Expression of intercellular adhesion molecule 1 (ICAM-1) on the human oviductal epithelium and mediation of lymphoid cell adherence

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The epithelium of the human oviduct expresses the major histocompatibility complex (MHC) class II and shows endocytic properties towards luminal antigens. Therefore, the epithelial cells might behave as antigen-presenting cells, inducing a local immune response. The activation of antigen-specific T cells not only requires presentation of the peptide antigen by MHC class II, but also the presence of co-stimulatory molecules in the antigen-presenting cells. Therefore, the expression of the intercellular adhesion molecule 1 (ICAM-1) was examined in the epithelium of the human oviduct. Most oviducts showed epithelial ICAM-1 expression, as assessed by immunocytochemistry, western blot analysis and RT–PCR assay, and the expression was restricted to the luminal border of ciliated and secretory cells. Interferon γ, interleukin 1 and lipopolysaccharide treatments increased the percentage of ICAM-1-positive cells in primary cultures, indicating that the expression of ICAM-1 in the oviduct might be upregulated in vivo by inflammatory cytokines or bacterial infections. Binding assays between allogenic phytohaemagglutinin-activated lymphocytes and epithelial monolayers expressing ICAM-1 demonstrated that this molecule stimulated lymphocyte adherence. The presence of ICAM-1, in addition to MHC class II, supports the putative role of the oviductal epithelium in antigen presentation. The exclusive apical distribution of ICAM-1 indicates that T-cell activation would occur in a polarized manner. Binding of lymphoid cells to the surface of the oviductal epithelium may help to retain these immune cells that are required for the clearance of pathogens.

Introduction

All the elements of the mucosal immune system have been identified in the human oviduct, including intraepithelial T lymphocytes (CD4+ and CD8+), T cells clustered in follicles or dispersed in the subepithelial tissue, B lymphocytes and macrophages (Cooper et al., 1987; Otsuki et al., 1989; Wollen et al., 1994; Givan et al., 1997; Cardenas et al., 1998). The epithelium of the oviduct also expresses the major histocompatibility complex (MHC) class II molecules (Bulmer and Earl, 1987; Edelstam et al., 1992; Imarai et al., 1998) and shows endocytic properties towards luminal antigens (Murakami et al., 1985; Imarai et al., 1998). These observations indicate that the oviductal epithelium might participate in the processing of antigens and in the local stimulation of lymphoid cells, as proposed for other mucosal epithelial cells (Bland and Warren, 1986; Tabibzadeh et al., 1986; Kaiserlian et al., 1989; Wira and Rossoll, 1995).

The induction of an immune response requires processing and presentation of the peptide antigen by MHC class II-positive antigen-presenting cells to antigen-specific T cells. It also requires cell–cell interactions that are not antigen specific between the antigen-presenting cells and the T cells. Those non-antigen-specific interactions include the binding of the intercellular adhesion molecule 1 (ICAM-1) on the antigen-presenting cell to the lymphocyte function-associated antigen molecules (LFA-1) on the T-cell surface. The binding of ICAM-1 to LFA-1 provides an important co-stimulatory signal for the T-cell receptor mediated activation (Dougherty et al., 1988; van Seventer et al., 1990), the absence of which leads to functional inactivation of the antigen-specific T cells (van Parijs and Abbas, 1998). In addition to its role in lymphocyte activation, ICAM-1 mediates leucocyte adherence and is involved in transependothelial and transepithelial migration during inflammation (Devine et al., 1986; Springer, 1994; Taguchi et al., 1998).

ICAM-1 is a membrane-bound glycoprotein expressed on non-haematopoietic cells such as vascular endothelial cells, thymic cells, certain epithelial cells, fibroblasts, and on
Materials and Methods

Antibodies

The following antibodies were used: mouse monoclonal anti-human ICAM-1 (DAKO Co, Carpinteria, CA) and mouse monoclonal anti-cytokeratin (DAKO Co.), goat polyclonal IgG against human ICAM-1 (Santa Cruz Biotechnology Inc, Santa Cruz, CA), biotinylated goat anti-mouse IgG (Pierce Chemical Co., Rockford, IL), affinity-purified mouse polyclonal anti-goat IgG (Pierce Chemical Co.) and alkaline phosphatase-conjugated anti-mouse IgG (Sigma Chemical Co., St Louis, MO).

