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Adhesion of Langerhans cells (LC) to keratinocytes is mediated by E-cadherin. IL-1, TNF-α, and LPS mobilize LC from epidermis and presumably attenuate LC-keratinocyte adhesion. To determine whether these mediators modulated LC E-cadherin-dependent adhesion directly, we characterized their effects on LC-like dendritic cells expanded from murine fetal skin (FSDDC). FSDDC were propagated from day 16 C57BL/6 fetal skin and isolated as aggregates (FSDDC-A) in which homophilic adhesion was mediated by E-cadherin. IL-1, TNF-α, and LPS induced dissociation of FSDDC-A that began within 4 to 8 h and was complete within 20 h. Anti-IL-1RI mAb inhibited disaggregation caused by IL-1α and IL-1β, but not that induced by TNF-α or LPS. Anti-TNF-α mAb inhibited the effect of TNF-α and LPS, but not that caused by IL-1α or IL-1β. Flow cytometry of FSDDC-A revealed that IL-1, TNF-α, and LPS induced increased expression of MHC class II, CD40, and CD86 and decreased E-cadherin expression that was temporally related to dissociation of aggregates. IL-1 and TNF-α caused a rapid reduction in FSDDC E-cadherin mRNA levels that preceded the decrease in E-cadherin surface expression. These results demonstrate that cytokines that induce LC emigration in vivo act directly on LC-like cells in vitro, reduce E-cadherin mRNA levels, down-regulate E-cadherin surface expression, and induce a loss of E-cadherin-mediated adhesion.

Studies of LC E-cadherin biology have been hampered by the lack of availability of large numbers of cells with appropriate characteristics. We recently described a primary culture system that allowed the expansion of LC-like DC (FSDDC) from fetal murine skin (17). FSDDC were propagated from day 16 C57BL/6 fetal skin in medium containing GM-CSF and CSF-1. Aggregates of E-cadherin− FSDDC (FSDDC-A) that resembled LC in terms of morphology, phenotype, and function and that exhibited E-cadherin-dependent homophilic adhesion were readily isolated (17). Like LC (18), FSDDC-A spontaneously matured into interdigitating DC-like cells in vitro, manifested by a characteristic surface phenotype, cytokine profile, and acquisition of potent allostimulatory activity. This in vitro maturation, like that of LC (4), was accompanied by decreased E-cadherin surface expression and by loss of E-cadherin-mediated adhesion (17). In the present study, we used this in vitro system to characterize effects of inflammatory mediators that activate and mobilize LC in vivo on E-cadherin-mediated adhesion in LC-like DC. Herein we demonstrate that the epidermal proinflammatory cytokines IL-1 and TNF-α act on LC-like DC to regulate E-cadherin function by decreasing steady-state E-cadherin mRNA levels, thereby reducing E-cadherin surface expression and attenuating E-cadherin-mediated adhesion.

Materials and Methods

Propagation of LC-like FSDDC

LC-like FSDDC were isolated as aggregates (FSDDC-A) from GM-CSF- and CSF-1-supplemented primary cultures of day 16 C57BL/6 murine fetal skin as previously described (17, 19). When necessary, FSDDC-A were dissociated with trypsin in EDTA (0.01% trypsin in calcium- and magnesium-free HBSS containing 1 mM EDTA for 30 min at 37°C) to allow enumeration of cells. FSDDC aggregates were subcultured in cytokine-supplemented DMEM at 5 × 10^5 cells/ml in T-25 flasks or 96-well plates as indicated.

Microscopy

FSDDC aggregates were subcultured for 18 h in T-25 flasks in the presence or absence of murine recombinant IL-1β (10 ng/ml; Genzyme, Cambridge, MA), TNF-α (10 ng/ml; Genzyme), or LPS (100 ng/ml) (Escherichia coli K-12-derived LPS (<0.008% protein), prepared as described (20), was produced by Dr. Robert Vogel, Uniformed Services University of the Health Services, Bethesda, MD.). Morphologic changes were documented using an inverted phase photomicroscope (Axiovert 405 M; Carl Zeiss, Oberkochen, Germany) equipped with Hoffman modulation contrast optics (Modulation Optics, Greenvane, NY).

