no evidence so far that Ly2 and Ly3 can be expressed independently of one another, i.e. no cells of Ly-2⁻3⁺ or Ly-2⁺3⁻ phenotypes have yet been identified, there are as yet only three well-defined Ly subclasses; Ly1, Ly23, and Ly123. In the following account we used the notation Ly1 to signify a population of T cells remaining after treatment with α Ly-2 or α Ly-3, plus C; and Ly23 to signify the equivalent population remaining after treatment with α Ly-1 plus C. These selected T-cell subclasses were obtained by treatment with Ly antisera plus C, either before or after column purification of T cells; both procedures yield T-cell subclasses with equivalent functional activities. The notation Ly-2⁺ is used to indicate the total population of T cells expressing the Ly-2 component, which comprises both Ly123 and Ly23 subclasses; as for example after positive selection on immunoabsorbent columns (see Materials and Methods).

II. The Participation of Different Ly⁺ T Cells in Primary and Secondary Antibody Responses In Vitro. Graded numbers of Ly1 cells and Ly23 cells were added to a constant number of B cells, together with 3×10^6 SRBC. After 5 days in culture, direct α SRBC PFC were enumerated. Ly1 cells generated helper activity roughly comparable to the same number of the unselected T population; Ly23 cells generated no appreciable helper activity (Table I). Two points are noteworthy: (a) Ly1 cells are the major source of helper activity in an unprimed T-cell population, and (b) the content of Ly1 cells in the selected Ly1 population is double that of the unselected T population, yet they have roughly the same helper activity; possible explanations include participation of some Ly-2⁺ cells in generating an optimal primary helper response, either by cooperation with Ly1 helpers, or by Ly123 \rightarrow Ly1 differentiation.

To measure helper memory activity, graded numbers of Ly1 cells and Ly23 cells from SRBC-primed donors were added to a constant number of spleen cells and SRBC. Again, Ly1 cells from SRBC-primed mice generated helper activity, but Ly23 cells did not (Table II). Further, in this case the helper activity of selected Ly1 cells, as compared with the unselected T-cell population, was substantially greater than that predictable from the twofold enrichment of Ly1 cells. This increment was directly proportional to the number of SRBC used to prime the T-cell donor mice. This suggested that removal of SRBC-activated Ly-2⁺ cells might result in augmented helper activity.

TABLE I
The Ability of Graded Numbers of T Subclass Cells to Help B Cells Produce a Primary α SRBC Response In Vitro

No. of T cells/culture ($\times 10^6$)*		PFC/Culture			Mean % control PFC	
Unselected T	Ly1	Ly23	Exp. 1	Exp. 2		Exp. 3
0.25			220			
0.5			465	447	450	
1.0			941	1,562	635	Control
1.5			2,063	1,905	800	
	0.25		185			84
	0.5		166	335	650	85
	1.0		870	1,110	510	81
	1.5		1,768	2,212	850	95
		0.25	0			0
		0.5	25	12	40	6
		1.0	110	160	40	12
		1.5	301	351	110	14

* The indicated T-cell population was added in graded numbers to 6×10^6 B cells, which in each case produced less than 50 PFC per culture alone.

III. Direct Evidence that SRBC-Activated Ly-2⁺ Cells Suppress the α SRBC Response. In section II of Results we showed that removal of Ly-2⁺ cells from the T-cell population of SRBC-primed mice augmented the α SRBC response in vitro. One explanation for this effect is that SRBC-activated Ly-2⁺ cells suppress the α SRBC response. The next experiments were designed to establish the effects of adding SRBC-activated Ly-2⁺ cells to α SRBC cultures.

Ly-2⁺ cells were positively selected by preincubation of T cells with Ly-2 antisera and passage through R α MFab columns (see Materials and Methods). The effluent fraction consisted almost entirely of Ly-2⁻ T cells while the fraction of adherent cells (after elution from the column with normal mouse [B6] serum [NMS]) consisted almost entirely of Ly-2⁺ T cells (Table III). The effluent fraction of T cells from donors given 10⁹ SRBC 5 days earlier demonstrated substantial helper activity (Table IV). The addition of eluted (Ly-2⁺) cells obtained from the same starting cell population resulted in substantial suppression of the SRBC response (Table IV).

