The Journal of Immunology
RESEARCH ARTICLE | JULY 01 1980

Staphylococcal protein A, a T cell-regulated polyclonal activator of human B cells. FREE

P E Lipsky

J Immunol (1980) 125 (1): 155–162.
https://doi.org/10.4049/jimmunol.125.1.155

0022-1767/80/1251-0155$02.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 125, No. 1, July 1980
Printed in U.S.A.

STAPHYLOCOCCAL PROTEIN A, A T CELL-REGULATED POLYCLONAL ACTIVATOR OF HUMAN B CELLS[1]

PETER E. LIPSKY[2]

From the Rheumatic Diseases Unit, Department of Internal Medicine, University of Texas Health Science Center, Dallas, Texas 75235

The capacity of staphylococcal protein A (SPA) to trigger the generation of immunoglobulin-secreting cells (ISC) from human peripheral blood mononuclear cells (PBM) was examined. SPA at low concentrations (≤1 μg/ml) induced polyclonal activation of human peripheral blood B cells, whereas higher concentrations (≥5 μg/ml) tended to suppress the response. SPA triggered the differentiation of both IgM- and IgG-secreting cells. Although the magnitude of the IgM response was comparable to that stimulated by pokeweed mitogen (PWM), SPA led to the differentiation of many fewer IgG-secreting cells than PWM. The response to SPA was absolutely T cell dependent. Moreover, the response was regulated by suppressor T cells. Inhibition of responsiveness observed with higher concentrations of SPA could be ascribed to the activity of suppressor T cells. In addition, PBM from normal individuals who responded poorly to lower concentrations of SPA were shown to have expanded suppressor cell activity. T cell suppression could be abolished by inhibiting T cell DNA synthesis with mitomycin C. SPA-triggered suppressor T cells were not specific in their action, since they were also able to suppress PWM-induced generation of ISC. In a number of individuals, suppressor T cells appeared to have different dose-response characteristics than helper T cells, in that they required higher concentrations of SPA to be activated. The ratio of SPA-responsive suppressor and helper T cells in PBM appeared to be quite low such that supplementation of B cell cultures with small numbers of T cells led to the expression of only help and little suppression. By using these two observations, it could be shown that although cell division was not necessary, SPA-induced helper T cell activity was augmented when the T cells were allowed to proliferate. These studies establish that SPA is a T cell-regulated activator of human peripheral blood B cells. Use of this probe should provide additional insights into the cellular interactions involved in the differentiation of antibody-forming cells in humans.

Staphylococcal protein A (SPA)[3] is a cell wall component synthesized by most strains of *Staphylococcus aureus* (1). It is a protein of 42,000 m.w. (2) that has been used extensively in various immunologic systems because of its capacity to bind immunoglobulins (Ig), especially IgG, from a number of species, including man (3). Recently, interest has also been directed toward the mitogenic potential of this molecule. Rodey *et al.* (4) originally reported that SPA stimulated human lymphocyte proliferation *in vitro*. Subsequent work has confirmed this observation and further delineated the characteristics of SPA-induced human lymphocyte activation (5–9). One important determinant of the capacity of SPA to stimulate various lymphocyte subpopulations seems to be the physical form of the mitogen. Thus, SPA bound to an insoluble matrix, such as the *Staphylococcus aureus* organisms itself or Sephadex or Sepharose beads, appears to specifically induce B cell proliferation (5–7). On the other hand, soluble SPA has been shown to stimulate both T cell activation (6, 8) and B cell DNA synthesis either directly (8, 9) or through a T cell-dependent mechanism (6).

S. aureus organisms (9–13) and SPA coupled onto Sepharose beads (9, 12) have also been shown to function as polyclonal activators of human B cells, inducing the differentiation of antibody-forming cells that secrete IgM antibodies of various specificities. The response of peripheral blood lymphocytes to *S. aureus* appears to be partially T cell independent, but nonetheless augmented by the addition of T cells (13). However, it is not clear that polyclonal activation of human blood B cells by *S. aureus* is related to the SPA content, since the Wood 46 strain of *S. aureus*, which does not contain SPA, still triggers the differentiation of antibody-forming cells in cultures of human spleen and blood lymphocytes (10). It has been claimed that soluble SPA functions as a polyclonal B cell activator of human spleen cells but is ineffective at triggering the differentiation of human peripheral blood B cells (9, 12). This has been taken as evidence that the subset of B cells capable of responding to soluble SPA with the generation of antibody-forming cells is not found in peripheral blood (12). Since PWM is an effective polyclonal activator of human peripheral blood B cells, these data suggested that different subsets of B cells might be stimulated by the two mitogens.

We therefore were interested in reexamining the capacity of soluble SPA to function as a polyclonal activator of human B cells. As opposed to previous results (10, 12), SPA at low concentrations was found to trigger differentiation of human

Received for publication January 24, 1980.
Accepted for publication March 17, 1980.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by United States Public Health Service Program Project Grant AM-09989 and by an Arthritis Foundation Clinical Center grant.

[2] Recipient of a National Institutes of Health Research and Career Development Award, 1-K04-AM00599.

[3] Abbreviations used in this paper: HBSS, Hanks' balanced salt solution; ISC, immunoglobulin-secreting cells; PBM, peripheral blood mononuclear cells; PWM, pokeweed mitogen; SPA, staphylococcal protein A; T_{mito}, mitomycin C-treated T cells; N-SRBC, neuraminidase-treated SRBC.

peripheral blood B cells into Ig-secreting cells (ISC). The cellular interactions involved in the generation and regulation of this reponse are described. SPA is a potent T cell-dependent, suppressor T cell-regulated activator of human B cells and, as such, should be a useful tool in dissecting the mechanisms involved in controlling the human immune response.

MATERIALS AND METHODS

Cell preparation. Peripheral blood mononuclear cells (PBM) were obtained from normal adult volunteers by centrifugation of heparinized venous blood on sodium diatrizoate/Ficoll cushions (Isolymph, Gallard-Schlesinger Chemical Mfg. Corp., Carle Place, N. Y.) as previously described (14). The cells were washed three times in Hanks' balanced salt solution (HBSS) before culture or further processing.

Mitogens. Pokeweed mitogen (PWM, Lot A665710) was purchased from Grand Island Biological Co., Grand Island, N. Y., and used at a final concentration of 10 μg/ml. SPA was obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. A 1 mg/ml solution of SPA contained no detectable endotoxin as judged by the Limulus lysate assay by using Lot EC-2 of the national standard *Escherichia coli* 0113 endotoxin as the assay standard.