Quantitative disaggregation assay

FSDDC aggregates were seeded into round-bottom 96-well plates at 10^5 cells/well and incubated for 18 h in the presence or absence of murine recombinant IL-1α, IL-1β, TNF-α, IL-6, or IL-10 (10 ng/ml each; Genzyme), human recombinant TGF-β1 (10 ng/ml; R&D Systems, Minneapolis, MN), LPS (100 ng/ml); or cytochalasin D (CCD; 100 μg/ml; Sigma) in a final volume of 0.2 ml/well. All aggregates contained less than 5 pg LPS/μg protein (LAL-test, BioWhittaker, Walkersville, MD). Well contents were subsequently harvested by limited trypsinization (0.01% for 15 min at 37°C) in the presence of calcium (which protects cadherins from trypsin digestion), added to 10 ml PBS, and particles were enumerated with a Coulter Counter (Coulter Electronics, Hialeah, FL). Counts obtained in wells without addition of cytokines (C_c) and in wells harvested with trypsin (0.01%) in 1 ml EDTA (C_100, representing maximal disaggregation) were determined in pentuplicate. Disaggregation (C_i) induced by cytokines, LPS, or CCD was determined in triplicate and expressed as a percentage of maximal disaggregation according to the following equation:

\[ X% = (C_c - C_i) \times (100 - C_c)^{-1} \times 100. \]

Quantitation of cytokine mRNA and protein

Total RNA was isolated from FSDDC-A after incubation for 18 h in the presence or absence of LPS (100 ng/ml) using RNAzol B (Tel-Test, Friendswood, TX). Cytokine and control mRNAs were quantitated using a multiprobe RNase protection assay system (RiboQuant; PharMingen, San Diego, CA) using the protocol provided by the manufacturer. Briefly, \(^{32}P\)UTP-labeled riboprobes were transcribed from cDNA templates using T7 polymerase (Promega, Madison, WI) and allowed to hybridize to sample mRNA overnight at 45°C. After treatment with RNase A and RNase T, protected fragments were resolved in denaturing 8% polyacrylamide gels and detected by autoradiography. Two cDNA template sets were used: mCK2 (IL-1α, IL-1β, IL-1RA, IL-6, IL-10, IL-12 (p35), IL-12 (p40), IFN-γ, and MIF) and mCK3 (IL-6, TNF-α, TNF-β, LTβ, IFN-γ, TNF-β1, and TGF-β2). Five micrograms of total RNA from FSDDC-A or from yeast (negative control) and 2.5 to 3 × 10^6 cpm of the radiolabeled probe was used per lane.

Spatotaneous and LPS-induced (or cytokine-induced) cytokine production was measured in FSDDC supernatants using ELISA kits for murine IL-1α, IL-1β, and TNF-α (Genzyme). FSDDC-A were subcultured in 24-well plates at 10^6 cells/ml in the presence or absence of LPS (100 ng/ml), IL-1α, IL-1β, or TNF-α (each at 10 ng/ml), and cell supernatants were obtained at 24 h. To quantitate cell-associated IL-1α, washed FSDDC were resuspended in PBS containing 2.5 mM EGTA, 2.5 μM n-propylthiouracil-p-guanidinobenzoate (Sigma), and Complete protease inhibitor mixture (Boehringer Mannheim, Indianapolis, IN) and subjected to four freeze/thaw cycles (21). Cell debris was sedimented (15,000 × g for 30 min), and the soluble fraction was recovered. FSDDC supernatants and lysates were stored at −70°C until analysis.

