POLYCLONAL ACTIVATION OF HUMAN PERIPHERAL BLOOD B LYMPHOCYTES BY FORMALDEHYDE-FIXED *SALMONELLA PARATYPHI* B

I. Immunoglobulin Production without DNA Synthesis*

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Human peripheral blood B lymphocytes can be activated by pokeweed mitogen (PWM)[1], *Staphylococcus aureus* Cowan I, Epstein-Barr virus, or *Nocardia* water-soluble mitogen (1–4). Of these polyclonal activators, PWM and *S. aureus* have been shown to stimulate both B and T cells; furthermore, the activation of peripheral blood B cells by PWM appears to be T cell dependent (5, 6). *Nocardia* mitogen, Epstein-Barr virus, and *S. aureus* Cowan I have been shown to be relatively T cell-independent B cell activators (2–4).

Several parameters can be used to evaluate the polyclonal "activation" of human peripheral blood B lymphocytes, including immunoglobulin (Ig) production in vitro, enumeration of Ig-secreting cells by reverse hemolytic plaque assay, and enumeration of intracytoplasmic Ig-containing cells in stimulated cultures. However, study of human peripheral blood B lymphocyte function in normal and disease states has been hampered by the failure of mature human B cells to respond to some classic activators of murine B cells, such as dextran sulfate, tuberculin (PPD), and *Escherichia coli* lipopolysaccharide (LPS) (7–10). Dextran sulfate activates relatively immature murine B cells and results predominantly in increased DNA synthesis; in contrast, PPD acts on relatively mature murine B cells and stimulates a marked increase in antibody production but only modest DNA synthesis (11). However, PPD and other polyclonal B cell activators (PBA) that activate mouse B cells do not have the same effects on human peripheral blood B lymphocytes.

The ability to stimulate human B cells selectively would be of considerable value for studies of immune function. We describe here a "new" PBA, i.e., formaldehyde-fixed *Salmonella paratyphi* B, which activates human peripheral blood B cells to produce large amounts of Ig but does not stimulate DNA synthesis as measured by incorporation of tritiated thymidine. This PBA apparently acts on relatively mature B cells

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[1] *Abbreviations used in this paper:* CVID, common variable immunodeficiency; E, sheep erythrocytes; LPS, lipopolysaccharide; PBA, polyclonal B cell activators; PBL, peripheral blood mononuclear cells; PPD, tuberculin; PWM, pokeweed mitogen; SRBC, sheep erythrocytes.

which differentiate into Ig-secreting cells without DNA synthesis. Furthermore, its action appears to be completely independent of T cells, since T-depleted cell populations respond well to formaldehyde-fixed *S. paratyphi* B.

Materials and Methods

Cell Preparations. Human peripheral blood mononuclear cells (PBL) were obtained on several occasions from heparinized blood of each of 12 normal adult volunteers and 5 adult patients with common variable immunodeficiency (CVID) without suppressor T cells, by centrifugation over a Ficoll-Hypaque gradient (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.) (12). The interface cells were washed twice with RPMI-1640 medium (Grand Island Biological Co., Grand Island, N. Y.). For preparation of lymphocyte subpopulations, PBL were suspended at 5×10^6/ml in RPMI-1640 medium supplemented with 20% heat-inactivated fetal calf serum (FCS); monocytes were partially removed by incubation of 10-ml aliquots in Falcon culture dishes (Falcon Labware, Div. of Becton, Dickinson and Co., Oxnard, Calif.) at 37°C in a 5% CO_2 in air incubator for 1 h. The nonadherent cells were harvested and rosetted with sheep erythrocytes (E) treated with neuraminidase (13). The E-rosetted cells and nonrosetted cells were separated by centrifugation on a Ficoll-Hypaque density gradient as described by Bentwich et al. (14). Cells that failed to form E rosettes (population E^-) were collected from the interface and washed. The E-rosetted cells (population E^+) in the pellet were exposed to distilled H_2O for 30 s to lyse the erythrocytes and then washed with RPMI-1640. The E^+ and E^- lymphocyte populations were rerosetted with neuraminidase-treated E and again separated as described above. In the E^+ preparation, 90–95% of the cells formed rosettes with neuraminidase-treated E; this population was designated as "T-enriched cells." In the E^- population, only 2–4% of the cells formed rosettes, and 90% or more were Ig-positive by indirect immunofluorescence using $F(ab')_2$ anti-B cell antiserum; this population was designated as "B-enriched cells." In additional experiments, Ig^+ B cells were isolated by rosetting of the E^- population with rabbit anti-human Ig antibody-coated human erythrocytes (15) followed by separation on Ficoll-Hypaque. The Ig^+ B cells in the pellet were exposed to distilled H_2O for 30 s to lyse the erythrocytes and then washed with RPMI-1640. The complement receptor-bearing B cell populations (C^+ B) were isolated as described by Hunt (16). Briefly, the E^--enriched populations were rosetted with sheep erythrocytes (SRBC) which had been coated with IgM rabbit anti-SRBC antibody followed with complement (EAC). The C^+ B cells, which formed rosettes with EAC, were then separated on Ficoll-Hypaque as described above.

