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The Role of Regulatory Components From Resident T Lymphocytes in Polyclonal B Cell Activation

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Resident T lymphocytes have been found to exert helper and suppressor regulatory influences with regard to polyclonal activation of murine splenic B lymphocytes elicited by lipopolysaccharide. In the normal adult spleen, only T cell helper influences are exercised over polyclonal B cell activation. This activity is a property of Lyt 1^+2^- T cells and does not appear to be subject to MHC restriction. Suppressive influence evidently is either latent or it exists at such a low level that its effects are difficult to detect. No regulatory activity can be recovered from the supernatants of T cells, cultured either with or without LPS. However, suppressor T cell function may be evoked by activating splenic T cells with Concanavalin A or by sonicating unstimulated splenic T cells in order to liberate a suppressive potential which 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^+2^- T cells, whereas suppressor activity is generated from Lyt 1^-2^+ T cells. The suppressive activity of T cell sonicates is not restricted by the MHC gene complex. 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 daltons, and the helper activity eluted with a molecular weight between 15,000 and 23,000 daltons. 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.

Key words: polyclonal B cell activation, suppression, T cells, regulation, lipopolysaccharide

Lymphocyte activation has been studied extensively in vitro by the use of polyclonal stimulants (mitogens), constituting a widely adopted model for the investigation of antigen-initiated lymphocyte proliferation and maturation. Moreover, the immune system is frequently confronted with polyclonal activators of microbial

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origin in many infectious disease situations; these activators are thought to play a role in the pathogenesis of certain diseases of autoimmunity, such as systemic lupus erythematosus. Because of the ubiquitous nature of such stimulants and the unusually large proportion of cells that respond to them, regulation of this arm of the humoral immune system is of considerable importance.

The ability of thymus-derived (T) lymphocytes to regulate the humoral immune response to specific antigen 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 which 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. 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 [12,13]. The T cells 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 the present communication 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 which 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 are discussed.

MATERIALS AND METHODS

Mice

C3H/St, C3H/HeN, C57BL/6J, and Balb/c male mice, 6–12 weeks of age, were obtained from the mouse breeding facility at Scripps Clinic and Research Foundation, La Jolla, CA. CBA/CaJ and SJL mice were purchased from the Jackson Laboratory, Bar Harbor, ME. All mice were maintained as described [14].

LPS

Bacterial lipopolysaccharide (LPS) 055:B5 was purchased from Difco Laboratories, Detroit, MI. 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 [15]. For measurement of polyclonal B cell activation, lymphocytes were incubated in plastic culture trays (No. 3008, Falcon Plastics, Oxnard, CA) at a cell density of 5×10^6

viable spleen cells/ml in 1.0 ml. Culture trays were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. Cultures were fed daily with 40 μl of nutritional cocktail [16]. The direct (PFC) response to SRBC or to 2,4,6-trinitrophenyl (TNP) was assayed with a modification of the hemolytic plaque assay of Jerne and Nordin [17]. TNP-conjugated SRBC were prepared after the method of Kettman and Dutton [18]. 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 [19]. Results are expressed as the arithmetic mean of triplicate cultures ± the standard error.

Preparation of B and T Cell-Enriched Populations

Spleen cell populations enriched for T lymphocytes were prepared by passage over nylon wool (NW) columns [20]. B lymphocyte-enriched populations were prepared by treating spleen cells with rabbit anti-mouse thymocyte serum (ATS) followed by C3H RBC-absorbed guinea pig complement (C) [21]. Anti-Lyt 1.1 and anti-Lyt 2.1 antisera were generously provided by Dr. F. Shen, Memorial Sloan-Kettering Cancer Center, New York, NY. 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 minutes at room temperature, followed by diluted C3H RBC-absorbed guinea pig complement. 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 l-ml volume on ice for 2 min. The sonicate was diluted with medium to the desired cell-equivalent concentration, centrifuged at 30,000g for 1 hr, and the soluble fraction either added to culture directly or subjected to gel chromatography.

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 PBS at 10 ml/hr; 1 ml fractions were collected.

