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Influence of proinflammatory cytokines on *Actinobacillus actinomycetemcomitans* specific IgG responses

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Objective: High levels of serum anti-*Actinobacillus actinomycetemcomitans* immunoglobulin G (IgG) correlate with reduced extent and severity of periodontal disease and the present study was undertaken to begin testing the hypothesis that proinflammatory cytokines are important in the induction of optimal anti-*A. actinomycetemcomitans* IgG responses.

Background: Studies with pokeweed mitogen indicate that interleukin-1α (IL-1α) and IL-1β are necessary for optimal IgG1 and IgG2 production and that prostaglandin E_2 (PGE_2) and interferon-γ (IFN-γ) selectively promote IgG2, which is a major component of the anti-*A. actinomycetemcomitans* response *in vivo*. The pokeweed mitogen results suggest that these proinflammatory cytokines would also be necessary for optimal production of IgG specific for *A. actinomycetemcomitans*.

Methods: Peripheral blood mononuclear cells from *A. actinomycetemcomitans*-seropositive subjects with localized aggressive periodontitis were stimulated with *A. actinomycetemcomitans* in immune complexes capable of binding follicular dendritic cells that participate in the induction of recall responses *in vivo*. Cultures were manipulated with anti-IL-1α, anti-IL-1β, anti-IFN-γ, anti-IL-12, anti-CD21, indomethacin, and PGE_2. *Actinobacillus actinomycetemcomitans* specific IgG production was monitored by enzyme-linked immunosorbent assay (ELISA).

Results: Addition of follicular dendritic cells to peripheral blood mononuclear cells cultures resulted in follicular dendritic cell-lymphocyte clusters and increased anti-*A. actinomycetemcomitans* IgG responses (3–40-fold increases) compared with controls lacking follicular dendritic cells. Anti-IL-1α, anti-IL-1β, anti-IFN-γ, anti-IL-12, anti-CD21 and indomethacin suppressed anti-*A. actinomycetemcomitans* IgG production by half or more. PGE_2 restored IgG responses suppressed by indomethacin.

Conclusions: The cytokines IL-1α, IL-1β, IFN-γ, IL-12, and PGE_2 were all necessary for optimal production of human anti-*A. actinomycetemcomitans and* the need for proinflammatory cytokines including the T helper 1 (Th1) cytokines is consistent with a response with a significant IgG2 component.

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High titers of serum antibody reactive with the serotype specific antigen of *Actinobacillus actinomycetemcomitans* serotype b correlate with reduced extent and severity of disease in early onset periodontitis patients (1, 2). Wilson and Hamilton first reported studies of the subclass distribution of IgG reactive with the serotype specific antigen of *A. actinomycetemcomitans* serotype b and indicated that the geometric mean of high-titer localized aggressive periodontitis sera was 136.5 μg/ml of immunoglobulin G2 (IgG2) vs. only 7.8 μg/ml of IgG1 (3). We reported a similar dominance of IgG2 and found that the dominant antigen was the serotype specific antigen (4). Ling *et al*. also analyzed the immunoglobulin subclass response to *A. actinomycetemcomitans* in patients and seropositive controls and also concluded that IgG2 was dominant (5). The reasons for the skewed IgG2 response to *A. actinomycetemcomitans* serotype b are not clear, but IgG2 is known to be a T helper 1 (Th1) subclass immunoglobulin dependent on interferon-γ (IFN-γ) (6).

A number of studies of IgG production have been done with peripheral blood mononuclear cells from localized aggressive periodontitis patients using pokeweed mitogen as a non-specific B cell activator. Pokeweed mitogen has important advantages over specific antigen for studies of IgG production *in vitro*. For example, monocytes or dendritic cells from one subject and T and B cells from other subjects can be used without concern for major histocompatability complex differences (7). In addition, the level of total IgG produced in polyclonal responses is high compared to antigen-specific responses and this makes IgG assays and analyses easier. It was also found that localized aggressive periodontitis peripheral blood mononuclear cells produced elevated levels of IgG2 and this corresponds with elevated serum IgG2 levels including high levels of IgG2 reactive with *A. actinomycetemcomitans* in localized aggressive periodontitis subjects (7, 8). Recent studies indicate localized aggressive periodontitis monocytes tend to become dendritic cells and these dendritic cells appear to promote elevated IgG2 production, which is dependent on Th1 cytokines (9). It was also discovered that both interleukin-1α (IL-1α) and IL-1β are necessary for optimal IgG1 and IgG2 responses and that prostaglandin E_2 (PGE_2) and IFN-γ are necessary for optimal IgG2 production (10–12). Nevertheless, it is obvious that pokeweed mitogen does not model antigen presentation or the numerous cell–cell interactions taking place in germinal centers of secondary lymphoid tissues where recall responses are initiated (13), prompting questions about the relevance of the pokeweed mitogen results. Accordingly, the present study was undertaken using *A. actinomycetemcomitans* as the relevant antigen for the localized aggressive periodontitis patients. IgG2 is a critical component of the anti-*A. actinomycetemcomitans* response in localized aggressive periodontitis patients, prompting the hypothesis that the proinflammatory cytokines including the Th1 cytokines would be critical for induction of optimal IgG specific for *A. actinomycetemcomitans*.

