EVIDENCE FOR A B-CELL-LIKE HELPER FUNCTION IN MIXED LYMPHOCYTE CULTURE BETWEEN IMMUNOCOMPETENT THYMUS CELLS*

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The proliferating cell in one-way mixed lymphocyte cultures (MLC)[1] is a T cell (1–7), and is found in peripheral lymphoid tissues and in a subpopulation of thymus cells (9). This immunocompetent thymus subpopulation (TH-2) (10) also contains cells active in graft-vs.-host reactions and carrier recognition in T-dependent antibody formation (9). It is characterized as having low buoyant density, low Thy-1 and high *H-2* membrane antigen content, and cortisone and X-ray resistance (8,9).

The experiments to be described establish that the TH-2 subpopulation does not proliferate or stimulate proliferation in MLC between allogeneic TH-2 cells, despite the presence of alloantigens on the cell surface. An accessory peripheral lymphoid cell is required in order for a proliferative MLC to occur in this circumstance. This defect in TH-2 cells is explored, and characteristics of the accessory cell are described which suggest that it is of B-cell lineage.

Materials and Methods

Mice. Female, 6-wk old mice of the strains C57BL/10Sn (B10), C57BL/10·BR (B10·BR), C57BL/10·A (B10·A), C57BL/10·AKM (AKM), and C57BL/10·D2 (D2) were obtained from Jackson Laboratories, Bar Harbor, Maine and kept in the animal rooms for at least 2 wk before use.

Physical Separation of Cell Populations. Subpopulations of lymphoid cells were obtained by a modification of the Dutton technique, (11) employing a discontinuous gradient of bovine serum albumin (BSA). Gradient separation of thymus cells was modified from the method used for

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[1] *Abbreviations used in this paper:* B cell, nonthymus processed lymphoid cell; BSA, bovine serum albumin; cAMP, adenosine 3′:5-cyclic monophosphoric acid, sodium salt; cGMP, guanosine 3′:5-cyclic monophosphoric acid, sodium salt; LD, lymphocyte-determined locus of *H-2*; LN, lymph node; LPS, lipopolysaccharide; MLC, mixed lymphocyte culture; PGE, PGF, prostaglandins; SPL, spleen; TH-1, immunoincompetent thymus cell; TH-2, immunocompetent thymus cell; [^{3}H]TdR, [^{3}H]thymidine; Thy-1 thymic antigen (theta).

peripheral lymphoid tissues (7); namely, the 26% layer was substituted by a 27% BSA layer. Density gradient separation yields bands of cells at the interfaces of adjacent BSA layers. These were: A layer, 10–23%; B layer, 23–26%; C layer, 26–29%; D layer, 29–35%; and a pellet, below 35%.

The subpopulation of thymus cells taken from the "B" layer is termed TH-2 in this paper, on the basis of prior studies; the subpopulation taken from the "D" layer is termed TH-1 (10). The notation TH-2_a/TH-$2_{(b)}$ will be used to describe the general case of a one-way MLC containing allogeneic or *H-2* locus-different congenic resistant mouse strains in which TH-2_a is the responder and TH-$2_{(b)}$ is the allogeneic or congenic mitomycin-blocked stimulator cell. Strain designations are substituted in the subscript notation in describing specific experiments. SPL indicates spleen cells, and LN signified lymph node cells.

Spleen cell subpopulations were also separated on the basis of adherence to glass. Cell populations were cultured in 100 mm diameter sterile glass petri dishes at a concentration of 100×10^6 cells in 10 ml RPMI-1640 per dish. After a 60-min incubation (37°C, 5% CO_2 atmosphere), the nonadherent cells were poured off, the plates washed twice, and the nonadherent cells pooled and washed again. Those cells still adhering to the glass surfaces were gently removed with a rubber policeman and washed. A similar procedure was used to prepare adherent and nonadherent cells cultured on plastic culture dishes. Spleen cells were also incubated in a nylon column (0.5 × 10 cm) for 30 min (37°C, 5% CO_2 atmosphere) and the nonadherent cells were subsequently collected. Viability determinations (trypan blue) were made of all cell subpopulations before use.

Hydrocortisone Treatment of Mice. Mice were injected i.p. with 2.5 mg hydrocortisone (Cortone; Merck, Sharpe, and Dohme, West Point, Pa.) per 20 g body weight, 2 days before each experiment.

Microplate Culture System. The method for the preparation of lymphoid cell suspensions and tissue cultures of mouse cells for MLC reactions has been previously described (7–9). The experiments described in this report have used the similar procedures with the exception that all cultures were performed in tissue culture microplates (Falcon Plastics, Div. of Bioquest, Oxnard, Calif.; [Microtest II plates] or Linbro Chemical Co., Inc., New Haven, Conn.) according to a modification of the method of Bach (2). This modification had the advantages of requiring fewer cells per culture and facilitating the processing of cultures at the end of the incubation period with the aid of the automatic cell harvester (Otto Hiller Co., Madison, Wis.). All cultures were made in quadruplicate. Cultures were maintained for 72 h, with 0.5 μCi[^{3}H]thymidine ([^{3}H]TdR) (Schwarz-Mann Div., Becton, Dickinson & Co., Orangeburg, N.Y.) being added per well during the last 20 h of culture. The contents of each well were processed as previously described (9).

