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Cells Involved in the Mitogen-Induced Helper Function which Facilitates the Blastogenic Response to *Actinomyces viscosus*

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Coculture of human peripheral blood lymphocyte suspensions, previously pulsed with either pokeweed mitogen (PWM) or an *Actinomyces viscosus* ultrasonicate supernatant fraction (AV), resulted in a blastogenic response that was greater than the sum of the responses of the two independently cultured lymphocyte suspensions. Selective cobalt-51 irradiation of the precultured lymphocytes prior to coculture revealed that PWM was inducing a helper activity which facilitated the blastogenic response to the poorly mitogenic AV. Subsequent experiments revealed that both B- and T-enriched lymphocyte populations were capable of providing such help, however, the B-cell help appeared to require T-cell interaction. As few as one PWM-activated helper cell per 150 AV-pulsed cells was sufficient to provide measureable help. In the presence of PWM helper cells, both B and T cells gave strong blastogenic responses to AV fractions. Results of this study suggest that lymphocytes can respond to poorly mitogenic substances from bacteria if helper cell activity is provided by a second, unrelated lymphocyte stimulant.

INTRODUCTION

Inflammatory lesions in the periodontium of man (1–4) and rodents (5) have been associated with the presence of certain species of *Actinomyces*. The prevalence of these organisms in mature dental plaque and their ability to activate macrophage and neutrophil degranulation (6), and stimulate lymphocytes from patients with periodontal disease (7–9) has implicated them in the etiology of gingivitis and periodontitis. It has been hypothesized that various host immunological responses to these organisms are responsible for initiating periodontal inflammation (7). Several laboratories (10, 11) have demonstrated B-cell mitogenic activities in extracts of *Actinomyces viscosus* (AV) using murine models. However, the mitogenicity of these substances for human lymphocytes from individuals free of gingival disease has, until recently, been discounted (12). In our previous report (13), we showed that AV pulsing of human lymphocytes from healthy individuals with such extracts, followed by stimulation with pokeweed mitogen (PWM), resulted in an amplified blastogenic response when compared to the sum of the responses to either AV or PWM. These studies showed that under those conditions, there was no mitogenic response to AV when cultured alone with human lymphocytes. However, it was not clear from our preliminary studies whether the amplified response resulted from AV amplifying the lymphocyte response to PWM, or by a PWM-stimulated helping activity permitting the lympho-

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cytes to respond blastogenically to AV. In this report, we describe experiments addressing this point which indicate that the amplification phenomenon is a result of PWM-induced help enhancing the blastogenic response to *A. viscosus*.

MATERIALS AND METHODS

Actinomyces viscosus Ultrasonicate Preparations

The preparation and characterization of the *A. viscosus* ultrasonicate preparation has already been reported (14, 15). Briefly, an isolate of *Actinomyces viscosus* (GA), obtained from a naturally occuring gingivitis site was kindly provided by Dr. Salam A. Syed. The cultures were grown under anaerobic conditions as previously described by Loesche, *et al.* (16). The cells were harvested from batch cultures by centrifugation at 12,000*g* for 30 min. The cell pellets were washed in sterile phosphate-buffered saline (PBS; 0.15 *M* NaCl, 0.05 *M* PO_4, pH 7.4) and finally resuspended in sterile distilled water. The washed cells were subjected to a total of 40 min of ultrasonic disruption while in an ice bath (Heat System Ultrasonics, Inc., Model W185D, 85 W), delivered in 5-min intervals with alternating periods of cooling. The cellular debris was removed by centrifugation at 12,000*g* for 30 min. The cell-free supernate (AV) was dialyzed against distilled water at 4°C and finally lyophilized.