Specific IgG responses in cultures of human peripheral blood mononuclear cells are seldom impressive and results are often reported as antibody forming cells where small amounts of antibody produced by single cells are detected. The low levels of specific IgG induced by oral pathogens, including *A. actinomycetemcomitans*, in peripheral blood mononuclear cells cultures has made studies of the regulation of such responses very challenging (14). We reasoned that use of follicular dendritic cells might facilitate *in vitro* studies of IgG responses against oral microorganisms by enhancing specific responses. Upon *in vivo* challenge for a recall response, antibody persisting from prior immunization(s) almost instantaneously converts the immunogen into antigen–antibody complexes. These immune complexes are transported to germinal centers where they are trapped by follicular dendritic cells (15, 16). The immune complexes activate the complement pathway and C3b and C4b fragments covalently bind to follicular dendritic cells through thio ester linkages and the immune complex bearing follicular dendritic cells label intensely with antibodies reactive with these complement components (17, 18). The C3b component on follicular dendritic cells degrades to become iC3b, C3d or C3dg and these fragments are ligands (CD21L) for complement receptor 2 or CD21. The follicular dendritic cell–immune complexes provoke potent recall responses and the production of memory B cells (19, 20). Engagement of CD21 in the B cell coreceptor complex by the complement-derived follicular dendritic cell–CD21L delivers a critical signal that dramatically augments signals delivered by engagement of B cell antigen receptor by antigen (19, 21). In addition, binding Ig-Fc in antigen–antibody complexes by follicular dendritic cell-*Fc*γRII decreases immunoreceptor tyrosine-based inhibition motif signaling in B cells, which may occur if immune complexes are permitted to cross-link B cell antigen receptor and B cell-*Fc*γRII. Thus, follicular dendritic cells minimize a negative signal to the B cell (19, 22). Follicular dendritic cells also protect B cells from apoptotic death and selectively stimulate B cells bearing high affinity receptors (23). None of these follicular dendritic cell–B cell interactions are major histocompatability complex or species restricted and this allows use of readily available murine follicular dendritic cells with human peripheral blood mononuclear cells to model the germinal center microenvironment (19, 24). In culture, lymphocytes cluster around the follicular dendritic cells and create a germinal center-like environment with specific B cells, immune complex bearing follicular dendritic cells, and specific T cells (25, 26). Follicular dendritic cells in these clusters provide antigen to engage B cell antigen receptor, and CD21L to engage CD21 in the B cell coreceptor complex, and antigen on *i*mmune *c*omplex *co*ated bodies (iccosomes) for B cells to process and present to obtain the necessary T cell help (19).

The results indicate that use of follicular dendritic cells to help recreate the germinal center microenvironment substantially enhanced anti-*A. actinomycetemcomitans* IgG responses. Furthermore, PGE_2, IL-1α, IL-1β, IL-12, and IFN-γ were all necessary for

optimal production of IgG anti-*A. actinomycetemcomitans* confirming and extending results obtained using pokeweed mitogen as a non-specific B cell activator.

Material and methods

Clinical subjects

This study was approved by the Institutional Review Board of Virginia Commonwealth University. Subjects with a history of localized aggressive periodontitis were obtained by the Clinical Research Center for Periodontal Disease, School of Dentistry, Virginia Commonwealth University Richmond, Virginia. These patients were less than 35 years old and had the localized pattern of severe periodontal destruction limited to the first molar or incisor teeth and up to two additional teeth.

Lymphocyte separation

Peripheral blood mononuclear cells were obtained from heparinized blood by density centrifugation using Lymphocyte Separation Medium (ICN, Aurora, OH, USA). Cells were centrifuged at 400 *g* for 20 min. Cells were collected from the interface, and washed three times in RPMI-1640 media (Cellgro, Herndon, VA, USA). After washing, the cells were suspended in RPMI-1640 supplemented with 10% of fetal calf serum and antibiotics for cell culture.

Antigen

Actinobacillus actinonomycetemcomitans serotype b ATCC 43718 (previously designated strain Y4) was obtained from the ATCC and was used in this study. The organism was grown to log phase in brain heart infusion medium (Difco Laboratories, Detroit, MI, USA) at 37°C in a 5% CO_2 atmosphere. The bacteria were frozen and stored at 1×10^8 organisms/ml and used in culture at 10^7/ml in most experiments. Immune complexes were made by adding 1 μg *A. actinonomycetemcomitans* specific serum antibody to 10^7 bacteria. To decorate *A. actinomycetemcomitans* with complement fragments, bacteria were incubated in fresh serum from a seronegative subject overnight at 37°C to facilitate lipopolysaccharide-mediated activation of the alternative complement pathway.

