CELLULAR INTERACTIONS IN THE PROLIFERATIVE RESPONSE OF HUMAN T AND B LYMPHOCYTES TO PHYTOMITOGENS AND ALLOGENEIC LYMPHOCYTES

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The cellular events leading to proliferation of cultured human lymphocytes after in vitro stimulation with phytomitogens or allogeneic cells are poorly understood. Little is known on the nature of the proliferating lymphocytes, or whether interactions between different cell types are required for blastogenic transformation of the eventually responding lymphocytes. Investigating these questions, attention has to focus primarily on monocytes, and the different lymphocyte subpopulations.

The role of the monocyte (or the monocyte-derived macrophage [1, 2], respectively) in antigen-induced lymphocyte activation continues to be of great interest. Lymphocyte response to many antigens requires the presence of monocytes. Removal of glass-adherent cells abolishes the in vitro antibody formation by mouse spleen cells against sheep erythrocytes (3) and antigen-induced transformation of human lymphocytes (4); addition of macrophages restores the lymphocyte reactivity (5, 6). Macrophage-lymphocyte interaction is also required for lymphocyte activation by allogeneic histoincompatible lymphocytes in vitro, the mixed leukocyte culture (MLC)[1] (7–11). In contrast, lymphocyte activation by plant mitogens such as phytohemagglutinin (PHA), concanavalin A (Con A), or pokeweed mitogen (PWM) is widely attributed to direct interaction of the soluble mitogen with lymphocyte membrane receptors (12–15). Opinions about the role of monocytes in the mitogen-induced lymphocyte proliferation are controversial: reports that phagocytic cells inhibit the lymphocyte response to PHA (16) conflict with others that removal of phagocytic cells decreases lymphocyte activation by this mitogen (17, 18). Alter and Bach described potentiation of PHA-induced lymphocyte proliferation by monocytes (11), whereas Oppenheim and co-workers (4) observed this effect only at suboptimal doses of PHA.

Lymphocytes can be separated into thymus-dependent (T) and thymus-independent (B) cells on the basis of ontogeny, function, and cell surface differentiation markers. Relatively small fractions of B or T cells from immunized animals will respond to the sensitizing antigens (19, 20), whereas a large fraction of the lymphocyte inoculum will be stimulated in vitro by phytomitogens (21, 22). Therefore, the lymphocyte response to phytomitogens is generally considered to be nonspecific,

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[1] *Abbreviations used in this paper:* Con A, concanavalin A; HBSS, Hanks' balanced salt solution; MLC, mixed leukocyte culture; N-SRBC, neuraminidase-pretreated sheep erythrocytes; PHA, phytohemagglutinin; PWM, pokeweed mitogen.

i.e., to be polyclonal as compared to the clonal lymphocyte proliferation during a specific immune response. Animal studies provide evidence that the polyclonal response to phytomitogens may be specific for certain lymphocyte subpopulations, so that they elicit either selective T- or B-cell proliferation. If a similar specificity of mitogens for lymphocyte subpopulations existed in man, this would provide a useful tool in the evaluation of immunologic responsiveness.

The response of lymphocytes from patients with selective immunodeficiencies to phytomitogens represents one approach to determine the specificity, if any, of any mitogen for human T or B cells. However, owing to the rarity of these diseases, and possible unrecognized defects of the apparently normal residual lymphocytes, such studies are limited in number, and their results cannot readily be generalized. Furthermore, interactions between T and B cells during the proliferative response in vitro may not be recognized under such conditions. However, the distinctive property of human T cells to form rosettes with unsensitized sheep erythrocytes (SRBC) (23, 24) offers a means to separate normal human peripheral blood T and B lymphocytes (25, 26), and thus to approach this problem.

