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IN VITRO IMMUNE RESPONSE OF HUMAN PERIPHERAL LYMPHOCYTES

VI. Distribution and Characterization of Precursors for PHA- and Protein A-Induced Colony-Forming B Cells[1]

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B cells from peripheral blood or cord blood formed colonies by stimulation with either PHA or protein A. On the other hand, tonsillar B cells did not form protein A-induced colonies, although PHA-induced colony formation was comparable to that observed in peripheral B cells. Lack of protein A-induced colony formation in tonsillar B cells was not due to the defect of helper T cells in preculture or to the presence of suppressor cells but was due to the absence of precursors for colony formation. The result showed that PHA- and protein A-induced colony-forming cells belonged to distinct subsets of B cells. Depletion of μ-bearing cells from peripheral B cells abrogated both PHA- and protein A-induced colony formation. Depletion of δ-bearing cells did not affect PHA- and protein A-induced colony formation and the population enriched with δ-bearing cells also showed colony formation. Depletion of complement receptor (CR)-positive cells removed precursors for both PHA- and protein A-induced colony formation. These results showed that precursor cells for PHA- and protein A-induced colony formation were IgM^+, IgD^+ and CR^+ or IgM^+, IgD^- and CR^+.

The semi-solid agar culture technique first described by Metcalf et al. (1) has been employed by several investigators (2–4) to probe murine B lymphocyte heterogeneity; the method revealed the absence of a certain subset of B cells in CBA/N mice (5) and the presence of abnormal B subset in NZB mice (6).

In our previous experiment (7), we established the method of colony formation in human B cells with PHA or protein A as a mitogen and suggested the presence of at least two distinct subsets of B cells. In this report, we studied the distribution of precursor cells for PHA- and protein A-induced colony forma tion in several organs and analyzed several surface characters of precursor cells.

The results show that precursor cells for protein A-induced colony formation are defective in tonsils, although they formed PHA-induced colonies comparable to that observed in peripheral B cells. The result confirms our previous suggestion that at least two distinct subsets of B cells are present in human lymphocytes.

MATERIALS AND METHODS

Reagents and culture medium. Agar Noble and PHA-P (lot 3110-56) were obtained from Difco (Detroit, MI). Protein A of *Staphylococcus aureus* (lot EA 11126 and EL 13791) was obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). Fetal calf serum (lot A 080522) was obtained from GIBCO (Grand Island, NY). Modified McCoy's 5a medium, MEM Na-pyruvate (100×), nonessential amino acid (100×), glutamin (200 mM), MEM vitamin mixture (100×), and 7.5% Na-bicarbonate were purchased from Flow Lab. (McLean, VA). L-asparagine and L-serine were obtained from Takara Kohsan Co. (Tokyo, Japan).

Antibodies. Anti-μ and anti-δ antisera were the same preparations as those used in our previous experiment (7) and they were specifically purified with IgM- or IgD-coupled Sepharose immunoabsorbent. FITC-conjugated $F(ab')_2$ fragments of anti-polyvalent Ig, anti-μ, anti-δ, and anti-γ antibodies were also the same preparations as those used in our previous article (7). 19S fraction of anti-SRBC antibody was obtained by 50% ammonium sulfate precipitation of rabbit anti-SRBC serum and Sephadex G-200 gel filtration and used for EAC-rosette formation.

Isolation of lymphocytes and separation of B and T cells. Human peripheral blood lymphocytes (PBL)[3] were separated from heparinized peripheral blood obtained from healthy adult volunteers by centrifugation on Ficoll-Paque (Pharmacia Fine Chemicals, Uppsala, Sweden) as described previously (8). Cord blood lymphocytes were also separated from heparinized cord blood obtained from the placental end of the cut cord immediately after delivery of the baby. Human palatine tonsils were obtained at tonsillectomy from 7- to 30-year-old patients with chronic tonsillitis and were separated into lymphocytes as described previously (9). B and T cells were separated by the rosette-forming method with neuraminidase-treated sheep erythrocytes as described previously (8). Rosette-forming cells were used as T cells and nonrosette-forming cells were used as B cells. Characterization of T and B cell populations was described previously (7). B cell fraction from tonsillar mononuclear cells contained 65 to 90% Ig-positive cells and 1 to 5%

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[3] Abbreviations used in this paper: FITC, fluorescent isothiocyanate; EAC, sheep erythrocytes sensitized with antibody and complement; PBL, peripheral blood lymphocytes; CR, complement receptor; TdR, thymidine.

