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Research Article

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Infection of Human Intestinal Epithelial Cells with Invasive Bacteria Upregulates Apical Intercellular Adhesion Molecule-1 (ICAM-1) Expression and Neutrophil Adhesion

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Abstract

The acute host response to gastrointestinal infection with invasive bacteria is characterized by an accumulation of neutrophils in the lamina propria, and neutrophil transmigration to the luminal side of the crypts. Intestinal epithelial cells play an important role in the recruitment of inflammatory cells to the site of infection through the secretion of chemokines. However, little is known regarding the expression, by epithelial cells, of molecules that are involved in interactions between the epithelium and neutrophils following bacterial invasion. We report herein that expression of ICAM-1 on human colon epithelial cell lines, and on human enterocytes in an in vivo model system, is upregulated following infection with invasive bacteria. Increased ICAM-1 expression in the early period (4–9 h) after infection appeared to result mainly from a direct interaction between invaded bacteria and host epithelial cells since it co-localized to cells invaded by bacteria, and the release of soluble factors by epithelial cells played only a minor role in mediating increased ICAM-1 expression. Furthermore, ICAM-1 was expressed on the apical side of polarized intestinal epithelial cells, and increased expression was accompanied by increased neutrophil adhesion to these cells. ICAM-1 expression by intestinal epithelial cells following infection with invasive bacteria may function to maintain neutrophils that have transmigrated through the epithelium in close contact with the intestinal epithelium, thereby reducing further invasion of the mucosa by invading pathogens. (*J. Clin. Invest.* 1996. 98:572–583.) Key words: *salmonella* • enterocytes • mucosal immunology • inflammation • bacterial pathogenesis

Introduction

Pathogenic bacteria that invade the host via the gastrointestinal tract must cross the epithelial surface lining to gain access to the underlying mucosa. In addition to forming a physical barrier to bacterial infection, intestinal epithelial cells can function as an integral component of the host's mucosal immune system. Thus, intestinal epithelial cells constitutively express, or can be induced to express, HLA class I and class II molecules as well as nonclassical HLA class Ib molecules (1, 2), produce components of the complement system (3, 4), express receptors for cytokines (4, 5), and can process and present antigens (1, 6). We recently reported that human colon epithelial cells can act as sensors for bacterial invasion since, in response to bacterial invasion, they rapidly upregulate the expression and secretion of an array of cytokines known to be important for the initiation of an acute inflammatory response (7, 8). The epithelial cytokine response is rapid, but short lived, and may be important for the initial recruitment and activation of neutrophils and mononuclear phagocytes at the site of bacterial infection (7).

Intercellular adhesion molecule-1 (ICAM-1, CD54)[1] is a cell surface glycoprotein that serves as a counter-receptor for the β2-integrins LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18) (9, 10). Interactions between ICAM-1 and the β2 integrins are known to have a central role in mediating intercellular adhesion and in signaling cells during the course of an inflammatory and immune response (11–14). ICAM-1 has a restricted tissue distribution and is constitutively expressed at low levels on subpopulations of hemopoietic cells, vascular endothelium, fibroblasts and various epithelia (e.g., bronchial, kidney, urinary tract, and skin) (15–19). Its expression is markedly upregulated at sites of inflammation (20) and cells that do not constitutively express ICAM-1 (e.g., epidermal keratinocytes) can upregulate its expression (19). The major agonists for ICAM-1 expression are proinflammatory cytokines (e.g., TNFα, IL-1, and IFN-γ) that are released at inflammatory sites (11, 12), as well as bacterial products such as LPS (16). Relevant to the studies herein, ICAM-1 is known to be expressed by several human colon epithelial cell lines and its expression can be upregulated in these cells by the cytokines TNFα, IL-1β, and IFN-γ (21–25), and is accompanied by enhanced binding of lymphocytes to the epithelial cells (22).

During the acute inflammatory response in infectious enterocolitis, the intestinal crypt epithelium is infiltrated by neutrophils, which transmigrate to the luminal side of the crypts (26–30). However, little is known regarding the molecules expressed on intestinal epithelial cells that are involved in that process and that mediate cellular interactions between intestinal epithelial cells and neutrophils during the course of acute intestinal mucosal inflammation. We report herein that expression of ICAM-1 both on human colon epithelial cell lines and human enterocytes in an in vivo model of bacterial infection is upregulated in response to bacterial invasion. More-

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1. *Abbreviations used in this paper:* CM, conditioned media; ICAM-1, intercellular adhesion molecule-1; SCID, severe-combined immunodeficient.

over, expression of ICAM-1 on epithelial cells was restricted to the apical side of the cells in vitro and in vivo, occurred in a patchy distribution in vivo, and was accompanied by increased neutrophil adhesion to epithelial cells in vitro. ICAM-1 expression by intestinal epithelial cells, in response to infection with invasive bacteria, may function to maintain neutrophils that have transmigrated through the epithelial layer in close contact with the intestinal epithelium, thereby reducing further invasion of the mucosa by invading pathogens.