Macrophage-Enriched Subpopulations. Peritoneal exudates were stimulated by an i.p. injection of thioglycollate (Difco Laboratories, Detroit, Mich.). 5–7 days later, the peritoneal cells were washed out with RPMI-1640 containing 1% fetal calf serum. The exudate cell population contained an average of 85% macrophages. Macrophage monolayers were prepared by seeding 1.25×10^4 peritoneal cells per culture well, containing 0.2 ml complete tissue culture medium. The cells were allowed to attach for 1 h, after which nonadherent cells were aspirated and fresh medium added. The cultures were maintained overnight, washed twice again to remove any additional nonadherent cells, and were then used in MLC as described.

Antisera. AKR anti-CBA-Thy-1 (θ) serum was prepared by procedures previously described (8). Goat antimouse immunoglobulin serum was purchased from Meloy Laboratories, Springfield, Va. All sera were titered and used at the highest dilution achieving maximum cytotoxicity.

Drugs. Mitomycin-C (Nutritional Biochemicals, Cleveland, Ohio) was used to block thymidine incorporation by stimulator and accessory cells, in a concentration of 50 μg/ml, as described elsewhere (7, 8). Cyclic AMP (adenosine 3′:5-cyclic monophosphoric acid, sodium salt), cyclic GMP (guanosine 3′:5-cyclic monophosphoric acid, sodium salt), and acetylcholine were purchased from Sigma Chemical Co., St. Louis, Mo. The prostaglandins PGE_1, PGE_2, and $PGF_{2\alpha}$ were a gift from Dr. J. E. Pike of the Upjohn Co., Kalamazoo, Mich.

Results

Failure of Allogeneic or Congenic Thymus Cells to Proliferate in a Th-2_a/TH-$2_{(b)}$ MLC Reaction. Table I gives data from a typical experiment which showed

TABLE I

One-Way Mixed Lymphocyte Cultures Between Various Thymus Cells and Thymus Cell Subpopulations and Peripheral Lymphoid Cells Derived from C57BL/10 and C57BL/10·BR Mice

Exp. no.	Origin of B10 responder cells	[^{3}H]TdR incorporation (mean cpm ± SE/10^6 responder cells) stimulated by [B10·BR]*					
		Thymus cell subpopulations				Spleen cells	No added cells
		Density gradient fraction		Cortisone resistant TH-2	Whole thymus		
		TH-2	TH-1				
1	Thymus density gradient fraction						
	TH-2	296±13	1,294±131	—	—	23,390±1,781	283±17
	TH-1	309±20	262±12	—	—	376±15	349±13
	Spleen	27,700±1,324	1,918±109	—	—	44,400±2,103	588±69
2	Thymus						
	Cortisone resistant‡	—	—	889±80	1,789±198	9,761±390	1,208±90
	Whole thymus	—	—	523±33	500±8	456±10	450±5
	Spleen	—	—	27,805±1,426	4,021±145	52,165±2,246	1,367±290

* Thymus or spleen cells from normal or cortisone-treated B10 and B10·BR mice were culture (1×10^6 responder and 1×10^6 mitomycin-treated stimulating cells in each culture) for 72 h and assayed for [^{3}H]TdR incorporation during the final 20 h of culture. Data given represent mean values of four cultures ± SE.

‡ Cortisone given 48 h before taking thymus cells (2.5 mg/20 g body weight).

that the immunocompetent thymus subpopulations (TH-2), derived either by density gradient separation or after prior hydrocortisone treatment, neither proliferated in one-way MLC nor stimulated congenic TH-2. In contrast, mitomycin-treated spleen cells induced proliferation in congenic TH-2 cells; and, conversely, mitomycin-treated TH-2 cells were fully stimulatory to congenic spleen cells. Similar data had been obtained with multiple allogeneic combinations in earlier experiments of this series, and are not reported here. The same results were also obtained in two-way MLC reactions between congenic TH-2 cells (Table II). The fact that proliferation did not occur in two-way TH-2_a/TH-2_b cogenic mixtures eliminates the possibility that inhibition is caused by mitomycin-C leakage from the blocked stimulator cell.