Culture medium. All cultures were carried out in medium RPMI 1640 (Microbiological Associates, Walkersville, Md.), supplemented with penicillin G (200 units/ml), gentamicin (10 μg/ml), L-glutamine (0.3 mg/ml), and 10% fetal bovine serum (FBS, Microbiological Associates).

Cell separation. PBM were separated into T cell-enriched and B cell-enriched populations by means of previously described techniques (15). Briefly, PBM were partially depleted of monocytes by incubating them on glass Petri dishes. The nonadherent cell population was harvested and incubated with neuraminidase-treated sheep red blood cells (N-SRBC) (16). The rosetting and nonrosetting cell populations were then separated by centrifugation on diatrizoate/Ficoll gradients. The nonrosetting cells were obtained from the interface and again rosetted with N-SRBC and centrifuged on the diatrizoate/Ficoll gradients to remove residual T cells. The resultant cell population contained less than 1% T cells as determined by N-SRBC rosetting, and more than 50% B cells as judged by staining for surface membrane-associated IgM or IgD with fluorescein-conjugated goat antisera to human IgM or IgD. The pelleted cells from the first centrifugation were treated with isotonic NH_4Cl to lyse the N-SRBC and then passed over a nylon wool column. The population eluted from the column was highly enriched for T cells (90 to 95% N-SRBC rosetting). In some experiments, T cells were treated with mitomycin C (T_{mito}) before culture. This was accomplished by suspending the T cells in HBSS at approximately 5×10^6/ml and incubating them on a rotator for 45 min at 37°C with mitomycin C (Sigma Chemical Co., St. Louis, Mo.) at a concentration of 40 μg/ml. Afterward, the cells were washed four times and suspended in culture medium for use.

Culture conditions for generations of ISC. Cells were cultured in microtiter plates with U-bottomed wells (Dynatech Laboratories, Alexandria, Va.). Routine cultures were carried out in triplicate with each microwell containing 1×10^5 PBM in 0.2 ml of culture medium. Mitogen dissolved in HBSS or an equivalent volume of HBSS (0.02 ml) as control was added to the wells, and they were incubated for 6 to 7 days at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. At the end of the incubation, cells from triplicate wells were pooled, washed, and resuspended in HBSS for assay. Cultures of separated B and T cell populations were similarly carried out with each microwell containing 2.5×10^4 B cells alone or supplemented with varying numbers of T cells.

Detection of ISC. ISC were detected with a previously described reverse hemolytic plaque assay (15, 17) that made use of SPA-coated SRBC. Plaques were developed with a polyvalent rabbit anti-human Ig [IgA + IgM + IgG] (Cappel Laboratories, Cochranville, Pa.) diluted 1:50. IgM-secreting cells were detected with a heavy chain-specific rabbit anti-human IgM antiserum (Cappel Laboratories) and IgG-secreting cells with a heavy chain-specific rabbit anti-human IgG Fc antiserum whose properties have been previously described (17). The complement source was a 1:20 dilution of guinea pig serum (Pel Freez Biologicals, Inc., Rogers, Ark.) that had previously been absorbed with SRBC. All data are expressed as the number of ISC per 10^6 cells initially cultured.

Assay of lymphocyte DNA synthesis. Cultural conditions used for the assay of lymphocyte ^{3}H-thymidine incorporation were identical to those used for the generation of ISC. The cells were incubated for 72 hr at 37°C with 1 μCi ^{3}H-thymidine (6.7 Ci/mM, New England Nuclear Co., Boston, Mass.) present for the last 18 hr. The cells were harvested onto glass fiber filter paper and ^{3}H-thymidine incorporation determined by liquid scintillation spectroscopy. All data are expressed as the difference in counts per minute between the means of triplicate mitogen stimulated and control cultures (Δcpm).

RESULTS

SPA stimulates the generation of ISC from human PBM. Initial experiments were carried out to determine whether SPA would stimulate the differentiation of ISC from human peripheral blood B cells. PBM were obtained from 11 normal individuals and cultured with varying concentrations of SPA. As shown in Figure 1, SPA triggered responses in each experiment, although in two experiments the number of ISC generated was modest (<1500 ISC per 10^6 PBM cultured). In most of the experiments, maximum responsiveness was observed with 0.5 or 1.0 μg/ml SPA with lower and higher concentrations yielding a decrease in the number of ISC generated. By contrast, when

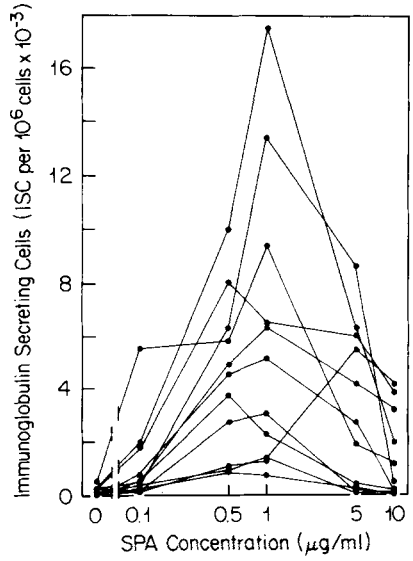

Figure 1. SPA-induced generation of ISC. PBM from 11 different individuals were prepared and cultured with various concentrations of SPA. After a 6- to 7-day incubation, the number of ISC generated was determined.

SPA-induced ^{3}H-thymidine incorporation was assayed by using cells from nine of these 11 individuals, responsiveness was found to vary directly with the concentration of SPA with no decrease in the magnitude of ^{3}H-thymidine incorporation observed with higher concentrations of SPA (Fig. 2).

On the basis of these results, PBM were obtained from a large number of individuals in order to compare responsiveness to SPA (1 μg/ml) with that stimulated by PWM, another polyclonal B cell activator. Figure 3 depicts data obtained from 142 separate experiments utilizing cells from 64 individual donors. In each experiment, PBM were stimulated with both PWM and SPA, and the magnitude of the response was compared. As can be seen, both mitogens stimulated the generation of ISC in most experiments. However, the magnitude of the PWM response (11, 340 ± 802 ISC per 10^6 cells, mean ± S.E.M) was nearly twice as great as the SPA response (5873 ± 481 ISC per 10^6 cells, mean ± S.E.M.). SPA stimulated the generation of more ISC than PWM in only 21 out of the 142 experiments. Moreover, SPA generated a minimal response (<2000 ISC per 10^6 cells) in 27% of the experiments, whereas minimal PWM responsiveness was found in only 8% of the experiments. Significant SPA-induced ^{3}H-thymidine incorporation was found in each experiment (data not shown).