Follicular dendritic cell isolation

BALB/c mice at 2–5 months of age were used and the mice were handled in accordance with Virginia Commonwealth University Animal Care and Use guidelines. Follicular dendritic cells were isolated from lymph nodes (brachial, axillary, inguinal, popliteal, mesenteric, and paraaortic) as previously described (25, 27). In brief, 3 days before follicular dendritic cell isolation, the mice were exposed to whole body irradiation (600 rad, Cesium^{137} source). The irradiation does not interfere with follicular dendritic cell accessory functions, but eliminates many of the B and T cells, which otherwise form clusters with follicular dendritic cells and decrease the follicular dendritic cell purity. Each lymph node capsule was opened using two 26-gauge needles, then the lymph nodes were placed in an enzymatic cocktail consisting of 1 ml of collagenase type VII (1 mg/ml, C-0773, Sigma, St Louis, MO, USA), 0.5 ml of DNaseI (5000 U/ml, D-4527, Sigma), and 0.5 ml RPMI-1640 supplemented with 20 mM HEPES, 2 mM glutamine, and 50 μg/ml Gentamicin. After 30 min at 37°C in a CO_2 incubator, the medium and released cells were removed and transferred to a 15-ml conical centrifuge tube containing 5 ml of RPMI-1640 with 20% fetal calf serum and placed at 4°C. The remaining tissue was subjected to a second 30 min digestion in a fresh aliquot of enzymes and the cells were collected as before. Isolated cells were centrifuged for 10 min at 400 *g* and the resulting cell pellet was gently resuspended to $1–2 \times 10^8$ cells/ml in RPMI-1640. Then 2 ml of this cell suspension was gently layered on a continuous 50% Percoll gradient and centrifuged for 25 min at 700 *g*. The low density (1.060–1.065 g/ml) fraction of cells containing the follicular dendritic cell was collected, washed by centrifugation at 400 *g*, and suspended in RPMI-1640 with 10% fetal calf serum then incubated at 37°C for 1 h in a small tissue culture dish (No. 3035, Costar, Cambridge, MA, USA) to deplete adherent cells. After incubation, the nonadherent cells containing 20–40% follicular dendritic cells were collected, washed, and suspended in RPMI-1640 with 10% fetal calf serum.

Cell culture

Human peripheral blood mononuclear cells were suspended at 10^6/ml in RPMI-1640 (Cellgro) supplemented with 10% heat-inactivated fetal calf serum (HyClone, Logan, UT, USA), 2 mM glutamine, and gentamycin 50 μg/ml (Gibco, Grand Island, NY, USA), cultured in 75-mm tubes (Falcon, Franklin Lakes, NJ) in a volume of 1 ml/tube and incubated at 37°C in a humidified atmosphere of 5% CO_2. In some cultures, follicular dendritic cells were added to a concentration of 2×10^5/culture and each experimental group was run in triplicate. After 7 days, the media were changed and the cultures were incubated for an additional 7 days when the supernatant fluids were collected after removing the cells by centrifugation at 400 *g* for 10 min. These fluids were assayed for *A. actinonomycetemcomitans* specific IgG and the values for the three cultures were used for statistical analysis. Reagents used to inhibit IgG production were added at the beginning of the peripheral blood mononuclear cells cultures. The monoclonal mouse anti-human IL-1α was obtained from Genzyme (Cambridge, MA, USA: catalog no. 1885-01). According to the manufacturer this antibody binds to natural and recombinant IL-1α with no detectable reactivity with human IL-1β. The monoclonal anti-IL-1β was obtained from R & D Systems (Minneapolis, MN, USA: catalog no. 8516-311) and according to the manufacturer this antibody neutralizes the biological activity of IL-1β but not IL-1α. The monoclonals reactive with human IFN-γ (catalog no. 554698) and human CD21 (catalog no. 30691A) were obtained from BD Pharmingen (San Diego, CA, USA), anti-IL-12 (catalog no. MAB 219) was obtained from R & D Systems, indomethacin was obtained from Sigma

and PGE_2 was obtained from Biomol (Plymouth Meeting, PA, USA).

Enzyme-linked immunosorbent assay for anti-*Actinobacillus actinomycetemcomitans* immunoglobulin G

Immunoglobulin G reactive with *A. actinomycetemcomitans* was quantified by enzyme-linked immunosorbent assay (ELISA). Briefly, *A. actinomycetemcomitans* at 50 μg/ml in pH 9.6 carbonate buffer was added to wells of 96-well microtiter plates. The plates were incubated overnight to coat the wells with *A. actinomycetemcomitans and* then washed to remove unbound *A. actinomycetemcomitans*. Supernatant fluids in various dilutions were added to the wells and the plates were incubated overnight at 4°C. After washing, 100 μl of appropriately diluted mouse anti-human monoclonal IgG1 and IgG2 were added to the wells and incubated at room temperature for 2 h. Once again the plates were washed and alkaline phosphatase conjugated goat anti-human IgG was added to each well. The plates were incubated at room temperature for 1 h, washed and 100 μl/well of substrate was added and the optical density (OD) was measured at 405 nm using a microplate reader. A serum pool with a known content of anti-*A. actinomycetemcomitans* was used to construct a standard curve for each plate and the standard curve was used to convert optical density into ng of anti-*A. actinomycetemcomitans* in wells with supernatant fluids from cultured cells.