The present studies were therefore designed to determine the mitogenic effects of plant mitogens, and allogeneic lymphocytes on separated human T and B cells in vitro, in order to obtain information on the initially mitogen-sensitive lymphocyte subpopulation(s) in man; furthermore, possible cellular interactions during the proliferative response were investigated. The present report will demonstrate that the most commonly used mitogens PHA, Con A, and PWM, as well as allogeneic lymphocytes stimulate separated human T but not isolated B cells. However, B cells can respond to phytomitogens but not to histocompatibility antigens in the presence of T cells; proliferation of T cells is not a requirement for this T-cell-mediated B-cell proliferation. We further demonstrate that two mechanisms contribute to T-lymphocyte activation by mitogens: direct lymphocyte-mitogen interaction, and stimulation of lymphocytes by mitogens processed by or bound to the surface of monocytes. The second, monocyte-dependent mechanism is more predominant with lower doses of mitogens.

Materials and Methods

Lymphocyte Separation.—Lymphocytes were prepared from heparinized venous blood of healthy adult donors by centrifugation over a Ficoll-Hypaque gradient (27). This preparation regularly contained more than 90% lymphocytes, the rest being monocytes and an occasional granulocyte (termed "monocyte-containing lymphocytes").

Preparation of Monocyte-Free Lymphocyte Suspensions.—To remove phagocytic cells, 50 ml heparinized whole blood were incubated with 250 mg sterile carbonyl iron powder in a 250-ml tissue culture flask (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.) for 60 to 90 min in a water bath at 37°C with continuous vigorous agitation. Engulfment of iron particles into all phagocytic cells (granulocytes and monocytes) was confirmed in stained smears before the

blood was centrifuged over a Ficoll-Hypaque gradient as above. Due to their greater specific gravity, iron-loaded monocytes are centrifuged to the pellet of the Ficoll-Hypaque layer; thus, this procedure yields lymphocyte preparations depleted of monocytes (less than 0.1% monocytes; termed "monocyte-depleted lymphocytes").

Preparation of Monocyte Layers.—Four parts heparinized whole blood were mixed with one part dextran (mol wt 115,000), and the red cells allowed to sediment for 45 min. White cell and differential counts on the white cell rich supernatant were done, the cells irradiated (2,500 rads), and adjusted with culture medium (see below) to various monocyte concentrations. 0.5-ml aliquots of these suspensions were added to glass culture test tubes, and incubated at 37°C for 6 days. As described by Hersh et al. (5), phagocytic cells (monocytes and granulocytes) will adhere firmly to the glass. Granulocytes disappear within 24–48 h after cultures are established, whereas monocytes gradually transform into a macrophage layer. After the 6-day incubation, the nonadherent lymphocytes were removed by vigorous agitation with two changes of culture medium (2 ml each). Cells remaining in the culture tubes after this procedure were almost exclusively monocyte-derived macrophages attached to the glass walls of the culture tubes, as confirmed in slides kept on the bottom of petri dishes under identical conditions.

Lymphocyte Surface Immunoglobulin Staining.—Direct immunofluorescent staining of lymphocyte surface immunoglobulins with a polyvalent fluorescein-conjugated goat antihuman immunoglobulin (Grand Island Biological Co., Grand Island, N.Y.) was performed as described previously (28).

Separation of Rosetting and Nonrosetting Lymphocytes.—Rosette formation between human T lymphocytes and sheep erythrocytes was performed using a modification of the method reported by Wybran et al. (25). Neuraminidase-pretreated sheep erythrocytes (N-SRBC) were used for rosette formation since they bind more firmly to human T lymphocytes than untreated SRBC (28, 29). SRBC (100,000/mm^3) in Hanks' balanced salt solution (HBSS) were incubated at 37°C for 30 min with vibrio cholerae neuraminidase (50 U/ml; obtained from Schwarz/Mann Div., Becton, Dickinson and Co., Orangeburg, N.Y.), washed twice, and resuspended to their original concentration in HBSS. 0.25 ml lymphocytes in HBSS (10,000/mm^3), 0.25 ml fetal calf serum (absorbed with SRBC; Microbiological Associates, Bethesda, Md.), and 0.5 ml N-SRBC (100,000/mm^3) were added to plastic test tubes, centrifuged for 8 min at 200 g, and further incubated at room temperature for 60 min. The gently resuspended pellets of all tubes were pooled, and centrifuged over a Ficoll-Hypaque gradient (30 min at 400 g). This procedure separates rosetting from nonrosetting cells owing to the greater specific gravity of the rosettes (rosetting cells form the pellet, whereas nonrosetting cells remain at the interface between the two layers). Morphologically, the pellet population (termed "rosetting cells" in this paper) consisted only of small lymphocytes (T cells). They were washed

