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CHARACTERIZATION OF A NON-T, NON-B HUMAN BLOOD LYMPHOCYTE THAT MEDIATES THE ENHANCING EFFECTS OF IMMUNE COMPLEXES ON LYMPHOCYTE BLASTOGENESIS[1]

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Human blood lymphocytes were fractionated by two separate approaches in studies to identify and characterize the cellular mediator of immune complex-induced enhancement of lymphocyte blastogenesis. Fractionated lymphocytes from subjects sensitized to keyhole limpet hemocyanin (KLH) were stimulated with antigen alone or IgG antibody complexed to KLH. Incorporation of ^{3}H-thymidine by these cells was compared with that of unseparated lymphocytes. Lymphocytes with Fc receptors for IgG found in the C3 receptor-negative subset of non-T, non-B cells, previously defined as "L" cells, were required for augmentation of blastogenesis. Tγ cells, B cells, and C3 receptor-positive null cells were ineffective mediators. The mode of action of "L" cells was to enhance T cell proliferation. Neither interaction with immune complexes nor co-culture with T cells induced "L" cells to proliferate. Enhancement of blastogenesis by "L" cells was monocyte-dependent, was unaffected by irradiation, and required KLH to be complexed to IgG antibody. Complexes containing an unrelated antigen had no effect.

"L" cells briefly exposed to KLH-IgG anti-KLH triggered a proliferative response 2-fold greater than similarly pulsed monocytes. However, unlike monocytes, "L" cells were unable to present uncomplexed antigen to sensitized cells. These studies have revealed that certain lymphocytes in the heterogeneous "L" cell fraction of blood lymphocytes may have an important regulatory role in lymphocyte blastogenesis. They appear to function as an accessory cell in a manner that can be distinguished from monocytes. One can consider "L" cells to be "auxiliary accessory cells." A perpetuation of T cell proliferation caused by "L" cells that have interacted with certain immune complexes may be an important factor in the chronicity of some human inflammatory diseases.

Immune complexes have well documented regulatory effects on immune responses. IgG complexed to specific antigens can enhance or suppress lymphocyte proliferation *in vitro* (1–8) and enhance or suppress antibody production *in vivo* (9–12). Recently, we have investigated factors affecting the magnitude of antigen-specific lymphocyte blastogenesis in man. Subjects were immunized with keyhole limpet hemocyanin (KLH),[4] and lymphocytes from these individuals were obtained. We described conditions in which IgG antibodies complexed to KLH elicited a proliferative response significantly greater than that observed with the optimal amount of uncomplexed antigen (13). This enhanced response was abolished when immune complexes were prepared with F(ab')$_2$ antibodies or when cell suspensions were first depleted of lymphocytes bearing receptors for the Fc portion of IgG.

The purpose of the present report is to identify the cell population required for an enhanced proliferative response and to determine the mechanism whereby this population exerts its effect. It will be shown that enhancement is mediated by a non-T, non-B cell found in the C3 receptor-negative subset of null cells, a fraction previously described as "L" cells. Studies have been conducted to learn whether these cells proliferate synergistically with T cells or simply enhance T cell reactivity. Evidence favoring the latter hypothesis has been obtained, and suggests an antigen-presentation mechanism of action. The accessory cell capabilities of "L" cells and macrophages will be compared.

MATERIALS AND METHODS

Source of sensitized lymphocytes. Seven healthy adult volunteers were immunized with KLH. The antigen preparation and immunization schedules have been described previously (13). In brief, the antigen was obtained as an ammonium sulfate precipitated slurry from Pacific Biomarine Supply Company, Venice, Calif., and was further purified by the methods of Campbell and co-workers (14). Subjects were immunized with 500 μg of KLH by injection intradermally and they were boosted at 2 weeks with 50 μg intradermally. All immunized subjects demonstrated delayed hypersensitivity.

Preparation of IgG anti-KLH. This has been described in detail elsewhere (13). In brief, rabbits were immunized with 4 mg of KLH in complete Freund's adjuvant and boosted 4 weeks later with 400 μg of KLH. The rabbits were bled 1 week after the last injection, and IgG fraction of this antisera was prepared by DEAE cellulose chromatography. The IgG fraction was filter-sterilized and stored at 4°C in a final concentration of 1.25

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[4] Abbreviations used in this paper: "L" cells, lymphocytes with membrane-*labile* immunoglobulin determinants, the C3 receptor negative subset of non-T, non-B cells that possess high avidity Fc receptors for IgG; KLH, keyhole limpet hemocyanin; E-RFC, lymphocyte-forming rosette with sheep erythrocytes; EAC, sheep erythrocytes sensitized with IgM antibody and mouse C.

mg/ml protein in phosphate-buffered saline (PBS) and hereafter shall be called IgG-anti-KLH.

