DEVELOPMENTAL AND COMMUNICATIVE INTERRELATIONS OF Ly123 AND Ly1 CELL SETS*

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In defining the relation of T-cell sets to one another, the prime task is to determine, for a given pair of cell sets jointly required for a particular function, whether one set is recruited from the other by differentiation (developmental sequence) or whether the relationship is entirely communicative (collaboration or induction).

The cell sets Ly123 and Ly1 are a case in point. From indirect evidence it has been inferred that Ly123 is the precursor set for Ly1 cells that act as helper-effectors (HE)[1] (Ly1:HE cells) (1) and for Ly1 cells that act as suppression-inducers (SI) (Ly1:SI cells) (2, 3), but an entirely communicative partnership has not been ruled out.

We now report direct evidence that the Ly1:HE and Ly1:SI cells are derived from the Ly123 set by differentiation, and further incidental evidence of communicative interactions involving Ly123 and Ly1 cells.

We obtained these data by using genetic markers (Lyt-1 allotypes of Lyt-1 congenic mice) to trace the origin of Ly1:HE and Ly1:SI cells from alternative, allotype-distinguished Ly123 cells, in the cortisone-resistant nylon-purified thymocyte (CRNPT)-macrophage (MΦ) test system (4) in which antigen-specific Ly1:HE (3) and Ly1:SI (3) cells are generated from CRNPT cultured on peritoneal MΦ from specifically primed mice.

Materials and Methods

Mice. C57BL/6 (B6) mice (phenotype Lyt-1.2:Lyt-2.2) were obtained from The Jackson Laboratory, Bar Harbor, Maine. Congenic B6-Lyt-1^a mice (phenotype Lyt-1.1:Lyt-2.2) were bred at the Sloan-Kettering Institute, New York.

Antigens. Preparation of group A Streptococcus vaccine (SAV) and its trinitrophenyl (TNP) conjugate (TNP-SAV) is described elsewhere (5, 6).

T-MΦ Culture. This procedure, in which CRNPT are cultured for 4 d on MΦ from donors primed with SAV, yielding mostly Ly1 cells (3), whose HE and SI functions are measured by plaque-forming cell (PFC) assay with T-depleted and intact spleen populations, respectively, is described elsewhere (3) and is briefly recapitulated in footnotes to Tables I and III.

Selection of Cell Sets from CRNPT. Ly2$^+$ cells (Ly123 and Ly23) were positively selected from B6 and B6-Lyt-1^a CRNPT as follows: Step 1: Incubation of CRNPT with α-Lyt-2.2 (7) for 30 min on ice (2.5×10^7 CRNPT/ml of 1:50 α-Lyt-2.2 with a cytotoxicity titer of 1:640 against B6 and B6-Lyt-1^a thymocytes, both B6 and B6-Lyt-1^a mice being Lyt-2.2). Step 2: Twice-washed antibody-coated cells from step 1 were applied to plastic dishes coated with immunoadsorbent-

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[1] *Abbreviations used in this paper:* B6, C57BL/6; C, complement; CRNPT, cortisone-resistant nylon-purified thymocyte(s); HE, helper-effector(s); MΦ, macrophage(s); PFC, plaque-forming cell; SAV, group A Streptococcus vaccine; SI, suppression-inducer(s); TNP, trinitrophenyl.

TABLE I

*Differentiation of Ly1:HE Cells from the Ly123 Cell Set**

Group	Culture on primed Mϕ: Ly1 alleles of CRNPT			Primed Ly1 cells transferred		Helper assay PFC index (experimental/standard)							
	All sets	Ly1 set	Ly123 set	Treatment (+C)	Ly1 allotype	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	Exp. 6	Exp. 7	Exp. 8
A	b	–	–	–	2				5.8				
B	a	–	–	–	1	15.4	6.0	9.9					
C	–	a	–	–	1	2.8	2.4	1.5					
D	–	b	–	–	2				1.3				
E‡	b	a	–	–	1‖ + 2‖	14.3	5.0	10.1	5.6				
F‡	b	a	–	α-1.2	1	3.9	1.7	2.3	1.0				
G‡	a	b	–	–	1¶ + 2¶	14.7	5.3	10.2	5.9				
H‡	a	b	–	α-1.2	1	17.5	6.6	10.6	6.0				
I	b	–	–	–	2					9.3	14.7	13.8	15.2
J	–	–	b	–	2					1.8	2.9		
K	–	–	a	–	1							3.2	5.7
L§	–	b	a	–	1** + 2**							16.2	17.4
M§	–	b	a	α-1.2	1							16.8	15.5
Standard (*PFC count*)						1.0 (*175*)	1.0 (*614*)	1.0 (*651*)	1.0 (*1,077*)	1.0 (*612*)	1.0 (*665*)	1.0 (*426*)	1.0 (*589*)