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monocytes. Separated T and B cells were resuspended in culture medium at a concentration of 1×10^6/ml.

Preculture of B cells in liquid medium. Two million B cells in 2 ml McCoy's modified medium with 15% FCS, 5×10^{-5} M 2-mercaptoethanol, and 100 units/ml of penicillin and 100 μg/ml of streptomycin were cultured in a 35 mm Falcon plastic dish with the same number of autologous or allogeneic T cells irradiated at a dose of 2000 rads and stimulated with PHA-P (1.25 μl/ml) or protein A (10 μg/ml). After 3 days' preculture at 37°C, cells were harvested, washed, and resuspended at a concentration of 5×10^6/ml in the medium for agar culture. Seventy five to 85% of the harvested cells were surface Ig-positive cells and 2 to 4% of the cells were E-rosette-forming cells.

Agar culture. A double layer method was employed as described previously (7). The lower layer was prepared with 1 ml of 0.5% agar in complete McCoy's medium in a 35 mm dish. An appropriate number of cells was seeded into the upper layer with 1 ml of 0.3% agar in complete McCoy's medium. Both layers contained equal concentrations of PHA or protein A. Complete McCoy's medium (10) was composed of 85% McCoy's 5a medium, 15% FCS, 2 mM L-glutamine, 16 μg/ml L-asparagine, 8 μg/ml L-serine, 1 mM sodium pyruvate, 0.8% MEM essential amino acid mixture (50×), 0.4% MEM nonessential amino acid mixture (100×), 0.6% sodium bicarbonate solution (7.5%), 5×10^{-5} M 2-mercaptoethanol, 100 units/ml of penicillin, and 100 μg/ml of streptomycin. This medium was prewarmed to 37°C, mixed with 1/10 volume of boiled 5% agar, and allowed to gel in a 35 mm dish. Then, medium warmed up to 37°C was mixed with 1/10 volume of boiled 3% agar, mixed with an appropriate number of cells and over-layered on 0.5% agar medium and allowed to gel. After seeding the cells, the dishes were incubated at 37°C in a fully humidified atmosphere of 10% CO_2 in air for 6 to 7 days and examined with a dissecting microscope (Olympus Co., Tokyo, Japan) for the presence of colonies. Only aggregates of more than 50 cells were counted as colonies.

Cell separation with anti-Ig coated dishes. μ-bearing or δ-bearing cells were separated by adherence to anti-μ- or anti-δ-coated dishes according to the method of Wysocki and Sato (11). Purified anti-μ or anti-δ antibody at 50 μg/ml in 10 ml of 0.05 M Tris-HCl (pH 9.5) buffer were employed for coating dishes. One to 2×10^7 B cells in 3 ml of 5% FCS in PBS were added onto antibody-coated dishes and held at 4°C for 70 min. After 40 min incubation, dishes were swirled to redistribute unattached cells. Nonadherent cells were harvested with two gentle washes by using 1% FCS in PBS. In order to recover adherent cells, dishes were washed gently 3 more times with 1% FCS in PBS, 10 ml of culture medium were added and cultured at 37°C for 1 hr. After incubation, adherent cells were harvested by flushing the entire surface of the plate with a Pasteur pipette. Percentages of B cells recovered in μ^+, μ^- and δ^+, δ^- populations after dish separation were 7.5%, 45.3%, 11.6% and 13.2%, respectively.