Methods

Cell lines. The human colon epithelial cell lines HT29 and Caco-2 were obtained from the American Type Culture Collection (Rockville, MD), and were grown in DMEM supplemented with 10% fetal calf serum, 2 mM glutamine and 25 mM Hepes. T84 were a gift from K. Dharmsathaphorn, and were grown in 50% DMEM and 50% Ham's F12 medium supplemented with 5% newborn calf serum, 2 mM glutamine and 25 mM Hepes.

Bacteria, cytokines and other reagents. The following bacteria were used in these studies: *Salmonella dublin* (31), *Yersinia enterocolitica* 08 (7), *Listeria monocytogenes* 4b (ATCC 19115), enteroinvasive *Escherichia coli* serotype O29:NM (ATCC 43892), *S. typhi* Ty2 *aro*A *aro*C (32), *E. coli* DH5α, and *Streptococcus bovis.* The recombinant human cytokines TNFα, GM-CSF, IL-8, and IL-1β were purchased from R & D Systems (Minneapolis, MN). Human recombinant IFN-γ was obtained from Biosource International (Camarillo, CA). Goat anti–human TNFα and goat anti–human IL-1β antibodies (IgG isotype) were obtained from R&D Systems. LPS from *E. coli* serotype O111 and normal goat IgG were obtained from Sigma Chemical Co. (St. Louis, MO). Antibodies to *Salmonella* common structural antigen-1 (CSA-1) were obtained from Dynatech Labs (Billinghurst, U.K.).

Infection of human colon epithelial cell lines. Colon epithelial cells were seeded into 24- or 48-well Costar tissue culture plates at a density of 2×10^5/ml in volumes of 0.5 or 0.25 ml per well, respectively, 24 h before agonist stimulation or infection with bacteria. At this seeding density, monolayers were subconfluent (e.g., 60–80% confluent) at the time of the experiment. Bacteria were grown overnight in trypticase soy broth at 37°C, with the exceptions of *Y. enterocolitica* which was grown in trypticase soy broth at room temperature, and *S. typhi* Ty2 *aro*A *aro*C which was grown in L-broth supplemented with a mix of aromatic amino acids (32) at 37°C. *S. dublin* and *S. typhi* Ty2 *aro*A *aro*C were recultured and grown to an optical density of 0.5 and 0.3–0.35 at 600 nm, respectively, to increase invasiveness. Bacteria were washed three times in isotonic saline, and resuspended in the medium used to grow the specific colon epithelial cell line used in each experiment. Bacteria at various concentrations were added in 100 or 200 μl volumes to epithelial cells grown in 24- or 48-well culture plates, respectively, and incubated for 1 h to allow bacterial entry to occur. Monolayers were washed three times to remove extracellular bacteria, and the cultures were further incubated for up to 12 h in the presence of 50 μg/ml gentamicin to kill the remaining extracellular bacteria (7). In experiments using killed bacteria, the bacteria were incubated with a high concentration of gentamicin (500 μg/ml) for 2 d at 37°C, washed three times, and used for the experiment. Killing was confirmed by the lack of colony growth after plating of the gentamicin-treated bacteria on tryptic soy agar plates and overnight incubation. To prepare conditioned media (CM) from bacteria-infected epithelial cell cultures, confluent HT29 or T84 monolayers in six-well plates were infected for 1 h with $1–5 \times 10^8$ *S. dublin* or *Y. enterocolitica*, bacteria were washed off, and cultures were further incubated with gentamicin for 8 h in a volume of 1 ml/well. Supernatants were removed, filtered through a 0.22-μm filter, and kept frozen at −80°C until use.

Flow cytometric analysis. The following monoclonal antibodies were used as primary antibodies for flow cytometry: Anti-CD54 (ICAM-1) (murine IgG1, AMAC, Inc., Westbrook, ME), anti-CD102 (ICAM-2) (murine IgG2a, Biosource International), anti-CD50 (ICAM-3) (murine IgG1, R&D Systems), anti-CD31 (PECAM-1 (murine IgG1, R&D Systems), anti-CD106 (VCAM-1) (murine IgG1, R&D Systems), and anti–human anti-IL-6 as a control antibody (murine IgG1, R&D Systems). We previously reported that the epithelial cell lines used herein do not express IL-6 (8). Monolayers of colon epithelial cells were detached by incubation with 0.25% trypsin and 0.25% EDTA in calcium- and magnesium-free PBS (pH 7.2). In control experiments it was found that this treatment did not interfere with the detection of the adhesion molecules tested in the present study. Cell viability was > 95%, as assessed by trypan blue dye exclusion. For flow cytometry, $\sim 5 \times 10^5$ cells were incubated with optimal concentrations of primary mouse monoclonal antibody at 4°C for 30 min. Subsequently, cells were washed, incubated with an optimal concentration of a R-phycoerythrin-labeled goat antibody against mouse IgG(H+L) (Southern Biotechnology Associates, Inc., Birmingham, AL) at 4°C for 30 min, and analyzed using a flow cytometer (FACScan, Becton Dickinson, Sunnyvale, CA).