Mononuclear Cell Isolation and Characterization

1. Mononuclear cell isolation. Mononuclear leukocytes were isolated from heparinized venous blood drawn from healthy volunteers between the ages of 21 and 40. The buffy coat layer was prepared from whole blood by centrifugation at 350*g* for 30 min, aspirated, and diluted in sterile PBS, and layered onto Ficoll–Hypaque (17) (Ficoll–Hypaque, Pharmacia Fine Chemicals, Piscataway, N.J.), and centrifuged at 500*g* for 30 min at 23°C. The mononuclear leukocytes, which were banded at the interface, were aspirated, washed three times in PBS, and resuspended in RPMI 1640 culture medium (Grand Island Biological Co., Grand Island, N.Y.), supplemented with 30 m*M* Hepes buffer, 2 m*M* glutamine, and gentamycin (50 μg/ml; Schering Corp., Kenilworth, N.J.).

2. Monocyte depletion. Mononuclear cell suspensions were depleted of monocytes by the G-10 Sephadex column method as described by Alonso *et al.* (18). Passage through such columns resulted in lymphocyte suspensions having less than 4% monocyte contamination as determined by latex particle uptake (19) and nonspecific esterase staining (20).

3. T- and B-lymphocyte isolation. T cells were prepared from monocyte-depleted leukocyte suspensions by rosetting with 2-aminoethylisothiouronium bromide (AET)-treated sheep erythrocytes (sRBCs) (21). Human T cells binding SRBCs were separated from nonrosetted cells by centrifugation through a Ficoll–Hypaque gradient as previously described. The nonrosetted cells banded at the gradient interface while the rosetted T cell pelleted in the gradient. The T cells were isolated by lysing the SRBCs with 0.85% NH_4Cl. Such T cell preparations were typically free of B cell and monocyte contamination (>99% T cells). The fraction of nonrosetting cells were typically > 90% B cells (T cell contamina-

tion was 1–3%). B lymphocytes were identified by rosetting with anti-human immunoglobulin-coupled polyacrylamide beads (22; Immunobeads, Bio-Rad laboratories, Richmond, Calif.), and by lack of nonspecific esterase staining (20).

Alternately, T and B lymphocytes were purified from monocyte-depleted lymphocyte suspensions by a modification of the method of Chess *et al.* (23). Briefly, 100×10^6 lymphocytes [20×10^6/ml in RPMI 1640, containing 5% newborn calf serum and 2.5 m*M* EDTA (starting buffer)] were incubated for 20 min at room temperature with 10 ml rabbit anti-human $F(ab')_2$–Sepharose 6B in a 15-ml plastic column. The nonadherent T lymphocytes were eluted with 15 ml of the starting buffer. Adherent B lymphocytes were then eluted in the following manner; a 5-ml aliquot of human γ globulin fraction II (Miles Laboratories, Inc., 10 mg/ml in RPMI 1640) was passed into the column and incubated for 15 min at room temperature. Following dispersal of the gel with a pipet, the cells were eluted with an additional 10-ml aliquot of the human γ globulin solution. This procedure was performed a second time and the eluants were combined and washed with RPMI 1640. The resultant B-cell population was at least 90% B lymphocytes, the balance being monocytes which adsorbed to the Sepharose matrix and were subsequently eluted with the lymphocytes. The T-cell population contained $<1\%$ B cells as determined by the immunobead reagent.

Lymphocytes Pulsing

Lymphocytes (2×10^6/ml) were cultured in RPMI 1640 media for 16 hr in the presence of AV (10–1000 μg/ml) or pokeweed mitogen (PWM; 1/10–1/1000 of stock, Grand Island Biological Co.). Following this culture period, each cell category was washed to remove excess cell stimulants and resuspended in RPMI 1640 media. Various cell suspensions were then subjected to irradiation by a cobalt source at a dose rate of 180 rad/min for a total of 1500 R. Following such treatment, cells were dispensed into microtiter wells, cultured an additional 3 days and processed as described below.