Statistics

Experiments were repeated a minimum of three times and the cultures were done in triplicate. The responses of different individuals varied considerably so representative experiments are generally presented. In some cases data are expressed in percentages to help normalize the results from a series of experiments with cells from different individuals. In these cases the positive control represents 100% and the effect of all treatments is indicated by the percentage of the positive control. Statistical significance was determined using ANOVA with $p < 0.05$ accepted as significant.

Results

Effect of follicular dendritic cells on anti-*Actinobacillus actinomycetemcomitans* immunoglobulin G responses

Peripheral blood mononuclear cells from *A. actinomycetemcomitans*-seropositive subjects with localized aggressive periodontitis were stimulated with *A. actinomycetemcomitans*–anti-*A. actinomycetemcomitans* (immune complexes) or *A. actinomycetemcomitans* alone in the presence or absence of follicular dendritic cell. Addition of follicular dendritic cell to peripheral blood mononuclear cells cultures resulted in lymphocytes clustering around follicular dendritic cells '*in vitro* germinal centers' and increased anti-*A. actinomycetemcomitans* IgG responses (3–40-fold increases) compared with controls lacking follicular dendritic cells. A typical experiment is shown in Fig. 1, which also illustrates that the IgG response was antigen dose-dependent. Statistically significant responses were obtained with 10^7 organisms and this dose was used in subsequent experiments. Follicular

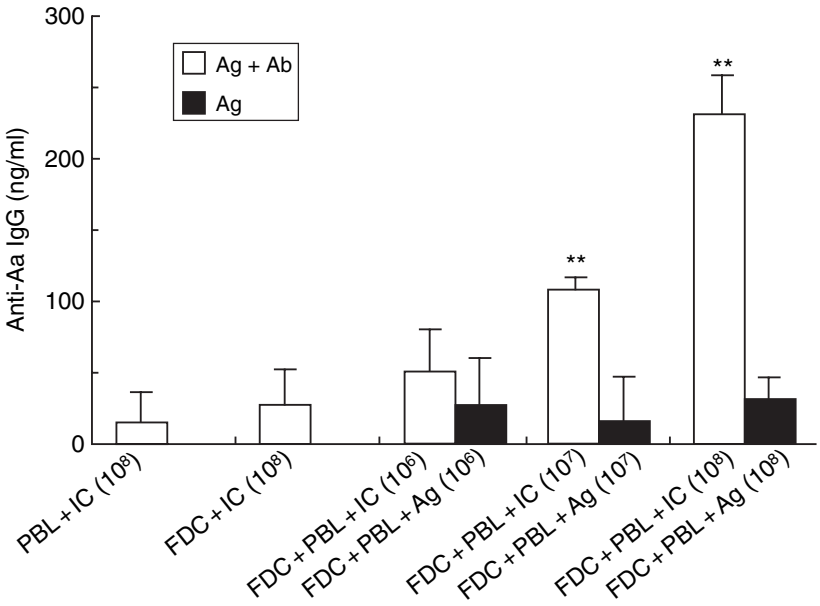

Fig. 1. Addition of murine follicular dendritic cells to human peripheral blood mononuclear cells cultures increased specific anti-*Actinobacillus actinomycetemcomitans* immunoglobulin G responses. The peripheral blood mononuclear cells from *A. actinomycetemcomitans*-seropositive subjects with localized aggressive periodontitis were stimulated with *A. actinomycetemcomitans*–anti-*A. actinomycetemcomitans* as an immune complex or *A. actinomycetemcomitans* alone in the presence of follicular dendritic cells from BALB/c mice (2×10^5/ml). The numbers in the parentheses indicate the number of bacteria in the culture. On day 7 the media were replaced with fresh media and on day 14 supernatant fluids were harvested and antibody production was measured. The murine follicular dendritic cells and immune complexes indicate the level of background antibody from the human antibody in immune complexes used to stimulate the responses. Other controls included examining the cultures for increased levels of anti-tetanus toxoid, which would be irrelevant in this system and the antibody could not be detected. Values represent the mean ± SE of three replicate cultures and these results are typical of three experiments of this type. **Represents $p < 0.01$ when compared with the peripheral blood mononuclear cells + immune complex. Aa: *Actinobacillus actinomycetemcomitans*; Ab: antibody; Ag: antigen; FDC: follicular dendritic cells; IC: immune complex; IgG: immunoglobulin G; PBL: peripheral blood mononuclear cells.

dendritic cells are known to deliver trapped immune complexes to B cells in the form of iccosomes that are endocytosed by specific B cells, which in turn process and present the antigen in the immune complexes to appropriate T cells that provide the B cells with necessary T cell help. *Actinobacillus actinomycetemcomitans* in the absence of specific antibody (peripheral blood mononuclear cells + follicular dendritic cells + *A. actinomycetemcomitans*) would not be trapped by follicular dendritic cell-Fc receptors and presented to the B cells by follicular dendritic cells. Indeed, addition of free *A. actinomycetemcomitans* resulted in minimal responses and generally failed to induce any detectable increase anti-*A. actinomycetemcomitans* IgG as illustrated in the experiment in Fig. 1. These results indicated that both follicular dendritic cell and antigen in immune complexes are important for optimal anti-*A. actinomycetemcomitans* responses.

