Expression of Major Histocompatibility Complex Class II and CD80 by Gingival Epithelial Cells Induces Activation of CD4⁺ T Cells in Response to Bacterial Challenge

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Received 13 April 2004/Returned for modification 3 May 2004/Accepted 18 October 2004

HLA-DR (major histocompatibility complex [MHC] class II) is often expressed by epithelial cells in gingival tissues with periodontal disease but not by cells in healthy gingival tissues. Confocal microscopic analyses revealed that gingival epithelial cells (GEC) from tissue with periodontal disease express both HLA-DR and B7-1 (CD80) costimulatory molecules. Rat GEC lines were established to elucidate the possible role of MHC class II and B7-1 expression by GEC. Stimulation of a rat GEC line with gamma interferon (IFN-γ) induced the expression of MHC class II, whereas the cell line constitutively expressed B7-1 costimulatory molecules as determined by reverse transcription-PCR and flow cytometry. Actinobacillus actinomycetemcomitans Omp29-specific CD4⁺ Th1 clone cells proliferated in response to pretreatment of GEC with fixed A. actinomycetemcomitans and IFN-γ. However, the Th1 cells did not respond to pretreatment of GEC with the bacteria alone or IFN-γ alone. The activation of Th1 clone cells induced by the GEC was inhibited by antibody to MHC class II or by CTLA4 immunoglobulin (CTLA4-Ig). Lymph node T cells did not demonstrate superantigen activity to A. actinomycetemcomitans, although both lymph node T cells and Th1 clone cells were sensitive to superantigen activity of staphylococcal enterotoxin A cultured in the presence of IFN-γ-treated GEC. These results suggested that GEC can take up bacterial antigen and consequently process and present the bacterial antigen to CD4⁺ T cells by MHC class II in conjunction with B7 costimulation. GEC appeared to play a role in the adaptive immune response by stimulating antigen-specific CD4⁺ T cells.

It is well documented that gingival epithelial cells (GEC) play an important role in the innate immune response by producing antimicrobial peptides and chemokines that recruit neutrophils (20). Although GEC in the lesions of periodontal disease express HLA-DR (major histocompatibility complex [MHC] class II) (6), the significance of MHC class II expression by GEC in the context of adaptive immune response is unclear. Evidence that the Th1 cytokine gamma interferon (IFN-γ) can induce MHC class II expression by human GEC (1, 30) is supported by the presence of physiologically significant IFN-γ in diseased lesions. In fact, prominent expression of IFN-γ mRNA by lymphocytes infiltrating inflamed gingival tissue is observed in periodontal disease (8, 34). The MHC class II expressed by epithelial cells in psoriasis can present some bacterial antigens such as superantigen (30). However, it is not known whether MHC class II expressed by GEC in periodontal diseased tissue can present bacterial antigen to T cells at the interface between densely infiltrating lymphocytes in the lamina propria and bacterial plaque in the periodontal pocket.

Antigen-specific T-cell activation by T-cell receptor/MHC class II engagement requires a costimulatory signal which is induced by the binding between CD28 on T cells and B7 costimulatory molecules expressed by antigen-presenting cells (APC) (2, 10, 23, 24). In general, two major costimulatory molecules, B7-1 (CD80) and B7-2 (CD86), are expressed by professional APC such as macrophages or dendritic cells. It is reported that infiltrating CD83⁺ mature dendritic cells in human gingival tissue express CD80 and CD86 costimulatory molecules (5) and that these mature dendritic cells appear to be associated with clusters of CD4⁺ T cells in the lamina propria (13, 14). Although nonprofessional APC including epithelial cells appear to be programmed not to express B7 costimulatory molecules, both B7-1 and B7-2 can be aberrantly expressed on the surface of alveolar and bronchiolar epithelial cells from patients with idiopathic pulmonary fibrosis and bronchiolitis (15). The expression of B7-2 by intestinal epithelial cells from patients with inflammatory bowel disease (29) further indicates that aberrant expression of B7 on epithelial cells may be circumstantially associated with the presence of inflammation. It is not clear if GEC can present bacterial antigen and if accompanying B7 can provide a proper costimulatory signal.

