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Studies on the Mechanism of Peptidoglycan- and Lipopolysaccharide-Induced Polyclonal Activation

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Peptidoglycan (PG) and lipopolysaccharide (LPS) are T cell-independent B cell mitogens and polyclonal activators in mice. The mechanism of in vitro proliferation and polyclonal activation of mouse splenocytes induced by PG from *Staphylococcus aureus* and LPS from *Escherichia coli* was further studied by using [^{3}H]thymidine incorporation and protein A hemolytic plaque assays. Concanavalin A-generated suppressor cells suppressed both polyclonal and proliferative responses induced by PG, LPS, and pokeweed mitogen. The suppression of the proliferative responses was similar for all these mitogens, but was significantly less pronounced than the suppression of the polyclonal antibody response. Polyclonal activation induced by LPS was the most susceptible to suppression by concanavalin A-generated suppressor cells, and the suppression was significantly greater than in the PG-induced polyclonal response. Also, PG-induced polyclonal activation was not susceptible to inhibition by polymyxin B, which is an inhibitor of other B cell mitogens and polyclonal activators. For optimal generation of immunoglobulin-secreting cells, PG or LPS had to be present for at least 48 h after the initiation of the cultures. Removal of the mitogens after 4 or 24 h of incubation resulted in a suboptimal response. For effective induction of the proliferative response, the mitogens had to be present in cultures for over 24 h. Polyclonal-activating properties of staphylococcal cell wall components were also compared. PG was by far the most potent inducer of polyclonal antibodies. Teichoic acid was not active as a polyclonal activator, whereas purified cell wall and protein A were very weak inducers of polyclonal antibodies. These studies demonstrate that PG, in addition to LPS, can be a useful probe for studies on polyclonal activation.

Cells of a number of microbial species as well as various bacterial products have been shown to possess mitogenic and polyclonal activating properties. Mitogens are substances which cause DNA synthesis, blast transformation, and ultimately division of lymphocytes. Interestingly, the majority of bacteria and bacterial products stimulate B lymphocytes (1, 2, 4, 6–9, 12, 14, 16, 19, 27, 30, 33, 39, 41, 42, 45), whereas plant mitogens stimulate primarily T lymphocytes (reviewed in reference 36). Most of the B cell mitogens (polyclonal activators) are also able to induce differentiation of lymphocytes resulting in the synthesis and secretion of polyclonal antibodies (1, 4, 8, 16, 17, 27, 32, 33, 39, 45). Both mitogens and polyclonal B cell activators can stimulate lymphocytes in a nonspecific manner, i.e., without involving antigen-specific receptors (the variable part of the immunoglobulin receptor in the case of B lymphocytes) (17). In mice, B-cell activation appears to be a result of a direct interaction of an activator with B lymphocytes, since no other cells are required for this process (8, 17, 32). In humans, antibody secretion resulting from activation of B lymphocytes from peripheral blood by commonly used polyclonal activators appears to require the help of both T lymphocytes and macrophages (23, 40, 42). In addition, some T cell mitogens have been shown to cause polyclonal B lymphocyte activation indirectly by inducing secretion of mitogenic factors from T cells (38).

Both mitogens and polyclonal B cell activators have been extremely useful in analyzing the mechanisms of lymphocyte activation and differentiation. These agents serve as (i) a model for the antigen-driven response (17, 32), (ii) probes of lymphocyte maturation or differentiation (or both) (17, 29), and (iii) indicators of lymphocyte function in a number of diseases affecting the immune system (13, 36). Therefore, to make meaningful comparisons between laboratory animals and humans, it is important to utilize mitogens and polyclonal activators that

are equally active in humans and rodents and whose mechanism of action and chemical structure are well characterized.

Whole bacterial cells are effective as mitogens and polyclonal activators (2, 39, 41). They are, however, less useful than purified products, because the presence of diverse components which act via different mechanisms may make interpretation of results difficult.