Microculture

Lymphocyte blastogenesis was assessed in a microtiter plate system as previously described (14). A total of 1×10^5 mononuclear leukocytes were added to each well and cultured in RPMI 1640 medium supplemented with 10% autologous plasma. Individual cell categories were cultured at 100,000 cells/well to simulate density effects. During the last 6 hr of culture, 2 μCi [*methyl*-^{3}H]thymidine (Amersham, TRA 120, 5 Ci/mmol) was added to each well. All cultures were harvested with a multiple-automated sample harvested (MASH II, Microbiological Associates) onto glass fiber filters. The filters were dried, placed in plastic vials filled with 3 ml scintillation cocktail (OCS, Amersham), and counted in a Packard Model 3320 liquid scintillation spectrometer. The "observed" counts per minute in the individually cultured cell categories represents one-half of the actual value obtained in order to indicate the contribution of each cell category (50,000 cells) to the coculture value (100,000 cells total).

TABLE 1
MONOCYTE INFLUENCE ON AV–PWM-ASSOCIATED AMPLIFICATION[a]

	Monocyte depleted	Unstimulated (cpm ± SE)	PWM stimulated (cpm ± SE)
P	–	1286 ± 168	118,124 ± 5137
P_{av}	–	1890 ± 699	187,030 ± 1413
P	+	1466 ± 322	57,861 ± 4184
P_{av}	+	1454 ± 352	250,956 ± 4727

[a] Monocyte-depleted or unfractionated lymphocyte suspensions (2×10^6/ml) were pulsed with AV (100 μg/ml) (P_{av}) or culture media (control) (P) for 16 hr. After washing, the cells (100,000 per well) were cultured in the presence or absence of PWM (1/100) for an additional 72 hr. These results are representative of five such independent experiments.

RESULTS

Identification of the Role of Actinomyces viscosus and PWM in the Amplification Phenomenon

In preliminary experiments (Table 1) the influence of monocytes on the amplification phenomenon was assessed. While amplification was apparent whether or not the monocytes were present, the magnitude of the enhancement was higher when they were removed. This is primarily a function of the blastogenic response to pokeweed mitogen, which is depressed when monocytes are removed. Therefore, monocyte depletion was performed in all subsequent studies to minimize the blastogenic response to PWM, and thus maximize the amplification phenomenon which occurred in the absence of monocytes.

Our previous report (14) indicated that while amplification could be enhanced by adding fresh autologous lymphocytes to AV-precultured cells prior to mitogen stimulation, neither the cell nor the stimulant mediating the phenomenon was apparent. In order to characterize the role of each stimulant, separate autologous monocyte-depleted lymphocyte suspensions were pulsed with either AV (100 μg/ml) or PWM (1/100) for 16 hr followed by washing and coculture for an additional 72 hr. As shown in Table 2, coculture of PWM-pulsed cell (P_{pwm}) with AV-pulsed cells (P_{av}) resulted in a blastogenic response which was (i) much greater than expected (calculated for this coculture category, and (ii) significantly ($P < 0.005$) greater than the response obtained by the control cocultures ($P + P_{av}$; $P + P_{pwm}$). To determine which category of pulsed cell (P_{av} or P_{pwm}) was undergoing enhanced blastogenesis, following preculture with either AV or PWM, the cells were irradiated to inhibit proliferation in the coculture yet leave radioresistant regulatory functions intact. Irradiation of the P_{av} category greatly reduced the amplified response (expected cpm = 9924, observed cpm = 11,362) in the $P_{av_i} + P_{pwm}$ coculture. In contrast, irradiation of PWM-pulsed cells had little effect on the coculture amplified response ($P_{av} + P_{pwm_i}$; expected cpm = 867, observed cpm = 22,780). These results therefore indicate AV-pulsed cells undergo increased proliferation when cocultured with PWM-pulsed cells.

