

CELL-MEDIATED IMMUNITY: DELAYED-TYPE  
HYPERSENSITIVITY AND CYTOTOXIC RESPONSES ARE  
MEDIATED BY DIFFERENT T-CELL SUBCLASSES\*

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The immune response has been divided into reactions mediated by antibody (humoral immunity) and reactions mediated by cells (cell-mediated immunity). The latter type of immune reaction consists of several distinct types of responses, which include delayed-type hypersensitivity responses (DTH), and the generation of cytotoxic lymphocytes. Although these two basic forms of cellular immunity probably play a central role in classical homograft reactions, e.g. skin graft rejection, we do not know whether DTH and cytotoxic responses are both properties of a single type of T cell or whether each is mediated by a distinct T-cell subclass.

We have recently found that peripheral T cells are composed of functionally distinct subclasses, each distinguished by characteristic cell surface patterns of Ly antigens (1, 2). Using antisera directed against three antigens, we have shown that helper function and killer function are mediated by separate subclasses of T cells which express Ly1 and Ly23, respectively. In the present study we have used Ly antisera to determine whether T cells that are programmed to generate killer activity are also active in the initiation of delayed-type hypersensitivity reactions. Our results indicate that they are not. Cells of the Ly1 subclass (which also mediate helper activity) are necessary and sufficient for the production and elicitation of DTH reactions, while cells of the Ly23 subclass, which are programmed to express cytotoxic activity, do not make a positive contribution to the DTH reaction and in some cases inhibit this response.

**Materials and Methods**

*Animals.* C57BL/6 (B6) mice 10-14 wk of age were obtained from The Jackson Laboratory, Bar Harbor, Maine. The congenic lines B6-Ly-1<sup>a</sup> and B6-Ly-2<sup>a</sup>, phenotypes Ly-1.1,2.2,3.2 and Ly-1.2,2.1,3.2, respectively, were produced and supplied by E. A. Boyse, Memorial Sloan-Kettering Cancer Center, New York.

*Antisera.* For details of the preparation and use of antisera to Ly1.2, Ly2.2, and Thy1.2 see references 1 and 3 which also provide a bibliography of the Ly systems.

*Isolation of Ly Subclasses.* 20-50 × 10<sup>6</sup> cells/ml were incubated with Ly antiserum at a final dilution of 1:40 in phosphate-buffered saline plus 10% fetal calf serum (PBS-FCS) for 1/2 h at 20°C.

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After washing once, the cells were brought up in 1 ml of freshly thawed selected rabbit serum (diluted 1:12 in PBS) as the source of complement (C), and were incubated for another  $\frac{1}{2}$  h at 37°C. Ly specificity was confirmed for each group of experiments by the absorption of activity from Ly antiserum by B6 cells and not by cells of the respective B6-Ly congenic lines (3).

*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<sup>-</sup>3<sup>+</sup> or Ly2<sup>+</sup>3<sup>-</sup> phenotypes have yet been identified (1), there are as yet only three well-defined Ly subclasses: Ly1, Ly23, and Ly123. Therefore, in the following account we have used the following notations: (a) Ly23 signifies the T-cell population remaining after treatment with anti-Ly1 plus C; (b) Ly1 signifies the selected T-cell population obtained by treating spleen cells or T cells with Ly2 or Ly3 antisera plus C. Since Ly1 cells obtained after treatment with Ly2 or Ly3 antisera give virtually identical results with respect to DTH activity, the results of treatment with Ly2 sera alone are shown.

*Purification of T Lymphocytes.* Nylon wool columns were used according to Julius et al. (4) to enrich for T cells. 80–90% of the recovered cells were Thy1<sup>+</sup> and 5–10% were Ig<sup>+</sup>.

*Methods to Measure DTH Reactions (5–7).* Three systems were used to study the participation of different Ly<sup>+</sup> subclasses in the DTH response:

(a) "B" mice thymectomized at 6 wk of age, lethally irradiated at 8 wk of age (750 R from a <sup>137</sup>Cs source), and protected with  $5 \times 10^6$  anti-Thy1-treated syngeneic bone marrow (BM) cells were reconstituted with different numbers of Ly1, Ly23, or unselected T cells from syngeneic donors along with  $5 \times 10^5$  sheep erythrocytes (SRBC) intravenously (i.v.). This relatively low dose was chosen because it permits the development of an optimal DTH response and does not produce an arthus reaction, as indicated by absence of early (3 h) foot pad swelling (8). 5 days later, these mice were challenged with  $6 \times 10^7$  SRBC subcutaneously (s.c.) in the right hind foot pad. 24 h later, the degree of foot pad swelling was measured and compared to that of unreconstituted B mice that had been immunized to SRBC in identical fashion (negative control).