Tissue samples

Protocols were approved by the Ethics Committee of the University of Santiago, Chile. Organs were obtained after informed consent from women aged 32–50 years that had undergone hysterectomy because of myoma or other neoplastic conditions not affecting the oviduct. A blood sample was taken on the day of surgery and concentrations of oestrogen and progesterone were measured by radioimmunooassay. Organs were received in sterile Dulbecco’s modified Eagle’s medium (DMEM; GIBCO BRL, Gaithersburg, MD), transported to the laboratory and processed within 2 h after surgery.

Immunohistochemistry

Pieces of ampullary segments of 1 cm in length from each oviduct were snap-frozen in liquid nitrogen and stored at –20°C until further processing. Tissue slices of 6 μm, obtained using a cryostat (Starlet Bright, Huntingdon), were fixed in cold 4% (w/v) paraformaldehyde for 20 min, followed by 70% (v/v) cold ethanol treatment for 10 min. After 30 min of incubation in 1% (v/v) BSA, sections were incubated overnight at 4°C with the mouse primary antibody against human ICAM-1 or against cytokeratin (1:100). The sections were washed several times and incubated with biotin-conjugated anti-mouse IgG (1:500) at room temperature for 30 min. Incubation with horseradish peroxidase–biotin complex (ABC-complex, Amersham Life Science Ltd; 1:100) was also carried out at room temperature for 1 h followed by dianimobenzidine (DAB) mixture (0.05% (w/v) DAB in 0.05 mol Tris–HCl 1, pH 8, plus hydrogen peroxide 0.003% (v/v) final concentration) for 7 min. Positive controls were the lymphoid cells present in the subepithelial tissue of most samples. Negative controls, in which the primary antibodies were omitted or replaced by non-immune serum, were run routinely in all experiments.

Primary epithelial cell culture

After washing the organ using PBS, the lumen was exposed through a longitudinal cut and strips of mucosal folds were dissected. The strips were washed in TC199 medium (Gibco BRL) and small pieces of about 1 mm in diameter were cut off and subsequently treated with 0.25% (w/v) trypsin (37°C, 10 min). The cell suspension was washed four times at 800 g for 8 min using Hank’s balanced salt solution (Gibco BRL). The final pellet was resuspended in TC199 containing 10% (v/v) fetal calf serum (Gibco BRL), insulin (5 mg ml–1), glutamine (1 mmol l–1), pyruvate (1 mmol l–1) and antibiotics (50 iu penicillin ml–1 and 50 μg streptomycin ml–1). Cells were seeded in culture plates and incubated at 37°C in 5% CO2 in air to allow early attachment of fibroblasts. After incubation for 1 h, the medium containing non-attached cells was removed and seeded in another plate. Cells were incubated for at least 3 days to obtain round colonies of epithelial cells which were identified routinely by cytokeratin immunostaining (Takeuchi et al., 1991).

Western blot analysis

Pieces of mucosal tissue or cultured epithelial cells were obtained from several oviducts. Cold RIPA buffer (1% (v/v) Nonidet P-40, 0.5% (w/v) sodium deoxycholate, 0.1% (w/v) SDS and 0.1 mg phenylmethylsulfonfluoride (PMSF) l–1 in PBS) and a mixture of antipain, chymostatin, leupeptin and pepstatin (final concentration 5 μg ml–1) were added to the samples, which were homogenized using a potter and incubated on ice for 30 min. Cell lysates were centrifuged at 14000 g for 20 min at 4°C. Protein concentration in the supernatant fluid was determined using the Bradford assay (Bradford, 1976). Protein samples (50 μg lane) and a molecular mass standard (BenchMark prestained protein ladder, GIBCO BRL) were subjected to 10% SDS-PAGE under reducing conditions and transferred to nitrocellulose (Sigma) by electoblotting (Harlow and Lane, 1988). Membranes were incubated overnight in blocking solution at 4°C (2% (w/v) BSA in PBS) and the goat polyclonal antibody against ICAM-1 was added (1:1000). After incubation for 2 h at room temperature, the blot was washed several times and exposed to the alkaline phosphatase-conjugated antibody against goat IgG (1:2000). Detection was carried out using the substrates 5-bromo-4-chloro-3 indolyl phosphate (BCIP;
Amresco, Solon, OH) and nitro blue tetrazolium (NBT; Amresco).