Given that follicular dendritic cells use complement receptors and Fc receptors to trap immune complexes, we reasoned that complement fragments might be sufficient to load *A. actinomycetemcomitans* on follicular dendritic cells and promote a productive response. To test this, *A. actinomycetemcomitans* was decorated with complement fragments via lipopolysaccharide-mediated activation of the alternative complement pathway by incubating *A. actinomycetemcomitans* overnight at 37°C with fresh serum from a seronegative subject. As illustrated in Fig. 2, the *A. actinomycetemcomitans* in complement was nearly as effective in promoting anti-*A. actinomycetemcomitans* IgG production as was *A. actinomycetemcomitans* incubated with specific antibody.

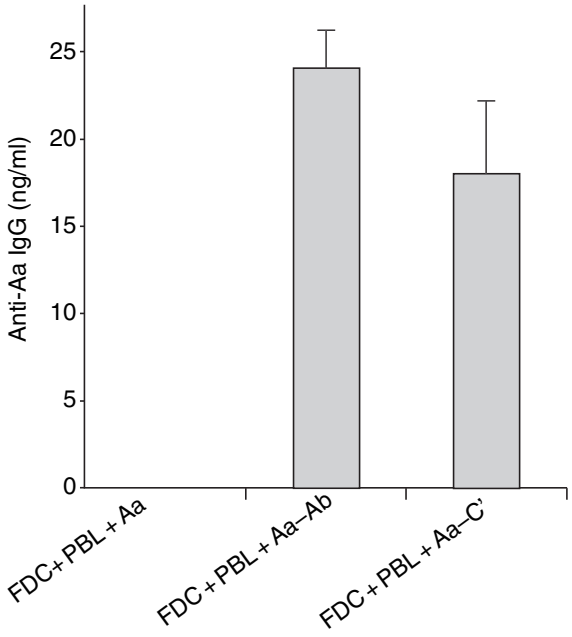

Fig. 2. Complement fragments on *Actinobacillus actinomycetemcomitans* were sufficient to allow follicular dendritic cells to handle *A. actinomycetemcomitans* productively. *Actinobacillus actinomycetemcomitans* was incubated overnight at 37°C in fresh serum from a seronegative subject to decorate the bacteria with complement fragments via lipopolysaccharide-mediated activation of the alternative complement pathway. The experimental set up with 10^7 bacteria was as in Fig. 1 except no *A. actinomycetemcomitans* specific antibody was used to facilitate loading of the bacteria on follicular dendritic cells. The results are typical of two experiments of this type. Aa: *Actinobacillus actinomycetemcomitans*; Ab: antibody; C′: complement fragment; FDC: follicular dendritic cells; IgG: immunoglobulin G; PBL: peripheral blood mononuclear cells.

Effect of anti-CD21, anti-interleukin-1, anti-interleukin-12, or anti-interferon-γ

Follicular dendritic cell-CD21L is known to bind to the coreceptor complex on B cells and promote activation (19). We reasoned that if this were important to the anti-*A. actinomycetemcomitans* response, then blocking the B cell coreceptor complex with murine anti-human CD21 should inhibit the adjuvant effect of adding murine follicular dendritic cells. As illustrated in Fig. 3, addition of anti-CD21 inhibited the anti-*A. actinomycetemcomitans* response by over half.

Studies of peripheral blood mononuclear cells from localized aggressive periodontitis patients stimulated with pokeweed mitogen indicate that both IL-1α and IL-1β are critical to optimal production of IgG (10). To determine if this is true for the specific IgG response to *A. actinomycetemcomitans*, we added anti-IL-1α or anti-IL-1β at the beginning of culture and, as indicated in Fig. 3, either antibody blocked production of the vast majority of IgG specific for *A. actinomycetemcomitans*.

IgG2 is the dominant anti-*A. actinomycetemcomitans* specific immunoglobulin *in vivo* and T cells polarized toward Th1 promote IgG2 (6, 28, 29). Given that Th1 polarization is promoted by IL-12, we reasoned that IL-12 might also be critical to the production of IgG2. This was tested using pokeweed mitogen as the stimulant and anti-IL-12 inhibited the IgG2 response by nearly 80% (data not shown). To determine if these Th1 associated cytokines are important for IgG specific for *A. actinomycetemcomitans,* we added anti-IL-12 or anti-IFN-γ at the beginning of culture and found that the majority of IgG anti-*A. actinomycetemcomitans* was blocked by either antibody (Fig. 4).