Cell separation with complement rosette (EAC-RFC) formation. Complement receptor (CR)-positive and CR-negative cells were separated by the method described by Bianco *et al.* (12). Two percent suspension of SRBC, in Hanks' balanced solution (BSS) was mixed with rabbit anti-SRBC IgM antibody diluted appropriately with BSS and incubated at 37°C for 30 min. Appropriately diluted A/He mouse serum (C) was added to the sensitized cells (EA) and incubated at 37°C for 30 min. The cells (EAC) were washed 3 times and adjusted to 1% suspension with BSS. One volume of B cell suspension (1 to 5 $\times 10^6$/ml) depleted of macrophages was mixed with two volume of 1% SRBC (EAC) suspension and one volume of heat-inactivated FCS absorbed with SRBC and centrifuged at 2000 rpm for 2 min, followed by incubation at 37°C for 15 min. After 15 min, cells were resuspended, centrifuged at 2000 rpm for 2 min, and incubated at 37°C for 45 min. After incubation, cells were resuspended, layered over Ficoll-Paque, and centrifuged at 400 × G for 30 min at room temperature. Cells collected from the interface (CR^--B) were washed and suspended in culture medium. Rosette-forming cells (CR^+-B) in the bottom of the gradient were collected, SRBC were lysed with Tris-ammonium chloride buffer, and the cells were suspended in culture medium. Depletion of macrophages was done by dish adherence (13) and the cells were examined for residual macrophages by peroxidase staining. The contamination of monocytes was less than 1%.

Measurement of ^{3}H-thymidine (^{3}H-TdR) uptake. 1×10^5 B cells in 0.2 ml of culture medium were cultured with 1×10^5 of 2000 rads-irradiated T cells or without irradiated T cells in a Micro Test II culture plate (Falcon Plastics Co., Oxnard, CA) and they were stimulated with 10 μg/ml of protein A. After 3 days' culture, 1 μCi of ^{3}H-TdR (New England Nuclear, Boston, MA) was pulsed for 4 hr and cells were harvested by Dynateck Automash cell harvester (Dynateck, England). The radioactivity was measured by a liquid scintillation counter with Bray's solution as a scintillator.

RESULTS

PHA- or protein A-induced colony formation in B cells from cord blood or tonsils. B colony formation was studied with B cells from cord blood or tonsils. B cells from cord blood or tonsils were precultured with PHA or protein A in the presence of irradiated T cells from cord blood or tonsils, respectively. After 3 days' preculture, B cells were seeded into agar with PHA or protein A. As shown in Table I, B cells from cord blood formed colonies by stimulation either with PHA or with protein A and the number of colonies was comparable to that observed in B cells from PBL. On the other hand, B cells from tonsils did not form any colonies by stimulation with protein A, although the number of colonies formed in the presence of PHA was comparable to that detected in B cells from PBL or cord blood. As shown in Table II, any concentrations of protein A (1 to 100 μg/ml) tested did not induce colony formation in tonsillar B

TABLE I

PHA- or protein A-induced colony formation in B cells from cord blood or tonsils

B Cells from	PHA-Induced Colonies/ 5×10^5 Cells	Protein A-Induced Colonies/5×10^5 Cells
Peripheral blood	144 ± 5[a]	314 ± 32
Cord blood	158 ± 12	264 ± 23
Tonsil	146 ± 13	0[b]

[a] Mean number of colonies/5×10^5 seeded cells ± SD.

[b] Five experiments were done in 5 different tonsils.

TABLE II

Dose response of protein A for the colony formation in B cells from tonsils

B Cells from	Colonies/5×10^5 Cells at Concentration of Protein A:[a]		
	1 μg/ml	10 μg/ml	100 μg/ml
Tonsil—1	2 ± 1[b]	1 ± 1	12 ± 3
Tonsil—2	0	0	0
Peripheral blood	220 ± 12	465 ± 19	435 ± 28

[a] The same concentration of protein A was employed in both liquid and agar culture.