twice in HBSS, and resuspended in culture medium (see below) to the concentration needed. In order to minimize manipulations of lymphocytes, no efforts were made to lyse or remove the N-SRBC present in this preparation. The interface cells were washed, re-rosetted and again centrifuged over a Ficoll-Hypaque layer as described above, in an effort to remove any residual rosetting cells. The interface cell population obtained after this second procedure (termed "nonrosetting cells" in this context) consisted of 80–85% small lymphocytes (B cells), 15–20% monocytes, and an occasional eosinophil or basophil granulocyte.

Lymphocyte Culture Methods.—1-ml cultures were set up in duplicate or triplicate. Culture medium consisted of 400 ml Eagle's minimal essential medium (MEM) supplemented with 75 ml decomplemented pooled human serum, 10 ml L-glutamine (200 mM), 10 ml streptomycin (5,000 U/ml)–penicillin (5,000 U/ml), and 5 ml nonessential amino acids (all obtained from Flow Laboratories, Rockville, Md.). The concentration of responding cells per culture was 2×10^5 when unseparated lymphocytes, or separated T or B lymphocytes were used; where T and B cells were recombined as responding cells, 2×10^5 of each were present per culture. Lymphocytes were stimulated by addition of different concentrations of mitogens in 0.1 ml MEM, or of 0.1 ml of a suspension of allogeneic histoincompatible lymphocytes (2×10^6/ml). To obtain one-way stimulation in MLCs, stimulating allogeneic lymphocytes were irradiated immediately before mixing them with the responding cells (2,500 rads at a dose rate of 1,666 rad/min from a cesium source [Kewaunee Scientific Equipment Corp., Adrian, Mich.] The cultures were maintained in a humidified 5% CO_2-air atmosphere at 37°C.

Lymphocyte stimulation was assessed from the uptake of tritiated thymidine ([^{3}H]TdR) into DNA during the last 4 h in culture. Phytomitogen-stimulated cultures were harvested on day 3, and MLCs on day 7, as described previously (22).

Mitogens.—The content of one vial of PHA-P (Difco Laboratories, Detroit, Mich.) was diluted in 5 ml MEM, and stored frozen in aliquots at −20°C until use. 0.005–0.0005 ml of this stock solution, diluted to 0.1 ml with MEM, was added per 1-ml culture. Similarly, the content of one vial PWM (Grand Island Biological Co., Grand Island, N.Y.) was dissolved in 10 ml MEM, and stored frozen at −20°C until use. 0.03 or 0.0015 ml of this stock solution, diluted to 0.1 ml with MEM, were added per culture. Con A (Jackbean phytohemagglutinin; Calbiochem, San Diego, Calif.) was diluted to 10 mg/ml in MEM, and stored frozen at −20°C until use. 2–50 µg Con A, diluted to 0.1 ml with MEM, were added per culture.

RESULTS

The procedure utilized in the present studies for separation of rosetting and nonrosetting mononuclear cells was highly effective: preparations of nonrosetting cells contained less than 1% rosetting lymphocytes; and less than 2%

of the rosetting lymphocytes carried detectable mounts of surface immuno-globulins.

Preparations of rosetting cells contained 30–35 N-SRBC per lymphocyte. Orienting experiments showed that lymphocyte stimulation by phytomitogens or allogeneic lymphocytes was unaltered by addition of N-SRBC in this ratio.

T cells did not stimulate allogeneic monocyte-depleted cells, a setting where as little as 1% monocytes are sufficient to elicit a lymphocyte response (Table II). Thus, functional criteria confirm the morphological finding (see under Methods) that they were monocyte depleted.

