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Polyclonal Activation of Human Peripheral Blood B Lymphocytes by *Fusobacterium nucleatum*

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The present study examined in vitro polyclonal human B-lymphocyte (B-cell) activation (PBA) by *Fusobacterium nucleatum*. Pokeweed mitogen, a well-studied PBA activator, was included in some experiments for comparison. PBA was determined by the total immunoglobulin A, G, and M concentrations in the culture supernatants as measured by micro-enzyme-linked immunosorbent assay. *F. nucleatum*, at concentrations between 1 and 10 μg/ml, stimulated optimal PBA in monocyte-depleted cultures, whereas the pokeweed mitogen response was optimal in unfractionated, monocyte-containing cultures. Immunoglobulin synthesis occurred primarily between days 6 and 8 after stimulation with *F. nucleatum*. T lymphocytes enhanced the PBA response to *F. nucleatum*, particularly at a T- to B-cell ratio of 1:1. Immunoglobulin production was greater in round-bottomed wells than in flat-bottomed wells at lymphocyte concentrations of 200,000 cells per well. The PBA response, however, increased dramatically in flat-bottomed wells containing higher lymphocyte concentrations, suggesting that PBA is enhanced by cell-to-cell contact. A delay in stimulation of the lymphocytes with *F. nucleatum* resulted in diminished immunoglobulin production. The results provide information on the regulation of in vitro PBA induced by *F. nucleatum*. The data also suggest that there may be differences in the mechanisms by which *F. nucleatum* and pokeweed mitogen stimulate PBA.

Fusobacterium nucleatum is an anaerobic fusiform bacterium frequently isolated from lesions associated with human oral diseases such as gingivitis (35), periodontitis (34, 40), periodontosis (22), and abcesses of alveolar bone (31) and dental pulp (41). In preliminary experiments, we found that *F. nucleatum* can induce polyclonal B-lymphocyte (B-cell) activation (PBA) in vitro. PBA represents the stimulation of multiple B-cell clones, each capable of producing a specific antibody. Bick et al. (3) screened 16 isolates of gram-negative bacteria associated with periodontal diseases and found three isolates of *F. nucleatum* to be extremely good stimulants of PBA. Whether *F. nucleatum*-induced PBA is responsible for any part of the tissue damage associated with periodontal diseases is undetermined. Activated B cells can release osteoclast-activating factor, a lymphokine involved in bone resorption (5). In addition, the chronic inflammatory periodontitis lesion is histologically characterized by a large infiltration of antibody-secreting plasma cells (33), which is suggestive of local B-cell activation and differentiation. This activation is speculated to be a consequence of interaction with plaque bacteria, such as *F. nucleatum*, having PBA-inducing properties (6; reviewed in reference 24).

In vitro studies in our laboratory are identifying the complex regulatory mechanisms involved in activation and suppression of PBA by oral bacteria. Such information may provide a better understanding of the role of PBA in oral diseases. We have found that careful definition of in vitro culture conditions is critical in obtaining optimal expression of PBA by a particular stimulant. Therefore, we examined several in vitro parameters of *F. nucleatum*-induced PBA, as measured by total immunoglobulin production. PBA by *F. nucleatum* was suppressed in the presence of monocytes and enhanced by T lymphocytes (T cells). In addition, the concentration of *F. nucleatum* or lymphocytes and the shape of the culture well determined the magnitude of the PBA response.