[b] Mean number of colonies/5×10^5 seeded cells ± SD.

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cells. Since depletion of macrophages or monocytes from B cell population did not affect the PHA- or protein A-induced colony formation in peripheral B cells (data not shown), defects of protein A-induced colony formation in tonsillar B cells were not due to the functional defect of macrophages or monocytes.

In order to study whether the lack of colony formation in tonsillar B cells with protein A was due to the absence of B-precursors for protein A-induced colony formation or due to functional defect of helper T cells in tonsils, tonsillar B cells were precultured with irradiated T cells from PBL in the presence of protein A and colony formation was studied. As shown in Table III, when B cells from PBL-1 were precultured with irradiated T cells either from PBL-2 or from tonsils and seeded into agar culture, the number of colonies formed was comparable to that obtained by preculture of B cells from PBL-1 with autologous T cells. On the other hand, tonsillar B cells precultured with T cells from PBL-1 did not form any colonies by stimulation with protein A. The result suggested that lack of protein A-induced colony formation in tonsillar B cells was due to the absence of B-precursors for protein A-induced colonies.

Because B cell population contained monocytes, the possibility that no colony formation in tonsillar B cells might be due to the suppressor cells in the B cell fraction should be considered. In order to exclude this possibility, the effect of the B cell fraction from tonsils on the colony formation of B cells from PBL was studied. As shown in Table IV, B cells from PBL or from tonsil were separately precultured with protein A in the presence of irradiated T cells. After 3 days' preculture, they were mixed and seeded into agar cultures. The mixture of peripheral and tonsillar B cells formed 116 colonies, which were about a half of that observed in the mixture of peripheral B cells from two donors, PBL-1 and PBL-2. The result showed that the B cell fraction from tonsils did not inhibit colony formation of peripheral B cells in agar culture. Experiments were also carried out to exclude the possibility that the B cell fraction from tonsils contained suppressor cells that exerted their suppressor effect on the activation of B cells in preculture. As shown in Table V, a half million B cells from PBL were mixed with the same number of B cells from tonsils and precultured with protein A in the presence of irradiated peripheral T cells. As a control, a mixture of peripheral B cells from two donors was precultured in the presence of the same T cells and protein A. After 3 days' preculture, they were seeded into agar culture with protein A. As shown in this table, the number of colonies observed in the mixture of peripheral and tonsillar B cells was about a half of that formed in the mixture of peripheral B cells from two donors. These results showed that lack of protein A-induced colony formation in tonsillar B cells was not due to the presence of suppressor cells but due to the lack of precursors for protein A-induced colony formation.

TABLE III

Helper function of peripheral or tonsillar T cells on protein A-induced colony formation or on the uptake of ^{3}H-TdR

Expt. No.	Cells from		Colonies/5×10^5 Cells	^{3}H-TdR Uptake (S.I.)[b]
	B cells	T cells[a]		
1	PBL-1	PBL-2	439 ± 37[c]	9.0
	PBL-1	Tonsil	416 ± 8	7.6
2	PBL-2	PBL-1	455 ± 37	32.1
	Tonsil	PBL-1	2 ± 2	42.2

[a] T cells were irradiated at the dose of 2000 rad, mixed with B cells and stimulated with 10 μg/ml protein A.

[b] Stimulation index (S.I.) was calculated by the following formula:

$$SI = \frac{^{3}\text{H-TdR uptake (cpm) in protein A-stimulated cells}}{^{3}\text{H-TdR uptake (cpm) in nonstimulated cells}}.$$

Cpm in nonstimulated cells was 2845 ± 401 and 2540 ± 146 in experiment 1 and 1470 ± 16 and 1417 ± 125 in experiment 2.

[c] Mean number of colonies/5×10^5 seeded cells ± SD.