Abs and flow cytometry

Anti-IL-1RI mAb (JAMA; Ref. 22), anti-IL-1α mAb (ALF; Ref. 23), and anti-IL-1β mAb (B122; Ref. 24) were provided by Drs. Robert Schreiber and David Chaplin (Washington University School of Medicine, St. Louis, MO), and anti-TNF-α mAb (hamster IgG) was purchased from Genzyme. The neutralizing capacity of each mAb was determined using recombinant cytokines. LPS contamination was <5 pg/100 μg protein (LAL-test; BioWhittaker). Hybridomas producing anti-I-Aβ (Y3P, mouse IgG2a) and anti-I-Aβ (MKD6, mouse IgG2a) were obtained from the American Type Culture Collection (Rockville, MD). Anti-CD16/CD52 (2D.10; ref. 22) was provided by Dr. Masatoshi Takeichi (Kyoto University, Kyoto, Japan). Anti-CD160/CD53 (10011, rat IgG2a) was provided by Dr. Dr. Masatoshi Takeichi (Kyoto University, Kyoto, Japan). Anti-CD40 (H40-3, hamster IgM), anti-CD45 (30F11.1, rat IgG2b), anti-CD86 (GL-1, rat IgG2a), and isotype-matched controls were purchased from PharMingen or FITC-modified mAb. Phycoerythrin-streptavidin was obtained from Tago (Burlingame, CA). Rat mAb were purified from ascites or hybridoma supernatants using immobilized protein G (Pierce, Rockford, IL). Mouse mAb were purified from supernatants using immobilized protein A (Pierce). Y3P and MKD6 were conjugated with FITC (Sigma) as described (25).

FSDDC-A were subcultured for 18 h in round-bottom 96-well plates in the presence or absence of LPS or cytokines. Before staining, FSDDC were dissociated in HBSS containing 1 mM EDTA (30 min at 37°C) in 10% chelated FBS (26). For multicolor flow cytometry, cells were suspended in cold PBS containing 5% FBS and 0.02% NaN₃, reincubated with saturating concentrations of FITC-mAb, Bio-mAb, and phycocyanin-conjugated mAb. For single-color flow cytometry, cells were incubated with hybridoma supernatants and FITC-modified, affinity-purified (Fab’), fragments of goat anti-rat IgG (Tago). Stained cells were analyzed using a FACScan flow cytometer equipped with Cellquest software (Becton Dickinson, Mountain View, CA). Propidium iodide-permeable (nonviable) cells were excluded from the analysis.

Northern blot analysis

Total RNA was isolated from FSDDC using RNAzol B, denatured, fractionated by formaldehyde/agarose gel electrophoresis (10 μg/lane), and transferred to nylon membranes (Hybond; Amersham, Arlington Heights, IL). Hybridization was performed overnight at 42°C with random-primed \(^{32}P\)IdCTP-labeled murine E-cadherin cDNA (Ref. 27; supplied by Dr. John Stanley, University of Pennsylvania, Philadelphia, PA) in 50% formamide, 10% dextran sulfate, 1% SDS, and 100 μg/ml salmon sperm DNA. Blots were subsequently stripped and probed with \(^{32}P\)IdCTP-labeled rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA (provided by Dr. Koichi Suzuki, National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD). Relative amounts of E-cadherin and GAPDH mRNA were estimated using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). For dose-response studies, FSDDC-A were incubated for 18 h with various concentrations of IL-1β or TNF-α before preparation of RNA. For time course studies, cytokines (10 ng/ml) were added at various times, and all cells were harvested simultaneously by vigorous trituration. An aliquot of each sample was prepared for flow cytometry as described above.
Statistical analysis
The statistical significance of differences in the disaggregation assay was calculated using the paired Student’s t test.

Results
Effects of proinflammatory cytokines and LPS on cell adhesion in FSDDC aggregates

Intact aggregates of FSDDC (FSDDC-A) were harvested from primary cultures of day 16 C57BL/6 fetal skin cells maintained for 2 wk in GM-CSF- and CSF-1-supplemented medium by limited trypsinization in calcium-containing medium and separated from single cells by 1 g sedimentation. Within 3 h after initiating subcultures, FSDDC-A adhered loosely to tissue culture flasks, and cells at the periphery of the aggregates began to exhibit sheetlike processes and dendrites. After 18 h, small numbers of cells with pronounced dendritic morphology were released from the aggregates. However, most cells remained tightly clustered (Fig. 1 A).

