BACTERIAL PATHOGENICITY

***N*-Acetyl-D-galactosamine specific lectin of *Eikenella corrodens* induces intercellular adhesion molecule-1 (ICAM-1) production by human oral epithelial cells**

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During the acute inflammatory response in periodontitis, gingival epithelial cells are considered to play important roles in the recruitment of inflammatory cells to the site of infection through the secretion of chemokines. However, little is known about the expression of molecules that are involved in the interaction between the epithelium and neutrophils following bacterial attachment. Earlier work reported that periodontopathogenic *Eikenella corrodens* strain 1073 up-regulated the expression and secretion of chemokines such as interleukin-8 (IL-8) from KB cells (a human oral epithelial cell line derived from a human oral epidermoid carcinoma). To elucidate the mechanism of the transmigration of neutrophils through the epithelium, the present study investigated the expression of adhesion molecules on KB cells in response to *E. corrodens* attachment. Adhesion molecule gene expression was assessed by RT-PCR and adhesion proteins expressed on KB cell surfaces were determined by cell-based ELISA and FACS. In RT-PCR, ICAM-1 mRNA levels were significantly increased within 1 h in response to exposure to *E. corrodens* and continued to increase over the 12-h period of study. In ELISA, increased surface ICAM-1 expression was paralleled by increased ICAM-1 mRNA levels. Furthermore, the increases in ICAM-1 expression on epithelial cells infected with *E. corrodens* were observed to be due to the *N*-acetyl-D-galactosamine (GalNAc) specific bacterial lectin-like substance of *E. corrodens* (EcLS), which was one of the adhesins of *E. corrodens*. This is the first study to report that a bacterial lectin-like substance increased the expression of ICAM-1 on gingival epithelial cells.

Introduction

Periodontitis is the inflammatory response in gingival and connective tissue elicited by bacterial colonisation in periodontal pockets. In this response, pocket epithelial cells may be the first cells of defence against periodontopathic bacteria and may be the primary source of the pro-inflammatory and inflammatory cytokine signals for initiation of the inflammatory response to periodontal pathogens. The initiation of periodontitis is often characterised by an influx of

neutrophils into the periodontal tissues across the epithelium [1]. When neutrophils transmigrate in large numbers, the resulting epithelial discontinuities are thought to represent precursor lesions for erosions and, subsequently, mucosal ulcers that develop in certain inflammatory periodontal diseases [2].

In the transmigration of neutrophils through gingival epithelial cells, cell–cell interactions play an important and probably central role. Tonetti *et al.* [3] reported the presence of high densities of ICAM-1 and interleukin-8 (IL-8)-positive cells in the most superficial layers of the junctional epithelium in periodontal pockets in periodontitis. Moreover, several studies [4–7] reported that the levels of ICAM-1 at the junctional epithelium were

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correlated with the intensity of the clinical conditions. These findings suggest that expression of cell adhesion molecules on gingival epithelial cells may participate in neutrophil homing and epithelial cell adhesion in periodontopathic bacteria-associated periodontal inflammation. Therefore, in periodontitis the production of chemokines and adhesion molecules could provide a means of recruiting and retaining inflammatory cells within the gingival epithelial layer, contributing to periodontopathic bacteria-mediated tissue injury.

Eikenella corrodens, a facultative gram-negative anaerobic rod, is found predominantly in subgingival plaque in patients with advanced periodontitis and may also cause extra-oral infections including abscesses, endocarditis, osteomyelitis, keratitis, conjunctivitis and cellulitis [8–11]. Earlier studies reported that *E. corrodens* 1073 has a cell-associated *N*-acetyl-D-galactosamine (GalNAc) specific lectin-like substance (EcLS) that mediates its adherence to various host tissue cell surfaces and oral bacteria [12–16]. It was also found that EcLS stimulates the proliferation of murine B cells [17]. EcLS is a large molecule and is composed of several components including 25-, 45- and 300-kDa proteins [18, 19]. Moreover, it has been shown that soluble products from *E. corrodens* 1073 induce the secretion and the expression of IL-8 by a human oral epidermoid carcinoma cell line (KB) [20, 21].