TABLE IV

Effect of the addition of precultured tonsillar B cells on protein A-induced colony formation of peripheral B cells

Expt. No.	Preculture[a]		Agar Culture[b]	Colonies/ 5×10^5 Cells
	B cells	T cells		
1	PBL-1	PBL-1	Precultured B-1 +	193 ± 30[c]
	PBL-2	PBL-1	Precultured B-2	
2	PBL-1	PBL-1	Precultured B-1 +	116 ± 12
	Tonsil	PBL-1	Precultured tonsillar B	

[a] B cells from PBL or tonsil were precultured with irradiated peripheral T cells and 10 μg/ml protein A for 3 days.

[b] The same number of precultured B1 and B2 or B1 and tonsillar B were mixed and seeded into agar culture.

[c] Mean number of colonies/5×10^5 seeded cells ± SD.

TABLE V

Effect of the addition of tonsillar B cell fraction into preculture on protein A-induced colony formation of peripheral B cells

Expt. No.	Preculture[a]		Colonies/Dish
	B cells	T cells	
1	PBL-1 + PBL-2	PBL-1	255 ± 51[b]
2	PBL-1 + tonsil	PBL-1	121 ± 20

[a] The same number of peripheral B cells from 2 donors (experiment 1) or the same number of peripheral and tonsillar B cells (experiment 2) were mixed and cultured with irradiated peripheral T cells and 10 μg/ml protein A. After 3 days' preculture, they were seeded into agar culture.

[b] Mean number of colonies/dish ± SD.

In spite of the lack of precursors for protein A-induced colony formation, tonsillar B cells could be stimulated to proliferation by protein A. As shown in Table III, when B cells from tonsils were stimulated with protein A in the presence of irradiated T cells, they showed the uptake of ^{3}H-TdR comparable to that of B cells from PBL. The result showed that colony-forming B cells account for only a small fraction of total B cells and this cloning procedure could detect the absence of protein A-induced colony-forming cells in tonsils. The result that tonsillar B cells formed colonies with PHA but not with protein A confirmed our previous suggestion that PHA and protein A stimulated distinct subsets of B cells into colony formation.

Isotype of surface Ig on precursor B cells for colony formation. In order to study the isotype of surface Ig on precursor B cells for PHA-or protein A-induced colony formation, B cells from PBL were separated by anti-μ or anti-δ-coated Petri dish and colony formation in a separated cell population was studied. Distribution of surface Ig isotypes in separated cell populations is summarized in Table VI. As shown in Table VII, depletion of μ-bearing cells in an anti-μ-coated Petri dish almost completely abrogated both PHA- and protein A-induced colony formation. On the other hand, the number of colonies obtained in the fraction enriched with μ-bearing cells, which was recovered from anti-μ-coated dish, was much higher than that observed in the unseparated cells, indicating that precursor cells for PHA- or protein A-induced colony formation had IgM on their

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TABLE VI

Distribution of surface Ig isotypes in the separated cell populations

Separation Procedure	Cell Type	% of Positive Cells[a]			
		sIg	sIgM	sIgD	sIgG
Control dish[b]	Unseparated	61.4	65.5	45.4	26.5
Anti-μ-coated dish	μ⁺B[c]	74.8	74.1	33.3	N.D.[d]
	μ⁻B[c]	36.7	2.7	18.4	52.5
Anti-δ-coated dish	δ⁺B[e]	70.6	42.9	74.4	N.D.[d]
	δ⁻B[e]	44.8	43.0	6.4	35.0

[a] Cells were stained after 24 hr culture.

[b] Separation procedure was performed by employing normal rabbit γ-globulin-coated dish.

[c] μ⁺B were the cells adhered to anti-μ-coated dish and μ⁻B were nonadherent cells.

[d] N.D., not determined.

[e] δ⁺B and δ⁻B represent the cells adhered or nonadhered to anti-δ-coated dish, respectively.