Neither alloantigen dose nor kinetic considerations appear to explain the failure of congenic TH-2_a/TH-$2_{[b]}$ MLC reactions. The ratio between responding and stimulating cells was varied over a wide range. For example, as shown in Fig. 1, TH-2_{B10} cells did not proliferate when mixed with cells at 0.5:1, 1:1, or 2:1 ratios. TH-2_{B10} cells did incorporate significant [^{3}H]TdR in combination with TH-$1_{[B10\cdot BR]}$ cells, and showed vigorous proliferation in response to $SPL_{[B10\cdot BR]}$ cells. The dose-response pattern of the TH-2_{B10}/$SPL_{[B10\cdot BR]}$ MLC is indistinguishable from that found for SPL_{B10}/$SPL_{[B10\cdot BR]}$ or LN_{B10}/$LN_{[B10\cdot BR]}$ (not shown). The time-course of the culture was also varied, and harvesting at 2, 3, 4, or 5 days did not yield a proliferative TH-2_{B10}/TH-$2_{[B10\cdot BR]}$ MLC (Fig. 2). It can, therefore, be reasonably concluded that TH-2 cells neither stimulate nor respond in a two-component MLC unless the other cell population in the mixture originates in the peripheral lymphoid tissue.

Triggering of Proliferative TH-2_a/TH-$2_{[b]}$ MLC by Addition of Mitomycin-Blocked Peripheral Lymphoreticular Cells. Spleen cells or LN cells were

TABLE II

*Two-Way Mixed Lymphocyte Cultures Between Thymus Cells or Thymus Cell Subpopulations or Spleen Cells from C57BL/10 and C57BL/10·BR Mice**

Exp. no.	B10 cell source	[^{3}H]TdR incorporation (mean cpm ± SE) upon adding B10·BR cell source					
		Thymus cell subpopulations				Spleen cells	No added cells
		TH-2	TH-1	Cortisone resistant	Whole thymus		
1	Thymus						
	TH-2	486±30	407±37	—	—	49,816±2,195	283±17
	TH-1	397±21	412±15	—	—	16,159±2,019	349±13
	Spleen	24,597±675	3,908±422	—	—	46,189±3,189	588±69
2	Thymus						
	Cortisone resistant‡	—	—	2,780±142	4,021±295	25,216±2,246	1,648±95
	Whole thymus	—	—	3,671±207	1,049±311	6,907±365	954±241
	Spleen	—	—	39,871±2,028	9,500±1,516	49,317±4,740	1,330±213

* Thymus or spleen cells from normal or cortisone-treated B10 and B10·BR mice were cultured (1×10^6 B10 cells and 1×10^6 cells per culture) for 72 h and assayed for [^{3}H]TdR incorporation during the final 20 h of culture. Data given represent mean values of four cultures ± SE.

‡ Cortisone given 48 h before taking thymus cells (2.5 mg/20 g body weight).

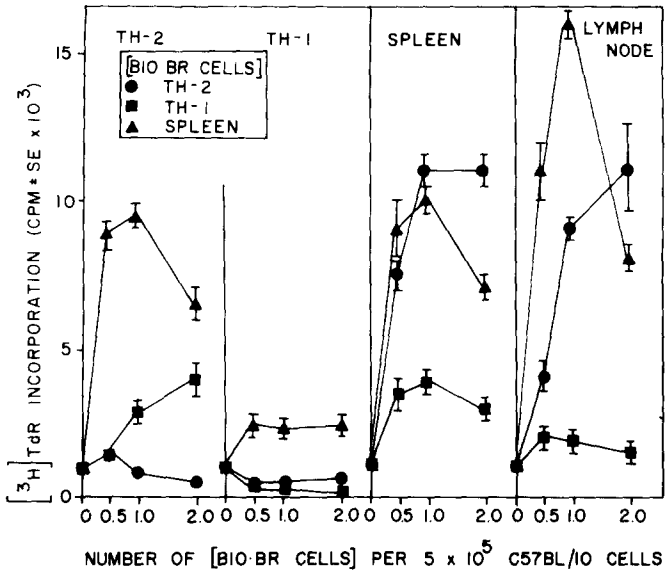

FIG. 1. Dose response of C57BL/10 lymphoid cells to various numbers of mitomycin-blocked C57BL/10·BR cells. Numbers of B10 cells per culture remained fixed at 5×10^5. Cultures were incubated at 72 h and [^{3}H]TdR incorporation was assayed during the final 20 h of culture. Each point represents the mean of four cultures ± SE.

satisfactory stimulator cell sources in MLC with TH-2_a responders, and were stimulated readily by TH-$2_{[b]}$. Therefore, experiments were performed in which TH-2_a/TH-$2_{[b]}$ MLC mixtures were supplemented with varying numbers of spleen or lymph node cells syngeneic with the TH-2_a or TH-$2_{[b]}$ cells in the MLC. Mitomycin-C treatment of the supplementing cells eliminated proliferative responses to the TH-$2_{[b]}$ population. Cells syngeneic with TH-$2_{[b]}$ did supple-

ment at low target cell ratios where no MLC response to these cells could be detected. Representative experiments in which various subpopulations and numbers of $SPL_{[B10]}$ cells were added to $TH\text{-}2_{[B10]}/TH\text{-}2_{[B10\cdot BR]}$ MLC are presented in Table III and Figs. 3–5. The data demonstrate that $TH\text{-}2_a/TH\text{-}2_{[b]}$ MLC do proliferate to an extent often equivalent to $TH\text{-}2_a/SPL_{[b]}$ MLC, when supplemented at a 1:10 ratio with $SPL_{[a]}$ cells; that is, cells syngeneic with the