Ig isotypes secreted in response to SPA and PWM. PBM was stimulated with either PWM or SPA, and after a 6-day incubation, the numbers of IgM- and IgG-secreting cells were determined. In 14 separate experiments, the number of IgM-secreting cells generated after culture with SPA was comparable to that found after stimulation with PWM (5493 ± 872 *vs* 5957 ± 746, respectively). By contrast, the number of IgG-secreting cells resulting from stimulation with SPA was significantly less than that triggered by PWM (1793 ± 341 *vs* 8129 ± 1118). As shown in Figure 4, the disparity between the number of SPA-induced IgG- and IgM-secreting cells was not a function of the length of culture, since more IgM-secreting cells were observed on each day throughout the period of incubation. It should be noted that in each experiment, SPA triggered the concurrent generation of IgM- and IgG-secreting cells.

T cell dependence of SPA responsiveness. B cells cultured alone were unable to generate significant numbers of ISC in response to either SPA or PWM (Table I). The small number of ISC found in these cultures was not significantly different from the number found when B cells were cultured without added mitogen (data not shown). Supplementation of B cell cultures with autologous T cells led to the generation of large numbers of ISC in response to both SPA and PWM.

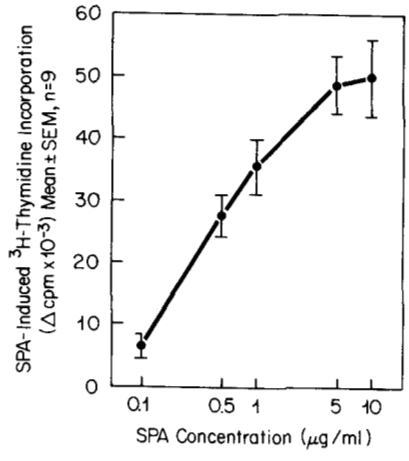

Figure 2. SPA-induced ^{3}H-thymidine incorporation. PBM from nine separate individuals were prepared and cultured with various concentrations of SPA. After a 72-hr incubation, ^{3}H-thymidine incorporation was determined. Each point represents the mean ± S.E.M. of the nine experiments.

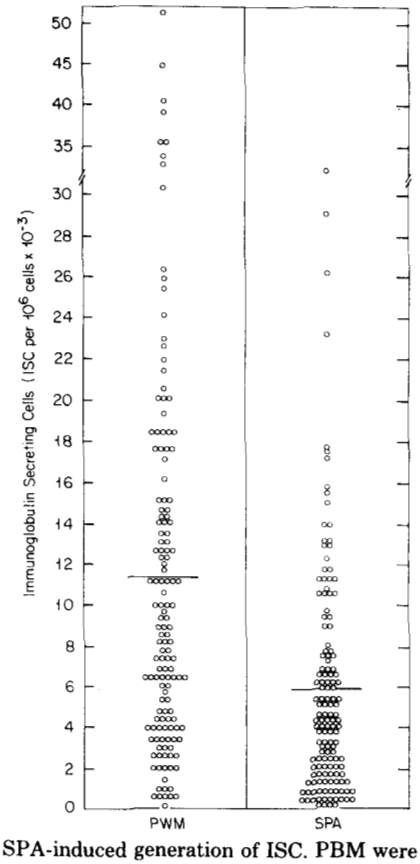

Figure 3. SPA-induced generation of ISC. PBM were obtained and cultured with either PWM (10 μg/ml) or SPA (1 μg/ml). After a 6- to 7-day incubation the number of ISC was determined.

SPA responsiveness is regulated by suppressor T cells. Initial experiments were carried out to determine whether exaggerated suppressor cell activity might explain the decreased SPA responsiveness manifested by PBM obtained from some normal individuals. In these experiments, PBM were obtained from four unrelated individuals, two of whom responded well and two poorly to SPA. These cells were either cultured alone with SPA, or PBM from two individuals were co-cultured with the mitogen, and the number of ISC generated was determined. As can be seen in Figure 5, nonresponder PBM inhibited the SPA response of responder PBM, whereas co-culture of PBM from the two responders led to no inhibition of responsiveness. Comparable results were obtained from two similar co-culture experiments. When SPA-induced ^{3}H-thymidine incorporation was examined, PBM from each individual were found to be comparably responsive (Δcpm = 27,256 ± 1176, mean ± S.E.M.). Moreover, SPA-induced ^{3}H-thymidine incorporation was not significantly affected when PBM from different individuals were co-cultured (data not shown).

These results suggested that polyclonal activation of B cells by SPA was regulated by the action of suppressor cells and that low responders had increased suppressor cell activity. In order to examine the role of suppressor cells in the SPA response further, experiments were undertaken by using purified populations of B and T cells. Since the suppressor T cells that inhibit PWM-induced generation of ISC are required to undergo cell division in order to express their suppressive activity (18–22),

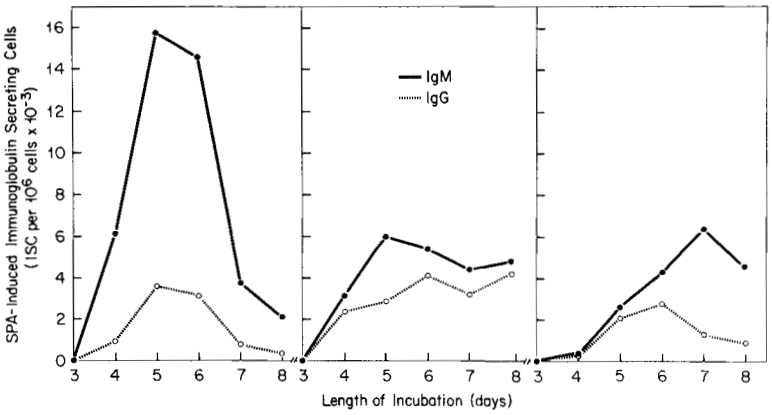

Figure 4. Kinetics of SPA responsiveness. PBM were obtained from three separate individuals and cultured with SPA (1 μg/ml). After various lengths of incubation, the number of IgM- and IgG-secreting cells was determined.