In the present study, we report clinical cases in which MHC class II and B7-1 were expressed by the same gingival epithelial cells from patients with periodontal disease. A syngeneic GEC line and bacterial antigen-specific CD4⁺ Th1 clone cells were employed to determine if GEC can take up bacterial antigen and present it to CD4⁺ T cells in an MHC class II- and/or B7-dependent fashion.

MATERIALS AND METHODS

Patients. Human gingival tissues were obtained from healthy subjects (n = 3; two males and one female) who were receiving treatment for cosmetic purposes (e.g., crown lengthening) and from patients with chronic periodontitis (n = 5; three males and two females; average age = 51 years; pocket depths, 4 to 8 mm) at surgery performed for therapeutic purposes. Healthy sites with no attach-
ment loss and no bleeding were selected, and diseased sites which exhibited moderate to severe periodontal attachment loss were also selected at random. Diseased individuals had at least one probing depth greater than 4 mm and bled on probing. The excised tissues were immediately embedded in optimal cutting temperature (OCT) compound (Tissue Tek) and stored at \(-80^\circ C\) until used for immunohistological analysis. Informed consent was obtained from all subjects before samples were collected.

**Immunofluorescent staining by double-color-imaging confocal microscopy.** Sections from diseased and healthy gingival tissues were fixed with acetone-ethanol (50:50, vol/vol) and treated with 1% rat serum in phosphate-buffered saline. After being washed, they were preincubated for 1 h at room temperature with mouse anti-human HLA-DR monoclonal antibody (MAb) (clone G46-6; PharMingen, San Diego, Calif.), anti-human CD80 MAb (clone MAB104; Immunotech, Marseilles, France), or isotype-matched control mouse MAb, PA20 (immunoglobulin G1 [IgG1]) (16). Each of these reagents was followed by biotinylated anti-mouse IgG antibody (Vector, Burlingame, Calif.) for 30 min, and the sections were stained with streptavidin-conjugated Texas Red-X (Molecular Probes, Eugene, Ore.). After being blocked with 5% normal mouse serum, the sections were further incubated with fluorescein isothiocyanate (FITC) conjugated anti-pancytokeratin MAb (clone C-11; Sigma, St Louis, Mo.) or FITC-conjugated mouse isotype-matched control (clone MOPC-21; Sigma). A laser-scanning confocal microscope (TCSNT; Leica Microsystems, Inc., Exton, Pa.) was used to assess the expression pattern of target molecules. Digital images were captured and saved in the computer system.

**Animals.** The animals used in the present studies were inbred Rowett rats (heterozygous/normal) that harbored a restricted flora (18). They were maintained under pathogen-free conditions in laminar-flow cabinets. All T-cell clones, spleen cells, GEC, and endothelial cells were derived from these Rowett strain rats.

**Bacteria.** *Actinobacillus actinomycetemcomitans* strain Y4 (ATCC strain 43718) was grown in pleuropneumonia-like organism broth (Difco, Detroit, Mich.) with glucose (3 g/liter) and sodium bicarbonate (1 g/liter) for 72 h at 37°C under a 5% CO2 atmosphere. The harvested bacteria were fixed with formalin and served as the T-cell antigen.

**T-cell clones.** Two *A. actinomycetemcomitans* Omp29-specific Th1-type T-cell clones (G21 and G23) and another Th1 clone reactive with an unknown antigen of *A. actinomycetemcomitans* other than Omp29 (G26) (18) were used in this study. These T-cell clones were maintained by weekly stimulation with irradiated (3,300 rads) syngeneic rat spleen APC and formalin-fixed whole *A. actinomycetemcomitans* antigen.

