Brief Definitive Report

INDEPENDENT DIFFERENTIATIVE PATHWAYS OF Ly1 AND Ly23 SUBCLASSES OF T CELLS

Experimental Production of Mice Deprived of Selected T-Cell Subclasses*

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The T-lymphocyte population comprises several subclasses, each programmed to express a particular set of Ly cell surface components and a particular set of immunologic functions. For example, the Ly1 subclass is programmed for the $Ly1^+Ly23^-$ surface phenotype and for helper function. The Ly23 subclass is programmed for the $Ly1^-Ly23^+$ phenotype and for killer/suppressor function (1, 2).

How are these two subclasses related to one another? Are they two stages of a single line of differentiation, in which case one of them must at some point give rise to the other? Or are they products of separate pathways of thymus-directed differentiation?

We have approached this question by isolating Ly1 and Ly23 cells and allowing them to populate syngeneic 'B mice', i.e. lethally irradiated thymectomized mice restored with T-cell-deficient bone marrow cells, for up to 6 mo.[1] We refer to these recipients as 'B-Ly1 mice' and 'B-Ly23 mice'. Similarly, we call B mice given unselected T cells 'B-T mice'.

Ly Subclass Notation. Because there is so far no evidence that Ly2 and Ly3 can be expressed independently of one another, i.e. no cells of $Ly2^-Ly3^+$ or $Ly2^+Ly3^-$ phenotypes have yet been identified, there are as yet only three well-defined Ly subclasses: Ly1, Ly23, and Ly123. Therefore in this report we use the following notation: Elimination of $Ly2^+$ cells with αLyt-2 (or αLyt-3) plus complement (C) removes the subclasses Ly123 and Ly23 (both being $Ly2^+$), leaving the subclass called Ly1. Elimination of $Ly1^+$ cells with αLyt-1 plus C removes the subclasses Ly123 and Ly1 (both being $Ly1^+$), leaving the subclass called Ly23. Therefore: (*a*) Ly1 signifies the T cells remaining after a given population has been treated with αLyt-2 (or αLyt-3) plus C. Because Ly1 cells selected with αLy2 have so far been indistinguishable from αLyt-3-selected Ly1 cells in regard to helper and killer criteria, only the results obtained by αLyt-2

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[1] This was suggested by the work of Howard and Wilson, who segregated populations of antigen-stimulated T cells in syngeneic thymectomized recipients (3).

selection are given in this report. (*b*) Ly23 signifies the T cells remaining after a given population has been treated with αLyt-1 plus C.

This terminology, and that for B mice repopulated with Ly1 or Ly23 cells (see below), will require revision if and when new Ly systems, such as Lyt-4 (formerly Ly5) (see 4), necessitate further subdivision of the Ly subclasses of T cells.

Materials and Methods

All Ly serology conformed to the account by Shen et al. (4). Ly subclasses were enumerated according to Cantor and Boyse (1). This paper and reference (2) also give details of all relevant functional assays together with the respective bibliography. Briefly, these procedures are:

Primary Helper Function. Primary helper function was measured by the ability of spleen cells from the different experimental mice to produce anti-sheep red blood cells (SRBC) plaque-forming cells (PFC) after stimulation in vitro by SRBC.

αSRBC Memory Function. αSRBC memory function was measured by the ability of spleen cells from SRBC-primed donors to (*a*) generate anti-SRBC PFC after in vitro stimulation with trinitrophenyl (TNP)-SRBC or (*b*) generate anti-TNP PFC after in vitro stimulation with TNP-SRBC.

Primary Alloreactive Killer Function. Primary alloreactive killer function was measured by the generation of specific cytotoxic activity after stimulation of B6 cells with irradiated BALB/c cells.

Alloreactive Killer Memory. Alloreactive killer memory was measured by the generation of anti-H-2^d-specific cytotoxicity after in vitro "boosting" of cells from B6 mice which have been skin grafted with BALB/c tail skin 3 wk before sacrifice.

Results

I. *Production and Testing of B-Ly1 and B-Ly23 Mice*

All recipients and donors were C57BL/6 (B6) mice.

STEP 1. Thymectomy at 6 wk of age.

STEP 2. Lethal irradiation (750 R; ^{137}Cs source) at 8 wk of age, followed immediately by intravenous restoration with 5×10^6 bone marrow (BM) cells pretreated with αThy-1 plus C.

STEP 3. 4–8 wk later: intravenous administration of $2–10 \times 10^6$ nylon-enriched T cells from pooled spleen and lymph node cells. Different groups of recipients were given: (*a*) Ly1 cells, making the B-Ly1 mouse, or (*b*) Ly23 cells, making the B-Ly23 mouse, or (*c*) the starting T-cell population untreated with any αLy serum, making the B-T mouse.

STEP 4. 4–28 wk later the mice were killed and their spleen and LNC tested for their ability to generate cytotoxic effector and antibody-forming cells. To determine whether antigenic stimulation might cause subclass conversion, some B recipients were given 5×10^5 SRBC together with the T-subclass cells, or were grafted with BALB/c tail skin 3 wk before reconstitution with T cells. A summary of our analysis of these mice is given in Table I.

