ENDOTOXIN PROTEIN IS A MITOGEN AND POLYCLONAL ACTIVATOR OF HUMAN B LYMPHOCYTES*

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The study of human B-lymphocyte function in normal and disease states has been hindered by the failure of these cells to be activated to proliferation by classical B-lymphocyte mitogens, such as lipopolysaccharide endotoxin (LPS,[1] dextran sulfate, or purified protein derivative (PPD) (1–4). Human peripheral blood lymphocytes (HPBL) are activated by phytohemagglutinin (PHA), pokeweed mitogen (PWM), and staphylococcal protein A (SpA) (5–10). Of these mitogens, PHA, PWM, and SpA all have been reported to stimulate both T and B lymphocytes, and the activation of B cells by PWM, and PHA appears to be thymus dependent (7, 9–11). Because these mitogens stimulate both major classes of lymphocytes, it is impossible to rapidly assess B-lymphocyte function in unseparated cell cultures.

The only method presently available for studying the function of human B lymphocytes is that of polyclonal activation (PCA) as determined by antibody production in vitro (12). However, this procedure requires rather lengthy periods of culture compared to proliferation techniques. PCA has been described in lymphocytes isolated from tonsils and peripheral blood (12–14). It has been shown that PWM is more stimulatory for these cells than LPS, PPD, and dextran sulfate (12, 13), but PCA by PWM was found to be highly T-cell dependent (14).

Recently a new protein mitogen has been described which is a potent activator of B lymphocytes of mice (15). This material which is found associated with the LPS in the outer membrane of Gram-negative bacteria is called endotoxin protein (EP). Furthermore, it stimulates the lymphocytes of rats, rabbits, guinea pigs and is also a mitogen for HPBL (16). In this paper, we report that it is the human B lymphocytes in the absence of T cells which are stimulated to DNA synthesis by EP. Furthermore EP is able to induce PCA in HPBL as shown by the production of antibodies against both sheep erythrocytes (SRBC), and 2,4,6 trinitrophenyl-conjugated SRBC (TNP-SRBC).

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[1] *Abbreviations used in this paper:* EP, endotoxin protein; HPBL, human peripheral blood lymphocytes; LPS, protein-free lipopolysaccharide endotoxin; LPS-B, Boivin lipopolysaccharide endotoxin; NWSM, nocardia water soluble mitogen; PBSA, phosphate-buffered saline with 10 mM sodium azide; PCA, polyclonal activation; PFC, plaque-forming cells; PHA, phytohemagglutinin-P; PPD, purified protein derivative; PWM, pokeweed mitogen; sIg, surface immunoglobulin; SpA, staphylococcal protein A; SRBC, sheep erythrocytes; TNP-SRBC, trinitrophenyl conjugated sheep erythrocytes.

Materials and Methods

Human Cell Suspensions. Heparinized venous blood was drawn from normal healthy adult volunteers, and purified lymphocytes were obtained by the method of Hypaque-Ficoll density gradient centrifugation (T7). Ficoll-Hypaque was purchased from Pharmacia Fine Chemicals, Inc., Piscataway, N.J. and sterile 50% hypaque sodium diatriozate solution was obtained from Winthrop Laboratories, New York. After separation, lymphocytes were washed twice in RPMI-1640 (Grand Island Biological Co., Grand Island, N.Y.) before use.

Lymphocyte Cultures. Human lymphocytes were cultured in RPMI-1640 supplemented with 100 IU/ml penicillin and 100 μg/ml streptomycin. Unless otherwise specified, cells were suspended at 8×10^6 viable cells per ml and 0.05 ml of the suspension was added per well of a microtiter plate (Flow Laboratories Inc., Rockville, Md.). An equal volume of mitogen in RPMI-1640 was added to the cell suspensions. In one experiment, 1 ml of cells was cultured at a concentration of 2×10^6 cells per ml in 12 × 75 mm plastic tubes (Beckman Instruments, Inc., Fullerton, Calif.) and 0.1 ml of mitogen diluted in RPMI-1640 was added to the cultures. Cells were normally cultured without serum, but in certain experiments 5% pooled human AB sera (615AB, Grand Island Biological Co.) or fetal calf serum (E064421, E172615, GIBCO) were used. All cultures were incubated at 37°C in an atmosphere of 10% CO_2 in humidified air for 72 h and 1 μCi of [^{3}H]thymidine (5Ci/mmole, Amersham Corp., Arlington Heights Ill.) was added for the last 20 h of culture. Cells cultured in microtiter plates were harvested on glass fiber filter papers (H. Reeve Angel & Co., Inc. Clifton, N.J., grade 934 AH) with an automated sampler (Otto Hiller Co., Madison, Wis.). Cells cultured in tubes were harvested on nitrocellulose ester filters by using a multimanifold sampler and washed with 15 ml of cold saline and 5 ml of cold trichloracetic acid. The samples were dried and counted by means of liquid scintillation with a Beckman LS-250 (Beckman Instruments, Inc.). Cell cultures were done in triplicate and replicate values did not differ by more than 5–10%. The results are reported as mean counts per minute or stimulation index (SI). The SI was calculated as the ratio of counts per minute of the stimulated cultures to counts per minute of the control cultures.