**Rat GEC and endothelial cells.** Rat GEC lines (REC-1 and REC-2) were established from two Rowett rats (2 weeks old) as palatal gingival explants described previously (24). Briefly, the palatal gingival explants were incubated in tissue culture plates containing Dulbecco Modified Eagle Medium plus 10% fetal bovine serum. After 2 weeks, epithelial cells grew out from the explants and were

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**FIG. 1.** Colocalization of cytokeratin and HLA-DR or B7-1 expression on human gingival epithelium from diseased or healthy gingival tissues as observed by confocal microscopy. Cytokeratin, an epithelial cell-specific phenotypic marker, is displayed as green (FITC) single exposure (A, D, G, and J). HLA-DR (B and H) and B7-1 (E and K) are displayed as red (Texas Red) single exposure. The colocalization of cytokeratin with HLA-DR (C and I) or cytokeratin with B7-1 (F and L) is displayed as a yellow color after double exposure of both FITC and Texas Red. Bars, 10 μm. The sections shown are representative of four diseased and three healthy periodontal tissues stained identically.
The subconfluent REC-1 or MAT-1 cells were stimulated with IFN-γ at which time the cell density was approximately 3 × 10^6 cells/well. The expression profile of each molecule on GEC was analyzed by an EPICS XL-MCL system (Coulter, Hialeah, Fla.) supplemented with epidermal growth factor (5 ng/ml; Serotec, Raleigh, N.C.) and bovine pituitary extract (30 to 50 μg/ml). The culture and characteristics of the endothelial cell clone (MAT-1) derived from a Rowett rat used herein have been previously described (17, 18). Briefly, MAT-1 was maintained in RPMI 1640 supplemented with 2.5% rat brain conditioned medium. Cells used for the following experiments were taken between passages 4 and 6.

Immunocytochemical analyses of cultured cells. Two rat GEC lines (REC-1 and REC-2) were fixed with 2% paraformaldehyde and then treated with 0.5% Triton X-100 for 1 h at room temperature. Mouse anti-pancytokeratin MAb (Sigma), which reacts with simple, cornifying, and noncornifying squamous epithelia, was employed as the first antibody, and FITC-labeled rat anti-mouse IgG (Jackson Immunoresearch Laboratories, Inc., West Grove, Pa.) was used as the secondary antibody. The staining pattern and morphological appearance of GEC were analyzed by fluorescent phase-contrast microscopy.

B7-1 and B7-2 detection by reverse-transcription-PCR. The methods for total RNA extraction and reverse transcription-PCR have been described previously (17). Primer pairs for rat B7-1 and B7-2 were as follows: B7-1 5’ primer, TGA AGCCATGTTCATAGTGGCCAG (sense, 12 to 36), and B7-1 3’ primer, CACCGTGAACATCTACTCAATGTA (antisense, 683 to 708) (31); B7-2 5’ primer, GCTCGTAGTATTTTGGCAGGACC (sense, 17 to 39), and B7-2 3’ primer, CGGGTATCCTTGCTTAGATGAGC (antisense, 331 to 353). cDNA amplification was accomplished for 26 cycles for B7-1 or B7-2 along with β-actin (25) as an internal control (94°C for 30 s, 60°C for 1 min, and 72°C for 1 min, followed by a single final elongation at 72°C for 10 min). The PCR products were separated in 1.7% agarose gels and stained with ethidium bromide.

Cell staining and flow cytometry. Single-cell suspensions of each cell line were incubated with anti-MHC class II MAb (OX6, IgG1; Serotec, Raleigh, N.C.), anti-CD80 MAb (3H5, IgG1; BD PharMingen) or isotype-matched control MAb at 10 μg/ml; followed by FITC-labeled rat anti-mouse IgG (Jackson Immunoresearch). The expression profile of each molecule on GEC was analyzed by an EPICS Altra Flow Cytometer (Beckman Coulter, Miami, Fla.).