Mitogens and Bacterial Suspensions. PWM and phytohemagglutinin (PHA) obtained from Grand Island Biological Co. were used in cell cultures at final dilutions of 1:100 and 1:40, respectively. LPS from *Salmonella typhosa* (trichloroacetic acid extract) purchased from Sigma Chemical Co. (St. Louis, Mo.) was used at the optimal concentration of 100 μg/ml. Bacteria isolated from hospitalized patients were kindly provided from the "bacterial library" of the Department of Microbiology, University of Illinois Medical Center. They were grown in Trypticase soy broth (Difco Laboratories, Detroit, Mich.) at 37°C for 18–24 h. After culture, bacterial suspensions were treated with 10% formaldehyde for at least 4 h at room temperature and then heat-killed at 80°C for 5 min. The bacteria were harvested and washed three times with sterile saline and resuspended as a 10% stock solution in phosphate-buffered solution.

Lymphocyte Cultures. For all stimulation experiments, PBL were resuspended at a concentration of 1×10^6/ml in RPMI-1640 supplemented with 10% heat-inactivated FCS, 5 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. Aliquots of 1 ml were cultured with various stimulators in plastic tubes (Falcon 2057; Falcon Labware, Div. of Becton, Dickinson and Co., Oxnard, Calif.) at 37°C in 5% CO_2 in an incubator.

Measurement of DNA Synthesis. Human PBL were cultured according to the method of DuBois et al. (17) in Nunc 1090 plastic tubes (Vangard Int., Neptune, N. J.). All cultures were pulsed with 0.5 μCi tritiated thymidine (6 Ci/mmol) 24 h before harvesting. Cells were harvested on day 3 when PHA was used as a stimulator and on day 5 when PWM, *S. aureus* Cowan I, or *S. paratyphi* B was used. In some experiments, cultures were harvested at different intervals as indicated.

Quantitation of Ig Secreted in Culture Supernatant Fluids. The amounts of IgG, IgM, and IgA in culture supernatant fluids were determined by laser nephelometry (18). These were examined without dilution using a commercially available system provided by Hyland Diagnostics, Div. Travenol Laboratories, Costa Mesa, Calif., according to the method described by Pryjma et al. (19). Briefly, the calibration standards were diluted 1:100 before mixing with antisera. The culture supernate was assayed undiluted, resulting in a 100-fold relative increase in the sensitivity of the method. The reference standards were adjusted to a range of concentration adequate to our needs. In the case of IgG determination, 100-μl aliquots of the culture supernate and only 50 μl of the calibration standard were used. This provided an additional twofold increase in sensitivity but only slight error, since these volumes were diluted in 1 ml of a mixture of antibody and antibody diluent or in blank solution.

Quantitation of Ig Binding to Bacteria. Ig was absorbed with bacteria according to the methods of Forsgren and Grubb (20). Briefly, 1 ml of the culture supernate or 10 μg myeloma IgG in PBS was added to 100 μl of bacterial suspension in PBS supplemented with 10% bovine serum albumin. After 30 min of incubation with continuous shaking at 37°C in a water bath, the bacteria were spun down and supernatant fluids were filtered through a 0.45-μm Millipore filter (Millipore Corp., Bedford, Mass.). The amount of Ig in the supernatant fluids after bacterial absorption was then quantitated by laser nephelometry (19).

Results

Dose Dependence and Kinetics of Ig Production Induced by Formaldehyde-fixed S. paratyphi B. PBL from normal adult donors were used to test responses to various strains of bacteria by measuring the amount of Ig secreted in stimulated cultures. After testing 30 strains, we found that *S. paratyphi* B stimulated the production of significantly larger quantities of IgM than did PWM or *S. aureus* Cowan I.