Preparation of immune complexes. As described previously (13), a quantitative precipitation curve with ^{125}I-labeled KLH was performed in order to calculate the amount of IgG required to prepare immune complexes at equivalence and to determine the antigen content of the precipitates. One hundred microliters of KLH (1 mg/ml) were mixed with 100 μl of IgG antibody (1.25 mg/ml). This mixture was incubated for 1 hr at 37°C and 24 hr at 4°C. The precipitate was centrifuged at 1000 × G for 10 min, and the pellet was resuspended in 3000 μl of PBS. Twenty-five microliters of this suspension, containing 8 μg of KLH, were added to 0.2 ml cultures of various cell suspensions in wells of microtiter plates to yield an equivalent concentration of 40 μg of KLH per milliliter of culture.

In order to identify the lymphocyte population that was binding immune complexes, KLH-IgG anti-KLH complexes were immobilized onto the plastic surfaces of flat-bottomed microplate wells. Previous studies with radiolabeled KLH had shown that incubation of 25 μg of KLH in 0.2 ml/well for 45 min at room temperature resulted in the binding to the plastic surface of an amount of KLH equivalent to a final concentration of 40 μg/ml in 0.2 ml cultures (13). To form immobilized immune complexes, 10 μg of IgG-anti KLH were subsequently incubated on the bound KLH antigen for 45 min, at room temperature, and unbound IgG was removed by thorough washing of the treated wells.

In other studies, plastic-immobilized bovine serum albumin (BSA)- anti-BSA complexes were made similarly. Solutions of BSA (Grand Island Biological Company, Grand Island, N. Y.) were prepared at 250 μg BSA/ml of PBS. Two-tenths milliliter (containing 50 μg of BSA) was added to the microwells for 45 min at room temperature and the plates were washed repeatedly to remove unbound antigen. Then 20 μg of rabbit IgG-anti BSA (Cappel Laboratories, Cochranville, Pa.) were incubated at the same temperature and time as for antigen binding and the wells were washed thoroughly to remove unbound IgG.

Preparation of mononuclear cell suspensions. Mononuclear cells were obtained from heparinized blood by Ficoll-Hypaque density gradient centrifugation with LSM (lymphocyte separation medium, Bionetics Laboratory Products, Kensington, Md.). Complete details of the cell separation procedures have been described previously (15, 16). The mononuclear cells were incubated at 37°C for 1 hr in human serum-free medium and washed in warmed RPMI 1640 to elute cytophilic IgG (15). One portion of these cells was used in studies as "unseparated mononuclear cells." The remaining cells were monocyte-depleted by incubation on 100 × 15 mm plastic Petri dishes for 45 min at 37°C. Approximately twenty million cells were cultured per dish in RPMI 1640 with 20% fetal bovine serum (Grand Island). This adsorption procedure was repeated another two times to separate adherent and nonadherent cells and both were harvested. At least 96% of the adherent cells were alpha-naphthyl esterase-positive, and shall be termed "monocytes" or "macrophages" interchangeably. After the third depletion step, less than 1% of the nonadherent cells were esterase positive.

Some lymphocyte suspensions were depleted of cells with Fc receptors for IgG by immunoadherence techniques (13). 8×10^6 lymphocytes in 2 ml of medium were added to 60 × 15 mm Petri dishes that had been previously coated with immobilized BSA-anti-BSA immune complexes, incubated for 45 min at room temperature, and the nonadherent cells were recovered. The immobilized complexes were prepared by first incubating 2 ml of BSA solution (5 mg/ml) in the Petri dishes for 45 min at 37°C. After extensive washing with PBS, 2 ml of rabbit IgG anti BSA (1:20 dilution) were added and incubated another 45 min at room temperature. The plates were then thoroughly washed. Less than 1% of the nonadherent lymphocytes formed rosettes with human RBC sensitized with Ripley IgG (17). As reported previously (18), this adsorption procedure did not remove B cells, which, however, have the capacity to form rosettes with ox cells coated with rabbit IgG. The adsorption procedure, therefore, removes only those lymphocytes with high avidity (or density) Fc-IgG receptors. Seventy percent (range 62 to 75%) of the lymphocytes were recovered after this immunoadherence depletion procedure.

In some experiments, Fc-receptor-bearing T or non-T cells were allowed to attach to KLH-anti KLH complexes immobilized to flat bottom microwells (Microtest II, Falcon No. 30401, Oxnard, Calif.); and the regulatory effects of these cells on blastogenesis was investigated. Since T cell preparations contained fewer cells with Fc receptors for IgG than non-T preparations, 0.2 ml of isolated E rosette-forming cells (E-RFC) or 0.1 ml of non-E-RFC (1×10^6/ml) was added to immobilized immune complexes in order to obtain similar numbers of attached cells. The plates were incubated for 1 hr at room temperature and the wells were washed five times to remove nonadherent cells. Then, Fc receptor-depleted lymphocytes (2×10^5/0.2 ml) were added and the microplates were cultured for 5 days. T cells were prepared by isolating E-RFC by the procedure of West and co-workers (19) except that the sheep erythrocytes used were not treated with neuraminidase. All T cell separation procedures were done twice. The T cell fraction contained 98% E-RFC, less than 1% Ig-positive cells, and less than 0.5% esterase-positive cells.

