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Research Article

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Characterization of a Non-T, Non-B Human Lymphocyte (L Cell) with Use of Monoclonal Antibodies

ITS REGULATORY ROLE IN B LYMPHOCYTE FUNCTION

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ABSTRACT These studies investigate the role of L lymphocytes in regulating terminal B lymphocyte differentiation. L cells have abundant Fc IgG receptors and comprise 10–15% of human peripheral blood mononuclear cells (PBMC). L cells lack the conventional markers of B and T lymphocytes and in culture, do not develop into B cells, T cells, or macrophages. Additionally, use of monoclonal antibodies failed to detect on L cells, cell surface antigens specific for B cells, T cells, and macrophages.

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In 14 individuals, L cells when co-cultured with PBMC, enhanced Ig synthesis by 83%±62 SD, and also enhanced cell proliferation. Radiated L cells lost enhancing properties. To study the role of their high density Fc IgG receptors, L cells pretreated with IgG antibody-sensitized erythrocytes were used (i.e., after lysis of rosettes). Such L cells significantly inhibited Ig synthesis (by >50%) despite promoting cell proliferation. Antibody-sensitized erythrocyte-rosetted macrophages did not inhibit Ig synthesis.

Thus, positive and negative influences can be mediated by the same cell, depending on the state of Fc-receptor stimulation. Such cells may play a more prominent role in "feed-back" regulation of Ig synthesis by virtue of having abundant Fc IgG receptors.

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INTRODUCTION

Delineation of normal immune-regulatory mechanism is currently a subject of great interest. Studies in both animals and humans have clearly defined the role of T lymphocytes in both help and suppression of immunoglobulin synthesis (1–4). However, the immune-regulatory role of other non-T lymphocyte subsets has received little attention.

Of particular interest is the immunoregulatory role of L lymphocytes, a non-T, non-B lymphocyte, that is uniformly rich in Fc IgG receptors (5–7). L cells comprise 10–15% of the total lymphocyte population in normal human blood and differ from other "third" population cells by their lack of complement (C3) receptors, their inability to develop membrane immunoglobulin in culture (like immature B cells) and their inability to develop into macrophages in culture (like promonocytes) (7–9, 16). This quantitatively prominent Fc IgG receptor-bearing subset of cells could be a likely candidate in immune regulation, especially in view of studies of Moretta et al. (4) demonstrating that T cells with Fc IgG receptors are suppressors of immunoglobulin synthesis.

Our study is aimed at investigating the functional role of human L cells in regulating B cell differentiation into plasma cells using an in vitro assay system that has previously been used to study both T "helper" and "suppressor" activity in humans (4, 10, 11).

METHODS

Isolation of peripheral blood mononuclear cells (PBMC).[1] PBMC were separated from fresh heparinized blood (50 U

[1] *Abbreviations used in this paper*: Con A, concanavalin A; EA, erythrocytes sensitized with anti-Rh antibody; EAC, erythrocyte sensitized with rabbit IgM antibody and fresh

beef heparin/ml blood) from healthy, adult donors by Ficoll-Hypaque density gradient centrifugation and then finally resuspended in RPMI 1640 (Grand Island Biological Co., Grand Island, N. Y.) containing 80 μg/ml glutamine, 50 μg/ml streptomycin, and 50 μg/ml penicillin (RPMI culture medium).

Identification of mononuclear subpopulations. B lymphocytes were identified by the presence of intrinsic membrane immunoglobulin using a fluorescein-conjugated (FITC) polyvalent goat antihuman immunoglobulin (Ig) (Hyland Diagnostics Div., Travenol Laboratories, Inc., Deerfield, Ill.). Extrinsic surface Ig present on non-B cells that could be confused for B cells were removed by a brief 37°C incubation as described (6). T lymphocytes were enumerated by two techniques: (*a*) binding of lymphocytes to unsensitized sheep erythrocytes to form rosettes (E rosettes) using described methods (6), and (*b*) by indirect immunofluorescence using a T cell monoclonal antibody (IgG2AK) produced by a murine hybridoma cell line (T_{101}, Hybritech Incorporated, La Jolla, Calif.). L lymphocytes were identified as non-T cells bearing Fc IgG receptors but lacking markers of B lymphocytes, i.e., C_3 receptors and surface Ig. Previously used markers of Fc IgG were used (7) i.e., (*a*) binding of cytophilic IgG and (*b*) binding of human O+ erythrocytes sensitized with anti-Rh antibody (EA). Cells with C_3 receptors were identified by a previously used technique (7), i.e., rosette formation with sheep erythrocytes sensitized with rabbit IgM antibody and fresh mouse serum as a source of complement. Macrophages were identified by ingestion of latex particles (0.81 μm Latex, Difco Laboratories, Detroit, Mich.) and by euchrysine staining of lysozymes.