To elucidate further the mechanisms of the trans-epithelial migration of neutrophils the present study investigated whether *E. corrodens* 1073 induces the expression of ICAM-1, one of the key adhesion molecules in the transepithelial migration of neutrophils, on gingival epithelial cells after infection with *E. corrodens*. Furthermore, the properties of the ICAM-1 induction factors from *E. corrodens* were characterised.

Materials and methods

Bacteria and growth conditions

E. corrodens 1073 was provided by S. S. Socransky (The Forsyth Institute, Boston, MA, USA) and was cultured at 37°C in tryptic soy broth containing KNO₃ 2 mg/ml and haemin 5 µg/ml under anaerobic conditions (N₂ 95%, CO₂ 5%).

Cell culture

The KB cell line (derived from a human oral epidermoid carcinoma) was provided by T. Okamoto (Hiroshima University School of Dentistry, Hiroshima, Japan). The KB cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Grand Island, NY, USA) supplemented with 2 mM L-glutamine, fetal bovine serum (FBS; JRH Biosciences, Lenexa, KA, USA) 10% v/v, penicillin 50 IU/ml and

streptomycin 50 µg/ml at 37°C in a water-saturated atmosphere of CO₂ 5% in air.

Infection of KB cells

Approximately 10⁵ KB cells in DMEM were seeded into wells of 24-well tissue culture plates and incubated until confluent monolayers developed. The bacteria were pelleted by centrifugation, washed twice in phosphated-buffered saline (PBS, pH 7.2), and suspended in DMEM without serum and antibiotics at a concentration of (1.0 × 10⁸)–(2.0 × 10⁸) cfu/ml. The bacterial concentrations were determined spectrophotometrically according to standard curves. KB cell layers were washed three times with Hanks's Balanced Salts Solution (Gibco), inoculated with 500 µl of the microbial suspension, and incubated at 37°C. As a control, interferon(IFN)-γ (Boehringer Mannheim, Tokyo, Japan) 40 ng/ml was added. For the kinetics studies, cells were incubated for 0.5, 1, 4, 6 and 12 h. At the end of these incubation periods, the culture medium was collected and centrifuged, and the supernate was stored at –20°C until assayed. RNA was extracted immediately from the cells as described below.

RNA extraction and cDNA preparation

Total RNA was extracted from KB cells, prepared as described above, with a Catrimox-14 (Iowa Biotechnology, Coralville, IA, USA) according to the manufacturer's instructions. RT-PCR was performed as described previously [22]. The following primers were used to amplify a fragment of ICAM-1 from cDNA: 5'-CGTGCCGCACTGAACTGGAC-3' (sense) and 5'-CCTCACACTTCACTGTACCT-3' (antisense). After pre-denaturation for 2 min at 94°C, the PCR conditions for ICAM-1 were as follows: denaturation at 94°C for 1 min, annealing at 60°C for 1 min and extension at 72°C for 1 min. The number of PCR cycles was 36 to ensure detection of low-abundance mRNA. The GAPDH housekeeping gene transcript was used as the control. A sample of each amplified product was subjected to electrophoresis in an agarose 1.5% gel (TaKaRa, Shiga, Japan), stained with ethidium bromide and visualised by UV illumination.

For negative controls, the Moloney murine leukaemia virus RT was omitted from the cDNA synthesis mixture to ensure amplification from genomic DNA.

The amount of ICAM-1 mRNA, compared with that of GAPDH mRNA in the controls, was semi-quantified by scanning densitometry of the gel with NIH Image 1.62, as reported by Darveau *et al.* [23].

Assay of sICAM-1 release

KB cells were infected with *E. corrodens* for 0.5, 1, 4, 6 and 12 h as described above. At each time point,

samples of cell culture supernate were removed and the concentration of sICAM-1 was measured by ELISA. A commercially available ELISA kit (Biosource, Camarillo, CA, USA) for the quantification of sICAM-1 was used as described in the manufacturer's instructions.

