T CELL REGULATION OF POLYCLONAL B CELL RESPONSIVENESS

III. Overt T Helper and Latent T Suppressor Activities from Distinct Subpopulations of Unstimulated Splenic T Cells*

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The ability of T lymphocytes to regulate the humoral immune response has been appreciated since Claman et al. (1) and Miller and Mitchell (2) described the absolute requirement for T helper cells in order for B cells to generate an antigen-specific response. Subsequently, modulation of the humoral response by T suppressor cells (3, 4) and T amplifier cells (5, 6) has been studied extensively. These regulatory cells have been characterized with respect to cell surface phenotype (7, 8) and physical characteristics (9); soluble factors have been obtained from both helper and suppressor cells that replace the function of T cells in the regulation of antigen-specific humoral responses (10, 11).

The regulatory effects of T lymphocytes on the nonspecific responses of B cells evoked by polyclonal B cell activators are much less thoroughly understood. Interest in these substances stems from the fact that the immune system of the body is frequently confronted with such activators in many infectious disease situations; moreover, polyclonal activation is thought to play a role in the pathogenesis of certain diseases of autoimmunity, such as systemic lupus erythymatosus. Although pokeweed mitogen is unable to evoke a polyclonal response from human B cells in the absence of T cells (12, 13), other mitogens such as protein A from *Staphylococcus aureus* stimulate polyclonal antibody production in the absence of T cells (14). An analogous situation pertains to murine splenic B lymphocytes, which are able to respond to most polyclonal stimulants in the total absence of T cells (15). Because of the ubiquitous nature of such stimulants and the unusually large proportion of B lymphocytes that respond to them (16), it appeared probable that this arm of the humoral immune system is subject to some type of regulatory influence.

Recent studies from this laboratory have demonstrated that addition of murine splenic T lymphocytes to B cell cultures results in marked enhancement of the polyclonal response to bacterial lipopolysaccharide (LPS)[1] (17, 18). The T cells

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[1] *Abbreviations used in this paper:* C, guinea pig complement; Con A, concanavalin A; LPS, bacterial lipopolysaccharide; NW, nylon wool; PFC, plaque-forming cells; PWM, pokeweed mitogen; RBC, erythrocytes; SRBC, sheep erythrocytes; TNP, trinitrophenyl.

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involved in this modulatory effect exert their activity early in the culture period, after which time their presence is no longer mandatory. The studies reported in this paper establish the susceptibility of B cells to negative or constraining influences as well as to enhancing signals. Suppressive activity is a latent attribute of a T cell subpopulation distinct from that responsible for mediating T cell helper effects. Separation and initial characterization of cell-free factors that exert helper or suppressor regulatory influence over the polyclonal B cell response are described and their roles as possible precursors to previously described antigen-specific and/or nonspecific regulatory T cell factors is discussed.

Materials and Methods

Mice. C3H/St and C3H/HeN male mice, 6–12 wk of age, were obtained from the mouse breeding facility at Scripps Clinic and Research Foundation, La Jolla, Calif. All mice were maintained as described elsewhere (17).

LPS. LPS 055:B5 was purchased from Difco Laboratories, Detroit, Mich. Concanavalin A (Con A) was obtained from Miles-Yeda, Ltd., Rehovot, Israel.

Lymphocyte Cultures. Preparation of spleen cell suspensions and constituents of the fetal calf serum-containing culture medium employed have been previously described (19). Single-cell suspensions of murine thymocytes were generated by screening the thymus through a steel mesh. Subsequent elimination of cell clumps and washing was identical to the procedure described elsewhere for spleen cells (20). For measurement of polyclonal B cell activation, lymphocytes were cultured (17) and cultures were fed daily (21) as described elsewhere. The direct plaque-forming cell (PFC) response to sheep erythrocytes (SRBC) and to 2,4,6-trinitrophenyl (TNP) was assayed with a modification of the hemolytic plaque assay of Jerne and Nordin (22). TNP-conjugated SRBC were prepared after the method of Kettman and Dutton (23). TNP-specific PFC were calculated by subtracting the response to SRBC from the TNP-SRBC response. All cultures were assayed at day 2 of culture (24).

Preparation of B Cell- and T Cell-enriched Populations. Spleen Cell populations enriched for T lymphocytes were prepared by passage over nylon wool (NW) columns (25). B lymphocyte-enriched populations were prepared by treating spleen cells with rabbit anti-mouse thymocyte serum followed by C3H erythrocyte (RBC)-absorbed guinea pig complement (C) (26). Anti-Lyt-1.1 and anti-Lyt-2.1 antisera were generously provided by Dr. F. Shen, Memorial Sloan-Kettering Cancer Center, New York. C3H/St spleen cells were treated at 30×10^6/ml with a 1:60 dilution of anti-Lyt-1.1 or with a 1:60 dilution of anti-Lyt-2.1 for 30 min at room temperature, followed by diluted C3H RBC-absorbed C. Viability was determined by trypan blue dye exclusion. Lysis of C3H splenic T cells by anti-Lyt-1.1 was 57%, and by anti-Lyt-2.1 was 27%.

Preparation of T Cell Sonicates. Splenic or thymic T cells were subjected to sonication in a 1-ml vol on ice for 2 min. The sonicate was diluted with medium to the desired cell-equivalent concentration, centrifuged at 30,000 *g* for 1 h, and the soluble fraction either added to culture directly or subjected to gel chromatography.