TABLE 2
CHARACTERIZATION OF THE ROLE OF AV AND PWM IN THE AMPLIFICATION PHENOMENON[a]

Categories	[*methyl*-^{3}H]Thymidine incorporation (cpm ± SE)		
	Calculated	Observed	Significance[b]
P	—	295 ± 171	
P_i	—	66 ± 25	
P_{pwm}	—	9,866 ± 751	
P_{pwm_i}	—	758 ± 294	
P_{av}	—	109 ± 53	
P_{av_i}	—	58 ± 30	
$P + P_i$	361	587 ± 198	
$P + P_{av_i}$	353	148 ± 30	
$P + P_{av}$	404	527 ± 81	
$P + P_{pwm}$	10,161	7,738 ± 427	$P < 0.005$
$P_{av} + P_{pwm}$	9,975	37,049 ± 5,747	
$P_{pwm} + P_i$	9,932	7,788 ± 647	$P < 0.05$
$P_{pwm} + P_{av_i}$	9,924	11,362 ± 1,184	
$P_{pwm_i} + P$	1,035	2,004 ± 109	$P < 0.001$
$P_{pwm_i} + P_{av}$	867	22,780 ± 412	

[a] Monocyte-depleted lymphocytes (2×10^6/ml) were cultured in the presence of pokeweed mitogen (P_{pwm}; 1/100 stock), AV (P_{av}; 100 μg/ml), or culture media alone (P) for 16 hr. After washing, the cells were irradiated ($_i$; 1500 R) as described. The indicated cell categories were cocultured for an additional 72 hr. Six hours prior to termination, 2 μCi [*methyl*-^{3}H]thymidine was added to each culture in order to assess DNA synthetic activity. The results are representative of six independent experiments.

[b] Significant difference between observed values (Student's *t* test).

Characterization of the Cells Responding to AV

Evidence from the previous experiment indicated that PWM was inducing a helping activity which facilitated the blastogenic response to AV. The ability of the PWM-induced helper to facilitate the AV-induced response over a range of AV concentrations was next assessed. Monocyte-depleted lymphocyte suspensions were pulsed with various concentrations of AV (0.1−1000 μg/ml) and then cocultured with irradiated PWM-pulsed autologous cells as previously described. As shown in Fig. 1, there is a dose-dependent increase in the lymphocyte response to AV, reaching a maximum at 100 μg/ml.

The cell type(s) responding to AV under these conditions was assessed in a similar manner. T- and B-enriched lymphocyte fractions were pulsed with AV (100 μg/ml) for 16 hr. Simultaneously, a monocyte-depleted lymphocyte fraction was pulsed with PWM (1/100) to serve as a source of helper activity. After washing and irradiation of the PWM-pulsed cells, the various cell categories were cocultured. As shown in Fig. 2, both B and T subfractions could be shown to respond to AV stimulation.

Characterization of the Cells Providing Helper Activity

Since PWM was previously shown to be capable of inducing helper activity, it

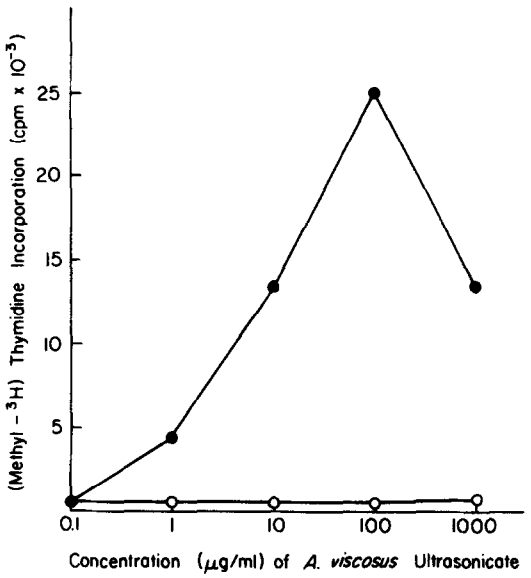

FIG. 1. Dose-dependent response to *A. viscosus* ultrasonicate. Lymphocyte suspensions (2×10^6/ml) were pulsed with AV (10–1000 μg/ml) for 16 hr. After washing, these cells (P_{av}) were cocultured with irradiated, PWM-pulsed (1/100, P_{pwm_i}) lymphocytes and cultured for an additional 72 hr. The blastogenic responses of [P_{av} + P_{pwm_i}] (●) are compared to that of the control cultures [P_{av} + P_i] (○).