Human Cell Separations. T and B lymphocytes were separated by the SRBC method of Jondal et al. (18). Briefly, lymphocytes were suspended in fetal calf serum at a concentration of 5×10^6 cells per ml and mixed with an equal volume of 1% SRBC in fetal calf serum. The cell mixture was pelleted by low speed centrifugation and incubated at 37°C for 15 min and then at 4°C for 60 min. The cell pellet was gently resuspended, layered on an equal volume of Ficoll-Hypaque and, spun at 700 *g* for 12 min. The cells at the interface were harvested, washed, and the separation procedure repeated. Enriched T lymphocytes were recovered from the first erythrocyte pellet by osmotic lysis (19), and enriched B lymphocytes were recovered from the interface layer of the second separation. Both populations of cells were washed twice in RPMI-1640 and after washing, the cells in both populations were resuspended, counted for viability by eosin dye exclusion, and cultured in microtiter plates as described above.

Immunofluorescence. HPBL were cultured for 72 h with or without mitogen before labeling with fluoresceinated goat anti-human Ig $F(ab')_2$ fragment (N.L. Cappel Laboratories, Inc. Cochranville, Pa.). After culture, the cells were centrifuged and the pellet resuspended in 0.1 ml of phosphate-buffered saline with 10 mM sodium azide (PBSA). Fluoresceinated antibody (0.5 ml), which had been diluted 1:2 in 5% bovine serum albumin containing 10 mM sodium azide, was added to 2×10^6 cells. The mixture was incubated at room temperature for 20 min and washed three times with PBSA. The pellet was brushed onto glass slides and Wright-Giemsa stained (20). The slides were counted and scored for blast cells and for fluorescence using a Zeiss fluorescent microscope (2-F1, West Germany).

Polyclonal Activation. PCA of human peripheral blood lymphocytes was performed as described by Fauci and Pratt (12, 13). Briefly, 2×10^6 cells were cultured with or without mitogen with fresh, heat inactivated (56°C for 30 min) pooled type AB or type A human serum, which had been SRBC absorbed (12). The cell cultures were incubated in an atmosphere of 5% CO_2 in humidified air at 37° for 6 d. At this time, the cells were harvested and plaque-forming cells (PFC) enumerated in a modified hemolytic plaque assay (21) against SRBC and TNP-SRBC (22).

Mitogens. Boivin LPS (LPS-B) was extracted from *Salmonella typhosa* 0-901, and *Salmonella typhimurium* W118-2 by trichloracetic acid (20). EP was prepared from these LPS-B preparations

TABLE I
Lymphocyte Activation by EP

Donors	Mitogen	[^{3}H]thymidine uptake Mean cpm (range)	Mean stimulation index (range)
Total*	—	1,002 (484- 2,968)	—
	PHA 1:800	25,468 (14,830-49,049)	28.4 (11.5-43.4)
	E. coli EP 25 μg‡	4,334 (1,776- 9,172)	5.8 (2.1-11.1)
	S. typhosa EP 25 μg§	11,320 (3,019-24,019)	12.7 (3.8-20.1)
Immunized‖	—	833 (579- 1,376)	—
	PHA 1:800	20,402 (15,817-31,923)	25.0 (21.9-27.7)
	E. coli EP 25 μg	5,781 (2,942- 8,027)	7.7 (2.1-11.1)
	S. typhosa EP 25 μg	10,856 (9,789-11,775)	14.5 (8.2-18.3)
Nonimmunized¶	—	1,142 (484- 2,968)	—
	PHA 1:800	27,494 (14,830-49,049)	29.8 (11.5-43.4)
	E. coli EP 25 μg	4,335 (1,776- 9,172)	5.8 (2.7- 8.9)
	S. typhosa EP 25 μg	12,484 (3,019-24,019)	12.0 (3.8-20.1)