TABLE VII

Effect of depletion of μ- or δ-bearing cells on PHA- or protein A-induced colony formation

Separation Procedure	B Cells	PHA-Induced Colonies/5 × 10⁵ Cells	Protein A-Induced Colonies/5 × 10⁵ Cells
Control dish	Unseparated	184 ± 24[a]	226 ± 16
Anti-μ-coated	μ⁺	287 ± 29	312 ± 48
dish	μ⁻	7 ± 7	10 ± 5
Anti-δ-coated	δ⁺	160 ± 8	78 ± 4
dish	δ⁻	148 ± 12	208 ± 24

[a] Mean number of colonies/5 × 10⁵ seeded cells ± SD.

surface, and surface IgM-negative cells, even if they had IgD or IgG on their surface (Table VI), did not form colonies. Depletion of δ-bearing cells did not affect either PHA- or protein A-induced colony formation. The fraction enriched with δ-bearing cells, which were recovered from anti-δ-coated dishes also formed colonies, but protein A-induced colony formation in this population was significantly less than that observed in the original cell population. The result showed that PHA- and protein A-induced colony-forming cells were derived from either δ⁺ or δ⁻ cells, but precursors for protein A-induced colonies were mainly in δ⁻ population (Table VII).

C3 receptors on precursors of colony-forming cells. In order to study if precursor cells for colony formation had C3 receptors on their surface, B cells were separated into CR-positive and CR-negative populations by EAC-rosette formation. As shown in Table VIII, CR-negative B cells formed only a few colonies by stimulation with either PHA or protein A. On the other hand, CR-positive B cells formed colonies more than the original B cell population, showing that PHA- or protein A-induced colony-forming cells were derived from CR-positive B cells.

DISCUSSION

The present study clearly showed that tonsillar B cells did not form any protein A-induced colonies, although they formed colonies by PHA-stimulation. The results also demonstrated that the absence of protein A-induced colony formation in tonsillar B cells was not due to the defect of helper function of T cells nor due to the presence of suppressor cells, but due to the lack of precursor B cells for colony formation. Our previous experiments (7) showed that protein A-induced colony-forming cells had cytoplasmic Ig, whereas B cells in PHA-induced colonies did not have any Ig in their cytoplasm. Moreover, PHA and protein A had an additive effect on the number of colonies induced, and 50% of colonies formed in the presence of both PHA and protein A had cytoplasmic Ig-positive cells. These results suggested that PHA and protein A might stimulate distinct subsets of B cells. The present result that tonsillar B cells contained precursors for PHA-induced colony formation but not for protein A-induced colony formation confirmed our previous finding that suggested the presence of at least two subpopulations of B cells. In murine B cells, the presence of several subsets has been demonstrated by the response against several T-dependent and T-independent antigens (14) and by several cell surface antigens (15). Lack of colony forming B cells in CBA/N mice also showed the presence of at least two subpopulations of B cells (5). In human B cells, however, few attempts have been made to distinguish subpopulations by the antibody against a certain cell surface glycoprotein (16) or by the alloantibody in the serum of a patient with Wiscott-Aldrich syndrome (17). In this context, this cloning procedure in semi-solid agar provides an useful and simple means for analysis of subpopulations of human B cells. Actually, in our accompanying paper (18), the presence of abnormal B cell subset in patients with juvenile rheumatoid arthritis (JRA) will be shown by employing this method.

In the present experiment, lack of precursors for protein A-induced colony formation was shown in tonsillar B cells, and the result suggested the presence of some differences between tonsillar and peripheral B cells. In several studies, Ig productions were shown in tonsillar as well as peripheral B cells by stimulation with polyclonal mitogens (19), antigens (20), or allogeneic helper factor(s) (21). Some differences of responsiveness were also reported between tonsillar and peripheral B cells. Fauci *et al.* (22) showed that T cell supernatants could reconstitute PWM-induced anti-SRBC PFC response of tonsillar B cells but not of peripheral B cells. Ringden *et al.* (23) showed that B cells from different organs, such as blood, spleen, adenoid, tonsil, and mesenteric lymph node, showed varying sensitivity to different polyclonal B cell activators. These results suggested that several subpopulations were present in human B cells and they showed a preferential localization in different lymphoid organs.