Velocity-Sedimentation Gradients. NW-purified splenic T cells were fractionated on velocity-sedimentation gradients by a modification of the technique of Tse and Dutton (9), using a 12.5–30% continuous Ficoll gradient. The gradient was removed in 2-ml fractions, which were pooled on the basis of cell number. Pool I contained fractions 1–4; pool II, fractions 5–8; pool III, fractions 9–11; and pool IV, fractions 12–14.

Gel Chromatography. T cell sonicates were chromatographed on a column of Sephadex G-200, 0.9 cm in diameter × 45 cm in height. The column was eluted with Dulbecco's phosphate-buffered saline at 10 ml/h; 1-ml fractions were collected.

Results

Augmentation of LPS-induced Polyclonal B Cell Activation by Supplemental T Cells. The capacity of T lymphocytes to alter the polyclonal response of B cells to LPS was

evaluated by coculture of B and T cell-enriched populations in varying proportions, in the absence or presence of optimal concentrations of LPS (100 μg/ml) (27); the direct PFC response to TNP was determined 48 h later. As the proportion of T cells increased, the magnitude of the B cell response also increased significantly (Fig. 1).

Suppression of the Polyclonal Response by Incremental Numbers of Splenic T cells. Incremental numbers of splenic T cells were added to cultures that contained 2.5×10^6 unseparated spleen cells in the presence or absence of LPS. Low numbers of supplemental T cells had no effect on polyclonal B cell activation (Fig. 2). However,

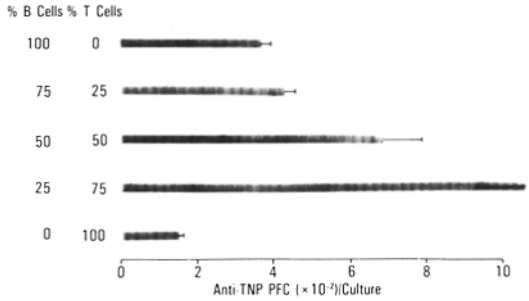

FIG. 1. Augmentation of LPS-induced polyclonal B cell activation by supplemental T cells. 5×10^6 viable C3H/St B cell-enriched and T cell-enriched lymphocytes, mixed in the proportions shown, were cultured in 1.0 ml of 5% fetal calf serum-containing medium in the presence or absence of 100 μg/ml LPS. The direct PFC response to TNP was assayed 48 h later. Results are expressed as the arithmetic mean of the difference of triplicate experimental and control cultures ± SE.

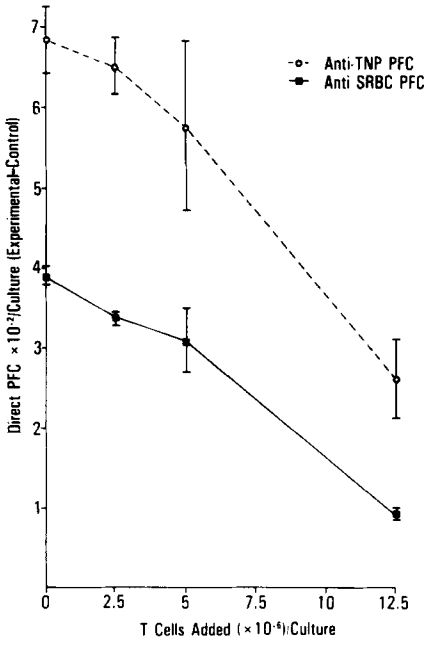

FIG. 2. Suppression of the polyclonal response by incremental numbers of splenic T cells. 2.5×10^6 viable C3H/St spleen cells were cultured in 1.0 ml of 5% fetal calf serum-containing medium in the presence of incremental numbers of viable, syngeneic splenic T cells either with or without 100 μg/ml LPS. The direct PFC response to SRBC was assayed 48 h later. Results are expressed as the arithmetic mean of the difference of triplicate experimental and control cultures ± SE.

when very high numbers of splenic T cells were used, significant suppression ensued. The ratio of T:B cells in these cases approached 10:1. Suppressed cultures, however, contained many more cells than did nonsuppressed cultures, and nonspecific factors such as cell crowding and exhaustion of nutrients in the medium may play a role in this phenomenon.

Effect of Con A-activated T cells on Polyclonal Activation of B Cells. Because of the crowded culture conditions in the experiments above, NW-purified splenic T cells were incubated with the T cell-specific lectin Con A to raise a population of nonspecific T suppressor cells (28, 29). Cells were harvested after 48 h and incremental numbers added to cultures that contained 2.5×10^6 splenic B cells. Significant suppression of the polyclonal B cell response ensued after addition of 1.25×10^6–2.5×10^6 T cells from Con A-activated cultures (Fig. 3). Thus, suppression can be evoked by Con A-activated T cells in numbers equal to the nonstimulated splenic T cells shown previously to generate optimal T cell helper function (17).