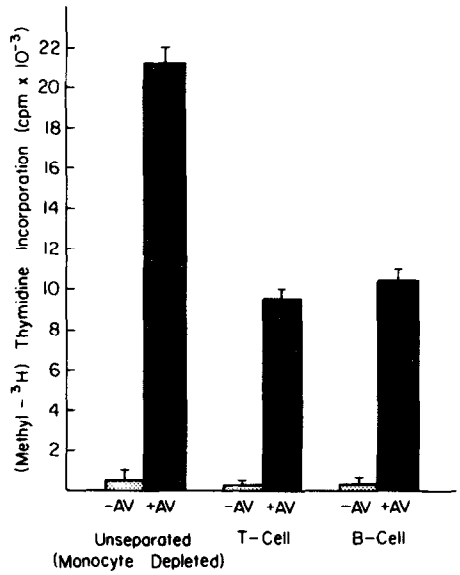

FIG. 2. Lymphocyte subpopulations responding to AV with PWM-induced helper activity. B and T lymphocytes purified by sheep RBC rosetting or unseparated cells were pulsed with AV (100 μg/ml) for 16 hr. After washing, they were cocultured with irradiated, PWM-pulsed (1/100 of stock) lymphocytes (unfractionated) and cultured an additional 72 hr.

was of interest to determine if such an effect was dose dependent, i.e., producing help at suboptimal concentrations and possibly suppression at the optimal and/or supraoptional concentrations. Concentrations of PWM between 1/1000 and 1/10 of the stock PWM were used to induce helper activity. As shown in Fig. 3, maximum helper activity occurred at 1/1000 of stock; however, at 1/10 (10 times greater than optimal concentration required for maximal blastogenic response), there is still enhancement.

The nature of the cells responsible for the helper activity was determined by culturing monocyte-depleted, T lymphocyte and B-enriched lymphocytes with PWM (1/100 stock) for 16 hr. After washing and irradiation, as previously described, they were cocultured with AV-pulsed monocyte-depleted lymphocytes. The results are shown in Table 3. PWM-pulsed, monocyte-depleted cells (unseparated) facilitated a significant ($P < 0.001$) amplification of the AV blastogenic response (38,888 vs 8963 cpm). Similarly, PWM-pulsed T lymphocytes produced an equivalent enhancement (34,576 vs 3778 cpm). PWM-pulsed B-enriched cells also produced enhancement of the AV blastogenic response (95,573 vs 44,774 cpm). Examination of the non-AV-pulsed controls (e.g., B_{pwm_i} + P) revealed that PWM-pulsed cells also stimulated a mitogenic activity in the control cells. The ability to produce such a mitogenic effect was greatest in PWM-pulsed B-enriched cells.

In the previous experiment we showed that PWM-induced helper activity could facilitate the blastogenic responses of both B and T lymphocytes. Since the experiment above revealed that both B and T lymphocytes were capable of provid-

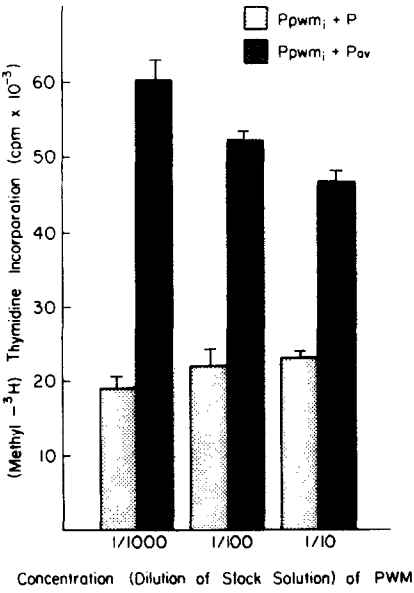

FIG. 3. Dose-dependent response to PWM: helper induction. Unfractionated lymphocytes suspensions (2×10^6/ml) were pulsed with PWM (1/10–1/1000 of stock) for 16 hr. After washing, they were irradiated (P_{pwm_i}) and cocultured with AV (100 μg/ml)-pulsed (P_{av}) or control (P) lymphocytes for an additional 72 hr. The blastogenic response of [P_{pwm_i} + P_{av}] is compared to [P_{pwm_i} + P] at each PWM concentration.