* Procedure. Step 1: Culture of macrophages from B6 mice injected with SAV (primed Mϕ). Step 2: Addition of CRNPT from B6 mice (Lyt-1 allele *b*) or B6-Lyt-1^a cogenic mice (Lyt-1 allele *a*), either without selection (all sets), or after negative selection with α-Lyt-2.2 serum + C (Ly1 set), or after positive selection with α-Lyt-2.2 serum (Ly123 set). Step 3: After 4 d, the harvested cells (mostly Ly1 [3]) were selected by cytolysis with α-Lyt-2.2 + C (primed Ly1 cells). Step 4: For the Groups indicated, the primed Ly1 cells were further selected by cytolysis with α-Lyt-1.2 + C (α-1.2), thus eliminating primed Ly1 cells of B6 origin (phenotype 1.2) but not of B6-Lyt-1^a origin (phenotype 1.1). Step 5: Helper assay (direct PFC) of 1 × 10^5 primed Ly1 cells (counted after selection with antiserum + C) per 1 × 10^7 T-depleted spleen cells. Standard, PFC count for T-depleted spleen cells alone.

‡ Ratio of unselected CRNPT (all sets)·Ly1 set cells is 3:1.

‖ Allotype 2 cells (origin *b*) exceed allotype 1 cells by 2:1.

¶ Allotype 1 cells (origin *a*) exceed allotype 2 cells by 2:1.

§ Ratio of Ly1 set cells:Ly123 set cells is 1:10.

** Allotype 1 cells (origin *a*) exceed allotype 2 cells by 3:1.

purified goat α-mouse Ig (gift of Richard K. Gershon, Department of Pathology, Yale University Medical School, New Haven, Conn.) (8). Step 3: Nonadherent cells were gently washed away, and adherent cells then released by vigorous pipetting. The cells recovered comprised ~50% of the population seeded. Cytotoxicity assays of this recovered population, denoted Ly123, showed 85–95% $Ly2^+$ cells (Ly123 and Ly23) and 2–6% Ly1 cells. Relevance of included Ly23 and residual contaminant Ly1 cells is discussed in context.

Ly1 cells were negatively selected from CRNPT by elimination of $Ly2^+$ cells with the same α-Lyt-2.2 serum plus complement (C) under predetermined optimal conditions for immune cytolysis.

Elimination of Lyt-1.2 Cells from the Primed Ly1 Population (Step 4 in Footnote to Table I). Primed Ly1 cells from step 3 were incubated with α-Lyt-1.2 serum plus C under predetermined conditions for optimal immune cytolysis.

Results and Discussion

Origin of Ly1:HE Cells from the Ly123 Set (Table I)

LY-123 CELLS ARE REQUIRED FOR THE GENERATION OF LY1:HE CELLS. This is established by experiments 1–4, which show that unselected CRNPT generate help (groups A and B), whereas Ly1 CRNPT alone do not (groups C and D).

THE GENOTYPE OF GENERATED LY1:HE CELLS IS THE SAME AS THAT OF THE LY123 CRNPT SET. This is established by groups E–H of experiments 1–4. In all four groups, the starting population of CRNPT included Ly123 cells marked by one Lyt-1 allotype, combined with Ly1 cells both of the concordant allotype (included in the unselected

CRNPT) and also of the discordant allotype (the added CRNPT Ly1 set). If Ly123 cells could induce conversion of Ly1 cells to Ly1:HE cells then Ly1:HE cells of both Lyt-1 allotypes should be produced. But this is not so: When Ly123-concordant cells were eliminated from the primed population, help was abolished (compare group F with control group E in experiments 1–4); but when Ly123-discordant Ly1 cells were removed, help was not affected (compare group H with control group G in experiments 1–4).