Lyt Phenotype of Cells Regulating the Polyclonal B Cell Response to LPS. C3H/St spleen cells were treated with either anti-Lyt-1.1 antiserum, anti-Lyt-2.1 antiserum, or both, followed by incubation with C; control cells were treated with C alone. The remaining cells, at a final concentration corresponding to 5×10^6 original cells/ml, were cultured in the presence or absence of LPS. Depletion of Lyt-1^+ cells from spleen cell populations resulted in a diminution of the polyclonal B cell response, usually by a factor of two or more (Table I). When both Lyt-1^+ and Lyt-2^+ cells were depleted from spleen cell populations, the response was again diminished. However, when only Lyt-2^+ cells were lysed, the resultant polyclonal response was not significantly altered from control levels. Thus, removal of an Lyt-1^+ helper T cell subpopulation lowers

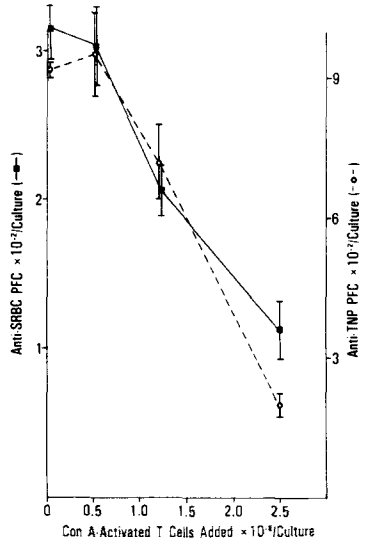

FIG. 3. The effect of Con A-activated T cells on polyclonal activation of B cells. 2.5×10^6 viable C3H/St B cells were cultured in 1.0 ml of 5% fetal calf serum-containing medium in the presence or absence of 100 μg/ml LPS. Incremental numbers of splenic T cells that had been activated for 48 h by exposure to 1 μg/ml Con A were added to these B cell cultures. T cells were washed extensively, first in medium that contained 0.1 M α-methyl mannoside, and then in medium that lacked it. The direct PFC responses to SRBC and TNP were assayed 48 h later. Results are expressed as the arithmetic mean of the difference of triplicate experimental and control cultures ± SE.

TABLE I

Lyt Phenotype of Cells Regulating the Polyclonal B Cell Response to LPS

Exp.	Spleen cell treatment*	Anti-SRBC PFC/culture‡	Anti-TNP PFC/culture‡
1	C	132 ± 20	496 ± 123
	Anti-Lyt-1 + Anti-Lyt-2 + C	ND§	ND
	Anti-Lyt-1 + C	77 ± 12	236 ± 25
	Anti-Lyt-2 + C	143 ± 6	453 ± 35
2	C	177 ± 9	663 ± 31
	Anti-Lyt-1 + Anti-Lyt-2 + C	47 ± 2	95 ± 30
	Anti-Lyt-1 + C	50 ± 14	12 ± 25
	Anti-Lyt-2 + C	183 ± 21	823 ± 25

* C3H/St spleen cells were treated as indicated, and the remaining cells, at a final concentration corresponding to 5×10^6 original cells/ml, were cultured in the presence or absence of 100 μg/ml 055:B5 LPS in a vol of 1.0 ml fetal calf serum-containing medium.

‡ The polyclonal response to LPS, assayed against SRBC and TNP after 2 d of culture, is presented as the difference of the arithmetic means of triplicate experimental and control cultures ± SE.

§ Not determined.

the polyclonal response to LPS, whereas removal of Lyt-2^+ cells fails to unmask any coexistent suppression.

Inability of T Cell-Culture Supernates to Regulate the Polyclonal B Cell Response to LPS. To learn if helper (or suppressor) activity was mediated by molecules secreted into the culture medium, T cells were cultured in the presence or absence of LPS for 24 h. Culture supernates were harvested and B cells cultured in them for 2 d. The supernate supported a normal polyclonal response to SRBC and TNP, but the magnitude of this response was not altered compared with control cultures (grown in medium incubated for 24 h without T cells) (data not shown). Thus, helper and suppressor effects do not appear to be mediated by soluble factors secreted into the culture supernate.

Regulation of the Polyclonal Response by Sonicates of Unactivated Splenic T Cells. Because regulation of the polyclonal B cell response was not found to be mediated by soluble factors secreted into the culture medium, it appeared likely that this phenomenon was attributable to cell-cell contact. Therefore, splenic T cells were separated into two parts, one left intact, and the other sonicated. Comparison of the effects of intact or the soluble fraction of sonicated T cells on the polyclonal response of B cell cultures to LPS (Fig. 4) indicated that whereas supplementation of these cultures with intact T cells provided help at a ratio of 1–3 T cells:B cell, the T cell sonicate provided T cell help at a ratio of 1 T cell:B cell; but at 3 T cells:B cell suppression was invariably observed.