Cytokine and lipopolysaccharide treatment

Epithelial cells (5 × 10⁶) isolated from each organ were seeded onto 12 mm round coverslips placed in 24-well plates (Nalge Nunc, Naperville, IL) and incubated at 37°C in an atmosphere of 5% CO₂ in air. After 2 days, cells were washed, recombinant human IFN-γ (10 iu ml-1; Biosource International SA, Camarillo, CA), IL-1 (50 and 1000 iu ml-1) or lipopolysaccharide (5 and 10 µg ml-1) were added to the plate and cells were grown for 2 days (Haraldsen et al., 1996). For immunocytochemistry, independent experiments using IFN-γ were performed with epithelial cell cultures from five patients, whereas experiments using IL-1 and lipopolysaccharide were performed using cell cultures from four patients. All treatments and controls were run in duplicate. Lipopolysaccharide was from E. coli 0122:38 (Sigma).

Immunocytochemistry and cell counts

After cytokine and lipopolysaccharide treatment, cells on coverslips were fixed in cold 2% (v/v) paraformaldehyde for 20 min and immunodetection of ICAM-1 and cytokeratine was performed in duplicate as described for tissue slices. Cells were counterstained with haematoxylin and coverslips were mounted in Kaiser’s glycerol gelatin (Merck, Darmstadt). Samples were viewed under the × 40 objective of an Olympus BX40 microscope. Positive cells were searched for over the entire coverslip, which usually contained at least 500 cells. If there was positive ICAM-1 staining, at least 100 cells were classified as positive or negative from three different fields selected randomly. Data are expressed as percentage of ICAM-1-positive cells.

RT–PCR assay

The cells were collected from culture dishes by trypsin–EDTA (Gibco BRL) treatment. After centrifugation, total RNA was prepared according to Chomczynski and Sacchi (1987). For cDNA synthesis, 0.5 µg RNA, 50 pmol oligo dT (Promega, Madison, WI), 0.5 mmol dNTPs 1⁻¹ (Promega), 5 mmol (dithiothreitol) DTT 1⁻¹, 2 µl reaction buffer (50 mmol KCl 1⁻¹, 10 mmol Tris–HCl 1⁻¹, pH 9.0, 0.1% (v/v) Triton-X100) and 200 U M-MLV reverse transcriptase (Promega) in 20 µl final volume were incubated for 1 h at 42°C. For PCR amplification, primers were designed according to sequences provided by the National Center for Biotechnology Information of the NIH. Primer sequences were: ICAM-1 sense sequence, 5’ GGG AGG CTC CGT GCT GGT GA 3’; ICAM-1 antisense sequence, 5’ TCA GTG CGG CAC GAG AAA TTG 3’; actin sense sequence, 5’ CTC ATC GTA CTC CTG CTT GCT G 3’; actin antisense sequence, 5’ GCT GTG CTA TGT TGC CCT AGA C 3’. PCR reaction mixture included 5 µl cDNA, 50 pmol of each primer, 0.2 mmol dNTPs 1⁻¹, 3 mmol MgCl₂ 1⁻¹ and reaction buffer (50 mmol KCl 1⁻¹, 10 mmol Tris–HCl 1⁻¹, pH 9.0, 0.1% (v/v) Triton-X100) and 2.5 U Taq polymerase (Promega) in a final volume of 25 µl. A total of 35 cycles of amplification were performed using the following protocol: denaturing at 92°C for 1 min, annealing at 60°C for 1 min, extension at 72°C for 1 min. Expected sizes of ICAM-1 and actin products were 447 and 448 base pairs (bp), respectively.

Activation and labelling of human lymphocytes

Blood was obtained from normal healthy volunteers and placed in heparinized tubes. Peripheral mononuclear cells were separated by centrifugation for 30 min at 900 g over Ficoll Hypaque (Pharmacia, Uppsala). Interfacial cells were recovered and washed in RPMI-1640 medium. Activation and labelling of cells were performed according to Cunningham and Kirby (1995). Briefly, cells were resuspended and cultured in RPMI supplemented with 10% (v/v) fetal calf serum, 1 mmol glutamine l⁻¹, 1 mmol pyruvate l⁻¹ and antibiotics, in the presence of 10 µg phytohaemaglutinin ml⁻¹ for 2–3 days at 37°C in an atmosphere of 5% CO₂ in air. Activated lymphocytes were collected and separated from dead cells using Ficoll Hypaque. Lymphocytes were labelled using 100 µCi Na₂¹⁰₉CrO₄ (NEN, Boston, MA) for 90 min at 37°C.