RNA extraction and Northern blot analysis. Cellular RNA was isolated using an acid guanidinium thiocyanate-phenol-chloroform method (Trizol; GIBCO BRL, Gaithersburg, MD). 10 μg of total RNA was size-fractionated on a formaldehyde/agarose gel, transferred to nitrocellulose, and probed with a ^{32}P-labeled cDNA fragment specific for human ICAM-1. The ICAM-1 cDNA fragment was prepared by RT-PCR amplification of RNA extracted from HT29 cells using primers that target a 398-bp fragment in the human ICAM-1 coding region (Stratagene, San Diego, CA). Hybridizations were performed at 42°C for 16 h using a solution of 50% formamide, 10% dextran sulfate, 5× SSC (1× SSC is 0.15 M NaCl, 15 mM sodium citrate), 50 mM Na_2HPO_4 (pH 7.0), 5× Denhardt's solution, 0.1% SDS, 250 μg/ml of salmon sperm DNA, and 5 μg/ml of polyuridylic acid. Nonspecifically bound radioactivity was removed by washing the blots twice in 0.1× SSC, 0.1% SDS at 60°C for 20 min, after which blots were exposed to x-ray film (Kodak XAR) at −80°C using an intensifying screen. Subsequently, blots were stripped in 0.1× SSC, 0.1% SDS at 85°C for 20 min, and re-hybridized to a ^{32}P-labeled cDNA fragment of human glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Human fetal intestinal xenografts. The human fetal intestinal xenograft model used in the present work has been described in detail previously (33). Briefly, human fetal small intestine (n = 6, gestational age 10–14 wk) was transplanted subcutaneously into C.B-17 severe-combined immunodeficient (SCID) mice. Xenografts were allowed to develop for a period of 10 wk before use, at which time the epithelium and underlying mucosa is fully differentiated (33). Xenografts were infected with $\sim 5 \times 10^7$ *S. typhi* Ty2 *aro*A *aro*C in DME/F12 medium in a 100 μl volume injected intraluminally by subcutaneous injection. Xenograft tissue was removed 6 h after infection, extensively washed, and snap frozen in OCT compound. Whole-mount pieces (5 × 5 mm) or 7 μm frozen sections of xenograft intestine were then fixed in methanol (−40°C) before staining by indirect immunofluorescence. Sections and tissues were counterstained with 5 μg/ml propidium iodide. The xenograft studies were performed with full approval from the Cambridge Local Ethics Committee and in accordance with the Home Office guidelines specified in the Polkinghorne Report (1989) (34).

Isolation of neutrophils and neutrophil adhesion assays. Peripheral blood obtained from healthy donors was anticoagulated with sodium heparin, and mixed with an equal volume of 6% dextran in PBS. After incubation at room temperature for 20 min, cells in the leukocyte-rich plasma were collected and resuspended in 0.9% NaCl, layered on Ficoll-Paque Plus (Pharmacia Biotech, Uppsala, Sweden) and centrifuged for 45 min at room temperature. Pellets were harvested and residual erythrocytes were lysed by hypotonic lysis. This procedure yielded > 95% viable neutrophils.

For the adhesion assay, neutrophils (5×10^6/ml) were resuspended in RPMI medium containing 2% human serum (Sigma

Chemical Co., St. Louis, MO). Neutrophils were added in a 200 μl volume to confluent HT29 monolayers grown in 48-well plates at a neutrophil:epithelial cell ratio of ~ 10:1, and the cultures were centrifuged at room temperature for 5 min at 50 *g*. Cultures were incubated for 10 min at 37°C, after which the monolayers were gently washed three times with HBSS to remove non-adherent neutrophils. For blocking ICAM-1, HT29 cells, previously incubated with agonists or infected with bacteria, were incubated for 30 min at room temperature with 20 μg/ml mouse anti–human CD54 (ICAM-1) (clone RR1, kindly provided by Dr. R. Rothlein) (9) or mouse anti–human CD4 (Becton Dickinson) as a control antibody. For blocking neutrophil counter-receptors for ICAM-1, neutrophils were incubated with 10 μg/ml mouse anti-human CD18 (LFA-1β) (Biosource International) for 30 min at 4°C before adding the cells to the epithelial monolayers. Antibodies were present throughout the subsequent incubation period.

Neutrophil adherence was quantitated by assaying myeloperoxidase activity in the adherent neutrophils. For myeloperoxidase assays, 100 μl HBSS containing 0.5% Triton X-100 was added to the epithelial cell/neutrophil co-cultures, followed by the addition of 10 μl of 1.0 M citrate buffer, pH 4.2. Lysates were centrifuged to remove debris, and enzymatic activity was determined by adding an aliquot of the cell lysates to a solution containing 1 mM ABTS (2,2′-azino-di-[3-ethyl]dithiazoline-6-sulfonic acid), 10 mM H_2O_2 in 100 mM citrate buffer (pH 4.2). Absorbance was determined at 405 nm.