II. *Retention of Ly Subclass Constitution by B-Ly1 and B-Ly23 Mice.* The question is: do B-Ly1 and B-Ly23 mice remain deprived of T cells of the Ly phenotype that was eliminated from the population used for T-cell reconstitution in step 3? This was ascertained by determining the proportions of $Ly1^+$ and $Ly2^+$ cells serologically as already described (1). To exclude radioresistant host T cells from analysis, B6 Ly1 cells were injected into thymectomized-irradiated

TABLE I

*Ly Phenotypes and Immune Functions of T Cells from B Mice Repopulated with Ly1 or Ly23 T-Cell Subclasses**

Parameter:		Experimental mice:			
		B	B-T	B-Ly1	B-Ly23
Ly phenotype					
Ly subclasses	Ly1⁺	<10	85	93	<10
(% Thy1⁺ cells)	Ly2⁺	<10	48	<10	72
Helper function:					
Primary helper	SRBC	<5	100‡	137	15
(% B-T PFC)	HRBC	<5	100§	288	16
SRBC memory	Direct	<5	100‖	136	22
(% B-T PFC)	Indirect	<5	100¶	104	19
TNP memory	Direct	<5	100**	95	20
(% B-T PFC)	Indirect	<5	100‡‡	106	17
Alloreactive killer function:					
Primary killer – spleen	2:1§§	ND	53	4	12
(% lysis)	5:1	ND	75	5	30
	10:1	0	88	8	48
Primary killer – LNC	2:1	ND	50	2	22
(% lysis)	5:1	ND	73	2	38
	10:1	0	81	3	47
Killer memory – spleen	2:1	ND	13	4	3
(% lysis)	5:1	ND	30	10	7
	10:1	0	50	13	11

ND, not done.

* Results expressed as percentages based on combined data. The raw data for the B-T mice, and numbers of mice per group, are given in the following footnotes. See text for time intervals between T-cell reconstitution and testing.

‡ Mean direct PFC/culture = 2,115, (range 1,560–2,250); 5 mice/group.

§ Mean direct PFC/culture = 275, (range 117–450); 4 mice/group.

‖ Mean direct PFC/culture = 1,760, (range 1,550–2,000); 4 mice/group.

¶ Mean indirect PFC/culture = 1,905, (range 1,040–2,800); 4 mice/group.

** Mean direct PFC/culture = 700, (range 480–845); 4 mice/group.

‡‡ Mean indirect PFC/culture = 595, (range 411–676); 4 mice/group.

§§ Ratio of attacker cells to target cells; 3–5 mice/group.

B6/Ly2.1,3.1 recipients, while B6 Ly23 cells were injected into thymectomized-irradiated congenic B6/Ly1.1 recipients.

As late as the 20th day after T-cell reconstitution B-Ly1 and B-Ly23 mice yielded T cells only of the Ly subclass originally administered. During this period, Ly1 cells evidently cannot give rise to Ly123 or Ly23 cells, nor can Ly23 cells give rise to Ly123 or Ly1 cells. Thus, according to the criterion of Ly surface phenotypes the genetic programs of Ly1 and Ly23 sets of T cells are independent and not sequential.

III. *Sustained Functional Restrictions of T Cells From B-Ly1 and B-Ly23 Mice.* We have seen that the T-cell subclass constitution of B-Ly1 and B-Ly23 mice is sustained for at least 20 days (II). The question now is: do the T-cell functions of B-Ly1 and B-Ly23 mice continue to be restricted to those that accord with the functions that have been assigned to the Ly1 and Ly23 subclasses in preceding studies?

This was assessed by testing B-Ly1 and B-Ly23 mice at increasing intervals of time for the presence of cells capable of helper and killer functions in vitro. T help is a characteristic function of Ly1 cells, and killing of Ly23 cells.

In the following account, the abbreviation 'interval' is used in reference to the periods elapsing between the administration of T cells to each B mouse and the subsequent testing of the recipient's T cells in the various assays. The data for each group have been combined in Table I because there was no demonstrable change with time.

The B-Ly1 Mouse

PRIMARY HELPER FUNCTION. In comparison with the standard B-T mouse, primary helper capacity of the B-Ly1 mouse was augmented in both αSRBC and αHRBC systems. Augmentation can be accounted for by the higher proportion of Ly1 cells entering the assay, which relates to the count of all T cells, or to lack of suppression by the missing $Ly2^+$ subclasses (5) (intervals 15, 18, 28, and 64 days).

HELPER MEMORY FUNCTION. This is measured by inclusion of 5×10^6 SRBC with the T-cell-reconstituting inoculum and subsequent measurement of αSRBC and αTNP-SRBC activities. By both criteria the helper memory function of the B-Ly1 mouse was at least equal to the B-T mouse standard (intervals 48, 51, and 64 days).

PRIMARY ALLOREACTIVE KILLER FUNCTION. Both the killer-effector cell and the prokiller cell from which the killer is generated belong to the Ly23 subclass (1, 6). No significant killer response could be elicited from T cells taken from B-Ly1 mice and challenged in vitro (intervals 28, 64, and 160 days).