Isolation of lymphocyte subpopulation. Freshly isolated PBMC in RPMI supplemented with 20% fetal calf serum (Grand Island Biological Co.) were initially depleted of macrophages by a single incubation (37°C, 1 h) in plastic petri dishes (10–20 × 10^6 cells/dish). With this technique, macrophage contamination was reduced to <10%. This initial macrophage depletion eliminated the problem of clumping of rosettes and nonrosetted cells that we encountered after overnight incubation in the cold. To isolate L cells, macrophage-depleted PBMC in Ca^{++} and Mg^{++} free Hanks' balanced salt solution (HBSS) were subjected to simultaneous rosetting with a mixture of EAC and E reagents. After an overnight incubation at 4°C, cells were gently resuspended and subjected to density gradient centrifugation with Ficoll-Hypaque. Cells remaining at the interface contained L cells (>80% pure). To isolate T cells, macrophage-depleted PBMC in HBSS were subjected to overnight rosetting with the E reagent. After an overnight incubation at 4°C, cells were gently resuspended and subjected to a slow density gradient centrifugation (200 *g* for 15 min) so that only strongly rosetted cells settled in the pellet. The pellet was then lysed with ammonium chloride to obtain T cells (>95% reform E rosettes). To obtain macrophages, nonadherent cells were removed from plastic dishes by vigorous washing, and then the adherent cells were gently scraped off with a rubber policeman. With this technique, macrophage purity was >83%. Viability of all cell subpopulations was >95% by trypan blue exclusion techniques.

Further purification of L cell subpopulation. L cells as isolated were contaminated with T lymphocytes (3–5% E rosettes) and macrophages (3–6%) but rarely with B lymphocytes (<1%). Removal of contaminating T cells from the L cell subpopulation was achieved by complement-dependent lysis of T cells. An IgG_2 mouse anti-T cell monoclonal antibody (Hybritech Incorporated) and rabbit serum (complement) was used in the cytotoxicity procedure.

Characterization of L cells with monoclonal antibodies. In these experiments, enriched L cell subpopulations were pretreated with the relevant monoclonal antibody, washed twice, stained with FITC-labeled horse anti-mouse IgG (Hybritech Incorporated), and then subjected to EA rosette formation at 4°C. These studies were performed in the presence of 0.02% sodium azide. The percentage of EA-rosetted cells (L cells) containing an FITC-labeled lymphocyte was then determined. For these determinations, freshly prepared wet mounts with cover slips compressed tightly were examined to optimize visualization of the centrally located lymphocyte. Monoclonal antibodies used had the following characteristics and specificities: (*a*) T_{101} specific for all peripheral blood T lymphocytes (Hybritech Incorporated), (*b*) anti-HLA-DR, nonpolymorphic determinants (clone L243, IgG2a, Becton, Dickinson & Co., Div. Falcon Labware, Rutherford, N. J.) (*c*) antimonocyte specific for monocytes but not lymphocytes or HLA-DR antigens (clone 63D3, IgG1-k, Bethesda Research Lab, Rockville, Md.), (*d*) antimonocyte specific for monocytes and a lymphocyte subpopulation (9, 24) (OKMI, Ortho Pharmaceutical Corporation, Raritan, N. J.).

Pokeweed mitogen (PWM)-induced production of intracytoplasmic Ig. In this assay, freshly prepared cells in RPMI containing 20% autologous human sera were incubated with PWM (Grand Island Biological Co., lot C477101) to achieve a final concentration of 1:100 of the stock solution of mitogen. PWM-treated cells were cultured for 8 d (5% CO_2, 37°C) in Falcon plastic tubes (Falcon Labware) and production of intracytoplasmic Ig was determined by making cytocentrifuge preparations of cells as described (12) and staining these preparations with goat antiserum to human Ig (polyvalent, Hyland Diagnostics Div.). Between 1,000 and 2,000 cells were counted to determine the percentage of cells with intracytoplasmic Ig.