* 14 individuals.
‡ EP lot 1176.
§ EP lot 1276.
‖ Four individuals immunized to *S. typhosa* from 1 to 6 yr previously.
¶ 10 unimmunized individuals.

and *Escherichia coli* 0127:B8 as previously described (15). Phenol extracted LPS was prepared by the method of Westphal et al. (23). All preparations were analyzed for protein by the method of Lowry et al. (24). LPS-B from *S. typhosa* 0-901 (lot 8178) and *S. typhimurium* W118-2 (lot 8578) contained 14.2% and 20.8%, protein, respectively. The phenol LPS from these organisms contained less than 1% protein. *S. typhosa* 0-901 EP contained 83% protein (lot 1276) or 85% protein (lot 9578); *E. coli* 0127:B8 EP (lot 1176) contained 88% protein and *S. typhimurium* W118-2 EP (lot 9778) was composed of 82.5% protein. Lyophilized PHA and PWM (Difco Laboratories, Detroit, Mich.) were reconstituted to their original volumes. PHA was used at the dilutions indicated and PWM was assayed at final dilutions of 1/50, 1/100, and 1/500. The optimal response of cells from each individual to these mitogens is reported. PPD (RT-33) was purchased from Statens Serum Institute, Copenhagen, Denmark and used as previously described (21).

Results

The ability of lymphocytes from normal healthy adults to respond to EP are summarized in Table I. Under serum-free conditions, all of the lymphocyte cultures were able to respond to PHA and both EP preparations, although the optimal dose of EP varied from 25 to 50 μg/well depending upon the individual. It was previously reported that *S. typhosa* 0-901 EP stimulated a greater incorporation of [^{3}H]thymidine than did *E. coli* 0127:B8 EP at all concentrations and in every lymphocyte preparation tested (16). This was so whether the lymphocytes were from individuals previously immunized to *S. typhosa* or from nonimmunized subjects (Table I). Furthermore there was no significant difference in stimulation between the normal and typhoid immunized groups to *S. typhosa* EP ($t = 0.98$, 13 degrees of freedom, $P > 0.2$), indicating that prior immunization to *S. typhosa* did not alter the lymphocyte response.

Culture Conditions. Under serum-free conditions, the optimum response of HPBL to these mitogens peaked at day 3 or 4 (Fig. 1) and decreased thereafter. However, in the presence of 5% human serum, the ability of HPBL to respond to stimulation by

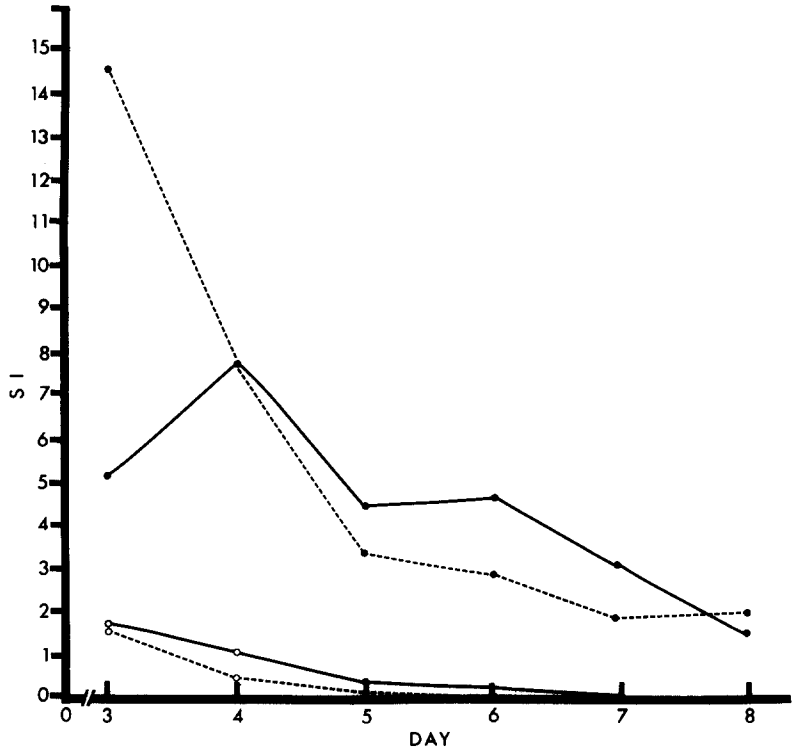

FIG. 1. SI of HPBL in response to EP at various days after initiation of culture, ●; donor 1 without serum; ○ donor 1 and 5% human serum; ●- - -●; donor 2 without serum; ○- - -○; donor 2 with 5% human serum.