To determine the optimal concentration of *S. paratyphi* B for the activation of PBL, various dilutions of the bacterial suspension were cultured with PBL for 7 d. The amount of Ig secreted was then determined by laser nephelometry. The highest Ig production was obtained with a 1:400 dilution of the stock bacterial suspension (~ 5×10^6 microorganism/ml). When this concentration was used, IgM was first detectable on day 4 or 5, depending on the donor and culture conditions. IgG and IgA were not measurable by laser nephelometry until day 6 or 7. Representative data from one of a dozen experiments are shown in Table I. IgM was usually detectable in cultures stimulated with *S. paratyphi* B 1 d earlier than in cultures stimulated with PWM or *S. aureus* Cowan I (Table I), and greater amounts of IgM were present at equivalent time points (days 6–10).

Lack of Mitogenic Effect of Formaldehyde-fixed S. paratyphi B. To determine whether *S. paratyphi* B would induce DNA synthesis (mitogenesis), various dilutions of bacterial suspension were cultured with PBL. The mitogenic activity was evaluated by tritiated thymidine incorporation. PBL cultures stimulated with various dilutions (from 1:400 to 1:2,000) of *S. paratyphi* B on day 5 or on days 1–7 in time-course studies (Table II) showed no significant increase in thymidine uptake. Thus, this bacterium is not mitogenic to PBL from healthy donors. Similarly, *S. paratyphi* B was not mitogenic to the B-enriched population isolated from PBL (Table III).

Comparison of Various Polyclonal B Cell Activators. The ability of *S. paratyphi* B to activate PBL to differentiate and mature into Ig-secreting cells was evaluated in comparison with PWM, *S. aureus* Cowan I, and LPS. Using PBL from 12 healthy donors, we evaluated Ig production in cultures stimulated with PWM, *S. aureus* Cowan I, *S. paratyphi* B, and LPS. Representative data for three subjects are summarized in Table IV. PBL stimulated with *S. paratyphi* B usually produced larger quantities of

TABLE I

Ig Production on Days 3–10 of Culture in Cultures of PBL Stimulated with Various Polyclonal B Cell Activators

Stimulant	Ig class	Amount of Ig						
		Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 10
		μg/culture						
None	IgM	0	0	0	0	0	0	0
	IgG	0	0	0	0	0	0	0
	IgA	0	0	0	0	0	0	0
S. paratyphi B	IgM	0	0	0.9	2.0	3.0	5.2	9.5
	IgG	0	0	0	0.8	1.2	1.5	4.3
	IgA	0	0	0	0	0.8	1.3	3.8
S. aureus Cowan I	IgM	0	0	0	0.8	2.0	3.4	5.7
	IgG	0	0	0	1.0	2.0	2.5	4.2
	IgA	0	0	0	1.2	1.3	2.2	3.0
PWM	IgM	0	0	0	1.0	1.8	2.4	3.4
	IgG	0	0	0	0.8	2.3	3.0	3.2
	IgA	0	0	0	1.2	1.3	2.2	2.8

TABLE II

Uptake of [^{3}H]Thymidine by PBL Stimulated with PHA, S. aureus Cowan I, and S. paratyphi B

Stimulant	[^{3}H]Thymidine uptake						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
				*cpm**			
None	80 ± 20	154 ± 30	324 ± 52	406 ± 32	428 ± 42	358 ± 40	380 ± 32
S. paratyphi B	75 ± 15	111 ± 24	389 ± 46	380 ± 46	442 ± 38	358 ± 150	408 ± 38
S. aureus Cowan I	ND‡	ND	ND	ND	8,950 ± 1,589	ND	ND
PHA	ND	ND	37,800 ± 570	ND	ND	ND	ND

* Mean of triplicate ± SD.

‡ ND, not done.