TABLE I

T cell dependence of SPA-induced generation of ISC

Supplemental T Cells[a]	Mitogen-Induced ISC	
	SPA	PWM
	ISC per 10^6 B Cells × 10^{-3} [b]	
−	0.4 ± 0.2	0.5 ± 0.1
+	29.7 ± 3.9	77.1 ± 10.3

[a] 2.5×10^4 B cells cultured with or without 1×10^5 T cells.
[b] Mean ± S.E.M. of 23 separate experiments.

experiments were carried out to determine whether inhibition of T cell proliferation would also alter suppression in the SPA system. In these experiments, B cells were cultured with autologous T cells or T cells that had been treated with mitomycin C to prevent DNA synthesis. As can be seen in Table II, the magnitude of the SPA response was greater in most of the experiments (1 through 11) when cultures were supplemented with T_{mito} rather than untreated control T cells. This was observed in experiments in which the PWM response was either augmented (expts. 1 through 7) or decreased (expts. 8 through 11) when B cell cultures were supplemented with T_{mito} instead of control T cells. In other experiments (12 through 16), cultures supplemented with T_{mito} generated fewer ISC in response to SPA than those containing control T cells. Of note was the finding that SPA stimulated the differentiation of more ISC than PWM in only two of the 16 experiments.

These results suggested that suppressor T cells played a role in regulating SPA responsiveness in cells obtained from some individuals and that cell division was required for suppressor T cells to exert their activity. It remained unclear whether the action of such suppressor T cells might be an important determinant of the SPA response in the other subjects. These experiments, however, had examined the response to a single concentration of SPA in cultures supported by only one density of added T cells. In the next experiments, therefore, larger numbers of T cells were utilized to determine whether suppressor T cell activity would become more apparent. As shown in Table III, the use of larger numbers of T cells (5×10^5/culture) markedly increased the degree of suppression seen in SPA-stimulated cultures. Both IgM and IgG responses were suppressed, and suppression was reversed by treating the T cells with mitomycin C.

Various concentrations of SPA were next used to explore further the role of suppressor T cells in determining the magnitude of the SPA response. In the experiments shown in Figure 6, B cells were cultured with autologous T cells or T_{mito} and stimulated with various concentrations of SPA. In each experiment, more ISC were generated in response to 5 or 10 μg/ml

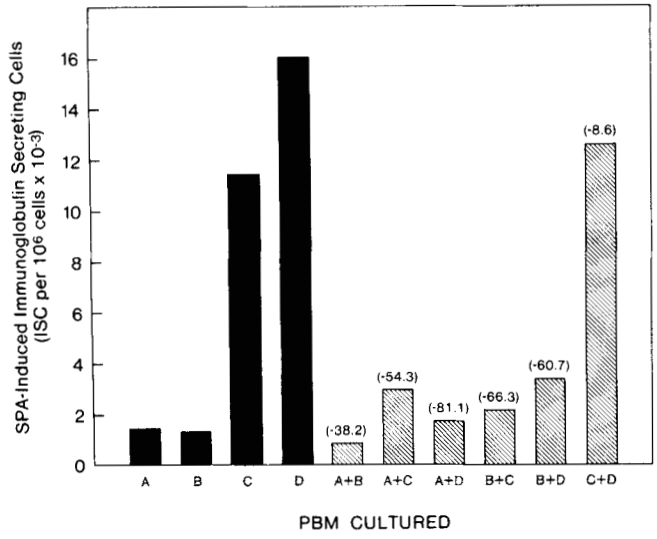

Figure 5. Co-culture of low responder and high responder PBM. PBM from four individuals were prepared. The PBM were either cultured alone (1×10^5/microwell) or mixed with another population of PBM as indicated and cultured. Co-cultures consisted of 0.5×10^5 of each cell population/microwell. After a 6-day incubation with SPA (1 μg/ml), the number of ISC was determined. The terms in parentheses indicate the percentage change from predicted that is calculated from the ratio of the number of ISC found in the co-cultures and the mean of the numbers of ISC found when each population was cultured alone.

SPA in cultures supplemented with T_{mito} than in those containing control T cells. For reference, the magnitude of the PWM response supported by T cells and T_{mito} (closed and open circles, respectively) is also shown. These results indicate that the decreased responsiveness of PBM seen with higher concentrations of SPA does not reflect mitogen-mediated inhibition of B cell function but rather the activity of suppressor T cells. When lower concentrations of SPA (<1 μg/ml) were employed, the response generated in the presence of T cells was found to be greater than or equal to that found in cultures supplemented with T_{mito}. These observations suggest the possibility that in some individuals helper T cells may be activated by lower concentrations of SPA than suppressor T cells. In addition, the data suggest that when helper T cells are preferentially triggered by low concentrations of SPA, their capacity to help is augmented when they are allowed to proliferate. As with PWM-stimulated helper T cell activity (18, 19, 21–23), however, cell division does not appear to be a prerequisite for the generation or expression of SPA-induced T cell help.

In order to examine these questions more fully, experiments were carried out in which both the number of supplemental T

TABLE II

Mitogen-induced generation of ISC: role of suppressor T cells

Experiment	Mitogen-Induced B Cell Activation[a]			
	SPA		PWM	
	T cells	T_{mito}	T cells	T_{mito}
	ISC per 10^6 B cells × 10^{-3}			
1	68.0	312.0	144.0	264.0
2	12.0	26.8	44.0	66.0
3	23.6	90.4	108.4	220.4
4	35.2	88.8	101.6	187.6
5	54.4	108.4	48.4	172.8
6	18.0	91.6	109.6	121.2
7	42.8	47.6	87.4	156.2
8	53.2	70.0	120.0	111.6
9	6.8	10.8	81.6	68.0
10	63.2	163.2	232.8	218.4
11	34.4	40.4	86.8	40.4
12	34.0	19.6	13.7	31.6
13	24.4	8.2	97.0	115.6
14	27.2	18.4	48.8	44.0
15	15.2	12.8	42.4	49.2
16	18.0	2.8	38.8	30.0

[a] 2.5×10^4 B cells were cultured in each microwell with either 2×10^5 autologous T cells or 2×10^5 autologous T_{mito}. After a 6-day incubation with either SPA (1 μg/ml) or PWM (10 μg/ml), the number of ISC generated was determined. The experiments have been grouped to facilitate examination of the data. The rank order does not reflect the time sequence of the experiments.