EP was totally suppressed (Fig. 1). Because human lymphocytes generally are cultured in the presence of serum, this phenomenon was investigated further. The results in Table II show that 5% pooled human serum significantly inhibits the response of HPBL to both *S. typhosa* and *E. coli* EP. In contrast, the response of these cells to PHA is increased at least 1.5–2 times that of unsupplemented cultures in terms of both cpm and stimulation indices. Fetal calf serum can also increase the stimulation of HPBL by PHA (Table III), but these particular lots do not change the response of human lymphocytes to EP.

To further investigate the role of cultural conditions on the response of HPBL to EP, the lymphocytes were cultured in plastic tubes at a concentration of 2×10^6 cells per ml in the absence of serum supplementation. In addition, two different lots of *S. typhosa* 0-901 EP (1276, 9578), one preparation of *S. typhimurium* W118-2 EP (9778), and Boivin preparations from both organisms were tested. The results shown in Table IV demonstrate that these conditions are able to support the proliferation of HPBL in response to EP and PHA. In addition, HPBL are stimulated by preparations LPS-B, but not by protein-free LPS (data not shown). The response of HPBL to 100 μg of LPS-B is in most cases, approximately equal to the response of these cells to 10 μg of EP and generally is ≈50% of the optimal EP response. However, in lymphocytes from two donors (G.G. and M.S.) the response to *S. typhosa* 0-901 LPS-B is ≈90% of that obtained with EP.

TABLE II

*Suppression of the Mitogenic Response of HPBL to EP by Pooled Human AB Serum**

Donor	Serum	Control	[^{3}H]thymidine uptake Mean cpm (SI)		*S. typhosa* EP§ 25 μg
			PHA 1:800	*E. coli* EP‡ 25 μg	
J. B.	−	1,376	31,923 (23.2)	2,924 (2.1)	11,289 (8.2)
	+	1,204	95,871 (79.6)	1,359 (1.1)	1,563 (1.3)
M. S.	−	1,306	39,904 (30.6)	9,175 (7.0)	24,019 (18.4)
	+	1,556	84,118 (54.1)	5,704 (3.7)	8,801 (5.7)
I. K.	−	750	22,624 (30.2)	5,162 (6.9)	15,104 (20.1)
	+	1,126	54,432 (48.3)	4,523 (4.0)	4,794 (4.3)
B. M.	−	889	22,313 (25.1)	3,797 (4.3)	9,407 (10.6)
	+	1,059	73,202 (69.1)	1,201 (1.1)	2,624 (2.5)
J. G.	−	593	25,710 (43.4)	2,151 (3.6)	3,019 (5.1)
	+	1,291	79,788 (61.8)	986 (0.8)	2,660 (2.1)
S. G.	−	2,748	20,008 (7.3)	9,809 (3.6)	15,453 (5.6)
	+	1,593	41,105 (25.8)	2,106 (1.3)	2,789 (1.7)

* Grand Island Biological Co. human serum (lot 615AB) used at a concentration of 5% after heat inactivation at 56°C for 30 min.
‡ EP lot 1176.
§ EP lot 1276.

TABLE III

Effect of Fetal Calf Serum on the Response of HPBL to S. typhosa EP‡*

Donor	Mitogen	[^{3}H]thymidine uptake Mean cpm (SI)		
		None	FCS 5%	FCS 10%
G. G.	—	1,473	1,226	1,119
	PHA, 1:800	13,429 (9.1)	31,758 (25.9)	33,434 (29.9)
	EP, 50 μg	14,888 (10.1)	16,741 (13.7)	14,230 (12.7)
G. C.	—	1,497	1,196	1,434
	PHA, 1:800	6,046 (4.0)	15,270 (12.8)	32,928 (23.0)
	EP, 50 μg	11,298 (7.5)	13,735 (11.4)	9,302 (6.5)
J. G.	—	1,803	870	1,100
	PHA, 1:800	4,396 (2.5)	9,355 (10.8)	29,715 (27.0)
	EP, 50 μg	6,493 (3.6)	6,136 (7.1)	6,226 (5.7)
M. S.	—	1,931	1,488	1,609
	PHA, 1:800	5,005 (2.6)	20,963 (14.0)	32,707 (20.3)
	EP, 50 μg	21,926 (11.4)	19,077 (12.8)	17,987 (11.2)