TABLE III

Uptake of [^{3}H]Thymidine by Separated Lymphocyte Populations Stimulated with PWM, S. aureus Cowan I, and S. paratyphi B

Lymphocyte population*	[^{3}H]Thymidine uptake after stimulation with			
	None	PWM	*S. aureus* Cowan I	*S. paratyphi* B
		cpm‡		
PBL	825 ± 98	17,369 ± 530	12,277 ± 621	902 ± 108
E^-	264 ± 46	520 ± 101	720 ± 61	248 ± 39
Ig^+	162 ± 25	469 ± 68	366 ± 52	202 ± 48
C^+	192 ± 35	423 ± 72	639 ± 121	217 ± 29

* PBL, peripheral blood mononuclear cells; E^-, E-rosette-negative cells enriched by two cycles of purification; Ig^+, Ig-positive cells enriched by rosetting of E^- cells with anti-Ig-coated human erythrocytes and separating on Ficoll-Hypaque; C^+, C-receptor-bearing cells enriched by rosetting of E^- cells with EAC and separating on Ficoll-Hypaque.

‡ Mean of triplicate ± SD.

TABLE IV
Comparison of Ig Production in Cultures of PBL Stimulated with PWM, S. aureus Cowan I, S. paratyphi B, and LPS

Donor	Stimulant	Amount of Ig		
		IgM	IgG	IgA
		μg/culture		
E.T.	None	0	0	0
	PWM	4.6	5.3	2.4
	S. aureus Cowan I	2.9	3.2	2.4
	S. paratyphi B	5.9	4.0	3.0
	LPS	0	0	0
W.C.	None	0	0	0
	PWM	3.3	3.9	2.8
	S. aureus Cowan I	4.4	5.9	3.4
	S. paratyphi B	9.4	2.8	3.0
	LPS	0	0	0
R.C.	None	0	0	0
	PWM	2.8	2.0	2.2
	S. aureus Cowan I	3.5	2.9	2.0
	S. paratyphi B	8.5	2.0	1.8
	LPS	1.6	1.0	1.2

IgM than IgG and IgA on day 7. By contrast, PBL stimulated with PWM or *S. aureus* Cowan I usually produced approximately equal amounts of IgM and IgG on day 7. Furthermore, the amount of Ig synthesized and released in culture supernates of PBL from most of the donors in response to *S. paratyphi* B was greater than that in culture supernates of PBL stimulated with PWM or *S. aureus* Cowan I. Only 2 of the 12 donors responded to LPS by production of significant amounts of Ig on day 7.

Polyclonal B Cell Activation by Formaldehyde-fixed S. paratyphi B. To determine whether the Ig secreted in cultures stimulated with *S. paratyphi* B consisted of specific antibodies against *S. paratyphi* B antigen, the culture supernate was absorbed with 2×10^9 *S. paratyphi* B cells as described in Materials and Methods. The amount of Ig was not decreased significantly after absorption, indicating little or no specific antibody against *S. paratyphi* B (14 vs. 13 μg of Ig after absorption). When 2×10^9 *S. aureus* Cowan I cells were used to absorb 10 μg of human myeloma IgG under the same conditions, 85% of the IgG was removed; since protein A on *S. aureus* Cowan I binds human IgG (21), this indicates that 2×10^9 *S. paratyphi* B should be enough to absorb almost all specific antibodies in the culture supernate. Taken together, our data indicate that *S. paratyphi* B is a polyclonal B cell activator which does not stimulate specific antibody production against bacterial antigens.

Response of B Cells to Formaldehyde-fixed S. paratyphi B. *S. paratyphi* B stimulated peripheral blood B lymphocytes to mature into Ig-secreting cells without DNA synthesis (Tables I and II), suggesting that cell proliferation is not necessary for B lymphocyte maturation after *S. paratyphi* B stimulation. In addition, the bacterium activated PBL to produce mainly IgM during the first days of cultures (Table I). Taken together, these data indicate that the B cell response to *S. paratyphi* B is T independent. To confirm this point, we isolated B lymphocytes from PBL and tested their response to *S. paratyphi* B. B-enriched cell cultures produced large amounts of

IgM when stimulated with *S. paratyphi* B but not when stimulated with PWM (Table V). Because more IgM was secreted by the B-enriched population than by unfractionated PBL stimulated with *S. paratyphi* B, these data indicate that the response of PBL to this PBA is indeed relatively T cell independent.

Effects of Polyclonal B Cell Activators on Cells from Patients with Intrinsic B Cell Defects. Patients with CVID have severe hypogammaglobulinemia involving all Ig classes. Waldmann et al. (22) have reported that lymphocytes from some CVID patients do not respond to PWM, and that T lymphocytes from some patients can suppress terminal differentiation of normal lymphocytes. More recently it has been reported that although suppression was demonstrable in some cases of CVID, it could not be a major factor in the majority (23).