Cell-based ELISA

KB cells (5×10^4 cells/well) were seeded into 96-well plates and cultured for 48 h. Then, KB cells were cultured with *E. corrodens* cells or EcLS for 10 h in the presence or absence of GalNAc. After incubation, KB cells were washed with PBS and were fixed with paraformaldehyde 4% in PBS. Then KB cells were blocked with bovine serum albumin (BSA) 2% for 1 h. KB cells were incubated for 30 min with anti-human ICAM-1 monoclonal antibody (MAb) (Ancell, Bayport, MN) at a dilution of 1 in 200. After washing twice, cells were incubated for 40 min with biotin-conjugated goat anti-mouse IgG (DAKO, Tokyo, Japan) at a dilution of 1 in 50 and incubated with avidin-conjugated horseradish peroxidase for 30 min. The assay was developed by addition of TMB peroxidase EIA substrate (BioRad, Tokyo, Japan).

Flow cytometric analysis

Monolayers of KB cells were detached by incubation with trypsin 0.25% and EDTA 0.25% in calcium- and magnesium-free PBS (pH 7.2). For single-label flow cytometric analysis of ICAM-1 expression, 2×10^5 cells/ml were incubated for 30 min on ice with $2.5 \mu\text{l}$ of anti-human ICAM-1 MAb in a total volume of $500 \mu\text{l}$ of PBS containing 0.05 mM EDTA, BSA 0.1% and FBS 0.05%. After washing twice, cells were incubated for 30 min on ice with $500 \mu\text{l}$ of a dilution (1 in 100) of fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG1 (DAKO). Cells were washed twice with PBS then the immunofluorescence of the FITC single label was measured with a flow cytometer (Coulter XL-MCL, Coulter Electronic, Hialeah, FL, USA).

Neutrophil adhesion assay

KB cells were seeded at a concentration of 2×10^5 cells/ml into 24-well culture plates and stimulated by *E. corrodens* for 10 h in the presence or absence of 50 mM GalNAc. As a positive control, parallel cultures were stimulated for the same duration with IFN- γ 40 ng/ml. After incubation for 10 h, medium containing bacteria or IFN- γ was removed and KB cells were washed twice with HBSS. For the neutrophil adhesion assay, neutrophils were resuspended in DMEM at a concentration of 1.0×10^6 cells/ml. Neutrophils were added to confluent KB cell monolayers infected with *E. corrodens* for 10 h. The cultures were centrifuged at room temperature for 5 min at 50 g. Cultures were incubated for 30 min at 37°C, after which monolayers

were gently washed three times with PBS to remove non-adherent neutrophils. Neutrophil adherence was quantified by assaying myeloperoxidase activity in the adherent neutrophils by the method of Huang *et al.* [22]. For myeloperoxidase assays, $450 \mu\text{l}$ of PBS containing Triton X-100 0.5% were added to the epithelial cell/neutrophil co-cultures, followed by the addition of $50 \mu\text{l}$ of 1.0 M citrate buffer, pH 4.2. Lysates were centrifuged to remove debris, and enzymic activity was determined by adding a sample of the cell lysates to a solution containing 1 mM ABTS (2,2'-azio-di-{3-ethyl} dithiazoline-6-sulphonic acid), 10 mM H_2O_2 in 100 mM citrate buffer (pH 4.2). Absorbance was determined with a microplate reader (BioRad) at 405 nm.

Purification of EcLS

EcLS was purified from *E. corrodens* 1073 cells on the basis of its haemagglutination activity [16]. *E. corrodens* 1073 cells were sonicated in the presence of Triton X-100 0.1% and 10 mM EDTA. The supernate was collected and dialysed against CaCl_2 0.1% (PBS-TC). The dialysate was applied to a galactosamine affinity column (Pierce, Rockford, IL, USA) and eluted with PBS-TC containing 10 mM GalNAc. The fraction eluted with 10 mM GalNAc was precipitated with ethanol and the precipitate was dialysed against distilled water and lyophilised.