* FCS, fetal calf serum. Grand Island Biological Co. lot E064421. Similar results seen with lot E172615.
‡ EP lot 1276.

Responding Cell. In mice, EP is a B-lymphocyte mitogen (15). Therefore it was essential to determine what class of lymphocytes in human lymphocytes cultures was responding to stimulation by EP. The data of Table V show that the majority of cells which had undergone blast transformation in response to EP were lymphocytes which possessed surface immunoglobulin (sIg). Even in cultures of enriched T lymphocytes, where only 6% of the cells were sIg positive 62% of the cells which were transforming

TABLE IV

*Response of HPBL Cultured in Tubes to EP**

Donor	B.M.‡	[^{3}H]thymidine uptake Mean cpm (SI)		G.C.§	J.B.§
		G.G.§	M.S.§		
Mitogen					
Con	960	1,642	1,398	1,221	988
PHA 1/800	10,645 (11.1)	43,774 (26.7)	54,323 (38.9)	22,849 (18.7)	24,475 (24.8)
1/1600	16,795 (17.5)	52,460 (31.9)	75,576 (54.1)	39,552 (32.4)	33,285 (33.7)
1/2400	ND‖	71,270 (43.4)	62,901 (45.0)	41,173 (33.7)	35,826 (36.3)
S. typhosa					
EP 10 μg/ml	3,578 (3.7)	7,303 (4.4)	5,315 (3.8)	2,482 (2.0)	3,327 (3.4)
50 μg/ml	9,709 (10.1)	8,396 (5.1)	7,757 (5.5)	4,900 (4.0)	4,361 (4.4)
100 μg/ml	13,592 (14.2)	8,389 (5.1)	8,197 (5.9)	6,328 (5.2)	4,757 (4.8)
LPS-B 100 μg/ml	ND	7,621 (4.6)	7,243 (5.2)	3,188 (2.6)	3,303 (3.3)
S. typhimurium					
EP 10 μg/ml	ND	6,751 (4.1)	5,880 (4.2)	2,149 (1.8)	2,921 (2.9)
50 μg/ml	ND	8,815 (5.4)	9,649 (6.9)	4,108 (3.4)	4,392 (4.4)
100 μg/ml	ND	8,984 (5.5)	9,221 (6.6)	4,826 (4.0)	4,360 (4.4)
LPS-B 100 μg/ml	ND	6,510 (4.0)	5,200 (3.7)	2,541 (2.1)	2,429 (2.5)

* Cells were cultured at 2 × 10^6 cells/ml.

‡ *S. typhosa* EP lot 1276.

§ *S. typhosa* EP lot 9578, *S. typhimurium* EP lot 9778; *S. typhosa* LPS-B lot 8178, *S. typhimurium* LPS-B lot 8578

‖ ND, not done.

TABLE V

Blastogenesis of HPBL in Response to EP

Cell type	sIg+ cells per 100 cells	Mean number of blast cells per 100 cells*				*S. typhosa* EP‡	
		Control		PHA			
		sIg−	sIg+	sIg−	sIg+	sIg−	sIg+
Unseparated	27	3.2	1.7	ND§	ND	2.7	13.3
B-enriched	92	1.1	8.4	3	12	0.9	36.4
T-enriched	5	7.6	0.5	38.8	1.2	4.7	7.6

* 400 cells per slide in triplicate were counted for each group for a total of 1,200 cells counted per group.

§ ND, not done.

‡ EP lot 1276.

in response to EP were sIg positive. In addition, the percent of Ig-positive transformed cells in unseparated preparations increased by more than two times the control value when the cells were cultured with EP. These data are strongly suggestive that EP stimulates human B lymphocytes.