Pretreatment of L cells with EA complexes or concanavalin A (Con A). Freshly isolated L cells (3 × 10^5) or macrophages (3 × 10^4) in 0.2 ml RPMI with 10% fetal calf serum were incubated with 0.025 ml of a 2% solution of the EA reagent or human O+ erythrocytes (control). After 1 h incubation at 37°C in 5% CO_2, the erythrocytes in the cell mixture were lysed with ammonium chloride and washed three times with RPMI before use in co-culture experiments. In these experiments, human erythrocytes used to prepare EA reagent were depleted of PBMC by Ficoll-Hypaque so as to prevent an "allogenic" effect.

Isolated lymphocyte subpopulations were pretreated with Con A as described (12). Briefly, 1 × 10^6 cells/ml were cultured in the presence of 32 μg/ml Con A (grade III, Sigma Chemical Co., St. Louis, Mo.). After 48 h, cultured cells were washed twice, then added to the appropriate responder cells.

Basic experimental design. 2 × 10^5 freshly isolated PBMC (nonmacrophage depleted) in 0.25 ml RPMI supplemented with 20% autologous sera were cultured alone (control) or co-cultured with 2 × 10^5 cells of an enriched subpopulation. Culture tubes were then exposed to PWM and allowed to proliferate in 5% CO_2 at 37°C for 8 d before determining intracytoplasmic Ig. In certain experiments, isolated subpopulations were either radiated (3,000 rad) or activated with EA-immune complexes before co-culturing with PBMC. At the end of 8 d, cell cultures were counted for approximate yield and for the percentage of cells with intracytoplasmic Ig. Cell viability at the end of 8-d culture was >90%. Extent of enhancement or suppression of Ig synthesis in co-cultures

mouse serum (complement); FITC, fluorescein-conjugated; HBSS, Hanks' balanced salt solution; PBMC, peripheral blood mononuclear cells; PWM, pokeweed mitogen.

was determined by calculating the total number of intracytoplasmic Ig-bearing cells for cell yields at the end of culture and then using these values in the following equation:

$$\frac{(\text{PBMC} + \text{subpopulation co-culture})}{(\text{PBMC alone}) + (\text{subpopulation alone})}$$

RESULTS

Characterization of L cells with monoclonal antibodies. In these experiments, enriched L cell subpopulations were pretreated with the relevant monoclonal antibodies (specific for either HLA-DR antigens, T cell, or monocyte antigen membrane determinants), stained with FITC horse anti-mouse IgG, and then subjected to EA rosette formation. The percentage of EA-rosetted cells (L cells) labeled with FITC was then determined. Data are depicted in Table I. L cells (EA rosette positive) from the nine subjects, whose cells were later used for immunoregulatory studies, rarely possessed T cell antigens (T_{101}, Hybritech Incorporated), DR antigens (Becton, Dickinson & Co.) and monocyte-specific antigen (BRL). However, a majority of L cells possessed the OKMI monocyte antigen (Ortho Pharmaceutical Corporation) that has been shown to be shared with T_γ cells (9, 24). Because of the latter observation, enriched L cell subpopulations from four subjects were kept in culture in RPMI containing either 20% fetal calf serum or human serum—a technique used by other investigators to check for promonocytes (22). Examination of cultures at varying intervals for up to 10 d did not reveal macrophages as determined by euchrysine staining, esterase staining, and latex bead ingestion.

The lack of stained EA-rosetted cells was not due to the monoclonal antibody complex on stained cells inhibiting rosette formation because the total percentage of EA rosettes using FITC-stained cells was identical to control cells not pretreated with monoclonal antibody. This is particularly relevant for the HLA-DR antigen as several investigators have demonstrated that antibodies to such antigens inhibit binding of complexes to Fc receptors on B lymphocytes (13). However, our studies and those of others (13) indicate that antibodies to HLA-DR antigens do not inhibit binding of EA to Fc receptors on L cells. This may well be explained on the basis that L cells lack HLA-DR antigens (14–16).