(b) Mice were lethally irradiated (850 R) and immediately reconstituted with  $5 \times 10^7$  syngeneic spleen cells which had been treated with various Ly antisera plus C. These irradiated hosts were immunized with SRBC, and tested for DTH activity as in (a). The negative control group consisted of irradiated mice injected with SRBC only (no spleen cells).

(c) To identify the cell active in the DTH response after immunization (7), different numbers of spleen cells from mice immunized 4 days previously with  $5 \times 10^5$  SRBC were treated with various Ly antisera or normal mouse serum (NMS) and inoculated into the foot pad of normal recipients along with  $6 \times 10^7$  SRBC. Foot pad swelling was measured 24 h later. A control group of recipient mice was inoculated with spleen cells from nonimmune donors along with  $6 \times 10^7$  SRBC.

*Foot Pad Measurement.* The right hind foot pad was measured immediately before and 24 h after antigenic challenge using a micrometer (Brown and Sharpe Mfg. Co., N. Kingston, R. I.). The mean of three foot pad readings was determined for each mouse. The mean increase of the control group was subtracted from the mean increase of each experimental group and the results were expressed in  $10^{-2}$  mm. Statistical significance (*P* values) of any differences in degree of DTH response were calculated according to Wilcoxon's Rank Test.

## Results

*The Ability of Selected Ly<sup>+</sup> T-Cell Subclasses to Adoptively Restore DTH Activity to B Mice (Fig. 1).* To determine which T-cell subclass is responsible for inducing DTH, we injected 2.5 and  $5 \times 10^6$  purified T cells i.v. into "B" mice together with  $5 \times 10^5$  SRBC. Mice injected with 2.5 and  $5 \times 10^6$  NMS-treated T cells (unselected T cells) developed significant DTH reactions. B mice reconstituted with the same numbers of Ly23 T cells did not develop significant foot pad enlargement, while B mice reconstituted with Ly1 T cells developed strong DTH reactions, which were more intense than those produced by B mice restored with unselected T cells. Finally, recipients of mixtures of Ly1 and Ly23 cells ( $2.5 \times 10^6$  plus  $2.5 \times 10^6$  and  $5 \times 10^6$  plus  $5 \times 10^6$ ) developed DTH responses that were significantly smaller than those produced by Ly1 cells alone. These findings

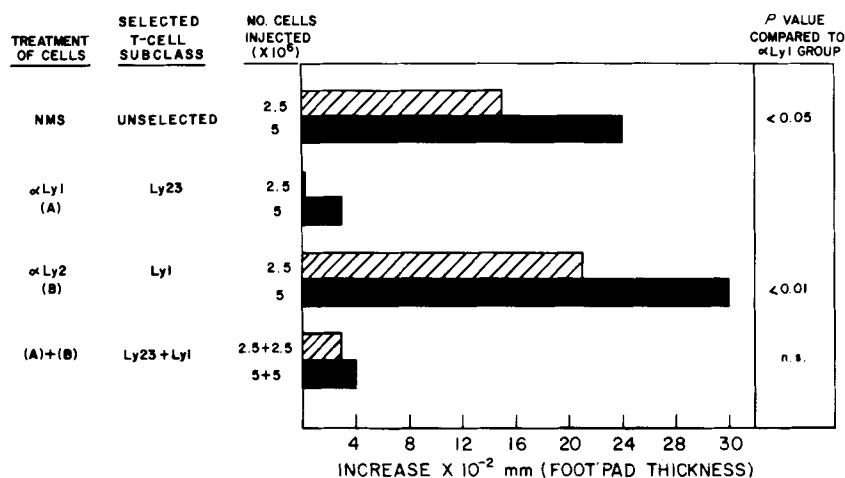


FIG. 1. Initiation of a DTH response by Ly1 cells in adoptive "B" hosts. Ly1 and Ly23 populations were isolated from B6 T cells and injected i.v. in the indicated numbers into syngeneic B mice (see Materials and Methods), along with  $5 \times 10^8$  SRBC. 5 days later, these mice were challenged s.c. in the right foot pad with  $6 \times 10^7$  SRBC; foot pad swelling was determined 24 h later, and compared with that of B mice restored with unselected T cells (positive control), and those receiving a mixture of Ly1 and Ly23 cells. Each group represents the mean response of six to eight recipients, obtained from three separate experiments. n. s., not significant.

indicate that cells of the Ly1 subclass are sufficient for the induction of DTH reactions, while Ly23 cells can suppress this response.