RESULTS

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 complement; control cells were treated with complement 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 2 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 the polyclonal response to LPS, whereas removal of Lyt 2^+ cells fails to unmask any coexistent suppression.

TABLE I. Effect of Anti-Lyt Treatment on T Cells Regulating the Polyclonal B Cell Response to LPS

Treatment[a]	% of C′ control PFC response[b]	
	SRBC	TNP
C′	100	100
αLyt 1 + C′	28	25
αLyt 2 + C′	103	108
αLyt 1 + αLyt 2 + C′	27	14

[a]C3H/St spleen cells were treated as shown and the remaining cells, at a final concentration corresponding to 5×10^6 original cells/ml, were cultured in 1.0 ml of 5% FCS-containing medium in the presence or absence of 100 μg/ml LPS.

[b]The polyclonal response to LPS, assessed against SRBC and TNP after 2 days of culture, is presented as a percent of the C′-treated cultures after subtraction of the background response (no LPS).

Ability of Histoincompatible Thymocytes to Provide Help for Polyclonal B Cell Activation

CBA/CaJ B cells were cultured without supplemental T cells or with thymocytes derived from strains with a variety of H-2 phenotypes. The ability of thymocytes to provide help comparable to splenic T cells has been demonstrated previously with regard to polyclonal responses [22]. Histocompatible (CBA/CaJ) H-2^k thymocytes very significantly augmented the B cell response to LPS. However, comparable help was provided by cells from C57BL/6J (H-2^b), SJL (H-2^s), and Balb/c (H-2^d) mice (Table II). The possibility that the help observed can be accounted for by a positive allogeneic effect is rendered unlikely by the length of culture (48 hours) and by the failure of the background to be elevated over the totally syngeneic situation. Moreover, whether the helper cell is derived from mice with Igh-1^a or Igh-1^b does not appear relevant to helper capacity.

Effect of Concanavalin A-Activated T Cells on Polyclonal Activation of B Cells

NW T cells were incubated with the T cell specific lectin Con A to raise a population of nonspecific T suppressor cells [23,24]. Cells were harvested after 48 hr and incremental numbers added to cultures containing 2.5×10^6 splenic B cells. Significant suppression of the polyclonal B cell response ensued after addition of 1.25 to 2.5×10^6 T cells from Con A-activated cultures (Table III). 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 [12].

Inability of T Cell Culture Supernatants 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 hr. Culture supernatants were harvested and B cells cultured in them for 2 days. The supernatant supported a normal polyclonal response to SRBC and TNP, but the magnitude of this response was not altered compared to control cultures (grown in medium incubated for 24 hr without T cells) (Table IV). Thus, helper and suppressor effects do not appear to be mediated by soluble factors secreted into the culture supernatant.

TABLE II. Ability of Con A-Activated T Cells to Suppress the Polyclonal B Cell Response to LPS

CBA/CaJ B cells[a]	Thymocytes	H-2	Igh-1	Anti-TNP PFC/culture −LPS	Anti-TNP PFC/culture +LPS
+	−			5 ± 3	215 ± 18
+	CBA/CaJ	k	a	15 ± 5	1445 ± 30
+	C57BL/6J	b	b	13 ± 3	1357 ± 68
+	SJL	s	b	15 ± 6	1400 ± 120
+	BALB/c	d	a	5 ± 5	1163 ± 73

[a]2.5 × 10^6 viable CBA/CaJ B cells were cultured with 2.5 × 10^6 viable thymocytes, derived from the strains indicated, in the presence or absence of 100 μg/ml LPS.