IV. Evidence that Ly23 Cells are a Major Source of Suppressor Activity in the Ly-2⁺ Population from Primed Donors. The experiments above do not indicate whether, after antigen stimulation, Ly-2⁺ suppressor cells belong to the Ly23 or Ly123 subclasses, or to both. We tested whether primed Ly23 cells are suppressive by adding them in increasing numbers to cultures containing constant numbers of normal spleen cells plus primed Ly1 cells. This gave proportionately increasing suppression which could not be ascribed to "crowding" because the addition of similar numbers of unselected primed T cells, or of primed Ly1 cells, produced an increment in activity (Table V). The degree of suppression obtained suggested that a substantial portion of Ly-2⁺ suppressive activity (section III of Results) can be accounted for by Ly23 cells.

TABLE II
The Influence of Ly⁺ Cell Subclasses on the In Vitro Secondary Response to SRBC

SRBC priming dose	No. of T cells/culture* ($\times 10^6$)			PFC Culture*			Mean % control PFC responses‡		
	Unselected T	Ly1	Ly23	Exp. 1	Exp. 2	Exp. 3			
10 ⁵	0.5			154			Control		
	1.0			1,186					
	2.0			2,774					
			0.5	294			191		
			1.0	1,500			126		
			2.0	3,421			123		
					0.5	0	0		
					1.0	169	14		
					2.0	412	15		
10 ⁷	0.5			1,860	—		Control		
	1.0			2,940	610				
	2.0			3,300	2,440				
			0.5	4,410	1,460		237		
			1.0	5,550	3,550		386		
			2.0	6,740	5,510		215		
					0.5	115	ND	6	
					1.0	360	ND	12	
					2.0	490	ND	15	
10 ⁹	0.5			2,320	460	810	Control		
	1.0			3,300	2,850	2,360			
	2.0			810	2,250	3,650			
			0.5	4,020	5,310	4,190	615		
			1.0	7,900	6,005	6,670	244		
			2.0	12,100	7,365	10,250	701		
					0.5	650	0	ND	14
					1.0	360	ND	ND	11
					2.0	320	640	ND	34

* Ly⁺ T cells were obtained from donors primed 5 days previously with SRBC and added in the indicated graded doses to 5×10^6 normal spleen cells (which produced between 2 and 400 PFC alone).

‡ % Control PFC responses, (PFC experimental)/(PFC control).

V. Evidence that Suppressive Activity of Ly23 Cells, after Immunization, is Antigen Specific. Suppression by Ly23 cells is antigen specific because the SRBC-primed Ly23 cells suppressed the response to SRBC but did not suppress the response to horse erythrocytes (HRBC), while HRBC-primed Ly23 cells suppressed the response to HRBC but not SRBC (Table VI). When both SRBC and HRBC were included in the cultures, Ly23 cells generally suppressed only the response to the priming antigen, although in some cases slight suppression to the second antigen was noted, suggesting that once specifically elicited, some of the suppressive activity may be nonspecific.

Similarly, the enhanced response obtained after removal of Ly-2⁺ cells from SRBC-primed T-cell populations (section II of Results) is specific because the

TABLE III
Positive Selection of Ly-2⁺ Cells on Immunoabsorbent Columns*

Characteristics of effluent and eluted cells	Pretreatment of T cells:			
	NMS		αLy-2	
	Effluent	Eluted	Effluent	Eluted
A. Recovery, %	94 ± 5	3	45 ± 5	40 ± 6
B. Cell Phenotypes (% of A)‡				
Thy-1 ⁺	86 ± 6		94 ± 5	90 ± 4
Ly-1 ⁺	70 ± 4	ND	82 ± 6	80 ± 8
Ly-2 ⁺	38 ± 3		2 ± 2	86 ± 4
C. Generation of killer activity	+		-	+
Generation of helper function	+		+	-

* T cells purified by passage through nylon were incubated with either αLy-2.2 or NMS for 30 min at 4°C, washed twice, and passed through a RαM Fab sephadex column. Retained cells were eluted by flushing the column with 20 ml of 10% NMS. The effluent and eluted populations were incubated overnight at 37°C and washed three times to remove any retained Ly-2.2 antibody. The proportions of Thy-1⁺, Ly-1⁺, and Ly-2⁺ cells (B) were then determined by the cytotoxicity assay (see below) and assayed for function (C). The table shows that the processed cells were not sensitive to C alone (B), and retained their expected functions (C), which justifies the conclusion that overnight incubation is effective in disposing of αLy-2.2 antibody originally attached to the Ly-2⁺ population.

‡ NMS + C controls (background analysis) ranged from 6 to 18% in these experiments. Figures for % lysis were derived from the following calculations: [cpm(antiserum + C) - cpm(NMS + C)]/[cpm(freeze-thaw) - cpm(NMS + C)].