In most experiments, B cells were prepared by negative selection. As described above, non T cells that did not adhere to immobilized BSA-anti-BSA monolayers were called B cells. In some experiments, sheep erythrocytes were sensitized with 19 S antibody and mouse C, and EAC rosette-forming cells were centrifuged through Ficoll-Hypaque as described previously (15). Ninety-six percent (±3%) of the pelleted cells were EAC rosette-forming cells. These preparations contained 85% Ig-positive B cells and 15% were null cells.

"L" cells were prepared by removing EAC rosette-forming cells from non T cell suspensions. EAC monolayers on plastic Petri dishes were prepared as described previously (13) and 8×10^6 cells in 2 ml were added to each dish. The "L" cell fraction contained 72 ± 6% of lymphocytes with high avidity Fc receptors, 7 ± 3% Ig-positive cells, 1% E-RFC, and 1% esterase-positive cells. Eighty percent of the maximal number of "L" cells were recovered.

Lymphocyte proliferative response to KLH and KLH-anti KLH complexes. Unless otherwise stated, 2×10^5 cells in 0.2 ml RPMI 1640 supplemented with antibiotics and 15% heat-inactivated pooled human AB serum (15) were cultured in triplicate in flat-bottomed microtiter plates (Microtest II, Falcon #30401). Culture wells contained antigen, or immune complexes, at a final antigen concentration of 40 μg per ml. The microplate cultures were incubated at 37°C in a humidified, 5% CO_2 atmosphere for 5 days, pulsed with 1 μCi of ^{3}H-thymidine (1.3 Ci/mM) for 4 hr, and harvested. Extent of blastogenesis was determined by liquid scintillation spectrometry.

In some experiments, lymphocytes were irradiated with 2000 rads from a ^{60}Co source (field size 12 × 25 cm, 1.82 rads/min). In other experiments, lymphocytes were pre-treated with mitomycin (Sigma Chemical Company, St. Louis, Mo.) at 25 μg/ml for 30 min. In each experiment cells from a single donor were used.

Pulse experiments with KLH or KLH-IgG anti KLH. 2 ×

10^6 T cells, "L" cells, or macrophages were incubated for 2 hr at 37°C with 20 μg of uncomplexed or complexed KLH. The cells were then washed twice and centrifuged over a cushion of FCS to remove unbound immune complexes. The pulsed cells were adjusted to 1×10^6/ml, and 5×10^4 T cells, "L" cells, or monocytes in 50 μl were added to microwells containing 2×10^5 mononuclear cells and cultured for 5 days.

RESULTS

Two approaches were used to determine which subset of lymphocytes with Fc receptors mediated the previously described (13) enhancement of antigen-specific blastogenesis induced by IgG antibody complexed to specific antigen.

The first procedure was to positively select Fc receptor-bearing T cells or non-T cells and to test each for mediation of the enhancement phenomenon. Some Tγ cells, as well as the non-T, non-B cells described as "L" cells, have Fc receptors capable of binding to insoluble immune complexes (18). Therefore, equivalent numbers of Tγ cells or "L" cells were attached to immobilized KLH-IgG anti-KLH immune complexes, and a separate suspension of Fc receptor-depleted lymphocytes supplemented with monocytes was added to the microwells. Table I shows that the non-T cell fraction adherent to immobilized immune complexes, but not Tγ, were able to mediate immune complex-induced enhancement. In some experiments, the lymphocytes that were allowed to attach to immobilized immune complexes had been previously irradiated with 2000 rads. No differences were observed between irradiated and nonirradiated Tγ and "L" cells.

In a second approach, "L" cells and B cells were enriched by negative selection and the effect of each population on T cell reactivity to antigen was examined. Table 2 shows that combinations of "L" cells and T cells (supplemented with 10% monocytes) reproduced the immune complex-enhancement phenomenon observed with unseparated cells. This effect was noted with physiologic proportions of T to "L" cells (7:1) and at lower T:"L" cell ratios (3:1). Although T cell and B cell mixtures responded as well or better to uncomplexed KLH as unseparated mononuclear cells, a further increase in ^{3}H-thymidine uptake induced by IgG antibody complexed to KLH was not observed. In other studies, B cells and C3-receptor-positive null cells were prepared by positive selection of EAC rosette-forming cells. Combinations of these positively selected C3 receptor-bearing cells and T cells did not mediate enhancement (results not shown). These studies provide direct evidence for the participation of the C3 receptor-negative subset of null cells in the enhancement phenomenon.