MATERIALS AND METHODS

F. nucleatum. Cultures of *F. nucleatum* Per-1, isolated from the subgingival plaque of a patient with chronic periodontitis, were supplied by Salam Syed, The University of Michigan Dental School. The bacteria were grown in Schaedler broth (Oxoid Ltd., Basingstoke, Hants., England) to stationary phase (2 to 3

days) at 37°C under anaerobic conditions (85% N_2, 10% H_2, 5% CO_2). The bacteria were centrifuged at 12,000 × g for 30 min, washed three times in sterile phosphate-buffered saline (PBS; 0.15 M NaCl, 0.05 M PO_4, pH 7.4), and finally suspended in sterile distilled water. The bacterial suspensions were cooled in an ice bath and sonicated for a total of 15 min delivered in alternate 5-min intervals of sonication and cooling. Microscopic examination of the bacteria indicated >99% disruption of intact cells. The pellet was washed and suspended in sterile distilled water. The supernatant and pellet fractions were lyophilized and used in the experiments on a weight-volume basis. Preliminary experiments showed greater PBA-stimulating activity in the pellet than in the supernatant fraction. Therefore, the pellet was used in the present study.

Mononuclear cell isolation. Donors, aged 20 to 33 years, with healthy gingiva were used in this study. Mononuclear leukocytes from heparinized venous blood were isolated by Ficoll-Hypaque density centrifugation as described previously (4, 19). The cells were suspended in RPMI 1640 culture medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 30 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer, 2 mM glutamine, and 50 μg of gentamicin (Schering Corp., Bloomfield, N.J.) per ml. The cells were counted in a hemacytometer chamber and adjusted to the desired concentration in RPMI 1640 medium. Viability was determined by trypan blue exclusion and always exceeded 95% in freshly isolated cell preparations.

Monocyte depletion. Mononuclear cell suspensions in 20% newborn calf serum–RPMI 1640 medium were depleted of monocytes by passage of the cells through a Sephadex G-10 column (2). The columns were incubated at 37°C under 5% CO_2 for a total of 30 min. The nonadherent cells were >99% free of nonspecific esterase-positive monocytes (10).

T and B lymphocyte isolation. T cells and B cells were isolated from monocyte-depleted leukocyte suspensions by rosetting with 2-aminoethylisothiouronium bromide-treated sheep erythrocytes as described previously (9, 16). Typically, T-cell preparations contained >95% T cells and 5% B or null cells. B-cell preparations contained 90% B or null cells, 4% T cells, and 6% monocytes as determined by sheep erythrocyte rosettes and nonspecific esterase staining.

In vitro immunoglobulin synthesis. Lymphocytes were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated (80°C, 10 min) fetal bovine serum (Hyclone lots 100185 and 100251; Sterile Systems Inc., Logan, Utah). Triplicate cultures were stimulated with *F. nucleatum* preparations in RPMI or with RPMI alone (unstimulated control) in a total volume of 0.2 ml per well in 96-well round-bottomed tissue culture plates (Costar, Cambridge, Mass.). The cultures were incubated under 5% CO_2 at 37°C and high humidity for 9 days unless otherwise noted.

Micro-ELISA. A micro-enzyme-linked immunosorbent assay (micro-ELISA) was used to measure immunoglobulin A (IgA), IgG, and IgM production and was described previously (19, 39). Polystyrene microtiter plates (Linbro; Flow Laboratories, McLean, Va.) were coated with 0.2 ml of heavy-chain-specific rabbit anti-human immunoglobulin (Bio-Rad Laboratories, Richmond, Calif.) (2 μg/ml) for 1 to 2 h at 37°C. Triplicate cultures were pooled and diluted in PBS containing 0.05% polyoxyethylene-(20)-sorbitan monolaurate (Tween 20) and 0.02% NaN_3 (PBS-Tween). We found that the addition of 0.002% phenol red to the PBS-Tween diluent at this step made pipetting into the microtiter plates easier to see, yet had no effect on the micro-ELISA results. A portion (0.1 ml) of each dilution was added to washed, duplicate assay wells. After 3 h in a humid chamber at 22°C, the plates were washed again, and 0.1 ml of heavy-chain-specific rabbit anti-human immunoglobulin conjugated with alkaline phosphatase was added. Sixteen h later, unbound conjugate was washed away, and 0.15 ml of alkaline phosphatase substrate (Sigma 104; Sigma Chemical Co., St. Louis, Mo.) in $MgCl_2$–sodium carbonate buffer was added. The plates were incubated for 30 min at 22°C, after which the absorbance at 405 nm was measured (Multiskan, Flow Laboratories). The concentration of immunoglobulin in the cultures was determined from a regression line calculated from dilutions of known amounts of IgA, IgG, and IgM.