When rosetting (T cells) and nonrosetting (B cells and monocytes) cells were stimulated with mitogens or allogeneic lymphocytes, B cells yielded negligible proliferative responses which may be attributed to residual contaminating T cells. In contrast, T cells showed high uptake of [³H]TdR (Table I). It was observed that unseparated lymphocytes, although containing fewer T lymphocytes per culture, responded with greater [³H]TdR uptake to all stimulants. When the unresponsive nonrosetting cells were recombined with T cells, the proliferative response was greatly in excess of that expected from the proliferation of the separated two fractions (Table I), indicating a synergy between cells in the two populations in response to the mitogens.

Two possible causes for this enhanced phytomitogen-induced lymphocyte transformation were considered: either, helper cells among the nonrosetting cells, themselves not capable of blastogenic transformation, processed mitogens for greater T-lymphocyte stimulation; and/or B cells were secondarily triggered into proliferative response in the presence of (activated?) T cells.

The latter possibility could be studied by irradiating either T or B cells (5,000 rads) before they were recombined for phytomitogen stimulation. Pro-

TABLE I

*Interactions between T and B cells in the Proliferative Response In Vitro**

Responding cells‡ (2 × 10⁶)	Stimulation with:			
	PHA (0.005 ml)	Con A (50 μg)	PWM (0.03 ml)	Allogeneic lympho-cytes§ (2 × 10⁵)
B	2,208 ± 497	1,957 ± 119	1,263 ± 340	2,048 ± 163
B irr	289 ± 30	198 ± 23	157 ± 27	296 ± 67
T	99,279 ± 3,583	44,843 ± 883	34,659 ± 1,319	6,197 ± 458
T irr	8,340 ± 1,105	313 ± 61	403 ± 4	188 ± 14
B + T	766,204 ± 21,330	602,632 ± 28,549	298,071 ± 25,776	19,756 ± 2,969
B + T irr	153,810 ± 17,729	136,788 ± 2,313	52,150 ± 7,986	1,073 ± 57
T + B irr	584,525 ± 51,670	471,850 ± 4,285	230,884 ± 30,884	18,433 ± 992
Unseparated Lymphocytes	254,655 ± 5,185	268,905 ± 2,690	119,717 ± 9,871	12,273 ± 3,204

* Responding cells were set up in 1-ml cultures, and stimulated with mitogens or allogeneic cells contained in 0.1 ml vol. Indicated are the means ±SD of the [³H]TdR uptake (dpm) in triplicate cultures.

‡ B, nonrosetting mononuclear cells; morphologically, 85% small lymphocytes, and 15% monocytes. B irr, irradiated (5,000 rads) nonrosetting cells. T, E-rosetting cells; morphologically, exclusively small lymphocytes. T irr, irradiated (5,000 rads) E-rosetting cells.

§ Prepared by centrifugation of whole blood over a Ficoll-Hypaque gradient; contained 8% monocytes.

TABLE II

*Effect of Monocytes on Blastogenic Transformation of Human Lymphocytes**

Stimulant	Monocyte-con-taining lympho-cytest (2 × 10⁵)	Responding cells			
		Monocyte-depleted lymphocytes‡ (2 × 10⁵) plus monocytes§			
		None	2 × 10²	2 × 10³	2 × 10⁴
PHA (0.005 ml)	403,300 ± 48,115	66,001 ± 2,362	61,114 ± 9,385	106,271 ± 16,215	432,050 ± 29,878
Con A (50 μg)	285,782 ± 65,179	99,940 ± 26,493	109,096 ± 7,463	151,307 ± 12,296	363,996 ± 50,819
PWM (0.03 ml)	141,096 ± 13,320	73,057 ± 1,487	67,323 ± 10,700	79,442 ± 736	173,937 ± 4,280
Allogeneic T cells (2 × 10⁶)	5,365 ± 2,234	828 ± 329	609 ± 83	1,297 ± 418	5,866 ± 2,478

$\vdash$———NS———$\dashv$

$\vdash$————$P < 0.01$————————$\dashv$

$\vdash$————————————$P < 0.001$——————$\dashv$

* See asterisk footnote in Table I.

‡ Monocyte-containing lymphocytes: 10% monocytes, and 90% small lymphocytes. Prepared by centrifugation of whole blood over a Ficoll-Hypaque gradient. Monocyte-depleted lymphocytes: monocytes removed by centrifugation over a Ficoll-Hypaque gradient after incubation with carbonyl iron powder (see Materials and Methods).