In the present experiment, depletion of μ-bearing cells almost completely abrogated both PHA- and protein A-induced colony formation. On the other hand, depletion of δ-bearing cells did not affect either PHA or protein A-induced colony formation and the population enriched with δ-bearing cells also formed colonies. The result also showed that CR-negative B cells did not form either PHA- or protein A-induced colonies. These results indicated that the precursor cells for PHA- or protein A-induced colony formation were sIgM⁺, sIgD⁻, CR⁺ or sIgM⁺, sIgD⁺, CR⁺. Moreover, the result showed that sIgG⁺ cells did not form colonies if they do not have sIgM on their surface. In murine colony system, Kincade *et al.* (24) showed that essentially all precursor cells for colony-forming B cells were sIgM-positive, and those μ-bearing precursors were divided into three

TABLE VIII

Distribution of colony-forming B cells in CR⁺ and CR⁻ cell populations

B Cells	PHA-Induced Colonies/5 × 10⁵ Cells	Protein A-Induced Colonies/5 × 10⁵ Cells
Unseparated	66 ± 8[a]	178 ± 22
CR⁺[b]	108 ± 4	260 ± 20
CR⁻[b]	13 ± 3	4 ± 2

[a] Mean number of colonies/5 × 10⁵ seeded cells ± SD.

[b] Percent of sIgM-positive cells in CR⁺ and CR⁻ cell population were 75 and 32%, respectively.

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categories on the basis of expression and function of sIgD, i.e., i) sIgD-negative, ii) sIgD-positive but not inhibited by anti-δ antibody, and iii) sIgD-positive and inhibited by anti-δ antibody. This result observed in a murine system coincides with our present result obtained in human B cells. However, anti-δ antibody did not inhibit but rather facilitated colony formation in human B cells as shown in our accompanying paper (18).