RESULTS

Stimulation of unfractionated and monocyte-depleted cultures. In previous experiments, using *Actinomyces viscosus* (19) or other gram-positive or -negative microorganisms (unpublished data) as stimulants, we consistently found that removal of monocytes from lymphocyte preparations was necessary to obtain human peripheral blood PBA. Therefore, we examined the effect of monocyte depletion (<1% esterase-positive cells) on *F. nucleatum*-induced PBA. Pokeweed mitogen (PWM), a well-studied polyclonal B-lymphocyte activator (7, 28), was included for comparison. The median IgM response to all concentrations of *F. nucleatum* tested typically was greater in monocyte-depleted cultures (Fig. 1). The difference was statistically significant in the cultures stimulated with *F. nucleatum* at 1 or 5 μg/ml. IgM production was significantly different ($P < 0.05$) from unstimulated controls at *F. nucleatum* concentrations of 1, 5, and 10 μg/ml. Concentrations of *F. nucleatum* greater than 10 μg/ml typically resulted in suboptimal IgM responses. Concentrations of *F. nucleatum* in excess of 10 μg/ml were found to be cytotoxic as determined by trypan blue exclusion, which most likely explains the lower immunoglobulin synthesis in these cultures. In contrast to *F. nucleatum*, PWM-induced immunoglobulin synthesis was greater in unfractionated cultures containing monocytes.

In some experiments, the total IgA and IgG concentrations were also measured in cultures stimulated with *F. nucleatum*. IgA and IgG concentrations correlated with, though were usually lower than, IgM concentrations in both unfractionated and monocyte-depleted cultures (Table 1). As with IgM, optimal IgG and IgA synthesis was obtained in monocyte-depleted

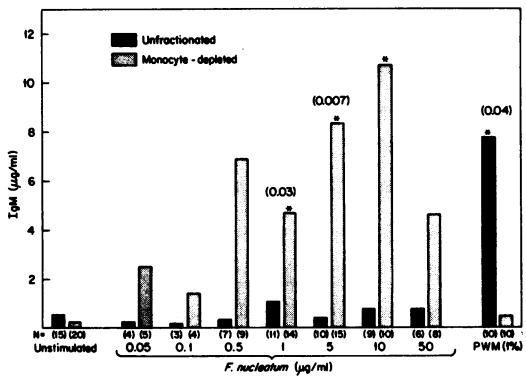

FIG. 1. IgM production in unfractionated and monocyte-depleted cultures stimulated with *F. nucleatum* and PWM. Results are expressed as the median response of *n* subjects. An asterisk (*) designates the responses at this concentration that are significantly different ($P < 0.05$; Mann-Whitney nonparametric analysis) from the unstimulated controls. The numbers in parentheses indicate a significant difference (*P* value indicated) between the responses in the unfractionated and monocyte-depleted cultures.

cultures. Only rarely did we find the IgG concentration equal to or slightly higher than the IgM concentration. IgA production was always less than IgG and IgM production. Therefore, IgM was routinely assayed as a measurement of PBA by *F. nucleatum*.

Lymphocyte concentration and culture well geometry. The shape of the culture vessel and the concentration of lymphocytes can influence PBA (13). Therefore, lymphocytes were added in graded doses to round- or flat-bottomed wells and then stimulated with *F. nucleatum* (Fig. 2) or PWM (Fig. 3). In unfractionated cultures stimulated with *F. nucleatum*, little, if any, IgM production was obtained regardless of the cell concentration or culture well geometry. In the monocyte-depleted cultures, however, *F. nucleatum* stimulated substantial IgM in both round- and flat-bottomed wells. The peak IgM production in round-bottomed wells was obtained at 200,000 cells per well, whereas an equivalent amount of IgM production in flat-bottomed wells required a cell concentration of 400,000 per well. In cultures (with or without monocytes) stimulated with *F. nucleatum* at concentrations of 0.05, 0.5, or 50 μg/ml (data not presented), the IgM responses paralleled the responses at the optimal stimulating dose (5 μg/ml) shown in Fig. 2.