§ Added as part of a suspension of autologous-irradiated (5,000 rads) lymphocytes; with the mitogens listed, none of the three monocyte preparations alone gave more than 800 dpm.

liferation under these conditions was attributed to the nonirradiated cell component. B lymphocytes, unresponsive to mitogens when purified, showed a considerable mitogen-induced proliferation in the presence of (irradiated or nonirradiated) T cells (Table I). This suggested that both T and B cells took part in the proliferative response to phytomitogens observed after stimulation of T + B cell mixtures, the T-cell proliferation contributing the greater proportion of the total proliferation. This conclusion was supported by the fact that the sum of [³H]TdR uptake after stimulation of B + T irr, and of T + B irr mixtures was almost identical to that of T + B mixtures (Table I). As little as 5 % T cells were sufficient to obtain mitogen-induced B-cell proliferation.

The existence of mitogen-processing "helper cells" was suggested by observations (Table I) that irradiated nonrosetting cells (consisting of B cells and monocytes) greatly enhanced the T-cell proliferative response. This enhanced T-cell proliferation was not observed (data not shown) when the nonrosetting cells had been prepared from monocyte-depleted lymphocytes, which strongly suggested monocytes to be the cause of the potentiated T-cell proliferation. The helper cells among the nonrosetting cells, which enhanced the T-cell response to plant mitogens, possessed phagocytic function: monocyte-depleted lymphocytes were combined with different numbers of autologous irradiated (5,000 rads) monocyte-containing or monocyte-depleted lymphocytes, and stimulated with various mitogens. As can be seen in Table II, the proliferative lymphocyte response to mitogens decreased markedly with removal of monocytes, but could be restored by addition of the irradiated monocyte-containing lymphocyte suspension. In contrast, addition of irradiated monocyte-depleted lymphocytes did not enhance lymphocyte reactivity (data not included in Table II).

The helper cells were conclusively shown to be monocytes in the following experiment: monocyte layers were established as described under Materials and Methods, whereupon autologous T cells and phytomitogens were added; monocytes enhanced the T cells' response to phytomitogens in a dose-dependent fashion, as listed in Table III.

T-lymphocyte proliferation with different concentrations of mitogens and monocytes was further investigated: T-cell stimulation in the presence of different concentrations of monocytes was compared to that observed in the absence of monocytes. It was found that monocytes enhanced the T-lymphocyte response to mitogens relatively more at lower concentrations of the three mitogens. Thus, addition of 10% monocytes increased the [^{3}H]TdR uptake into T cells 30-fold (from 11,938 +/−480 to 363,151 +/−20,829 dpm) after stimulation with 0.001 ml PHA; but [^{3}H]TdR uptake increased only fivefold (from 93,300 +/− 780 to 461,431 +/− 39,883 dpm) following stimulation with 0.005 ml PHA. Similar data were obtained with Con A and PWM. 0.1% monocytes did not increase the proliferative response of purified T cells, but 10% monocytes greatly enhanced the T-cell proliferation; 1% monocytes had an intermediary but significant effect (Tables II, III).

Irradiation of monocytes with doses as high as 8,000 rads did not affect their capacity to enhance the response of T lymphocytes to plant mitogens (Table IV); their function to promote MLC reactivity was reduced only at radiation doses greater than 6,500 rads (Table V).

DISCUSSION

Rosette formation with SRBC, considered as a distinctive feature of human T lymphocytes (24, 30), was utilized in the present studies for effective separation of rosetting (T) and nonrosetting (B) peripheral blood lymphocytes, as determined by surface differentiation markers, and by the completely different responses to phytomitogens. Similarly efficient separation of human T and B cells by E-rosette sedimentation was recently reported by other investigators (26). The purity of the T- and B-cell preparations therefore allowed us to determine their respective responsiveness to phytomitogens and allogeneic cells.