TABLE IV
Comparison of the In Vitro SRBC Responses of Separated and Recombined Ly-1⁺ and Ly-2⁺ T-Cell Populations from the same Primed T-Cell Pool

Groups	No. T cells added* (×10 ⁶)			αSRBC response (PFC/well)			
	Unselected	Ly1	Ly-2 ⁺	Exp. 1		Exp. 2	
				Direct	Total	Direct	Total
A	0.25			330	500	200	400
	0.5			1,150	1,200	600	840
	1.0			1,900	2,000	900	1,000
B		0.125		900	1,000	1,200	1,400
		0.25		2,800	2,900	1,850	1,600
		0.5		3,200	3,200	2,600	2,800
C			0.5	150	100	200	250
			1.0	200	200	100	100
D		0.5	0.5	1,700	1,400	750	1,000
		-	-	200	150	250	100

Interpretations: The numbers of Ly1 cells contained in the unselected population (group A) are roughly equal to the numbers of Ly1 cells in group B (see Table III). Therefore, comparing groups A and B, the removal of Ly-2⁺ cells is seen to augment considerably the helper activity of the Ly1 population. When the Ly-2⁺ population (group C) is recombined with the Ly1 population, the helper activity of the reunited population (group D) is reduced to that of the original unselected T-cell population (group A).

* T cells from SRBC-immune donors (10⁶). SRBC 5 days earlier were separated into Ly-2⁺ and Ly-2⁻ (Ly1) fractions on immunoabsorbent columns (see Table III) and added in graded numbers to 2.5 × 10⁶ normal spleen cells.

TABLE V
Suppression of T-Helper Activity by Ly23 Cells from Primed Donors

No. of primed T cells ($\times 10^6$)*		Exp. 1		Exp. 2		% Decrease in PFC response†		Helper index‡	
Group	Ly1	Ly23	Unselected	Direct	Total	Direct	Total		
A	—	—	—	200	250	300	350	Control	1
	0.25	—	—	2,600	2,000	2,100	3,000		
	1	—	—	5,000	3,400	4,100	4,200		
	2	—	—	8,600	11,800	4,200	6,100		
	3	—	—	10,400	13,100	4,600	8,000		
B	1	0.4	—	1,800	2,200	2,000	3,900	64, 51 35, 7	0.33
	1	1.0	—	1,600	2,000	1,400	3,000	68, 66 40, 29	0.25
	2	1.0	—	2,300	3,100	2,000	3,400	73, 52 74, 44	0.20
	—	—	1	3,200	4,400	ND			
	—	—	2	2,800	3,200	ND			

* The indicated T-cell population was obtained from donors primed 5 days previously with 10^8 SRBC and added in graded doses to 5×10^6 normal spleen cells.

† % Decrease PFC = $100 - (100 \times \text{experimental control})$.

‡ The "helper index" is A/B, where (B) is the number of Ly1 cells indicated in group B and (A) is the number of Ly1 cells that would generate the same number of direct PFC in the absence of Ly-2⁺ cells (extrapolated from a dose-response curve drawn from the data of group A).

TABLE VI
Specificity of suppression by Ly23 Cells from Donors Primed with SRBC or HRBC

Ly23 cells* primed to:	Antigen in culture:	PFC response against:	% Decrease PFC response†			Mean % decrease PFC response
			Exp. 1	Exp. 2	Exp. 3	
S	S	S	85	78	59	76
S	H	H	10	0	18	9
H	H	H	—	79	100	90
H	S	S	—	28	0	14
S	S + H	S	81	77	45	68
		H	43	47	20	37
H	S + H	S	—	10	0	5
		H	—	65	90	73

* T cells from mice primed with 5×10^6 SRBC or HRBC 5 days previously were treated with α Ly-1 or α Ly-2 plus C. $5-8 \times 10^5$ selected Ly23 cells primed with the indicated RBC (S or H) were added to cultures containing 3×10^6 normal spleen cells plus $5-8 \times 10^5$ Ly1 cells. The PFC response after stimulation with either SRBC or HRBC, was assayed after a 5 day induction period.

