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Mature Dendritic Cells Infiltrate the T Cell-Rich Region of Oral Mucosa in Chronic Periodontitis: In Situ, In Vivo, and In Vitro Studies[1]

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Abstract

Previous studies have analyzed the lymphoid and myeloid foci within the gingival mucosa in health and chronic periodontitis (CP); however, the principal APCs responsible for the formation and organizational structure of these foci in CP have not been defined. We show that in human CP tissues, $CD1a^+$ immature Langerhans cells predominantly infiltrate the gingival epithelium, whereas $CD83^+$ mature dendritic cells (DCs) specifically infiltrate the $CD4^+$ lymphoid-rich lamina propria. In vivo evidence shows that exacerbation of CP results in increased levels of proinflammatory cytokines that mediate DC activation/maturation, but also of counterregulatory cytokines that may prevent a Th-polarized response. Consistently, in vitro-generated monocyte-derived DCs pulsed with *Porphyromonas gingivalis* strain 381 or its LPS undergo maturation, up-regulate accessory molecules, and release proinflammatory (IL-1β, PGE_2) and Th (IL-10, IL-12) cytokines. Interestingly, the IL-10:IL-12 ratio elicited from *P. gingivalis*-pulsed DCs was 3-fold higher than that from *Escherichia coli*-pulsed DCs. This may account for the significantly ($p < 0.05$) lower proliferation of autologous $CD4^+$ T cells and reduced release of IFN-γ elicited by *P. gingivalis*-pulsed DCs. Taken together, these findings suggest a previously unreported mechanism for the pathophysiology of CP, involving the activation and in situ maturation of DCs by the oral pathogen *P. gingivalis*, leading to release of counterregulatory cytokines and the formation of T cell-DC foci.

The oral mucosa is colonized by commensal and pathogenic flora the mass and diversity of which rival that of the lower bowel (1, 2). This presumably represents a formidable challenge to the host in maintaining immune homeostasis, yet surprisingly little is known of how local immune cells respond to this flora. Specific pathogens within the plaque biofilm induce a strong humoral immune response during periodontitis (3), and this response is apparently derived from both systemic and mucosal immune systems (4). The oral pathogen *Porphyromonas gingivalis* is uniquely equipped to cause chronic periodontitis (CP),[3] infecting and invading through the oral epithelium (1), resisting phagocytosis (5), and

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[3]Abbreviations used in this paper: CP, chronic periodontitis; DC, dendritic cells; GCF, gingival crevicular fluid; LP, lamina propria; LC, Langerhans cell; MDDC, monocyte-derived DC; MOI, multiplicity of infection; OLF, oral lymphoid follicles.

infecting dendritic cells (DCs) in vitro and in situ (6). In the rat model of periodontitis, introduction of Ags from another oral pathogen, *Actinobacillus actinomycetemcomitans* directly into the gingival mucosa induces a rapid and protective humoral immune response in the rat model (7). This suggests that the gingiva may be a significant site of recruitment of cells capable of capturing and presenting Ags locally or in the lymphoid organs. Many studies have addressed the local T cell response in periodontitis (8 –14), but scant evidence exists in humans on how these responses are initiated and regulated.

Of particular significance for initiating the immune response within respiratory (15) and gastrointestinal mucosa (16 –21) are DCs. In their immature state, DCs are efficient Ag capture cells, but as they mature, they undergo phenotypic changes that facilitate their migration toward lymphoid organs and their unique ability to prime naive T cells (22–24). The presence of the proinflammatory cytokines IL-1β and TNF-γ promotes DC migration and maturation (23–25) and also enhances the immune response by promoting clonal expansion and differentiation of Ag-activated $CD4^+$ T cells (26). In contrast, the presence of IL-10 is associated with reduced DC migration (27), immunosuppression (28), conversion of DCs into macrophages (29), and an unfavorable outcome of diseases, such as leprosy, caused by intracellular pathogens (30).

Here we show differential localization of DC subpopulations in gingival mucosa, with immature $CD1a^+$ localized to the epithelium in health and disease and mature $CD83^+$ DCs restricted to the T cell-rich lamina propria (LP) in CP. The presence of elevated levels of cytokines IL-1β, PGE_2, and IL-10 in the local milieu of active CP suggests a counterregulatory microenvironment supportive of DC maturation, but inhibitory of an effective cell-mediated response. In vitro evidence indicates that *P. gingivalis* and its LPS induce DCs to mature but stimulate the release of counterregulatory cytokines (as in vivo) and promote an inefficient T cell response. We propose a novel model for the pathophysiology of CP whereby pathogen- and cytokine-driven DC maturation in situ results in formation of oral lymphoid foci.