Further cell separation studies were done to verify the original data. Six individuals were tested and the results (Fig. 2) demonstrate that after the removal of T lymphocytes, the remaining B-enriched cells (>94% sIg positive) were able to respond to EP, but were not stimulated by PHA. The T-enriched cells (>94% sIg negative) were able to proliferate when cultured with PHA. There was variation in the response to EP and PHA from individual to individual; however, in all cases, the stimulation of B-enriched cells by EP was significantly above background (SI > 4.5).

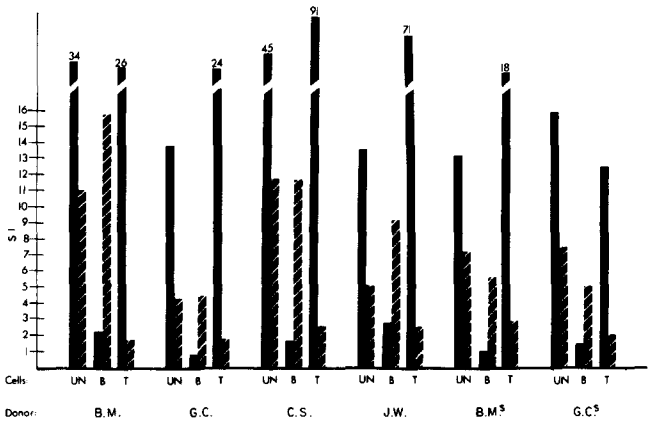

FIG. 2. Response of HPBL to an optimal concentration of PHA (■) and EP (▨). Numbers over the solid lines are stimulation indices which are off scale. Un = unseparated, B = B-enriched, T = T-enriched. All cells were cultured in the presence of 5% fetal calf serum; a superscript "s" denotes cells which were cultured without serum.

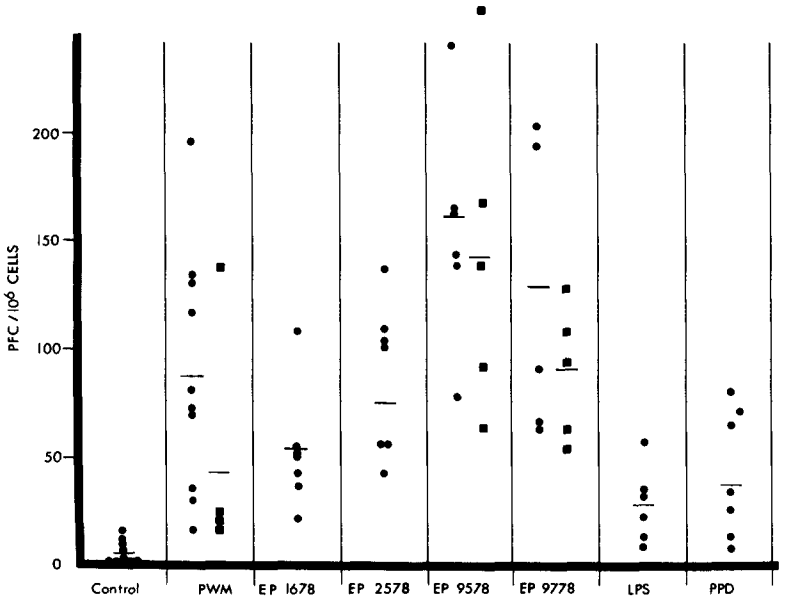

FIG. 3. Polyclonal activation of EP. The results are reported for the optimal doses of PWM. All other mitogens were tested at 100 μg/ml. ● anti-SRBC plaques; ■ anti-TNP-SRBC plaques. Background for anti-TNP-SRBC plaques was 0.

Although all of this evidence suggests thymus-independent cells are proliferating in the presence of EP, the sine qua non of B-cell activation is antibody production. Therefore, if EP could nonspecifically activate clones of B lymphocytes to make antibody to diverse antigens, then it would be assured that EP was stimulating human B lymphocytes. The results of PCA experiments are shown in Fig. 3. Three different *S. typhosa* EP preparations (1678, 2578, 9578) and *S. typhimurium* EP 9778 were able to stimulate PCA. In addition, PWM stimulated PCA in most of the individuals tested. More interestingly, EP preparations 9578 and 9778 stimulated approximately twice

as many PFC than did PWM when assayed against SRBC and TNP-SRBC. LPS and PPD also stimulated nonspecific antibody production, but to a lesser degree than any of the other mitogens, confirming the results of Fauci and Pratt and Fauci et al. (12, 13).