Because PWM is a T-dependent PBA, its use as a test substance would not permit the distinction of a "pure" B cell defect from a suppressor effect in patients with CVID. By contrast, *S. paratyphi* B appears to be a T-independent PBA, and its use should allow a much clearer delineation of the level of the B cell defect. If a patient with CVID has PBL which are unable to respond because of the absence of efficient macrophages, inadequate T cell help, or excessive suppressor T cells, the B cells should still be activated polyclonally by *S. paratyphi* B, whereas PWM should produce limited or no stimulation, as has often been observed.

We conducted a preliminary analysis of the responses to PWM, *S. aureus* Cowan I, and *S. paratyphi* B by PBL from CVID patients. Of six adult patients with CVID seen by us in the past few months, five had no evidence of suppressor T cells or suppressor monocytes and were willing to return for repeated study. As shown in Table VI, their PBL did not respond to *S. paratyphi* B by measurable Ig production. In contrast, PBL from two of the patients responded well to *S. aureus* Cowan I, which has been shown to be relatively T independent (19), and responded partially to PWM. When B cells isolated from the PBL of patients 1, 3, and 5 were tested, no Ig production by the enriched B cells was detectable, even in the presence of T cells isolated from normal adults. Thus, the response to *S. paratyphi* B provided better discrimination between normal and CVID B cells than did PWM or *S. aureus* Cowan I; furthermore, no morphologic changes in the CVID B cells were seen after stimulation.

TABLE V

Effects of Depletion of T and B Lymphocytes on the Response to PWM and S. paratyphi B

Cell population	Stimulant tested	Amount of Ig		
		IgM	IgG	IgA
		μg/culture		
PBL	None	0	0	0
PBL	PWM	2.0	1.5	2.1
PBL	*S. paratyphi* B	2.2	1.0	2.0
B-enriched cells	PWM	0.8	0	0
B-enriched cells	*S. paratyphi* B	3.2	0	2.2
B and T cells*	PWM	9.0	1.6	3.5
B and T cells	*S. paratyphi* B	3.2	0.9	2.0
T-enriched cells	PWM	0	0	0
T-enriched cells	*S. paratyphi* B	0	0	0

* B and T cells at equal concentration.

TABLE VI

Responses of PBL from Five Patients with Common Variable Immunodeficiency to PWM, S. aureus Cowan I, and S. parathyphi B

Patient	Stimulant	Amount of Ig		
		IgM	IgG	IgA
		*μg/culture**		
Case 1	None	0	0	0
	PWM	0.6	0	0
	S. aureus Cowan I	2.1	0	0
	S. paratyphi B	0	0	0
Case 2	None	0	0	0
	PWM	2.0	0	1.2
	S. aureus Cowan I	3.8	0	1.5
	S. paratyphi B	0	0	0
Case 3	None	0	0	0
	PWM	0	0	0
	S. aureus Cowan I	0	0	0
	S. paratyphi B	0	0	0
Case 4	None	0	0	0
	PWM	0	0	0
	S. aureus Cowan I	0.8	0	0
	S. paratyphi B	0	0	0
Case 5	None	0	0	0
	PWM	0	0	0
	S. aureus Cowan I	0	0	0
	S. paratyphi B	0	0	0
Control (5 pooled)	None	0	0	0
	PWM	4.1	2.2	2.8
	S. aureus Cowan I	4.0	2.0	2.1
	S. paratyphi B	5.8	1.8	1.7

* Determined in 7-d cultures.

Discussion

Our results indicate that formaldehyde-fixed *S. paratyphi* B, a new PBA, activates normal human peripheral blood B lymphocytes to produce large amounts of Ig in culture without a significant increase in DNA synthesis and with no requirement for T cell collaboration. This is the first description of a human PBA which does not induce lymphocyte DNA synthesis. Our findings support the dissociation of cell proliferation from polyclonal activation (Ig synthesis), as has been reported in mice (24) and proposed for human PBL (25–27).