TABLE 3

CHARACTERIZATION OF PWM-INDUCED HELPING ACTIVITY WHICH FACILITATES THE BLASTOGENIC RESPONSE TO *A. viscosus*[a]

Categories	Control precultured (cpm ± SE)		Significance[b]	PWM precultured (cpm ± SE)		Significance
	Calculated	Observed		Calculated	Observed	
P	—	103 ± 48		—	—	
P_{av}	—	2,158 ± 97		—	—	
P_i/P_{pwm_i}[c]	—	61 ± 40		—	2,354 ± 200	
P_i/P_{pwm_i} + P	164	27 ± 1	$P < 0.001$	2457	8,963 ± 156	$P < 0.001$
P_i/P_{pwm_i} + P_{av}	2219	1,037 ± 202		4512	38,883 ± 2052	
T_i/T_{pwm_i}	—	245 ± 42		—	556 ± 112	
T_i/T_{pwm_i} + P	348	80 ± 27	$P < 0.001$	659	3,778 ± 461	$P < 0.001$
T_i/T_{pwm_i} + P_{av}	2403	1,538 ± 117		2714	34,576 ± 1265	
B_i/B_{pwm_i}	—	97 ± 49		—	318 ± 87	
B_i/B_{pwm_i} + P	200	226 ± 59	$P < 0.001$	421	44,774 ± 1001	$P < 0.001$
B_i/B_{pwm_i} + P_{av}	2255	2,747 ± 181		2476	95,573 ± 2665	

[a] Unfractionated, monocyte-depleted lymphocytes were precultured with pokeweed mitogen (P_{pwm}; 1/100 stock), AV (P_{av}; 100 μg/ml), or culture media alone (P) for 16 hr. Similarly, SRBC-fractionated T cells and B cells were precultured with pokeweed mitogen (T_{pwm}, B_{pwm}) or culture media (T, B). After washing, pokeweed-stimulated and the respective unstimulated control cells were irradiated ($_i$; 1500 R) and cocultured with nonirradiated P or P_{av} cells for an additional 72 hr. Six hours prior to termination, 2 μCi [*methyl*-^{3}H]thymidine was added to each culture in order to assess DNA synthetic activity. The results are representative of three such independent experiments.

[b] Significant difference between observed values (Student's *t* test).

[c] Slash designation indicates cultures contained either irradiated control-precultured or pokeweed mitogen-precultured cells.

ing help, we examined the ability of PWM-stimulated T and B cells to amplify AV-stimulated T and B cells. As shown in Table 4, while PWM-pulsed T cells provided help to AV-pulsed T cells, PWM-pulsed B cells were not able to facilitate a B-cell response to AV. However, both combinations (B_{pwm_i} + T_{av}) and (T_{pwm_i} + B_{av}) resulted in an amplified response, suggesting that a T cell is involved in the mediation of B-cell-associated help.

In order to determine whether a T-cell contaminant in the B-cell suspension was responsible for the B-associated help, the number of helper cells required to facilitate the AV response was assessed. In these experiments, graded numbers of irradiated, PWM-pulsed helping cells (unfractionated, and anti-$F(ab')_2$ column-purified B and T lymphocytes) were added to cultures containing 75,000 AV (100 μg/ml)-pulsed unfractionated lymphocytes. As shown in Fig. 4, in all cases, as few as 500 monocyte-depleted lymphocytes (1:150, P_{pwm_i}:P_{av}) produced significant ($P < 0.05$) enhancement of the AV-induced response. Such enhancement was dose dependent, i.e., with increasing numbers of PWM-pulsed cells there were increased levels of enhancement.