We have previously shown that intercellular adhesion in these aggregates is mediated by the homophilic adhesion molecule E-cadherin and that cells in FSDDC aggregates can adhere to other cells via an E-cadherin-dependent mechanism (17). E-cadherin-mediated adhesion of LC to keratinocytes must be attenuated before LC can emigrate from epidermis after activation by Ag. Because proinflammatory cytokines (IL-1 and TNF-α) and LPS have been implicated in LC activation and in the mobilization of LC (and other DC) from nonlymphoid tissues (7, 9–14, 16), we investigated the effects of these agents on E-cadherin-mediated adhesion in FSDDC-A. Treatment of FSDDC-A with IL-1β (Fig. 1B), TNF-α (Fig. 1C), or LPS (Fig. 1D) for 18 h resulted in almost complete dissociation of FSDDC aggregates.

The ability of IL-1, TNF-α, and LPS to modulate E-cadherin-mediated adhesion in FSDDC-A was confirmed using a Coulter counter-based disaggregation assay (Fig. 2A). In addition, we determined that the effects of these epidermal proinflammatory cytokines were selective in that other cytokines (including IL-6, IL-10, and TGF-β1) did not induce significant dissociation. As expected, CCD, an inhibitor of actin polymerization (28), also induced complete dissociation of FSDDC-A. This is consistent with earlier studies that demonstrated that classical cadherins mediate adhesion only if they are physically linked to a functional actin-myosin cytoskeleton (29). Dose-response studies revealed that half-maximal dissociation of FSDDC was induced by 0.2 ng/ml IL-1β and 1 ng/ml IL-1α, TNF-α, and LPS, with maximal dissociation occurring at 10 to 100 ng/ml (Fig. 2B). Time course studies demonstrated that cytokine- and LPS-induced dissociation began after a lag period of ~4 h, was half maximal at 12 to 16 h, and was complete by 20 h (Fig. 2C). In contrast, CCD induced almost complete dissociation within 4 h (Fig. 2C).

Cytokine profile of FSDDC-A
LPS induces several cytokines in cells of the DC lineage (30–33). To characterize the cytokine profile of FSDDC-A, we utilized a

![FIGURE 1. Disruption of E-cadherin-dependent adhesion in FSDDC aggregates by IL-1β, TNF-α, and LPS. FSDDC-A were seeded in chamber slides in the presence or absence of IL-1β (10 ng/ml), TNF-α (10 ng/ml), or LPS (100 ng/ml). After 18 h, morphologic changes were documented by phase contrast photomicroscopy (Hoffman modulation optics, ×400). A, Control; B, IL-1β; C, TNF-α; D, LPS.](http://www.jimmunol.org/)

![FIGURE 2. Quantitation of effects of cytokines and LPS on adhesion in FSDDC aggregates. A, Adhesion in FSDDC-A was quantitated using a Coulter counter-based disaggregation assay (see Materials and Methods) after 18 h of treatment with various cytokines (10 ng/ml), LPS (100 ng/ml), or CCD (10 µg/ml) (*p < 0.001 as compared with FSDDC without treatment). B, FSDDC-A were exposed to various concentrations of IL-1α, IL-1β, TNF-α, and LPS for 18 h, and intercellular adhesion was quantitated. C, Cytokines (10 ng/ml), LPS (100 ng/ml), and CCD (10 µg/ml) were added to FSDDC-A at various times, and intercellular adhesion in all samples was quantitated simultaneously on termination of the experiment. Results of each experiment depict the mean ± SD of triplicate determinations and are representative of three similar experiments (n = 3).](http://www.jimmunol.org/)
To characterize the involvement of IL-1 and TNF-α by anti-cytokine and anti-cytokine receptor mAb into the medium. and TNF-α detected in FSDDC-A supernatants, even after LPS treatment. Signal (Table I). In contrast, little if any IL-1 accumulation of FSDDC-A induced by IL-1, TNF-α (mean ± 6 SD for n = 5 (†) and n = 2 (**) experiments). ND, not determined.

