TWO Ly-1 T HELPER CELL SUBSETS DISTINGUISHED BY Qa-1 PHENOTYPE

The Priming Environment Determines Whether One or Both Subsets Will Be Generated*

By J. S. McDOUGAL, F. W. SHEN, S. P. CORT, AND J. BARD

From the Cellular Immunology Branch, Center for Infectious Diseases, Centers for Disease Control, U. S. Public Health Service, U. S. Department of Health and Human Services, Atlanta, Georgia 30333; and Memorial Sloan-Kettering Cancer Center, New York 10021

Several investigators have provided evidence that more than one Ly-1 T cell subset is involved in helper effector (HE)[1] function for the B cell response to T-dependent antigens. Distinctive functional Ly-1 subsets have been defined by additional phenotypic markers (notably I-A, I-J, and Qa-1) (1–5), adherence properties (3), priming environment (6–9), major histocompatibility complex restrictions (4), antigen specificity (4, 7), and theoretical interpretations of the slope of dose-response curves at limiting effector cell input (log antibody response vs. log effector cell number) (6, 7).

This report resolves a major discrepancy we have had in relation to other reports as to whether or not the Qa-1 immunogenetic marker distinguishes two functional Ly-1: HE subsets. We find that isolated T cells primed with antigen-pulsed macrophages generate appreciable HE activity only in the Ly-1:Qa-1$^-$ T cell subset. However, an Ly-1:Qa-1$^+$ T cell subset associated with HE function can be generated if priming takes place (in vivo or in vitro) in an environment containing B cells.

Materials and Methods

Mice. C57BL/6 (B6) mice (H-2^b:Lyt-1.2:Lyt-2.2:Qa-1$^-$) were obtained from The Jackson Laboratory (Bar Harbor, ME). Congenic B6-T1a^a (H-2^b:Lyt-1.2:Lyt-2.2:Qa-1$^+$) were bred at Sloan-Kettering Institute, New York.

Antigens. Preparation of group A streptococcus vaccine (SAV) and its trinitrophenyl conjugate (TNP-SAV) is described elsewhere (10).

SAV-primed Ly-1 T Cells. The thymocyte-macrophage (T-Mø) culture system, in which cortisone-resistant nylon-purified thymocytes (CRNPT) are cultured for 4 d on macrophages from SAV-primed donors, has been described in detail (11, 12). This system yields mostly Ly-1 cells, which are harvested at 4 d, treated with anti-Lyt-2.2 serum plus complement (C), and assayed for HE activity by their effect on the anti-TNP-SAV plaque-forming cell (PFC) response of T-depleted spleen cell cultures from unprimed mice (10). Splenic T cells were primed in vitro either as the whole spleen cell population or as nylon wool-purified splenic T cells exactly as is done with CRNPT. Experiments reported here were done at least three times.

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[1] *Abbreviations used in this paper:* C, complement; CRNPT, cortisone-resistant nylon wool-purified thymocytes; FBSI, feedback suppression inducer; HE, helper effector; MHC, major histocompatibility complex; PFC, plaque-forming cells; SAV, group A streptococcus vaccine; T-Mø, thymocyte-macrophage culture system; TNP, trinitrophenyl.

Selection of Cell Sets from Primed T Cell Cultures. Ly-1 cells were obtained by negative selection by a two-stage procedure with anti-Lyt-2.2 sera and selected rabbit C under predetermined conditions for optimal immune cytolysis; antisera, procedures, and specificity controls have been described elsewhere (13).

Reaction of antiserum B6 × A-Tla^b anti-A strain leukemia ASL-1 with B6-T1a^a peripheral T cells defines the Qa-1 system (14). Qa-1$^+$ cells were eliminated by using a 1:30 dilution of this antiserum (2.5 × 10^7 cells/ml), as in the Lyt-negative selection (above). Specificity controls consisted of testing the sera on congenic strains that are either positive or negative for the Qa-1 allele (B6-Tla^a, B6) (15). A monoclonal TL antibody with no anti-Qa-1 activity was used to confirm that the activity of the anti-Qa-1 serum was related to Qa-1 rather than TL (anti-TL activity is present in anti-Qa-1 sera); details are given elsewhere (13).

