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T-Cell Regulation of Polyclonal B-Cell Activation Induced by Extracts of Oral Bacteria Associated with Periodontal Diseases

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These studies were designed to examine the role of regulatory T cells in the polyclonal antibody response of human peripheral blood lymphocytes to extracts of bacterial isolates commonly associated with periodontal disease. Polyclonal antibody responses to the organisms tested were found to be T cell dependent, as are most of the B-cell activators in the human system. Functional T helper activity was resistant to 1,500 rads of irradiation. Optimal polyclonal antibody responses to the bacterial extracts occurred at a 3:1 T-cell-to-B-cell ratio, whereas pokeweed mitogen-induced responses peaked at a 1:1 ratio, suggesting a difference in T-cell regulatory influences in response to these activators. Purified populations of T helper and supressor cells exerted potent regulatory control of the responses to the bacterial extracts. These findings support the conclusion that regulatory T lymphocytes exert a potent modulating influence over the polyclonal response to periodontally associated bacteria and may play an important role in regulating the lymphocyte response in the diseased site.

The periodontal diseases are inflammatory conditions which may include sufficient destruction of the periodontium to result in loss of teeth. Their etiology is generally agreed to be bacterial (20, 27). Although the pathogenicity of certain species has been suggested (28), the associated floras are very complex, including more than 260 distinct types or species (18), leaving many unresolved questions about the role of given species in pathogenesis. There are several potential mechanisms whereby bacteria might directly initiate or perpetuate certain signs of disease, but host response factors, including immunological reponses, may be equally important in determining the progression and severity of disease (19, 20). The histopathology of established or advanced periodontal lesions is dominated by B lymphocytes and plasma cells, suggesting that the periodontal diseases may be primarily B-cell lesions. Also, a number of findings suggest that polyclonal activation of B cells induced by bacteria in the resident flora may be an important pathogenetic mechanism.

Peripheral blood lymphocytes (PBLs) from normal subjects have been shown to support good polyclonal antibody synthesis when stimulated by extracts of both gram-negative (2, 26) and gram-positive (5, 26) periodontally associated bacteria. In addition, several investigators have found no differences between diseased and control individuals in the proliferative response of their PBLs stimulated by various periodontal sonic extracts (6, 12, 16, 29; K. M. Resmini, P. Stashenko, S. S. Socransky, and A. D. Haffajee, J. Dent. Res. **61**:220, 1982). Mitogenic activity of a broad range of bacterial sonic extracts has been demonstrated in cultures of lymphocytes from germfree mice, cord blood, and nude mice (30; J. G. Tew, S. Donaldson, and P. H. Bick, J. Dent. Res. **61**:317, 1982). These findings argue against antigen-mediated events as being the sole pathogenic mechanisms and support the hypothesis that polyclonal B-cell activation contributes to the disease process.

The present study was designed to examine the regulatory aspects of the polyclonal B-cell activation induced by oral bacterial isolates in cultures of human PBLs. Antibody production by human lymphocytes in response to the majority of polyclonal B-cell activators has been found to be dependent upon T cells (31). Therefore, we wanted to determine whether lymphocyte activation induced by periodontally associated bacteria demonstrated a similar T-cell requirement, and we wanted to dissect this T-cell regulation further with regard to T-cell subpopulations. In addition, studies were performed to assess the ability of human PBLs to produce immunoglobulin G (IgG) after stimulation with periodontopathic organisms; our previous studies had examined the IgM response (2).

The results presented here confirm and extend our previous findings. Selected gram-negative and gram-positive isolates were found to polyclonally induce significant levels of IgG and IgM in culture supernatants from PBLs. This antibody production was dependent upon T lymphocytes, and T-cell subsets were found to exert significant regulatory control in IgG and IgM antibody production.

MATERIALS AND METHODS

Bacterial strains. The strains tested included gram-negative and gram-positive species isolated from samples of periodontal microflora and characterized by W. E. C. Moore and L. V. Holdeman of Virginia Polytechnic Institute and State University, Blacksburg, Va. These were *Fusobacterium nucleatum* D30A9 and D43B2F, *Capnocytophaga ochracia* D28B7, *Actinomyces viscosus* serotype II (strain D34B26), *Streptococcus sanguis* II (strain D24B19), *Bacteroides buccae* D3A6, and an undescribed species of nonsaccharolytic bacilli with polar flagella, currently designated *Wolinella* HVS D16B17 in their laboratory. In addition, two strains of *Actinobacillus actinomycetemcomitans*, Y4 and N27, obtained from A. Tanner, Forsyth Dental Center, Boston, Mass, were tested.

Bacterial extract preparation. The bacterial strains were harvested from broth cultures in peptone-yeast extract broth with appropriate additives to support growth and were washed and suspended in 1 part of glycerol to 2 parts of phosphate-buffered saline (PBS) at approximately 0.1 g of cells per ml. The suspensions were stored frozen (−20°C) until sonicated for use.

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Cell wall sonic extracts from the various bacterial isolates were prepared by the method of Baker et al. (1) as previously reported (26). The sediment, consisting largely of cell wall fragments, was the extract used to stimulate PBL cultures in all the assays reported. Protein concentrations were determined by the method of Lang (14).

In vitro culture methods. Human PBLs were cultured by the method of Fauci and Pratt (8). All studies were performed with normal human PBLs obtained from the venous blood of individuals 19 to 29 years of age who did not have periodontitis as defined by loss of connective tissue attachment. Heparinized venous blood (25 U of heparin per ml, preservative free; Abbott Laboratories, North Chicago, Ill.) was obtained from normal healthy volunteers. Blood was mixed with an equal volume of Hanks balanced salt solution, and 30 ml of this mixture was layered over 15 ml of lymphocyte separation medium (LSM) solution (Litton Bionetics, Kensington, Md.) in a sterile Falcon 50-ml conical tube (Becton Dickinson Labware, Oxnard, Calif.). The tubes were centrifuged at 400 × *g* for 35 min in a swinging-bucket rotor. The mononuclear cell fraction was recovered from the LSM-Hanks balanced salt solution interface, washed three times in RPMI 1640, and suspended in 2 to 5 ml of medium. Cell viability was assessed by phase-contrast microscopy. Cells were suspended in complete medium consisting of RPMI 1640 supplemented with 2.0 mM L-glutamine, 100 U of penicillin per ml, 100 μg of streptomycin per ml (all components from GIBCO Laboratories, Grand Island, N.Y.), and 10% heat-inactivated fetal calf serum (MA Bioproducts, Kensington, Md.) For studies employing the sheep erythrocyte (SRBC) rosetting procedure, the fetal calf serum was absorbed twice with SRBCs. Unless otherwise noted, 10^6 PBLs were then added in 0.95 ml to Falcon plastic capped culture tubes (12 by 75 mm), to which was added 0.05 ml of medium (control), 0.05 ml of bacterial sonic extracts (experimental), or pokeweed mitogen (PWM; GIBCO Laboratories; positive control) to yield the appropriate final concentration of activator. Each group was cultured in triplicate for 7 days at 37°C in a humidified atmosphere of 5% CO_2 in air.

Preparation of T and B cells. T cells were isolated by rosetting with SRBCs treated with 2-aminoethylisothiuronium bromide (AET-SRBCs) and were prepared by the method of Kaplan and Clark (11). A 1% solution of AET-SRBCs was mixed with an equal volume of human PBLs at 4 × 10^6 cells per ml in complete medium, centrifuged at 100 × *g* for 10 min, and incubated at 37°C for 5 min and at 4°C for 2 h or overnight. After incubation, the cells were suspended gently, layered over LSM, and centrifuged for 35 min at 400 × *g* in a swinging-bucket rotor. The rosette-positive cells were obtained from the cell pellet, and the attached SRBCs were lysed by treatment with 2 to 3 ml of a Tris-ammonium chloride buffer for 5 min. The rosette-positive cells were pooled, washed three times in RPMI 1640, counted for viability, and suspended in complete medium. The cell fraction was then characterized by esterase staining, by surface immunoglobulin determination with direct immunofluorescence, and by a second rosetting procedure with AET-SRBCs. This T-cell fraction routinely contained 97 to 99% rosette-positive cells, 1 to 2% surface immunoglobulin-positive cells, and 1% or less esterase-positive cells. If the T-cell fraction was found to contain 3% or more nonrosetting cells, the AET-SRBC rosetting procedure was repeated.