Enhancing effect of L cells on PWM-induced differentiation of B cells. Optimal differentiation of human B cells into plasmacytoid cells with PWM stimulation requires T cells and macrophages (4, 10, 17). Hence, to investigate the immunoregulatory role of L cells in this model system, highly purified L cells were co-cultured with PBMC in the presence of PWM. In 14 normal adults studied, L cells consistently augmented both PBMC proliferation (as determined by cell yields) and B cell differentiation into plasmacytoid cells. Representative experiments are exemplified in Table II and Fig. 1. Optimal augmentation was observed when the ratio of L cells to PBMC was 1:1 (Fig. 1). In L cells containing co-cultures, cell yields increased (enhancement ratios varied from 1.4:1 to 2:1), while differentiation into plasmacytoid cells increased by a mean of 83%±62 SD.

In contrast, an E rosette-purified T cell subpopulation either enhanced or suppressed B cell differentiation when co-cultured with PBMC at a ratio of 1:1 (Table II). This variable response with the T cells was, however, abolished when the T cell subpopulation was irradiated before co-culturing with PBMC. Radiated T cells, when co-cultured at a ratio of 1:1 consistently led to enhancement of Ig synthesis, thus confirming previous observations demonstrating that radiation inactivates a subset of suppressor T cells (11). Under the culture conditions used, the degree of enhancement of B cell differentiation by radiated T cells was similar to that obtained with L cells (Table II). However, unlike T helpers, which are radioresistant, L cell enhancement was always abolished (eight experiments) with L cell irradiation.

Enhancement of Ig synthesis by L cells was not class specific. In nine subjects studied, L cells enhanced

TABLE I
Characterization of L Cells Using Monoclonal Antibodies in Double-marker Studies

	Percent EA rosettes with FITC-labeled lymphocytes			
Subjects	*T cell antibody (T_{101})	HLA-DR antibody	Monocyte antibody (BRL)	‡Monocyte antibody (OKMI)
P.L.	0	12	0	85
G.P.	2	8	0	76
O.G.	0.5	0.5	0	78
J.G.	0.5	0.5	0	83
H.M.	0	2	0	70
C.S.	1	0	0.5	68
M.M.	1	1	0	77
R.A.	0.5	0	0	80
J.S.	0	0	0	81

Enriched L cell subpopulations not further depleted of contaminating T cells with monoclonal antibody were pretreated with the relevant monoclonal antibodies, stained with FITC-conjugated horse anti-mouse IgG, and then subjected to EA-rosette formation at 4°C. The percentage of EA rosettes that had FITC-staining lymphocytes was then determined after carefully examining 100 rosetted cells.

* Characteristics of various monoclonal antibodies are detailed in Methods.

‡ OKMI monocyte Antibody is reactive with monocytes, granulocytes; and $T\gamma$ lymphocytes (9, 24).

TABLE II
PWM-induced polyclonal Ig Synthesis of PBMC Effect on Ig Synthesis of Co-culturing PBMC with L Cells

Cells cultured	Intracytoplasmic Ig-staining cells per 1,000 cells recovered						
	P.L.	G.P.	L.B.	O.G.	J.G.	H.M.	C.S.
PBMC alone	220	70	62	45	6	42	6
L alone	10	3	12	17	5	3	1
PBMC + L	200 (1.14)	110 (2.0)	83 (1.55)	72 (1.69)	31 (4.5)	60 (1.76)	15 (2.93)
PBMC + L_{EA}	57 (0.32)	8 (0.14)	90 (1.68)	8 (0.19)	4 (0.58)	5 (0.15)	2 (0.39)
PBMC + T	52 (0.39)	68 (1.62)	56 (1.39)	ND	43 (8.3)	76 (2.99)	0.5 (0.13)
PBMC + T_{Rad}	240	106	97	ND	42	83	22

2×10^5 PBMC were co-cultured with equal numbers of autologous L or T cells in the presence of PWM. Cultures were harvested at 8 d and checked for intracytoplasmic Ig and total cell yields. L cell subpopulations were purified by a cytotoxicity assay to remove T cells. L_{EA} are L cells pretreated with EA complexes. In all subjects, L_{EA}, when cultured alone, had <10 plasmacytoid cells per 1,000 cultured cells recovered. Values of co-cultures containing L cells pretreated with O+ human cells (control) were very similar to that of co-cultures with untreated L cells. T_{Rad} are T cells irradiated with 3,000 rad. Cell yields for irradiated T cells when cultured alone was <20%. Figures in parentheses are ratios to determine extent of enhancement or suppression of Ig synthesis. This is determined from total yields as described in Methods.

both IgM and IgG synthesis, although in two of the nine subjects, there was preferential enhancement of a particular Ig class (Table III).