Lipopolysaccharide (LPS), a protein-free endotoxin from gram-negative bacteria, has been the most extensively studied B cell mitogen and polyclonal B cell activator in mice (reviewed in references 17, 29, 32, and 36). However, the structure of LPS is extremely complex and can vary even within the same species (28). Moreover, it has not been widely used for studies in humans, since it does not readily activate human peripheral blood lymphocytes under standard culture conditions (16, 35, 37). Only some preparations of LPS were able to activate human lymphocytes when special culture conditions were employed (26, 31, 45).

The use of peptidoglycans (PG), heteropolymers present in cell walls of all bacteria (43), offers the possibility of overcoming these difficulties and adds another useful tool to the immunologist's armamentarium. The chemical structure of PG is very well established, and it is also relatively constant (43). Staphylococcal PG is a B cell mitogen and polyclonal activator which possesses equally high activity in both mice and humans and is equally effective in the induction of DNA synthesis and secretion of polyclonal antibodies (8–10; A. I. Levinson, A. Dziarski, B. Zweiman, and R. Dziarski, Arthrit. Rheumat. **24**:577, 1981; A. I. Levinson, A. Dziarski, B. Zweiman and R. Dziarski, submitted for publication). PG-induced mitogenic and polyclonal activation of murine B lymphocytes is T cell independent and, to a large extent, macrophage independent (8, 9). PG is not mitogenic for murine T lymphocytes (9). By contrast, in cultures of human peripheral blood lymphocytes both T and B lymphocytes are activated, and induction of polyclonal antibody secretion in B lymphocytes is T cell dependent (Levinson et al., submitted for publication).

In this study we further investigated the mechanism of PG-induced proliferation and polyclonal activation of murine splenocytes and compared it to LPS-induced activation in terms of sensitivity to concanavalin A (ConA)-induced suppressor cells, sensitivity to polymyxin B, and the time of exposure to PG that is required for activation of lymphocytes. We also evaluated polyclonal activating properties of other staphylococcal cell wall fractions, i.e., teichoic acid (TA), protein A, and purified cell wall (PG-TA complex), since some of these products were shown to act as mitogens or polyclonal activators (or both) of human lymphocytes (10, 14, 27, 42). The reports of their mitogenic or polyclonal activating properties in murine lymphocytes, however, are controversial (10, 33, 34, 46).

MATERIALS AND METHODS

Mice. Female BALB/c mice 6 to 8 weeks old were obtained from Flow Laboratories, McLean, Va., or Ace Animals, Inc., Boyertown, Pa.

CW fractions, polyclonal activators, and mitogens. Cell wall (CW), PG, and TA were obtained from *Staphylococcus aureus* 845 as previously described (11). Briefly, to obtain purified CW (PG-TA complex), cells were disrupted in a Mickle disintegrator, and proteins and nucleic acids were removed from the crude CW preparation by trypsin and RNase and DNase digestion. TA and PG were separated by trichloroacetic acid extraction. Analysis of the chemical composition of TA revealed the presence of alanine, glucosamine, and ribitol (anhydroribitol); PG contained lysine, glutamic acid, glycine, alanine, glucosamine, and muramic acid; whereas CW contained all of the above components (11). Accidental contamination with exogenous endotoxins was ruled out by toxicity tests in adrenalectomized mice (9). Before use, PG and CW were suspended in sterile phosphate-buffered saline, treated with ultrasonication, heated at 70°C, and tested for sterility (8). Staphylococcal protein A was obtained from Pharmacia Fine Chemicals (Piscataway, N.J.). *Escherichia coli* O127:B8 LPS, prepared by a phenol-water extraction method, was obtained from Sigma Chemical Co. (St. Louis, Mo.), and pokeweed mitogen (PWM) was purchased from GIBCO Laboratories (Grand Island, N.Y.).

Cell cultures. Spleen cell suspensions and cultures were prepared as previously described (8–10) in RPMI 1640 medium supplemented with 10% (for polyclonal activation) or 5% (for mitogenicity) fetal calf serum, antibiotic-antimycotic mixture, and 10 mM HEPES (*N*-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer (for polyclonal activation only). For polyclonal activation, replicate cultures of 5×10^5 cells (0.5 ml each) in 5-ml plastic tubes were established. For mitogenicity, quadruplicate cultures of 2×10^5 cells (0.2 ml each) were established in 96-well flat-bottom tissue culture plates. Appropriate concentrations of polyclonal activators or mitogens were added at the initiation of the cultures, which were then incubated at 37°C in a humidified atmosphere with 5% CO_2 and 95% air.