Antigen presentation to T-cell clones and lymph node lymphocytes by GEC and spleen cells. REC-1 or MAT-1 cells (endothelial cell clones) were seeded at 5 × 10^5 cells/well on 96-well plates and cultured until they reached confluence, at which time the cell density was approximately 4 × 10^6 cells/well. The subconfluent REC-1 or MAT-1 cells were stimulated with IFN-γ (500 U/ml; R&D Systems, Minneapolis, Minn.) in the presence or absence of formalin-fixed A. actinomycetemcomitans (10^9 cells/well) for 3 days in advance. REC-1 and MAT-1 were further treated for 45 min with mitomycin C (MMC; Sigma) at 25 μg/ml on day 0. T-dense cells (10^5 cells/well) were then added with or without anti-MHC class II MAb (10 μg/ml; Serotec, Raleigh, N.C.). Therefore, the real ratio of REC-1 to T cells was 3:1 to 4:1 as T cells were applied to confluent REC-1 cells. In some experiments, CTLA4-Ig fusion protein or control L6 fusion protein was also added (10 μg/ml each) to the culture. T-cell proliferation was assessed by measuring [3H]thyidine incorporation during the last 16 h of a total of 3 days of culture.

T-cell response to superantigen. Fresh lymph node lymphocytes were isolated from cervical lymph nodes, and the T-cell population was enriched by passing the lymphocytes through glass wool and nylon wool columns as previously reported (17). Irradiated (3,300 rads) syngeneic rat spleen cells or MMC-treated REC-1 cells that were precultured with or without IFN-γ and antigens 3 days in advance were used as APC. Fixed A. actinomycetemcomitans (10^9 well) or staphylococcal enterotoxin A (SEA; Sigma) at appropriate doses were utilized as antigens. Lymph node T cells (10^5/well) or G21 T clone cells (10^5/well) were cocultured with APC as described above.

RESULTS

Expression of HLA-DR and B7-1 on human gingival epithelium from patients with chronic periodontitis. A positive staining pattern of cytokeratin, an epithelial cell-specific molecular marker (21), was observed in the basal or suprabasal cells of the gingival epithelium (green areas in the left panel of Fig. 1). Also shown is Texas Red-labeled single staining (red areas in the center panel of Fig. 1) for HLA-DR (Fig. 1B and H) or for B7-1 (Fig. 1E and K). The sections stained with Texas Red-labeled antibodies were further double exposed in laser-scanning confocal microscopy in conjunction with the FITC-labeled cytokeratin staining (yellow or green areas in the right panel in Fig. 1). Consequently, colocalization of cytokeratin and HLA-DR (Fig. 1C) or cytokeratin and B7-1 (Fig. 1F) was revealed by the yellow- and green-positive cells in the diseased gingival tissues. However, in the gingival tissue from healthy subjects, cytokeratin staining did not overlap with either HLA-DR or B7-1 (Fig. 1I and L, respectively), as demonstrated by the lack of yellow- or green-positive cells. Among the gingival tissue samples from the three patients that showed HLA-DR expression, 20 to 30% of GECs, especially in the basal membrane layer, were positive for HLA-DR. It is noteworthy that HLA-DR-positive (but cytokeratin-negative) cells were observed in the basal layers of epithelium in both diseased and healthy gingival tissues (Fig. 1C and I [white arrows]). These HLA-DR-bearing cells may be Langerhans’ cells or dendritic cells based on their configuration. Similarly, we examined two ad-
ditional identically stained healthy tissues (three in all) and four tissues from patients with chronic periodontitis, by confocal microscopy. Healthy tissues did not demonstrate either HLA-DR or B7-1. By contrast, the examination of four diseased tissues from the patients with chronic periodontitis showed that three stained for HLA-DR and three stained for B7-1.

Identification of rat GEC. Rat GEC lines from gingival tissues were established and maintained as closely packed epithelial cell-like cells with a so-called “cobblestone appearance” (Fig. 2). Cells grown for between four and six passages were used for all experiments. Immunoreactivity for cytokeratins was observed in the cytoplasm of all cultured GEC cell lines (Fig. 2B), and similar results were obtained when REC-2 cells were identically stained (data not shown). Thus, these results confirmed that the cell lines from rat gingival tissue were epithelial cells that express cytokeratin.