Effect of pretreatment of L cells with EA complexes. An important characteristic of all L cells is the presence of high density, high avidity Fc IgG receptors (6, 18, 19). It therefore became important to modulate these receptors with immune complexes and determine whether L cell function was affected. Techniques similar to those of Moretta et al. (4) to study the function of Fc IgG receptors on T cells was used. Hence, L cells were initially subjected to rosetting with either O+ human erythrocytes (control) or EA complexes, and then treated with ammonium chloride to lyse the erythrocytes and free the L cells. Addition of such L cells (i.e., pretreated with EA complexes) to PBMC culture led to marked suppression of B cell differentiation in 13 of the 14 subjects studied. Data on seven subjects is depicted in Table II. Significant inhibition (>50%) was noted even when the ratio of L cells to PBMC was 0.1:1. The observed suppression in B cell differentiation was associated with enhancement in proliferation as determined by cell recovery at the end of the culture. Cell yield enhancement ratios varied from 1.2:1 to 1.8:1. After irradiation, L cells pretreated with EA complexes were neither inhibitory nor enhancing.

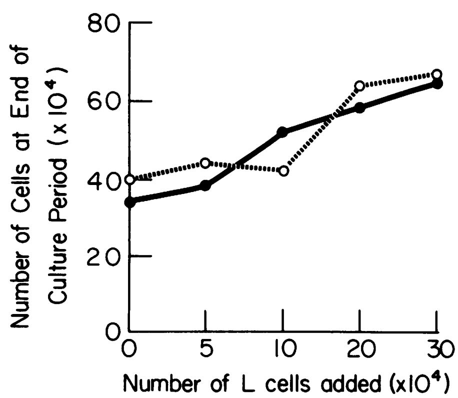

FIGURE 1 Varying number of L cells were co-cultured with 2×10^5 PBMC in the presence of PWM. Cell yields at the end of 8 d are depicted on this figure. Cell yields of L cells when cultured alone decreased and varied between 70 and 85% of the starting number of cells. Subjects: P.L., ○; J.G., ●.

Because there was some macrophage contamination (2–6%) in the L cell subpopulation, it became important to determine whether the observed inhibition with the EA complexes was due to activation of suppressor macrophages as described (20, 21). Hence, highly purified macrophages (an adherent population scraped off plastic dishes) were subjected in a similar fashion to EA complexes and then added to PBMC. As exemplified in Table IV, addition of EA-pretreated macrophages to PBMC did not lead to increased suppression of B cell differentiation. In these experiments, 2×10^4 macrophages were co-cultured with 2×10^5 PBMC as macrophage contamination in the L cell subpopulation was always <10%. Of note, adherent macrophages (nonpretreated with immune complexes) in certain individuals suppressed B cell dif-

Table III

Effect on Ig Subclasses of Co-culturing L or T Cells with Autologous PBMC Subjected to PWM Stimulation

	Plasmacytoid cells per 1,000 cells recovered					
	Subject J.G.			Subject G.P.		
Cells co-cultured	Poly	IgM	IgG	Poly	IgM	IgG
PBMC	57	19	38	30	15	16
PBMC + L	120	60	60	80	66	14
PBMC + T_{Rad}	183	73	110	66	19	47

Details of experiments as in Table II.

ferentiation, while in others the opposite effect was noted (Table IV).

Effect of pretreatment of L cells with Con A. Since the mitogen Con A can preferentially activate T lymphocytes that suppress Ig synthesis, it became intriguing to determine whether this mitogen affected L cells in a similar fashion. Hence, L cells, T cells, and radiated T cells were precultured for 48 h with or without Con A, and then these subpopulations were cocultured with PBMC. The rather surprising observation in all six experiments was that L cells consistently developed spontaneous suppressive activity when precultured without the mitogen (Table V). This contrasted with nonirradiated T cells where development of spontaneous suppressive activity was observed in three of the six subjects. Addition of Con A to precultures did not augment the induced suppressive activity of preculturing L cells, but nonetheless induced suppressive activity in T cells of all subjects. Irradiated T cells maintained their augmenting effect despite Con A in the precultures.