TABLE III

Mitogen-induced ISC: role of suppressor cells

Expt.	Supplement to B Cell Cultures[a]	Mitogen-Induced ISC			
		SPA		PWM	
		IgM	IgG	IgM	IgG
		ISC per 10^6 B cells × 10^{-3}			
1	Nil	0	0.4	0	0
	1×10^5 T cells	13.6	23.6	39.6	84.8
	5×10^5 T cells	1.6	2.0	28.8	5.6
	1×10^5 T_{mito}	29.2	18.8	76.0	69.6
	5×10^5 T_{mito}	87.2	38.8	126.4	74.0
2	Nil	0	0	0.4	0
	1×10^5 T cells	26.2	2.4	23.2	30.0
	5×10^5 T cells	6.0	0	ND[b]	ND
	1×10^5 T_{mito}	30.4	8.0	24.0	64.0
	5×10^5 T_{mito}	46.4	13.2	ND	ND

[a] 2.5×10^4 B cells were mixed with the number of T cells or T_{mito} indicated and cultured with either SPA (1 μg/ml) or PWM (10 μg/ml). After a 6-day incubation the number of IgG- and IgM-secreting cells was determined.

[b] ND, not done.

cells and the concentration of SPA were varied. Data from two such experiments are shown in Figure 7. In the first experiment, helper T cell and B cell reactivity is observed with low concentrations of SPA (0.1 and 0.5 μg/ml). On the other hand, suppressor cell activity is not seen when cultures are stimulated with these low concentrations of SPA, but becomes apparent only when 1 μg/ml or more of SPA is used. In the second experiment, helper and suppressor T cells appear to be stimulated by all concentrations of SPA above 0.1 μg/ml. Evidence for suppressor T cell activity includes the finding that the number of ISC generated decreases as the number of T cells added to culture is increased and the observation that responsiveness is augmented in cultures supplemented with T_{mito} rather than control T cells.

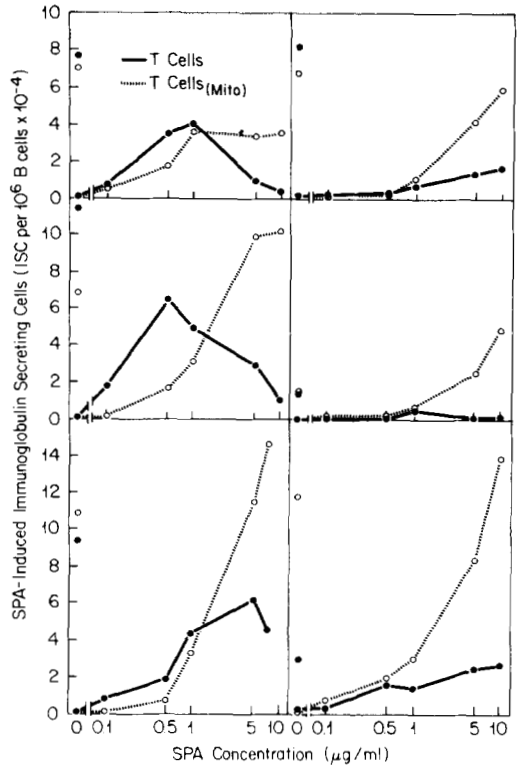

Figure 6. Suppressor T cell regulation of the SPA response. Purified B and T cell populations were obtained from six individuals. B cells (2.5×10^4/microwell) were then cultured with 2×10^5 T cells or T_{mito} and the number of ISC determined after a 6-day incubation with various concentrations of SPA. Closed circles indicate the magnitude of the PWM (10 μg/ml) response in cultures supported by T cells and open circles the PWM response supported by T_{mito}.

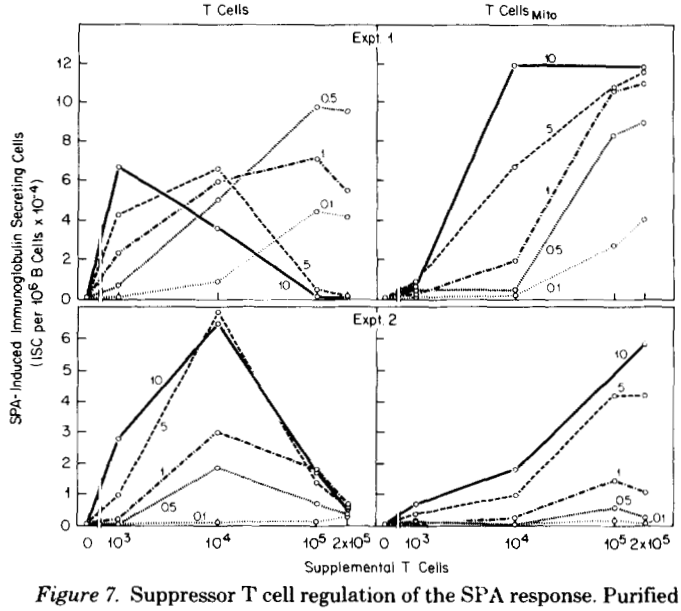

Figure 7. Suppressor T cell regulation of the SPA response. Purified B and T cell populations were obtained from two individuals. B cells (2.5×10^4/microwell) were then cultured with various numbers of T cells (*left panels*) or T_{mito} (*right panels*) and stimulated with various concentrations of SPA as indicated.

When cultures are supplemented with small numbers of T cells, responses to all concentrations of SPA are augmented when control T cells rather than T_{mito} are used. Suppression is

only observed in cultures containing larger number of T cells. In the first experiment, suppression is seen with as few as 10^4 T cells when 10 μg/ml of SPA is used, whereas more T cells are required for suppression to become apparent with lower concentrations of SPA. In the second experiment, 10^5 T cells are required to cause suppression with all concentrations of SPA. These observations suggest that the ratio of SPA responsive suppressor to helper T cells is low in peripheral blood. Moreover, the data indicate that when suppressor T cell activity is limited either by using lower concentrations of SPA, as in the first experiment, or by using small numbers of T cells to supplement the cultures, helper function is facilitated when the T cells are allowed to proliferate.

SPA inhibits PWM responsiveness. The following experiments were carried out to determine whether SPA-induced suppressor T cells specifically inhibited SPA responsiveness or were also able to inhibit responses triggered by another polyclonal B cell activator. Figure 8 presents data in which PBM from six separate individuals were cultured with varying concentrations of SPA with or without optimal concentrations of PWM (10 μg/ml). As can be seen, the addition of even low concentrations of SPA (0.5 μg/ml) usually inhibited PWM responsiveness. Higher concentrations of SPA were inhibitory in five of the six experiments. In experiment C, little suppression was observed when cultures were stimulated with SPA alone or with the mixture of SPA and PWM. Of interest, inhibition of PWM responsiveness was often seen with concentrations of SPA below that yielding maximal SPA responsiveness.