Results

Qa-1 Phenotype of In Vitro Generated Ly-1:HE. We found very little synergy between Ly-1:Qa-1$^+$ and Ly-1:Qa-1$^-$ subsets generated in vitro from CRNPT in the T-Mø system. Fig. 1 shows that the Ly-1:Qa-1$^-$ subset is somewhat less effective in HE function than equal numbers of unselected Ly-1 cells (about half of which are Qa-1$^-$). Positive selection of Ly-1:Qa-1$^+$ cells is technically difficult. Therefore, the question whether the Ly-1:Qa-1$^+$ subset has independent HE activity has not been directly tested by assaying Ly-1:Qa-1$^+$ cells for HE function. An alternative approach has been to assay serially proportioned combinations of primed Ly-1:Qa-1$^-$ cells with unselected (Qa-1$^+$ and Qa-1$^-$) primed Ly-1 cells (1, 2). The synergy in PFC generation is very minor (data not shown) compared with the synergy reported for combined Qa-1$^+$- and Qa-1$^-$-primed Ly-1 cells by Cantor et al. (1) and Stanton et al. (2).

The discrepancy between those studies study and ours is not due to the different sources of T cells (spleen as opposed to CRNPT), because we find the same low level of synergy when using nylon wool-purified splenic T cells in place of CRNPT in the T-Mø culture. Also discounted is the possibility that the synergy was masked in our B cell assay culture because of the presence of residual T cells with which Ly-1:Qa-1$^+$ cells (the phenotype of the feedback suppressor inducer) might interact to induce

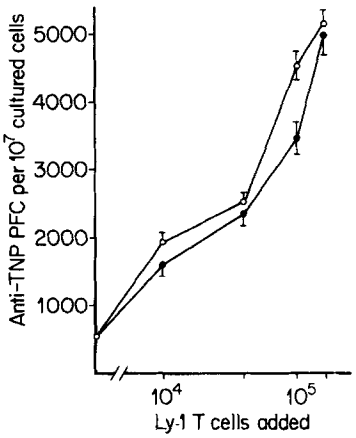

FIG. 1. HE activity of primed Ly-1-Qa-1$^-$ cells compared with unselected primed Ly-1 cells. SAV-primed T-Mø culture cells were selected with either anti-Lyt-2.2 + C (Ly-1 set) (○) or with anti-Lyt-2.2 plus anti-Qa-1 + C (Ly-1:Qa-1$^-$ subset) (●), and titrated (equal numbers of viable cells) for HE activity with T-depleted spleen cells. Results are expressed as anti-TNP PFC response after 4 d.

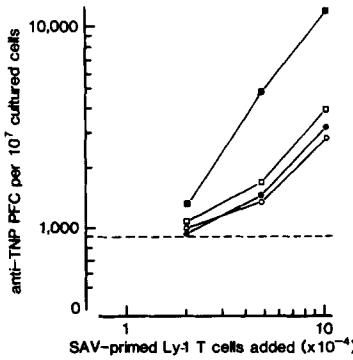

FIG. 2. HE activity of splenic Ly-1 T cells primed in vivo (boxes) or in vitro as isolated T cells (circles). SAV-primed Ly-1 cells (closed circles and boxes) and the SAV-primed Ly-1:Qa-1$^-$ subset (open circles and boxes) were titrated for HE activity with T-depleted spleen cells. (Dotted line shows background anti-TNP PFC response of T-depleted spleen alone.)

concomitant help and suppression (1, 2, 10, 16). Assay cultures constructed with B cells prepared by anti-Thy-1.2 plus C treatment (the usual method), anti-Ly-1 and Lyt-2 plus C treatment, anti-Ig adherent B cells (17), or a sequential combination of the above gave similar results. In addition, the assay culture has in fact been used to demonstrate synergy by using appropriately primed T cells.

Qa-1 Phenotype of In Vivo Primed Ly-1:HE. The difference in degree of synergy found with in vitro-primed Ly-1:HE as compared with others may lie in the conditions used for priming. For example, synergy is readily detected if in vivo primed splenic T cells are assayed, whereas only a low level of synergy can be demonstrated if splenic T cells are primed in vitro on macrophages in the absence of B cells (Fig. 2).