DISCUSSION

Using the recently developed monoclonal antibodies, it is now evident that the L cell subpopulation is a homogenous subpopulation distinct from B or T cells, or even macrophages. L cells possess high density Fc IgG receptors but lack C_3 receptors, intrinsic membrane Ig, T cell-specific antigen, and do not develop into macrophages. Perhaps the previous confusion in the literature with regard to membrane markers present on L cells (reviewed 8) may be explained on the basis of utilization of nonspecific markers and the use of heteroantisera. The latter may explain the high incidence (40–50%) of DR-positive antigen previously noted on noncultured L cells (8). Our data and those of others indicate that the majority (>90%) of noncultured L cells lack the DR antigen (14–16).

These data demonstrate that L cells, like T cells and macrophages, also possess immunoregulatory properties. Although it is highly unlikely that B cell differentiation into plasma cells is L cell dependent as has been demonstrated with T cells, our data provide evidence that L cells can exert both positive or negative influences on terminal B cell differentiation, depending on the state of activation of their Fc IgG receptors. That a potentially single subset of cells can exert both positive and negative influences on the immune system has not been described before. Previous studies have clearly demonstrated, at least with regard to T cells, that different T cell subsets are involved

Table IV

Effect on Ig Synthesis of Co-culturing Immune-Complex-pretreated Macrophages with Autologous PBMC

	Plasmacytoid cells per 1,000 cells recovered				
Cells co-cultured	P.L.	G.P.	J.G.	H.M.	P.L.
PBMC	110	30	122	42	100
PBMC + 10% Macs	80	30	53	47	170
PBMC + 10% $Macs_{EA}$	76	72	60	51	182
PBMC + EAlysed	110	ND*	118	ND*	ND*

Cells adherent to plastic dishes despite vigorous washing served as a source of macrophages. 80–86% of such cells ingested latex particles and also contained lysozymes that stained red with euchyrine. Pretreatment of macrophages with EA and design of co-culture experiments as described in legend of Table II. 2×10^5 PBMC were co-cultured with 2×10^4 macrophages. Cells recovered at the end of 8 d originated from the PBMC population as macrophages die after 2 d. No significant difference in cell yields was observed with the various cell mixtures.

* Not done.

Table V

Effect on B Cell Differentiation of Pretreatment of L Cells and T Cells with Culture Media Alone or Con A before Co-culture Experiments

		Plasmacytoid cells per 1,000 cells recovered		
Cells co-cultured	Pretreatment of subpopulation	G.S.	M.M.	P.L.
PBMC alone		102	110	50
PBMC + L	Media	26	6	4
PBMC + L	Con A	38	13	7
PBMC + T	Media	14	91	38
PBMC + T	Con A	11	32	16
PBMC + Rad T	Media	302	94	140
PBMC + Rad T	Con A	311	111	170

Subpopulations were precultured with or without Con A in RPMI with 20% autologous serum for 48 h, washed twice and then added to co-cultures. 2×10^5 PBMC were co-cultured with 2×10^5 subpopulations.

with enhancement or suppression of Ig synthesis (1, 4, 11, 21). Evidence suggesting that a single subset of L cells is responsible for both enhancement and suppression was derived from the following observations. Firstly, all L cells have Fc IgG receptors, and when added to PBMC are always stimulatory, unlike that observed with T cells. Depending on the ratio of T suppressors to T helpers, a T cell subpopulation could either exert a positive or negative signal on B cell differentiation as was observed by others and also in our experiments (Table II) (11, 21). Secondly, after Fc IgG activation with immune complexes, only suppression was observed. Thirdly, irradiated L cells lost both enhancing and suppressor properties, unlike that observed with T cells (11). Finally, the L cell subpopulation appears to be homogenous with regard to the presence or absence of membrane receptors or determinants.

The augmenting (or inhibitory) effect of L cells on B cell differentiation cannot be explained on the basis of contaminating T cells. T cells were removed from the L cells in a cytotoxicity assay using a T cell-specific monoclonal antibody. Additionally, unlike T helper cells, the L cells enhancing effect was radiosensitive, even when used in co-culture at ratios >1:1.