For purification of a B-lymphocyte-rich fraction, the nonrosetting cells were retrieved from the LSM-medium interface after centrifugation of the AET-SRBC-treated lymphocytes over LSM. The cells were pooled, washed three times in RPMI 1640, counted for viability, and suspended in complete medium. This nonrosetting fraction was characterized for esterase staining cells, surface immunoglobulin-positive cells, and rosette-positive cells. Routinely, this fraction contained 30 to 42% esterase-positive cells, 60 to 70% surface immunoglobulin-positive cells, and 1 to 2% rosette-positive cells. Although this nonrosetting fraction contained a significant proportion of monocytes, it was referred to as B cells.

RIA. An indirect solid-phase radioimmunoassay (RIA) was used for the determination of immunoglobulin production in cultures of human PBLs. For coating the solid phase, IgG-fraction goat anti-human IgG or IgM (Cappel Laboratories, Cochranville, Pa.) was diluted in bicarbonate-carbonate coupling buffer (pH 9.6) at a concentration of 10 μg of antibody protein per ml. Quantities of this solution (1 ml) were then added to Falcon plastic tubes (12 by 75 mm) and incubated overnight at room temperature.

For each experiment, culture supernatants and a standard IgM or IgG preparation were tested under identical experimental conditions. For preparation of the standard curve, chromatographically purified IgG or pooled myeloma IgM (both from Cappel Laboratories) were diluted in 0.15 M PBS, pH 7.1, at 5 mg/ml and then were diluted further in 0.15 M PBS–10% newborn calf serum (NCS) to yield a linear standard curve. Each culture supernatant was prepared in duplicate at two dilutions in 0.15 M PBS–10% NCS. Culture supernatants, standard, or buffer alone (negative control) were added to the antibody-coupled tubes and were incubated on a shaker platform for 4 h and then overnight at 4°C. The tubes were then decanted and washed in tap water 20 times.

^{125}I-labeled goat anti-human IgG or IgM (prepared by the chloramine-T technique to a specific activity of 10 to 20 μCi/mg Ab protein) was diluted in 0.15 M PBS–10% NCS, and 1 ml was added to each tube and incubated overnight at 4°C. The excess radiolabel was then decanted, and the tubes were again washed in tap water 20 times. Bound radioactivity was determined by counting the tubes in an LKB CompuGamma. The data were calculated by utilizing a counts per minute versus log concentration spline function.

Determination of antibody specificity. Absorption experiments were performed for assessment of antibody specificity. PBL cultures were stimulated with 200 μg of *A. actinomycetemcomitans* Y4 per ml for 7 days, and the culture supernatants were harvested. The quantity of IgG produced was determined by the RIA. *A. actinomycetemcomitans* antigen was coupled to the plastic tubes by diluting it to 25 μg/ml in a bicarbonate-carbonate coupling buffer (pH 9.6) and adding 1 ml to the plastic tubes at room temperature overnight. Coupled tubes were then decanted, washed 20 times in tap water, and stored at 4°C. To demonstrate that this protocol resulted in sufficient binding of the bacterial sonic extract to the tubes, the standard RIA was performed with known positive serum samples for *A. actinomycetemcomitans*. These serum samples were obtained from two patients with juvenile periodontitis and were positive for precipitating antibody to this bacterium as determined by Ouchterlony analysis (21). Dilutions of these serum samples were mixed in PBS–10% NCS and added to the culture tubes previously coupled with *A. actinomycetemcomitans*. Incubation proceeded for 4 h on a shaker platform and overnight at 4°C. The tubes were then washed 20 times with tap water, and 1 ml of ^{125}I-labeled goat anti-human IgG was added to each tube. After an overnight incubation at 4°C, these tubes were washed free of excess radiolabel, and the bound

radioactivity was determined in a gamma counter. It was found that the tubes coupled with *A. actinomycetemcomitans* bound approximately 30 to 40% of the counts per minute added. Based on this level of radiolabel binding, the absorption tubes coupled with *A. actinomycetemcomitans* were capable of binding a minimum of 1 mg of specific anti-*A. actinomycetemcomitans* Y4 antibody.

Separation of lymphocytes on a solid-phase immunoadsorbent. A manual cell-sorting technique or panning was performed to functionally examine subpopulations of human T cells by utilizing the Orthoclone monoclonal antibodies. The methods of Wysoki and Sato (32) and of Kotzin et al. (13) were utilized with modifications. T cells were obtained by SRBC rosetting, and the panning technique was employed to separate these T cells into T-helper/inducer ($OKT4^+$) and T-suppressor/cytotoxic ($OKT8^+$) populations. The monoclonal antibodies OKT4 and OKT8 were incubated at a 1:20 to 1:40 dilution with T cells suspended in RPMI 1640 at 5×10^6 cells per ml for 30 min at 4°C with agitation every 10 min. The antibody-treated cells were washed twice in 5% fetal calf serum–PBS and were suspended in 5% fetal calf serum–PBS to a concentration of 6.6×10^6 cells per ml. Three milliliters of this cell suspension were poured onto anti-immunoglobulin-coated bacteriological-grade petri dishes (Lab-Tek Products, Naperville, Ill.; 15 by 100 mm). Absorbent plates were coated with affinity-purified goat anti-mouse IgG (Tago, Inc., Burlingame, Calif.) at 10 μg/ml in 0.5 M Tris buffer, pH 9.5, and incubated at room temperature for 4 h. These plates were incubated with antibody-treated cells for 2 h at 4°C with gentle swirling of the plates after 1 h. The dishes were then gently washed five times with 5 ml of 1% fetal calf serum–PBS at each wash to recover the nonadherent cells. Recovery of the bound cells was accomplished by vigorously pipetting 5 to 10 ml of PBC–1% NCS across the plate surface and collecting the dislodged cells. Both the positively and negatively selected cell fractions were washed once in RPMI 1640, counted for viability, and suspended to a suitable concentration for culturing.

The purity of these fractions was assessed by an indirect immunofluorescence technique. The selected $OKT4^+$ or $OKT8^+$ cell populations were approximately 95% positive for the selected markers, whereas the OKT4- or OKT8-depleted cell populations contained only 4 to 5% contamination by the reciprocal population.

RESULTS

Initial studies were designed to quantitate immunoglobulin production by human PBLs. We were especially interested in examining IgG levels since Mackler et al. have reported a predominance of IgG-producing plasma cells in periodontal lesions (15). For the assessment of immunoglobulin production in response to periodontal bacterial isolates, PBLs from normal individuals were cultured in the presence of bacterial sonic extracts at three doses (50, 100, and 200 μg/ml). PWM was utilized as the positive control activator, and medium alone was added to unstimulated control cultures. At the termination of a culture period, supernatants were assayed for both IgM and IgG in the solid-phase indirect RIA. Preliminary kinetic studies demonstrated significant immunoglobulin levels of IgM and IgG which peaked on days 7 to 10. Therefore, all culture supernatants were tested after a 7-day incubation.

Pooled data from a number (8 to 11) of healthy individuals at the maximum stimulatory dose of activator are shown in Fig. 1. PWM and all bacterial isolates, excluding one strain of *F. nucleatum* (D43B27), stimulated significant levels of IgG synthesis in culture. Immunoglobulin levels ranged from 2,534 ng/ml for *A. viscosus* to 5,380 ng/ml for *Wolinella* HVS. Similar data for IgM production are shown in Fig. 2. As with IgG, significant IgM production was induced in PBLs from normal donors by all bacterial isolates, excluding one strain of *F. nucleatum*. For IgM production, the levels of immunoglobulin ranged from 2,238 ng/ml for *B. buccae* to 441 ng/ml for *C. ochracea*. IgG production exceeded that of IgM, although this trend was apparent only when results from a large number of experiments were pooled. There appeared to be individual high and low responders to these bacterial sonic extracts and individual patterns of reactivity existed for both the amount and the predominant isotype of the antibody produced in response to a particular stimulant. From these data, it was concluded that the gram-positive and gram-negative bacterial isolates tested stimulated significant levels of both IgM and IgG in cultures of normal PBLs.