**FIGURE 3.** Cytokine mRNA profile of FSDDC. Relative levels of 14 cytokine mRNAs in FSDDC cultured in the presence or absence of LPS were determined using a multiprobe-RNase protection assay (see Materials and Methods) (n = 3). Lane 1, control FSDDC RNA; lane 2, RNA from LPS-treated FSDDC; lane 3, yeast RNA. NS, nonspecific; mL32, murine ribosomal protein L32; mGAPDH, murine GAPDH.

**FIGURE 4.** Effects of neutralizing anti-IL-1α, anti-IL-1β, anti-IL-1RI, and anti-TNF-α mAb on the cytokine- and LPS-induced dissociation of FSDDC aggregates. FSDDC-A were preincubated as indicated with anti-IL-1α, anti-IL-1β, anti-IL-1RI, and anti-TNF-α mAb before addition of IL-1α, IL-1β, TNF-α, or LPS (3 ng/ml). Adhesion in FSDDC-A was measured after an additional 18-h incubation (mean ± SD of triplicate determinations; n = 3). *p < 0.05 as compared with the dissociation induced by stimuli in the absence of neutralizing mAb.

**Inhibition of cytokine- or LPS-induced FSDDC-A disaggregation by anti-cytokine and anti-cytokine receptor mAb**

To characterize the involvement of IL-1 and TNF-α in the dissociation of FSDDC-A induced by IL-1, TNF-α, and LPS, we assessed the ability of mAb directed against IL-1α, IL-1β, IL-1RI, and TNF-α to block cytokine- and LPS-induced dissociation of FSDDC-A. FSDDC-A were preincubated as indicated with anti-IL-1α, anti-IL-1β, anti-IL-1RI, and anti-TNF-α mAb before addition of IL-1α, IL-1β, TNF-α, or LPS (3 ng/ml). Adhesion in FSDDC-A was measured after an additional 18-h incubation (mean ± SD of triplicate determinations; n = 3). *p < 0.05 as compared with the dissociation induced by stimuli in the absence of neutralizing mAb.

**Effects of IL-1, TNF-α, and LPS on FSDDC surface phenotype**

Recently, we demonstrated that cells in FSDDC aggregates display a surface phenotype similar to that of freshly obtained LC (17). Upon subculture for 3 to 5 days, FSDDC-A release single cells with a pronounced dendritic morphology and a surface phenotype analogous to that of interdigitating DC (17). Dissociation of FSDDC-A with IL-1, TNF-α, or LPS also gave rise to cells that were highly dendritic (see Fig. 1). To determine whether dissociation was also accompanied by a change in surface phenotype, we