In the PWM-stimulated cultures (Fig. 3), IgM production was optimal in unfractionated cultures at a concentration of 300,000 cells per well. As with *F. nucleatum* stimulation, PWM-induced PBA was optimal in round-bottomed wells. In the monocyte-depleted cultures, a moderate IgM response was obtained in round-bottomed wells, primarily at high cell concentrations, whereas little IgM was produced in flat-bottomed wells at any cell concentration tested.

Effect of delayed stimulation with *F. nucleatum*. To determine the optimal time at which to stimulate PBA in vitro, we added *F. nucleatum* to monocyte-depleted cultures at intervals. To minimize pipetting errors and volume effects, we added the stimulant in 0.1 ml to 0.9-ml cultures in round-bottomed tubes. A delay in the addition of *F. nucleatum* to the lymphocyte cultures resulted in diminished levels of IgM (Fig. 4). Stimulation of lymphocytes 8 h after the start of in vitro culture resulted in a 55% decrease in IgM secretion compared with cultures receiving *F. nucleatum* at time 0. IgM production was greatly reduced (>90%) after the cells had been in culture for 18 h.

TABLE 1. IgA, IgG, and IgM production stimulated by *F. nucleatum* and PWM[a]

Stimulant (μg/ml)	Unfractionated[b]			Monocyte depleted[b]		
	IgM	IgG	IgA	IgM	IgG	IgA
Control	56 ± 29	157 ± 25	153 ± 15	132 ± 28	75 ± 30	0
F. nucleatum						
0.005	77 ± 34	181 ± 23	165 ± 23	156 ± 23	149 ± 53	0
0.05	78 ± 15	143 ± 44	200 ± 35	258 ± 57	299 ± 220	0
0.5	68 ± 43	326 ± 104	196 ± 51	1,395 ± 574	935 ± 191	218 ± 96
5	77 ± 30	235 ± 24	177 ± 14	3,073 ± 216	2,875 ± 310	1,392 ± 83
50	170 ± 16	548 ± 18	274 ± 78	521 ± 114	723 ± 173	0
PWM (1%)	2,512 ± 497	4,731 ± 650	1,723 ± 126	1,277 ± 243	2,598 ± 778	1,526 ± 675

[a] Unfractionated and monocyte-depleted lymphocyte preparations were cultured for 9 days in round-bottomed wells at a concentration of 200,000 cells per well. Results are from a single donor and are representative of at least five similar experiments.

[b] Results expressed as nanograms per milliliter ± standard error of the mean of triplicate wells, each assayed in duplicate by micro-ELISA.