TABLE III. Ability of Con A-Activated T Cells to Suppress the Polyclonal B Cell Responses to LPS

Number of Con A-activated T cells (× 10^{-6})/culture[a]	Anti-SRBC PFC/culture[b] −LPS	Anti-SRBC PFC/culture[b] +LPS	Anti-TNP PFC/culture[b] −LPS	Anti-TNP PFC/culture[b] +LPS
None	23 ± 7	338 ± 22	135 ± 18	1053 ± 19
0.5	45 ± 15	348 ± 30	160 ± 50	1110 ± 90
1.25	25 ± 8	232 ± 19	147 ± 22	868 ± 81
2.50	23 ± 8	135 ± 20	120 ± 10	317 ± 26

[a]2.5 × 10^6 viable C3H/St B cells were cultured in 1.0 ml of 5% FCS-containing medium in the presence or absence of 100 μg/ml LPS. Incremental numbers of splenic T cells, activated with 1 μg/ml Con A for 48 hr, were added to culture.
[b]The direct PFC responses to SRBC or TNP were assessed 48 hr later. Results are expressed as the arithmetic mean of triplicate cultures ± the SE.

TABLE IV. Inability of T Cell Culture Supernatants to Regulate the Polyclonal B Cell Response to LPS

Supernatant[a] T cells	Supernatant[a] LPS	Anti-SRBC PFC/culture[b]	Anti-TNP PFC/culture[b]
−	−	29 ± 14	157 ± 104
−	+	132 ± 11	1062 ± 190
+	−	25 ± 9	159 ± 81
+	+	108 ± 20	825 ± 198

[a]15 × 10^6 viable C3H/St T cells were cultured in 3.0 ml of FCS-containing medium in the presence or absence of 100 μg/ml 055:B5 LPS. The culture supernatant was harvested after 24 hr and 2.5 × 10^6 viable C3H/St B cells were cultured in the supernatant for 2 days.
[b]The polyclonal response to LPS, assayed against SRBC and TNP after 2 days of culture, is presented as the arithmetic mean of triplicate cultures ± the SE.

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 T cells with those of the soluble fraction of sonicated T cells

TABLE V. Ability of Resident T Cells and T Cell Sonicates to Modulate Polyclonal B Cell Responses

Composition of culture[a]		αTNP PFC/culture[b] when T cells are:	
B cells	T cells	Intact	Sonicated
100%	0%	418 ± 14	418 ± 14
75%	25%	412 ± 26	569 ± 69
50%	50%	840 ± 114	1107 ± 70
25%	75%	960 ± 28	207 ± 8
0%	100%	83 ± 3	0 ± 0

[a]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 sonicated cell equivalents) were cultured in 1.0 ml of 5% FCS-containing medium in the presence or absence of 100 μg/ml LPS.

[b]The direct PFC responses to TNP were assayed after 48 hours of culture. Results are expressed as the arithmetic mean of triplicate cultures ± SE.

on the polyclonal response of B cell cultures to LPS (Table V), indicated that whereas supplementation of these cultures with intact T cells provided help at a ratio of 1-3 T cells per B cell, the T cell sonicate provided T cell help at a ratio of 1 T cell per B cell; at 3 T cells per 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 per 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 due to toxicity, B cells were co-cultured with incremental amounts of T cell sonicate with LPS, and B cell viability evaluated after 2 days of culture (Table VI). The results indicated that no selective loss of viability occurs.

Residence of Helper and Suppressor Activities in T Cell Subpopulations Bearing Distinct Lyt Surface Phenotypes

In order 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^+2^- T cells to B cell cultures enhanced the polyclonal B cell response (Table VII). This was true whether the cells were intact or sonicated. However, when Lyt 1^-2^+ 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 Lyt 1^-2^+ T cell equivalents markedly suppressed the response.

Suppression of Polyclonal B Cell Responsiveness Is Not Restricted by the MHC

In order to learn if MHC restriction played a role in T cell suppression of the polyclonal B cell response to LPS, T cell sonicates were prepared from mice of a

TABLE VI. Effect of T Cell Sonicates on B Cell Viability

B cells[a]	Splenic T cell equivalents[b]	Viable cells recovered[c]
2.5×10^6	0	1.28×10^6 (± 0.08)
2.5×10^6	2.5×10^6	1.47×10^6 (± 0.03)
2.5×10^6	12.5×10^6	1.35×10^6 (± 0.03)

[a] 2.5×10^6 viable C3H/St B cells were cultured in 1.0 ml of FCS-containing medium in the presence of 100 μg/ml 055:B5 LPS.
[b] The indicated number of soluble sonicated T cell equivalents were added at the onset of culture.
[c] B cell viability was determined by the trypan blue dye exclusion test after 48 hr of culture. Results are presented as the arithmetic mean of triplicate cultures ± the SE.