Although L cells have been shown to possess a marker that is present on T_γ cells and monocytes (OKMI), there is compelling evidence to indicate that these cells are not promonocytes or monocytes (9, 22, 23). L cells lack the intracytoplasmic enzymes present in monocytes, do not ingest latex particles, and in culture do not develop into macrophages (7–9). Secondly, unlike L cells, nonimmune complex-pretreated macrophages, when added to PBMC, either augmented or inhibited B cell differentiation (Table IV). Others have made similar observations and have further demonstrated that macrophage-induced suppression is radioresistant (20, 21). Finally, pretreatment of macrophages with EA complexes did not lead to increased suppression of B cell differentiation (Table IV). Presence of monocyte antigen on L cells could indicate that L cells share a similar myeloid lineage with monocytes or that L cells are precursors of promonocytes and require more fastidious in vitro culture techniques to make them develop into macrophages.

More recent studies indicate that the majority of T_γ cells are indeed non-T cells as they lack T cell-specific antigens detected by monoclonal antibodies (9, 24). Could T_γ cells, therefore, be L cells that form E rosettes? Firstly, both cells are morphologically similar (25). Secondly, T_γ cells, like L cells, possess the marker that is present on myeloid precursors (9, 24), and in addition, develop into suppressors of B lymphocyte differentiation only after immune-complex activation (4). However, it is unclear whether T_γ, when nonactivated by immune complexes, actually augment B cell differentiation as observed with L cells. Hayward and Lydyard (26) provide preliminary data indicating that human newborn T_γ cells, when nonactivated by immune complexes, can enhance B cell differentiation into plasma cells. However, more data is required to substantiate whether T_γ cells belong to the L cell lineage.

Suppression induced by immune-complex activation of L cells and T_γ cells is distinct from the spontaneous suppression observed with another T cell subset (21, 26–28). This latter T cell subset possesses surface antigens detected by the OKT5 monoclonal antibody (29) and rather surprisingly, also has Fc IgM receptors (9, 24). Additionally, suppression of B cell differentiation induced by T cells in the absence of complexes is also associated with marked suppression of proliferation (28). Our data and that of others clearly indicate that L cells and T_γ cells lead to enhancement of proliferation even after immune-complex activation of these cells (4, 30).

A surprising observation was the loss of helper and development of suppressor activity by L cells but not helper T cells after in vitro incubation and in the absence of mitogenic stimulation. This observation is in keeping with that of others (31) who also demonstrated that human nylon nonadherent lymphoid cells (which are invariably enriched for L and T cells) (5, 7) develop suppressor activity after in vitro incubation. Suppression of the in vitro PWM-induced B cell differentiation is, however, greater with co-cultures containing Con A-pretreated PBMC than with PBMC subjected only to in vitro incubation (12). It therefore seems reasonable to assume that the augmented suppression observed with Con A pretreatment of PBMC is due to activation of a subset of cells distinct from L cells or the radioresistant T helper cells. Perhaps Con A activates suppressor cells within the T_μ subset, which do not depend on immune complexes to activate their suppressive potential. Hayward et al. (32) have data to demonstrate that this may indeed be the case. However, more studies are needed to define whether synergism is required between various subsets for the induction of suppressors after mitogenic stimulation.

The concept of a potentially single subset of cells exerting both a positive and negative influence on terminal B cell differentiation is an intriguing possibility. However, these in vitro observations cannot be easily reconciled with our current understanding of the immune regulation of B cell differentiation. It may well be that those subsets of cells that can spontaneously augment or suppress the immune response possess antigenic specificity and are directly under genetic control and, hence, are important in the primary response to an antigenic stimulation. Immune regulation of B cell differentiation mediated by L cells and perhaps T_γ cells may lack genetic control, but be dependent on IgG immune complexes. L cells may therefore operate in the feed-back control of the secondary immune response. Such a hypothesis may help explain the presence of T suppressors in the T_μ subset of cells because control of the initial antibody response, which is mainly IgM, may require antigen specificity. This may also explain the absence of HLA-DR antigens on L cells, as such antigens may not be essential for the secondary immune response.

Deficiency of L cells has been described in active sarcoidosis (33). In this disease hypergammaglobulinemia is commonly present (34), despite the presence of excessive macrophages that inhibit B cell differentiation (35). Deficiency of L cells may explain this hypergammaglobulinemia as perhaps such patients lack the nonspecific feed-back regulation of terminal B cell differentiation; hence, the need for increased suppressor macrophages in this disease.

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