Assay of lymphocyte binding to the oviductal epithelium

Binding of lymphocytes to epithelial cells was examined using the method of Ikuta et al. (1991). Confluent epithelial monolayers in 24 well-plates, with or without IFN-γ pre-treatment (48 h), were incubated with 0.5 ml ⁵¹Cr-labelled lymphocytes (10,000 cells per well) for 1 h at 37°C. After washing to remove non-adherent lymphocytes, the remaining cells were lysed by the addition of 0.5 ml of 10% (v/v) Triton-X100 (Sigma) for 10 min. The amount of ⁵¹Cr released was measured using a Packard Cobra II gamma-counter, and the percentage of adherent cells was calculated as follows: (c.p.m. in lysate–100)/(c.p.m. in maximum control). A concentration of 10 µg ml⁻¹ of the anti-ICAM-1 monoclonal antibody (Dako) was added to epithelial cells 30 min before the addition of lymphocytes to investigate the role of ICAM-1 in lymphocyte adherence to epithelial cells. Experiments were performed in triplicate.

Statistical analysis

The Mann–Whitney U test was used to compare the mean serum concentration of oestradiol and progesterone in patients whose oviducts expressed ICAM-1 with those in patients whose oviducts did not express ICAM-1. The paired t test was used to examine cytokine and lipopolysaccharide treatment effects. Data on ICAM-1-dependent adherence of lymphocytes to epithelial cells were analysed by multiple comparisons using ANOVA followed by Duncan’s test. A P value of < 0.05 was considered significant.
Results

Expression of ICAM-1 in the human oviduct

Immunohistochemical staining using a monoclonal antibody raised against ICAM-1 was performed in ampullary segments. Eight of 14 specimens showed ICAM-1 expression (Table 1) along the epithelium, which was always restricted to the luminal border of both ciliated and secretory cells (Fig. 1). ICAM-1 staining was also observed in a population of stromal cells probably consisting of mononuclear leucocytes. Sex steroid concentrations influence gene expression in receptor-bearing organs like the oviduct, thus the expression of ICAM-1 was correlated with the oestradiol and progesterone serum concentration in the patients. Serum oestradiol concentrations in the patients whose oviductal epithelia expressed ICAM-1 were significantly lower (Table 1; median 99 pmol l⁻¹, range 71–596) compared with those that did not (Table 1; median 508 pmol l⁻¹, range 108–840) (P < 0.05). No difference was found in serum progesterone concentrations between the groups (Table 1). ICAM-1 expression was also determined in primary cultures of oviductal epithelial cells from ten patients. After 6 days of culture, epithelial cell monolayers contained more than 95% of cells expressing cytokeratine 18 (that is, epithelial cells). Nine of ten cell cultures expressed variable amounts of ICAM-1 immunostaining, ranging from 0 to 80% of positive cells (data not shown).

Western blot analyses were also used to examine ICAM-1 expression in the oviductal mucosae and epithelial cells. A specific protein band of approximately 70 kDa was present in protein extracts prepared from mucosae (not shown) or epithelial cells (n = 3; Fig. 2). The signal was greatly reduced when blots were incubated with a mixture of the polyclonal antibody and the ICAM-1 blocking peptide (Santa Cruz Biotechnology Inc.) (Fig. 2), indicating that the band of 70 kDa was ICAM-1.

Table 1. Epithelial expression of intercellular adhesion molecule 1 (ICAM-1) in the human oviduct