† Percent decrease PFC = $100 - [100 \times \text{experimental (Ly1 + Ly23)}/\text{control (Ly1 alone)}]$. In these experiments, the cultures supplemented with Ly1 cells alone (controls) produced between 870-2,940 α SRBC PFC and 330-1,980 α HRBC PFC after stimulation with SRBC and HRBC, respectively.

response to SRBC, but not to HRBC, was enhanced after depletion of Ly-2⁺ cells. These findings: (a) indicate a greater degree of specificity at the suppressor cell level than at the helper cell level, where some cross-reactivity for the two antigens is present, (Table VII); and (b) confirm the conclusion of section II of Results, namely that the enhanced response seen after removal of Ly-2⁺ cells is not due simply to enrichment of Ly1 helper activity.

TABLE VII
Specificity of Enhancement after Removal of Ly-2⁺ Cells from SRBC-Primed T-Cell Populations

No. SRBC-primed T cells ($\times 10^6$)*		PFC response [‡]			
		Exp. 1		Exp. 2	
Unselected	Ly1	SRBC	HRBC	SRBC	HRBC
0.5		1,750	1,090	950	1,050
1.0		3,050	1,400	1,700	1,500
2.0		2,450	1,550	2,000	1,700
	0.5	4,150	900	2,600	850
	1.0	5,100	1,100	4,200	1,100
	2.0	7,350	1,000	5,950	1,200
—	—	850	315	500	225

* The indicated numbers of Ly1 or unselected T cells from SRBC-primed donors (10^8 SRBC) were added to 5×10^6 normal spleen cells plus 3×10^6 SRBC or HRBC.

[‡] Direct α SRBC or α HRBC PFC per culture after stimulation with SRBC or HRBC, respectively.

VI. Suppression by Ly23 Cells In Vivo. The data above indicate that Ly1 cells help antibody responses in vitro, while Ly23 cells suppress. To test whether these observations in vitro reflect cellular mechanisms governing the magnitude of the antibody response in vivo, we tested the influence of antigen-activated Ly subclasses on antibody production in adoptive hosts. For this purpose, B mice (21 days after reconstitution) received intravenously 5×10^8 SRBC together with graded numbers of SRBC-primed Ly subclass cells. As was the case in vitro, (see section I of Results) Ly23 cells provided no help, and the α SRBC response of given numbers of primed Ly1 cells alone was considerably greater than that of the same number of Ly1 cells included in the unselected population (Table VIII). In the next set of experiments, B mice received intravenously 5×10^8 SRBC plus a constant number of primed Ly1 cells, together with graded numbers of primed Ly23 cells. As was the case in vitro (see section IV of Results), the α SRBC response was progressively diminished by increasing numbers of primed Ly23 cells.

Discussion

The Ly components of the T-cell surface were used here to investigate further whether helper and suppressor activities can be assigned to distinct T-cell subpopulations. Clearly, cells of the Ly1 subclass develop only helper activity after stimulation by antigen, whereas cells of the Ly-2⁺ subclasses, in particular Ly23 T cells, express suppressor activity (as well as cytotoxicity [4]).

These data have two important implications: (a) The generation of T-cell suppressor activity is an invariable consequence of priming with high doses of erythrocyte antigen. (b) Immunosuppression after stimulation by antigen is mainly confined to a subclass of T cells that also expresses cytotoxic function but is distinct from helper T cells. The fact that the degree of suppression is directly

TABLE VIII
The influence of SRBC-Primed Ly23 Cells Upon an In Vivo SRBC Response

	No. of SRBC-primed T cells injected into B mice ($\times 10^6$)			PFC/spleen		% Decrease in PFC response	
	Unselected	Ly1	Ly23	Direct	Total	Direct	Total
Exp. 1	1			12,500	14,000		
	2			22,852	32,356		
	6			25,600	55,000		
	12			27,000	80,000		
			1	20,600	64,100		
			2	27,800	93,600		
			6	50,000	85,100		
			12	46,100	75,400		
			1	0	0		
			2	0	0		
			6	2,000	4,200		
			12	4,000	6,500		
Exp. 2		2	—	33,100	90,400		
		2	2	10,600	28,200	68	69
		2	4	4,900	18,000	85	80
		0	4	2,100	2,800		
Exp. 3		1.5	—	5,900	18,880		
		3.0	—	12,670	29,400		
		1.5	1.5	2,180	10,900	63	42
		1.5	3.0	2,079	4,764	65	75

See text for protocol.

proportional to the amount of immunizing antigen also suggests that suppression normally serves to prevent excessive or unduly prolonged responses to antigen.