Next, studies were undertaken to define the cellular mechanism involved. The first question was whether "L" cells can interact with T cells by themselves or whether they require macrophage participation. Figure 1 shows the mean of three separate experiments and provides evidence that macrophages are required. Highly purified T cells containing less than 0.1%

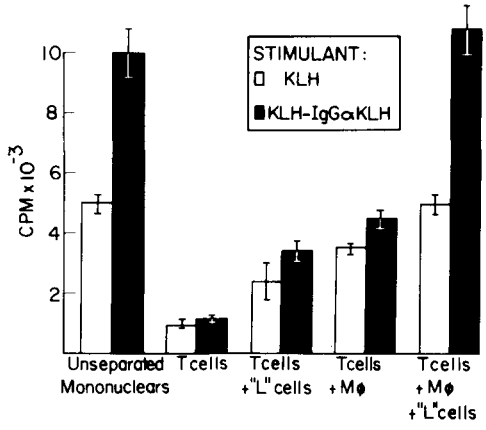

Figure 1. Immune complex-elicited enhancement of antigen-specific blastogenesis requires a combination of T cells, monocytes, and "L" cells. T cells (1.5×10^5) were incubated alone or with 2.5×10^4 "L" cells and/or monocytes (Mφ) and stimulated with uncomplexed KLH or IgG-anti-KLH containing 40 μg/ml of antigen (see *Materials and Methods*). Values indicate the mean ± S.E.M. of three separate experiments.

TABLE I

Non-T lymphocytes are required for enhancement of blastogenesis mediated by immune complexes

Step 1[a]	Step 2[b]	KLH	Immobilized KLH αKLH
		cpm ± S.E.M.	
Expt. A			
None	Unseparated mononuclear cells	7,929 ± 137	15,933 ± 2,402
None	Fc-IgG receptor lymphocyte depleted	6,557 ± 402	8,538 ± 554
Tγ cells adherent to immobilized immune complexes	Fc-IgG receptor lymphocyte depleted		8,821 ± 200
Non-T cells adherent to immobilized immune complexes	Fc-IgG receptor lymphocyte depleted		16,276 ± 1,190
Expt. B			
None	Unseparated mononuclear cells	4,243 ± 276	8,397 ± 149
None	Fc-IgG receptor lymphocyte depleted	3,640 ± 311	4,914 ± 166
Tγ cells adherent to immobilized immune complexes	Fc-IgG receptor lymphocyte depleted		5,118 ± 134
Irradiated Tγ cells adherent to immobilized immune complexes	Fc-IgG receptor lymphocyte depleted		5,239 ± 205
Non-T cells adherent to immobilized immune complexes	Fc-IgG receptor lymphocyte depleted		8,044 ± 633
Irradiated non-T cells adherent to immobilized immune complexes	Fc-IgG receptor lymphocyte depleted		8,006 ± 480

[a] Step 1 consisted of allowing purified T cells or non-T cells with Fc-IgG receptors to adhere to KLH-IgG αKLH complexes immobilized in microwells and removing the nonadherent cells.

[b] Step 2 consisted of the addition of 2×10^5 Fc-IgG receptor-depleted cells and 2×10^4 monocytes to the microwells. In Expt. B some lymphocytes were irradiated with 2000 rads.

monocytes were prepared for these studies. "L" cell-T cell mixtures responded poorly to antigen alone or to immune complexes in comparison with unseparated mononuclear cells. The addition of monocytes to T cells restored reactivity to uncomplexed KLH almost to control values, but enhanced stimulation with immune complexes was not observed. A combination of "L" cells, monocytes and T cells completely restored the enhancing effect of immune complexes.

The ineffectiveness of the monocyte- T cell combinations might be explained by insufficient numbers of functional monocytes. Additional studies were performed in which increasing numbers of monocytes were added to T cells. Although 75% of these monocytes prepared for these experiments had Fc receptors detected by rosette formation with human RBC sensitized with Ripley IgG, Figure 2 shows that cultures containing up to 20% monocytes did not respond to KLH-anti-KLH as well as unseparated mononuclear cells.

The next question considered was the blastogenic capabilities of "L" cells. This information was needed to learn whether the increased ^{3}H-thymidine elicited by immune complexes was a result of "L" cell proliferation. Although "L" cells alone could not be induced to proliferate by KLH or KLH-anti KLH (Table II), they might be activated when a combination of immune complexes and the products of stimulated monocytes and/or T cells were present. Studies with mitomycin, however, revealed that "L" cells do not proliferate (Table III).

A third question we addressed was whether specific anti-KLH antibody was required for an augmented response to KLH or whether an unrelated immune complex could also boost the cellular response to uncomplexed KLH. Unseparated mononuclear cells were cultured in microwells containing immobilized BSA-anti-BSA immune complexes. Table IV shows that these immobilized complexes could not induce a proliferative response or enhance T cell reactivity to KLH. Only IgG-anti KLH complexed to KLH had this effect. Thus, a nonspecific immune complex did not provide the appropriate signal to enhance T cell reactivity. The precise amount of either anti-BSA or anti-KLH that bound to the corresponding immobilized antigen was not determined. We estimated that the amount of antibody bound to BSA was at least as much and probably more than that bound to KLH on a molar basis. In fact, the number of Fc receptor cells attaching to immobilized BSA-anti BSA complexes was identical with the number binding to immobilized KLH-anti KLH.