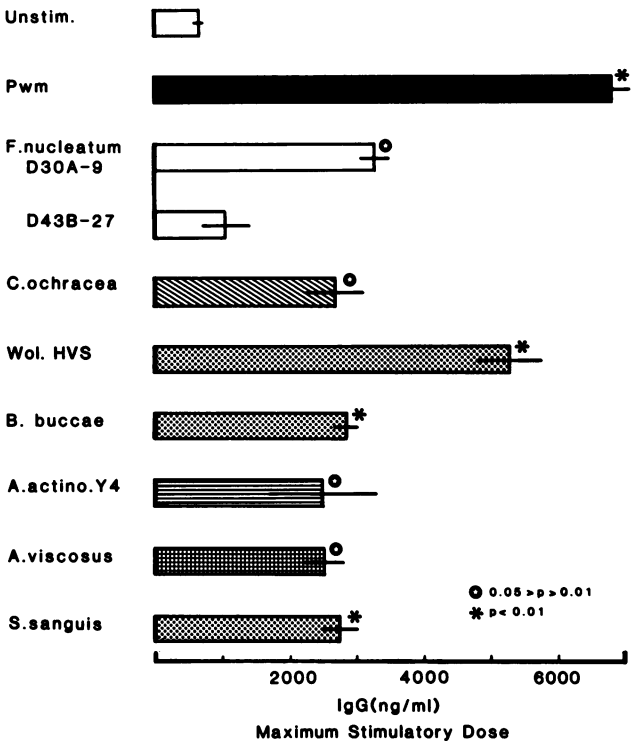

FIG. 1. IgG levels stimulated by periodontal isolates. Normal human PBLs were cultured in triplicate for 7 days in the presence of medium alone (unstimulated), PWM (diluted 1:100, 1:200, or 1:1,000), or sonic extracts from various gram-negative and gram-positive bacteria (50, 100, or 200 μg/ml. Culture supernatants were assessed for IgG by RIA. For statistical comparison, the maximal responses at the optimum stimulatory dose were pooled from all experiments (n = 8 to 11). The mean ± standard error of each bacterial extract group and of the unstimulated group were compared by the Student t test.

Demonstration of the polyclonal nature of the antibody response. Although significant antibody production had been shown both in the present study and in previous reports using the plaque-forming cell assay (2, 5), the polyclonal nature of the antibody response had not been accurately estimated. Therefore, experiments were designed to determine the relative proportions of antigen-specific and polyclonal antibody after stimulation with the bacterial extracts. Since the majority of the antibody produced in response to a polyclonal activator is not specific for the stimulating orga-

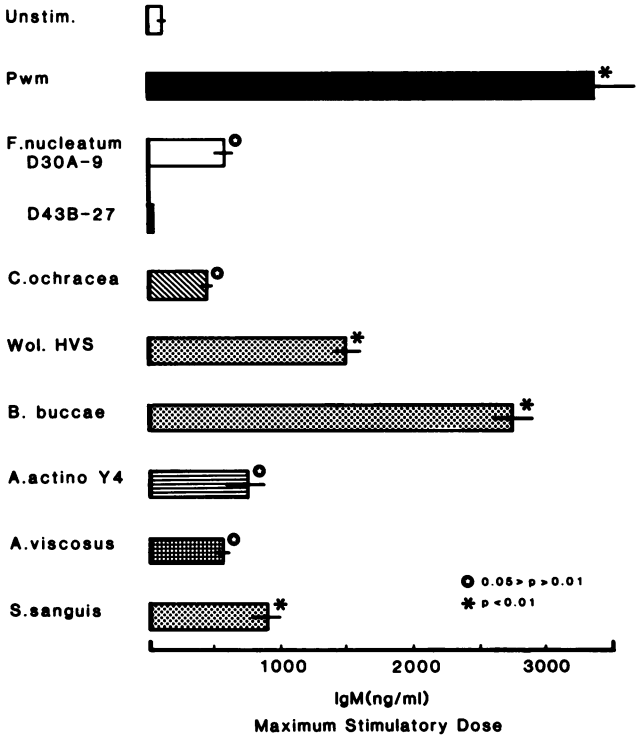

FIG. 2. IgM levels stimulated by periodontal isolates. Normal human PBLs were cultured as described in the legend to Fig. 1. Culture supernatants were assessed for IgM by RIA. Statistical comparisons were made as described in the legend to Fig. 1 (n = 8 to 11). The mean ± standard error of each bacterial extract group and of the unstimulated group were compared by the Student t test.

nism, the experiments were designed to demonstrate the degree of nonspecificity of the immunoglobulin produced in culture.

A series of absorption experiments was performed to answer this question. PBLs from four individuals were stimulated *in vitro* with three doses of sonicated *A. actinomycetemcomitans* Y4 (50, 100, and 200 μg/ml), and the amount of total IgG present in the culture supernatants was assessed by the standard RIA. These supernatants were then incubated in culture tubes coated with the stimulating bacterial sonic extract to deplete any specific antibody. After absorption, another IgG quantitation was performed to determine the percentage of immunoglobulin which had been depleted. Cultures stimulated with *A. actinomycetemcomitans* Y4 were utilized because positive human serum for this antibody was available and could be used to demonstrate binding of the bacterial sonic extract to the absorption tube. In Table 1, results of these experiments showing representative supernatants from cultures stimulated with 200 μg of *A. actinomycetemcomitans* Y4 per ml are presented. The amount of specific IgG in culture supernatants which could bind to an *A. actinomycetemcomitans* Y4-coupled tube was 9% of the total IgG present. However, considering that chromatographically purified IgG was nonspecifically depleted by 10%, the actual level of specific *A. actinomycetemcomitans* Y4 antibody may have actually been less than 10%. Supernatants from cultures stimulated with 50 and 100 μg extracts of sonic extracts per ml produced similar results (data now shown). From these experiments, it was concluded that less than 10% of the IgG obtained in the culture supernatants was specific for the stimulating organism, suggesting that this bacterium stimulated a polyclonal response.

T-cell regulation of the polyclonal antibody response. Studies were designed to assess the requirement for T-cell help in the generation of IgG and IgM in culture supernatants. T cells were depleted by the AET-SRBC rosetting procedure. Unseparated and T-cell-depleted PBLs from normal individuals were stimulated with various gram-negative isolates at three doses. A comparison of the responses of unseparated and T-cell-depleted PBLs is presented in Table 2. The maximal responses at the optimum stimulatory dose were pooled from several (five to seven) experiments for each bacterial extract and were evaluated. There was a significant decrease in the IgG and IgM antibody levels in all cultures stimulated by both PWM and the bacterial sonic extracts after T-cell depletion. Therefore, it was concluded that T cells are required for the generation of both IgM and IgG in cultures of human PBLs activated with extracts of various periodontal organisms. In addition to the data shown, several other gram-negative and various gram-positive isolates were tested, and antibody production was always found to be dependent upon T cells.

Since depletion of T cells was clearly shown to inhibit the production of IgM and IgG stimulated by PWM and periodontopathic organisms, further studies were needed to demonstrate that T lymphocytes could restore the response to depleted cell preparations. To accomplish this, unfractionated PBLs were separated into B-cell-rich and T-cell-rich fractions by SRBC rosetting. To a constant number of B cells (5×10^5), various amounts of T cells were added (from 2.5×10^5 to 3×10^6 T cells). Reconstituted cultures were stimulated with *S. sanguis*, *B., buccae*, *Wolinella* HVS, and PWM at three doses and were cultured for 7 days. Culture supernatants were then assessed for IgG and IgM immunoglobulin levels. Representative experiments from one normal donor are presented in Fig. 3 (the experiments shown in Fig. 4 were performed with a different donor). Three individuals were tested by this experimental protocol. Figure 3 presents the IgM levels in culture supernatants from cultures in which graded numbers of T cells were added back to 5×10^5 B cells. The response to *B. buccae* was below control level in the absence of T cells. However, as increasing numbers of T cells were restored to culture, there was a peak in the IgM level with the addition of 5×10^5 T cells. The immunoglobulin level gradually declined until it returned to control level at 3×10^6 T cells added. It is difficult to

TABLE 1. Lack of specificity of the IgG response induced by *A. actinomycetemcomitans* Y4

Culture supernatant	IgG produced (ng/ml)[a]		% Depleted[b]
	Before absorption	After absorption	
Unstimulated	453 ± 45	545 ± 31	0
A. actinomycetemcomitans Y4 stimulated	1,026 ± 33	929 ± 42	9
	1,097 ± 37	980 ± 34	9
IgG control	11.4 ± 0.6	10.9 ± 0.5	10

[a] IgG produced in culture supernatants was determined by RIA. Culture supernatants were absorbed on tubes coated with *A. actinomycetemcomitans* Y4 and then were assayed in IgG-coated tubes to determine the amount of specific antibody depleted.