From animal studies and clinical observations in selective immunodeficiency diseases, PHA, and allogeneic lymphocytes are generally considered to induce proliferation of T lymphocytes (reviewed by Daguillard [31]). The present studies confirm the human T cell as the target cell of PHA: this mitogen elicited a proliferative response of purified T cells, whereas purified B cells were unresponsive. The data in Table I further identify T cells as the responding lymphocyte subpopulation in human MLCs, which is in accordance with findings in mice (32, 33) and rats (34); it is also consistent with results derived from a different experimental system that demonstrated that T but not B cells can recognize foreign histocompatibility antigens (35).

From animal studies, there is general agreement that T but not B cells are directly activated by soluble Con A (12, 36–40). The present studies extend

TABLE III

Enhancement of Mitogen-Induced T-Cell Proliferation on Monocyte Layers*

Stimulant	Responding cells					
	2 × 10⁶ T lymphocytes‡ plus monocytes§					Unseparated lymph-ocytes‖ (2 × 10⁶)
	None	2 × 10²	2 × 10³	2 × 10⁴	2 × 10⁶	
None	457 ± 55	523 ± 52	735 ± 111	555 ± 177	513 ± 52	2792 ± 248
PHA (0.0025 ml)	7,802 ± 1,370	9,254 ± 1,533 (1.2)	20,593 ± 1,862 (2.6)	182,904 ± 16,311 (23.4)	658,155 ± 46,594 (84.4)	167,490 ± 9,954
Con A (20 µg)	10,835 ± 1,395	7,201 ± 1,957 (0.7)	28,907 ± 5,813 (2.7)	508,635 ± 30,971 (46.9)	ND	117,097 ± 10,121
PWM (0.0015 ml)	11,778 ± 612	12,178 ± 316 (1.1)	118,803 ± 9,971 (10.1)	247,155 ± 36,288 (21.0)	247,397 ± 33,170 (21.0)	178,991 ± 5,264

NS P < 0.005 P < 0.001 P < 0.001

* Indicated are the means ± SD of the [³H]TdR uptake (dpm) in triplicate cultures. Figures in parentheses indicate the stimulation ratio over cultures containing no monocytes (first column).

‡ T cells: mononuclear cells forming rosettes with unsensitized, neuraminidase-treated sheep erythrocytes (morphologically, exclusively small lymphocytes).

§ Prepared as monolayers; for details, see Materials and Methods. With the doses of mitogens listed, all three of the monocyte layer preparations yielded less than 900 dpm in the absence of T lymphocytes.

‖ Prepared by centrifugation of whole blood over a Ficoll-Hypaque gradient; contained 11% monocytes.

TABLE IV

Effect of Irradiation on Monocyte-Induced Enhancement of the T-Cell Response to Mitogens *

| Radiation dose‡ (rads) | Stimulation with: | |
	PHA (0.005 ml)	Con A (50 μg)
None	461,431 ± 39,883	ND
4,000	383,814 ± 29,969	139,504 ± 10,979
5,500	411,059 ± 62,407	123,960 ± 13,815
6,500	349,814 ± 120,129	129,384 ± 11,759
8,000	392,078 ± 5,234	135,889 ± 1,801

* A mixture of 2×10^5 T cells and 2×10^4 monocytes (total vol 1 ml) was stimulated with the indicated mitogens. Monocytes were added as part of autologous-irradiated (5,000 rads) nonrosetting mononuclear cells (17% monocytes, 83% small [B] lymphocytes). Indicated are the means ± SD of the [³H]TdR uptake (dpm) in triplicate cultures.

‡ Radiation dose applied to the monocytes before they were added to the T cells.

TABLE V

Effect of Monocyte Irradiation on MLC Reactivity *

Monocyte radiation dose	dpm
None	75,854 ± 4,784
2,500 rads	72,010 ± 18,270
4,500 rads	67,234 ± 12,126
6,500 rads	76,336 ± 8,544
8,000 rads	48,437 ± 6,112
10,000 rads	38,362 ± 3,835

* Allogeneic histoincompatible, monocyte-depleted T cells were used as stimulating and responding cells, respectively (2×10^5 of each; total culture vol 1 ml). 2×10^4 monocytes, autologous to the responding cells and contained in a preparation of nonrosetting cells, were irradiated as indicated, and added to triplicate cultures. Indicated are the means ± SD of the [³H]TdR uptake (dpm).

these findings to man: they indicate that T cells are the primary Con A responsive cells among human peripheral blood lymphocytes, whereas purified B lymphocytes are unresponsive to this mitogen.