Materials and Methods

Human subject use

The Institutional Review Board approved all protocols involving human subjects. Informed consent was obtained from all subjects before commencement of the study. All subjects were in good general health and were not on antibiotic, anti-inflammatory, or anticoagulant therapy during the month preceding the baseline exams. Also excluded from the study were subjects with a history of drug allergies, rheumatic fever or other conditions requiring prophylactic antibiotic treatment, gross caries or other dental pathology, fewer than 20 teeth, and pregnant or nursing females.

Collection and handling of clinical specimens, disease categories, clinical protocols

Gingival tissue specimens from 29 adult subjects, including 8 periodontally healthy, 7 with gingivitis, and 14 with CP, were excised under nerve block anesthesia by sharp dissection. The clinical criteria for these disease categories have been described previously (5, 31, 32). Gingival tissue was properly oriented in OCT medium by inserting a tooth landmark (3-mm strip of filter paper) alongside the tissue specimen, was flash frozen, and then was stored at −80°C. Cryostat sections measuring 7 μm thick were cut and fixed in cold acetone for 10 min. Sections were stained with H&E to confirm the clinical diagnosis by histological means (not shown). The experimental design for the induction of active (n = 12) or “quiescent” (n = 20) gingival inflammation (Table I) in subjects with preexisting mild CP (minimum of three probing pocket depths of ≥ 5 mm, clinical attachment levels of ≥ 5 mm,

bleeding on probing, and horizontal bone loss at the same sites), as well as the technique for sampling gingival crevicular fluid (GCF), have been described previously (32). All four periopaper strips from each tooth were placed in one glass screw-capped vial and stored at −80°C.

Analysis of GCF cytokines by ELISA

Sterile saline (400 μl) was added to vials containing strips, and proteins were eluted by placing the vials on a rotator (Roto-Mix; Barnstead/Thermolyne, Dubuque, IA) at 250 ± 10 rpm for 1 h in a 4°C cold room. Initial studies (not shown) established the dilution of test samples that yielded sufficient volume and sensitivity for detection of the four cytokines within the linear range of their respective standard curves (32). Dilutions ranged from 1/2.5 to 1/5 v/v. The diluted samples then were aliquoted into new vials and frozen at −80°C for subsequent analysis. Commercial ELISA kits (R&D Systems, Minneapolis, MN.) were used to analyze the levels of IL-1β, IFN-γ, IL-10, and PGE_2 within the GCF samples. All reagents were brought to room temperature, and assay diluent (50 μl) was added to each well. The standard or unknown sample then was added to the wells (200-μl volume), and analysis was performed as described by manufacturer. Color development and intensity of the color were measured with an ELISA plate reader (Molecular Devices, Sunnyvale, CA.). A standard curve was prepared, plotting the optical density vs the concentration of the cytokine expressed as pg/30 s.

Single and double immunohistochemistry (enzyme-linked)

Single immunohistochemistry was performed on prefixed frozen sections by indirect method, as described previously (33). Sections were stained by the biotin-streptavidin-peroxidase method (Vectastain ABC Elite kit, Burlingame, CA). The substrate was 3-3′diaminobenzidine tetrahydrochloride (Vector Laboratories, Burlingame, CA). The primary Abs used are listed (Table II). The specificity was confirmed by substituting the respective isotype controls for the primary Abs. The sections were counterstained with hematoxylin. For double staining, after the first staining, sections were washed and labeled by the biotin-streptavidin-glucose oxidase method (Vectastain ABC GO kit). The sections were counterstained with Vector MethylGreen (Vector Laboratories). The specificity of the secondary Ab was confirmed by substituting the respective isotype control for the secondary Abs. A blinded examiner using light microscopy enumerated the immunoreactive cells. The area of the grid was calculated to obtain the number of cells per square millimeter. Two different areas were randomly selected by a blinded examiner in the epithelium and two in the LP to determine the number of cells per square millimeter. The number of positively stained cells in the epithelium and LP were also compared with each other. Statistical analysis is described below.

DC cultures, multiparameter flow cytometry analysis

Monocyte-derived DCs (MDDCs) were generated as described by Palucka et al. (34). Briefly, monocytes were isolated from mononuclear fractions of peripheral blood by negative selection and seeded in the presence of GM-CSF and IL-4 ($1–2 \times 10^5$ cells/ml) for 6– 8 days, after which flow cytometry (see Fig. 3) was performed to confirm the immature DC phenotype ($CD14^-CD83^-CD1a^+$). Cell surface markers of DCs were evaluated by four-color immunofluorescence staining with the following mAbs: CD1a-FITC (BioSource International, Camarillo, CA); CD40-PE (Coulter, Seattle, WA and Coulter-Immunotech, Westbrook, ME); CD80-PE (BD Biosciences, Mountain View, CA); CD83-PE (Immunotech); CD86-PE (BD PharMingen, San Diego, CA); HLA-DR-PerCP (BD Biosciences); and CD14 APC (Caltag Laboratories, Burlingame, CA). After 30 min at 4°C and washing with staining buffer (PBS, pH 7.2, 2 mM EDTA, and 2% FBS), cells were fixed in 1% paraformaldehyde. Analysis was performed with FACSCalibur (BD

Biosciences). Marker expression was analyzed as the percentage of positive cells in the relevant population defined by forward scatter and side scatter characteristics. Expression levels were evaluated by assessing mean fluorescence intensity indices calculated by relating mean fluorescence intensity noted with the relevant mAb to that with the isotype control mAb for samples labeled in parallel and acquired by using the same setting.