The Whole Spleen Cell Population, but Not Isolated Splenic T Cells Primed In Vitro, Generates Both Ly-1:Qa-1$^-$:HE and Ly-1:Qa-1$^+$ Help-associated Cells. To determine whether the difference between in vivo- and in vitro-primed cells could be reproduced totally in vitro, cultures containing the whole spleen cell population were primed in vitro. After 4 d, the cells were nylon wool purified, selected for Ly-1 phenotype, and assayed for HE function. These responses were compared with those induced by Ly-1 cells derived from cultures containing nylon wool-purified splenic T cells rather than whole spleen (Fig. 3). Selection of the primed Ly-1 cells with anti-Qa-1 sera plus C after induction and before assay reveals that Ly-1:Qa-1$^+$ cells contribute substantially to the helper effect, whereas only a minimal amount of synergy is found in the Ly-1 population primed as isolated T cells.

CRNPT Primed In Vitro in the Presence of B Cells Generate Both Ly-1:Qa-1$^-$:HE and Ly-1:Qa-1$^+$ Help-associated Cells. Returning to the original culture system, we have tried to prime CRNPT on antigen-pulsed macrophages in the presence of B cells (anti-Ig adherent spleen [17]). Ly-1 T cells from these cultures are more effective helpers than T cells primed without B cells present; and, in elimination experiments like that done in Figs. 2 and 3, Ly-1:Qa-1$^+$ cells account for the enhanced helper effect (Table I). However, the increase is not as dramatic as for splenic T cells primed as whole spleen, nor, in our hands, have the results been as consistent. In only 7 of 12 experiments have Ly-1 cells from CRNPT primed with B cells been consistently superior in their helper effect when examined over a dose range of 10^4–5×10^5. We cannot yet distinguish whether this represents an inherent difference or the relative immaturity

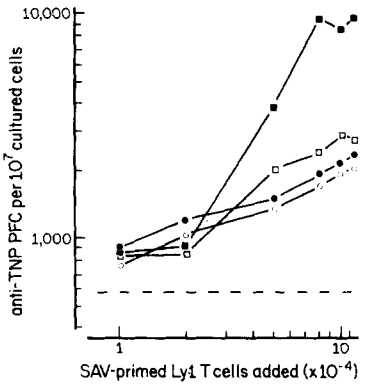

FIG. 3. HE activity of SAV-primed Ly-1 cells obtained from cultures primed as whole spleen (boxes) or as isolated splenic T cells (circles). SAV-primed Ly-1 cells (closed circles and boxes) and the SAV-primed Ly-1:Qa-1$^-$ subset (open circles and boxes) were titrated for HE activity with T-depleted spleen cells. (Dotted line shows background anti-TNP PFC response of T-depleted spleen alone.)

TABLE I

Priming CRNPT in the Presence of α-Ig-adherent B Cells Generates Ly-1:Qa-1$^+$ Help-associated Cells

Group	Initial components of T-Mø culture*	SAV-primed Ly-1 cells from T-Mø culture‡		HE assay§ (PFC index)‖				
		Further selected (+ C)	Remaining subset	Experiment 1	Experiment 2	Experiment 3	Experiment 4	Experiment 5
Ai	CRNPT + B cells	None	Ly-1	10.4	4.7	3.0	7.3	5.1
ii	CRNPT + B cells	α-Qa-1	Ly-1:Qa-1$^-$			2.2	5.8	2.0
Bi	CRNPT	None	Ly-1	4.0	2.5	2.5	5.2	2.6
ii	CRNPT	α-Qa-1	Ly-1:Qa-1$^-$			2.3	4.8	2.7
	Standard			1.0	1.0	1.0	1.0	1.0
	PFC count			550	1,121	498	808	1,010

* Cell numbers: 5×10^5 SAV-primed Mø 10^7 CRNPT, 10^7 α-Ig-adherent B cells.
‡ Selected with α-Ly-2.2 + C.
§ Assay 1×10^6 Ly-1 cells on T-depleted spleen culture for α-TNP PFC to TNP-SAV. Mouse strains: experiments 1 and 2, B6; 3–5, B6Tla^a.
‖ Experimental/standard.

of CRNPT as opposed to splenic T cells, the requirement for another cell that is adequately represented in whole spleen but not in the separated cell populations, or simply suboptimal cell ratios or concentrations used for priming (we have tried multiple ratios and concentrations).