Assay for IgS PFC. After 4 days of incubation with or without polyclonal activators (unless otherwise indicated), each culture was washed with 5 ml of basal medium Eagle (GIBCO) and suspended for the plaque assay. In some experiments cultures were additionally washed two times with basal medium Eagle to remove products (e.g., protein A) which might interfere with the plaque assay. Numbers of viable cells were determined by the trypan blue dye exclusion method. Numbers of immunoglobulin-secreting plaque-forming cells (IgS PFC) were determined as described previously (8) by using the protein A hemolytic plaque-forming cell (PFC) assay. Briefly, staphylococcal protein A was coupled to sheep erythrocytes with $CrCl_3$,

and the protein A-sheep erythrocytes were mixed with appropriately diluted lymphocyte suspensions and 1.12% agarose at 43°C. The mixtures were plated in 5-cm petri dishes precoated with a layer of 2.5% agarose. The plates were incubated in a humidified atmosphere (5% CO_2–95% air) at 37°C for 30 min, and 1 ml of appropriately diluted polyvalent rabbit anti-mouse immunoglobulin serum specific to γ-heavy and κ- and λ-light chains (Cappel Laboratories, Inc., Cochoranville, Pa.) was added. After 1.5 h of further incubation, the anti-immunoglobulin serum was replaced with 1 ml of guinea pig complement, and the plates were then incubated for an additional hour. Complement lyses the erythrocytes surrounding immunoglobulin-secreting lymphocytes and results in the formation of a plaque. The application of polyvalent anti-mouse immunoglobulin serum allows detection of all IgS PFC (8). Plaques were counted under a magnifying glass and dark background illumination. Because of the differences in the numbers of viable cells recovered from cultures after incubation with some mitogens, in most experiments the results were expressed both as IgS PFC/10^4 cultured cells and IgS PFC/10^4 viable recovered cells.

Mitogenicity assay. After 24 h of incubation with or without mitogens each culture was pulsed with 0.5 μCi of [^{3}H]thymidine (specific activity, 6.7 Ci/mmol). After an additional 18 h of incubation cells were collected on glass microfiber filters with an automated cell harvester, and radioactivity was determined in a liquid scintillation counter (9, 10). The results (incorporation of radioactivity into DNA) were expressed as counts per minute per culture.

Inhibition by polymyxin B. Polyclonal activators were mixed with equal volumes of appropriately diluted polymyxin B sulfate (7,900 U/mg; Sigma), and after 30 min at room temperature the mixtures were added to the cell cultures.

ConA-induced suppressor cells. Suppressor cells were generated as described by Krakauer et al. (25), with minor modifications. Spleen cells were cultured with 3 μg of ConA (Calbiochem, San Diego, Calif.) per ml in RPMI 1640 medium, supplemented with 10% fetal calf serum, 10 mM HEPES, and an antibiotic-antimycotic mixture, at a concentration of 10^6 cells per ml in 2.5-ml samples in 24-well flat-bottom tissue culture plates (Linbro Scientific, Inc., Hamden, Conn.), at 37°C in a humidified 5% CO_2–95% air atmosphere. After 24 h cells were harvested, washed three times with Hanks balanced salt solution, and suspended in tissue culture medium. Control cells were cultured under the same conditions, but without ConA. For mitogenicity experiments, control cells and ConA-generated suppressor cells were, in addition, treated at 37°C for 30 min with 25 μg of mitomycin C (Sigma), an irreversible inhibitor of DNA synthesis, per ml. The cells were then washed three times and resuspended in tissue culture medium. Control cells or ConA-generated suppressor cells were mixed with equal numbers of spleen cells (5×10^5 cells in 0.5 ml for polyclonal activation, or 1×10^5 cells in 0.1 ml for mitogenicity), and cultured with appropriate polyclonal activators. IgS PFC or proliferation were then assayed as described above. The results are expressed as a percentage of control, which equals the following: (number of IgSPFC or [^{3}H]thymidine incorporation in cultures with ConA-generated suppressor cells × 100) ÷ (numbers of IgS PFC or [^{3}H]thymidine incorporation in cultures with control cells).