Purification of autologous $CD4^+$ cells

$CD4^+$ cells were purified from PBMC of the same donor that were used for DC generation (autologous), as described previously (34). Cells bearing CD4 Ag were isolated from mononuclear fraction through positive selection with anti-CD4 mAb and goat anti-mouse IgG-coated microbeads (Miltenyi Biotec, Gladbach, Germany). Isolation of $CD4^+$ cells was achieved with Minimacs separation columns (Miltenyi Biotec) as described by the manufacturer. In all of the experiments, the isolated cells were 80–90% $CD4^+$ as determined by staining with FITC-conjugated anti- CD4 mAb followed by flow cytometry analysis (not shown).

LPS purification

The methodology for isolation and purification of LPS from *P. gingivalis* 381 and *E. coli* American Type Culture Collection (ATCC, Manassas, VA) type strain 25922 was as described previously in our laboratory (35). Briefly, whole cell pellets were subjected to hot-phenol water extraction; the aqueous phase was subjected to extensive dialysis against distilled water, followed by lyophilization and then isopyknic density gradient centrifugation. The LPS-containing fractions were dialyzed extensively against distilled water, lyophilized, and subjected to biochemical analysis for purity (34).

Cytokines from DCs and T cells

To study cytokines produced by MDDCs, culture supernatants were collected at 24 h from DC pulsed with *P. gingivalis* 381*, E. coli* ATCC type strain 25922 (25:1 bacteria-to-DC ratio), or 100 ng/ml of *P. gingivalis* or *E. coli* LPS. For T cell cytokines (IFN-γ), culture supernatants were collected after DCs were cocultured with autologous $CD4^+$ T cells for 5 days. The production of cytokines (IL-10, IL-12 p70, IL-1β, PGE_2, and IFN-γ) was determined in triplicate by using commercial ELISA Kits (R&D Systems) and by following the manufacturer's instructions. The values shown in Table III represent the mean of 10 separate analyses.

T cell proliferation assay

The ability of bacteria-pulsed DCs to stimulate autologous T cell proliferation was performed as described previously (36). Day 6 MDDCs were pulsed with either *P. gingivalis* (25:1 multiplicity of infection (MOI)); *E. coli* (25:1 MOI); 100 ng/ml *P. gingivalis* LPS; or 100 ng/ml *E. coli* LPS, for 24 h at 37°C. DCs in complete RPMI with no bacteria or LPS were used as controls. DCs were washed extensively and cultured at graded doses (5000 DC/200 μl, 1000 DC/200 μl, and 300 DC/200 μl) in complete RPMI 1640 medium with 10% human AB serum with autologous $CD4^+$ T cells (50,000 cells/200 μl). Peak proliferative response was achieved with 1000 DC, so this number was used for subsequent assays. Proliferation was determined after 5 days by uptake of tritiated thymidine (1 μCi/well for the last 16 h). Assay was repeated 10 times on separate days, and the mean results are shown in Table III.

Statistical analyses

Descriptive statistics, means, and SE for the numbers of immunoreactive cells with each cell surface marker in healthy, gingivitis, and CP tissues (see Fig. 2) were calculated and

analyzed for statistical significance by Tukey's multiple comparisons test ($p < 0.05$; Minitab, State College, PA). Differences within epithelium and LP for each individual cell marker were analyzed by Student's *t* test ($p < 0.05$). Descriptive statistics, means, and SE of the clinical indices (not shown) and GCF cytokine levels (Table I) were calculated by using SAS 6.12 (Cary, NC) and Proc Mixed with experimental group and genotype as the factors and the baseline levels as the covariate, as described previously (31). Proc Mixed covariate analysis was used to determine which means were statistically significant, with the output being least squares means and least significant difference. Differences in IL-10, IL-1β, PGE_2, and IFN-γ between-group means were declared only if the *p* value for the F statistic in the analysis of covariance was ≤0.05, and the least significant difference also was significant at ≤0.05. Results of in vitro cytokine levels and T cell proliferation (Table III) were analyzed by Kruskall-Wallis test ($p < 0.05$; Minitab).