Discussion

Despite numerous reports of distinctive functional or phenotypic T helper cell subsets (1–9), we have tried without success to demonstrate synergy with in vitro-primed Ly-1:HE using a number of approaches. We (18) and others (7) have not been able to confirm the reported synergy (3) between nylon wool-adherent and nonadherent subsets. Slope analysis of dose-response curves has been difficult to interpret—a problem we attribute to the readout assay. The in vitro PFC response

does not usually result in the 3–4 log range of increasing PFC responses before a plateau is reached, thereby making reliable slope calculations difficult. Nor have we been able to demonstrate an idiotypic or IgV_H-gene restriction in the effector function of Ly-1:HE generated in the T-Mø culture (19). When Cantor and colleagues (1) found that a combination of Ly-1:Qa-1^+ and Ly-1:Qa-1^- cells was more efficient in HE function than the Ly-1:Qa-1^- subset alone (2), we could not confirm this in our usual system (Fig. 1).

We were, however, able to demonstrate synergy using in vivo-primed Ly-1 cells (Fig. 2). This suggested to us that our failure to demonstrate synergy was not because of technical limitations of the induction or assay systems but because the T-Mø system in fact generates only one type of functional helper cell: an antigen-specific, MHC-restricted, Ly-1:Qa-1^-:IJ^- T cell (10–12, 16) that is clearly capable of substantial helper activity on its own (Fig. 1).

The inference that priming T cells in vitro in the absence of B cells may not favor the generation of a second, help-associated cell was prompted by observations made in several systems: the demonstration of Ly-1 help-associated cells that are Ig recognizing or at least functionally restricted to help for idiotype (20–28), allotype (29), and isotype (30), and particularly the observations by Janeway and colleagues (6, 7, 31) and Bottomly et al. (26), and more recently by Nutt et al. (8) and L'Age-Stehr (9), that B cells or their gene products are important determinants in the priming environment. Indeed, priming T cells in vitro in the presence of B cells either as whole spleen or as separated B cells does generate a second, Ly-1^+:Qa-1^+ population capable of augmenting the response stimulated by Ly-1:Qa-1^- cells (Fig. 3, Table I). Although these data favor the notion that the Ly-1:Qa-1^+ cell requires B cells for its induction, we have not ruled out the possibility that other cells are responsible. Comparable implication of B cells or Igh-gene products is provided by Nutt et al. (8) and L'Age-Stehr (9) as well as indirectly in other systems (6, 7, 26, 29, 31–36). As a group, Ig-recognizing, Ig-requiring, Ig-inducible, or Ig-restricted Ly-1 helper cells have been defined functionally. Our data assign a membrane phenotype, Qa-1, which discriminates a help-associated, B cell-requiring subset from the classic MHC-restricted, antigen-specific Ly-1 helper set in our system. We hope these data will provide a distinguishing marker in other systems as well, and this has in fact been suggested or inferred by others (31, 34) although, as far as we know, not formally demonstrated.

The Ly-1:Qa-1^+ subset is more appropriately termed "help-associated" rather than "HE" because, until we are able to independently assay this subset (by such means as positive selection and assay for HE function), we cannot distinguish whether it acts directly on B cells or by acting on Ly-1:Qa-1^- cells. This distinction is critical to understanding the recognition structure involved as well. We are only on the threshold of evaluating the Qa-1^+ help-associated cell in terms of antigen specificity, priming sequence and requirements, MHC and Igh gene restrictions, and idiotypic recognition. It is quite likely that our previous inability to demonstrate an Igh or idiotypic restriction in the function of Ly-1:HE was because our usual priming environment did not generate the Ly-1:Qa-1^+ help-associated cell. Although our system lends itself quite readily to the analysis of Igh and V_H gene restrictions using allotype-congenic strains (19), we have had some trouble accurately analyzing individual idiotypic responses. This difficulty, the problem with reproducibility of the Ly-1:Qa-1^+ gener-

ation using CRNPT (Table I), and the problem of positively selecting Ly-1:Qa-1$^+$ cells, should not be too difficult to resolve. If so, we will be in a position to make these evaluations.