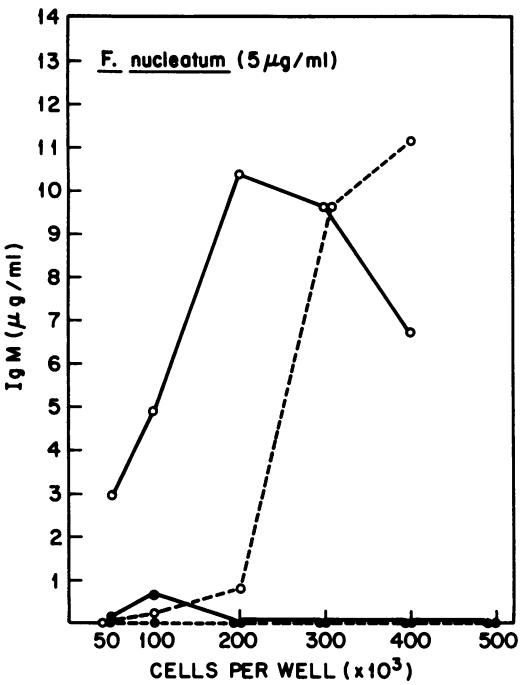

FIG. 2. Effect of lymphocyte concentration and culture well geometry on IgM production stimulated by *F. nucleatum*. Unfractionated (●) or monocyte-depleted (○) lymphocytes were added to round (solid line)- or flat (dashed line)-bottomed wells. Results are the pooled average of triplicate test cultures (minus the unstimulated control value) and are representative of three similar experiments.

Kinetics of IgM secretion. The release of IgM into the culture medium was measured over a 14-day period (Fig. 5). In monocyte-depleted cultures, IgM secretion was first detected on day 4, increased rapidly between day 4 and day 8, and thereafter leveled off. IgM production in unfractionated cultures paralleled the kinetics of monocyte-depleted cultures. The release of IgG and IgA followed similar time courses (data not presented).

T-lymphocyte effect. Polyclonal activation of human B cells is commonly enhanced by helper activity provided by T cells (7, 11, 15, 23, 32). The effect of T cells on *F. nucleatum*-induced PBA was determined by adding sheep erythrocyte-rosetted T cells to B-cell-enriched, monocyte-depleted cultures. In experiments in which the cell concentration was held constant while varying the ratio of B cells to T cells, IgM production increased with increasing numbers of T cells (Fig. 6). However, when the number of T cells exceeded the number of B cells in the cultures (i.e., >1:1 ratio), IgM production was depressed. By using the data in Fig. 6, the average amount of IgM secreted per B cell can be estimated. B- to T-cell ratios of 25:1, 3:1, 1:1, 1:4, and 1:10 yielded 0.03, 0.20, 0.39, 0.18, and 0.08 ng of IgM per ml per B cell, respectively. These calculations again indicate the enhanced IgM production in cultures containing equal numbers of B and T cells. Similar results were obtained when various numbers of T cells were added to a constant number of B cells (data not presented). Furthermore, the IgM concentration was greater than the IgG or IgA concentration in the *F. nucleatum*-stimulated cultures, regardless of the B- to T-cell ratio (data not presented). Since the B-cell preparations contained approximately 4% T cells, the absolute requirement for T cells in *F. nucleatum*-induced PBA was not determined.

DISCUSSION

The results of this study indicate that *F. nucleatum* possesses a potent polyclonal activator of human peripheral blood B cells. The PBA factor appears to be located primarily in the pellet fraction after sonication of the whole cell. Studies to characterize the cellular location and physicochemical nature of this PBA are currently in progress.

Immunoglobulin production induced by *F. nucleatum* was enhanced by the removal of monocytes (<1% esterase-positive cells) from the lymphocyte cultures. Similar suppressive

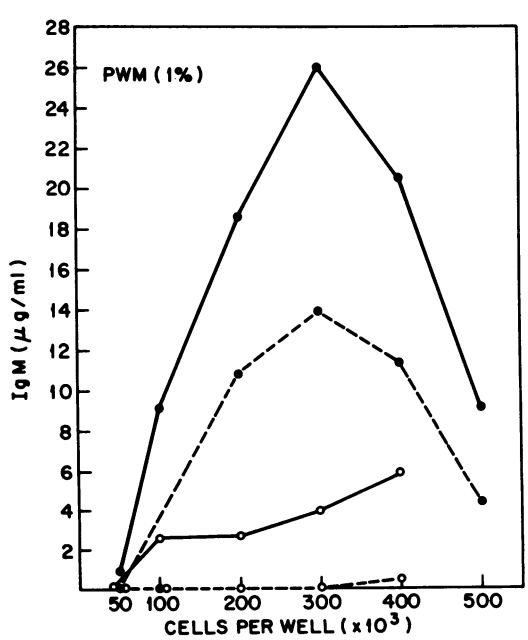

FIG. 3. Effect of lymphocyte concentration and culture well geometry on IgM production stimulated by PWM. Symbols and culture conditions are the same as described in the legend to Fig. 2.