Results

Mature DCs specifically infiltrate the T cell-rich LP in CP

We have enumerated immature $CD1a^+$ Langerhans cells (LCs) and mature $CD83^+$ DCs by using immunohistochemistry (Fig. 1) within epithelium and LP of the gingiva in health, gingivitis, and CP (Fig. 2*A*). Macrophage/myeloid cells ($CD14^+$) and lymphocyte subsets also were enumerated within these same tissue compartments for comparison (Fig. 2*B*).

Microscopic analysis of healthy epithelium revealed the presence of large numbers of $CD1a^+$ LCs, but only a few $CD83^+$ mature DCs or $CD14^+$ cells (Fig. 2A). In gingivitis, there was a slight increase in the numbers of $CD1a^+$-labeled cells in the epithelium. However, the transition from health to CP was associated with a significant increase (over 3-fold increase, from 90 cells/mm^2 to 312 cells/mm^2; $p < 0.05$) in numbers of LCs in the epithelium. Moreover, the transition from health to CP was associated with a significant increase (6-fold increase, from 8 cells/mm^2 to 48 cells/mm^2; $p < 0.05$) in numbers of mature DCs in the LP.

In healthy epithelium, lymphocytes also comprise a large number of cells (Fig. 2*B*) and, relative to the epithelium, the LP contains a denser resident population of lymphocytes. Transition from health to CP was marked by a significant increase ($p < 0.05$) in numbers of $CD4^+$ T cells and $CD45RO^+$ T cells, both of which were predominantly present in LP, the zone of mature DC infiltration. $CD8^+$ T cells also increased in CP, but this increase was only significant ($p < 0.05$) in the epithelium, where LCs reside. B cells were present in large numbers (≅150 cells/mm^2) at all times and always localized to the LP, similar to the pattern observed by $CD14^+$ myeloid cells.

Increased levels of DC-mobilizing/maturing cytokines are released in active periodontitis in vivo

We next analyzed the levels of cytokines that might influence DC redistribution/maturation and T cell activation within this tissue. We designed a clinical study in human subjects with preexisting mild CP to determine the level of these cytokines in the GCF in active vs quiescent disease. The results (Table I) indicate that disease exacerbation is accompanied by significant changes in the local cytokine microenvironment, including significant ($p < 0.05$) increases in the levels of IL-1β, PGE_2, and IL-10. IFN-γ was present in the GCF of all CP subjects but did not increase significantly in active disease.

The oral mucosal pathogen *P. gingivalis* or its LPS activates DCs to undergo maturation and up-regulate costimulatory molecule expression in vitro

Recent in situ studies of periodontitis in rats (7) and humans (37) suggest that costimulatory molecule expression on gingival APCs might play a role in alveolar bone loss and the local

T cell response, respectively. Here, human immature MDDCs (Fig. 3*A*) were pulsed with the oral pathogen *P. gingivalis* for from 4 to 14 h. Previous studies from our laboratory (6) as well as unpublished observations established the optimum MOI (25:1) for internalization of *P. gingivalis* by DCs, as confirmed by trifluorochrome phagocytosis microassay (5) and electron microscopy (data not shown). As our previous studies indicate that the LPS of *P. gingivalis* is an immunodominant Ag capable of inducing a strong IgG response in human subjects with CP (31), MDDCs were also pulsed with LPS (100 ng/ml) from *P. gingivalis.* The gut commensal *E. coli* ATCC type strain 25922 and its purified LPS served as the controls. Our results show that *P.* gingivalis (Fig. 3, *D* and *E*), its LPS (Fig. 3*F*), or LPS of *E. coli* (Fig. 3*G*) induced DCs to undergo maturation in a time-dependent manner, as evidenced by up-regulation of CD83. Moreover, expression of costimulatory molecules required for efficient Ag presentation, including CD80, CD86, CD40, and HLA-Dr also were up-regulated. The morphologic changes as immature DCs become matured by LPS also are shown (Fig. 3, *B* and *C*).

P. gingivalis-matured DCs release relevant cytokines and stimulate a limited T cell response

Based on in situ, in vivo, and in vitro correlates detailed above, we had reason to expect that DCs matured by the oral pathogen *P. gingivalis* would be potent at stimulating T cell responses, as determined by IFN-γ production and proliferation. However, we had reservations in this regard because of the GCF evidence that IFN-γ did not increase significantly in active CP. This was possibly attributable to the presence of counterregulatory Th2-biasing cytokines, such as IL-10 (27) and PGE_2 (38). Therefore, we analyzed the supernatants from the DCs pulsed as above with *P. gingivalis, E. coli* and their LPS moieties for proinflammatory cytokines (IL-1β, PGE_2), the Th2-biasing cytokine IL-10, and the Th1-biasing cytokine IL-12. DCs pulsed with either *P. gingivalis* or *E. coli* release IL-1β, PGE_2, IL-10, and IL-12, although *P. gingivalis*-pulsed DCs released significantly less ($p < 0.05$) of all four cytokines (Table III). The ratio of IL-10:IL-12 elicited by *P. gingivalis* was 3-fold higher than that by *E. coli* (7:1 vs 2:1, respectively). This elevation in IL-10:IL-12 ratio induced by *P. gingivalis* may be reflected in the significantly ($p < 0.05$) lower IFN-γ levels from $CD4^+$ T cells cocultured with DC-*P. gingivalis*, relative to DC-*E. coli* (35 vs 1031 pg/ml, respectively) and in the limited proliferation of autologous T cells elicited by *P. gingivalis*-pulsed DCs as compared with *E. coli*-pulsed DCs (Table III).