REFERENCES

1. Metcalf, D., N. L. Warner, G. J. V. Nossal, J. F. A. P. Miller, K. Shortman, and E. Rarbellino. 1975. Growth of B lymphocyte colonies *in vitro* from mouse lymphoid organs. Nature 255:630.
2. Metcalf, D., G. J. V. Nossal, N. L. Warner, J. F. A. P. Miller, T. E. Mandel, J. E. Layton, and G.A. Gutman. 1975. Growth of B-lymphocyte colonies *in vitro*. J. Exp. Med. 142:1534.
3. Kincade, P. W., P. Ralph, and M. A. S. Moore. 1976. Growth of B-lymphocyte clones in semisolid culture is mitogen dependent. J. Exp. Med. 143:1265.
4. Kincade, P. W., and P. Ralph. 1977. Regulation of clonal B lymphocyte proliferation by anti-immunoglobulin or anti-Ia antibodies. Cold Spring Harbor Symp. Quant. Biol. 41:245.
5. Kincade, P. W. 1977. Defective colony formation by B lymphocytes from CBA/N and C3H/HeJ mice. J. Exp. Med. 145:249.
6. Kincade, P. W., G. Lee, G. Fernandes, M. A. S. Moore, N. Williams, and R. A. Good. 1979. Abnormalities in clonable B lymphocytes and myeloid progenitors in autoimmune NZB mice. Proc. Natl. Acad. Sci. 76:3436.
7. Muraguchi, A., T. Kishimoto, T. Kuritani, T. Watanabe, and Y. Yamamura. 1980. In vitro immune response of human peripheral lymphocytes. V. PHA- and protein A-induced human B colony formation and analysis of the subpopulations of B cells. J. Immunol. 125:564.
8. Hirano, T., T. Kuritani, T. Kishimoto, and Y. Yamamura. 1977. *In vitro* immune response of human peripheral lymphocytes. I. The mechanism(s) involved in T cell helper functions in the pokeweed mitogen-induced differentiation and proliferation of B cells. J. Immunol. 119:1235.
9. Watanabe, T., K. Yoshizaki, T. Yagura, and Y. Yamamura. 1974. *In vitro* antibody formation by human tonsil lymphocytes. J. Immunol. 113:608.
10. Kincade, P. W., C. J. Paige, R. M. E. Parkhouse, and G. Lee. 1978. Characterization of murine colony-forming B cells. I. Distribution, resistance to anti-Ig antibodies, and expression of Ia antigens. J. Immunol. 120:1289.
11. Wysocki, L. J., and V. L. Sato. 1978. "Panning" for lymphocytes: a method for cell selection. Proc. Natl. Acad. Sci. 75:2844.
12. Bianco, C., R. Patrick, and V. Nussenzweig. 1970. A population of lymphocytes bearing a membrane receptor for antigen-antibody-complement complexes. I. Separation and characterization. J. Exp. Med. 132:702.
13. Mosier, D. E. 1967. A requirement for two cell types for antibody formation *in vitro*. Science 158:1573.
14. Mosier, D. E., I. M. Zitron, J. J. Mond, A. Ahmed, I. Scher, and W. E. Paul. 1977. Surface immunoglobulin D as a functional receptor for a subclass of B lymphocytes. Immunol. Rev. 37:89.
15. Huber, B., R. K. Gershon, and H. Cantor. 1977. Identification of a B-cell surface structure involved in antigen-dependent triggering: absence of this structure on B cells from CBA/N mutant mice. J. Exp. Med. 145:10.
16. Wang, C. Y., S. M. Fu, and H. G. Kunkel. 1979. Isolation and immunological characterization of a major surface glycoprotein (gp54) preferentially expressed on certain human B cells. J. Exp. Med. 149:1424.
17. Brouet, J. C., C. Grillot-Courvalin, and M. Seligmann. 1980. Human antibody reacts with a B-cell subset in man to induce B-cell differentiation. Nature 283:668.
18. Muraguchi, A., T. Kishimoto, T. Kuritani, and Y. Yamamura. *In vitro* immune response of human peripheral lymphocytes. VII. Effect of anti-μ and anti-δ antibodies on B colony formation and detection of abnormal B cells in patients with juvenile rheumatoid arthritis. *J. Immunol.* 125:2638.
19. Janossy, G., and M. Greaves. 1975. Functional analysis of murine and human B lymphocyte subsets. Immunol. Rev. 24:12.
20. Fauci, A. S., and K. R. Pratt. 1976. Activation of human B lymphocytes. I. Direct plaque-forming cell assay for the measurement of polyclonal activation and antigenic stimulation of human B lymphocytes. J. Exp. Med. 144:674.
21. Chiorazzi, N., S. M. Fu, and H. G. Kunkel. 1979. Induction of human antibody responses *in vitro* with emphasis on allogeneic helper factors. Immunol. Rev. 45:219.
22. Fauci, A. S., K. R. K. Pratt, and C. Whalen. 1976. Activation of human B lymphocytes. II. Cellular interactions in the PFC response of human tonsillar and peripheral blood B lymphocytes to polyclonal activation by pokeweed mitogen. J. Immunol. 117:2100.
23. Ringden, O., B. Rynnel-Dagöö, T. Kunori, C. I. E. Smith, L. Hammarström, A. Freijd, and E. Möller. 1979. Induction of antibody synthesis in human B lymphocytes by different polyclonal B cell activators: evaluation by direct and indirect PFC assays. Immunol. Rev. 45:195.
24. Kincade, P. W., G. Lee, M. P. Scheid, and M. D. Blum. 1980. Characterization of murine colony-forming B cells. II. Limits to *in vitro* maturation, Lyb-2 expression, resolution of IgD$^+$ subsets, and further population analysis. J. Immunol. 124:947.

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