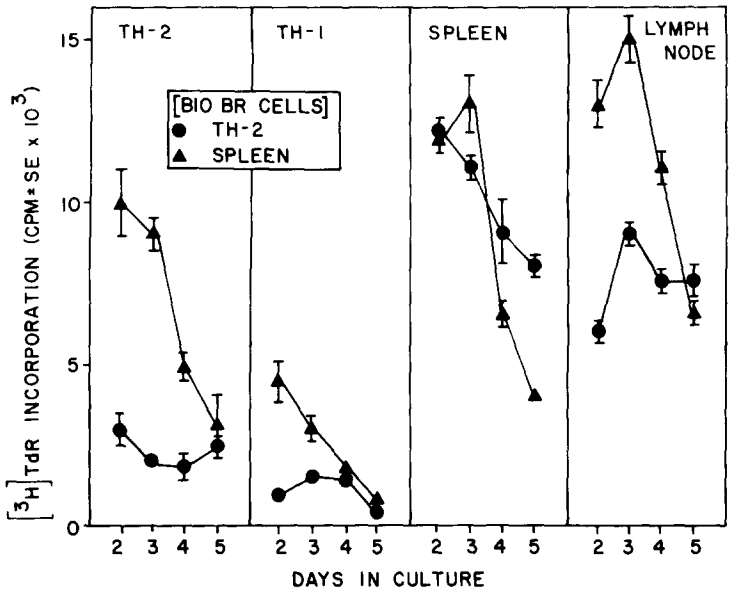

FIG. 2. Time-course of MLC response of C57BL/10 cells to mitomycin-blocked B10·BR cells. Each culture consisted of equal numbers (5×10^5) of B10 responder cells and B10·BR mitomycin-blocked stimulating cells. Cultures were maintained for the number of days indicated and assayed for [^{3}H]TdR incorporation during the final 20 h of culture. Each point represents the mean of four cultures ± SE.

TABLE III

*Effect of Adding Macrophages on Proliferation in a $TH\text{-}2_{B10}/TH\text{-}2_{[B10\cdot BR]}$ MLC**

B10 responder cell	B10 macrophages added	[^{3}H]TdR incorporation (mean cpm ± SE/10^6 responder cells when B10·BR stimulator cells added		
		TH-2	Spleen	No cells added
TH-2	+	126 ± 9	7,210 ± 601	269 ± 21
	−	55 ± 4	12,513 ± 1,196	99 ± 2
Spleen	+	24,200 ± 1,340	38,688 ± 1,969	807 ± 139
	−	29,197 ± 1,522	29,191 ± 509	1,083 ± 41

* TH-2 or spleen cells from B10 and B10·BR mice were cultured on peritoneal cell monolayers established the day before (see Materials and Methods) and were cultured (1×10^6 responding cells plus 1×10^6 mitomycin-blocked stimulating cells) for 72 h; cultures were assayed for [^{3}H]TdR incorporation during the final 20 h of culture. Data given represent mean value of four cultures ± SE.

responding cell. A similar supplementing effect was seen when 1:10 ratios of $SPL_{[b]}$ were added (data not shown).

The successfully supplementing spleen cell subpopulation was further characterized, in a series of experiments, to be of low density (Fig. 3), nonadherent to glass, plastic or nylon (Fig. 5), and resistant to the cytotoxic effects of anti-Thy-1 and complement in most experiments. In some experiments (e.g. Fig. 4) slight reduction in supplementing effect after anti-Thy-1 treatment was observed. Pretreatment of the supplementing cell population with antimouse Ig and

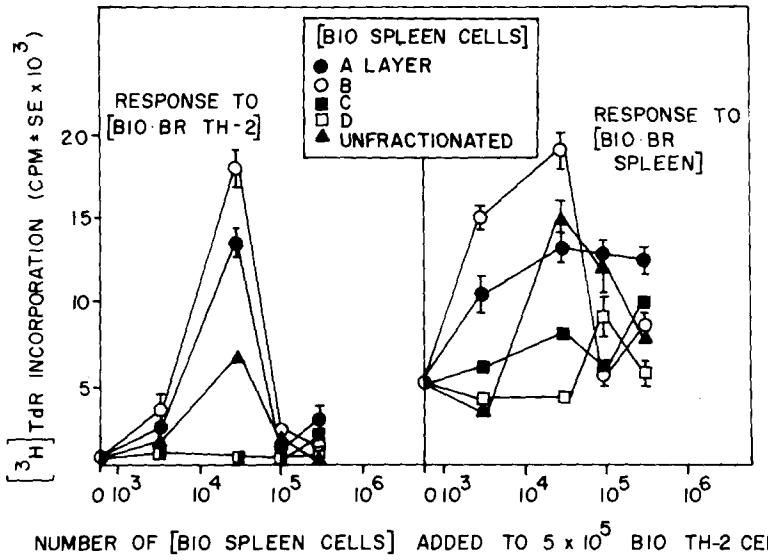

FIG. 3. MLC response of C57BL/10 TH-2 cells to either mitomycin-blocked B10·BR TH-2 or spleen cells when gradient-separated B10 spleen cells (mitomycin-blocked) are also added to the cultures. Each culture consisted of equal numbers (5×10^5) of B10 TH-2 cells and mitomycin-blocked B10·BR cells. The mitomycin-blocked B10 spleen cell subpopulations were added per culture at the numbers indicated. All cultures were maintained for 72 h and assayed for [^{3}H]TdR incorporation during the final 20 h of culture. Each point represents the mean of four cultures ± SE.