[b] Amount of IgG bound to the tube coated with *A. actinomycetemcomitans* Y4 as a percentage of the total IgG present.

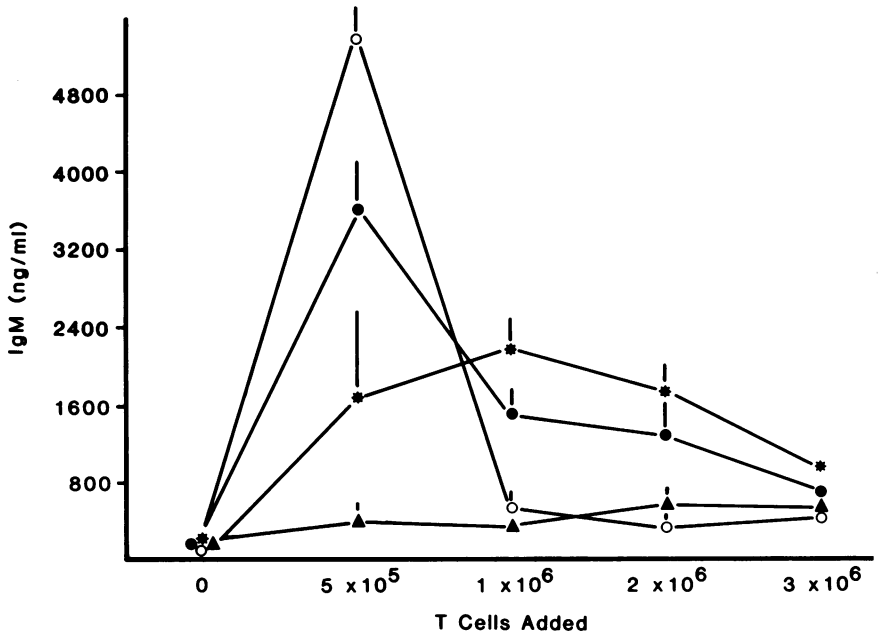

FIG. 3. Effect of restoring T cells to B-cell-enriched cultures on IgM production. Unfractionated PBLs were separated into T-cell-rich and B-cell-rich fractions. Increasing numbers of T cells were added to a constant number of B cells (5×10^5). Cultures were stimulated with medium alone (▲), PWM (1:200 dilution; ○), or sonic extracts (50 and 200 μg/ml) of *B. buccae* (●) or *S. sanguis* (✱) and were incubated for 7 days. Supernatants were harvested and assayed for IgM by an RIA. Immunoglobulin levels at the optimal stimulatory dose for each experimental group from triplicate cultures were used for determination of the mean ± standard error.

evaluate the data at this final point (3×10^6 T cells) because of cell density considerations. The response to *S. sanguis* appeared similar to that seen with *B. buccae*, but the curve was less dramatic. As with all activators used, there was no response with B cells alone, but with the addition of T cells there was a restoration of the IgM response to *S. sanguis* with a peak observed at 10^6 T cells added. The level then gradually declined until it was near control levels at 3×10^6 T cells added. The response to PWM appeared more dramatic than that seen with the bacteria. From a nonsignificant IgM level in B cells alone, there was a dramatic peak in the response with 5×10^5 T-cells added, and the response sharply declined upon the addition of 10^6 or more T cells. Although all individuals tested also demonstrated an optimal PWM response when 5×10^5 T cells were added, the sharp decline noted in this figure upon the addition of 10^6 or more T cells was not as dramatic in every experiment. Two individuals also showed significant antibody production upon the addition of 10^6 T cells and (consistent with Fig. 3) demonstrated suppression of immunoglobulin levels with the restoration of 2×10^6 to 3×10^6 T cells. A number of workers have noted similar findings with PWM (3, 23, 31).

IgG responses were evaluated by the same experimental protocol used for Fig. 3 (Fig. 4). The general patterns observed for IgM were also seen with IgG production, although a number of differences were apparent. The response to *Wolinella* HSV was restored from base-line levels with the addition of 5×10^5 T cells. However, the response did not peak until 2×10^6 T cells were added, and then it gradually declined. The *S. sanguis*-induced and PWM-induced IgG responses were significantly above the control level with 2.5×10^5 T cells added; however, the *S. sanguis* response did not peak until 10^6 T cells were restored to cultures. In contrast, the PWM response peaked sharply with 5×10^5 T cells and then gradually declined. The response was never totally inhibited, as was seen for IgM production.

In summary, these data demonstrate that both the IgM and IgG responses in cultures of normal B cells stimulated with bacterial isolates and PWM can be restored with the addition of T cells. Although PWM and bacterium-stimulated cultures could produce significant levels of immunoglobulin upon the addition of 5×10^6 T cells, there were clear differences in the T-cell requirement for optimal antibody production. PWM-induced IgG and IgM levels peaked with the addition of 5×10^5 T cells, whereas cultures stimulated with *S. sanguis*, *B. buccae*, and *Wolinella* HSV did not produce optimal antibody levels until greater numbers of T cells were added. This difference was more apparent for IgG production (Fig. 4) than for IgM production (Fig. 3).

Optimal T-cell-to-B-cell ratios for polyclonal activation by bacterial sonic extracts. To further dissect the T-cell requirements for the polyclonal response induced by the periodontal bacterial isolates, another series of experiments examined the T-cell-to-B-cell ratios required for an optimal response. One important charcteristic distinguishes these experiments from those shown in Fig. 3 and 4. In these experiments, the total cell density was maintained at a constant level (10^6 cells per ml). Therefore, these experiments could assess the optimal T-cell-to-B-cell ratios in our standard culture system (10^6 cells per culture), thus avoiding any nonspecific in vitro effects of varying the cell densities.

For these experiments, PBLs from healthy controls were obtained from whole blood by using LSM and were separated into B-cell-rich and T-cell-rich fractions with the SRBC rosetting procedure. B-cell-rich fractions contained approximately 30 to 42% monocytes and 60 to 70% B cells; T-cell-rich fractions contained 98% T cells and 2% contaminating non-SRBC-rosetting cells. These fractions were added back to cultures at different ratios, maintaining the total cell

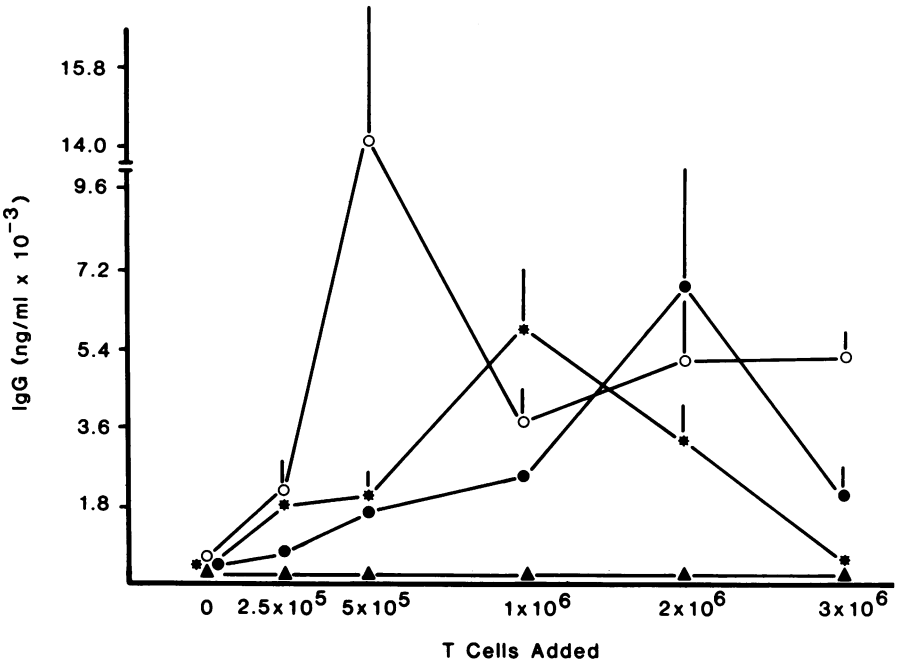

FIG. 4. Effect of restoring T cells to B-cell-enriched cultures on IgG production. Cultures were established as described in the legend to Fig. 3 and were stimulated with medium alone (▲), PWM (○), or sonic extracts of *Wolinella* HVS (●) or *S. sanguis* (✱). Supernatants were harvested and assayed for IgG by an RIA. Immunoglobulin levels at the optimal stimulatory dose for each experimental group from triplicate cultures were used for determination of the mean ± standard error.