Statistical methods. Student's *t*-test was used to determine the significance of the observed differences.

RESULTS

Polyclonal activating properties of staphylococcal cell wall components. Numbers of IgS PFC induced in cultures of splenocytes incubated with various concentrations with PG, CW, TA, and protein A were compared (Table 1). PG, at its optimal concentration determined in previous experiments (8), was the most effective polyclonal activator. CW and protein A induced a small (ca. 10 times smaller than PG), but statistically significant, increase in the numbers of IgS PFC, as compared with control cultures incubated without any stimulants. The optimal concentrations were 15 μg/ml for CW and 1,000 μg/ml for protein A. TA did not induce polyclonal activation of immunoglobulin secretion. In cultures incubated with high concentrations of CW and TA a decrease in the numbers of PFC was observed due to the toxicity of these preparations. In kinetics experiments (Fig. 1), the numbers of IgS PFC peaked on day 4 in both protein A- and PG-stimulated cultures, whereas in CW-stimulated cultures they peaked on day 2 of incubation. TA (data not shown) did not induce polyclonal activation (higher than in control cultures) despite a prolonged 6-day incubation period.

Inhibition of polyclonal activation by polymyxin B. Polymyxin B, a cationic antibiotic, binds to LPS and other B cell mitogens and inhibits their polyclonal activating properties (5, 21, 44). Polymyxin B, as expected, inhibited the ability of LPS to induce polyclonal antibodies, but it did not significantly ($P > 0.05$) inhibit PG-induced polyclonal activation (Fig. 2). Similar results were obtained when, after incubation of PG or LPS with polymyxin B, the mixtures were extensively dialyzed to remove unbound polymyxin B, and then the dialysates were added to the lymphocyte cultures (data not shown).

Sensitivity to ConA-generated suppressor cells. ConA-generated suppressor cells can non-specifically suppress a variety of both cell-mediated and humoral immune responses, including polyclonal and specific in vitro antibody responses and mitogen-induced stimulation (18, 22, 24). Sensitivity to the suppression by ConA-generated suppressor cells of PG-, LPS-, and PWM-induced secretion of polyclonal antibodies and DNA synthesis were compared (Table 2). The suppression of polyclonal antibody response in cultures incubated with all three mitogens was significantly greater ($P < 0.05$ for PG and PWM; $P < 0.001$ for LPS) than the suppression of the proliferative response. The suppression of LPS-

TABLE 1. Polyclonal activation of splenocytes by staphylococcal cell wall components[a]

Expt	Stimulant added	Concn (μg/ml)	IgS PFC/10^4 cells	
			Cultured	Viable recovered
1	None		15 ± 2.0	52 ± 7.0
	PG	400	371 ± 7.5	827 ± 17
	CW	1	22 ± 3.2	91 ± 13
		5	35 ± 6.0	110 ± 19
		15	37 ± 5.0	134 ± 18
		50	15 ± 1.8	100 ± 12
		150	1.6 ± 0.7	11 ± 4.9
	TA	1	11 ± 3.6	54 ± 17
		10	5.6 ± 2.1	19 ± 7.0
		50	4.8 ± 1.4	15 ± 4.1
		150	0.8 ± 0.1	3.6 ± 0.8
	Protein A	1,000	46 ± 3.7	123 ± 12
2	None		7.1 ± 0.2	41 ± 1.1
	PG	400	319 ± 14	735 ± 32
	CW	15	27 ± 0.9	112 ± 3.6
	Protein A	1	8.9 ± 0.5	39 ± 2.3
		10	12 ± 0.3	46 ± 1.2
		100	16 ± 2.4	66 ± 9.7
		1,000	32 ± 5.3	98 ± 17

[a] Cultures of 5×10^5 splenocytes were incubated for 4 days with the indicated products and assayed for IgS PFC by the protein A hemolytic plaque assay. The results from two representative experiments are shown; the values are means ± standard errors of the means determined from four cultures.