Discussion

Our results show a marked change in the tissue localization of DCs from health to CP. Indeed, immature $CD1a^+$ DCs (by definition, Ag capture cells) increased significantly in numbers in the diseased epithelium, whereas $CD83^+$ mature DCs (by definition, APCs) increased in numbers within the lymphoid-rich diseased LP. Interestingly, when the data for all the markers was expressed as a percentage of change from health to CP (not shown), the following cells changed by >100%: mature DCs in LP (↑164%); immature DCs in LP (↓198%); and $CD8^+$ cells in epithelium (↑169%).

Although these percentages were not tested statistically, we have interpreted the increase in mature DCs and decrease in immature DCs in the same tissues as evidence for local redistribution of LCs to LP, and subsequent maturation in situ (Fig. 4); however, the actual source of mature DCs is unknown presently. Our results further show that although certain cytokines released in this microenvironment (i.e., IL-1β and PGE_2) could promote in situ DCs activation/maturation and T cell expansion, the counterregulatory cytokine IL-10 also is released. Finally, the oral pathogen *P. gingivalis* is able to induce cultured MDDCs to undergo maturation and to release relevant proinflammatory (i.e., IL-1β and PGE_2) and Th

cytokines (IL-10 and IL-12), but elicits a limited T cell response compared with *E. coli*-pulsed DCs.

Based on these results, we have developed a novel working model for the pathophysiology of CP involving the formation of what we have termed “oral lymphoid follicles” or OLF (Fig. 4) within the LP around the dentition. We acknowledge that the term “follicle” might imply a proliferating B cell lymphoid organ and hence may be misleading, but nonetheless propose this terminology. We propose that the development of OLF is initiated in gingivitis by LCs homing to the gingival epithelium in response to bacteria/Ags in the oral biofilm (39), as well as a source of the earliest signals for lymphoid/myeloid trafficking, i.e., epithelium/keratinocytes (40). That the $CD1a^+$ cells enumerated in this study were in fact LCs was confirmed by scanning laser confocal microscopy and double immunofluorescence with CD1a and the LC-specific marker of Birbeck granules, LAG (41), as well as single immunoenzyme staining with langerin (Ref. 43; data not shown). Earlier studies have shown the dynamics of LCs trafficking in the respiratory mucosa in response to bacterial Ags (15), as well as LCs in oral mucosa/gingiva in response to plaque/allergen accumulation (10, 39, 42, 44, 45). One study identified increased numbers of LCs in the gingival epithelium of CP patients and also identified *P. gingivalis* (i.e., *Bacteroides gingivalis*) and *Actinobacillus actinomycetemcomitans* within the same tissues by immunohistochemistry (47). Our previous studies have shown by scanning laser confocal microscopy that LCs are infected with *P. gingivalis* in situ (6). In this same study, the pathophysiology of chronic gingivitis/mild CP was equated with a bacterially induced contact hypersensitivity response (46), wherein Ag (i.e., *P. gingivalis*)-sensitized gingival epithelial LCs engage $CD8^+$ T cells homing to the gingival epithelium (6). This supposition is consistent with the cellular infiltrates within the epithelium but is too simplistic to account for all of our findings, particularly those observed within the LP. The foci of myeloid and lymphoid cells within the LP of the diseased periodontium have been characterized by other investigators (8–14). Some have interpreted the predominance of $CD4^+$ activated/memory T cells and B cells within the LP as evidence that the CP “lesion” is in fact a tertiary lymphoid organ (48). It is necessary to differentiate, within OLF, expression of primary immune response markers from those consistent with recall responses expected within a tertiary lymphoid organ.

We propose that OLF are not fully developed unless and until sufficient antigenic challenge is provided by specific pathogens/commensals in the subgingival flora such as *P. gingivalis* and *Fusobacterium nucleatum* (1, 2, 40). In like manner, lymphoid follicles in Peyer’s patches do not develop normally in germ-free animals, but are restored when animals are monoinfected (49, 50). Both *P. gingivalis* and *F. nucleatum* have been shown to stimulate human gingival epithelial cells in vitro to produce β-defensins, proinflammatory cytokines, and chemokines in vitro that are required for leukocyte recruitment (40). We further propose that although T cell expansion and IFN-γ production (Table I) occurs in OLF, the development of a protective cell-mediated response is limited by the presence of elevated IL-10 and PGE_2 levels in vivo (Table III).