concentration at 10^6 cells per ml. The T-cell-to-B-cell ratios were set up to provide increasing numbers of T cells; however, decreased numbers of B cells and macrophages were needed to maintain a constant cell number. The ratios of T cells to B cells were 1:1, 2:1, 3:1, and 7:1. After a 7-day activation, culture supernatants were harvested by centrifugation, and the quantities of IgM and IgG were determined by RIA. The results of a representative experiment (one of five individuals tested) measuring IgG production are presented in Fig. 5. Cultures stimulated with *Wolinella* HVS

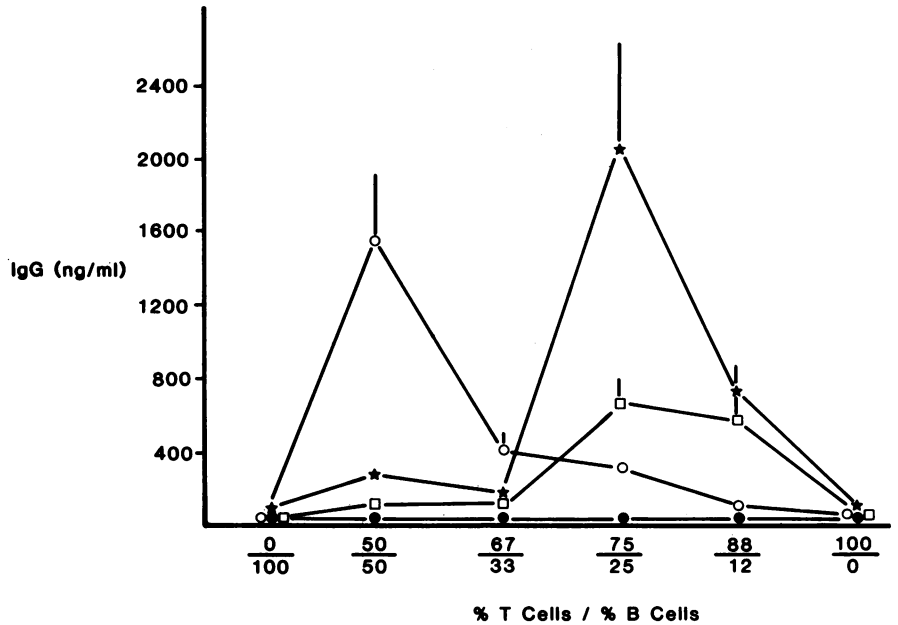

FIG. 5. Optimal T-cell-to-B-cell ratios for IgG production in response to PWM and bacterial sonic extracts. Unfractioned PBLs were separated into T-cell-rich and B-cell-rich fractions. T cells were restored to B-cell fractions maintaining a cell density of 10^6 cells per ml. Cultures were stimulated with medium alone (●), PWM (1:10 dilution; ○), or sonic extracts (50 and 200 μg/ml) of *Wolinella* HVS (★) or *S. sanguis* (□) and were incubated for 7 days. Supernatants were harvested and assessed for IgG by RIA. Immunoglobulin levels at the optimal stimulatory dose for each experimental group from triplicate cultures were used for determination of the mean ± standard error.

TABLE 2. Effect of T-cell depletion on antibody production

Stimulator[a]	Immunoglobulin produced (ng/ml) in cultures:			
	IgG		IgM	
	Unfractionated	T-cell depleted[b]	Unfractionated	T-cell depleted
Control	259 ± 67	93	37 ± 7	2 ± 1
PWM	1,042 ± 176	216 ± 21[a]	2,520 ± 367	18 ± 5[c]
F. nucleatum				
D30A-9	12,027 ± 547	149 ± 21[c]	4,560 ± 987	3 ± 1[c]
D43B-27	395 ± 72	74 ± 3[d]	30 ± 16	2 ± 1[d]
Wolinella HVS	4,000 ± 333	248 ± 44[c]	6,107 ± 539	7 ± 2[c]
B. buccae	3,168 ± 754	111 ± 37	1,875 ± 90	4 ± 1[c]
Bacteroides melaninogenicus subsp. *intermedius*	1,873 ± 378	96 ± 5[c]	8,213 ± 787	5 ± 1[c]
C. ochracea	723 ± 103	113 ± 22[c]	189 ± 31	3 ± 1
A. actinomycetemcomitans	707 ± 49	83 ± 7[c]	1,256 ± 22	5 ± 2[c]

[a] Stimulators were tested at three doses, and the maximum stimulatory dose is represented in each case.
[b] T-cell-depleted fractions were prepared by rosetting with SRBC and contained 2 to 3% rosette-positive cells.
[c] $P < 0.01$ (comparison with unfractionated cultures; Student t test).
[d] $0.05 > P > 0.01$ (comparison with unfractionated cultures; Student t test).

showed peak responses ($P < 0.01$) at the 3:1 T-cell-to-B-cell ratio, whereas cultures stimulated with *S. sanguis* peaked at ratios of 3:1 or 7:1. Neither *Wolinella* HVS nor *S. sanguis* stimulated significant IgG production at ratios of 1:1 or 2:1. In contrast, PWM cultures showed a distinct difference in the optimal T-cell-to-B-cell ratios. There was a sharp peak in IgG production at the 1:1 ratio, and the peak declined until it was nonsignificant at the 7:1 ratio. All individuals tested demonstrated similar patterns of response to both PWM and periodontal isolates. Therefore, the optimal T-cell-to-B-cell ratio for PWM-driven IgG synthesis was found to be 1:1 (2:1 in a few individuals). In contrast, the optimal IgG level in response to stimulation by the bacterial sonic extracts was observed at 3:1 or 7:1. Several investigators (3, 31) have proposed that the optimal responsiveness of PWM at a 1:1 ratio coupled with a declining response at higher ratios of T cells to B cells indicates that PWM induces greater T-suppressor cell activity.

Effect of irradiation on optimal T-cell-to-B-cell ratios. To examine more directly the possible role of T-suppressor cells and to further define the T-cell helper requirement for B-cell responses to periodontal bacterial sonicates, purified T lymphocytes were irradiated with 1,500 rads and added back to purified B-cell-rich fractions. Irradiation was used because a number of investigators have reported that T-suppressor cell function can be differentiated from T-helper cell function by a sensitivity to ionizing radiation (9, 23, 24). The experimental design was identical to the protocol previously described (Fig. 5), except that all T cells were irradiated. The IgG response of reconstituted cultures with irradiated T cells is shown in Fig. 6. The amount of IgG produced was increased when the cultures were restored with irradiated T cells at all T-cell-to-B-cell ratios tested. With the addition of irradiated T cells, the optimal ratio for the PWM-stimulated IgG response shifted to 2:1. This has been found to be true for all individuals tested. In addition, from the peak observed at the 2:1 ratio, the response curve decreased very gradually, so that at a 3:1 ratio a significant PWM IgG response remained (3,819 ng/ml). In contrast, the response to PWM at the 3:1 ratio with normal T cells was 334 ng/ml (Fig. 5). The optimal T lymphocyte ratios for *S. sanguis* and *Wolinella* HVS responses remained the same with irradiated and with normal T cells (Fig. 6). In response to all of the activators tested, the absolute amount of IgG increased with the addition of irradiated T cells. The PWM optimal response shifted from a T-cell-to-B-cell ratio of 1:1 to 2:1. In contrast, the optimal response to both bacterial sonic extracts remained at the same ratio. It should be emphasized that with all activators, irradiated T cells were able to restore the immunoglobulin response, indicating that T-cell division was not required for the provision of T-cell help. A similar pattern was also observed for IgM production in the majority of individuals tested (data not shown).