Results

Co-culture of colon epithelial cells with invasive bacteria upregulates ICAM-1 expression. To characterize the expression of adhesion molecules on intestinal epithelial cells in response to infection with invasive enteric bacteria, we used the human colon epithelial cell lines HT29, T84, and Caco-2 as a model system. These cells are known to constitutively express variable levels of surface ICAM-1 (21, 22), whereas little is known about the expression of other adhesion molecules by these cells. Subconfluent monolayers of these cells were co-cultured with a range of invasive and non-invasive gram negative bacteria, and the expression of adhesion molecules was quantitatively assessed by flow cytometry. As shown in Fig. 1, *A* and *B* and Table I, the invasive bacteria *Y. enterocolitica*, *S. dublin*, and enteroinvasive *E. coli* (serotype O29:NM) markedly upregulated surface ICAM-1 expression on HT29 and T84 cells. Similarly, ICAM-1 levels were increased, although to a lesser extent, following co-culture with an invasive gram positive bacteria, *L. monocytogenes* (Table I). In contrast to the findings with invasive bacteria, infection with the noninvasive gram negative bacteria *E. coli* DH5α or gram positive bacteria *S. bovis*, or stimulation with bacterial LPS, did not upregulate ICAM-1 expression. As a comparison, the known agonists of ICAM-1 expression, TNFα and IFN-γ, were moderately more potent stimulators of surface ICAM-1 expression by HT29 and T84 cells than the invasive bacteria *Y. enterocolitica* and *S. dublin* (Table I). GM-CSF, an agonist known to increase ICAM-1 expression on several other cell types (35–37), did not stimulate surface ICAM-1 expression on HT29 cells when tested in doses ranging from 1 ng to 100 ng/ml (data not shown). Unlike the human colon epithelial cell lines T84 and HT29, Caco-2 cells constitutively expressed high levels of surface ICAM-1, and surface ICAM-1 levels were only minimally upregulated (< twofold) in this cell line in response to co-culture with invasive bacteria or stimulation with the agonists TNFα or IFN-γ (data not shown).