complement essentially eliminated the supplementing effect (Fig. 4). $LN_{[a]}$ cells were equally effective in restoring the $TH\text{-}2_a/TH\text{-}2_{[b]}$ MLC (data not given). Macrophage-enriched peritoneal exudate cells were completely ineffective (Table III), as were spleen cells selected for capacity to adhere to glass or plastic (Fig. 5).

The dose-response aspects of this supplementing effect were explored in further experiments in which both the number of added cells and the density fraction were varied over a wide range, as shown in Fig. 3. Only spleen cells of low density (A and B fractions) were effective in supplementing the MLC response; the cells of higher net density (C and D fractions) had little or no effect. Unexpected was the very limited range of ratios of supplementary to responding cells effective in restoring one-way $TH\text{-}2_a/TH\text{-}2_{[b]}$ MLC proliferation. When low density supplementary spleen cells were similarly added to one-way $TH\text{-}2_a/SPL_{[b]}$ MLC, proliferative responses were enhanced (Fig. 3). The dose-response

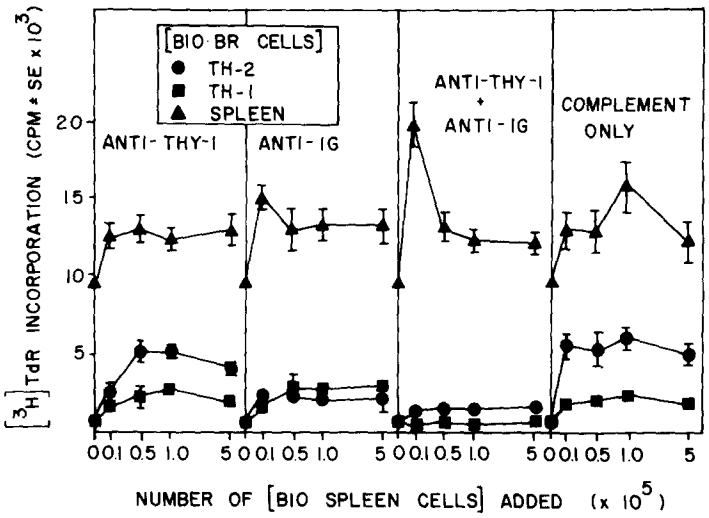

FIG. 4. Effect of adding antiserum plus complement-pretreated C57BL/10 spleen cells (mitomycin-blocked) on MLC response of C57BL/10 TH-2 cells to mitomycin-blocked C57BL/10·BR cells. Each culture consisted of equal numbers of (5×10^5) of responder B10 TH-2 cells and mitomycin-blocked B10·BR stimulating cells. Antiserum-pretreated B10 spleen cells were added per culture at the numbers indicated. All cultures were maintained for 72 h and assayed for [^{3}H]TdR incorporation during the final 20 h of culture. Each point represents the mean of four cultures ± SE.

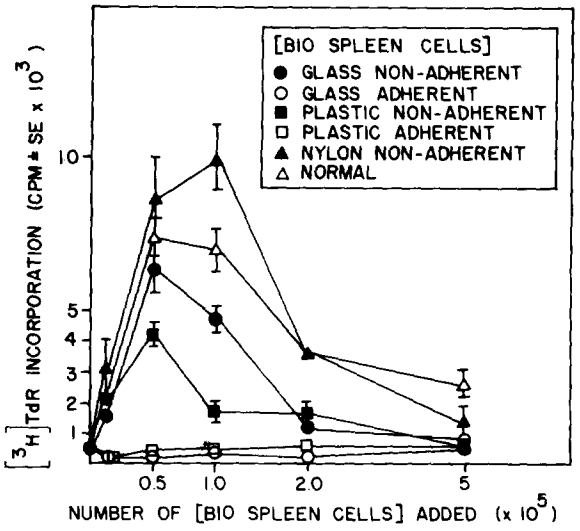

FIG. 5. Effect of adding adherent or nonadherent C57BL/10 spleen cells (mitomycin-blocked) on MLC response of C57BL/10 TH-2 cells to mitomycin-blocked B10·BR cells. Each culture consisted of equal numbers (5×10^5) of responder B10 TH-2 cells and mitomycin-blocked B10·BR stimulating cells. Adherent or nonadherent B10 spleen cells were added at the numbers indicated. All cultures were maintained for 72 h and assayed for [^{3}H]TdR incorporation during the final 20 h of culture. Each point represents the mean of value of four cultures ± SE.

relationships were similarly restricted to a relatively narrow range of effective ratios, although this range was somewhat broader than that found for TH-2_a/TH-$2_{[b]}$ MLC.