Effect of prostaglandin E_2 on immunoglobulin G2

Studies of peripheral blood mononuclear cells from localized aggressive

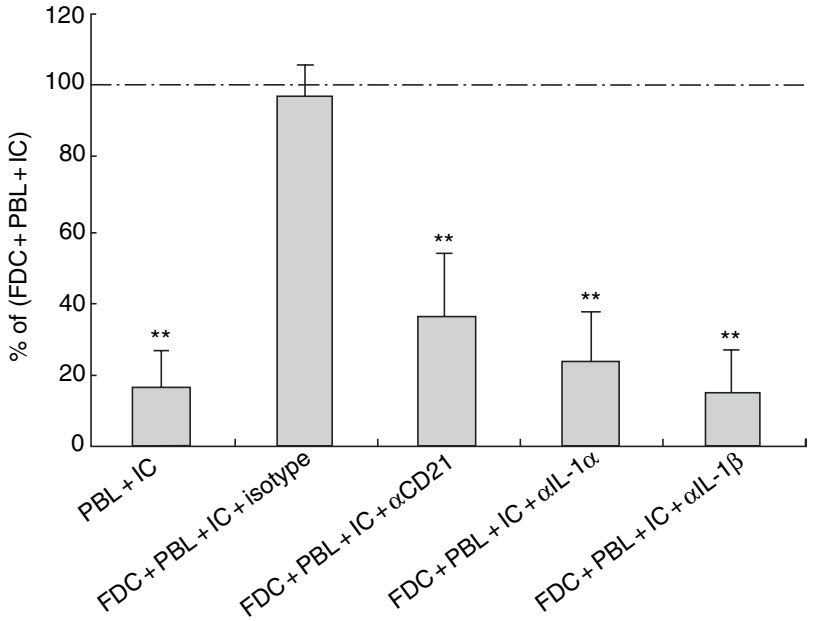

Fig. 3. Blocking B cell CD21 or neutralizing interleukin-1 (IL-1) inhibited production of anti-*Actinobacillus actinomycetemcomitans* immunoglobulin G (IgG). Antibodies reactive with CD21 or IL-1 were present at 20 μg/ml in the cultures. The results were normalized by representing data as percentage of IgG response in peripheral blood mononuclear cells + follicular dendritic cells + immune complex culture. Values are mean ± SE of four experiments and the mean 100% response was 134 ng of anti-*A. actinomycetemcomitans* IgG/ml with individual results ranged from 57 to 253. **Represents $p < 0.01$ when compared with the peripheral blood mononuclear cells + follicular dendritic cells + immune complex + isotype control. FDC: follicular dendritic cells; IC: immune complex; IL-1α: interleukin-1α; IL-1β: interleukin-1β; PBL: peripheral blood mononuclear cells.

periodontitis patients with pokeweed mitogen indicate that PGE_2 is critical to optimal production of IgG2 (11). To determine if this is true for the IgG response to *A. actinomycetemcomitans*, peripheral blood mononuclear cells from *A. actinomycetemcomitans*-seropositive subjects with localized aggressive periodontitis + follicular dendritic cells were stimulated with *A. actinomycetemcomitans*-anti-*A. actinomycetemcomitans* immune complex in the presence of indomethacin and/or PGE_2. Indomethacin clearly suppressed anti-*A. actinomycetemcomitans* IgG and addition of PGE_2 (100 nM) restored production of specific IgG as has been shown for IgG2 using pokeweed mitogen (Fig. 5).

Discussion

The present study was undertaken in an effort to confirm and extend results using a relevant specific antigen in place of the non-specific activator used in previous studies. Our experience with follicular dendritic cells provided us with a tool that made it possible to study IgG responses to *A. actinomycetemcomitans*. Follicular dendritic cells handle antigen productively in the form of immune complexes. Immune complexes form in primary responses as soon as antibody is produced and almost instantaneously in recall responses when antigen combines with antibody persisting from the initial priming. These immune complexes are trapped by follicular dendritic cells and the immune complex bearing follicular dendritic cells are required for development of fully functional germinal centers (15, 30, 31). Critical events in germinal centers include immunoglobulin class switching, production of plasmablasts that home to bone marrow and produce large amounts of specific antibody, somatic hypermutation, induction of high affinity IgG involved in affinity maturation, and production of B memory cells with high affinity receptors (13, 20, 31–36). In addition to providing intact antigen to engage B cell antigen receptor, follicular dendritic cells bear CD21L, which provides a potent cosignal through the B cell-CD21/CD19/CD81 coreceptor complex (19). These follicular dendritic cell–B cell interactions are not major histocompatability complex or even species restricted and this allows the use of murine follicular dendritic cell with human peripheral blood mononuclear cells (24). We also attempted to use the human follicular dendritic cell-like cell line HK, but the immune complexes did not appear to be effectively trapped and our attempts were unsuccessful (unreported observation). Although T cells must be major histocompatability complex compatible with B cells for processed antigen to be recognized, follicular dendritic cells present intact antigen to B cell antigen receptor. B cells must then process and present antigen to T cells to obtain help, and follicular dendritic cells facilitate this step by providing antigen in the form of iccosomes for B cells to process (16, 37, 38). The B cells appear to find iccosomes palatable and readily process the follicular dendritic cell-derived antigen (16). The results reported here indicate that addition of follicular dendritic cells to human peripheral blood mononuclear cells from seropositive subjects increased anti-*A. actinomycetemcomitans* IgG responses and facilitated studies of IgG regulation. Optimal follicular dendritic cell function required that immune complexes be trapped and presented by follicular dendritic cells, and immune complex trapping was mediated by either specific antibody or by complement fragments deposited on *A. actinomycetemcomitans*. The adjuvant effect of follicular dendritic cells was inhibited by anti-human-CD21, indicating cosignaling via follicular dendritic cell–CD21L was important.