Discussion

It is clear from the data presented that EP is a mitogen and polyclonal activator of human B lymphocytes. The mitogenic stimulation by EP is optimal at 2–4 d, and may be suppressed by the addition of pooled human AB serum. In studies to be reported elsewhere, it has been demonstrated that the inhibition of the response of HPBL to EP is not due to antibody directed against EP. In contrast to human serum, fetal calf serum does not suppress the proliferative response of HPBL to EP.

That the response of HPBL to EP is nonspecific has been demonstrated in several ways. First, the response of individuals who had been deliberately immunized against typhoid was not significantly different from that of nonimmunized controls. Second, the optimal mitogenic response to EP appears early in culture for all the individuals tested, unlike the response to specific antigens which takes 6–7 d (25–27). Finally, EP was able to stimulate polyclonal activation in HPBL, which by definition is nonspecific.

In mice, the dissociation of proliferation from polyclonal activation has been demonstrated (27–29). That this may be so in human lymphocytes as well is suggested by the difference between the kinetics of proliferation and that of polyclonal activation. As with PWM, EP activates peak mitogenesis in HPBL at 3 d, while PCA is not optimal until 6 d (13). Additional evidence in support of the dichotomy of response is that although pooled AB serum suppressed the proliferative response to EP, it was required for activation of PFC. In addition, lymphocytes from only $\approx$70% of the individuals tested were stimulated to produce antibody by PWM, even though the cells from all donors were able to proliferate when cultured with this mitogen (A. Fauci and G. Whalen, personal communications). Apparently in humans as in mice, nonspecific activation of PFC is not dependent upon proliferation.

In contrast to the numerous reports in the literature of the inability of LPS to stimulate proliferation i. HPBL, EP, which is found associated with LPS in the bacterial outer membrane, is active. It is interesting to note that in our hands LPS-B, but not LPS, can activate HPBL. This suggests that the stimulation of HPBL by a commercially prepared LPS described by Hsu (30) might be due to protein contamination of these preparations. Activation of human lymphocytes by LPS-B, but not by protein-free LPS, supports our position that the protein content of all LPS preparations should be determined so that the component responsible for lymphocyte activation is clearly defined.[2]

We observed that different EP preparations have various activities on human lymphocytes (16). For example, the mitogens EP 1176, 1276, 9578, and 9778 were extracted from bacteria grown in shaker flasks, but two other preparations, 1678 and 2578, were grown in 90-liter fermentation tanks. Although the latter preparations are not able to induce proliferation in human lymphocytes, they can stimulate PCA. Thus, different bacterial growth conditions result in EP preparations with different

[2] Goodman, G. W., and B. M. Sultzer. 1979. Further studies on the activation of lymphocytes by endotoxin protein. *J. Immunol.* In press.

activities on human lymphocytes. Additionally, Schnaitman has shown that different conditions of bacterial growth result in differences in the composition of the proteins found in the outer membrane (31), an observation confirmed by us using polyacrylamide gel electrophoresis (16). In studies in progress, different EP preparations are being separated into their polypeptide components. We believe that these experiments will reveal which of the proteins in this heterogeneous complex is responsible for activating HPBL.

At present, there has been only one other mitogen, *Nocardia opaca* water soluble mitogen (NWSM), which has been described as a nonspecific activator of human B lymphocytes but not T cells (32, 33). NWSM is a peptidoglycan which activates lymphocytes from primates, mice and rabbits, as well as man (34, 35). However, there appears to be a number of functional differences between this mitogen and EP. PCA induced by NWSM is considerably less than that induced by EP and peak mitogenic stimulation by NWSM is observed at 5 d, whereas the peak proliferative response induced by EP is seen at 3 d. It will be interesting to compare these materials in parallel and to determine whether the B lymphocytes subpopulations that are activated are the same.

In conclusion, the application of EP in the comparative analysis of human B-cell function in the normal and disease states appears to be promising.

Summary

Endotoxin protein (EP) has been shown to be a mitogen and polyclonal activator of human peripheral blood lymphocytes. EP stimulates proliferation of B lymphocytes in the absence of T cells, and this activation is nonspecific by a number of parameters. Additionally, EP mitogenesis, but not polyclonal activation, is inhibited in the presence of human serum, suggesting that these events are dissociable. In these studies, EP appears to be equivalent to or better than pokeweed mitogen in stimulating nonspecific antibody production in vitro.

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