In order to show that SPA was inhibiting PWM responsiveness by stimulating suppressor T cell activity, experiments with purified B and T cells were carried out. As shown in Table IV, SPA inhibited PWM responsiveness only in B cell cultures supplemented with T cells and not in those supported by T_{mito}. It should be noted that even when suppressor T cell activity was ablated, stimulation with the mixture of SPA and PWM resulted in nonadditive responses that were comparable in magnitude to those triggered by one or the other of the mitogens alone.

DISCUSSION

The use of polyclonal B cell activators has been instrumental in developing an understanding of the cellular interactions involved in the generation and control of antibody production (24, 25). PWM has been used most extensively in human studies (25), whereas a number of such stimuli have been utilized in murine models to dissect in greater detail the mechanisms controlling B cell activation (24).

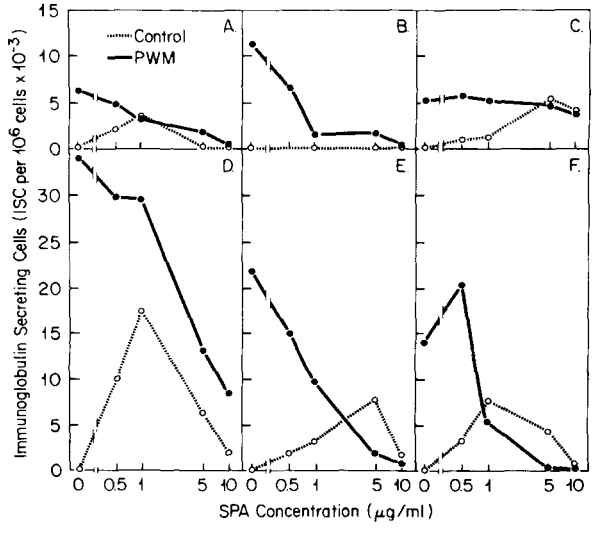

Figure 8. SPA inhibition of PWM responsiveness. PBM from six individuals were prepared and cultured with or without PWM (10 μg/ml) and with or without various concentrations of SPA. After a 6-day incubation the number of ISC was determined.

TABLE IV

Mitogen-induced generation of ISC: role of suppressor T cells

Expt.	Supplement to B Cell Cultures[a]	Mitogen-Induced ISC		
		SPA	PWM	SPA + PWM
		ISC per 10^6 B cells × 10^{-3}		
1	T cells	40.0	76.2	21.1
	T_{mito}	35.6	70.0	64.4
2	T cells	82.0	94.0	21.6
	T_{mito}	124.4	109.2	134.8
3	T cells	29.1	114.1	11.7
	T_{mito}	99.2	53.7	93.9
4	T cells	1.6	13.8	0.4
	T_{mito}	48.8	14.6	38.8

[a] 2.5×10^4 B cells were cultured with either 1×10^5 T cells or 1×10^5 T_{mito}. Cultures were stimulated with either PWM (10 μg/ml), SPA (1 μg/ml), or a mixture of the two mitogens. In expt. 3, 5 μg/ml SPA was used and in expt. 4, 10 μg/ml SPA was used.

Recently, this approach has become feasible in man, with the demonstration that a number of different materials, such as Epstein-Barr virus (26, 27), Nocardia opaca water soluble mitogen (28), and bacterial lipopolysaccharide (10, 29), can stimulate human B cell differentiation. Because of the observation that intact *S. aureus* organisms (9–13) or SPA coupled to insoluble beads (9, 12) might trigger the generation of antibody-forming cells, we examined the possibility that soluble SPA might also function as a polyclonal activator of human B cells.

The studies presented here examined the response to SPA by evaluating the total number of ISC generated by using a reverse hemolytic plaque assay; the number of cells secreting antibody against specific antigenic determinants was not examined. Since the reverse hemolytic plaque assay employed made use of SPA-coated SRBC, it was possible that the observed response to SPA was a specific antibody response and not the result of activation of multiple clones of B cells with different antigenic specificities. A number of observations make this possibility unlikely. First, the magnitude of the response was many times greater than that found by other laboratories when the number of specific antibody-forming cells was evaluated after either polyclonal activation with PWM (30) or stimulation with a specific antigen (31, 32). Second, the magnitude of the SPA response was identical when anti-human Ig coated SRBC (17) or SPA-coated SRBC were used in the assay. Third, no hemolytic plaques were found unless developing anti-Ig antiserum was used. Since many of the ISC generated in response to SPA secreted IgM, the formation of hemolytic plaques in the absence of developing antiserum would have been expected if the antibody secreted had been directed against SPA. Finally, *S. aureus* organisms have been shown to be polyclonal activators of human peripheral blood and splenic B cells (9–13), and soluble SPA and SPA coupled onto insoluble beads triggers polyclonal activation of human splenic B cells (9, 12). These observations all support the conclusion that the ISC generated by SPA in the current studies represent the result of activation of multiple clones of antigen specific B cells.

SPA was found to be a potent stimulus for the generation of ISC from human peripheral blood B cells. Responsiveness was absolutely dependent on the activity of helper T cells and regulated by the activity of suppressor T cells. Moreover, polyclonal B cell activation by SPA required the active participation of monocytes (unpublished observation). In these regards, SPA responsiveness was similar to that triggered by PWM, although important differences between the two mitogens were seen. The magnitude of the SPA response was usually less than that stimulated by PWM. Although there was considerable individual variation, much of the discrepancy could be accounted for by the decreased number of IgG-secreting cells that was generated in response to SPA. This diminished IgG response could not be explained by the action of suppressor T cells, since the maximum number of IgG-secreting cells induced by SPA was less than that stimulated by PWM even when suppressor cell activity was inhibited by treating the T cells with mitomycin C (Table III). Moreover, increasing T cell help by adding very large numbers of T_{mito} (5×10^5/well) to B cell cultures did not lead to SPA-induced IgG responses that were comparable in magnitude to those seen in PWM-stimulated cultures containing 5-fold fewer T cells. A number of previous observations (17, 19, 33) have indicated that PWM activates separate subsets of B cells to differentiate into IgM- and IgG-secreting cells rather than stimulating a single population of B cell precursors to synthesize IgM initially and then switch to IgG secretion. SPA is comparable to PWM, in that each mitogen stimulates concurrent but not sequential IgM and IgG secretion. This suggests that SPA may be similar to PWM in stimulating the differentiation of IgM- and IgG-secreting cells from individual B cell subsets. Therefore, the diminished number of IgG-secreting cells induced by SPA compared with PWM is likely to reflect a decreased capacity of this mitogen to activate the B cell precursors of IgG-secreting cells. Otherwise, there appears to be considerable overlap between the B cells activated by SPA and PWM. This conclusion is supported by the observation that minimal additivity was seen when cultures of B cells supported by T_{mito} were stimulated by both mitogens simultaneously.