*Successful Transfer of DTH Activity into Irradiated Hosts after Removal of Ly2<sup>+</sup> Cells (Fig. 2).* In a second approach, lethally irradiated mice were reconstituted with spleen cells that had been treated with various Ly antisera. In this system, irradiated hosts given  $5 \times 10^7$  spleen cells (which were required for hematopoietic recovery) did not develop substantial DTH reactions. However, recipients of spleen cells that had been depleted of Ly2<sup>+</sup> cells consistently produced strong DTH reactions.

*Transfer of Immune Cells into the Foot Pad of Normal Mice (Fig. 3).* The data above indicate that the resting T-cell population, before stimulation by SRBC, already contains Ly1 cells that are programmed to initiate DTH responses, as well as Ly2<sup>+</sup> cells that are not so programmed. To determine whether the Ly1 phenotype remains unchanged during sensitization by SRBC, we analyzed the Ly phenotype of T cells present in an immune cell population responsible for transfer of DTH: Spleen cells obtained from mice immunized 5 days previously with  $5 \times 10^5$  SRBC i.v., were treated with Ly antisera and injected into the foot pad of normal animals together with  $6 \times 10^7$  SRBC. DTH effector activity was present in the Ly1 immune population and absent in the Ly23 population. Again, the enhanced response of the selected Ly1 population was lost when this population was combined with selected Ly23 cells. These data indicate that (a) during sensitization, whereby DTH-reactive T cells are generated from antecedent Ly1 cells already committed to DTH function, there is no demonstrable change in the Ly phenotype, and (b) Ly23 cells may exert inhibitory effects upon both the induction and expression of DTH.

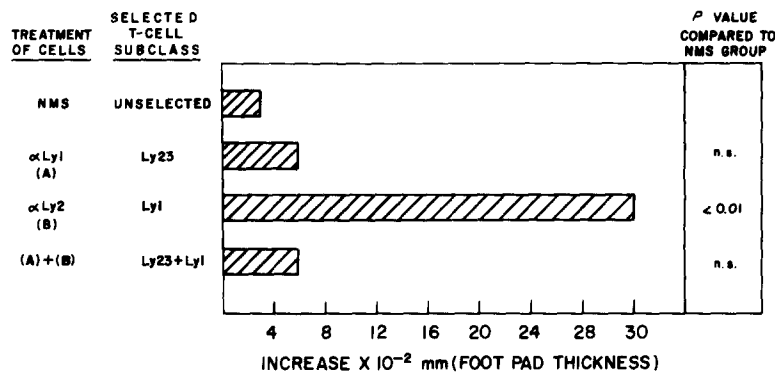


FIG. 2. Initiation of a DTH response by Ly1 cells in adoptive irradiated hosts. Ly1 and Ly23 populations were selected from B6 spleen cells and injected ( $50 \times 10^6$  viable spleen cells) into irradiated hosts along with  $5 \times 10^5$  SRBC. 5 days later, these mice were challenged s.c. in the right foot pad with  $6 \times 10^7$  SRBC; foot pad swelling was determined 24 h later and compared with that of irradiated recipients that had been restored with unselected spleen cells or a mixture of Ly1 and Ly23 cells ( $25 \times 10^6$  plus  $25 \times 10^6$ ). Each group represents the mean response of six to eight recipients, obtained in three experiments. n. s., not significant.

### Comment

We have asked whether cytotoxic and DTH activities, two forms of cell-mediated immunity, are mediated by the same Ly subclass. The findings are that: (a) the provision of Ly1 cells to thymus-deprived hosts restores their ability to generate a DTH response and (b) the provision of Ly23 cells to these hosts does not. Our interpretation of these data is that the ability to generate DTH reactions, at least in this system, is confined to cells of the Ly1 subclass. Ly23 cells clearly make no positive contribution to this response, and in sufficient numbers, can suppress both the generation and expression of DTH responses. Possibly the inability to induce DTH with higher concentrations of SRBC (5, 9) may reflect preferential activation of this suppressive T-cell subclass (9, 10). These data do not directly indicate whether Ly123 cells, a third major T-cell subclass (1), contribute to the DTH response. Our results show that they are not essential to the response, but do not rule out the possibility that these cells may play a regulatory role.