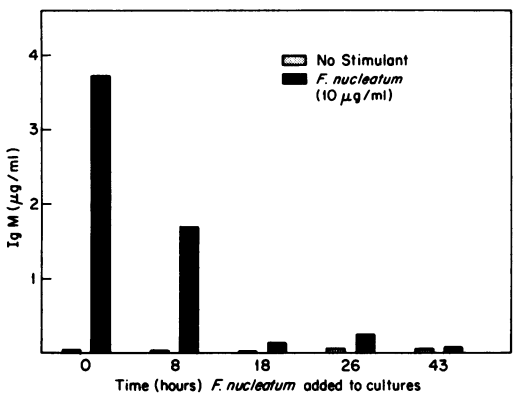

FIG. 4. Effect of delayed stimulation on IgM production induced by *F. nucleatum*. Monocyte-depleted lymphocytes (2×10^6) in 0.9 ml of 10% fetal bovine serum–RPMI 1640 medium were cultured in round-bottomed tubes. At the time indicated, 0.1 ml of *F. nucleatum* in RPMI 1640 medium or RPMI 1640 medium alone (no stimulant) was added to the tubes. Results are the pooled average of duplicate tubes and are representative of two similar experiments.

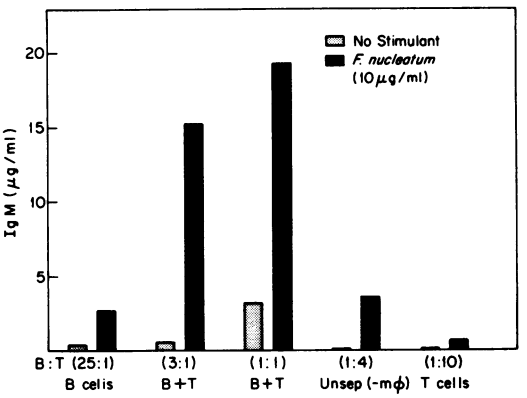

FIG. 6. Effect of B- to T-cell ratio on IgM production stimulated by *F. nucleatum*. T cells, B cells, mixtures of T and B cells, or unseparated, monocyte-depleted lymphocytes (unsep −mφ) were cultured at 100,000 cells per round-bottomed well. Cultures received either *F. nucleatum* or RPMI 1640 medium (unstimulated control). Ratios of B to T cells are indicated for each culture condition. Results are the pooled average of triplicate cultures and are representative of three similar experiments.

effects of monocytes on PBA are found in cultures stimulated by *A. viscosus* (19), lipopolysaccharide (11), staphylococcal protein A (20), tetanus toxoid (8), keyhole limpet hemocyanin (8), and extracts of other gram-positive and -negative microorganisms (personal unpublished data). The mechanism of monocyte suppression is undetermined. The monocytes may inactivate the PBA factor via phagocytosis or enzymatic degradation. Furthermore, monocytes secrete biologically active compounds including prostaglandins, cyclic AMP, and interferon, which inhibit lymphocyte functions (1) and which may be released in the stimulated cultures. Alternatively, the enhancement in *F. nucleatum*-induced PBA after depletion of monocytes may result from the concomitant depletion of suppressor lymphocytes. After lidocaine elution of the adherent cells from the Sephadex G-10 columns (27), roughly 20 to 40% of the cells lack nonspecific esterase staining characteristic of monocytes (10).