Further information was gained by estimating the proportions of Lyt-1.1 and Lyt-1.2 cells, by cytotoxicity assay, in primed Ly1 populations generated from starting populations comprising appropriate combinations of allotype-marked CRNPT. If Ly1:HE cells can be generated only from Ly123 cells, and if the starting population comprises Ly123 cells of one allotype together with Ly1 cells of both allotypes, as in groups E to H, then the primed population should contain more Ly1 cells of the Ly123-concordant than of the Ly123-discordant allotype. And indeed counts of Lyt-1.1 and Lyt-1.2 cells in primed populations so generated contained roughly twice as many Ly123-concordant Ly1 cells as Ly123-discordant Ly1 cells (groups E and G; in which the ratio of unselected CRNPT:Ly1 cells in the starting population was 3:1). In group L the starting population contained only Ly123-discordant Ly1 cells (except for residual contaminants), yet the Ly123-concordant Ly1 cells in the primed population exceeded the Ly123-discordant Ly1 cells by >3:1 (ratio of Ly123:Ly1 cells in the starting population was 10:1).

The ratios of Lyt-1.1:Lyt-1.2 cells in the primed Ly1 populations reflected the proportions of CRNPT of the respective allotypes in the starting population. Thus hypothetical overgrowth by Ly123 cells competing with fewer discordant Ly1 CRNPT cannot account for the concordance of Ly123 and Ly1:HE allotypes.

Fig. 1 confirms that Ly1:HE cells can be derived only from Ly123 cells, because when Ly123-concordant Ly1 cells are eliminated from the primed Ly1 population (group F, Table I) the number of primed Ly1 cells required for optimal help is raised by a factor of at least 20 in comparison with elimination of Ly123-discordant Ly1 cells (group H).

GENERATION OF Ly1:HE CELLS REQUIRES NOT ONLY PRECURSOR Ly123 CELLS BUT ALSO Ly1 CELLS. THESE ARE NOT THE PRECURSOR OF Ly1:HE CELLS. Participation of the Ly23 set, which is included with Ly123 in the positive selection procedure with α-Ly2 serum, can be discounted because there is no conversion of Ly23 cells to Ly1 cells (thymectomized B mice given Ly23 cells do not generate Ly1 cells [9]), and because extensive studies have shown no contribution of Ly23 cells to helper function.

Ly123 cells alone cannot generate Ly1:HE cells (Table I, groups J and K); existing Ly1 cells are required (groups L and M). But allotype-discordant Ly1 cells (groups L and M) are as effective as allotype-concordant Ly1 cells (groups A, B, and I) in the generation of Ly1:HE cells from Ly123 cells. Therefore the relation of these Ly1 cells to the Ly123 cells is communicative. Both these Ly1 and Ly1:HE cells are inductive, the former inducing Ly123 cells and the latter B cells; whether the use of other phenotypic markers will distinguish them as separately programmed Ly1 sets remains to be seen.

It may be asked whether the communicative function here ascribed to an Ly1 subset could be a result of contaminant Lyt-null cells. We consider this unlikely for the following reason: Positive selection of $Ly2^+$ cells varied in efficiency, yielding

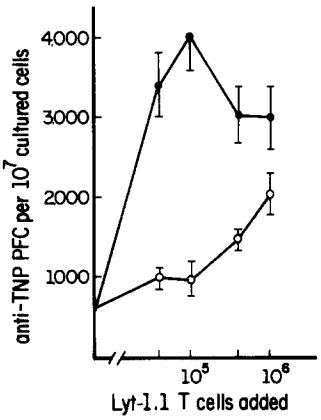

FIG. 1. Stimulation of the response of T-depleted spleen cell cultures to TNP-SAV by SAV-primed Ly1 cells from B6-Lyt-1^a (Lyt-1.1). B6-Lyt-1^a Ly1 cells were induced by SAV-pulsed MΦ in T-MΦ cultures that contained Ly123 cells of B6-Lyt-1^a origin (●) or of B6 origin (○). Experimental protocol as in Table I, experiment 3, groups F and H. Anti-TNP PFC response ± SD of triplicate cultures, assayed after 4 d.