Regulatory effects of T-cell subpopulations upon polyclonal antibody responses to bacterial sonic extracts. To assess the regulatory influences of T lymphocytes more directly, the role of T-cell subpopulations as defined by the OKT monoclonal reagents was examined. The manual cell sorting or panning technique was used for these experiments. PBLs from three normal individuals were separated into B-cell-rich and T-cell-rich fractions by the AET-SRBC rosetting procedure. T cells were then separated into T-helper/inducer ($OKT4^+$) and T-suppressor/cytotoxic ($OKT8^+$) populations after being mixed with the appropriate monoclonal antibody and binding the antigen-positive cells to petri plates coated with goat anti-mouse immunoglobulin. Cultures were established in which each T-cell population was depleted of either T-helper or T-suppressor cells, and the remaining T cells were added back to B cells at the optimal T-cell-to-B-cell ratio for PWM (1:1) or bacterial sonic extracts (3:1). In addition, T-suppressor cells ($OKT8^+$) were added back to cultures depleted of this population at physiological propor-

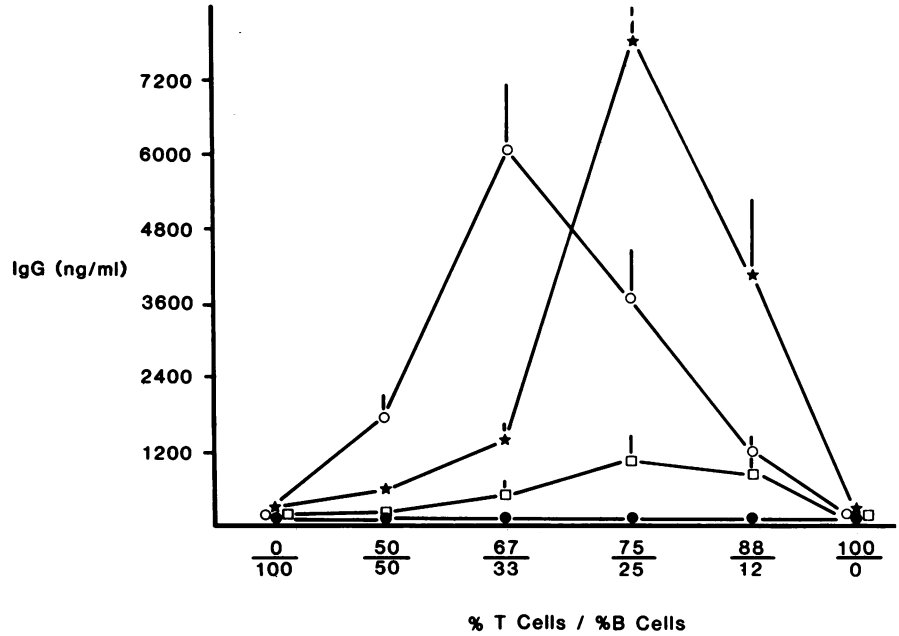

FIG. 6. Effect of irradiation on the optimal T-cell-to-B-cell ratios for IgG production. Cultures were established as described in the legend to Fig. 5. with irradiated T cells and were stimulated with medium alone (●) or with PWM (○) or sonic extracts of *Wolinella* HVS (★) or *S. sanguis* (□). Supernatants were harvested and assessed for IgG by an RIA. Immunoglobulin levels at the optimal stimulatory dose for each experimental group from triplicate cultures were used for determination of the mean ± standard error.

tions (35% of T cells). Figures 7 and 8 present the data produced for the IgM and IgG responses, respectively, when these experimental protocols were used. The data demonstrate that the PWM-stimulated IgM response (Fig. 7) was significantly depressed after depletion of $OKT4^+$ helper T cells when compared with the unseparated and B-cell-plus-T-cell reconstituted cultures. With the depletion of T-suppressor cells ($OKT8^+$), the response dramatically increased; however, PWM-induced immunoglobulin production was suppressed after reconstitution of the cultures with $OKT8^+$ cells. Cultures stimulated with *Wolinella* HVS and *S. sanguis* cultures demonstrated a similar pattern of responsiveness. After the depletion of $OKT4^+$ T cells, the response was significantly decreased to below that seen in the unseparated and B cell plus T cell reconstituted cultures. Upon removal of $OKT8^+$ T cells, the response to both bacterial sonic extracts dramatically increased. This significant increase was ablated after the restoration of $OKT8^+$ cells to the culture. Therefore, both PWM and bacterial cultures were significantly modulated by T-cell subpopulations. The PWM-stimulated response in the OKT8-depleted cultures was significantly greater than both *Wolinella* HSV-induced and *S. sanguis*-induced responses, but this was not seen in other cultures. This may suggest that the PWM response was more susceptible to T-suppressor cell effects than the cultures stimulated with bacteria.

The trends seen in the IgM responses are repeated in the IgG responses (Fig. 8). With the depletion of T-helper cells there was a decrease in the responses to PWM, *Wolinella* HSV, and *S. sanguis*. Upon depletion of $OKT8^+$ cells, all three activators demonstrated a significant increase in antibody levels. However, there was no difference between the effect on the PWM response compared with bacterial stimulation as was observed in the IgM response (Fig. 7). After restoration of the cultures with $OKT8^+$, all responses decreased, although the decreases seen in the PWM and *Wolinella* HVS responses were not statistically significant when compared with the OKT8-depleted cultures.

DISCUSSION

The presence of significant levels of IgM and IgG in culture supernatants of PBLs from normal individuals stimulated in vitro with periodontal sonic extracts lends further strength to the polyclonal B-cell activating properties of oral bacteria isolated from periodontally affected sites. Six gram-negative and two gram-positve isolates were tested, and with one exception, all stimulated significant levels of both IgM and IgG isotypes. IgM levels in cultures stimulated with bacterial sonic extracts varied from 441 to 2,238 ng/ml, depending upon the organism used, whereas IgG levels ranged from 1,038 to 5,380 ng/ml. PWM-stimulated IgM production demonstrated a mean level of 2,850 ng/ml; PWM-induced IgG levels averaged 6,850 ng/ml. Thus, the most potent bacterial isolates produced levels of immunoglobulin comparable to those stimulated by the well-known polyclonal B-cell activator, PWM. It must be emphasized that the levels of IgG and IgM stimulated by PWM were significantly greater than has been reported by other investigators using comparable doses. Waldmann and Broder (31) have obtained mean IgG levels of 1,380 ng/ml in 7-day cultures, as compared with 6,850 ng/ml in our system after 7 days. Our levels were greater than the quantity of IgG these workers obtained after a 21-day culture period. The mean IgM levels for 7-day cultures in our system was 2,850 ng/ml, whereas Waldmann and Broder reported 2,344 ng/ml. In addition, PWM-activated B cells in this study produced greater levels of IgG than Mangan and Lopatin (17) have reported. Clearly, our culturing system provided optimal conditions for PWM antibody production. Since we have observed suppression of IgM and IgG production by monocytes present in our PBL fractions (4), the quantities of immunoglobulin most likely would have

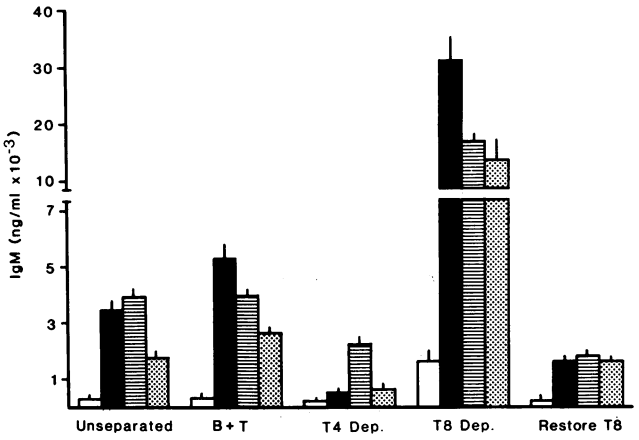

FIG. 7. Effect of altering subpopulations of human T cells on IgM production. Human PBLs were rosetted with AET-SRBCs and were separated into B-cell-rich and T-cell-rich fractions. The T cells were further separated by panning into $OKT4^+$ and $OKT8^+$ populations. Experimental groups were set up in which $OKT4^+$ and $OKT8^+$ cells were depleted. In addition, $OKT8^+$ cells were restored to T-cell populations depleted of $OKT8^+$ cells. Cultures were set up at the optimal T-cell-to-B-cell ratios for PWM (1:1) and bacterial sonic extracts (3:1) and were stimulated with medium alone (□) or with PWM (1:200 dilution; ■) or sonic extracts (50 and 200 μg/ml) of *Wolinella* HVS (▤) or *S. sanguis* (▦). After a 7-day incubation, supernatants were harvested and assayed for IgM by RIA. The data are from a representative experiment from one individual. Three individuals were tested, and all gave similar results. Immunoglobulin levels at the optimal stimulatory dose for each experimental group from triplicate cultures were used for the determination of the mean ± standard error.