DTH responses may take several forms, e.g. cutaneous basophil hypersensitivity (CBH) (11), contact sensitivity (8), and the more classical form described by Bloom and Chase (8). The DTH response of mice to SRBC has characteristics of both the classical form of delayed hypersensitivity as well as several properties of the CBH response. Nonetheless, the characteristic feature of these different forms of DTH is that all are specifically initiated by sensitized T cells, which serve to attract inflammatory cells to the site of antigen. These subclassifications of DTH probably reflect differences in the types of nonimmune cells that are attracted to the site of inflammation by sensitized T cells rather than the type of T cell that initiates the response. Thus Ly1 cells are also responsible for specific "trapping" of isotope-labeled bone marrow cells after immunization with both SRBC (B. Huber and H. Cantor, unpublished observation) and fowl gamma globulin (J. Miller, personal communication); this trapping effect prob-

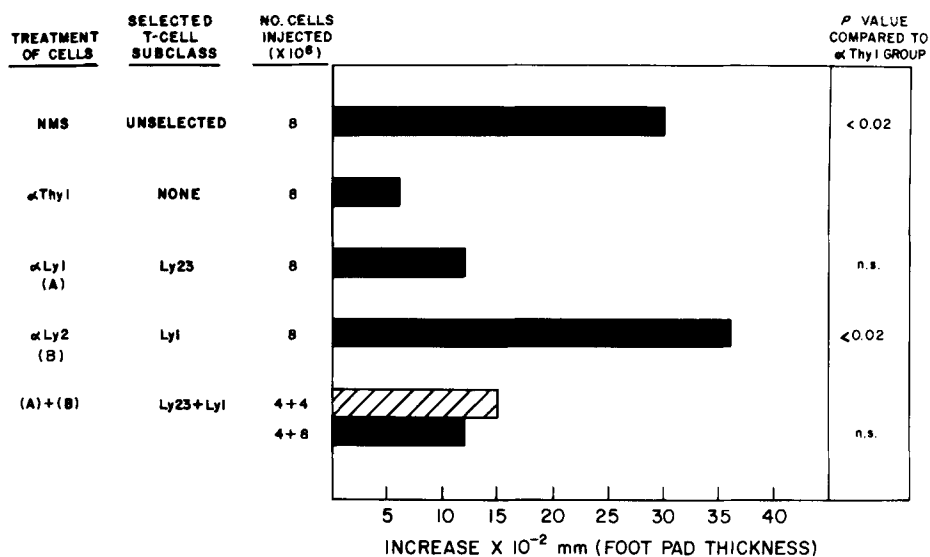


FIG. 3. Stability of Ly1 phenotype on DTH initiator T cells after antigen activation. Ly1 and Ly23 populations were selected in the usual way from spleen cells obtained from donors primed 5 days previously with  $5 \times 10^8$  SRBC. The indicated numbers of viable cells were injected s.c. into the foot pad of normal (unprimed) mice along with  $6 \times 10^7$  SRBC; the degree of foot pad swelling was measured 24 h later, and compared with that of mice inoculated with Thy1-treated cells (negative control) or unselected T cells (positive control). Each group represents the mean of four to six recipients obtained from two experiments. n. s., not significant.

ably represents a necessary step in the induction of DTH, but is not in itself sufficient for expression of DTH (R. K. Gershon, unpublished observation).

The central implication of these findings is that the broad division between humoral immune responses and cellular immune responses does not correspond to the division of labor among T-cell subclasses. While cytotoxic activity, one form of cell-mediated immunity, is a property of the Ly23 subclass, a second form of cell-mediated immunity, DTH, is a property of another T-cell subclass, one that also stimulates B cells to produce antibody. These findings support the idea that cells of the Ly1 subclass may be particularly equipped to interact with Ia-associated antigen on the surface of macrophages in the generation of antibody (T-B cooperation) and initiation of DTH (1, 10).

Our experiments indicate that two major components of the cell-mediated immune response, the generation of cytotoxic cells and the initiation of DTH reactions, reflect the separate activities of two distinct T-cell subclasses. The contribution of these two subclasses to the homograft response is not yet established.

### Summary

Cell-mediated immunity includes both the generation of cytotoxic cells and initiation of delayed-type hypersensitivity (DTH). The resting T-cell population, before stimulation by antigen, already contains cells of the Ly1 subclass that are programmed to initiate DTH (and helper function) but not cytotoxic responses,

as well as Ly23 cells which can generate killer activity (and suppressive function) but not DTH. The central implication of these findings is that the broad division between humoral and cell-mediated immune responses does not precisely correspond to the division of labor among T-cell subclasses. The relative contribution of DTH-competent Ly1 cells and cytotoxic Ly23 cells to the classical homograft response remains to be determined.

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