<table>
<thead>
<tr>
<th>Age of patient (years)</th>
<th>Uterine pathology</th>
<th>Serum oestradiol (pmol l⁻¹)</th>
<th>Progesterone (nmol l⁻¹)</th>
<th>ICAM-1</th>
</tr>
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<tbody>
<tr>
<td>35 IEN</td>
<td>71</td>
<td>3.4</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>36 IEN</td>
<td>92</td>
<td>1.0</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>40 Myoma</td>
<td>92</td>
<td>1.0</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>50 Myoma</td>
<td>92</td>
<td>1.3</td>
<td>+</td>
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</tr>
<tr>
<td>42 Myoma</td>
<td>106</td>
<td>2.0</td>
<td>+</td>
<td></td>
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<tr>
<td>46 Myoma</td>
<td>163</td>
<td>3.1</td>
<td>+</td>
<td></td>
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<tr>
<td>38 Myoma</td>
<td>284</td>
<td>34.7</td>
<td>+</td>
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</tr>
<tr>
<td>45 Myoma</td>
<td>596</td>
<td>4.2</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>99</td>
<td>2.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>43 Myoma</td>
<td>108</td>
<td>9.3</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>42 Myoma</td>
<td>138</td>
<td>5.9</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>46 Myoma</td>
<td>319</td>
<td>2.2</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>44 Myoma</td>
<td>696</td>
<td>2.0</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>32 IEN</td>
<td>710</td>
<td>2.4</td>
<td>–</td>
<td></td>
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<tr>
<td>48 Myoma</td>
<td>840</td>
<td>25.4</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>508</td>
<td>4.2</td>
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IEN: intraepithelial neoplasia. a versus b, P < 0.05 (Mann–Whitney U test).

Fig. 1. Immunohistochemical detection of intercellular adhesion molecule 1 (ICAM-1) in the epithelium of the human oviduct by a peroxidase-based staining system (brown) and haematoxylin counterstaining (blue). Positive staining for ICAM-1 appeared along the epithelium and was restricted to the luminal border of the cells (arrows). E: epithelium; L: lumen. Scale bar represents 50 μm.
Effect of IFN-γ, IL1 and lipopolysaccharide on the expression of ICAM-1

Incubation of oviductal epithelial cells with 10 IU IFN-γ ml⁻¹ (n = 5) or with inflammatory mediators such as IL-1 (1000 IU ml⁻¹; n = 4) and lipopolysaccharide (10 μg ml⁻¹; n = 4) for 48 h augmented the percentage of ICAM-1-positive cells detected by immunocytochemistry (Figs 3 and 4). The induction was observed in all cases studied. IFN-γ also induced an increase in ICAM-1 mRNA in all cases examined (n = 6; P < 0.03), as assessed by RT–PCR (Fig. 5).

Role of ICAM-1 in lymphocyte adherence to oviductal epithelial cells

Binding assays between allogenic phytohaemagglutinin-activated lymphocytes isolated from peripheral blood and epithelial monolayers were performed to determine whether epithelial ICAM-1 in the human oviduct promotes lymphocyte adherence. The assays were performed twice and the results were similar on both occasions. Treatment of epithelial cells with 10 IU IFN-γ ml⁻¹ for 2 days, which induced the expression of ICAM-1, also produced a 17-fold increase of lymphocyte binding to epithelial cells (Fig. 6). Anti-ICAM-1 antibodies caused a 78% inhibition of the lymphocyte binding to the epithelial cells (Fig. 6), indicating that this molecule mediated the adherence of lymphocytes to the oviductal epithelium.

Fig. 2. Western blot analysis for detection of the intercellular adhesion molecule 1 (ICAM-1) protein in epithelial cells of the human oviduct. Lane 1: BenchMark prestained protein ladder (GIBCO BRL). Lane 2: whole protein extract from cultured epithelial cells of a human oviduct. The blot was incubated with the ICAM-1-specific polyclonal antibody. Lane 3: whole protein extract from cultured epithelial cells of a human oviduct. The blot was incubated with a mixture of the ICAM-1 peptide used to raise the polyclonal antibody and the ICAM-1-specific antibody (5:1).

Fig. 3. Effect of interferon γ (IFN-γ) on intercellular adhesion molecule 1 (ICAM-1) expression in cultured epithelial cells of the human oviduct. Immunohistochemical detection of the protein without (a) and with (b) IFN-γ treatment. Interferon γ increased the number of ICAM-1-positive cells (brown). Scale bar represents 10 μm.
Discussion

This is the first study to demonstrate the expression of ICAM-1 in the epithelium of the human oviduct, both in ciliated and secretory cells. The expression of ICAM-1 in the human oviduct was confirmed by three independent methods: immunocytochemistry, western blot analysis and RT–PCR, which revealed the presence of the protein and the mRNA. The oviductal ICAM-1 is a protein of approximately 70 kDa, which differs from the 85–114 kDa ICAM-1 described in other types of cell, probably due to differences in the extent of the N-linked glycosylation of the 55 kDa core protein (Dustin et al., 1986; Diamond et al., 1991). The expression of ICAM-1 has been described in the human endometrium (Tabibzadeh and Poubouris, 1990; Tawia et al., 1993; Thomson et al., 1999) and, as was found in the present study in the oviduct, ICAM-1 was localized in the apical surface of the luminal epithelium but also in the glandular epithelium and stromal fibroblasts (Thomson et al., 1999).