been higher if monocytes had been depleted from all PBLs before culturing. Mangan and Lopatin (17) have utilized this technique and have achieved greater levels of IgG and IgM in monocyte-depleted cultures stimulated with *A. viscosus*. Thus, when we consider that a culturing system was utilized in which PWM immunoglobulin systhesis was optimized but conditions for bacterial cultures were not optimal (though adequate), it becomes even more striking that a number of bacterial isolates stimulated IgM and IgG production very close to the levels stimulated by PWM. Thus, the significant levels of polyclonal IgG and IgM coupled with the highly significant plaque-forming cell responses induced by these bacterial isolates (2, 5) strengthen the finding that periodontal organisms are potent stimulators of polyclonal B-cell activation.

After confirmation of the polyclonal B-cell activating ability of bacterial sonic extracts, studies were designed to assess the T-cell regulation of this B-cell activation. Both IgG and IgM synthesis were found to be clearly T cell dependent. This was consistent with the literature since very few PBAs have been shown to be independent of T cells in humans (10, 31). The requirement for T-helper function for IgG production was clearly demonstrated. All cultures showed a restoration of immunoglobulin synthesis when T cells were added to B cells alone, and T-cell help was radiation resistant. There were differences seen in the T-cell requirements for cultures stimulated by the bacteria and those activated with PWM. PWM-induced IgG synthesis was restored to optimal levels upon the addition of 5×10^6 T cells (a 1:1 ratio) and gradually declined as more T cells were added. In contrast, *B. buccae*-stimulated cultures were optimally restored with 10^6 T cells, and *S. sanguis*-activated cultures did not produce optimal levels of IgG until 2×10^6 T cells were added. Therefore, cultures activated with bacterial sonic extracts required a greater number of T cells than PWM-stimulated PBLs did, indicating a greater T-cell helper requirement. These data correlated well with the experiments in which optimal T-cell-to-B-cell ratios at a constant cell culture density were determined. Here again, the PWM response required fewer T lymphocytes than were needed in cultures stimulated with the periodontopathic organisms. PWM synthesis peaked at a 1:1 ratio, whereas bacterial cultures did not peak until ratios of 3:1 (*Wolinella* HVS) or 7:1 (*S. sanguis*) were reached. Factors other than an increased T-cell helper requirement may explain the differences between PWM and bacterial responses. As alluded to earlier, the number of B cells and monocytes decreased as the number of T cells increased. Considering the clearly different regulatory role of monocytes in this system, the decreased number of monocytes in cultures reconstituted to a 3:1 or 7:1 T-cell-to-B-cell ratio may have provided the optimal milieu for significant B-cell activation. However, if the data are considered together, a differential T-cell helper requirement would seem more likely. Mangan and Lopatin (17) have also demonstrated an increased T-cell helper requirement in cultures stimulated by *A. viscosus* as compared with PWM-activated PBLs. Confirmation of an increased T-helper cell requirement was not observed in the T-cell subset experiments with a single T-cell-to-B-cell ratio because no differential requirement for the $OKT4^+$ population was seen in the cultures stimulated by *Wolinella* HSV or *S. sanguis*. Further experiments are needed to demonstrate an increased T-cell helper requirement by periodontal isolates.

A number of investigators have suggested that PWM induces the generation of T-suppressor cells (3, 31) and the data presented here lend further support to this suggestion. The majority of individuals tested produced optimal quantities of immunoglobulin upon PWM activation when the T-cell-to-B-cell ratio for IgG was 1:1. As the number of T-cells

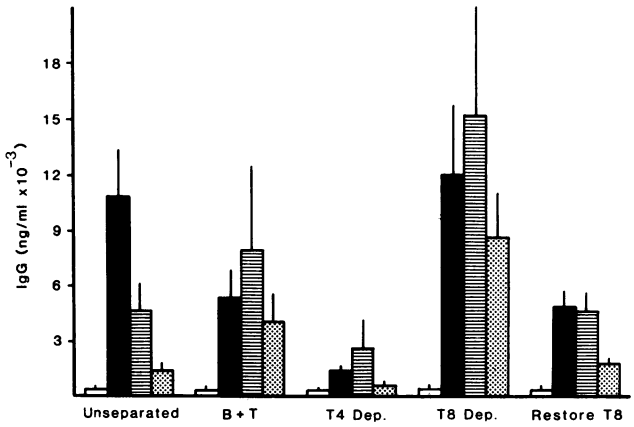

FIG. 8. Effect of altering subpopulations of human T cells on IgG production. Cultures were established as described in the legend to Fig. 7. Cultures were stimulated with medium alone (□), PWM (■), or sonic extracts of *Wolinella* HVS (▤) or *S. sanguis* (▦). Supernatants were harvested and assayed for IgG by RIA. The data are from a representative experiment from one individual. Three individuals were tested and gave similar results. Immunoglobulin levels at the optimal stimulatory dose for each experimental group from triplicate cultures were used for the determination of the mean ± standard error.

was increased, immunoglobulin production decreased. Upon the depletion of T-suppressor cells by irradiation, the optimal T-cell-to-B-cell ratio increased to 2:1, suggesting a significant role for T-suppressor cells. In cultures stimulated by periodontal isolates, the optimal immunoglobulin response for IgG was observed at the 3:1 or 7:1 T-cell-to-B-cell ratios indicating that more T cells provided an optimal response. When T cells were irradiated to remove T-suppressor cell activity, the optimal ratio for IgG production did not change. These experiments suggested that immunoglobulin production in PBL cultures stimulated with the gram-negative or gram-positive periodontal sonic extracts was not as susceptible to the effects of T-suppressor cells as antibody production in cultures activated with PWM. Suppressor cells, however, appeared to mediate an effect on the bacterium-stimulated responses because the level of immunoglobulin produced at all ratios for both bacteria and PWM was significantly increased after T-cell irradiation. In examining the T-suppressor cell subset directly by the panning technique, no difference was seen in regulation by the $OKT8^+$ population in cultures stimulated by PWM and in the bacterial extracts. These experiments do not exclude a differential regulation since any fine differences in T-suppressor cell requirements may not have been appreciated in examining the $OKT8^+$ population as a whole.

In summary, the present data indicate that T cells have an important modulatory role in the regulation of immunoglobulin synthesis stimulated by both periodontal isolates and PWM. PBLs stimulated with periodontal isolates required more T-helper cells than did PBLs stimulated with PWM. This was more apparent for production of IgG than of IgM and was consistent with the literature since IgG responses are generally more T cell dependent than IgM production is (22). The mechanism for the greater T-cell requirement is not known. It could be postulated that PWM, a known polyclonal T-cell activator, stimulates a greater number of T lymphocytes, thus necessitating the addition of fewer T cells. In addition, it is possible that different B-cell subpopulations (having different T-helper cell requirements) are preferentially activated by PWM and the bacterial sonic extracts. In support of this, two distinct B-cell subpopulations have been described in mice which differ in their T-helper cell interactions and genetic restrictions (25). Moreover, the differences between bacterial sonic extract and PWM in regard to T-cell regulation may simply reflect a different balance between suppressor and helper influences. Fauci and Haynes (7) have emphasized that the response to PWM in cultures of normal human PBLs is dependent upon complex regulatory interactions involving various lymphocyte subpopulations. The periodontal isolates may simply require a greater number of T-helper cells coupled with a decreased susceptibility to suppressor-cell activity.