Our in vitro results indicate that DCs matured by *P. gingivalis* are able to stimulate a very limited autologous $CD4^+$T cell response, as compared with DCs matured by *E. coli*. Our efforts are now being directed to understanding the mechanism(s) of this disparity. One possibility being investigated in the human and murine (51) systems is that *P. gingivalis* or its LPS induce a Th2-biased response in DCs. This is supported by the data in Table III showing an elevation in IL-10:IL-12 ratio from *P. gingivalis*-pulsed DCs, relative to *E. coli*-pulsed DCs (7:1 vs 2:1), but confirmation of a Th2-biased response requires more rigorous investigation.

In conclusion, our results support the role of DCs in the pathophysiology of CP through their activation and in situ maturation in the T cell-rich LP under the influence of oral pathogens and a cytokine milieu that may be counterregulatory to a protective T cell-mediated response.

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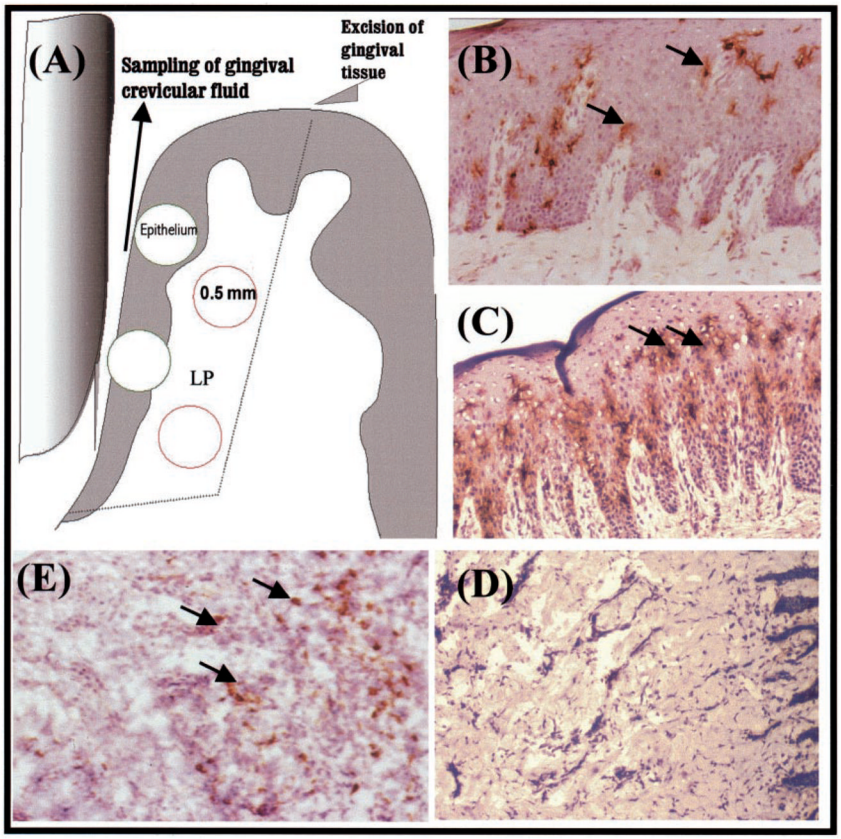

FIGURE 1.

Source of clinical specimens, representative stainings. *A,* GCF was sampled from the gingival sulcus, as described in *Materials and Methods*. Gingival tissue was surgically excised, snap frozen, and serial sectioned (0.7μm) with a cryostat. Quantitation of immunoreactive cells in epithelium (epi) and LP was performed by light microscopy (×40 objective) with an ocular grid (see circles), as described in *Materials and Methods*. Shown is representative immunoenzyme staining (arrows) achieved using: the mAb NA1/34 (anti-CD1a) on a healthy (*B*) and CP (*C*) specimen and the mAb HB15A (anti-CD83) on a healthy (*D*) and CP (*E*) specimen. Isotype controls run in parallel revealed no staining (not shown).