DISCUSSION

We previously reported (14) that preculture of human lymphocytes with ultrasonicated preparations of *Actinomyces viscosus* (AV), followed by stimulation of those cells with pokeweed mitogen (PWM) resulted in a synergistic or amplified blastogenic response when compared to the sum of the individual responses to AV and PWM. The nature of the amplification was not clear at that time. In this

TABLE 4
INFLUENCE OF PWM-INDUCED HELPER ACTIVITY ON *A. viscosus*-PULSED B AND T LYMPHOCYTES[a]

Categories	[*methyl*-^{3}H]Thymidine incorporation (cpm ± SE)			
	Control precultured	Significance[b]	PWM precultured	Significance
T_i/T_{pwm_i}[c] + T	110 ± 62	$P < 0.05$	2,369 ± 275	$P < 0.001$
T_i/T_{pwm_i} + T_{av}	841 ± 228		14,742 ± 514	
T_i/T_{pwm_i} + B	572 ± 183	NS	19,738 ± 259	$P < 0.001$
T_i/T_{pwm_i} + B_{av}	927 ± 48		31,408 ± 842	
B_i/B_{pwm_i} + B	282 ± 182	NS	876 ± 217	NS
B_i/B_{pwm_i} + B_{av}	740 ± 257		1,020 ± 79	
B_i/B_{pwm_i} + T	50 ± 35	$P < 0.005$	23,870 ± 883	$P < 0.001$
B_i/B_{pwm_i} + T_{av}	1200 ± 195		49,672 ± 2373	

[a] SRBC-fractionated T cells (T) or B cells (B) (2×10^6/ml) were cultured in the presence of pokeweed mitogen (T_{pwm}, B_{pwm}; 1/100 stock), AV (T_{av}, B_{av}; 100 μg/ml), or cultured media alone (T,B) for 16 hr. After washing, the PWM-stimulated and the respective control cells were irradiated ($_i$; 1500 R) as described. The indicated cell categories were cocultured for an additional 72 hr. Six hours prior to termination, 2 μCi [*methyl*-^{3}H]thymidine was added to each culture in order to assess DNA synthetic activity. The results are representative of two independent experiments.

[b] Significant difference between control and test categories (Student's *t* test).

[c] Slash designation indicates cultures contained either irradiated control-precultured or PWM-precultured cells.

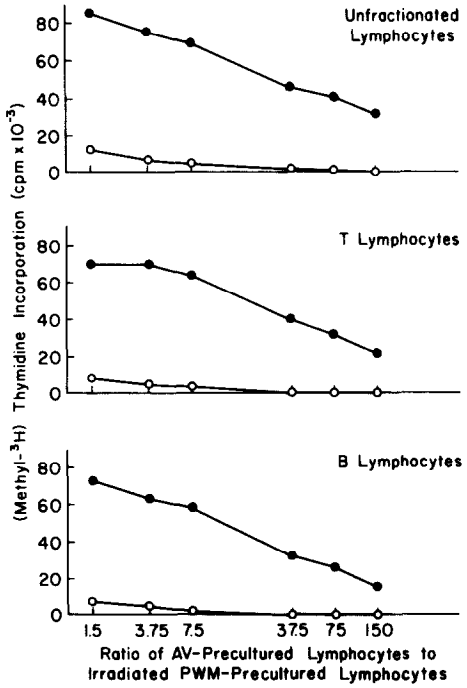

FIG. 4. Helper cell titration. To a constant number (75,000) of unfractionated AV-pulsed (100 μg/ml) (●) or media control (○) lymphocytes was added increasing numbers of irradiated PWM-pulsed (1/100) unfractionated, or anti-F(ab')$_2$ column purified T or B lymphocytes. The blastogenic response of the coculture cells was assessed after an additional 72 hr in culture.

report, we described further studies which indicate that PWM pulsing of lymphocytes induces a helping activity which facilitates the blastogenic response to AV. The cells responding to AV were shown to be of the B and T-lymphocyte subclasses. Studies using murine culture systems also describe the polyclonal activation of B and T cells by AV preparations (10, 11).