Because DNA synthesis is not required for B cell activation by *S. paratyphi* B, it could be argued that the bacterium may simply enhance Ig production by preexisting Ig-secreting cells. If this were the case, however, Ig production would be detectable as early as day 1 or day 2 after stimulation, and there would not be a significant increase in the number of plaque-forming cells in later days. Our data show that Ig production in cultures stimulated with *S. paratyphi* B was first detectable only on day 4. In addition, IgM plaque formation was first detected on day 3 and reached a maximum on day 6 (2,100 ± 300 and 12,960 ± 480, respectively). On the other hand, IgM was detectable in cultures stimulated with *S. paratyphi* B 1 d earlier than in cultures stimulated with PWM or *S. aureus* Cowan I (Table I), and our preliminary data

indicate that significant numbers of IgM plaque-forming cells appeared 1 d earlier than in cultures stimulated with PWM or *S. aureus* Cowan I. These data suggest that the cells responding to *S. paratyphi* B are more differentiated toward Ig-secreting cells than are the cells responding to PWM or *S. aureus*. Thus, the responding cells may be immediate precursors of Ig-secreting cells.

The mechanism by which *S. paratyphi* B induces Ig synthesis by human B cells is not clear. Our data (Table IV) indicate that the PBL response to LPS differs from the response to *S. paratyphi* B, and most strains of G(−) bacteria containing LPS that we tested did not activate human PBL (unpublished data). Thus, it is likely that activation is due to components of the cell wall other than LPS. In addition, *S. paratyphi* B binds all human peripheral blood B lymphocytes (Dr. M. Teodorescu, personal communication), and it may be that activation is due to their binding to the bacterium. Our preliminary data show higher Ig production in cultures centrifuged to promote rosette formation between the bacteria and lymphocytes than in mixtures incubated without centrifugation, suggesting that close contact between this bacterium and B lymphocytes is indeed important for the activation process.

We have shown that the B cell response to *S. paratyphi* B is relatively T independent (Table V). Our preliminary data further confirmed that human cord blood lymphocytes responded strongly to this bacterium but not to PWM. Because human cord blood lymphocytes contain large numbers of suppressor cells (28, 29), the response of cord blood lymphocytes to *S. paratyphi* B indicates that the responding cells are not influenced by suppressor T cells. By contrast, in five patients with CVID without elevated suppressor cell activity in their PBL, no Ig was detected on day 7 in cultures stimulated with *S. paratyphi* B. The number of T cells with Fc receptors for IgG in the patients' PBL cultures was virtually nil, and no suppressor effects by autologous T cells were detectable by Waldmann's technique (22). In addition, the numbers of T and B cells in our five patients with CVID were within the normal range. Thus, it is possible that CVID patients lack the B cell subpopulation capable of responding to *S. paratyphi* B.

In conclusion, formaldehyde-fixed *S. paratyphi* B appears preferable to other available PBA for the comparative analysis of human B cell function in normal and disease states, in that it is relatively T cell independent and stimulates higher levels of Ig secretion than do other PBA. Furthermore, this new PBA should facilitate investigations of (*a*) B cell defects manifesting clinically as "agammaglobulinemia," and (*b*) the ontogeny of the human humoral immune response.

Summary

A "new" polyclonal activator of human peripheral blood B cells, formaldehyde-fixed *Salmonella paratyphi* B, is described. This bacterium does not stimulate cell proliferation as measured by incorporation of tritiated thymidine but does stimulate a subpopulation of B cells to secrete large amounts of IgM, IgG, and IgA in 7-d cell cultures. The immunoglobulins (Ig) produced by cells responding to *S. paratyphi* B are not specific antibodies against the bacterial antigens. In comparison with other B cell activators (pokeweed mitogen, *Staphylococcus aureus* Cowan I, and lipopolysaccharide), *S. paratyphi* B stimulation produced greater amounts of IgM but less IgG than pokeweed mitogen (PWM) or *S. aureus* Cowan I; lipopolysaccharide failed to stimulate

significant Ig production on day 7 in most cases. In addition, the response to *S. paratyphi* apparently did not require T cell collaboration. These results suggest that the B cell subpopulation(s) responding to *S. paratyphi* B may be more differentiated B cells than those responding to either PWM or *S. aureus* Cowan I. Peripheral blood mononuclear cells from five patients with common variable immunodeficiency without evidence of abnormal suppressor T cells or monocytes failed to respond to *S. paratyphi* B, whereas cells from two of the same patients responded well to *S. aureus* Cowan I and partially to PWM. Thus, *S. paratyphi* B appears to be superior to other B cell activators for studies of B cell function in normal and abnormal states.

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