In experiments in which the number of responding B cells was kept constant and the number of supplemental intact or sonicated T cells was varied, helper activity could be observed at ratios up to 2 sonicated T cell equivalents:B cell (data not shown). At ratios greater than this, however, suppression was again observed with sonicates (but not with intact T cells, which still provided help). Thus, sonication appears to reveal the presence of latent suppressor activity not manifested in intact, unstimulated splenic T cells. To determine if the suppressor activity was a result of toxicity, B cells were cocultured with incremental amounts of T cell sonicate with

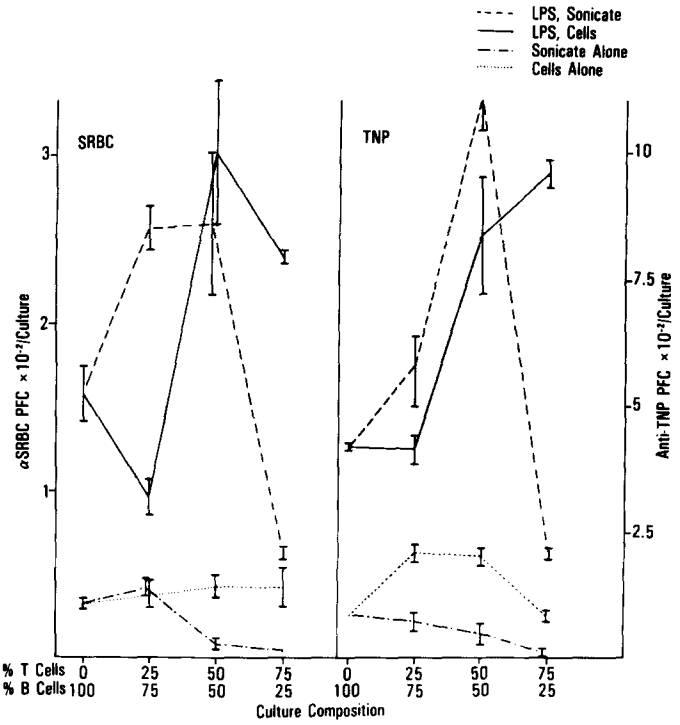

FIG. 4. Regulation of the polyclonal response by the sonicated fraction of unactivated splenic T cells. C3H/St B cell-enriched populations were mixed in varying proportions with syngeneic T cell-enriched populations (either as intact cells or as the soluble fraction of T cell sonicates) and 5×10^6 of the mixed cells (or cells plus cell equivalents) were cultured in 1.0 ml of 5% fetal calf serum-containing medium in the presence or absence of 100 μg/ml LPS. The direct PFC responses to SRBC (left panel) and TNP (right panel) were assayed 48 h later. Results are expressed as the arithmetic mean of triplicate cultures ± SE.

LPS, and B cell viability evaluated after 2 d of culture (data not shown). The results indicated that no loss of viability occurs.

Residence of Helper and Suppressor Activities in T Cell Subpopulations Bearing Distinct Lyt Surface Phenotypes. To determine whether the suppression described above was attributable to excess help or represented an activity distinct from help, splenic T cells were depleted either of Lyt-2.1- or Lyt-1.1-bearing cells. The remaining cells were either left intact or used for the preparation of T cell sonicates. Addition of Lyt-1^+23^- T cells to B cell cultures enhanced the polyclonal B cell response (Table II). This was true whether the cells were intact or sonicated. However, when Lyt-1^-23^+ splenic T cells were added to B cell cultures, no help was observed with either intact or sonicated preparations. Furthermore, although intact cells failed to suppress the polyclonal response significantly, addition of an equal number of sonicated T cell equivalents markedly suppressed the response.

Regulation of the Polyclonal B Cell Response by Intact and Sonicated Preparations of Physically Distinct Splenic T Cells. Splenic T cells were fractionated on a Ficoll velocity-sedimentation gradient and were pooled as described in Materials and Methods. Microscopic examination revealed that pool I contained predominantly small lymphocytes, whereas pool IV was composed primarily of large lymphocytes. Cells in pools II and

TABLE II

Effect of Whole and Sonicated Lyt-1$^+$23$^-$ and Lyt-1$^-$23$^+$ T Cells on Polyclonal B Cell Responses

B cells*	T cells‡			Anti-SRBC PFC/ culture§
	Number	Lyt phenotype	Condition	
2.5×10^6	0	—	—	140 ± 4
2.5×10^6	2.5×10^6	Lyt-1$^+$23$^-$	Intact	290 ± 16
2.5×10^6	2.5×10^6	Lyt-1$^+$23$^-$	Sonicated	290 ± 28
2.5×10^6	2.5×10^6	Lyt-1$^-$23$^+$	Intact	185 ± 34
2.5×10^6	2.5×10^6	Lyt-1$^-$23$^+$	Sonicated	44 ± 14

* 2.5×10^6 viable C3H/St B cells were cultured in 1.0 ml of fetal calf serum-containing medium in the presence or absence of 100 μg/ml 055:B5 LPS.

‡ C3H/St T cells were treated with anti-Lyt sera and C as indicated, and 2.5×10^6 resultant cells added to culture either intact or as the soluble fraction of the T cell sonicate.

§ The polyclonal response to LPS, assayed against SRBC after 2 d of culture, is presented as the difference of the arithmetic means of triplicate experimental and control cultures ± SE.