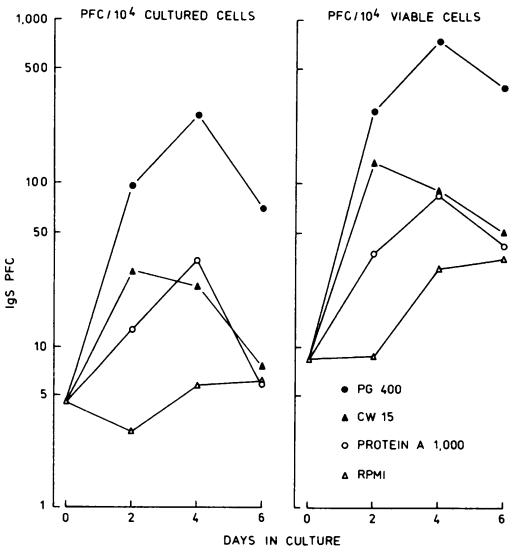

FIG. 1. Kinetics of polyclonal activation of splenocytes cultured with the indicated concentrations (micrograms per milliliter) of PG, CW or protein A, or in medium alone (RPMI). Cultures were prepared and assayed on the indicated days as described in footnote a of Table 1. The results are means determined from eight cultures in two experiments; standard errors were lower than 15% and are not indicated.

induced IgS PFC response was significantly greater ($P < 0.005$) than the PG-induced response, whereas the suppression of the PWM-induced IgS PFC response was intermediate, but not significantly different ($P > 0.05$) from the LPS- or PG-induced PFC response. These differences were not due to selective loss of viability of the cells in some groups of cultures, as very similar results were obtained when these data were expressed as IgS PFC per 10^4 cultured cells (data not shown).

Time of exposure of lymphocytes to PG or LPS required for activation. The requirement for and the role of the continuous presence of a stimulant in lymphocyte activation was studied. The response of cells cultured with PG or LPS for 96 h (optimal period for the induction of IgS PFC) was compared with the responses of cultures from which PG or LPS were removed after 48, 24, or 4 h of incubation by repetitive washing in culture medium and then culturing the cells for the remaining periods of time without adding any stimulants. As an additional control, to account for any possible damage to the cells during the washing procedure, we used cultures which were washed in the same way after 24 h of incubation with PG or LPS, and to which the same mitogens were then added back and the incubation was continued for the remaining 72 h.

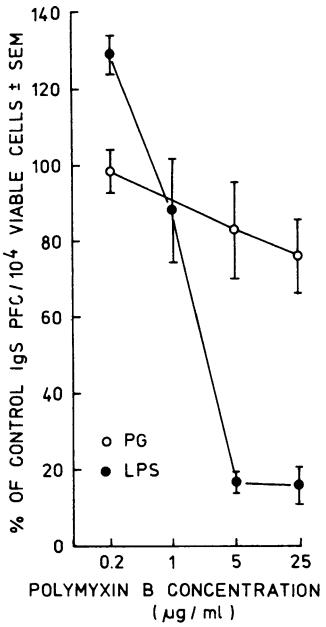

FIG. 2. Effect of polymyxin B on PG- and LPS-induced polyclonal activation. PG or LPS was mixed with polymyxin B and then added to the splenocyte cultures to give the indicated final concentrations of polymyxin B and 400 μg of PG per ml or 50 μg of LPS per ml. Cultures were incubated and assayed as in Table 1. The values are means determined in four experiments, in each experiment three cultures were pooled before the assay. Control cultures incubated only with PG and only with LPS yielded 407 ± 128 and 240 ± 58 IgS PFC/10^4 viable cells.

For the optimal generation of IgS PFC, PG, or LPS had to be present for at least 48 h after initiation of the cultures (Table 3). The removal of PG or LPS after 24 or 4 h of incubation resulted in a suboptimal response.