Failure to Demonstrate a Reconstituting Soluble Product of Supplementary Spleen Cell Subpopulations. It seemed possible that the supplementing effect of peripheral lymphoid cells in TH-2_a/TH-$2_{[b]}$ MLC could be due to some soluble factor released into the medium by the added $SPL_{[a]}$ cells, permitting or triggering a nonspecific proliferative response in the presence of allogeneic cells. Table IV gives results representative of many experiments designed to detect

TABLE IV

*Effect of Supernates from Spleen Cell MLC on One-Way MLC by TH-2 Cells**

Spleen MLC supernate‡	[^{3}H]TdR incorporation (mean cpm ± SE) by C57BL/10 TH-2 cells in one-way MLC: [B10·BR cells]		[C57BL/10 cells]		No cells
	TH-2	Spleen	TH-2	Spleen	
B10/[BR]	156 ± 12	6,116 ± 854	203 ± 19	204 ± 9	65 ± 5
B10/[B10]	182 ± 23	4,482 ± 332	127 ± 5	220 ± 22	67 ± 4
B10/[BR] + [B10]§	102 ± 6	2,153 ± 154	66 ± 4	96 ± 5	72 ± 6
[B10] alone	75 ± 10	353 ± 20	53 ± 5	51 ± 8	42 ± 3
None	155 ± 11	2,466 ± 71	58 ± 5	138 ± 8	151 ± 9

* Thymus or spleen cells from B10 and B10·BR mice were cultured (5×10^5 responder cells plus 5×10^5 mitomycin-blocked stimulating cells in each culture) for 72 h and assayed for [^{3}H]TdR incorporation during the final 20 h of culture. Data given represent mean value of four cultures ± SE.

‡ Cell-free supernates were collected from 72 h cultures of B10 and B10·BR cells (1×10^6 responder cells plus 1×10^6 mitomycin-blocked stimulating cells). Parenthesis indicate the cells present during the 72 h incubation period. 50 lambda (of total vol equals 0.2 ml) of each supernate were added per culture.

§ This group of SPL_{B10}/$SPL_{[B10 \cdot BR]}$ MLC cultures also contained 10% $SPL_{[B10]}$ to approximate the conditions of a TH-2_{B10}/TH-$2_{[B10 \cdot BR]}$ MLC which has had $SPL_{[B10]}$ added (Fig. 3).

such a soluble product. Supernatant fluids taken from SPL_a/$SPL_{[b]}$ MLC enhanced TH-2 cell proliferation in TH-2_a/$SPL_{[b]}$ MLC, but had no detectable effect on the TH-2_a/TH-$2_{[b]}$ cultures. The supplementary effect appears, therefore, not to have the qualities of a blastogenic factor but to require that the $SPL_{[a]}$ cell subpopulation be present and intact in order that TH-2_a cells may proliferate. All attempts to trigger proliferation in TH-2_a/TH-$2_{[b]}$ MLC by add- a wide range of doses of LPS or of a variety of pharmacologic agents known to affect lymphocyte behavior, including acetylcholine, cyclic AMP, cyclic GMP, PGE_1, PGE_2, and $PGF_{2\alpha}$ were unsuccessful.

Contributions of Different Regions of the H-2 Locus to TH-2_a/TH-$2_{[b]}$ MLC Reactions. A possible way in which the supplemental cell population might reconstitute TH-2_a/TH-$2_{[b]}$ proliferative responses is by providing some product of the proposed LD (or MLC) locus, apparently required to activate the proliferative phase of MLC reactions (2, 12). To test this possibility, TH-2_a/$SPL_{[b]}$ MLC reactions were examined which involved cells taken from congenic resist-

ant mice, in combinations in which differences were limited to the *D* end of the *H-2* region, or the *K* and *I* regions. The results (Table V) indicate that, although the ability of TH-$2_{[b]}$ cells to stimulate SPL_a cells does not appear to be related to *D*, *D-S*, or *K-I* differences, the capacity of $SPL_{[b]}$ cells to stimulate TH-2_a cells is evidently strongly favored by *K-I* differences. These results are consistent with the interpretation that the supplementing spleen cell subpopulation has the function of providing to the MLC some product, determined at the *K-I* end of the *H-2* locus, which permits TH-2_a cell proliferation. Work in progress, utilizing other congenic mouse strains, is aimed at isolating further the *H-2* region sublocus which contributes to the capacity of peripheral lymphoid cells to trigger a proliferative TH-2_a/TH-$2_{[b]}$ MLC.