Statistical analysis

All statistical analyses were performed by the unpaired Student's *t* test. Differences in the data were considered significant when the probability value was $<5\%$ ($p < 0.05$).

Results

Effect of *E. corrodens* on ICAM-1-specific mRNA in KB cells

To assess the specific induction of ICAM-1 at the mRNA level, RT-PCR was performed with the RNA isolated from KB cells after bacterial stimulation for 8 h, and the amounts of ICAM-1 mRNA were semi-quantified by scanning densitometry of the gel with the NIH Image 1.62 analysis software program, as reported by Darveau *et al.* [23]. As shown in Fig. 1, infection of KB cells with *E. corrodens* 1073 significantly increased the level of ICAM-1-specific mRNA compared with those from uninfected control cells. IFN- γ was used as a positive control.

Time-course analysis of ICAM-1 mRNA expression following infection with *E. corrodens*

A time-course analysis of ICAM-1 mRNA expression by KB cells is shown in Fig. 2. The expression of ICAM-1 mRNA levels increased within 30 min after

Fig. 1. ICAM-1 mRNA expression by KB cells after stimulation with *E. corrodens* 1073. RNA was isolated from KB cells after bacterial stimulation for 10 h. cDNA synthesis and RT-PCR were performed as described in *Materials and methods*. The GAPDH housekeeping gene was used as the control. Lane 1, positive control (IFN- γ); 2, *E. corrodens* 1073; 3, control (medium alone); M, markers. The amounts of ICAM-1 mRNA, compared with those of GAPDH mRNA in the controls, were semi-quantified by scanning densitometry of the gel with NIH Image 1.62.

Fig. 2. Time-course analysis of ICAM-1 gene expression induced from KB cells by *E. corrodens* 1073 stimulation. RNA was isolated from KB cells after exposure to bacterial stimulation for 0.5, 1, 4, 6 or 12 h. cDNA synthesis and RT-PCR were performed as described in *Materials and methods*. The GAPDH housekeeping gene was used as a control. The amounts of ICAM-1 mRNA, compared with those of GAPDH mRNA in the controls, were semi-quantified by scanning densitometry of the gel with NIH Image 1.62.

infection with *E. corrodens* and continued over the 12-h study period.

Effect of E. corrodens on surface ICAM-1 expression by KB cells

To determine whether the enhanced expression of ICAM-1 mRNA in KB cells was associated with the increased expression of surface expression of ICAM-1, the effects of *E. corrodens* on the expression of ICAM-1 on KB cell surfaces were examined by cell-based

ELISA and flow cytometry. KB cells were incubated with *E. corrodens* 1073 for 10 h and the expression levels of ICAM-1 on cell surfaces were measured by cell-based ELISA. Although surface ICAM-1 levels were little increased in the absence of *E. corrodens* infection, IFN- γ increased the expression of surface ICAM-1 on KB cells by two-fold compared with the uninfected controls (Fig. 3a). After infection of KB cells by *E. corrodens* 1073, the expression of surface ICAM-1 levels on KB cells was significantly up-regulated by 45% compared with non-stimulated cells (control) (Fig. 3a).

Monolayers of KB cells were co-cultured with *E. corrodens* 1073 for 10 h and the quantitative expression of ICAM-1 on KB cells was determined by flow cytometry. In the absence of bacteria (control), the KB cells were found to constitutively express low levels of ICAM-1; the expression of surface ICAM-1 levels on KB cells was markedly increased compared with the controls (Fig. 3b). The distribution of ICAM-1 expression by KB cells following infection with *E. corrodens* 1073 was unimodal and narrow as judged by flow cytometry. This suggests that KB cells are uniform in their potential for up-regulation of cell surface ICAM-1 expression in response to *E. corrodens* 1073.