TABLE VII. Immunoregulation of Polyclonal B Cell Responses by Whole and Sonicated Lyt 1^+ and Lyt 2^+ T Cells

	T cells[b]			Anti-SRBC PFC response
B cells[a]	Number	Lyt phenotype	Condition	(% of B cell response)[c]
2.5×10^6	0	–	–	100 ± 3%
2.5×10^6	2.5×10^6	Lyt 1^+	intact	207 ± 11%
2.5×10^6	2.5×10^6	Lyt 1^+	sonicated	207 ± 20%
2.5×10^6	2.5×10^6	Lyt 2^+	intact	132 ± 24%
2.5×10^6	2.5×10^6	Lyt 2^+	sonicated	31 ± 10%

[a] 2.5×10^6 viable C3H/St B cells were cultured in 1.0 ml of FCS-containing medium in the presence or absence of 100 μg/ml LPS.
[b] C3H/ST cells were treated with anti-Lyt antisera and C′ as shown, and 2.5×10^6 resultant cells were added to culture either intact or as the soluble fraction of a T cell sonicate.
[c] The polyclonal response to LPS, assayed against SRBC after 2 days of culture, is presented as the % of the B cell response of the difference of the arithmetic means of experimental and control cultures ± the SE.

TABLE VIII. Suppression of Polyclonal B Cell Responsiveness Is Not Restricted by the MHC

C3H/St B cells[a]	T cell sonicate	(H2)	% of B cell PFC response[b]
+	–		100%
+	C3H/St	(k)	23%
+	SJL	(s)	35%
+	C57BL/6J	(b)	30%
+	BALB/c	(d)	17%

[a] 2.5×10^6 C3H/St B cells were cultured with 8×10^6 T cell equivalents, derived from the strains indicated, in the presence or absence of 100 μg/ml LPS.
[b] Anti-SRBC PFC were assayed after 48 hr of culture. Results are presented as the percent of B cell control represented by triplicate experimental-control cultures.

variety of H-2 phenotypes. When syngeneic T cell sonicate was added (at a 3:1 ratio) to B cell cultures, the B cell response was significantly suppressed, as before. However, in addition, the soluble fraction of T cell sonicates prepared from SJL mice ($H\text{-}2^s$), C57BL/6J mice ($H\text{-}2^b$), or Balb/c mice ($H\text{-}2^d$) were all effective suppressors of the polyclonal B cell response. The Igh-1 allotype does not appear to

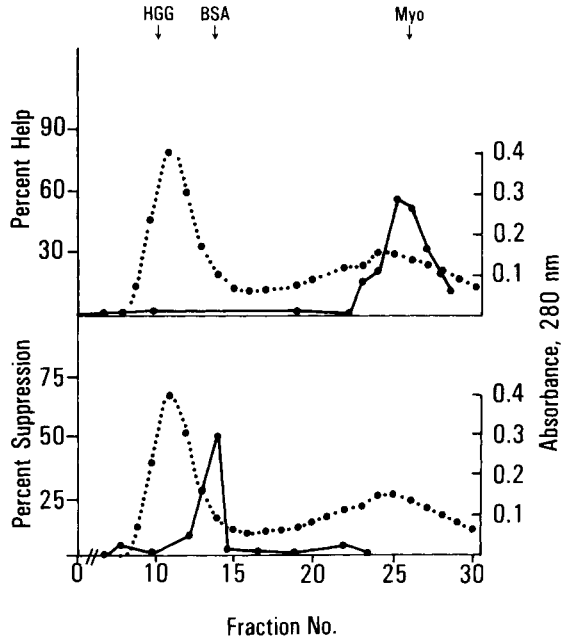

Fig. 1. Separation of helper and suppressor activities from splenic T cell sonicates. Splenic T cell sonicates were chromatographed over a column of Sephadex G-200 as described in Materials and Methods. 2.5 × 10^6 viable C3H/St splenic B cells in the presence of 100 μg/ml 055:B5 LPS were supplemented with 50 μl of the column fraction to be assayed (5–10 × 10^6 T cell equivalents). The direct PFC response to SRBC (solid line) was assayed 48 hours later. Results are expressed as the arithmetic mean of triplicate cultures from three separate experiments. The molecular weight markers human gamma globulin in (HGG), bovine serum albumin (BSA), and myoglobin (MYO) are indicated by the arrows at the top of the figure.