TABLE V
*H-2 Locus Contributions to One-Way MLC Reactions**

Exp. no.	Responder + [stimulator]	*H-2* difference	[^{3}H]TdR incorporation (mean cpm ±SE/10^6 responder cells) when stimulator cells added		
			TH-2	Spleen	No cells added
1	B10·BR [B10·AKM]	*D*			
	TH-2		128 ± 20	2,860 ± 31	313 ± 6
	Spleen		24,516 ± 887	11,553 ± 577	818 ± 62
2	B10·BR + [B10·A]	*D,S*			
	TH-2		163 ± 6	3,230 ± 212	313 ± 6
	Spleen		30,063 ± 734	30,950 ± 604	818 ± 62
3	B10·A + [B10·D2]	*K,I*			
	TH-2		64 ± 2	14,087 ± 1,388	39 ± 1
	Spleen		22,922 ± 499	45,833 ± 606	1,347 ± 20

* TH-2 or spleen cells from the various mouse strains were cultured (1×10^6 responder cells plus 1×10^6 mitomycin-blocked stimulating cells) for 72 h and assayed for [^{3}H]TdR incorporation during the final 20 h of culture. Data given represent mean values of four cultures ± SE.

Discussion

The data presented in this paper adds to other evidence (2, 6, 9, 10, 12–19) indicating the complexity of interactions between T lymphocytes and alloantigen-bearing cells in the MLC. The TH-2 thymus subpopulation differs slightly from the peripheral T cell in density profile and in net content of T-cell membrane antigens, but has the similar alloantigen content, and is immunocompetent both in vivo and in vitro in terms of T-cell functions (7–9). The experiments described indicate that this subpopulation cannot, however, initiate a proliferative response to serologically defined alloantigens on the surface of allogeneic or congenic TH-2 cells. The MLC is stimulatory only when one member of the cell mixture is a peripheral lymphoid cell or when a critical ratio of nonproliferating peripheral lymphoid cells, syngeneic to either responder or target, is added. The supplementing or helper cell has been defined thus far as of relatively low net

density, not adherent to glass or nylon, insusceptible to the cytotoxic effect of anti-Thy-1 (θ) but completely susceptible to anti-Ig and complement. The supplementary activity appears to depend upon neither a soluble mediator nor cell proliferation. Experiments in progress are designed to define further the *H-2* locus through which this helper cell effect is expressed.

The experimental design employed here differs from most studies of MLC in that the immunocompetent cell subpopulation has been isolated from the thymus, and its behavior in MLC has been examined in relation to other thymus cells of similar origin and to that of peripheral lymphoid cells. Demonstration of a helper cell effect in MLC between immunocompetent thymus cells should, therefore, provide a better-defined model through which to explore the cellular and molecular mechanisms underlying alloantigen recognition and T-cell triggering in MLC. T-cell target cell and helper roles can be delineated in a way not possible with the heterogeneous or contrived cell populations which have been heretofore employed.

The characteristics of the supplementing or helper subpopulation, as defined thus far, fit some criteria for cells of B-cell lineage. Curiously, however, they are not removed by nylon column passage. It is not known whether this same subpopulation is capable of antibody production or if it has Fc or C3 receptors. Since it is probable that multiple B-cell subclasses exist, each with differing arrays of these individual markers of functions, apparent discrepancies between observed helper cell behavior and net B-cell characteristics should be anticipated. It is possible that the helper function is the property of a special B-cell subclass.

This model and the data derived thus far still present major complexities which must be considered in developing any hypothesis explaining mechanisms involved. Presumably, both of the TH-2 cell subpopulations in TH-2_a/TH-$2_{|b|}$ MLC contain cell subsets which carry T-cell receptors specific to the alloantigenic specificity on the other cell, although the character of such T receptors is unresolved. The putative B-cell ($SPL_{|a|}$) helper component, required to trigger TH-2_a/TH-$2_{|b|}$ proliferation, may be assumed also to bear a subset of cells bearing receptors specific for the alloantigen complex on the TH-$2_{|b|}$ component. These receptors are likely, however, to resemble immunoglobulins.

Although mitomycin-C blocks eliminate cell proliferation of both TH-$2_{|b|}$ and the supplementing cell subpopulation, thus isolating the responding population, it presumably does not interfere with cell interactions involving either T or B receptors not requiring DNA synthesis, such as specific receptor ligand interactions; short-term membrane alterations may follow this such as endocytosis, shedding, or receptor mobility. It is conceivable, for example, that the triggering process could involve the interactions of TH-$2_{|b|}$/$SPL_{|a|}$ or, conversely, TH-2_a/$SPL_{|b|}$, as well as the TH-2_a/TH-$2_{|b|}$ interaction upon which attention is usually focussed.

In view of these complexities, it is appropriate to examine the constraints the data reported impose upon any explanatory working hypothesis of the helper cell effect rather than to deal with the possibilities they leave open. These constraints are: (*a*) TH-2_a/TH-$2_{|b|}$ is a nonproliferative MLC; (*b*) the known properties of the helper peripheral lymphoid cell resemble those of a B cell; (*c*) a critical cell ratio is required for helper cell function; and (*d*) neither TH-2_a/$SPL_{|a|}$

nor SPL_a/TH-$2_{[a]}$ combinations are of themselves, proliferative combinations, although submitogenic interactions cannot be excluded.