The present study has provided additional support for the theory that the potent B-cell activation is induced by periodontal isolates. Significant levels of both IgG and IgM have been observed in culture supernatants from PBLs stimulated with selected bacterial sonic extracts. IgG produced after stimulation with *A. actinomycetemcomitans* was proven to be polyclonal since less than 10% was specific for the stimulating organism. The role of T cells was explored, and they were found to exert crucial regulatory effects on the B-cell activation observed. In fact, the modulatory function of T lymphocytes may be the important regulator of the polyclonal B-cell activation induced by these periodontal isolates. Further studies are required to define in greater detail the interaction of T-cell subsets in this response.

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LITERATURE CITED

1. **Baker, J. J., S. P. Chen, S. S. Socransky, J. J. Oppenheim, and S. E. Mergenhagen.** 1976. Importance of *Actinomyces* and certain gram-negative anaerobic organisms in the transformation of lymphocytes. Infect. Immun. **13**:1363–1368.
2. **Bick, P. H., A. B. Carpenter, L. V. Holdeman, G. A. Miller, R. R. Ranney, K. G. Palcanis, and J. G. Tew.** 1981. Polyclonal B-cell activation induced by extracts of gram-negative bacteria isolated from periodontally diseased sites. Infect. Immun. **34**:43–49.
3. **Broder, S., R. L. Edelson, M. A. Lutzner, D. L. Nelson, R. P. MacDermott, M. E. Drum, C. K. Goldman, B. D. Meade, and T. A. Waldmann.** 1976. The Sezary syndrome. A malignant proliferation of helper T-cells. J. Clin. Invest. **58**:1297–1306.
4. **Carpenter, A. B., E. C. Sully, K. G. Palcanis, and P. H. Bick.** 1982. Role of monocytes in polyclonal immunoglobulin production stimulated by sonicates of periodontally associated bacteria. Infect. Immun. **42**:853–862.
5. **Donaldson, S. L., P. H. Bick, W. E. C. Moore, R. R. Ranney, J. A. Burmeister, and J. G. Tew.** 1982. Polyclonal B-cell activating capacities of gram-positive bacteria frequently isolated from periodontally diseased sites. J. Periodontal Res. **17**:569–575.
6. **Donaldson, S. L., R. R. Ranney, J. A. Burmeister, and J. G. Tew.** 1981. Blastogenic responses by lymphocytes from periodontally healthy populations induced by periodontitis-associated bacteria. J. Periodontal. **53**:743-751.
7. **Fauci, A. S., and B. F. Haynes.** 1979. Pokeweed mitogen-induced PFC responses of human peripheral blood lymphocytes: regulation of B-cell triggering, p. 17–34. *In* A. S. Fauci and R. Ballieux (ed.), Antibody production in man. In vitro synthesis and clinical implications. Academic Press, Inc., New York.
8. **Fauci, A. S., and K. R. Pratt.** 1976. Activation of human B-lymphocytes. 1. Direct plaque-forming cell assay for the measurement of polyclonal activation and antigenic stimulation of human B-lymphocytes. J. Exp. Med. **144**:674–684.
9. **Fauci, A. S., K. R. Pratt, and G. Whalen.** 1978. Activation of human B. lymphocytes. VIII. Differential radiosensitivity of subpopulations of lymphoid cells involved in the polyclonally induced PFC responses of peripheral blood B Lymphocytes. Immunology **35**:715–720.
10. **Geha, R. S.** 1979. Regulation of human B-cell activation. Immunol. Rev. **45**:275–305.
11. **Kaplan, M. E., and C. Clark.** 1974. An improved rosetting assay for detection of human T-lymphocytes. J. Immunol. Methods **5**:131–135.
12. **Kiger, R., W. H. Wright, and H. Creamer.** 1974. The significance of lymphocyte transformation to various stimulants. J. Periodontol. **45**:780–785.
13. **Kotzin, B. L., C. J. Benike, and E. G. Engleman.** 1981. Induction of immunoglobulin-secreting cells in the allogeneic mixed leukocyte reaction: regulation by helper and suppressor lymphocyte subsets in man. J. Immunol. **127**:931–935.
14. **Lang, C. A.** 1958. Simple microdetermination of Kjeldahl nitrogen in biologic materials. Anal. Chem. **30**:1692–1694.
15. **Mackler, B. F., T. C. Waldrop, P. Schur, P. B. Robertson, and B. M. Levy.** 1978. IgG subclasses in human periodontal disease. I. Distribution and incidence of IgG subclass bearing lymphocytes and plasma cells. J. Periodontal Res. **13**:109–119.
16. **Mangan, D. F., B. E. Laughon, B. Bower, and D. E. Lopatin.** 1982. In vitro lymphocyte blastogenic responses and titers of humoral antibodies from periodontitis patients to oral spirochete isolates. Infect. Immun. **37**:445–451.
17. **Mangan, D. F., and D. E. Lopatin.** 1981. In vitro stimulation of

immunoglobulin production from human peripheral blood lymphocytes by a soluble preparation of *Actinomyces viscosus*. Infect. Immun. **31**:236–244.

18. **Moore, W. E. C., R. R. Ranney, and L. V. Holdeman.** 1982. Subgingival microflora in periodontal disease: cultural studies, p. 13–26. *In* R. J. Genco and S. E. Mergenhagen (ed.), Host-parasite interactions in periodontal diseases. American Society for Microbiology, Washington, D.C.
19. **Page, R. C., and H. E. Schroeder.** 1981. Current status of the response in chronic marginal periodontitis. J. Periodontol. **52**:477–491.
20. **Ranney, R. R., B. F. Debski, and J. G. Tew.** 1981. Pathogenesis of gingivitis and periodontal disease in children and young adults. Pediatr. Dent. **3**:89–100.
21. **Ranney, R. R., N. R. Yanni, J. A. Burmeister, and J. G. Tew.** 1982. Relationship between attachment loss and precipitating serum antibody to *Actinobacillus actinomycetemcomitans* in adolescents and young adults having severe periodontal destruction. J. Periodontol. **53**:1–7.
22. **Rosenberg. Y. J.** 1982. Isotype-specific T-cell regulation of immunoglobulin expression. Immunol. Rev. **67**:33–58.
23. **Saxon, A., H. Stevens, and R. F. Ashman.** 1977. Regulation of immunoglobulin production in human peripheral blood leukocytes: cellular interactions. J. Immunol. **118**:1872–1879.
24. **Siegal, F. 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**:642–647.
25. **Singer, A., Y. Asano, M. Shigeta, K. S. Hathcock, A. Ahmed, C. G. Fathman, and R. J. Hodes.** 1982. Distinct B-cell subpopulations differ in their genetic requirements for activation by T-helper cells. Immunol. Rev. **64**:137–160.
26. **Smith, S., P. H. Bick, G. A. Miller, R. R. Ranney, P. L. Rice, G. H. Lalor, and J. G. Tew.** 1980. Polyclonal B-cell activation: severe periodontal disease in young adults. Clin. Immunol. Immunopathol. **16**:354–366.
27. **Socransky, S. S.** 1977. Microbiology of periodontal disease. Present status and future considerations. J. Periodontol. **48**:497–504.
28. **Socransky, S. S., A. C. R. Tanner, A. D. Haffajee, J. D. Hillman, and J. M. Goodson.** 1982. Present status of studies on the microbial etiology of periodontal diseases, p. 1–12. *In* R. J. Genco and S. E. Mergenhagen (ed.), Host-parasite interactions in periodontal diseases. American Society for Microbiology, Washington, D.C.
29. **Tew, J. G., G. S. Miller, E. J. Greene, P. L. Rice, W. P. Gordon, K. G. Palcanis, and R. R. Ranney.** 1981. Immunological studies of young adults with severe periodontitis. II. Cellular factors. J. Periodontal Res. **16**:403–416.
30. **Tew, J. G., R. R. Ranney, and S. L. Donaldson.** 1982. Blastogenic responsiveness in periodontally healthy subjects. Evidence for mitogenic activity in oral bacteria. J. Periodontal Res. **17**:466–468.
31. **Waldmann, T. A., and S. Broder.** 1982. Polyclonal B-cell activators in the study of the regulation of immunoglobulin synthesis in the human system. Adv. Immunol. **32**:1–63.
32. **Wysoki, L. G., and V. L. Sato.** 1978. Panning for lymphocytes: a method for cell selection. Proc. Natl. Acad. Sci. USA. **75**:2844–2848.