Generation of ISC was seen over a fairly narrow range of SPA concentrations. Suppressor T cell-mediated inhibition of B cell differentiation was observed at higher concentrations of SPA. The suppressor T cells had some functional characteristics in common with those stimulated by PWM, concanavalin A, or prolonged *in vitro* incubation without mitogenic stimulation (18–22). In each of these systems suppressor T cell function was ablated when T cell proliferation was inhibited. It is not clear whether these various stimuli activate the same or different subpopulations of suppressor T cells.

The magnitude of the SPA response appears to be dependent on the balance between helper and suppressor influences. Although there was considerable variation among individuals, a number of general principles emerged. First, the actual number of suppressor T cells appeared to be much smaller than the number of helper T cells, although the effect of suppressor cells was often dominant over help. This was most easily shown when B cell cultures were supplemented with a very small number of T cells. Under these conditions, helper T cell activity was still apparent, but suppressor cell activity appeared to have been diluted out. When B cell cultures were supplemented with larger numbers of T cells, the effect of suppressor cells was uniformly seen. These data suggest that the pool of helper T cells in peripheral blood is much larger than that of suppressor T cells. The finding that the ratio of the number of suppressor and helper T cells in normal human peripheral blood is quite low agrees with results obtained in other systems. For example, the subpopulation of T cells that express Fc receptors for IgG and can suppress the PWM response is considerably smaller than the subset of IgM Fc receptor bearing T cells possessing helper activity (21). Similarly, Dosch *et al.* (34) have examined the generation of antigen-specific plaque-forming cells and found that the suppressor cell pool is small compared with the helper cell population responsive to the same antigen.

A second feature of the SPA system that affected the magnitude of the response generated involved the relative dose-response characteristics of helper and suppressor T cells. Although both helper and suppressor T cell activity appeared to increase as a function of the concentration of SPA, low concentrations of SPA appeared to stimulate helper T cells specifically in many experiments, whereas higher concentrations of SPA were needed to induce suppressor T cell function. At the highest concentrations of SPA, suppression was dominant in most experiments. Thus, two different variables affected the balance of helper and suppressor influences that played an important role in determining the magnitude of the SPA response. These were the ratio of suppressor to helper cells and the relative SPA dose-response characteristics of these two functional T cell subpopulations. Alterations in either or both of these characteristics markedly affected the expression of SPA responsiveness.

PWM responsive helper T cells are not thought to require cell division to express their activity maximally (18, 19, 23). This conclusion derives from studies that explored the effect of inhibition of T cell proliferation on the expression of helper T cell activity. However, most of these studies examined responsiveness in cultures supported by large numbers of irradiated or mitomycin C-treated T cells. Although clearly showing that help does not require T cell division, these studies have not been able to determine whether help is facilitated when T cell proliferation is allowed to proceed. The utilization of SPA under conditions where helper T cells can be specifically activated makes it possible to compare the capacity of T cells and T_{mito} to provide help in the absence of suppressor cell activity. When lower concentrations of SPA or small numbers of T cells were used to restrict the influence of suppressor T cells, help was augmented in cultures containing T cells capable of undergoing cell division. These results indicate that, although not necessary, SPA-induced helper activity is facilitated when T cell proliferation is permitted.

The reason that previous investigators (9, 12) have been unable to demonstrate polyclonal activation of human peripheral blood B cells by soluble SPA is unclear. It is possible that SPA does not trigger the specific blood B cell precursors of the anti-fluorescein isothiocyanate-SRBC antibody-forming cells assayed in the previous studies but does stimulate the remainder of the polyclonal response. It seems more likely, however, that the cultural conditions employed or the concentrations of SPA used accentuated suppressor cell activity that precluded the development of ISC. Regardless, soluble SPA is a potent activator of human peripheral blood B cells. In preliminary studies, we have confirmed that SPA activates human splenic B cells (9, 12) and found that the magnitude of the B cell response to SPA is comparable in blood and spleen. Thus, we have found no evidence to support the idea that SPA-responsive B cells reside in spleen but not blood. Moreover, the data suggest that SPA responsiveness cannot be used to define a subset of human B cells distinct from PWM responsive cells, since no additivity is observed when B cells are stimulated with

both mitogens. It is more likely that SPA responsiveness is a characteristic of most PWM-responsive precursors of IgM-secreting cells and some precursors of IgG-secreting cells.

In summary, SPA is a T cell-regulated polyclonal activator of human B cells. As with PWM, the magnitude of the response induced by SPA appears to be regulated by a balance of positive and negative T cell influences. SPA appears to differ from PWM in a number of ways, including its greater capacity to trigger suppressor cell function. This conclusion is supported by the observations that more normal individuals were hyporesponsive to SPA than to PWM and that decreased responsiveness usually resulted from accentuated suppressor cell activity. In addition, suppressor cell function was uniformly seen when higher concentrations of SPA were employed, even when suppression in the PWM system was minimal. As a T cell-dependent activator of human B cells and especially because of its capacity to stimulate suppressor T cells, SPA should prove to be useful in developing a better understanding of the cellular mechanisms controlling antibody formation in man.

Acknowledgments. The expert technical assistance of Ms. Patricia A. Thompson and Ms. Rona K. Nakamoto is greatly appreciated. I would also like to thank Dr. Fred Finkelman for preparing the anti-human IgG Fc developing antiserum, Drs. J. A. Cuthbert and S. A. Rosenberg for reviewing the paper, and Ms. Debbie McInnis for preparing the manuscript.