In similar experiments, induction of DNA synthesis in lymphocytes cultured for 48 h was studied (Table 4). A 24-h incubation period with PG or LPS was sufficient for the induction of almost optimal proliferative responses. The removal of PG or LPS after 4 h of incubation resulted in a greatly reduced mitogenic response.

DISCUSSION

Our results indicate that PG is the most active part of staphylococcal CW and is mainly responsible for the induction of polyclonal antibodies in mice. This is in agreement with our previous findings of the dominant role played by PG in the induction of the proliferative response (10). These and previous (10) results indicate that TA, when bound to PG, renders the CW cytotoxic and inhibits both the polyclonal activating and mitogenic properties of the CW. TA was not active as a polyclonal activator. Protein A and CW, in our culture system, were very weak polyclonal activators of mouse splenocytes, although the former is quite effective as a polyclonal activator in humans (27). This finding underscores the differences between the rodents and humans in the ability to respond to polyclonal activators. Moller and Landwall (33) reported a somewhat higher polyclonal activating effect of protein A in mice than that obtained in our studies. This discrepancy is most probably due to the use of serum-free media in their cultures, which resulted in low background levels of PFC of unstimulated cells. The lack of polyclonal activating properties of protein A in mice reported by Nakano et al. (34) was most likely due to the use of protein A at concentrations that were too low.

A number of polyclonal B cell activators in mice are, like LPS, polyanionic structures, and their activity can be inhibited by polymyxin B (5, 21, 44). PG-induced polyclonal activation was not inhibited by this antibiotic, which indicates that polyanionic structures binding polymyxin B are either absent or unavailable on the PG molecule, and that these structures are not required for a substance to be a polyclonal B cell activator. Our recent findings also indicate that

TABLE 2. Sensitivity to suppression by ConA-generated suppressor cells of PG-, LPS- and PWM-induced activation of splenocytes[a]

Stimulant added[b]	% of control	
	IgS PFC/10^4 viable cells	DNA synthesis[c]
PG	45 ± 4[d]	63 ± 5
LPS	20 ± 4	55 ± 4
PWM	34 ± 7	67 ± 12

[a] Cultures were prepared, incubated, and assayed as described in footnote *a* of Table 1. For DNA synthesis assays cultures were incubated for 24 h and labeled with [^{3}H]thymidine for an additional 18 h. Each culture received 50% of ConA-activated cells or 50% of cells cultured without ConA (control).

[b] PG, 400 μg/ml; LPS, 50 μg/ml (PFC assays) or 100 μg/ml (proliferation); PWM, 1:100 (vol/vol).

[c] In proliferation experiments ConA-activated or control cells were treated with mitomycin C.

[d] The values are means ± standard errors of the mean obtained in four experiments. Control cultures had the following yields when incubated as follows: with PG, 976 ± 224 IgS PFC/10^4 viable cells and 19,072 ± 1,037 cpm; with LPS, 734 ± 106 IgS PFC/10^4 viable cells and 16,064 ± 920 cpm; with PWM, 83 ± 13 IgS PFC/10^6 viable cells and 6,671 ± 692 cpm; and without any stimulants, 24 ± 2.6 IgS PFC/10^4 viable cells and 2,366 ± 282 cpm.

TABLE 3. Time of the exposure of lymphocytes to PG or LPS required for polyclonal activation[a]

Stimulant added[b]	Time of incubation with stimulant (h)[c]	IgS PFC/10^4 cells[d]	
		Cultured	Viable recovered
None	0–96	8 ± 0.2	24 ± 4.5
PG	0–96	354 ± 151	563 ± 144
LPS	0–96	193 ± 23	341 ± 32
PG	0–24; 24–96	294 ± 98	558 ± 128
LPS	0–24; 24–96	299 ± 105	498 ± 113
None	0–48	12 ± 6.1	19 ± 9.3
PG	0–48	306 ± 84	518 ± 122
LPS	0–48	137 ± 40	237 ± 41
None	0–24	11 ± 2.3	43 ± 18
PG	0–24	100 ± 17	227 ± 38
LPS	0–24	87 ± 17	202 ± 20
None	0–4	28 ± 8.3	54 ± 12
PG	0–4	61 ± 11	131 ± 29
LPS	0–4	53 ± 8.2	93 ± 16