Surface ICAM-1 expression and neutrophil adhesion to E. corrodens-infected KB cells

The possibility that ICAM-1 expressed on KB cells enhanced the binding of neutrophils to KB cells was then tested. In the absence of bacteria (control), the binding of neutrophils to KB cells was little increased but IFN- γ significantly increased the binding of neutrophils to KB cells by two-fold compared with the controls. After the infection of KB cells by *E.*

Fig. 3. Surface ICAM-1 expression on KB cells following infection with *E. corrodens*. Confluent monolayers of KB cells cultured in 24- or 96-well plates were cultured with *E. corrodens* for 10 h. ICAM-1 expression on cell surfaces was measured by cell-based ELISA (a) and flow cytometry (b) as described in *Materials and methods*. Representative findings are shown as the means and SD from three independent experiments. *Significant difference ($p < 0.001$) versus control values.

corrodens 1073, the binding of neutrophils to KB cells was significantly up-regulated by 45% compared with non-stimulated cells (control) (Fig. 4).

Effect of GalNAc on expression of ICAM-1 on KB cells after infection with E. corrodens

Previous work demonstrated that GalNAc inhibited the adhesion of *E. corrodens* to KB cells and slightly down-regulated the IL-8 and IL-6 mRNAs expressed in KB cells in response to *E. corrodens* 1073 [20]. To clarify the mechanism of ICAM-1 expression on KB cells after bacterial stimulation and to test the possibility that EcLS are inducers of ICAM-1 expression, the present study observed the effect of GalNAc on the expression of ICAM-1 on KB cells.

In cell-based ELISA (Fig. 5a) and flow cytometric analysis (Fig. 5b), 50 mM of GalNAc down-regulated the expression of ICAM-1 on KB cells after infection

Fig. 4. Neutrophil adhesion assays. KB cell monolayers in 24-well plates were incubated with *E. corrodens* or IFN-γ (40 ng/ml) as described in *Materials and methods* for 10 h. Then, monolayers were washed and 1×10^6 freshly prepared peripheral blood neutrophils per well were added and allowed to adhere for 30 min at 37°C. Total lysates were analysed for myeloperoxidase activity as a measure of the number of neutrophils adhering to monolayers. Neutrophil adherence is expressed as the percentage of adhesive cells compared with 100% deposited cells. Representative findings are shown as the means and SD from three independent experiments. *Significant differences ($p < 0.001$) versus control values.

with *E. corrodens* 1073 to the control level (Fig. 5c). Moreover, adhesion of neutrophils to KB cells infected with *E. corrodens* 1073 was inhibited to the control level. These findings strongly suggest that EcLS may play an important role in the expression of ICAM-1 on KB cells after infection with *E. corrodens* 1073.

Effect of EcLS on expression of ICAM-1 mRNA and surface ICAM-1 on KB cells

To confirm the role of EcLS in the expression of ICAM-1 induced by *E. corrodens* 1073, the present study determined whether EcLS could increase the expression of ICAM-1 on KB cells. In RT-PCR, EcLS enhanced the expression of ICAM-1 mRNA in a dose-dependent manner and the addition of GalNAc completely abolished the expression of ICAM-1 mRNA (Fig. 6). In cell-based ELISA and flow cytometric analysis, EcLS increased the expression of surface ICAM-1 on KB cells to levels equivalent with that of *E. corrodens* whole cells and the addition of GalNAc to the culture mixture reduced the increase of surface ICAM-1 expression to the control level (Fig. 7a and b). In neutrophil adhesion assays, EcLS increased the binding of neutrophils to KB cells and the addition of GalNAc to the culture mixtures also decreased the adherence of neutrophils to the control level (Fig. 7c). These findings appeared to be similar to those observed in experiments with *E. corrodens* whole cells. Taken together, these findings suggest that EcLS may be the crucial factor that induces the expression of ICAM-1 expression on gingival epithelial cells infected with *E. corrodens*.