Suppression generated after exposure to a large number of SRBC or of HRBC was highly specific, and required the presence of the priming antigen for induction. This lack of cross-reactivity between SRBC and HRBC, on the part of suppressor T cells, indicates a higher degree of specificity than that exhibited by helper T cells, where there is some cross-reactivity for the two antigens (Table VIII and reference 6). However, once specific suppression was elicited by the priming antigen *in vitro*, there was in some instances partial inhibition of a simultaneous response to the second antigen. Whether this decreased specificity of expression is also apparent *in vivo* has not yet been established.

These findings, taken together with previous results, indicate that thymus-dependent differentiation results in the autonomous formation of at least three functionally distinct T-cell subclasses: T helpers (T_H), T cytotoxic and suppressor cells (T_{CS}), and early appearing or immature T cells (T_E). The properties of these subclasses are summarized in Table IX.

The observation that a substantial part of suppressor activity after immuniza-

TABLE IX
T-Cell Subclasses*

Characteristics:	T _H	T _{CS}	T _E	References
Ly phenotype	1	23	123	2-4, 11-14, footnote 2
Helper activity (T-B, T-T)				
Primary response	+		?	2, 4, 11, Table I
Secondary response	+			12, Table II
Suppressor activity				
Primary response		?	?	
Secondary response (specific)		++	+	Tables II-IV
Allotype suppression		+		Footnote 2
Polyclonal induction		+		2
Killer activity				
Prekiller		+	?	4, 11
Killer-effector		+		4, 11-13
Delayed-type hypersensitivity	+			14

* T_{CS}, T cytotoxic/suppressor cell; T_E, T early appearing or immature cell; and T_H, T-helper cell.

tion is mediated by cells of the Ly23 subclass requires comment. Cells of this subclass also generate killer function after stimulation by allogeneic cells, responding to *H-2K/D* differences in mixed lymphocyte cultures, while cells of the Ly1 subclass respond preferentially to *I*-region differences (4). If both killer and suppressive activities are mediated by the same Ly23 cells, one can envisage that T_S activity could be mediated by specific elimination of antigen-reactive T_H or B cells, or of antigen-bearing macrophages. This is particularly suggested by reports that cells bearing a foreign antigen closely associated with *H-2K* or *H-2D* determinants can be lysed by syngeneic T cells (7, 8), especially if the antigen-bearing target cells are activated. Cells bearing antigen associated with self-Ia determinants are probably not lysed by killer cells, but may instead trigger T_H cells (4). On the other hand, the future use of new immunogenetic markers like Ly may show that the Ly23 population is divisible into two subclasses with discrete killer and suppressor functions.

Our experiments indicate that the net helper response after immunization with SRBC is determined by the relative proportions of SRBC-specific T_H activity and T_S activity present in the primed cell population. This implies: (a) that the level of response to a given antigenic determinant may reflect the relative proportions of T_S and T_H cells with specificity for that determinant, and (b) that unresponsiveness to a given antigenic determinant may reflect a preponderance of antigen-specific T_S cells rather than a lack of specifically reactive T_H cells. In view of recent indications that genetic unresponsiveness to antigen may in some cases be caused by preferential activation of antigen-specific T_S cells (9, 10) it is possible that at least some immune response (*Ir*) genes may act by channeling the differentiation of antigen-reactive T-cell clones towards the T_H or the T_S pathways, high-responder *Ir* alleles favoring the former, and low-responder alleles the latter.

² Herzenberg, L. A., K. Okumura, H. Cantor, V. L. Sato, E. A. Boyse, F. W. Shen, and L. A. Herzenberg. Allotype-specific T-cell cooperation in antibody formation: removal by suppressor T cells and genetic control in Ig congenic mice. Manuscript in preparation.

Summary

Cells of the Ly1 subclass generate helper activity in both primary and secondary responses to sheep erythrocytes (SRBC). In contrast, after priming with SRBC, cells of the Ly-2⁺ subclasses, in particular Ly23 cells, have suppressive activity. The degree of Ly23-mediated suppression is directly proportional to the amount of antigen (SRBC) used for priming. Suppression by Ly23 cells is specific, because Ly23 cells from SRBC-primed animals do not suppress the response to horse erythrocytes, and vice versa. Thus, both cytotoxic and specific suppressor functions are mediated by T cells of a subclass, provisionally designated T_{CS}, which can be distinguished from helper T cells (T_H), by their Ly phenotypes. It remains to be determined whether killing and suppression are functionally interrelated properties of a single Ly23 subclass, or whether the Ly23 population comprises two subclasses whose surface phenotypes are not yet distinguishable by immunogenetic criteria.

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