The relative molecular mass of the molecule in the endometrium has not been reported (Tabibzadeh and Poubouris, 1990; Tawia et al., 1993; Thomson et al., 1999). An explanation for the lower level of protein glycosylation might be found in the study of Diamond et al. (1991), who...
epithelial peptide–MHC class II complex. In most mammals anergy rather than activation will be the outcome of the basolateral and basal membrane of the epithelium, T-cell that unless other co-stimulatory molecules are present in the basolateral surface of the oviductal epithelium, indicating that antigen processing occurs only when antigen is endocytosed from surface, where MHC class II is expressed, whereas antigen processing occurs only when antigen is endocytosed from the epithelial apical surface. ICAM-1 is not detectable in the basolateral surface of the oviductal epithelium, indicating that unless other co-stimulatory molecules are present in the basolateral and basal membrane of the epithelium, T-cell activation rather than activation will be the outcome of the interaction between intraepithelial or stromal T cells and the epithelial peptide–MHC class II complex. In most mammals the transit of spermatozoa and embryos throughout the oviduct exposes the local immune cells to the challenge of allogenic antigens, thus efficient regulatory mechanisms of the immune response must operate to avoid harmful allogenic reactions. Induction of T-cell anergy might be one of these mechanisms.

In addition to ICAM-1, B7 molecules (CD80/CD86) binding to CD28 may provide accessory function for an efficient T-cell response (Croft and Dubey, 1997). Expression of the co-stimulatory molecule B7.2 was not detected in the epithelium of oviductal tissue sections, although positive stromal cells were frequently observed (Cardenas et al., 1998). The presence of B7.1 and a number of other co-stimulatory receptor–ligand pairs in the oviductal epithelium requires further investigation.

The results of the present study demonstrate that ICAM-1 in oviductal epithelial cells meets the requirement for one of the classical roles of this cell adhesion molecule, that is, the specific binding of leucocytes. The adhesive interactions between lymphocyte ligands and ICAM-1 in the epithelium of the human oviduct might help to retain these immune cells, which are required for clearance of luminal pathogens. To date, there are no reports of the presence of leucocytes bound to the epithelium in the lumen of the oviduct in vivo; however, migration of leucocytes into the lumen of different organs appears to occur in healthy humans and other species (Heatley and Bienenstock, 1992; Kennedy et al., 1995; Borgenovo et al., 1997). In the oviduct, leucocytes from the stroma (Cooper et al., 1987; Otsuki et al., 1989; Wollen et al., 1994; Givan et al., 1997; Cardenas et al., 1998) could migrate into the lumen and be retained by the epithelium. Alternatively, because the human oviduct is open to the peritoneal cavity, it is possible that leucocytes present in this compartment (Oosterlynck et al., 1994) migrate into the reproductive tissues and also bind to the oviductal and uterine ICAM-1. Moreover, the binding of leucocytes to ICAM-1 could facilitate a putative leucocyte transepithelial migration in the oviduct, as has been described for T cells bound to ICAM-1 in the epithelial cells of the retina and airway (Devine et al., 1986; Taguchi et al., 1998). Finally, binding of cells to ICAM-1 in the oviductal epithelium might also include the embryo. Owing to the apical distribution of ICAM-1 in the tissue section, although positive stromal cells were frequently observed (Cardenas et al., 1998) some authors have suggested a possible role for ICAM-1 in embryo uterine implantation. Although there is no direct evidence for such a role, it is possible that ICAM-1 has a role in ectopic implantation, which occurs most commonly in the oviduct.