We have, however, examined two other functional Ly-1 subsets which, like the Ly-1 help-associated cell described here, are Qa-1$^+$:IJ$^+$: the Ly-1 feedback suppression inducer (FBSI) and the Ly-1 subset that induces the generation of Ly-1:HE from Ly-123 precursors (10, 15, 18, 19, 37). These cells are distinguished functionally by the mode in which they are assayed, their requisite cell interactions, and their precursor and inducer requirements, but have not as yet been distinguished phenotypically (1, 10, 15, 16, 18, 19, 33–37). Whether these functional subsets involve the same or different populations capable of diverse inducer functions remains to be determined. The FBSI is antigen specific and V_H gene restricted in its activity (although both the antigen and Igh restrictions can be overcome by manipulation) (10, 18, 19, 33, 36). The FBSI can be induced by antigen-pulsed macrophages (10), aggregated immunoglobulin (18), antigen-antibody complexes (35), B cells or their products (18, 36), and B cell blasts (34). In the last case, the same induction protocol that generates FBSI will also induce Igh-restricted helper function if assayed in the absence of suppressor cell precursors (9, 34). The recognition of Ig-associated structures by Ly-1 cells may be highly specific for certain V_H structures (idiotypes). Although this has not been demonstrated using an individual idiotypic system in the FBSI or Qa-1$^+$ help-associated cell systems, there is certainly precedent for specialized Ly-1 helper T cells that either bear idiotype (20, 21, 25, 28, 38), are induced by anti-idiotype (20, 22, 28), bind to idiotype (23, 24, 27), or selectively activate B cells bearing idiotype (21–27). It will be essential to determine the relationship of Ig recognition, antigen recognition, and MHC recognition to each functional cell set, their interacting cell sets, and the ultimate target of their effects, the B cell. The question of whether one Ly-1:Qa-1$^+$ cell type can be multifunctional and whether the various functional Ly-1:Qa-1$^+$ cell sets are antigen, MHC, idiotype, or anti-idiotype specific will probably ultimately be answered using cloned cell lines. We are currently trying this approach as well.

Summary

The Qa-1 cell surface phenotype reportedly distinguishes two Ly-1 T cell subsets conjointly required for T helper effector activity. Ly-1 cells, obtained from several different priming regimens, were negatively selected with anti-Qa-1 plus complement and compared with unselected Ly-1 cells for helper cell activity. Priming isolated T cells on antigen-pulsed macrophages in the absence of B cells favors the generation of the Ly-1:Qa-1$^-$ subset, which is capable of efficient helper activity in the absence of the Ly-1:Qa-1$^+$ subset. Priming T cells in an environment containing B cells generates both Ly-1:Qa-1$^-$ helper effector cells and Ly-1:Qa-1$^+$ cells which contribute to the helper effect. Whether Ly-1:Qa-1$^+$ cells are capable of independent helper activity cannot be determined, and, as such, Ly-1:Qa-1$^+$ cells are more appropriately termed "help associated" rather than "helper effector." Our results assign a membrane phenotype, Qa-1, which distinguishes an Ly-1 help-associated B cell requiring subset in our system and may prove to be a general marker in a number of systems for Ly-1 inducer cell subsets which functionally require or recognize B cells or their products.