ALLOREACTIVE KILLER MEMORY FUNCTION. This is tested by priming in vivo with an H-2-incompatible skin homograft and then testing by challenge in vitro (cell-mediated cytotoxicity). No significant memory response was elicited from the T cells of B-Ly1 mice. But neither was there a significant response from B-Ly23 mice for reasons discussed below (interval 160 days).

The B-Ly23 Mouse

PRIMARY HELPER AND HELPER MEMORY FUNCTIONS. There was little response from T cells of B-Ly23 mice in either the former assay (intervals 15, 18, 28, and 64 days) or the latter (intervals 48, 51, and 64 days).

PRIMARY ALLOREACTIVE KILLER FUNCTION. Ly1 cells amplify the killer function that is generated from Ly23 pro-killer cells but do not themselves acquire killer function (4). T cells of B-Ly23 mice generated substantial killer function (intervals 28, 64, and 160 days) but not so great as those of B-T mice. The lesser response is in keeping with the known amplification of killer function by Ly1 cells during the initiation phase of immunization, but the present experiments do not exclude a role for Ly123 cells (6).

ALLOREACTIVE KILLER MEMORY FUNCTION. The weak reaction of T cells from B-Ly23 mice in this assay (intervals 160 days) was no greater than that of B-Ly1 mice and greatly inferior to the B-T mouse.

These findings suggest that, in the absence of other T-cell subclasses, after antigen activation Ly23 cells may (*a*) actively suppress regeneration of a cytotoxic response or (*b*) undergo 'exhaustive differentiation' after in vivo stimulation by the $H\text{-}2^d$ graft. We favor the first possibility because of reported evidence that previously activated Ly23 cells can suppress the generation of Ly23 cytotoxic effector cells (2).

Discussion

Evidently B-Ly1 mice are equipped for helper function, primary and remembered, but not for killer function, and B-Ly23 mice are equipped for primary alloreactive killer function but not for helper function. The defective killer memorization of B-Ly23 mice accords with the previously recognized amplification of Ly23 killer activity by Ly1 cells (6) which has been attributed to the recognition of *I*-region antigen by Ly1 cells during the initial phase of immunization. However, the experiments described in the present report do not exclude the possibility that previously activated Ly23 cells can themselves suppress new generation of cytotoxic effector cells, a finding noted previously (2).

All this is compatible with the hypothesis that the Ly1 and Ly23 T-cell phenotypes always signify actual and potential immune functions, because the immune functions of B-Ly1 and B-Ly23 mice concur with the Ly subclasses which they possess.

A further important conclusion follows: Even after prolonged residence of Ly1 cells in hosts that have been deprived of Ly23 cells, and in which therefore all physiological controlling mechanisms must be set to favor expansion of the latter population, there is no appreciable generation of Ly23 from Ly1 cells in B-Ly1 mice. Reversely, there is no appreciable stock of Ly1 cells in B-Ly23 mice. Clearly, Ly1 and Ly23 cells must each have exercised a differentiative option that bars them from giving rise to one another. In other words, these two subclasses belong to different lines of differentiation and are not sequential stages of a single progression.

What bearing does this have on models of T-cell differentiation? Assuming that the TL^+Ly123 thymocyte is the precursor of all three subclasses (which is not finally proven, but is not essential to the point in question) the following models can be discarded:

$$\text{TL}^+\text{Ly123} \rightarrow \text{Ly123} \quad \begin{cases} (a) \rightarrow \text{Ly1} \rightarrow \text{Ly23} \\ \text{or} \\ (b) \rightarrow \text{Ly23} \rightarrow \text{Ly1} \\ \text{or} \\ (c) \rightarrow \text{Ly1} \leftrightarrow \text{Ly23} \end{cases}$$

This leaves two models:

$$\text{[Previous nomenclature} \quad \text{T}_0 \rightarrow \text{T}_1 \rightarrow \text{T}_2\ (7, 8)\text{]}$$

$$(d) \qquad \text{TL}^+\text{Ly123} \rightarrow \text{Ly123} \rightarrow \begin{cases} \text{Ly1} \\ \text{Ly23} \end{cases}$$

or

$$(e) \qquad \text{TL}^+\text{Ly123} \rightarrow \begin{cases} \text{Ly123} & (\text{T}_1) \\ \text{Ly1} & (\text{T}_2) \\ \text{Ly23} & (\text{T}_2) \end{cases}$$

At the moment we favor model (*d*) in view of recent evidence that at least after antigen stimulation some Ly123 cells → Ly23 cells (9). If this model is correct the factors regulating the formation of mature Ly1 and Ly23 T cells from Ly123

precursors must play a central role in generating and regimenting the total population of T cells.

Summary

When B mice are supplied with Ly1 or Ly23 cells they acquire, over the next 6 mo, only the immune functions associated with each of these T-cell subclasses, respectively. The T-cell population of these 'B-Ly1' and 'B-Ly23' mice also remains restricted to the Ly1 and Ly23 subclass phenotypes. Thus the Ly1 and Ly23 populations are derived from two separate lines of differentiation and are not sequential stages of a single differentiative pathway.

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