Fig. 5. Inhibitory effects of GalNAc on the expression of ICAM-1 by KB cells following infection with *E. corrodens* 1073. KB cell monolayers in 24- or 96-well plates were cultured with *E. corrodens* 1073 in the presence or absence of GalNAc (50 mM) for 10 h. (a) ICAM-1 expression levels on the cell surfaces were measured by cell-based ELISA as described in *Materials and methods*. Representative findings are shown as the means and SD from three independent experiments. (b) Cell suspensions were then prepared and were labelled for 30 min at 4°C with FITC-conjugated anti-human ICAM-1 MAb and analysed by flow cytometry as described in *Materials and methods*. (c) KB cell monolayers in 24-well plates were cultured with *E. corrodens* 1073 with or without GalNAc (50 mM) for 10 h. Neutrophil adherence is expressed as the percentage of adhesive cells compared with 100% deposited cells. Representative findings are shown as the means and SD from three independent experiments. *Significant differences ($p < 0.001$) versus control values.

Fig. 6. Induction of the expression of ICAM-1 mRNA on KB cells stimulated with EcLS. KB cells were incubated with the indicated concentration of EcLS for 10 h in the presence or absence of GalNAc (50 mM). cDNA synthesis and RT-PCR were performed as described in *Materials and methods*.

Discussion

Neutrophils within the gingival crevice provide the first cellular host defence mechanism against periodontopathic bacteria [1]. Half the leucocytes infiltrating junctional epithelium and 90% of the leucocytes isolated from crevicular fluid are neutrophils [24, 25]. The concentration of neutrophils in the periodontal tissues exceeds the concentration of neutrophils in blood [1]. Thus, it has been suggested that neutrophils play important roles in the establishment of periodontal lesions. While the complex events related to transendothelial migration of neutrophils are being widely addressed, those related to transepithelial migration have received less attention. However, such transepithelial migration of neutrophils may be clinically important in the establishment of periodontal lesions [26].

Tonetti *et al.* reported that neutrophil access into the junctional epithelium was not random but rather a highly regulated process able to selectively enrich neutrophils, and the establishment of a gradient of ICAM-1 expression across the junctional epithelium and the expression of IL-8 in its superficial layers probably represent important regulatory mechanisms leading to neutrophil migration into the gingival sulcus [3].

To elucidate those regulatory processes for neutrophil migration into the gingival tissue a recent study demonstrated that *E. corrodens* surface proteins and products secreted into the culture supernate are able to activate the epithelial cells to secrete pro-inflammatory cytokines, such as IL-6 and IL-8, which are a prerequisite for transmigration and accumulation of neutrophils into gingival epithelium and crevices [20]. The findings of the present report demonstrate that KB cells up-regulate both the expression of ICAM-1 mRNA (Fig. 1) and the expression of membrane ICAM-1 (Fig. 3) in response to *E. corrodens* infection. Moreover, the expression of ICAM-1 in response to *E. corrodens* infection of primary cultures of gingival epithelial cells appeared to be very similar to that observed in experiments with KB cells (data not shown). Taken together, these findings suggest that *E. corrodens* appears to be capable of establishing of a gradient of ICAM-1 and IL-8 expression across the junctional epithelium.

A previous study with cell-free *E. corrodens* culture supernates demonstrated that the direct contact of *E. corrodens* 1073 with oral epithelial cells was not necessarily required for the stimulation of IL-6 and IL-8 secretion [20]. In contrast, cell-free *E. corrodens* culture supernates did not increase the expression of ICAM-1. This finding suggests that the activation of ICAM-1 expression in gingival epithelial cells may require the direct contact of *E. corrodens* cells and gingival epithelial cells. Therefore, the present study aimed to determine whether EcLS, which was a GalNAc-specific lectin-like adhesin of *E. corrodens*, played a crucial role in the expression of ICAM-1 on KB cells. As expected GalNAc, which competitively inhibited adhesion of *E. corrodens* to epithelial cells mediated by EcLS [15], reduced the expression of ICAM-1 on KB cells by infection with *E. corrodens* (Fig. 5a and b). This finding strongly indicated the involvement of EcLS in the expression of ICAM-1 on KB cells infected with *E. corrodens*. To further confirm the involvement of EcLS, the study determined whether purified EcLS increased the present expression of ICAM-1 on KB cells. EcLS increased the expression of ICAM-1 mRNA (Fig. 6), cell surface ICAM-1 expression on KB cells (Fig. 7a and b) and neutrophil adhesion (Fig. 7c). These findings confirm that EcLS plays a crucial role in the expression of ICAM-1 on KB cells infected with *E. corrodens*. Eckmann *et al.* [27] reported that galactose/GalNAc-specific lectin of *Entamoeba histolytica* increased the expression of inflammatory cytokines by cultured human epithelial cells. Therefore, the present study determined whether EcLS was the key factor in the expression of IL-8 by epithelial cells infected with *E. corrodens*. In contrast to expectation, EcLS increased neither the expression of IL-8 mRNA nor the secretion of IL-8 protein by KB cells (data not shown). Therefore, this is the first study to report that bacterial lectin enhanced the expression of ICAM-1 on epithelial cells but did not increase IL-8.