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References

1. Cantor, H., J. Hugenberger, L. McVay-Boudreau, D. D. Eardley, J. Kemp, F. W. Shen, and R. K. Gershon. 1978. Immunoregulatory circuits among T-cell sets. Identification of a subpopulation of T-helper cells that induces feedback inhibition. *J. Exp. Med.* **148:**871.
2. Stanton, T. H., C. E. Calkins, J. Jandinski, D. J. Shendel, O. Stutman, H. Cantor, and E. A. Boyse. 1978. The Qa-1 antigenic system. Relation of Qa-1 phenotypes to lymphocyte sets, mitogen responses, and immune functions. *J. Exp. Med.* **148:**963.
3. Tada, T., T. Takemori, 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.
4. Swierkosz, J. E., P. Marrack, and J. W. Kappler. 1979. Functional analysis of T cells expressing Ia antigens. I. Demonstration of helper T cell heterogeneity. *J. Exp. Med.* **150:** 1293.
5. Okada, M., T. H. Stanton, R. C. Kuppers, and C. S. Henney. 1981. The differentiation of cytotoxic T cells in vitro. IV. Interleukin-2 production in primary mixed lymphocyte cultures involves cooperation between Qa-1^+ and Qa-1^- "helper" T cells. *J. Immunol.* **126:** 1635.
6. Janeway, C. A., R. A. Murgita, F. I. Weinbaum, R. Asofsky, and H. Wigzell. 1977. Evidence for an immunoglobulin-dependent antigen-specific helper T cell. *Proc. Natl. Acad. Sci. U. S. A.* **74:**4582.
7. Janeway, C. A., D. L. Bert, and F. W. Shen. 1980. Cell cooperation during in vivo anti-hapten antibody responses. V. Two synergistic $Ly1^+23^-$ helper T cells with distinctive specificities. *Eur. J. Immunol.* **10:**231.
8. Nutt, N., J. Haber, and H. W. Wortis. 1981. Influence of Igh-linked gene products on the generation of T helper cells in the response to sheep erythrocytes. *J. Exp. Med.* **153:**1225.
9. L'Age-Stehr, J. 1981. Priming of T helper cells by antigen-activated B cells. B cell-primed Lyt-1^+ helper cells are restricted to cooperate with B cells expressing the Igh phenotype of the priming B cells. *J. Exp. Med.* **153:**1236.
10. McDougal, J. S., F. W. Shen, and P. Elster. 1979. Generation of T helper cells in vitro. V. Antigen-specific $Ly1^+$ T cells mediate the helper effect and induce feedback suppression. *J. Immunol.* **122:**437.
11. McDougal, J. S., and D. S. Gordon. 1977. Generation of T-helper cells in vitro. I. Cellular and antigen requirements. *J. Exp. Med.* **145:**676.
12. McDougal, J. S., and S. P. Cort. 1978. Generation of T helper cells in vitro. IV. F_1 T helper cells primed with antigen-pulsed parental macrophages are genetically restricted in their antigen specific helper activity. *J. Immunol.* **120:**445.
13. Shen, F. W., E. A. Boyse, and H. Cantor. 1975. Preparation and use of Ly antisera. *Immunogenetics.* **2:**591.
14. Stanton, T. H., and E. A. Boyse. 1976. A new serologically defined locus, Qa-1, in the Tla region of the mouse. *Immunogenetics.* **3:**525.
15. McDougal, J. S., F. W. Shen, S. P. Cort, and J. Bard. 1980. Feedback suppression: phenotypes of T cell subsets involved in the Ly1 T cell induced immunoregulatory circuit. *J. Immunol.* **125:**1157.
16. Shen, F. W., J. S. McDougal, J. Bard, and S. P. Cort. 1981. T cell development: precursors and inducer requirements of helper effector and feedback suppression inducer cell sets. *J. Exp. Med.* **154:**935.
17. Wysocki, L. J., and V. L. Sato. 1978. "Panning" for lymphocytes: a method of cell selection. *Proc. Natl. Acad. Sci. U. S. A.* **75:**2844.
18. Nicholson, J. K. A., and J. S. McDougal. 1981. Aggregated IgG-induced suppression of in vitro antibody responses: definition of the cellular target(s). *J. Immunol.* **126:**2382.
19. McDougal, J. S., J. K. A. Nicholson, S. P. Cort, F. W. Shen, and J. Bard. 1981.

Immunoglobulin-related induction, recognition, or restriction in the immunoregulatory function of in vitro generated Ly1 cells. *In* Immunoglobulin Idiotypes and Their Expression. C. Janeway, E. E. Sercarz, H. Wigzell, and C. Fox, editors. Academic Press, Inc., New York. 649.