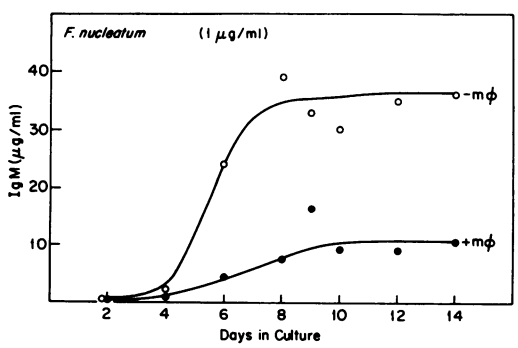

FIG. 5. Kinetics of IgM production in unfractionated (+mφ) and monocyte-depleted (−mφ) cultures. Cultures received *F. nucleatum* or RPMI 1640 medium (unstimulated control) on day 0 and were removed from the incubator on the days indicated and then frozen. All supernatants were assayed at the same time. Results are expressed as the pooled average of triplicate cultures (minus the unstimulated control value) and are representative of four similar experiments.

In contrast to PBA by *F. nucleatum*, activation induced by PWM was optimal when monocytes were present in the lymphocyte cultures (Fig. 1). This finding has been reported by others (8, 28, 29), although some investigators reported a suppressive effect of monocytes on the generation of PWM-induced immunoglobulin-secreting lymphocytes (7, 20). Recent evidence suggests that monocytes release interleukin-1, which mediates PWM-triggered B-cell differentiation into plasma cells (29). The difference in the mechanism(s) of monocyte regulation of PBA by PWM and *F. nucleatum* remains to be studied.

The pellet fraction from sonicated *F. nucleatum* Per-1 is the most potent PBA stimulant we have isolated from microorganisms associated with periodontal disease. Bick et al. (3) screened 16 isolates of gram-negative bacteria from subgingival plaque and similarly found that the pellet fractions of three isolates of *F. nucleatum* were potent activators of B cells. The optimal stimulatory concentration of *F. nucleatum* in the

study by Bick et al. is slightly higher (50 to 100 μg/ml) than the optimal concentration determined in the present study (1 to 10 μg/ml). These differences are most likely due to differences in (i) the bacterial strains used, (ii) the preparation of the bacterial extracts, (iii) bacterial or lymphocyte cultures in vitro, (iv) the assay used to measure PBA (antibody plaque-forming cells versus total IgM secretion), or (v) the responsiveness of the lymphocytes of the subjects. The optimal PBA concentration that we found for our crude preparation of *F. nucleatum* is 50 to 100 times less than the stimulatory concentration found for a crude soluble preparation of *A. viscosus* (19), 10-fold less than a water-soluble extract from *Nocardia* sp. (14), 3-fold less than purified protein derivative of tuberculin (23), and similar to the concentrations of staphylococcal protein A (15), tetanus toxoid (13), and keyhole limpet hemocyanin (13) which induce PBA.

The shape of the culture vessel determined the magnitude of the polyclonal B-cell response. At a concentration of 200,000 lymphocytes per well, round-bottomed wells allowed optimal total IgM production after stimulation with *F. nucleatum* or PWM. This finding parallels the results of Lane et al. (13) with polyclonal immunoglobulin secretion induced by tetanus toxoid, keyhole limpet hemocyanin, and PWM. Compared with flat-bottomed wells, the round-bottomed wells provide increased lymphocyte cell-to-cell contact, which appears to support enhanced nonspecific immunoglobulin synthesis (13). Similarly, the release of lymphokines is also greatly enhanced in round-bottomed versus flat-bottomed tubes (25).

Lymphocyte cultures became progressively refractive to stimulation by *F. nucleatum* after an 8-h incubation in vitro. This suggests that, in the absence of stimulation, the B cells lose the ability to differentiate into antibody-secreting plasma cells. The decrease in B-cell responsiveness may result from the in vitro environment either directly or indirectly causing alterations in cellular factors necessary for differentiation (e.g., membrane receptors, enzymes, etc.). Furthermore, since T-cell interaction enhances B-cell activation (Fig. 6), culturing in vitro might also decrease T-cell helper activity or increase suppressor activity.