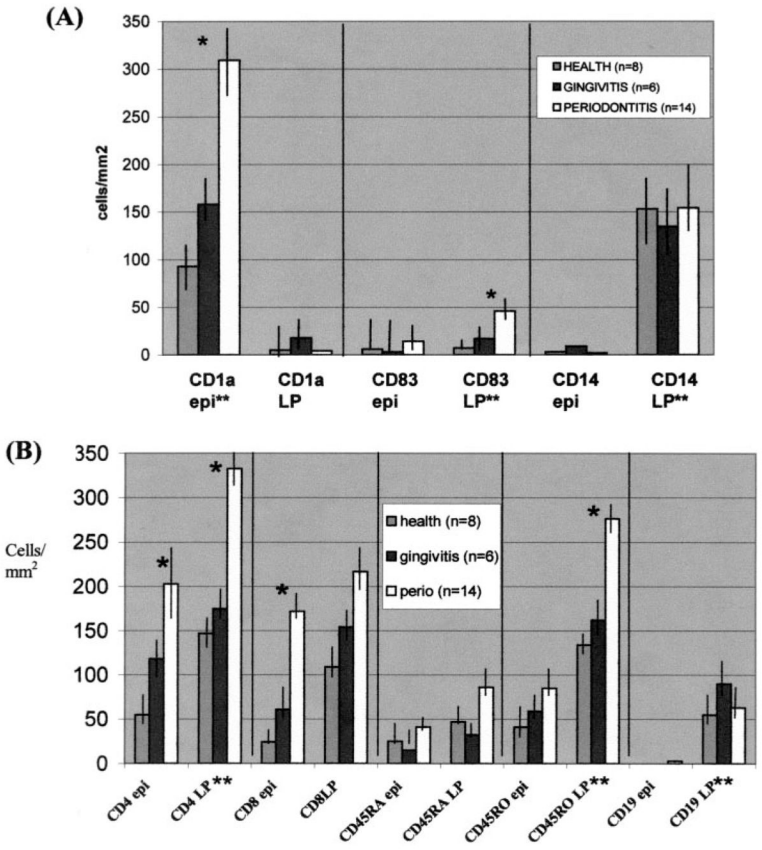

FIGURE 2.

Increased numbers of $CD83^+$ mature DCs infiltrate the T cell-rich LP of diseased mucosa. Shown are the mean numbers (+ SE) of immunoreactive DC/myeloid subpopulations (*A*) and lymphoid subpopulations (*B*) in the epithelium (epi) and LP per square millimeter of gingival tissue from subjects in each clinical category. *, Statistically significant differences ($p < 0.05$) relative to gingivitis and health (Tukey's multiple comparisons test). **, Statistically significant differences ($p < 0.05$), comparing epithelium to connective tissue compartment or vice versa (Student's *t* test). Data are representative of at least three serial sections from each subject, which were stained as described in *Materials and Methods*.

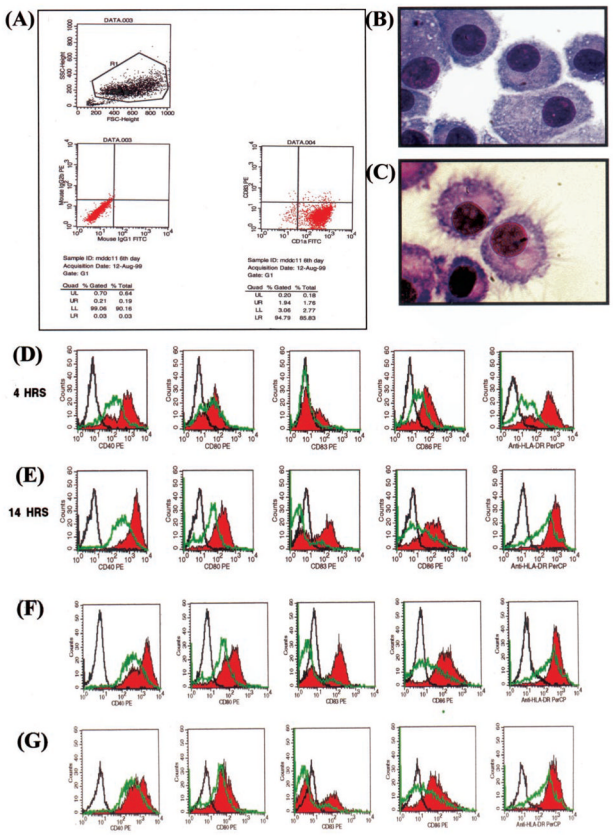

FIGURE 3.

The oral mucosal pathogen *P. gingivalis* or its LPS activate DCs to undergo maturation and up-regulate costimulatory molecule expression in vitro. Day 6 immature MDDCs were generated as described in *Materials and Methods. A,* Immature MDDC phenotype ($CD1a^+$, $CD83^+$), as confirmed by multiparameter flow cytometry analysis. CD14 expression (not shown) was typically 80–95% negative. *B,* Immature MDDCs stained by H&E. *C,* LPS-matured MDDCs stained by H&E. *D* and *E,* FACS histograms showing up-regulation (red histogram) of, from *left* to *right*, CD40, CD80, CD83, CD86, and HLA-Dr on MDDCs after coculture with *P. gingivalis* whole cells for 4 and 14 h, respectively. *F,* MDDCs cocultured with *P. gingivalis* LPS (100 ng/ml) for 14 h. *G,* MDDCs cocultured with *E. coli* LPS (100 ng/ml) for 14 h. Green histograms are DC controls and black histograms are isotype controls. The primary mAbs used are given in Table II and in *Materials and Methods*.