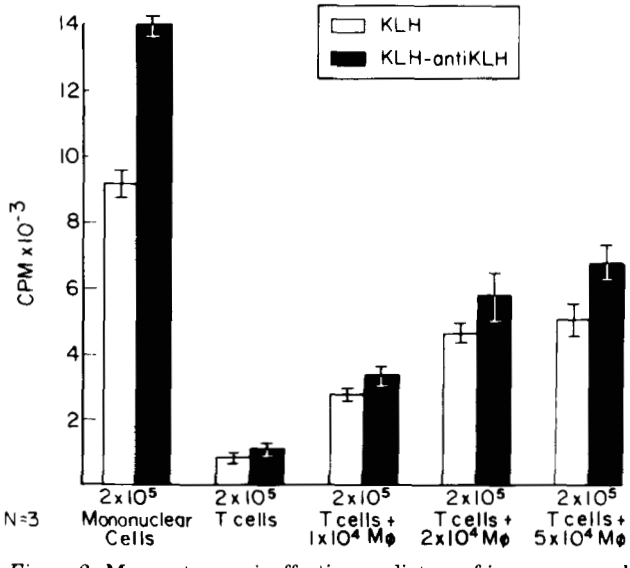

Figure 2. Monocytes are ineffective mediators of immune complex-elicited enhancement. The bars indicate the mean ± S.E.M. of three separate experiments (N = 3).

TABLE II

Blastogenesis of lymphocyte subpopulations stimulated by KLH or KLH-IgG anti-KLH

Population	Unstimulated	KLH	KLH αKLH	p Value[a]
	cpm ± S.E.M.			
Unseparated mononuclear cells	928 ± 50[b]	6,748 ± 1,081	16,732 ± 1,354	<0.001
Purified cells				
T cells	804 ± 54	3,616 ± 378	4,740 ± 394	N.S.[c]
B cells	925 ± 115	1,038 ± 173	1,188 ± 240	N.S.
L cells	538 ± 169	727 ± 170	804 ± 259	N.S.
Cell combinations				
Ratio T to non-T 3:1				
T + B cells	2,128 ± 351	10,489 ± 554	12,782 ± 615	N.S.
T + L cells	1,307 ± 209	6,859 ± 527	16,227 ± 1,233	<0.01
Ratio T to non-T 7:1				
T + B cells	1,584 ± 318	9,134 ± 216	11,134 ± 188	N.S.
T + L cells	1,170 ± 346	6,473 ± 173	15,386 ± 589	<0.01
Other—ratio 1:1				
B + L cells	865 ± 74	940 ± 93	1,048 ± 130	N.S.

[a] Cpm KLH has been compared with cpm KLH αKLH with the use of the Student *t*-Test.

[b] Values represent the mean of four separate experiments. Each well contained 2×10^5 cells and an additional 2×10^4 (10%) monocytes.

[c] Not significant.

TABLE III

L cells enhance T cell reactivity: effect of mitomycin on blastogenic response[a]

Populations	KLH	KLH αKLH
Unseparated mononuclear cells	5,706 ± 208[b]	15,234 ± 1,231
T cells	2,724 ± 860	4,249 ± 125
T + "L" cells	4,931 ± 298	18,614 ± 452
T + "L"$_m$[c]	4,554 ± 946	16,537 ± 507
T_m + "L"	433 ± 70	46 ± 6

[a] Values indicate the mean of three separate experiments. 2×10^4 monocytes were added to cultures of fractionated lymphocytes.

[b] Cpm of unstimulated cultures have been subtracted from the cpm of stimulated cultures. Unstimulated values ranged from 819 cpm (unseparated cells) to 189 cpm (T + L_m cells).

[c] The subscript denotes treatment with mitomycin. The ratio of T cells to L cells was 7:1.

TABLE IV

IgG antibody must be complexed to specific antigen for enhancement of blastogenesis

Additive	Cpm ± S.E.M.
KLH	6,297 ± 698[a]
KLH-IgG anti-KLH	15,909 ± 125
BSA-IgG anti-BSA	54 ± 28
BSA-IgG anti-BSA + KLH	5,229 ± 583

[a] The values shown are after the cpm of unstimulated cells (954 cpm) have been subtracted. Unseparated mononuclear cells were stimulated with the additives indicated above.