Distinct helping activities were shown to be manifested by the two lymphocyte cell types. A T-cell-mediated helping activity, able to be activated by PWM, was shown to facilitate the AV response. In similar studies (13) a mitogen-activated T-helping activity was also shown to facilitate the blastogenic response of human lymphocytes to lipopolysaccharide (LPS). The B-cell-enriched population also appeared to be capable of providing help for the response to AV preparations. At a ratio of one B-enriched cell per 150 AV-precultured cells, a significant level of help was measured (Fig. 4). However, since the level of T-cell contamination in the B-cell-enriched suspension ranged 1–3%, there remains the possibility that as many as 15 T cells could be providing help to the AV-precultured lymphocytes. The potential of T-cell involvement must be considered in the following discussion of a possible B-cell role in the help effect. PWM activation of the B-enriched population produced two phenomena. First, enhancement of the AV response to levels greater than attained by PWM-stimulated helper T cells was measured. This enhancement was in addition to a second nonspecific enhancement of proliferative activity which was independent of the response to AV. The ability to exert such

activity in other lymphocyte fractions was much less and appeared to be related to the B-cell content of the PWM-pulsed fraction, i.e., B > monocyte-depleted, unseparated cells > T.

The nature of B-cell helper activity is still uncertain. In the experiments shown in Table 4, [B_{pwm_i} + B_{av}] did not result in enhancement, while [T_{pwm_i} + T_{av}; T_{pwm_i} + B_{av}; and B_{pwm_i} + T_{av}] did. This suggests that the B helping activity either does not act on AV-pulsed B cells, or that its generation requires the presence of a T cell either in the helping cell fraction or resident in the AV-pulsed cell fraction.

Helping cell activity has been shown to facilitate other poorly mitogenic substances of bacterial origin (24, 25). The requirement for a radioresistant T cell for B lymphocyte response to mitogens has been described by MacDermott *et al.* (26). Miller *et al.* (27) have reported that long-term culture was required to activate a T helper cell which could facilitate the blastogenic response of human peripheral blood lymphocytes to purified LPS. The presence of helper cell populations have been described by DuBois *et al.* (28), whereby irradiated autologous or allogenic cells were capable of reconstituting *in vitro* proliferative responses, even if the reconstituting cells themselves were unresponsive to the stimulant.

The mechanism by which the B cell (as well as T cell) facilitates the blastogenic response to AV remains to be elucidated. However, several modes of action have been described by others. Lymphokine synthesis by B cells, independent of blastogenesis, has been described (8). One such B lymphokine is reported to possess mitogenic activity. Another method of amplification may result from an increase in the rate of spontaneous proliferation, or background, which has been suggested as an important factor in the response of lymphocytes to certain stimulants (29). The ability of B-cell produced lymphokines to enhance the sensitivity of lymphocytes to stimulation may be an important factor in perpetuating inflammatory lesions associated with specific microorganisms. The relationship between the nonspecific mitogenesis and amplification of the AV response is not clear at present, and in fact, may be distinct phenomena. The existence of regulatory B cells which influence immune responses to substances such as LPS (30) and picryl chloride (31) have also been documented. While these cells have been characterized as supressor cells in those studies, presence of helper B cells, in our cultures, cannot be discounted.

The generation of mitogenic substances during inflammation by neutrophils (32), monocyte and macrophages (33), or the inherent mitogenic factors in bacterial and fungal products (34), may provide sufficient triggering of nonspecific helping T- and B-cell activity to facilitate the immune response to normally "silent" mitogenic substances as characterized by *A. viscosus*. Under nondisease situations, T-cell regulation, especially help, may predominate because of the low levels of circulating B lymphocytes relative to T cells. However, in inflammatory lesions, characterized by high numbers of B cells and plasma cells (35), B-cell regulation may play a significant role in facilitating immune responses to certain bacterial products.

Work in progress in our laboratory is evaluating the nature of T- and B-cell help of immune responses to bacterial substances. Especially of interest is the nature of the nonspecific mitogenicity associated with the mitogen-pulsed B cell and its relationship to B-cell help.

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