Expression of B7-1 mRNA by GEC. Total RNA was isolated from REC-1, REC-2, and MAT-1 cells after incubation in the presence or absence of IFN-γ for 3 days. Total RNA was also isolated from lipopolysaccharide (LPS)-stimulated spleen cells (24 h) as a positive control. LPS-treated spleen cells expressed both B7-1 and B7-2 mRNA. Both REC-1 and REC-2 cells, but not MAT-1 cells, expressed B7-1 mRNA irrespective of stimulation with IFN-γ. Although the LPS-stimulated spleen cells expressed B7-2 mRNA, the three cell lines, REC-1, REC-2, and MAT-1, did not show B7-2 mRNA expression (Fig. 3A).

Antigen-specific T-cell proliferation induced by IFN-γ-treated REC in comparison to spleen APC. In order to evaluate if B7-1-expressing REC cells can stimulate T cells by MHC class II-mediated antigen presentation, REC-1 cells were preincubated with IFN-γ and cocultured with G21 Th1 clone cells (10⁴ cells/well) in the presence or absence of A. actinomycetemcomitans (Aa). Serially diluted MMC-treated spleen cells were used as a source of professional APC to stimulate G21 cells (10⁴ cells/well) in the presence of A. actinomycetemcomitans. The coculture of G21 cells was incubated for 3 days, and [³H]thymidine was applied to the coculture during the last 16 h of the total culture period * significantly different from A. actinomycetemcomitans (−) control; **, significantly different from T-cell proliferation induced by REC-1 in the presence of A. actinomycetemcomitans). Note that spleen APC at 3,000 cells/well did not show any difference from the [³H] incorporation induced by 30,000 REC-1 cells/well with A. actinomycetemcomitans (+). (C) Flow cytometry analyses of B7-1, B7-2, and MHC class II expression by REC. Subconfluent REC-1, REC-2, and rat aorta endothelial cell clone MAT-1 were cultured in the presence or absence of IFN-γ (500 U/ml) for 3 days. Isolated single cell suspension was reacted with MAbs anti-MHC class II (OX-6), anti-B7-1 (3H5), or anti-B7-2 (24F), followed by FITC-conjugated anti-mouse IgG. Profiles of control MAb (PA20; open pattern) and specific MAbs (solid pattern) are shown on a logarithmic scale. LPS-treated spleen cells (24 h) were employed as a positive control.
in an antigen-specific manner. The \[^{3}H\]thymidine incorporation of G21 induced by REC-1 (30,000 cells/well) with \textit{A. actinomycetemcomitans} was comparable to that for spleen APC at 3,000 cells/well, indicating that the efficiency of antigen presentation by spleen APC was at least 10 times higher than that by REC-1.

**Flow cytometry analyses of MHC class II, B7-1, and B7-2 on rat GEC.** Surface expression of MHC class II, B7-1, and B7-2 on rat GEC lines (REC-1 and REC-2) was analyzed by flow cytometry (Fig. 3C). The expression of MHC class II was induced on both REC-1 and REC-2 cells after stimulation with IFN-\(\gamma\) (500 U/ml) for 3 days. Irrespective of the IFN-\(\gamma\) treatment, REC-1 and REC-2 cells expressed B7-1 constitutively, but B7-2 was not detected. Spleen cells that were treated with LPS for 24 h upregulated MHC class II, B7-1, and B7-2 expression significantly. Although the MAT-1 endothelial cell clone expressed MHC class II in the presence of IFN-\(\gamma\), little or no B7-1 or B7-2 expression was detected. It is noteworthy that the level of MHC class II expression by LPS-treated spleen cells was apparently higher than that by REC-1 or REC-2 cells stimulated with IFN-\(\gamma\). The discrepancy in intensity of MHC class II expression appeared to be responsible for the difference in the efficiency of antigen presentation between APC and REC (Fig. 3B).