TABLE II

*Generation of Ly1:HE Cells by Combined Ly123 and Ly1 Sets is Not Subject to Lyt-1 Restriction**

Group	Culture on primed Mϕ: Ly1 alleles of		Primed Ly1 cells transferred		Helper assay PFC index experimental/ standard
	Unselected CRNPT	Splenic Ly1 set	Treatment (+C)	Ly1 allotype	
A	–	a	α-1.2	a	3.8
B	a	a	α-1.2	a	7.4
C	b	a	α-1.2	a	6.7
Standard (*PFC count*)					1.0 (*1,077*)

* All procedures as in footnotes to Table I, except for Step 2: Addition of 2×10^6 unselected B6 (Lyt-1 allele *b*) or B6-Lyt-1^a (allele *a*) CRNPT plus 1×10^7 Ly1 cells selected from B6-Lyt-1^a nylon-purified splenic T cells with α-Lyt-2.2 + C.

various degrees of contamination with Lyt-null and Ly1 cells, which were distinguished by cytotoxicity assay with Lyt-1 antiserum; we found that occasional generation of help without added Ly1 cells was correlated with excess contamination with Ly1 cells and not with excess contamination with Lyt-null cells.

A final possibility, which Table I does not exclude, is that Ly1 cells can be induced by Ly123 cells to become Ly1:HE cells, provided that there is compatibility for Lyt-1 allotypes (Lyt-1 restriction):

$$\text{Ly123} \rightarrow (\text{communication}^*) \rightarrow \text{Pro-Ly1:HE} \rightarrow \text{Ly1:HE},$$

where * means subject to Lyt-1 allotype restriction. Table II rules out such an interpretation: This demonstration required use of the splenic Ly1 set, because this has native helper activity (Table II, group A, PFC index 3.8). Help was amplified equally by unselected CRNPT that were concordant (group B, PFC index 7.4) or discordant (group C, PFC index 6.7) with the splenic Ly1 cells. This lack of Lyt-1

TABLE III

*Differentiation of Ly1 Suppressor-Inducer Cells from the Ly123 Cell Set**

Group	Culture on primed Mϕ: Ly1 alleles of CRNPT			Primed Ly1 cells transferred		Suppressor assay PFC index experimental/standard			
	All sets	Ly1 set	Ly123 set	Treatment (+C)	Ly1 allotype	Exp. 1‡	Exp. 2‡	Exp. 5‡	Exp. 6‡
E‡	b	a	–	–	1 + 2	0.09	0.14		
F‡	b	a	–	α-1.2	1	0.73	0.55		
G‡	a	b	–	–	1 + 2	0.05	0.18		
H‡	a	b	–	α-1.2	1	0.10	0.20		
I‡	b	–	–	–	2			0.11	0.08
J‡	–	–	b	–	2			0.04	0.11
Standard						1.00	1.00	1.00	1.00
(*PFC count*)						(*8,692*)	(*10,596*)	(*3,607*)	(*3,058*)

* All procedures as in footnotes to Table I, except for Step 5: Suppressor assay (direct PFC) of 1×10^6 primed Ly1 cells, counted after selection with antiserum + C, per 1×10^7 unselected spleen cells. Standard, PFC count for unselected spleen cells alone.

‡ Experiment numbers and group letters correspond to Table I, which indicates that the same primed Ly1 population was assayed both for help (Table I) and for suppression (Table III).

restriction signifies that the role of the Ly1 CRNPT in question is communicative and that these Ly1 CRNPT are not the precursors of Ly1:HE.

With peripheral T cell sets, Feldmann et al. (10) found both Ly123 and Ly1 cells to be necessary for the generation of Ly1:HE cells (antigen KLH). The contribution of the Ly123 set was effective across a cell-impermeable barrier, which implies that Ly123 cells supply a signal for acquisition of Ly1:HE function. The question whether Ly1:HE cells are directly differentiated from Ly123 cells was not studied. Thus there is no conflict with our data, which, by the use of genetic markers, clearly show that Ly1:HE cells differentiate from Ly123 cells.