REFERENCES

1. Goding, J. W. 1978. Use of staphylococcal protein A as an immunological reagent. J. Immunol. Methods 20:241.
2. Sjöquist, J., B. Meloun, and H. Hjelm. 1972. Protein A isolated from *Staphylococcus aureus* after digestion with lysostaphin. Eur. J. Biochem. 29:572.
3. Forsgren, A., and J. Sjöquist. 1966. Protein A from *S. aureus*. I. Pseudoimmune reaction with human gammaglobulin. J. Immunol. 103:828.
4. Rodey, G. E., T. Davis, and P. G. Quie. 1972. Response of peripheral blood lymphocytes from normal, hypogammaglobulinemic, and chronic lymphocytic leukemic patients. J. Immunol. 108:178.
5. Forsgren, A., A. Svedjelund, and H. Wigzell. 1976. Lymphocyte stimulation by protein A of *Staphylococcus aureus*. Eur. J. Immunol. 6:207.
6. Romagnani, S., A. Amadori, M. G. Giudizi, R. Biagiotti, E. Maggi, and M. Ricci. 1978. Different mitogenic activity of soluble and insoluble staphylococcal protein A (SPA). Immunology 35:471.
7. Sirianni, M. C., F. Pandolfi, F. Aiuti, and H. Wigzell. 1979. Protein A-positive *Staphylococci* serve as a selective B cell mitogen for lymphocytes from primary immunodeficiency patients. Clin. Exp. Immunol. 36:107.
8. Sakane, T., and I. Green. 1978. Protein A from *Staphylococcus aureus*—a mitogen for human T lymphocytes and B lymphocytes but not L lymphocytes. J. Immunol. 120:302.
9. Ringdén, O., and B. Rynnel-Dagöö. 1978. Activation of human B and T lymphocytes by protein A of *Staphylococcus aureus*. Eur. J. Immunol. 8:47.
10. Ringdén, O., and B. Rynnel-Dagöö, E. M. Waterfield, E. Möller, and G. Möller. 1977. Polyclonal antibody secretion in human lymphocytes induced by killed Staphylococcal bacteria and by lipopolysaccharide. Scand. J. Immunol. 6:1159.
11. Rynnel-Dagöö, B., O. Ringdén, H. Alfredsson, and E. Möller. 1978. The use of bacteria for the functional characterization of human lymphocyte subpopulations in various lymphoid organs. Scand. J. Immunol. 8:369.
12. Ringdén, 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.
13. Kim, Y. T., A. Schwartz, J. Blum, and M. E. Weksler. 1979. The plaque-forming cell response of human blood lymphocytes. 1. PFC response of lymphocytes to formalin-treated Staphylococci. Cell. Immunol. 48:308.
14. Lipsky, P. E., and M. Ziff. 1977. Inhibition of antigen and mitogen-induced human lymphocyte proliferation by gold compounds. J. Clin. Invest. 59:455.
15. Rosenberg, S. A., and P. E. Lipsky. 1979. Monocyte dependence of pokeweed mitogen-induced differentiation of immunoglobulin secreting cells from human peripheral blood mononuclear cells. J. Immunol. 122:926.
16. Galili, U., and M. Schlesinger. 1974. The formation of stable E rosettes after neuraminidase treatment of either human peripheral blood lymphocytes or of sheep red blood cells. J. Immunol. 112: 1628.
17. Ginsburg, W. W., F. D. Finkelman, and P. E. Lipsky. 1978. Circulating and mitogen-induced immunoglobulin secreting cells in human peripheral blood: evaluation by a modified reverse hemolytic plaque assay. J. Immunol. 120:33.
18. Siegal, F. P., and M. Siegal. 1977. Enhancement by irradiated T cells of human plasma cell production: dissection of helper and suppressor function *in vitro*. J. Immunol. 118:642.
19. Saxon, A., R. H. Stevens, and R. F. Ashman. 1977. Regulation of immunoglobulin production in human peripheral blood leukocytes: cellular interactions. J. Immunol. 118:1872.
20. Haynes, B. F., and A. S. Fauci. 1977. Activation of human B lymphocytes. III. Concanavalin A-induced generation of suppressor cells of the plaque-forming cell response of normal human B lymphocytes. J. Immunol. 118:2281.
21. Moretta, L., S. R. Webb, C. E. Grossi, P. M. Lydyard, and M. D. Cooper. 1977. Functional analysis of two human T cell subpopulations: help and suppression of B cell responses by T cells bearing receptors for IgM or IgG. J. Exp. Med. 146:184.
22. Lipsky, P. E., W. W. Ginsburg, F. D. Finkelman, and M. Ziff. 1978. Control of human B lymphocyte responsiveness: enhanced suppressor T cell activity after *in vitro* incubation. J. Immunol. 120: 902.
23. Keightley, R. G., M. D. Copper, and A. R. Lawton. 1976. The T cell dependence of B cell differentiation induced by pokeweed mitogen. J. Immunol. 117:1538.
24. Gronowicz, E., and A. Coutinho. 1975. Functional analysis of B cell heterogeneity. Transplant. Rev. 24:3.
25. Janossy, G., and M. Greaves. 1975. Functional analysis of murine and human B lymphocyte subsets. Transplant. Rev. 24:177.
26. Rosen, A., P. Gergely, M. Jondal, G. Klein, and S. Britten. 1977. Polyclonal Ig production after Epstein-Barr virus infection of human lymphocytes *in vitro*. Nature 267:52.
27. Kirchner, H., G. Tosato, R. M. Blaese, S. Broder, and I. T. Magrath. 1979. Polyclonal immunoglobulin secretion by human B lymphocytes exposed to Epstein-Barr virus *in vitro*. J. Immunol. 122:1310.
28. Brochier, J., C. Bona, R. Ciorbaru, J-P. Revillard, and L. Chedid. 1976. A human T-independent B lymphocyte mitogen extracted from Nocardia opaca. J. Immunol. 117:1434.
29. Finkelman, F. D., and P. E. Lipsky. 1978. Immunoglobulin secretion by human splenic lymphocytes *in vitro*: the effects of antibodies to IgM and IgD. J. Immunol. 120:1465.
30. 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.
31. Dosch, H-M., and E. W. Gelfand. 1977. Generation of human plaque-forming cells in culture: tissue distribution, antigenic, and cellular requirements. J. Immunol. 118:302.
32. Delfraissy, J-F., P. Galanaud, J. Dormont, and C. Wallon. 1977. Primary *in vitro* antibody response of human peripheral blood lymphocytes. J. Immunol. 118:630.
33. Hammarström, L., A. G. Bird, S. Britton, and C. I. E. Smith. 1979. Pokeweed mitogen induced differentiation of human B cells: evaluation by a protein A haemolytic plaque assay. Immunology 38:181.
34. Dosch, H-M., A. Shore, and E. W. Gelfand. 1979. Regulation of specific plaque-forming cell response in man: restraint of B cell responsiveness. Eur. J. Immunol. 9:702.