**B7-1 expression appears to be responsible for antigen-specific T-cell clone proliferation induced by IFN-\(\gamma\)-treated GEC.** G21 T clone cells were cocultured for 3 days with MMC-treated REC-1 cells that had been previously treated with IFN-\(\gamma\) and/or antigen. These T clone cells showed significant proliferation in response to the IFN-\(\gamma\) and \textit{A. actinomycetemcomitans}-treated REC-1 compared to single treatment with IFN-\(\gamma\) or \textit{A. actinomycetemcomitans} alone, as measured by \[^{3}H\]thymidine incorporation (Fig. 4A). Endothelial clone cells (MAT-1) did not induce antigen-specific proliferation of G21 CD4\(^{+}\) T clone cells. The proliferation of T clone cells induced by IFN-\(\gamma\) and antigen-treated REC-1 was abrogated by the presence of anti-MHC class II mAb or CTLA4-Ig (Fig. 4B). These findings suggested that B7-1 expressed by GEC could provide a functional costimulatory signal along with MHC class II signaling to the antigen-specific T cells, also indicating that epithelial cells expressing MHC class II and B7-1 can successfully present antigen to T cells.

**Lack of superantigen activity in \textit{A. actinomycetemcomitans} antigen for GEC antigen presentation to T cells.** It is well known that bacterial superantigen can be presented on MHC class II without processing in the APC, because superantigen binds directly to the external structure of MHC class II and bridges with the T-cell receptor (30). To examine if bacterial antigen presentation by GEC is due to superantigen activity from \textit{A. actinomycetemcomitans}, the staphylococcal superantigen SEA was used in the T-cell proliferation assay to ascertain the nature of a superantigen response (Fig. 5). Both G21 T clone cells and lymph node T cells responded to SEA by proliferating when cocultured with spleen APC (Fig. 5A). Although splenic APC combined with \textit{A. actinomycetemcomitans} antigen induced the proliferation of G21 T clone cells, such splenic APC did not stimulate lymph node T cells (Fig. 5A), indicating that lymph node T cells did not contain \textit{A. actinomycetemcomitans}-specific T cells and that G21 T clone cells were sensitive to SEA-mediated superantigen activity. When REC-1 cells were utilized as APC, both G21 T clone cells and lymph node T cells demonstrated significantly elevated proliferation in response to SEA whereas \textit{A. actinomycetemcomitans} induced only the antigen-specific proliferation of G21 Th1
cloned cells but no antigen-specific proliferation of lymph node T cells. Since REC-1 cells express MHC class II in the presence of IFN-γ (Fig. 3B) and since lymph node T cells are susceptible to superantigen-mediated signaling from MHC class II, if A. actinomycetemcomitans contains superantigen, the column indicated by the symbol in Fig. 5A and B should be significantly higher than that for medium alone. However, the particular columns were not different from the medium-alone groups, demonstrating that REC-1-mediated proliferation of G21 T cells was not due to the superantigen activity of A. actinomycetemcomitans. Taking these results together, it is highly plausible that internally incorporated and processed A. actinomycetemcomitans antigen is presented by MHC class II of REC-1.

**DISCUSSION**

We examined the potential role of GEC in antigen presentation to Th1 clone cells in a syngeneic rat model. Very importantly, rat GEC lines in the present study constitutively expressed B7-1, but not B7-2, reproducing the features of human GEC in the periodontal disease lesion (Fig. 1). We found that IFN-γ and A. actinomycetemcomitans antigen-stimulated rat GEC could induce specific proliferation of Th1 clone cells in an MHC class II and B7-1-dependent manner. This finding suggested that bacterial antigens are incorporated into rat GEC and are presented by MHC class II after undergoing enzymatic processing into small peptides, as has been demonstrated for epithelial cells (11, 26–28). Therefore, GEC appeared to be involved not only in innate immune responses (20) but also in directing adaptive immune responses (3).

Epithelial cells have previously been demonstrated to phagocytose and digest extracellular debris, erythrocytes, or even microorganisms such as Candida albicans (7), Mycobacterium leprae (28), and A. actinomycetemcomitans (26, 27). A. actinomycetemcomitans appears to gain entry into GEC through a mechanism of phagocytosis associated with F-actin rearrangement (4, 27). The existence of intestinal epithelial cell-mediated bacterial antigen processing and presentation is well documented (11). However, until the present study, the question whether GEC can present cytoplasm-incorporated bacterial antigen to T cells has not been addressed. In this study, fixed A. actinomycetemcomitans antigen seemed to be presented by MHC class II expressed on rat GEC. We have demonstrated live A. actinomycetemcomitans internalization into REC-1 cells as determined by the bacterial entry assay system of Meyer and colleagues (4, 27; unpublished data). Since fixed bacteria were used in the present study, it would be intriguing to investigate if only dead or fixed bacteria can be processed and presented by MHC class II and whether live bacteria can avoid this host GEC function. Of course, this would be of particular interest in assessing GEC recruitment of T cells in periodontal disease.