The fundamental hypothesis we sought to test was that a number of proinflammatory cytokines including

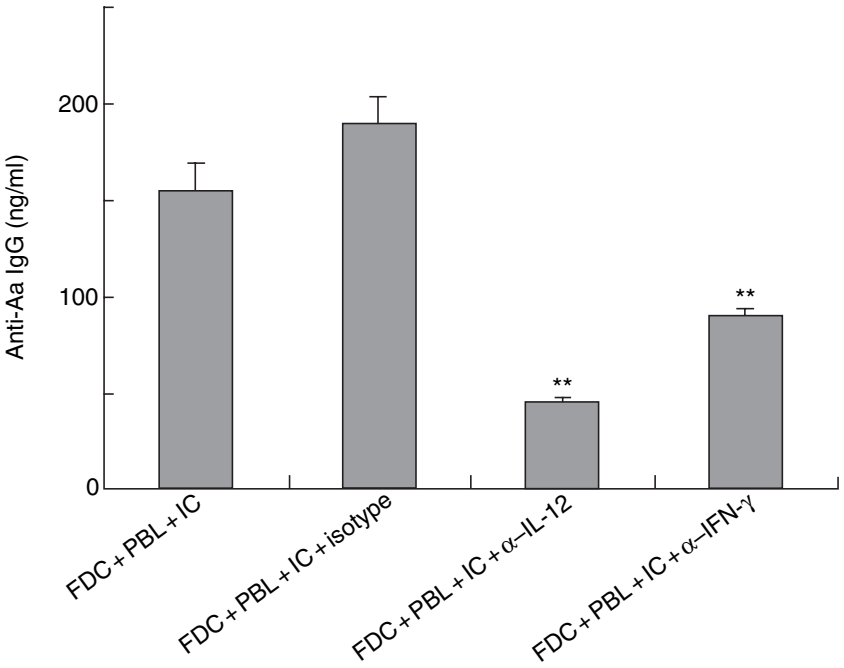

Fig. 4. Anti-interferon-γ (IFN-γ) or anti-interleukin-12 (IL-12) suppressed anti-*Actinobacillus actinomycetemcomitans* immunoglobulin G (IgG). The peripheral blood mononuclear cells from *A. actinomycetemcomitans*-seropositive subjects with localized aggressive periodontitis and follicular dendritic cells were stimulated with *A. actinomycetemcomitans*–anti-*A. actinomycetemcomitans* immune complex in the presence of anti-IFN-γ or anti-IL-12 at 50 μg/ml and 20 μg/ml, respectively. The data are representative of three separate experiments and are expressed as mean ± SE. **Represents $p < 0.01$ when compared with the peripheral blood mononuclear cells + follicular dendritic cells + immune complex + -isotype. Aa: *Actinobacillus actinomycetemcomitans*; FDC: follicular dendritic cells; IC: immune complex; IgG: immunoglobulin G; PBL: peripheral blood mononuclear cells.

Th1 cytokines known to be important for polyclonal activators are critical for optimal IgG responses against specific antigens on *A. actinomycetemcomitans*. Work with pokeweed mitogen indicated that optimal IgG responses require IL-1α, IL-1β and that optimal IgG2 responses require the Th1 cytokines IL-12, IFN-γ, and PGE_2 (6, 10–12). The present results are consistent with the pokeweed mitogen data, indicating that optimal induction of human anti-*A. actinomycetemcomitans* requires the same proinflammatory cytokines including the Th1 cytokines. B cells and plasma cells are present in large numbers in the inflammatory cell infiltrate observed in periodontal lesions and the presence of these cells may be correlated with tissue destruction. Generally, antibody responses are thought of as being Th2 dominated. However, the anti-*A. actinomycetemcomitans* response is Th1 dominated and this specific antibody in combination with the activities of cytokines in Th1-dominated response may be more effective in attacking pathogens such as *A. actinomycetemcomitans* than cytokines in Th2-dominated responses. This would be consistent with the observation that this Th1-dominated antibody is associated with a reduction in the extent and severity of disease in patients with early onset disease (1, 2).