Our finding that separated T but not B cells respond to PWM may add to the controversy which exists about the target cells of PWM. Strong evidence exists that in various animal species isolated T and B cells do both respond directly to PWM (36, 41, 42). However, thymic dependence of PWM-stimulated cells in rats (43) and mice (44) has also been reported. If, as our data indicate, human T cells are the only directly PWM-sensitive cells, PWM-induced proliferation of human B cells observed by other investigators (45, 46) has to be attributed to T-cell-mediated B-cell triggering (see below). Support for our results comes from studies in children with thymic aplasia whose lymphocytes respond poorly or not to PWM (Lawton A. III, personnal communication), from the demonstration of a normal lymphocyte response to PWM in B-cell-depleted children with congenital sex-linked agammaglobulinemia (47, 48), and from observa-

tions of decreased lymphocyte reactivity to PWM in T-cell deprived animals (49).

A most interesting finding was that B cells, although unresponsive when purified, underwent considerable phytomitogen-induced proliferation in the presence of as little as 5% T cells. This T-cell-mediated B-cell proliferation occurred whether T cells proliferated or not. These data emphasize that the cellular proliferative events occurring after mitogenic stimulation of purified lymphocyte subpopulations are not necessarily identical to those that are observed after stimulation of mixed T + B cell populations with the same mitogens: cellular cooperation may lead to proliferation of cells which by their nature are not responsive when they are isolated. Recent studies in experimental animals, reporting participation of B cells in the proliferative response to "T cell" stimulants such as PHA (50–53), Con A (50), and allogeneic lymphocytes (51, 52, 54), as well as the above-mentioned reports of PWM-induced proliferation of human B cells in unseparated lymphocyte preparations (45, 46), have most likely to be explained by such T-cell-mediated B-cell proliferation. B-cell proliferation under these conditions may be attributed to the release of soluble mediators by T cells, enabling B cells to respond to the stimulants (55), or to focussing of the mitogens on T-cell surfaces (50, 56, 57). It is noteworthy that, in contrast to animal studies (52–54, 58), the present studies did not reveal a similar T-cell-mediated B-cell proliferation in human MLCs.

Our data confirm earlier reports (7–11) that monocytes play an essential role in the initiation of MLC-reactivity: monocyte-depleted lymphocytes were not stimulated by allogeneic T cells, but this nonreactivity was converted to strong stimulation by 1–10% monocytes (Table II). The function of monocytes to promote lymphocyte reactivity in MLC is highly radioresistent, as shown in Table V.

Radioresistent and phagocytizing cells among the nonrosetting cells, identified to be monocytes, enhanced mitogen-induced T-cell proliferation in a dose-dependent fashion. Monocyte-derived macrophage layers, exposed to phytomitogens in vitro and then washed free of extracellular soluble mitogen, induce blastogenic transformation of autologous lymphocytes (59; Lohrmann, Novikovs, and Graw, unpublished observation), which demonstrates the capacity of monocytes to transfer a mitogenic stimulus to the responsive lymphocytes. Inhibition of mitogen-induced lymphocyte proliferation, as described by Folch and co-workers (60) in rat lymphocyte cultures at macrophage concentrations greater than 5%, was not observed in the present studies.

The controversy in previous reports on the role of monocytes in the response of lymphocytes to soluble phytomitogens (5, 11, 16, 17) may well be explained by varying degrees of monocyte contamination of the "purified" lymphocyte preparations utilized by the different investigators, as pointed out by Alter and Bach (11). The purified T-cell preparations used in the present studies, containing insufficient monocytes to allow MLC activation, showed a pro-

liferative response to all three tested phytomitogens. This implies that direct T-cell-mitogen interaction is efficient to stimulate T cells, and that monocytes are not an essential part of mitogen-induced lymphocyte activation. However, the potentiation of the T-cell response in the presence of monocytes implies that a second, monocyte-dependent mechanism for lymphocyte activation exists. Both mechanisms contribute to the total lymphocyte response when the usual Ficoll-Hypaque-separated, monocyte-containing lymphocyte preparations are stimulated with phytomitogens. This monocyte-dependent lymphocyte activation is relatively more effective with lower concentrations of phytomitogens.