In previous experiments, *E. corrodens* whole cells slightly induced the expression of TNF- α on KB cells but did not increase IL-1 β . As the expression of ICAM-1 on epithelial cells is known to be up-regulated by pro-inflammatory cytokines including IL-1 β and TNF- α [28–31], the present study determined whether EcLS could induce the production of TNF- α from epithelial cells and whether TNF- α secreted from epithelial cells infected with *E. corrodens* might

Fig. 7. Enhancement of the expression of surface ICAM-1 and neutrophil adherence on KB cells stimulated with EcLS. KB cell monolayers in 24-well plates were cultured with EcLS (0.8 $\mu\text{g}/\text{ml}$) with or without GalNAc (50 mM) for 10 h. **(a)** ICAM-1 expression on the cell surfaces was measured by cell-based ELISA. Representative findings are shown as the means and SD from three independent experiments. **(b)** Cell suspensions were prepared and then ICAM-1 levels on harvested cells were measured by flow cytometry as described in *Materials and methods*. **(c)** Neutrophil adherence is expressed as the percentage of adhesive cells compared with 100% deposited cells. Representative findings are shown as the means and SD from three independent experiments. *Significant differences ($p < 0.001$) versus control values.

participate in the expression of ICAM-1. In RT-PCR, EcLS did not induce IL-1 β or TNF- α in KB cells (data not shown). This finding suggests that EcLS may directly increase the expression of ICAM-1 on gingival

epithelial cells but not by mediating the production of TNF- α from gingival epithelial cells.

In a variety of inflammatory and immune disorders,

including periodontitis, it has been reported that levels of ICAM-1 in body fluids correlate with the intensity of the clinical condition [32–34]. Schmal *et al.* [35] reported that sICAM-1 enhanced production of MIP-2 and TNF- α by macrophages and intensified lung injury after intrapulmonary disposition of immune complexes. In periodontal tissues, as in lung tissues, sICAM-1 might play an important role in tissue destruction but little is known about the mechanisms of the destruction of the periodontal tissues *via* sICAM-1. Several studies reported that in crevicular fluid, sICAM-1 levels were higher for patients with inflammation, and the elevated sICAM-1 levels in crevicular fluid may represent increased shedding of this molecule in the interstitial fluid as a result of membrane-bound ICAM-1 up-regulation on ICAM-1 gingival-bearing cells in relation to plaque accumulation and inflammation [36–38]. Therefore, the present study examined whether infection of KB cells by *E. corrodens* increased sICAM-1 levels in culture supernate. In contrast to expectations, *E. corrodens* was not able to increase the level of sICAM-1 in the culture supernates. Jarvis *et al.* [39] reported that *Neisseria gonorrhoeae* could up-regulate the expression of ICAM-1 on epithelial cells but did not enhance the sICAM-1 in culture medium. To shed membrane-bound ICAM-1, certain proteinases might be required.

In conclusion, the findings of the present study demonstrated that EcLS from *E. corrodens*, GalNAc adherence lectin, directly induces up-regulation of bioactive ICAM-1 expression on gingival epithelial cells and promotes adhesion of neutrophils to epithelial cells. The exact nature of the gingival epithelial cell receptor that is implicated in the activation remains unknown and further investigations will be necessary to characterise more precisely the different molecules involved in the signalling pathway.

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