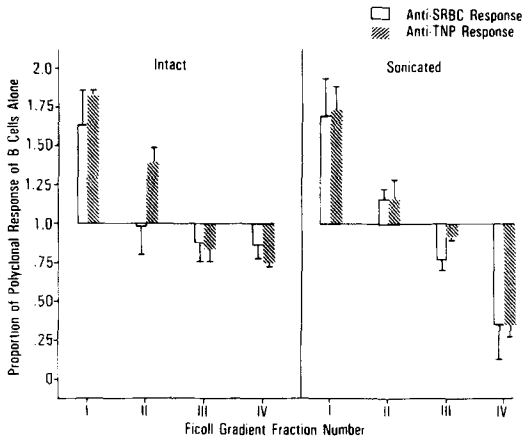

FIG. 5. Regulation of the polyclonal B cell response by intact and sonicated preparations of physically distinct splenic T cells. C3H/St splenic T cells were fractionated on a Ficoll gradient and pooled as described in Materials and Methods. 2.5×10^6 of these cells (either as intact cells or as the soluble fraction of cell sonicates) were added to 2.5×10^6 syngeneic splenic B cells in the presence or absence of 100 μg/ml LPS. The direct PFC response to SRBC (open bars) and TNP (hatched bars) was assayed 48 h later. Results are expressed as the proportion of the polyclonal response of B cells alone mounted by triplicate experimental cultures ± SE.

III were intermediate in size. All pools were assayed for their effects on the polyclonal response (Fig. 5). T cell-helper effects were most marked in the slowly sedimenting pool of T cells (pool I) and its sonicates. Helper activity in pool II was less marked and somewhat variable. Suppressor activity was generally of low magnitude and of marginal significance in intact cells from pools III and IV; when the cells from pool IV were sonicated, however, significant suppression of the polyclonal response was consistently observed. Thus, T cells mediating helper activity can be separated from those responsible for suppressive effects both by physical means and by cell surface phenotypes.

Inability of Intact and Sonicated Thymocytes to Suppress the Polyclonal Response. Helper activity for polyclonal B cell responses can be rendered by thymocytes as well as

splenic T cells (30). To determine whether thymocytes were also capable of exercising suppressive influences, incremental numbers of intact or sonicated thymocytes were added to B cell cultures (Fig. 6). Whether intact or sonicated, thymocytes (even in large excess) served only to enhance the polyclonal B cell response to LPS. The suppressive effect of 10×10^6 sonicated splenic T cell equivalents per culture is shown at lower right (Fig. 6). It therefore appears that although the thymus contains T cell helper activity, it is devoid of T cell suppressor potential.

Separation of Helper and Suppressor Activities from Splenic T Cell Sonicates. Physical separation of helper and suppressor factors present in splenic T cell sonicates was undertaken by subjecting these sonicates to gel chromatography on a column of Sephadex G-200. Splenic B cells in the presence of LPS were supplemented with 5×10^6–10×10^6 cell equivalents of each fraction. The suppressor activity eluted far ahead of the helper activity (Fig. 7). Molecular weight estimates based on three similar elution profiles ranged from 68,000–84,000 for the suppressive factor, and 15,000–23,000 for the helper factor. No relationship of activity to the protein elution profile was apparent.

Discussion

Polyclonal responsiveness of murine splenic B cells to nonspecific activators typified by LPS is a phenomenon regulable by the action of T cells. In the normal spleen, this regulation appears to be biased in the direction of T helper activity without significant constraint imposed by suppressor cells. However, splenic T lymphocytes possess latent suppressor capacity that can be evoked experimentally.

The helper activity of T cells for polyclonal activation is optimal when T cells are present in modest excess relative to B cells. We have previously found that this activity

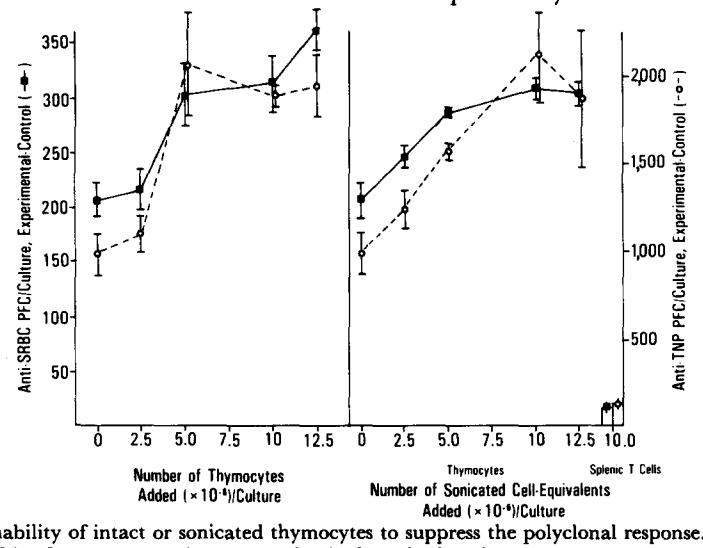

FIG. 6. Inability of intact or sonicated thymocytes to suppress the polyclonal response. 2.5×10^6 viable C3H/St B cells were cultured in 1.0 ml of 5% fetal calf serum-containing medium in the presence of incremental numbers of viable, syngeneic thymocytes, either as intact cells or as the soluble fraction of cell sonicates, in the presence or absence of 100 μg/ml LPS. The direct PFC responses to SRBC (solid line) and TNP (dashed line) were assayed 48 h later. Results are expressed as the arithmetic means of the difference of triplicate experimental and control cultures ± SE. For purposes of comparison, the results obtained by adding 10×10^6 sonicated splenic T cell equivalents to these B cell cultures is presented in the lower right-hand corner.