We considered the possibility that the "L" cell fraction might have an antigen presentation function analogous to that of macrophages. In order to test this possibility, separate suspensions of T cells, monocytes and "L" cells were pulsed with either KLH, or KLH-IgG anti-KLH for 2 hr, washed, and then mixed with fresh mononuclear cells. Figure 3 shows the mean of four separate experiments. In agreement with findings of

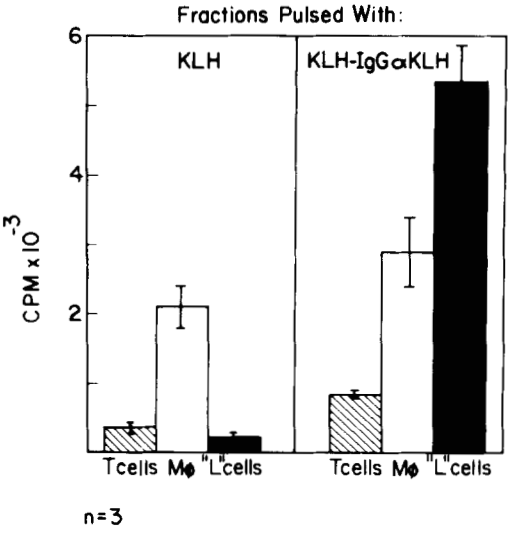

Figure 3. A comparison of the antigen-presenting capacities of monocytes and "L" cells. Five × 10^4 monocytes (Mϕ), "L" cells, or T cells were briefly exposed to either KLH alone or KLH-IgG anti-KLH, washed, and added to microwells containing 2 × 10^5 unstimulated, autologous mononuclear cells (see *Materials and Methods*). Values shown indicate the mean ± S.E.M. of three separate experiments. Cpm of simultaneous cultures not stimulated with antigen have been subtracted from cpm of stimulated cultures.

others in experimental animals (20), human monocytes briefly exposed to KLH stimulated sensitized autologous lymphocytes to proliferate. In sharp contrast, "L" cells pretreated with antigen alone were incapable of triggering a blastogenic response, but capacity of "L" cells pulsed with immune complexes to perform this function was twice that of similarly treated blood monocytes.

DISCUSSION

These studies provide further evidence that three separate cell types contribute to the blastogenic response of human lymphocytes to antigen. Besides various T cell subsets (21–23) and monocytes (24, 25), non-T cells are important determinants in the magnitude of blastogenesis (15, 26). We have studied the modulating properties of immune complexes on antigen-specific blastogenesis and have shown that antigen complexed to specific IgG antibody can accelerate and augment the blastogenic response (13). Lymphocytes with Fc receptors found in the C3 receptor negative subset of non-T, non-B cells (called "L" cells) mediate this effect apparently by presenting antigen to helper T cells. Other subsets of T cells, B cells, and C3 receptor-positive null cells lack this capacity. "L" cells bind the Fc portion of the complexed antibody molecule and may present antigen to T cells as well as to clusters of T cells and macrophages.

A possible relationship between "L" cells and monocytes deserves comment. Both do not proliferate (15). The accessory cell function of monocytes is radiation resistant (27) as is that of "L" cells shown in these studies. These two cell types have previously been distinguished on the basis of ultrastructure, alpha-naphthyl esterase staining, and functional properties (15). In these studies, the two cell types could be distinguished functionally on the basis of their interaction with T cells in antigen presentation: 1) "L" cells could not substitute for monocytes in enabling purified T cells to respond to uncomplexed antigen. 2) Unlike monocytes, "L" cells lacked the capacity to bind uncomplexed antigen to the cell membrane, as demonstrated by their inability to stimulate T cell DNA synthesis after a brief exposure to KLH. 3) The capacity of "L" cells pulsed with KLH-anti-KLH immune complexes to induce a blastogenic response was twice that of similarly treated monocytes. These findings suggest that "L" cells can function as accessory cells in antigen-specific blastogenesis by T cells.

Macrophages have a major antigen-presentation function that includes antigen "processing." The "antigen presentation" function of "L" cells is clearly different. It is macrophage dependent. One may conceive of "L" cells as "auxiliary accessory cells" when antigen complexed to specific IgG antibody is present.

Other workers have established the macrophage requirement for antigen-induced lymphocyte blastogenesis (28). It has also been established that macrophages interiorize most of the surface-bound KLH and leave only a small amount on the cell membrane (29). "L" cells are nonphagocytic (15) and may concentrate a large amount of complexed antigen on the cell membrane. This presumed (but not proven) quantitative difference in membrane-associated antigen may explain the greater capacity of "L" cells pulsed with KLH-IgG/anti-KLH to stimulate a blastogenic response.

The relationship between "L" cells and Tγ cells is unclear. Both possess Fc receptors for IgG that are qualitatively similar (18) and both can mediate antibody-dependent cellular cytotoxity and natural cytotoxity (30, 31). At this time, two functional differences have been described: 1) Tγ cells proliferate in response to concanavalin A (32); "L" cells are non-responsive (15) and 2) only Tγ cells can mediate mitogen-induced cellular cytotoxity (30). The finding that "L" cells but not Tγ cells mediate the enhancement phenomenon described in the present report provides additional evidence that each population has distinct functional properties.