The accumulation of IgM in cultures stimulated by *F. nucleatum* peaked between days 7 and 8. These responses parallel PBA induced by *A. viscosus* (19), staphylococcal protein A (21, 26), tuberculin purified protein derivative (23), *Nocardia* sp. (14), keyhole limpet hemocyanin (13), and PWM (21, 26, 30). The production of *F. nucleatum*-induced IgM, IgG, and IgA followed similar time courses, although IgG and IgA concentrations were lower than that of IgM (data not presented). IgM production is also typically greater than IgG or IgA production in cultures stimulated with *Nocardia* extracts (14), PWM (30, 38), staphylococcal protein A (15), keyhole limpet hemocyanin (38), and *A. viscosus* (19). However, PBA induced by tetanus toxoid is characterized by IgG synthesis greater than that of IgM (37).

IgM production stimulated by *F. nucleatum* was greatly enhanced in cultures containing equal numbers of T and B cells. Stimulation of B cells from human peripheral blood commonly requires T-cell help (7, 11, 15, 23, 32). Cultures with excess numbers of T cells (e.g., unfractionated suspensions) yielded decreased IgM production. Saxon et al. reported similar T-cell concentration effects, using cultures stimulated with PWM (32). The reason for PBA suppression by high concentrations of T cells is as yet undetermined. That the lower immunoglobulin production reflects a dilution of B cells by T cells is not substantiated by the results of experiments in which increasing numbers of T cells were added to a constant number of B cells. In these experiments, the PBA response was similarly diminished in the presence of excess numbers of T cells. Thus, the data may suggest that the peripheral blood T-cell population contains a minority of cells with PBA-suppressive activity. When excess numbers of T cells are added to B cells, the effect of these suppressor cells is demonstrated. At low T- to B-cell ratios, insufficient numbers of the suppressor cells are present to affect B-cell activation.

A comparison of the *F. nucleatum*- and PWM-induced responses in this study suggests one or both of the following: (i) these stimulants induce PBA by different mechanisms, or (ii) these stimulants induce PBA which is regulated by different mechanisms. PWM-induced PBA appears to be enhanced by, though not completely dependent upon, monocytes, whereas *F. nucleatum*-induced PBA appears to be highly monocyte-independent (Fig. 1). Furthermore, the response profiles of *F. nucleatum*- and PWM-stimulated cultures are different in round- or flat-bottomed wells containing various lymphocyte concentrations (Fig. 2 and 3). Examination of similarities and differences in PBA induced by *F. nucleatum* and PWM may provide a better understanding of the mechanisms involved in PBA.

The magnitude of the IgM response in cultures stimulated with *F. nucleatum* is highly suggestive of PBA. In most experiments, we found that the magnitude of the *F. nucleatum*-induced response was equal to or higher than the response elicited by PWM, a commonly employed polyclonal B-cell activator. Nevertheless, it is possible that the IgM produced after stimulation with

F. nucleatum is specific for *F. nucleatum*. However, in parallel experiments (D. F. Mangan, T. Won, and D. E. Lopatin, manuscript submitted for publication), specific IgM antibody responses in *F. nucleatum*-stimulated cultures were determined. The maximum anti-*F. nucleatum* IgM response was typically less than 100 ng/ml. Since PBA responses were 10- to 100-fold higher than the specific responses, the total IgM measured in the present study represents the stimulation of multiple B-cell clones (i.e., PBA).

This report along with several others (3, 18, 19, 36) indicates that microorganisms found in dental plaque contain potent B-cell stimulants. Chronic periodontitis is histologically characterized by a substantial accumulation of immunoglobulin-secreting plasma cells which may indicate localized B-cell differentiation. Recent studies have determined that local immunoglobulin synthesis occurs in diseased, plasma cell-rich gingival tissue, but does not occur in healthy histologically normal gingival tissue (12, 17). The role of microbial PBA in this immunoglobulin production has yet to be determined. The close juxtaposition of the plasma cells in the gingival tissue with potent PBA-stimulating microorganisms in subgingival plaque implicates these bacteria in the localized B-lymphocyte responses.

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