**Table I.** Cytokine production by FSDDC aggregates

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Cytokines in Supernatants</th>
<th>Cytokines in Lysates</th>
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<tr>
<td></td>
<td>IL-1α</td>
<td>IL-1β</td>
</tr>
<tr>
<td>None*</td>
<td>&lt;15</td>
<td>&lt;15</td>
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<tr>
<td>LPS (100 ng/ml)*</td>
<td>&lt;15</td>
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<tr>
<td>IL-1α (10 ng/ml)**</td>
<td>ND</td>
<td>&lt;15</td>
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<tr>
<td>IL-1β (10 ng/ml)**</td>
<td>&lt;15</td>
<td>ND</td>
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<tr>
<td>TNF-α (10 ng/ml)**</td>
<td>&lt;15</td>
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* FSDDC-A were incubated in the presence or absence of LPS, IL-1α, IL-1β, or TNF-α, and amounts of immunoreactive TNF-α, IL-1α, and IL-1β in 24-h supernatants were determined by ELISA. Cell-associated IL-1 was measured in freeze/thaw lysates. Values reported represent pg of cytokine produced per 10⁶ cells per 24 h (mean ± SD for n = 5 (†) and n = 2 (**) experiments). ND, not determined.
performed multicolor flow cytometry. Cells were stained with FITC-anti-CD45 and the indicated mAb, and CD45<sup>+</sup> cells (>90% of all cells present) were selected for analysis. Treatment of FSDDC-A with IL-1α, IL-1β, TNF-α, and LPS for 18 h induced increased expression of MHC class II Ag, CD40, CD80, and CD86 (Fig. 5). Effects on CD86 expression were especially dramatic. Incubation of FSDDC-A with IL-6, IL-10, or TGF-β1 did not result in a change in FSDDC surface phenotype (data not shown). Data presented in Fig. 6 demonstrate that treatment of FSDDC-A with IL-1β or TNF-α also caused down-regulation of cell surface E-cadherin expression and that down-modulation of E-cadherin and up-regulation of MHC class II Ag occurred with similar time courses. Similar results were obtained after stimulation with LPS (data not shown). These data, when considered in conjunction with data presented in Fig. 2, indicate that loss of FSDDC cell surface E-cadherin (Fig. 6) and decreased E-cadherin-mediated adhesion (Fig. 2) are temporally linked.

E-cadherin mRNA expression in FSDDC

To determine whether cytokine-induced decreases in cell surface E-cadherin levels reflected alterations in steady-state mRNA levels, FSDDC E-cadherin mRNA levels were quantitated by Northern blotting. Concentrations of IL-1β and TNF-α that down-regulated FSDDC cell surface E-cadherin also decreased E-cadherin mRNA levels in a dose-dependent fashion (Fig. 7A). Time course studies revealed that IL-1β and TNF-α each rapidly down-regulated FSDDC E-cadherin mRNA levels, with a ~50% decrease occurring about 2 h after cytokine addition (Fig. 7B). Flow cytometric data obtained in parallel (Fig. 6) demonstrated that cytokine-induced changes in cell surface E-cadherin levels were minimal at 2 h and became evident only considerably later. These results suggest that IL-1 and TNF-α cause a reduction of E-cadherin mRNA steady-state levels leading to reduced E-cadherin surface expression, which, in turn, results in a loss of E-cadherin-mediated adhesion and dissociation of FSDDC-A.

Discussion

Previous in vivo studies implicated IL-1, TNF-α, and LPS in the activation and mobilization of LC and other nonlymphoid DC
from their tissues of origin but did not allow mechanisms, or even
targets, of action of these inflammatory mediators to be precisely
determined (7, 9–14, 16). To facilitate studies of the biochemistry
and cell biology of LC/DC cell activation and maturation, as well
as studies of E-cadherin-mediated adhesion involving cells of the
dC lineage, we defined culture conditions that permit LC-like cells
to be expanded from murine fetal skin (17). LC from adult epi-
dermis are not suitable for these kinds of studies for several rea-
sons. First, the spontaneous activation and maturation that accom-
pnies preparation of LC from epidermis precluded in vitro studies
of the LC-stimulatory properties of proinflammatory cytokines.
Second, routine isolation of keratinocyte-free LC in quantities suf-
cient to permit the experiments described in this report to be
carried out was not possible.

The present study demonstrates that inflammatory mediators
that mobilize LC from epidermis act directly on LC-like DCs ex-
spended from murine fetal skin (FSDDC) in vitro and regulate E-
cadherin expression and function in these cells. Because these
same stimuli did not influence keratinocyte E-cadherin expression
(our unpublished observations), we conclude that cadherin expres-
sion and function in leukocytes and epithelial cells can be differ-
entially regulated. Loss of E-cadherin-mediated adhesion in
FSDDC was accompanied by the development of dendritic mor-
phology, up-regulation of class II MHC Ag and costimulatory mol-
ecules, and decreased expression of E-cadherin on FSDDC sur-
faces. Activation of LC in vivo results in similar changes in surface
phenotype within a similar time frame (7, 9, 14). Changes in cel-
ular morphology and in surface phenotype did not occur in
FSDDC that were disaggregated by mAb that blocked E-cadherin
homophilic interaction (ECCD-1 (34)), indicating that loss of E-
cadherin-mediated adhesion in aggregates is a consequence of ac-
tivation and maturation of FSDDC and not a trigger of this process
(our unpublished observations).