act as a factor determining the suppressive potential of these sonicates, as was the case for helper activity in Table II.

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 × 10^6 cell equivalents of each fraction. The suppressor activity eluted far ahead of the helper activity (Fig. 1). Molecular weight estimates based on three similar elution profiles ranged from 68,000–84,000 daltons for the suppressive factor, and 15,000–23,000 daltons 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 which can be evoked experimentally. We have previously found that the helper activity of T cells for polyclonal activation is optimal when T cells are present in modest excess relative to B cells. This activity may be removed by treatment with an antithymocyte serum and complement [12]. Theofilopoulos et al have since made parallel observations regarding the effect of T cells in polyclonal responses of normal and autoimmune murine strains [25].

Normal splenic T cells exhibit little or no suppressor activity under non-activating circumstances. The fact that removal of helper cells with the anti-Lyt 1.1 antiserum failed to unmask any latent constraining effects with regard to αLyt 1 and αLyt 2-treated cells 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 to abrogation of helper activity attributed to αLyt 1 + C′, treatment with αLyt 2 + C′ was essentially without effect.

T cell helper activity for the polyclonal response does not appear to be restricted by the MHC. Thymocytes from strains of a diversity of H-2 phenotypes all provided comparable degrees of help. This observation accords with the lack of H-2 restriction observed for non-antigen-specific T cell helper factors, such as interleukin-II [26] and some antigen-specific factors [27].

The polyclonal response is subject to suppressive regulation as well as helper effects. 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 and co-workers [28] but at variance with that of Nespoli et al [29]. Reasons for the discrepant results of the latter group are not clear.

Investigation of the means by which T cell regulatory signals are communicated to B cells revealed first that neither helper not 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 to 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 per B cell; at a ratio of 3 T cells per 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, since 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 after sonication caused potent suppression. These observations are in accord with those of Tse and Dutton, who found that the cells exerting helper and suppressor activities for the primary humoral response to SRBC in vitro are Lyt 1^+ and Lyt 2^+, respectively [8].

The suppressive activity present in the soluble fraction of T cell sonicates is not restricted by elements encoded by the MHC. Thus, cell sonicates from mice representing a diversity of H-2 phenotypes all provided comparable degrees of suppression. Lack of H-2 restriction has been reported for both antigen-specific T suppressor factors [33] and for non-antigen-specific suppressor factors [38] in other systems.

The helper and suppressor activities of splenic T cell sonicates appear to be distinct molecular moieties which can be separated 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 prior to generation of antigen-nonspecific regulatory activity. However, if they are components of other factors, their participation may involve antigen-specific as well as nonspecific T cell regulatory factors. The molecular weight characterization of the helper activity is compatible with a role as a component of interleukin-II [26], allogeneic effect factor [30], and the non-antigen-specific mediator of Kappler and Marrick [31], or a precursor to the MIF-like factor of Rocklin et al [32]. The suppressive activity in splenic T cell sonicates could exist as a precursor to antigen-specific suppressive factors, such as that of Takemori and Tada [11] or that of Waltenbaugh et al [33]. However, it well may be either a component of a precursor to (or identical to) the inhibitor of DNA synthesis of Namba and Waksman [34,35], the inhibitor of DNA polymerase of Lee and Lucas [36], soluble immune response suppressor [37], or the immunoglobulin binding factor of Fridman [38]. More extensive physicochemical and immunological characterization of these T cell regulatory activities will be necessary in order to establish their precise relationship to these previously described factors.

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