It is relatively difficult to determine if A. actinomycetemcomitans Omp 29 antigen is processed and presented by MHC class II molecules in rat GEC, especially since it is also unclear if only the Omp 29 is taken up by GEC or if whole bacteria are phagocytized and processed by GEC. However, the evidence that fixed intact A. actinomycetemcomitans used in this study did not possess superantigen activity (Fig. 5) indicated that Omp 29 is incorporated into GEC, probably processed in the GEC, and presented to Omp 29-specific T clone cells. Petit and Stashenko also reported that periodontal bacteria including A. actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, and Prevotella nigrescens did not have detectable superantigen activities (32).

B7 costimulatory molecules are programmed for expression by professional APC, which include macrophages, dendritic cells, Langerhans’ cells, and B cells. Professional APC that express B7-1 and B7-2 also provide positive costimulatory signals through binding to CD28 expressed on T cells (2, 10, 22, 33). On the other hand, nonprofessional APC usually do not
express B7 costimulatory molecules (12). However, we and others have reported that epithelial cells in some tissues express aberrant B7-1 (9, 15, 29, 33). To investigate the significance of the aberrant B7-1 expression on epithelial cells in gingival tissues of chronic periodontitis, we took advantage of syngeneic antigen-specific Th1 clone cells and the GEC from Rowett rat cell lines (REC). Since the affinity between the gingival tissues of chronic periodontitis, we took advantage of others have reported that epithelial cells in some tissues express B7 costimulatory molecules (12). However, we and others have reported that epithelial cells in some tissues express B7 costimulatory molecules (12). However, we and others have reported that epithelial cells in some tissues express B7 costimulatory molecules (12). However, we and others have reported that epithelial cells in some tissues express B7 costimulatory molecules (12).

Considering that IFN-γ is the most consistently up-regulated cytokine in diseased gingival tissue (34), it is plausible that the IFN-γ produced in the diseased lesion is responsible for the up-regulation of MHC class II expression on the gingival epithelium in vivo. Current findings have demonstrated that activated T cells can up-regulate the expression of the osteoclast differentiation factor RANKL (35–37). Therefore, GEC-mediated T-cell activation may be associated with the up-regulation of RANKL expression and promotion of bone resorption. However, professional APC in the center of the inflammation may be located closer to alveolar bone than the GECs are, and they appear to be much more potent in activation of T cells by antigen presentation with MHC class II. GECs which are more closely exposed to the bacteria in the gingival crevice would have better access to the bacterial antigens. Additional study will address these questions and should further elucidate the significance of antigen presentation by GECs in periodontal disease.

ACKNOWLEDGMENTS

We thank Peter Linsley for CTLA4-Ig and L6. We also thank Michael Glogauer and Jerome Haber for provision of patient gingival disease. Considering that IFN-γ is the most consistently up-regulated cytokine in diseased gingival tissue (34), it is plausible that the IFN-γ produced in the diseased lesion is responsible for the up-regulation of MHC class II expression on the gingival epithelium in vivo. Current findings have demonstrated that activated T cells can up-regulate the expression of the osteoclast differentiation factor RANKL (35–37). Therefore, GEC-mediated T-cell activation may be associated with the up-regulation of RANKL expression and promotion of bone resorption. However, professional APC in the center of the inflammation may be located closer to alveolar bone than the GECs are, and they appear to be much more potent in activation of T cells by antigen presentation with MHC class II. GECs which are more closely exposed to the bacteria in the gingival crevice would have better access to the bacterial antigens. Additional study will address these questions and should further elucidate the significance of antigen presentation by GECs in periodontal disease.

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