One may speculate how monocytes enhance the phytomitogen-induced T-cell proliferation. They may take up the mitogens into their cytoplasm, transforming them into a more mitogenic form, before transferring them to responsive lymphocytes. Such a mechanism has been demonstrated for specific antigens (61). Alternatively, monocytes may concentrate mitogens locally on their surfaces; such mitogen focusing appears to represent a potent stimulus for responsive lymphocytes (56, 57). This would explain why monocytes enhance lymphocyte activation better at low concentrations of mitogens: multiple-point attachment of mitogens to adjacent surface receptors with subsequent cross-linking of surface receptor sites apparently is required for lymphocyte activation (K. Lindahl-Kiessling; quoted by Greaves and Bauminger [57]). At low mitogen concentrations, monocytes may still concentrate mitogens on their surface in a density sufficient for lymphocyte activation, when soluble mitogens fail to stimulate lymphocytes by direct mitogen-lymphocyte interaction. Potentiation of lymphocyte response to PHA bound to the surface of lymphocytes and erythrocytes (62), although not seen under the conditions of our experiments, may indicate that mitogen-focusing is not a specific function of monocytes, but rather that monocytes are particularly effective in this regard.

Finally, macrophage-derived soluble mediators may be responsible for the potentiated mitogen-induced lymphocyte proliferation observed in the presence of monocytes, as suggested by Gery et al. (63). These authors observed a mitogenic effect of such "lymphocyte-activating factors"; in contrast, there was no lymphocyte activation on macrophage layers observed in our studies when mitogens were absent, although the same layers greatly enhanced T lymphocyte proliferation after stimulation with mitogens (Table III). Thus, the nature of the monocyte-macrophage action on T lymphocytes remains to be identified.

SUMMARY

In vitro studies were performed to determine the proliferative responsiveness of human peripheral blood thymus-dependent (T) and thymus-independent (B) lymphocytes to phytomitogens and allogeneic lymphocytes. Recombination of T and B cells, with selective inhibition of proliferation of one of the two populations, was used to identify cellular interactions which may contribute to

cell proliferation. The distinctive feature of human T lymphocytes to form rosettes with unsensitized sheep erythrocytes was utilized to separate human peripheral blood lymphocytes into highly purified rosetting (T) and non-rosetting (B) cells. The proliferative response of these separated lymphocyte subpopulations to various stimulants was assessed from the uptake of tritiated thymidine into DNA.

Phytohemagglutinin, concanavalin A, pokeweed mitogen, and allogeneic lymphocytes stimulated separated T cells, whereas no proliferation was observed with the T-cell-depleted B-cell population. This suggests that it is the human T cell which is activated directly by these stimulants. In the presence of T cells (proliferating or nonproliferating), B cells were capable of proliferation following stimulation with phytomitogens, but not in response to histocompatibility antigens. Thus, T-cell-mediated B-cell proliferation contributes to the overall lymphocyte response in phytomitogen-stimulated T + B cell mixtures, but not in human mixed leukocyte cultures. T-cell activation by allogeneic cells required the presence of monocytes; in contrast, the three tested phytomitogens stimulated T cells in the absence of monocytes. This indicates that direct interaction of mitogens with lymphocyte membrane receptors is sufficient to trigger T cells into proliferative response. However, monocytes considerably enhanced the proliferative response of T cells in a dose-dependent fashion; this monocyte-dependent mechanism of T-cell activation was predominant at lower concentrations of phytomitogens, and contributed relatively less at higher mitogen doses. Both, the direct, monocyte-independent, and the indirect, monocyte-dependent T-lymphocyte activation contribute to the total in vitro response of lymphocyte preparations to phytomitogens.

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