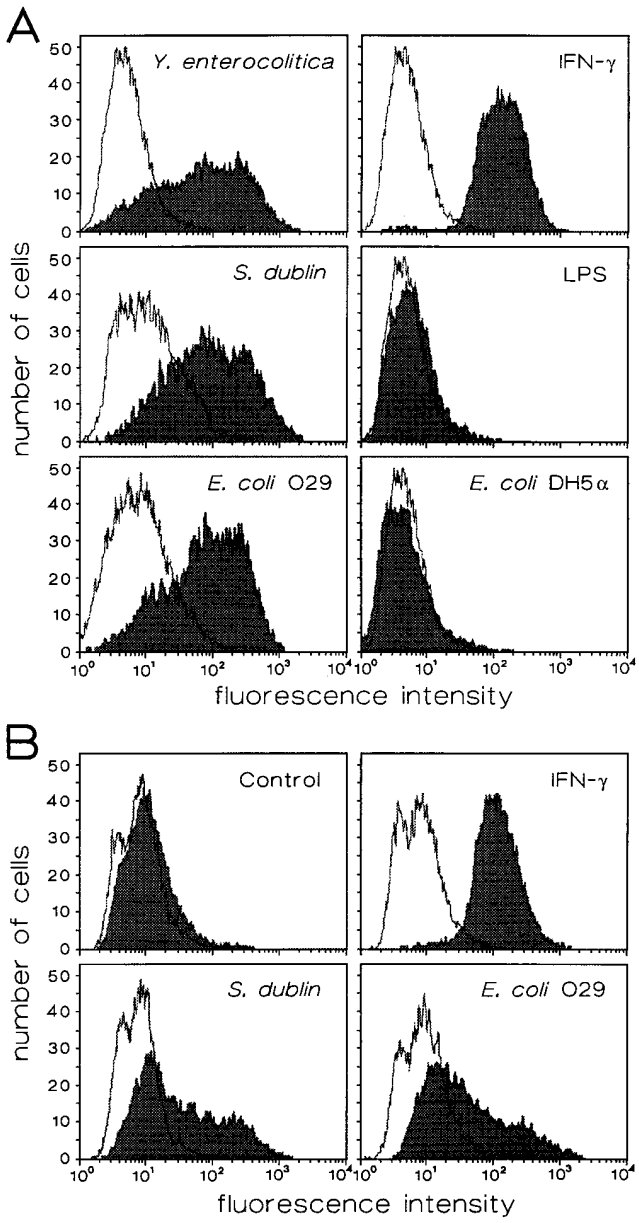

Figure 1. Flow cytometric analysis of ICAM-1 expression by colon epithelial cell lines infected with invasive bacteria. (*A*) Subconfluent monolayers of HT29 colon epithelial cells in 24-well plates were infected for 1 h with 5×10^8 bacteria/well (except *S. dublin* which was used at 10^8/well) in a 1-ml volume, washed, and further incubated in the presence of 50 μg/ml gentamicin for 9 h. Parallel cultures were stimulated for 9 h with 10 μg/ml LPS or 40 ng/ml IFN-γ. Cells were detached from the plates, stained for ICAM-1 by indirect immunofluorescence, and analyzed by flow cytometry. Shaded areas represent ICAM-1 expression in agonist stimulated or bacterially infected cultures and lines depict ICAM-1 expression in parallel unstimulated control cultures. Data are from a representative experiment. Quantitative data from several independent experiments are presented in Table I. (*B*) Culture and infection conditions were as in Fig. 1 *A*, with the exception that the bacterial inocula were fivefold lower. Cells in control wells were neither agonist-stimulated nor bacterially infected. Shaded areas are results using anti-ICAM-1 as the primary antibody and lines are results using an isotype-matched control monoclonal antibody (murine IgG1 anti–human IL-6) as the primary antibody. The secondary antibody was R-phycoerythrin-labeled goat anti-IgG as described in Methods. As shown, unstimulated control cells constitutively expressed low levels of ICAM-1.

Table I. Increased ICAM-1 Expression by Colon Epithelial Cell Lines in Response to Infection with Invasive Bacteria

Additions to culture	ICAM-1 surface levels*	
	HT29 cells	T84 cells
Gram-negative bacteria		
Y. enterocolitica	9.2±1.1	4.9±0.2
S. dublin	6.0±0.7	4.4±0.9
E. coli O29:NM (enteroinvasive)	8.5±1.4	1.7±0.1
E. coli DH5α (nonpathogenic)	1.1±0.1	1.2±0.1
Gram-positive bacteria		
L. monocytogenes	2.1±0.4	2.9±0.2
S. bovis	0.9±0.1	1.0±0.1
Other stimuli		
LPS	1.1±0.1	1.2±0.1
TNFα	15.5±2.3	11.9±1.7
IFN-γ	18.5±2.2	14.6±1.7
None	1.0	1.0

Monolayers of HT 29 and T84 cells were cultured in 1 ml volumes in 24-well plates and infected with 5×10^8 bacteria/ml (except *S. dublin* which was used at 10^8/ml) for 1 h. Cultures were washed to remove extracellular bacteria, and incubated for an additional 9 h in the presence of 50 μg/ml gentamicin. Parallel cultures were stimulated for 9 h with 10 μg/ml LPS, 50 ng/ml TNFα, or 40 ng/ml IFN-γ. Cells were removed from the plates, and incubated with mouse anti-human ICAM-1, followed by incubation with R-phycoerythrin-labeled goat anti–mouse IgG and analyzed by flow cytometry as described in Methods. *Surface levels of ICAM-1 are expressed as the ratio of the median fluorescent intensity of stimulated cells to that of control cells. Values represent means± SEM of the results of three or more independent experiments.

In addition to ICAM-1, the HT29 colon epithelial cell line, but not T84 or Caco-2 cells, constitutively expressed low levels of ICAM-2. However, the expression of ICAM-2 was not upregulated on HT29 cells in response to infection with *S. dublin* or *Y. enterocolitica*, or stimulation with TNFα or IFN-γ (data not shown). In contrast, ICAM-3, VCAM-1, and PECAM-1 were not detected on unstimulated HT29, T84, or Caco-2 cells, or after stimulation with the identical stimuli.

Relationship of bacterial inoculum and surface ICAM-1 levels. Since ICAM-1 was the only adhesion molecule among those tested that was expressed and upregulated in response to infection with invasive bacteria, the subsequent studies focused on the mechanisms that govern the regulated expression of this adhesion molecule. ICAM-1 expression, as shown in Fig. 2, *A* and *B*, increased with increasing bacterial inocula. Furthermore, as shown in Fig. 2 *A* for *S. typhi* infection, at increased bacterial inocula, increased ICAM-1 expression was mostly due to a greater proportion of ICAM-1 positive cells, since the intensity of ICAM-1 staining on ICAM-1 positive cells was similar at the different bacterial inocula tested. Infection with as few as, on average, 2–5 *S. dublin* per cell increased the median fluorescent intensity of ICAM-1 staining of HT29 and T84 cells (Fig. 2 *B*). In comparison, infection with *Y. enterocolitica* required 10- to 100-fold higher inocula for maximal ICAM-1 induction, although 10–20-fold higher numbers of *Y. enterocolitica* than *S. dublin* can be recovered from HT29 and T84 cells infected with identical inocula of these bacteria (8).

Upregulation of ICAM-1 surface expression required infection with live organisms, since killed *S. dublin* or *Y. entero-*