[a] Cultures were prepared and assayed after 96 h of incubation as described in footnote *a* of Table 1.
[b] PG, 400 μg/ml; LPS, 100 μg/ml.
[c] Stimulants were removed at the end of indicated time periods by repetitive washing of lymphocytes.
[d] The values are means ± standard errors of the mean determined in three experiments; in each experiment three cultures were pooled before the assay.

both mitogenic and polyclonal activating properties of PG are inhibited by anti-PG antibodies when PG is reacted with these antibodies before being added to the cultures (R. Dziarski and A. Zeiger, unpublished results). These antibodies did not block the polyclonal activating properties of LPS or the mitogenic activity of several mitogens. Taken together, these data suggest that lymphocyte-activating properties of PG and LPS may depend on structurally different parts of their molecules and therefore possibly involve interactions with different receptors on the lymphocyte surface. However, further studies are required to verify this hypothesis.

TABLE 4. Time of the exposure of lymphocytes to PG or LPS required for induction of DNA synthesis[a]

Stimulant added[b]	Time of incubation with stimulant (h)[c]	Net cpm[d] ± SEM
PG	0–48	53,401 ± 719
LPS	0–48	20,840 ± 228
PG	0–24; 24–48	30,673 ± 1,093
LPS	0–24; 24–48	21,348 ± 1,068
PG	0–24	28,081 ± 3,964
LPS	0–24	16,611 ± 477
PG	0–4	4,521 ± 304
LPS	0–4	5,065 ± 363

[a] Cultures were prepared and assayed after total 48 h of incubation as described in footnote *a* of Table 2.
[b,c] See footnotes *b* and *c* of Table 3.
[d] Net cpm, Difference in counts per minute between stimulated and unstimulated cultures. The values are means determined from four cultures in this representative experiment.

It has been suggested that in certain diseases affecting the immune system, e.g., systemic lupus erythematosus or juvenile rheumatoid arthritis, one of the possible immunologic defects involves suppressor cells (reviewed in reference 24). We decided to study the sensitivity to suppression by ConA-generated suppressor cells of PG-, LPS-, and PWM-induced lymphocyte activation, because polyclonal activators are used in in vitro studies to evaluate lymphocyte function in patients with immunological diseases (13). Moreover, polyclonal activation by microbial products in vivo has been implicated in the pathogenesis of these diseases (20). Some polyclonal activators of bacterial origin, e.g., PG, may in fact contribute to the development and exacerbation of rheumatoid arthritis (3; I. Pardo, R. Dziarski, and A. I. Levinson, Clin. Res. **29**:488A, 1981; and I. Pardo, R. Dziarski, and A. I. Levinson, submitted for publication). It seemed possible, therefore, that polyclonal activation induced by PG had a different susceptibility to suppressor cells than polyclonal activation induced by other agents. Indeed, PG-induced secretion of polyclonal antibodies was significantly less susceptible to the suppression by ConA-generated suppressor cells than LPS-induced polyclonal activation. These results provide a basis for future studies on the role of PG-induced polyclonal activation and suppressor cells in immunological diseases.

If polyclonal activation by bacterial products plays any role in vivo, one of the factors influ-

encing this phenomenon may be the requirement for prolonged exposure of lymphocytes to these stimulants. As we have shown in this paper, this prolonged exposure is in fact necessary for effective induction of polyclonal antibodies in vitro. Optimal polyclonal activation of lymphocytes by both PG and LPS required the presence of these mitogens for the initial 48 h of incubation. Since bacterial cell wall components, including PG, can persist in tissues for long periods of time (reviewed in references 11 and 15), they could provide a continuous stimulus required for such an activation. Indeed, my recent study (R. Dziarski, J. Immunol., in press) demonstrated sizeable polyclonal lymphocyte activation by PG and LPS in vivo. Moreover, a large proportion of both in vitro- and in vivo-induced polyclonal antibodies were autoantibodies, which may have important pathological implications.

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