LPS treatment of FSDDC-A stimulated increases in steady-state
levels of mRNAs encoding IL-1α, IL-1β, and IL-1RA measured at
18 h, while TNF-α mRNA levels were comparable in unstimulated
and LPS-treated cells. Quantitation of proinflammatory cytokines
in supernatants revealed that FSDDC-A released small amounts of
TNF-α spontaneously and considerably more after LPS treatment.
Although immunoreactive IL-1 was not detectable in supernatants
conditioned by unstimulated or LPS-stimulated FSDDC, signifi-
cant amounts of IL-1α and IL-1β were present in freeze-thaw lys-
ates of LPS-treated cells. IL-1α did not induce TNF-α production,
and TNF-α did not stimulate IL-1 release. Results of Ab inhibition
studies were consistent with the cytokine release data. The activity
of LPS on E-cadherin-mediated adhesion involving FSDDC was
dependent on TNF-α but not on IL-1. Furthermore, anti-IL-1 re-
agents did not inhibit the action of TNF-α, and anti-TNF-α mAb
did not inhibit the action of IL-1.

Northern blot analysis indicated that IL-1 and TNF-α treatment
of FSDDC-A induced a rapid reduction in steady-state E-cadherin
levels that preceded decreases in cell surface E-cadherin. E-cad-
herin mRNA levels decreased by ~50% within 2 h after addition
of IL-1 or TNF-α, while cell surface E-cadherin protein levels
began to decrease only several hours later. Subsequent changes in
FSDDC E-cadherin levels occurred with a time course compatible
with the 5-h half-life previously reported for completely processed
E-cadherin in epithelial cells (35). This implies that E-cadherin
expression and function in FSDDC may be primarily (or entirely)
regulated at the mRNA level. Currently, we cannot differentiate
effects of IL-1 and TNF-α on E-cadherin transcription from those
on mRNA stability. Based on prior studies, regulation at the tran-
scriptional level seems likely.

In the past several years, cis-acting elements that regulate ex-
pression of murine and human E-cadherin genes have been iden-
tified and significant homologies have been noted. Particular em-
phasis has been placed on an upstream palindromic sequence
termed E-pal, and E-pal binding proteins have been demonstrated
in epithelial as well as mesenchymal cells. Deletion experiments
suggest that E-pal binding proteins act as transcriptional activators
in epithelial cells and repressors in mesenchymal cells, resulting in
tissue-specific expression of E-cadherin (36, 37). Other studies
indicate that the E-cadherin promoter is often silenced in carcinoma
cells by hypermethylation of GC-rich regions (38, 39). Overexpression
of the transmembrane tyrosine kinase-encoding oncogene
ERBB2 has been shown to decrease E-cadherin in mammary epi-
theial cells by inhibiting E-cadherin transcription, but it is not
clear whether this effect is mediated through DNA methylation
(40). Selective activation of AP-1 transcription factors (c-fos and
c-jun) in mammary epithelial cells also results in decreased E-
cadherin expression and/or function (41–43). Because AP-1 is an
important component of ERBB2, IL-1, TNF-α, and LPS signal
transduction pathways, it is possible that increased expression or
activation of this transcription factor complex in FSDDC is res-
ponsible for the down-regulation of E-cadherin expression that we
have observed. It will be of interest to determine whether any
or all of these pathways are activated in FSDDC by IL-1, TNF-α,
and LPS, or whether FSDDC E-cadherin expression is controlled
via novel regulatory mechanisms.

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