Moretta and co-workers (33) have reported that Tγ cells are induced to become suppressor cells after interaction with immune complexes and that these suppressors are radiosensitive. We considered the possibility that the failure to detect "L" cell-like "enhancers" in the Tγ preparations was due to activated suppressor cells that masked enhancement. However, irradiation of the Tγ cells did not produce enhancement. Since there is evidence that cell proliferation may not be essential for the development of suppressor activity (21–23), the experiments with irradiated cells reported here cannot exclude a role for co-existing suppressor cells in Tγ cell preparations.

It is clear that the enhancement phenomenon is not simply a nonspecific "on signal" triggered by IgG immune complexes that bind to Fc receptors. BSA-anti-BSA immune complexes had no effect on augmenting KLH-specific blastogenesis. Moreover, KLH-anti-KLH complexes could not trigger lymphocytes from nonimmunized subjects to proliferate (13). Whereas Berman and Weigle (34) have reported that free Fc fragments can induce mouse lymphocytes to proliferate, such effects appeared to be directed at mouse B cells, whereas in the present studies, immune complexes did not stimulate purified B cells to proliferate. Although T and B cell synergism in the response to uncomplexed antigen was observed, addition of immune complexes did not enhance ^{3}H-thymidine uptake.

The antigen-presentation role of "L" cells will require further study. It is apparent that this mechanism is more complex than simply the aggregation of antigenic determinants on a cell surface. Aggregation of KLH by F(ab′)$_2$ antibodies or KLH immobilized to plastic surfaces induced stimulation of T cells only slightly more than soluble KLH. One might speculate that passively bound antigen closely linked to Ia-like structures present on certain "L" cells (16) may serve as recognition units

for sensitized cells. Alternatively, immune complexes may stimulate "L" cells to release a soluble factor that enhances T cell proliferation through a macrophage intermediate. This would account for the finding that each of the three cell types must be present for the maximum expression of these modulating effects.

Last, one may speculate about the significance of the phenomenon we have described to human disease. "L" cells or "Third population" cells have been found in the synovial membrane of patients with rheumatoid arthritis (35), and the presence of IgG immune complexes in these synovial fluids is well established (36). There is considerable evidence that T cells play an important role in chronicity of rheumatoid arthritis, but the factors which perpetuate T cell activation are unknown. It is possible that "L" cells bind certain IgG immune complexes and present them to T cells. This cellular interaction between T cells and "L" cells in turn may lead to a perpetuation of T cell proliferation and chronic inflammation.