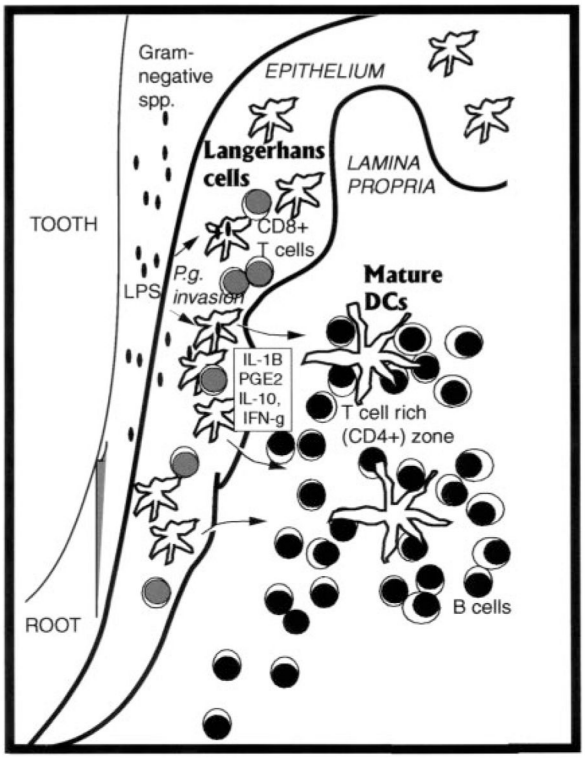

FIGURE 4.
Working model of DC/lymphoid foci and local milieu in CP.

Table I

List of primary Abs

Ab	Clones	Working Dilution	Source
CD1a	NA1/34	1/100	DAKO
CD4	13B8.2	1/100	Immunotech
CD8	DK25	1/100	DAKO
CD14	RM052	1/100	Immunotech
CD45RA	2H4	1/100	Coulter
CD45RO	UCHL-1	1/50	DAKO
CD83	HB15A	1/100	Immunotech
CD19	SJ25C1	1/100	BD Biosciences

Table II

Active disease results in increased levels of DC-maturating/mobilizing and counterregulatory cytokines[a]

	Disease State	
Cytokines (mean pg/30s ± SE)	**Active (*n* = 12)**	**Quiescent (*n* = 20)**
IL-1β	224 ± 26[b]	80 ± 27
PGE_2	396 ± 44[b]	228 ± 44
IL-10	21.34 ± 6[b]	8.13 ± 6
IFN-γ	46 ± 12	31 ± 6

[a]The experimental protocol is described in *Materials and Methods*.

[b]Significant increase, as determined by Proc Mixed covariate analysis ($p < 0.05$).

Table III

Cytokine release and autologous T cell proliferation by DCs pulsed with *P. gingivalis* (Pg) A7436 or *E. coli* 25922 and their LPS[a]

Groups	IL-1β (pg/ml) ±SE	PGE_2 (pg/ml) ±SE	IL-10 (pg/ml) ±SE	IL-12 p70 (pg/ml) ±SE	IFN-γ (pg/ml) ±SE	T Cell Proliferation (incorporation of [^{3}H]thymidine into $CD4^+$ autologous T cells)
DC + Pg	48 ± 1.8[b]	358 ± 34[b]	88 ± 54[b]	12 ± 7[b]	35 ± 35	4,964 ± 2,328[b]
DC + *E. coli*	272 ± 7.2	863 ± 9.8	600 ± 208	267 ± 98	1,031 ± 941	12,985 ± 2,328
DC + *Pg*LPS	ND[c]	ND	137 ± 78	30 ± 11	42 ± 42	3,451 ± 1,927
DC + *E. coli* LPS	ND	ND	178 ± 95	30 ± 15	19 ± 111	10,133 ± 3,516
DC	0[d]	42 ± 23	0[d]	0[d]	0[d]	142 ± 30
PHA						68,336 ± 34,193

[a] Means ± SE of 10 separate experiments. Cytokine levels from DCs (IL-1β, PGE_2, IL-10, and IL-12) and T cells (IFN-γ) were analyzed by ELISA as described in *Materials and Methods*.

[b] Statistically significant differences as assessed by Kruskall-Wallis test ($p < 0.05$).

[c] ND, not done. DC and T cell viability was determined by trypan blue exclusion and did not differ between the groups (not shown).

[d] Not detectable.