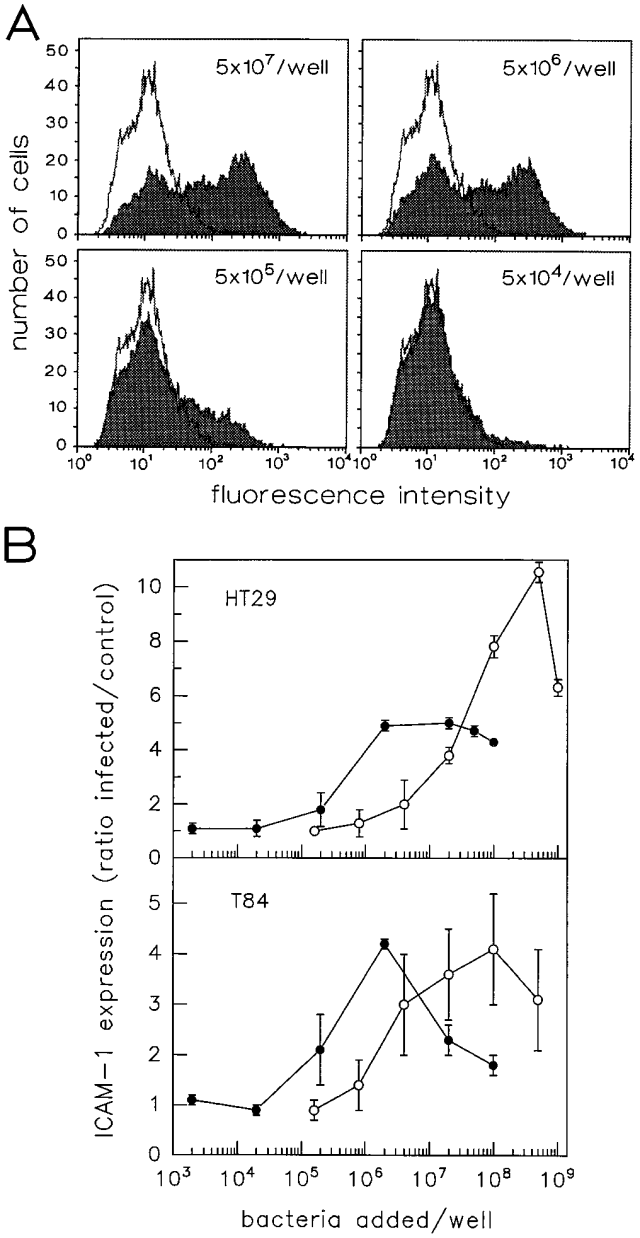

Figure 2. Relationship between bacterial inoculum and increased ICAM-1 expression in bacteria-infected colon epithelial cell lines. (*A*) Subconfluent monolayers of HT29 cells in 24-well plates were infected for 1 h with different numbers of *S. typhi* Ty2 *aro*A *aro*C (5×10^4–5×10^7/well) as indicated, washed, and further incubated in the presence of gentamicin for 9 h. Cells were stained for ICAM-1 and analyzed by flow cytometry as described in the legends for Fig. 1 *A* and Table I. Shaded areas represent ICAM-1 expression in bacterial-infected cultures and lines depict ICAM-1 expression in parallel unstimulated control cultures. Cultures contained ~ 1–2 × 10^5 cells/well. (*B*) Subconfluent monolayers of HT29 and T84 cells were infected with different numbers of *S. dublin* (●) or *Y. enterocolitica* (○) as in *A*. ICAM-1 surface levels were determined by flow cytometry as in Fig. 1 *A* and Table I, and are given as a ratio of the median fluorescent intensity of infected cells relative to that in uninfected control cells. Cultures contained approximately 1–2 × 10^5 cells/well. Data points represent means±SEM of the results of three independent experiments.

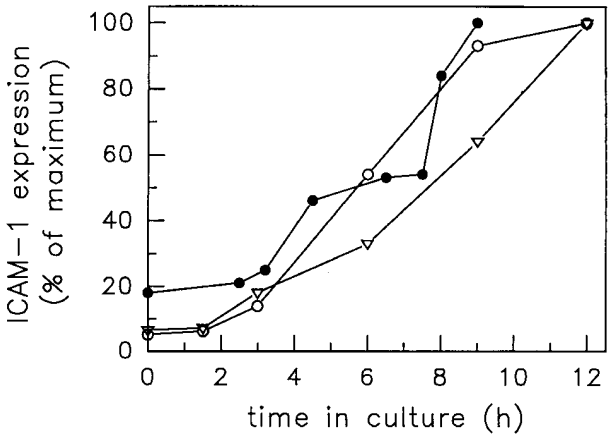

Figure 3. Time course of increased ICAM-1 expression by HT29 colon epithelial cells after *S. dublin* infection or agonist stimulation. Subconfluent monolayers of HT29 cells in 24-well plates were infected with *S. dublin* (●), or stimulated with TNFα (○) or IFN-γ (▽), as described in Table I. At different times after infection, cells were removed from the plates, and analyzed for ICAM-1 surface levels by flow cytometry. Results for each time point are calculated as a ratio of the median fluorescent intensity of stimulated to control cells, and are depicted in the figure as a percentage of the maximum ICAM-1 surface levels observed for *S. dublin* or each agonist during the course of the experiment. Data from a representative experiment are shown. Similar results were obtained in an additional experiment.

colitica did not increase surface expression of ICAM-1 on HT29 cells (the ratio of ICAM-1 expression on cells co-cultured with 1×10^8/well and 5×10^8/well killed bacteria to that on control cells was 0.97±0.1, and 1.1±0.1 for *S. dublin* and *Y. enterocolitica*, respectively, as determined in three separate experiments). Thus, induction of ICAM-1 required viable bacteria, but was not strictly related to the number of intracellular bacteria, which may reflect differences in the bacterial route of entry or the intracellular localization of bacteria.