Given the known association of PGE_2 with Th2 responses and IgG2 with IFN-γ, we were surprised to discover that production of IgG2 is selectively enhanced by the addition of PGE_2 to pokeweed mitogen-stimulated peripheral blood mononuclear cells cultures (11). Furthermore, indomethacin treatment clearly inhibits pokeweed mitogen-stimulated IgG2, and PGE_2 restores production by pokeweed mitogen (12). Remarkably, addition of IFN-γ also restores indomethacin-suppressed IgG2 but not IgG1 and we found that indomethacin suppressed IFN-γ production and that PGE_2 restores IFN-γ levels (12). A need for PGE_2 for IFN-γ production was not anticipated, but it has been reported that subnanomolar concentrations of PGE_2 can act on primed Th1 cells and enhance production of IFN-γ (39). Consistent with this relationship, we found that indomethacin-mediated suppression could be reversed by addition of IFN-γ, but not by addition of IL-12 (12). These results suggest that PGE_2 is acting on Th1 cells and not on the accessory cells. The PGE_2-stimulated Th1 cells would increase IFN-γ production and this would explain how PGE_2 could promote IFN-γ dependent IgG2 responses in localized aggressive periodontitis patients. Given this background, it is not surprising that indomethacin was a potent inhibitor of anti-*A. actinomycetemcomitans* in the present study and that PGE_2 fully restored the anti-*A. actinomycetemcomitan* response (Fig. 5).

The ability to mount high IgG responses to *A. actinomycetemcomitan* and *P. gingivalis* is thought to be important because high levels of IgG2 and IgG1 specific for these organisms appear to minimize the extent and severity of periodontal destruction (2). Thus Th1 responses that help clear the pathogens in localized aggressive periodontitis may be helpful. However, proinflammatory processes associated with Th1 can lead to more tissue destruction (40, 41). It appears that the protective anti-*A. actinomycetemcomitans* response relates only to patients with high levels of specific antibodies that should best clear the pathogen (2). Low responses that fail to clear a significant portion of the bacteria could help sustain the Th1 response and promote tissue injury. Thus, it may be critical that we understand the regulation of anti-*A. actinomycetemcomitans* responses. *In vitro* studies of how IgG anti-*A. actinomycetemcomitans* responses are regulated have been hampered by the inability to induce adequate responses, but it appears that addition

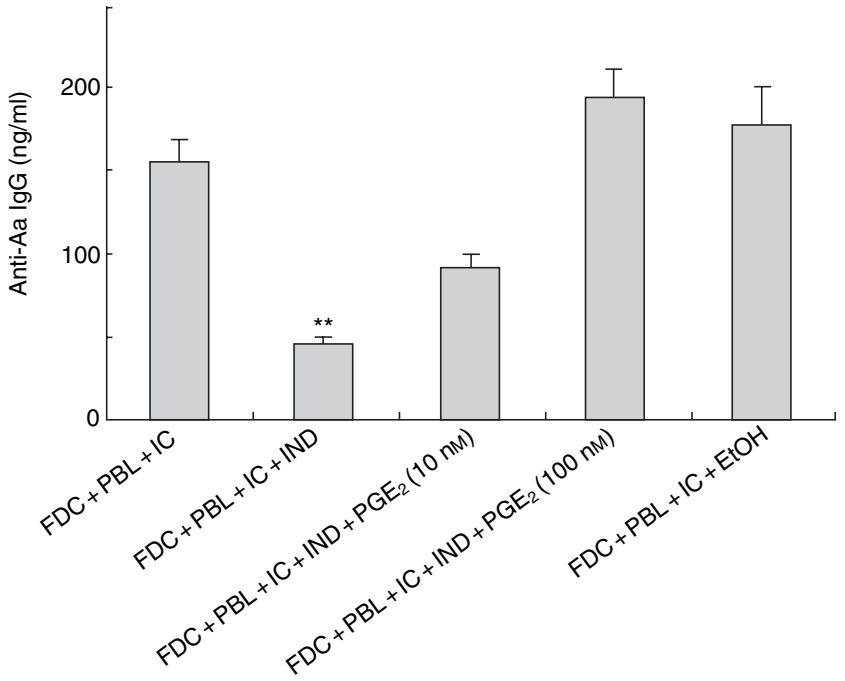

Fig. 5 Indomethacin-mediated suppression of anti-*Actinobacillus actinomycetemcomitans* immunoglobulin G responses and the restorative effect of prostaglandin E_2. Peripheral blood mononuclear cells from anti-*A. actinomycetemcomitans*-seropositive subjects with localized aggressive periodontitis and follicular dendritic cells were stimulated with immune complex (*A. actinomycetemcomitans*–anti-*A. actinomycetemcomitans*) in the presence of indomethacin (5 μg/mi) and/or prostaglandin E_2. A small amount of ethanol (0.05% final culture concentration) was used to get the indomethacin in solution and the final column represents this vehicle control. The data are representative of three separate experiments and are expressed as mean ± SE. **Represents $p < 0.01$ when compared with the peripheral blood mononuclear cells + follicular dendritic cells + immune complex. Aa: *Actinobacillus actinomycetemcomitans*; EtOH: ethanol; FDC: follicular dendritic cells; IC: immune complex; IgG: immunoglobulin G; IND, indomethacin; PBL: peripheral blood mononuclear cells; PGE_2: prostaglandin E_2.

of follicular dendritic cell to peripheral blood mononuclear cells cultures helps recreate the *in vivo* microenvironment in germinal centers and results in substantially higher IgG responses. This allows study of the regulation of specific responses under controlled conditions *in vitro*. These studies may offer important insight into how the *in vivo* IgG responses to *A. actinomycetemcomitans* might be optimized and we look forward to results from such studies.

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