In this context, explanations should first be explored as to why the TH-2_a/TH-$2_{[b]}$ MLC fails to proliferate. Four possibilities seem feasible within the constraints of the data. These are: (*a*) T receptors on TH-2 cells are of low concentration or low combining strength resulting in a TH-2_a/TH-$2_{[b]}$ T-receptor-alloantigen interaction frequency insufficient to trigger proliferation; (*b*) rapid T-receptor shedding at such a high rate that T receptor-alloantigen interaction fails to trigger proliferation; (*c*) a required nonspecific cell-to-cell proliferation signal is absent in TH-2 cells; and (*d*) steric interference to effective cell-to-cell interactions is eliminated in some way by the helper cell.

Data presented here does not permit a clear choice between these alternatives. Evidence exists both for a high rate of T-receptor shedding by splenic T cells (20), and for a low concentration of T receptors on peripheral T cells (21–26). In hypothesis 1, the helper cell requirement might be related to a need to focus or aggregate widely scattered T receptors in order to permit proliferation. In hypothesis 2, the helper requirement could conceivably be related to a need to stabilize T receptors as a preamble to proliferation, or, conversely, to greatly increase the rate of receptor shedding, thus creating a rate of receptor turnover which by itself triggers cell division. Until better markers are defined which can be used to accurately measure T-receptor turnover, it is difficult to test this hypothesis further. No currently available evidence eliminates completely possibilities 3 and 4, other than well-known experiments concerning microenvironmental requirements for cell proliferation or differentiation.

Hypothesis 1 has further ramifications in terms of the possible mechanism of the helper cell effect. Assuming, (*a*), that a subset of the helper cells ($SPL_{[a]}$) has Ig receptors specific to TH-$2_{[b]}$, and, (*b*), that Fc receptors are present on TH-2_a, as has been recently reported for peripheral T cells by Hudson (27), then a triggering mechanism can be envisioned as follows: Ig receptors on $SPL_{[a]}$ interact with the specific alloantigen ligand upon TH-$2_{[b]}$. This could permit the Fc region of the Ig receptor to engage Fc receptors on the TH-2_a surface. This interaction could have the effect of stabilizing T-receptor-alloantigen ligand interactions in both directions between TH-2_a and TH-$2_{[b]}$ and/or provide the necessary membrane triggering event. Dickler and Sachs (28) and others (29–32) have postulated an analogous mechanism involving peripheral T cells recently. The critical 1:8 to 1:10 ratio of helper to responding cells may relate to limitation on the number of Fc receptor sites on TH-2 cells, or possibly to competitive inhibition of T-receptor-alloantigen ligand interactions, if excess Ig-type receptors are added to the cell mixture and bind these ligands. The Fc receptor of the TH-2 subpopulation may thereby be related to the elusive LD or MLC locus product. The function of the Ig-bearing cell would be to provide Fc-bearing Ig receptors, which can, after interacting in a competitive way with specific alloantigen ligands, engage T-cell Fc receptors and trigger MLC proliferation.

The experiments described pertain to TH-2 cells and not necessarily to alloantigen-responsive peripheral T cells. No known data rule out similar cell MLC requirement for spleen or lymph node T cells in combination with B-cell-enriched peripheral lymphoid cells. T cells are known to be minimally or non-stimulating

target cells in mouse (7, 12, 33, 34) and human (15,35) systems; and, it has recently been found (12,33) that the optimal target cell is a B cell. This conceivably may reflect not a defective property of the T-target cell per se, but the helper cell effect described here.

Summary

The immunocompetent subpopulation by mouse thymus cell (TH-2) was isolated by buoyant density centrifugation and by hydrocortisone pretreatment. TH-2 cells undergo a proliferative one-way or two-way mixed lymphocyte culture (MLC) response only when cultured with allogeneic or congenic peripheral lymphoid cells. However, mixtures of allogeneic TH-2 cells alone do not proliferate in either one-way or two-way MLC reactions. Such MLC mixtures are proliferative only if mitomycin-blocked peripheral lymphoid cells are also present in the mixture. The peripheral helper cell has been found to be of low net density, nonadherent, insensitive to anti-thy-1 serum cytotoxicity, but sensitive to the cytotoxic effects of anti-immunoglobulin serum plus complement. The helper effect does not depend on proliferation nor does it appear to involve demonstrable soluble mediators. The nature of failure of MLC between TH-2 subpopulations appears to be dependent on the expression of some product of the *K, I* regions of the *H-2* locus. Possible mechanisms by which a B-cell-like helper cell triggers TH-2 proliferation are discussed in terms of the present knowledge of specific alloantigen receptor on T and B cells, and the immunoglobulin Fc region receptors on T cells.

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