20. Black, S. J., G. J. Hammerling, C. Berek, K. Rajewsky, and K. Eichmann. 1976. Idiotypic analysis of lymphocytes in vitro. I. Specificity and heterogeneity of B and T lymphocytes reactive with anti-idiotypic antibody. *J. Exp. Med.* **143:**846.
21. Hetzelberger, D., and K. Eichmann. 1978. Recognition of idiotypes in lymphocyte interactions. I. Idiotype selectivity in the cooperation between T and B lymphocytes. *Eur. J. Immunol.* **8:**846.
22. Bottomly, K., B. J. Mathieson, and D. E. Mosier. 1978. Anti-idiotypic induced regulation of help for the response to phosphorylcholine in adult BALB/c mice. *J. Exp. Med.* **148:**1216.
23. Adorini, L. M., M. Harvey, and E. E. Sercarz. 1979. The fine specificity of regulatory T cells. IV. Idiotypic complementarity and antigen bridging interactions in the anti-lysozyme response. *Eur. J. Immunol.* **9:**906.
24. Woodland, R., and H. Cantor. 1978. Idiotype-specific T helper cells are required to induce idiotype-positive B memory cells to secrete antibody. *Eur. J. Immunol.* **8:**600.
25. Benca, R. J., J. Quintans, J. F. Kearney, P. M. Flood, and H. Shreiber. 1980. Studies on phosphorylcholine-specific T cell idiotypes and idiotype specific immunity. *Mol. Immunol.* **17:**823.
26. Bottomly, K., C. A. Janeway, B. J. Mathieson, and D. E. Mosier. 1980. Absence of an antigen-specific helper T cell required for the expression of the T 15 idiotype in mice treated with anti-μ antibody. *Eur. J. Immunol.* **10:**159.
27. Gleason, K., S. Pierce, and H. Kohler. 1981. Generation of idiotype-specific T cell help through network perturbation. *J. Exp. Med.* **153:**924.
28. Miller, G. G., P. I. Nadler, Y. Asano, R. J. Hodes, and D. H. Sachs. 1981. Induction of idiotype-bearing, nuclease-specific helper T cells by in vivo treatment with anti-idiotype. *J. Exp. Med.* **154:**1981.
29. Herzenberg, L. A., K. Okumura, H. Cantor, V. L. Sato, F. W. Shen, E. A. Boyse, and L. A. Herzenberg. 1976. Demonstration of allotype-specific helper cells and their specific removal by suppressor T cells. *J. Exp. Med.* **144:**330.
30. Kishimoto, T., and K. Ishizaka. 1973. Regulation of antibody response in vitro. VI. Carrier-specific helper cells for IgG and IgE antibody response. *J. Immunol.* **111:**720.
31. Janeway, C. A., K. Bottomly, E. Dzierzak, D. Eardley, S. Drum, and R. K. Gershon. 1981. The development and functions of immunoglobulin dependent T cells. *J. Supramol. Struct. Cell Biochem.* **5s:**93.
32. Black, S. J., and L. A. Herzenberg. 1979. B-cell influences on the induction of allotype suppressor T cells. *J. Exp. Med.* **150:**174.
33. Eardley, D. D., F. W. Shen, H. Cantor, and R. K. Gershon. 1979. Genetic control of immunoregulatory circuits. Genes linked to the Ig locus govern communication between regulatory T-cell sets. *J. Exp. Med.* **150:**44.
34. L'Age-Stehr, J., H. Teichmann, R. K. Gershon, and H. Cantor. 1980. Stimulation of regulatory T cell circuits by immunoglobulin-dependent structures on activated B cells. *Eur. J. Immunol.* **10:**21.
35. Rao, V. S., J. A. Bennett, F. W. Shen, R. K. Gershon, and M. S. Mitchell. 1980. Antigen-antibody complexes generate Lyt1 inducers of suppressor cells. *J. Immunol.* **125:**63.
36. Zubler, R. H., B. Benacerraf, and R. N. Germain. 1980. Feedback suppression of the immune response in vitro. II. IgV_H-restricted antibody-dependent suppression. *J. Exp. Med.* **151:**681.
37. Shen, F. W., J. S. McDougal, J. Bard, and S. P. Cort. 1980. Developmental and communicative inter-relations of Ly123 and Lyl cell sets. *J. Exp. Med.* **151:**566.
38. Julius, M. H., H. Cosenza, and A. A. Augustin. 1978. Evidence for the endogenous production of T cell receptors bearing idiotypic determinants. *Eur. J. Immunol.* **8:**484.