Time course analysis of epithelial ICAM-1 expression following infection with invasive bacteria. A time course analysis of ICAM-1 expression by HT29 cells is shown in Fig. 3. Surface ICAM-1 levels increased within 3–6 h after *S. dublin* infection or stimulation with TNFα or IFN-γ, and continued to increase over the 9–12 h period of study. Furthermore, when HT29 cells were infected with *S. typhi aro*A *aro*C, which invades the cells but does not replicate inside, and analyzed for ICAM-1 expression 9 and 31 h after infection, ICAM-1 expression was increased to a similar extent at both time points, indicating that ICAM-1 expression remained increased over prolonged periods following infection. Similar kinetics for increased ICAM-1 expression were found in *S. dublin*-infected T84 cells (data not shown).

Increased surface ICAM-1 expression is paralleled by increased ICAM-1 mRNA levels. To further characterize the mechanisms that underlie increased epithelial ICAM-1 expression in response to infection with invasive bacteria, ICAM-1 mRNA levels were determined by Northern blot analysis. As shown in Fig. 4 for HT29 cells, following *S. dublin* infection, ICAM-1 mRNA levels increased by 2 h, peaked at 3–5 h with a

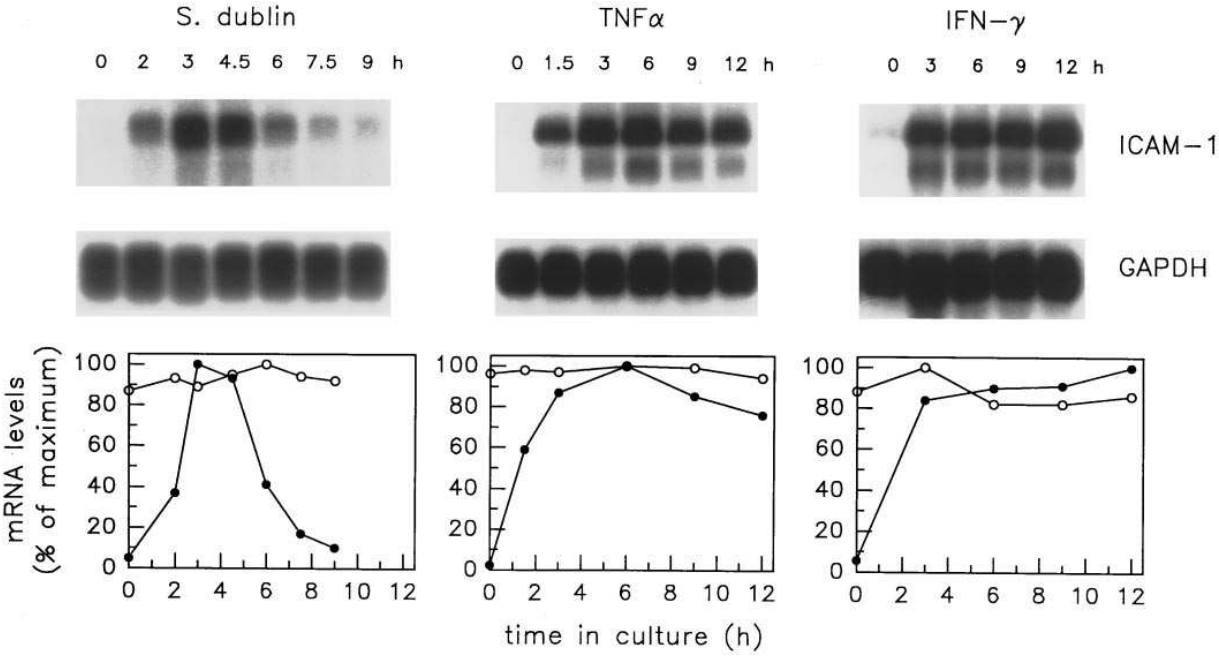

Figure 4. Northern blot analysis of increased ICAM-1 expression by HT29 colon epithelial cells in response to *S. dublin* infection or agonist stimulation. HT29 monolayers were infected with *S. dublin*, or stimulated with TNFα or IFN-γ, as described in Table I. At different times after infection, cellular RNA was extracted, 10 μg RNA was size-fractionated on a formaldehyde/agarose gel, and transferred to a nitrocellulose membrane. Blots were probed with radioactively labeled cDNA fragments of the genes for human ICAM-1 and, after stripping of the blots, human GAPDH. Blots were exposed to x-ray film for optimal periods of time (*top panel*), and the films were analyzed by scanning densitometry (*bottom panel*). Scanning results are expressed as a percentage of the maximum mRNA levels for ICAM-1 (●) and GAPDH (○) observed during the course of the experiment. The Northern blots for ICAM-1 show two mRNAs of 3.4 and 2.5 kb, as expected from previous reports (10). The Northern blots for GAPDH shows a single band of 1.4 kb. Sizes of mRNAs were derived from comparison with ribosomal RNAs.