Origin of Ly1:SI Cells from the Ly123 Set (Table III)

We next investigated whether the Ly1 cell that induces specific suppression of antibody production (Ly1:SI) is also derived from the Ly123 set by differentiation. The test system was the same, except that the spleen cell culture assay was geared to show suppression, by the use of unselected rather than T-depleted spleen cells, and by raising the number of primed Ly1 cells transferred from 1×10^5 to 1×10^6 (3).

THE GENOTYPE OF THE GENERATED LY1:SI CELLS IS THE SAME AS THAT OF THE LY123 CRNPT. This is established by groups E-H of experiments 1 and 2 of Table III. In all four groups, the starting population included Ly123 cells marked by one Lyt-1 allotype combined with Ly1 cells of both the concordant allotype (from the unselected CRNPT) and also of the discordant allotype (added CRNPT Ly1 set). If Ly123 cells could induce conversion of Ly1 cells to Ly1:SI cells, then Ly1:SI cells of both allotypes should be produced. But this is not so: When Ly123-concordant Ly1 cells were eliminated from the primed population, suppression was drastically reduced (compare group F with control group E); but when Ly123-discordant Ly1 cells were removed, suppression was not affected (compare group H with control group G).

FOR THE GENERATION OF LY1:SI CELLS, IN CONTRAST TO LY1:HE CELLS, LY1 CELLS ARE NOT REQUIRED. This is evident from experiments 5 and 6, which show that suppres-

sion generated from CRNPT that lack the Ly1 set (group J) was as great as that generated by unselected CRNPT (group I). (The corresponding experiment numbers and group letters in Tables I and III indicate that the same pools of primed Ly1 cells were assayed both for help [Table I] and for suppression [Table III].) It should be noted that in group J of Table I, in which Ly1 CRNPT were excluded, there was no help, but suppression was fully manifest (group J, Table III). Thus Ly1:SI and Ly1:HE must be separate populations.

As regards the generation of Ly1:HE cells, reasons were given above for discounting participation by passenger Ly23 CRNPT. In the case of Ly1:SI, Ly23 CRNPT are again excluded from a precursor role on the basis of allotype markers (groups E-H, Table III), but communicative participation remains to be investigated.

The Ly123 set is clearly the source of cell sets with several distinct immunological functions (11). A prime question in the developmental history of the T cell lineage is whether each functionally distinct progeny set is derived from a separately programmed Ly123 subset, or whether instructive signals (as shown here for generation of Ly1:HE cells, and probably for Ly23 suppressors [2]) determine the options exercised by a uniformly programmed Ly123 set. The decision between these alternative explanations should certainly be possible by the use of further markers such as Qa-1 and I-J. For such studies the CRNPT population has the advantage that it is unlikely to have been affected by modifying agents encountered by T cells that have entered the general circulation.

Summary

To distinguish and define the differentiative and communicative relations of Ly123 and Ly1 cells in generating specific helper-effector (HE) (Ly1:HE) and specific suppression-inducing (SI) (Ly1:SI) cells, these two functional sets were generated from various combinations of congenic genetically marked sets of cortisone-resistant nylon-purified thymocytes (CRNPT) by culture on antigen-primed macrophages (MΦ) (the T-MΦ culture system).

It was thus shown that Ly1:HE and Ly1:SI cells are produced by differentiation from antecedent Ly123 cells. Ly1:HE and Ly1:SI are separate Ly1 populations; generation of Ly1:HE cells requires the presence of Ly1 cells, whereas the generation of Ly1:SI cells does not. Although the Ly23 CRNPT set, which is included when Ly123 cells are positively selected with Lyt-2 antiserum, is ruled out as a precursor source of Ly1:SI cells, the possibility of a communicative role for Ly23 cells in generating Ly1:SI cells remains to be investigated.

The role of the Ly1 set required for the generation of Ly1:HE cells from CRNPT is communicative, not differentiational; and it is not a precursor source of Ly1:HE or Ly1:SI cells in the CRNPT population.

It remains to be seen whether the use of additional phenotypic markers will distinguish subsets of Ly123 and Ly1 cells engaged in these several functions.

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