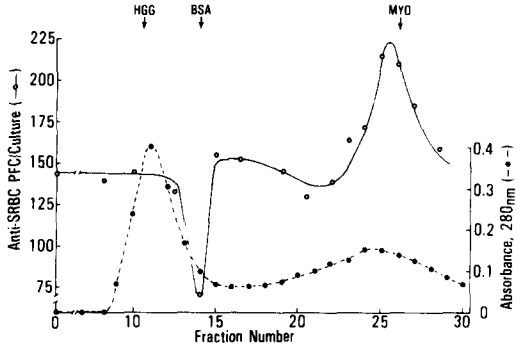

FIG. 7. Separation of helper and suppressor activities from splenic T cell sonicates. Splenic T cell sonicates were chromatographed over a column of Sephadex G-200 as described in Materials and Methods. 2.5×10^6 viable C3H/St splenic B cells in the presence of 100 μg/ml 055:B5 LPS were supplemented with 50 μl of the column fraction to be assayed (5×10^6–10×10^6 T cell equivalents). The direct PFC response to SRBC (solid line) was assayed 48 h later. Results are expressed as the arithmetic mean of triplicate cultures from three separate experiments. The molecular weight markers human gamma globulin (HGG), bovine serum albumin (BSA) and myoglobin (MYO) are indicated by the arrows at the top of the Figure.

may be removed by treatment with an anti-thymocyte serum and complement (17). Theofilopoulos et al. (31) have made parallel observations regarding the effect of T cells on polyclonal responses of normal and autoimmune murine strains. Moreover, the response of human PBL to *S. aureus* is also enhanced by addition of T cells to culture (14).

Addition of large numbers of splenic T cells to cultures of C3H spleen cells (at ~10:1, T:B ratio) depressed their ability to mount a polyclonal response to LPS. This effect has been observed using pokeweed mitogen (PWM) in the human system by Janossy et al. (12). Under these crowded cell culture conditions, overcrowing, exhaustion of nutrients, and cell death may play a part in the suppression observed. This possibility is made less likely, however, by the failure to observe similar suppression when large numbers of thymocytes were added to B cell cultures.

The polyclonal response is subject to suppressive regulation under nonlimiting cell culture conditions. Con A-activated T cells suppress the polyclonal B cell response, whereas equal numbers of nonactivated T cells provide help. These experiments also demonstrate that the B cell itself is subject to suppression in the absence of T helper cells, in accord with data presented by Primi et al. (32) but at variance with that Nespoli et al. (33). Reasons for the discrepant results of the latter group are not clear.

Normal splenic T cells exhibit little or no suppressor activity under nonactivating circumstances. That removal of helper cells with the anti-Lyt-1.1 antiserum failed to unmask any latent constraining effects implies that although such effects can be demonstrated, as in the Con A experiments, suppressive activity must be evoked in order to be observed. In contrast, the human peripheral blood B cell response to specific antigen normally is subject to T cell restraint (34).

Investigation of the means by which T cell regulatory signals are communicated to B cells revealed first that neither helper nor suppressor influences are transmitted by soluble factors secreted into the culture medium. Second, sonicates of normal, unactivated splenic T cells are capable of transmitting not only helper T cell signals to B cells undergoing a polyclonal response, but also suppressive regulatory influences.

This was most dramatically illustrated in experiments in which the effects of whole and sonicated splenic T cells were compared directly. Optimal T cell helper effects were seen using intact cells at ratios of 1–3 T cells per splenic B cell. When B cells were supplemented with concentrations of sonicated splenic T cells equivalent to the number of intact T cells used, parallel helper effects could be observed at ratios of 1 T cell:B cell; at a ratio of 3 T cells:B cell, however, potent suppressor effects supervened. From these experiments it appears that T cell signals regulating polyclonal B cell activation are transmitted either by direct cell to cell contact or by secretion of factors at very close proximity to the B cell (perhaps implying rapid inactivation after secretion). Helper influences are exerted by intact T cells as well as by T cell sonicates. Suppressor activity, however, is latent and must be unmasked (as by sonication) to enable one to observe its effects. The suppressor effects of T cell sonicates cannot be attributed to adverse effects on B cell viability, because sonicates fail to alter viability at either T-helper or T-suppressor concentrations.

The existence of both helper and suppressor activities in T cell sonicates is compatible with mediation of both regulatory activities either by a single molecule in different quantities or by two distinct molecular species. Elimination of Lyt-1^+ or Lyt-2^+ cells from splenic T cell populations demonstrated that help and suppression are mediated by distinct subpopulations of T cells: Lyt-1^+ T cells, intact or sonicated, mediated T-helper activity; intact Lyt-2^+ cells had no modulatory effect on the polyclonal response to LPS but caused potent suppression after sonication.

The helper and suppressor activities of splenic T cell sonicates can be further distinguished by physical characterization of their cells of origin. A subpopulation of slowly sedimenting splenic T cells was shown to mediate T cell helper effects whether the cells were intact or sonicated, whereas rapidly sedimenting T cells revealed their suppressive capacity only when sonicated. These observations are in accord with those of Tse and Dutton (9), who distinguished regulatory T cell activities for the primary antibody response to SRBC in vitro by such velocity-sedimentation gradients. Moreover, the same authors found that the cells exerting helper and suppressor activities for this antigen-specific response are Lyt-1^+ and Lyt-2^+, respectively (8).