Several cytokines regulate the expression of ICAM-1 in different cells (Dustin et al., 1986; Myers et al., 1992; Elgavish, 1993; Haraldsen et al., 1996; Huang et al., 1996). In the human oviduct, epithelial ICAM-1 was induced by IFN-γ and IL-1. These cytokines are produced by, and secreted in, the human oviduct (Srivastava et al., 1996), indicating that they probably play a role in regulating ICAM-1 synthesis in the epithelium, therefore fostering the interaction between the epithelium and lymphoid cells. Lipopolysaccharide was also able to induce expression of ICAM-1 in cultured oviductal epithelial cells, indicating that bacterial infection will also stimulate epithelial ICAM-1 expression in the reproductive tract. Invasion of

![Fig. 6. Intercellular adhesion molecule 1 (ICAM-1)-dependent adherence of lymphocytes to epithelial cells from the human oviduct.](Image)

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**Percentage of lymphocyte adherence**

<table>
<thead>
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<th>Treatment</th>
<th>Percentage of lymphocyte adherence</th>
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<tbody>
<tr>
<td>C</td>
<td>a</td>
</tr>
<tr>
<td>Anti-ICAM-1</td>
<td>b</td>
</tr>
<tr>
<td>IFN</td>
<td>c</td>
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<tr>
<td>IFN + anti-ICAM-1</td>
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**Table:**

<table>
<thead>
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<th>Treatment</th>
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<tr>
<td>C</td>
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<td>c</td>
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<tr>
<td>IFN + anti-ICAM-1</td>
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**Figure 6:** Intercellular adhesion molecule 1 (ICAM-1)-dependent adherence of lymphocytes to epithelial cells from the human oviduct. 51Cr-labelled adherent cells were lysed and measured in a gamma counter. The treatment of epithelial cells with interferon γ (IFN-γ) produced an increase in lymphocyte binding to epithelial cells (a versus c, P < 0.001). The binding was inhibited by anti-ICAM-1 antibodies (c versus d, P < 0.001). Multiple comparisons were carried out by ANOVA followed by Duncan’s test.

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**Figure:**

- **A:** Interleukin-1 (IL-1)
- **B:** Interferon-γ (IFN-γ)
- **C:** Anti-ICAM-1 antibodies

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**Legend:**

- **C:** Control
- **a:** Anti-ICAM-1
- **b:** IFN-γ
- **c:** IFN-γ + anti-ICAM-1

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**References:**

- Heatley and Bienenstock, 1992
- Kennedy et al., 1995
- Borgenovo et al., 1997
- Cooper et al., 1987
- Otsuki et al., 1989
- Wollen et al., 1994
- Givan et al., 1997
- Cardenas et al., 1998
- Oosterlynck et al., 1994
- Devine et al., 1986
- Taguchi et al., 1998
- Dustin et al., 1986
- Myers et al., 1992
- Elgavish, 1993
- Haraldsen et al., 1996
- Huang et al., 1996
- Srivastava et al., 1996
human mucosal epithelial cell lines by *Neisseria gonorrhoeae* upregulates the expression of ICAM-1 (Jarvis et al., 1999). Since *N. gonorrhoeae* infects the human reproductive mucosa, it is possible that infection of the reproductive tract in *vivo* induces upregulation of ICAM-1, which might function to recruit leucocytes at the site of infection. Differences in cytokine concentrations or subclinical or recent infections in the patients in the present study may explain the presence or absence of ICAM-1 expression in the oviducts. Finally, not only cytokines and bacterial components can modulate ICAM-1 expression. Oestradiol also has modulatory effects (down- and up-regulation) on endothelial expression of ICAM-1, which appear to depend upon cytokine induction of the molecule (Cid et al., 1994; Aziz and Wakefield, 1996; Dickens et al., 1999). Expression of ICAM-1 in the epithelium of the human oviduct appears to be related to oestradiol serum concentrations. Therefore, the present study also examined whether oestradiol modulates ICAM-1 expression in cultured oviductal epithelial cells. Preliminary results indicated that oestradiol had no effect either on basal or IFN-γ-induced ICAM-1 expression (E. Utreras, unpublished).

In summary, this study demonstrated apical ICAM-1 expression in the epithelium of the human oviduct, which was inducible by IFN-γ, IL-1 and lipopolysaccharide. The results support a role for ICAM-1 in lymphocyte binding *in vitro*, which may be important for mucosal immunity *in vivo*.

This study was supported by Fondecyt 1950272 and by Dicyt-USACH.

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