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REFERENCES

1. Harris, G. 1968. Further studies of antigen stimulation of deoxyribonucleic acid synthesis in rabbit spleen cell culture. II. The effects of specific antibody. J. Immunol. 14:415.
2. Leikin, S., and J. J. Oppenheim. 1971. Differences in transformation of adult and newborn lymphocytes stimulated by antigen, antibody and antigen-antibody complexes. Cell. Immunol. 1:468.
3. Banks, K. 1973. The effect of antibody on antigen-induced lymphocyte transformation. J. Immunol. 110:709.
4. Thorbecke, G. Y., and G. W. Siskind. 1973. Effect of specific antibody on the antigen-induced proliferative response of rabbit lymph node cells. J. Immunol. 110:648.
5. Oppenheim, J. J. 1972. Modulation of *in vitro* lymphocyte transformation by antibodies: enhancement by antigen-antibody complexes and inhibition by antibody excess. Cell. Immunol. 3:341.
6. Jacobs, R. P., R. M. Blaese, and J. J. Oppenheim. 1972. Inhibition of antigen-stimulated *in vitro* proliferation of thymic dependent chicken spleen cells by specific antibody. J. Immunol. 109:324.
7. Hertel-Wulff, B., and B. Rubin. 1976. Lymphocyte proliferation *in vitro* induced by soluble protein antigens. II. Cellular requirements. Eur. J. Immunol. 6:418.
8. Moller, G. 1969. Induction of DNA synthesis in normal human lymphocyte cultures by antigen-antibody complexes. Clin. Exp. Immunol. 4:65.
9. Fitch, F. W. 1975. Selective suppression of immune responses. Regulation of antibody formation and cell-mediated immunity by antibody. Prog. Allergy 19:195.
10. Houston, W. E., C. E. Pedersen, F. E. Cole, and R. D. Spertzel. 1974. Effects of antigen-antibody complexes on primary immune-response in rhesus monkeys. Infect. Immun. 10:437.
11. Morgan, E. L., and C. H. Templis. 1978. The requirement for the Fc portion of antibody in antigen-antibody complex-mediated suppression. J. Immunol. 120:1669.
12. Sinclair, N. R. StC. 1969. Regulation of the immune response. I. Reduction in ability of specific antibody to inhibit long-lasting immunological priming after removal of the Fc fragment. J. Exp. Med. 129:1183.
13. Carvalho, E. M., J. S. Davis, and D. A Horwitz. 1979. Conditions required for Fc-dependent immune complex enhancement of antigen-specific lymphocyte blastogenesis. J. Immunol. 124:5650.
14. Garvey, J. S., N. E. Cremer, and D. H. Sussdorf. 1977. Methods in Immunology. 3rd ed. W. A. Benjamin, Inc., Reading, Mass. Pp. 125–139.
15. Horwitz, D. A., and M. A. Garrett. 1977. Distinctive functional properties of human blood L lymphocytes: a comparison with T lymphocytes, B lymphocytes, and monocytes. J. Immunol. 116:437.
16. Horwitz, D. A., P. Niaudet, M. F. Greaves, J. Dorling, and O. Deteix. 1978. Surface markers and electron microscopy of human blood L cells: a comparison with T and B lymphocytes. J. Immunol. 121:678.
17. Lobo, P. I., and D. A. Horwitz. 1976. An appraisal of Fc receptors on human blood B and L lymphocytes. J. Immunol. 117:939.
18. Horwitz, D. A., M. Cooper, and E. Carvalho. 1979. Binding characteristics of Fc receptors for IgG on human peripheral blood $T\gamma$ lymphocytes and "L" lymphocytes: a technical report. Clin. Immunol. Immunopathol. 14:159.
19. West, W. H., S. M. Payne, J. L. Weese, and R. B. Herberman. 1977. Human T lymphocyte subpopulations: correlation between E-rosette-forming affinity and expression of the Fc receptor. J. Immunol. 119:548.
20. Rosenthal, A. S., P. E. Lipsky, and E. M. Shevach. 1975. Macrophage-lymphocyte interaction and antigen recognition. Fed. Proc. 34:1743.
21. Kurnick, J., T. C. Bell, and H. M. Grey. 1976. PHA induced activation of suppressor cells in normal human peripheral blood lymphocytes. Scand. J. Immunol. 5:771.
22. Shou, L., S. A. Schwartz, and R. A. Good. 1976. Suppressor cell activity after concanavalin A treatment of lymphocytes from normal donors. J. Exp. Med. 143:110.
23. Sakane, T., and I. Green. 1977. Human suppressor T cells induced by concanavalin A. Suppressor T cells belong to distinctive T cell subclasses. J. Immunol. 119:1169.
24. Oppenheim, J. J., G. G. Levanthal, and E. M. Hersh. 1968. The transformation of column-purified lymphocytes with nonspecific and specific antigenic stimuli. J. Immunol. 101:262.
25. Rosenstreich, D. L., and A. S. Rosenthal. 1974. Peritoneal exudate lymphocyte. III. Dissociation of antigen-reactive lymphocytes from antigen-binding cells in a T lymphocyte enriched population in the guinea pig. J. Immunol. 112:1085.
26. Lohrman, H. P., L. Novikovs, and R. G. Graw, Jr. 1974. Cellular interactions in the proliferative response of human T and B lymphocytes to response of human T and B lymphocytes to phytomitogens and allogeneic lymphocytes. J. Exp. Med. 139:1553.
27. Schmidtke, J. R., and F. J. Dixon. 1972. The functional capacity of x-irradiated macrophages. J. Immunol. 108:1624.
28. Seeger, R. C., and J. J. Oppenheim. 1970. Synergistic interaction of macrophages and lymphocytes in antigen-induced transformation of lymphocytes. J. Exp. Med. 132:44.
29. Unanue, E. R. 1971. Antigen binding cells. I. Their identification and role in the immune response. J. Immunol. 107:1168.
30. Pichler, W. J., F. W. Gendelman, and D. L. Nelson. 1979. Fc-receptors on human T lymphocytes. II. Cytotoxic capabilities of human $T\gamma$, $T\mu$, B and L cells. Cell. Immunol. 43:410.
31. Kay, H. D., G. D. Bonnard, W. H. West, and R. B. Herberman. 1977. A functional comparison of human Fc-receptor-bearing lymphocytes active in natural cytotoxicity and antibody-dependent cellular cytotoxicity. J. Immunol. 118:2058.
32. Moretta, L., M. Ferrarini, M. C. Mingari, A. Moretta, and S. R. Webb. 1976. Subpopulations of human T cells identified by receptors for immunoglobulins and mitogen responsiveness. J. Immunol. 117:2171.
33. Moretta, L., S. R. Webb, C. E. Grossi, P. M. Lydyard, and M. D. Cooper. 1977. Functional analysis of two human T-cell subpopulations: help and suppression of B-cell responses by T cells bearing receptors for IgM or IgG. J. Exp. Med. 146:184.
34. Berman, M. A., and W. O. Weigle. 1977. B-lymphocyte activation by the Fc region of IgG. J. Exp. Med. 146:421.
35. Abrahamsen, T. G., S. S. Froland, and J. B. Natvig. 1975. Elution and characterization of lymphocytes from rheumatoid inflammatory tissue. Scand. J. Immunol. 4:823.
36. Winchester, R. J., V. Agnello, and H. G. Kunkel. 1970. Gamma globulin complexes in synovial fluids of patients with rheumatoid arthritis. Partial characterizations and relationship to lowered complement levels. Clin. Exp. Immunol. 6:689.