Like splenic T cells, the thymus contains T cells with helper activity for polyclonal responses. In contrast, however, thymic T cells do not contain latent suppressor activity. This observation is in concordance with the reported absence of Lyt-23^+ T cells in the thymus (35). The implication of these observations is that Lyt-123^+ cells leave the thymus and subsequently lose the Lyt-1 cell surface antigen and develop a latent capacity to exert nonspecific suppressive activity. In similar observations, Rocklin et al. (36) described a histamine-induced suppressor factor present in spleen and lymph node T cells, but not in thymus. Additionally, Pahwa et al. (37) found that even after exposure to Con A neither human thymocytes nor their supernates were able to suppress the response of human peripheral blood lymphocytes to PWM.

The helper and suppressor activities of splenic T cell sonicates appear to be distinct molecular moieties separable by gel chromatography. It is reasonable to suspect that these factors, isolated from unstimulated splenic T lymphocytes, may well prove to be either precursors to or structural components of other T cell regulatory factors described in the literature. As factor precursors, they may be subject to enzymatic cleavage before generation of antigen-nonspecific regulatory activity. However, if they are components of other factors they may be involved in the generation of antigen-

specific as well as nonspecific T cell regulatory factors. More extensive physiocochemical and immunological characterization of these T cell regulatory activities will be necessary to establish their precise relationship to previously described factors.

Summary

Polyclonal activation of murine splenic B lymphocytes by lipopolysaccharide was found to be subject to regulation by helper and suppressor influences from T lymphocytes. In the normal adult spleen, only helper influences were exercised over polyclonal B cell activation; this influence is a property of Lyt-1^+23^- slowly sedimenting T cells. Suppressive influence evidently is latent, for it exists at such a low level (or the cells are so few in number) that its effects are difficult to detect. Suppressor T cell function may be evoked by culturing spleen cells at high ratios of T:B cells, by activating splenic T cells with concanavalin A, or by sonicating unstimulated splenic T cells to liberate a suppressive potential that is not expressed by these unstimulated cells when intact. The soluble fraction of resident splenic T cell sonicates exerts both helper and suppressor regulatory influences. The soluble helper activity is derived from Lyt-1^+23^- slowly sedimenting T cells, whereas suppressor activity is generated from a distinct subpopulation of Lyt-1^-23^+ rapidly sedimenting T cells. The thymus contains cells capable only of helping but not of suppressing polyclonal activation of splenic B cells. Helper and suppressor activities contained in splenic T cell sonicates were separated by gel chromatography; the suppressive activity was found to elute with a molecular weight between 68,000 and 84,000 and the helper activity eluted with a molecular weight between 15,000 and 23,000. The data indicate that helper and suppressor activities are distinct molecular entities derived from distinct splenic T lymphocyte subpopulations. The possibility that these molecules are precursors to or components of antigen-specific or nonspecific helper and suppressor factors described in the literature is discussed.

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References

1. Claman, H. N., E. A. Chaperon, and R. F. Triplet. 1966. Thymus-marrow cell combinations. Synergism in antibody production. *Proc. Soc. Exp. Biol. Med.* **122:**1167.
2. Miller, J. F. A. P., and G. S. Mitchell. 1967. The thymus and the precursors of antigen reactive cells. *Nature (Lond.).* **216:**659.
3. Gershon, R. K., and K. Kondo. 1971. Infectious immunological tolerance. *Immunology.* **21:** 903.
4. Gershon, R. K. 1974. T cell control of antibody production. *Contemp. Top. Immunobiol.* **3:**1.
5. 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.
6. Muirhead, D. Y., and G. Cudkowicz. 1978. Subpopulations of splenic T cells regulating an anti-hapten antibody response. II. Distinct functions of, and sequential requirement for, helper and amplifier cells. *J. Immunol.* **121:**130.

7. Cantor, H., and E. A. Boyse. 1977. Lymphocytes as the models for the study of the mammalian cellular differentiation. *Immunol. Rev.* **33:**105.
8. Tse, H. Y., and R. W. Dutton. 1977. Separation of helper and suppressor T lymphocytes. II. Ly phenotypes and lack of DNA synthesis requirement for the generation of concanavalin A helper and suppressor cells. *J. Exp. Med.* **146:**747.
9. Tse, H., and R. W. Dutton. 1976. Separation of helper and suppressor T lymphocytes on a Ficoll velocity sedimentation gradient. *J. Exp. Med.* **143:**1199.
10. Taussig, M. J. 1974. T cell factor which can replace T cells *in vivo*. *Nature (Lond.).* **248:**234.
11. Takemori, T., and T. Tada. 1975. Properties of antigen-specific suppressive T-cell factor in the regulation of antibody response of the mouse. I. In vivo activity and immunochemical characterizations. *J. Exp. Med.* **142:**1241.
12. Janossy, G., E. Gomez de la Concha, A. Luquetti, M. J. Snajdr, M. J. Waxdal, and T. A. E. Platts-Mills. 1977. T cell regulation of immunoglobulin synthesis and proliferation in pokeweed (Pa-1)-stimulated human lymphocyte cultures. *Scand. J. Immunol.* **6:**109.
13. Insel, R. A., and E. Merler. 1977. The necessity for T cell help and for human tonsil B cell responses to pokeweed mitogen: induction of DNA synthesis, immunoglobulin and specific antibody production with a T cell helper factor produced with pokeweed mitogen. *J. Immunol.* **118:**2009.
14. Ringden, O., and B. Rynnel-Dagoo. 1978. Activation of human B and T lymphocytes by protein A of *Staphylococcus aureus*. *Eur. J. Immunol.* **8:**47.
15. Andersson, J., O. Sjoberg, and G. Möller. 1972. Induction of immunoglobulin and antibody synthesis *in vitro* by lipopolysaccharides. *Eur. J. Immunol.* **2:**349.
16. Andersson, J., A. Coutinho, W. Lernhardt, and F. Melchers. 1977. Clonal growth and maturation to immunoglobulin secretion *in vitro* of every growth-inducible B lymphocyte. *Cell.* **10:**27.
17. Goodman, M. G., and W. O. Weigle. 1979. T cell regulation of polyclonal B cell responsiveness. I. Helper effects of T cells. *J. Immunol.* **122:**2548.
18. Goodman, M. G., and W. O. Weigle. Regulation of the immune response. *In* Handbook of Cancer Immunology. Vol. 7. H. Waters, editor, Garland Publishing, Inc., New York. In press.
19. Goodman, M. G., and W. O. Weigle. 1977. Nonspecific activation of murine lymphocytes. I. Proliferation and polyclonal activation induced by 2-mercaptoethanol and αthioglycerol. *J. Exp. Med.* **145:**473.
20. Goodman, M. G., J. M. Fidler, and W. O. Weigle. 1978. Nonspecific activation of murine lymphocytes. IV. Proliferation of a distinct, late maturing lymphocyte subpopulation induced by 2-mercaptoethanol. *J. Immunol.* **121:**1905.
21. Mishell, R. I., and R. W. Dutton. 1967. Immunization of dissociated spleen cell cultures from normal mice. *J. Exp. Med.* **126:**423.
22. Jerne, N. K., and A. A. Nordin. 1963. Plaque formation in agar by single antibody producing cells. *Science (Wash. D. C.).* **140:**405.
23. Kettman, J., and R. W. Dutton. 1970. An *in vitro* primary immune response to 2,4,6-trinitrophenyl substituted erythrocytes: response against carrier and hapten. *J. Immunol.* **104:**1558.
24. Coutinho, A., E. Gronowicz, W. W. Bullock, and G. Möller. 1974. Mechanism of thymus-independent immunocyte triggering. Mitogenic activation of B cells results in specific immune responses. *J. Exp. Med.* **139:**74.
25. Julius, M., E. Simpson, and L. A. Herzenberg. 1973. A rapid method for the isolation of functional thymus-derived murine lymphocytes. *Eur. J. Immunol.* **3:**645.
26. Doyle, M. V., D. E. Parks, and W. O. Weigle. 1976. Specific, transient suppression of the immune response by HGG tolerant spleen cells. II. Effector cells and target cells. *J. Immunol.* **117:**1152.

27. Goodman, M. G., D. E. Parks, and W. O. Weigle. 1978. Immunologic responsiveness of the C3H/HeJ mouse: differential ability of butanol-extracted lipopolysaccharide (LPS) to evoke LPS-mediated effects. *J. Exp. Med.* **147**:800.
28. Rich, R. R., and C. W. Pierce. 1974. Biological expressions of lymphocyte activation. III. Suppression of plaque-forming cell responses *in vitro* by supernatant fluids from concanavalin A activated spleen cell cultures. *J. Immunol.* **112**:1360.
29. Dutton, R. W. 1975. Suppressor T cells. *Transplant. Rev.* **26**:39.
30. Goodman, M. G., and W. O. Weigle. 1980. Inability of T cells from mice with the CBA/N B-cell defect to amplify polyclonal B-cell responses to bacterial lipopolysaccharide. *Clin. Immunol. Immunopathol.* **15**:375.
31. Theofilopoulos, A. N., D. L. Shawler, R. A. Eisenberg, and F. J. Dixon. 1980. Splenic immunoglobulin-secreting cells and their regulation in autoimmune mice. *J. Exp. Med.* **151**: 446.
32. Primi, D., L. Hammarstrom, and C. I. E. Smith. 1979. Regulation of thymus-independent responses by concanavalin A-activated spleen cells. *Cell. Immunol.* **42**:90.
33. Nespoli, L., G. Möller, D. Waterfield, and R. Ekstedt. 1977. Experimental conditions for obtaining suppressor and helper effects on the primary in vitro immune response by lymphocytes activated by polyclonal T-cell activators. *J. Exp. Med.* **145**:631.
34. Dosch, H.-N., and E. W. Gelfand. 1979. Specific *in vitro* IgM responses of human B cells: a complex regulatory network modulated by antigen. *Immunol. Rev.* **45**:243.
35. Cantor, H., and E. A. Boyse. 1975. Functional subclasses of T lymphocytes bearing different Ly antigens. I. The generation of functionally distinct T-cell subclasses as a differentiative process independent of antigen. *J. Exp. Med.* **141**:1376.
36. Rocklin, R. E., D. K. Greineder, and K. L. Melmon. 1979. Histamine-induced suppressor factor (HFS): further studies on the nature of the stimulus and the cell which produces it. *Cell. Immunol.* **44**:604.
37. Pahwa, S., L. Shou, R. Pahwa, E. Smithwick, and R. A. Good. 1979. Influence of human thymocytes on B lymphocyte differentiation in man. *Cell. Immunol.* **44**:433.