Table II. Release of TNFa or IL-1β Is not Important for Increased ICAM-1 Expression by Colon Epithelial Cells after Bacterial Invasion

Cells	Stimulus added*	ICAM-1 surface levels				
		Controls	+ Anti-TNF*a*‡	+ Anti-IL-1β‡	+ Anti-TNFα + anti-IL-1β‡	+ Control Ig‡
HT29	CM from *Y. enterocolitica*-infected cells	1.7±0.2§	1.1±0.1	1.4±0.1	1.0±0.1	1.8±0.5
	CM from *S. dublin*-infected cells	2.0±0.2	1.3±0.1	1.5±0.2	1.2±0.1	1.5±0.1
	Y. enterocolitica	11.6±0.7	7.9±0.7	13.0±1.1	9.4±0.3	11.0±0.9
	S. dublin	7.6±1.3	5.6±1.1	6.3±0.1	5.7±0.3	6.2±1.0
	TNFα	17.3±1.3	0.9±0.1	n.d.	n.d.	17.2±2.0
	IL-1β	2.2±0.3	n.d.	1.0±0.1	1.0±0.2	2.2±0.1
	None	1.0	n.d.	n.d.	n.d.	n.d.
T84	CM from *Y. enterocolitica*-infected cells	1.0±0.2	0.9±0.1	n.d.	n.d.	n.d.
	CM from *S. dublin*-infected cells	1.4±0.4	0.9±0.2	n.d.	n.d.	n.d.
	Y. enterocolitica	4.6±0.5	5.4±0.5	n.d.	n.d.	n.d.
	S. dublin	4.5±0.5	4.2±0.4	n.d.	n.d.	n.d.
	TNF*a*	9.8±0.8	0.7±0.1	n.d.	n.d.	n.d.
	None	1.0	n.d.	n.d.	n.d.	n.d.

*Conditioned media (CM) were prepared from bacteria-infected epithelial cell monolayers as described in the Methods. Fresh epithelial cells were cultured in 100% CM. Parallel cultures were stimulated with 25 ng/ml TNFα or IL-1β for 12 h. Subsequently, cells were removed from the dishes, stained for ICAM-1 expression by indirect immunfluorescence, and analyzed by flow cytometry. ‡Polyclonal goat antibodies (20 μg/ml) against human TNFα and IL-1β, as well as a control antibody (normal goat IgG), were present throughout the infection period and incubation period for the bacteria-infected cultures, and throughout the stimulation period for the agonist-stimulated cultures. For cultures containing CM, antibodies were not present during the preparation of the CM, but were added at the time of CM addition to fresh monolayers. §Levels of surface ICAM-1 expression are given as a ratio of the median fluorescent intensity of stimulated cells to that of control cells. Numbers represent mean±SEM of at least three experiments. *n.d.*, not done.

10- to 20-fold increase over controls, and returned to near baseline levels at 9 h after infection. Similar results were obtained with *S. dublin*-infected T84 cells (data not shown). Thus, infection with invasive bacteria induced a relatively rapid but transient increase in epithelial ICAM-1 mRNA levels. In comparison, increased mRNA levels following TNFα or IFN-γ stimulation of HT29 cells followed similar kinetics in the first 4 h after stimulation. However, in contrast to bacterial infection, mRNA levels following TNFα stimulation decreased only slightly over the 12 h observation period. Furthermore, after IFN-γ stimulation, ICAM-1 mRNA levels continued to increase up to 12 h after stimulation, a finding which is consistent with a previous observation that IFN-γ increases ICAM-1 mRNA stability (38).

Role of TNFα or IL-1β release in increased expression of ICAM-1 by colon epithelial cells after bacterial invasion. We previously reported that bacterial invasion of HT29 and T84 cells by *S. dublin* resulted in increased TNFα mRNA expression and secretion, and increased, albeit low, levels of IL-1β mRNA (8). Since those cytokines can stimulate increased ICAM-1 expression by epithelial cells, we asked whether increased ICAM-1 expression by HT29 and T84 cells following bacterial invasion was simply due to the release of TNFα or IL-1β by these cells. As shown in Table II, ICAM-1 expression was upregulated by 1.7 to 2-fold when CM from HT29 cells infected with *Y. enterocolitica* or *S. dublin* were added to fresh cultures of HT29 cells, an increase that was only 15–26% of that seen after infection of HT29 cells with the same bacteria. Also shown in Table II, the ability of CM to increase ICAM-1 expression by HT29 cells was mostly due to TNFα and not IL-1β, as indicated in blocking studies using anti-TNFα and anti–IL-1β antibody. In contrast to HT29 cells, CM from bacteria-infected T84 cells did not significantly induce ICAM-1 expression by these cells when added to fresh monolayers (Table II), a finding which is consistent with the previous observation that T84 cells secrete only very low levels of TNFα (< 6 pg/ml) following infection with invasive bacteria (8).

In a further approach to determine the importance of released TNFα or IL-1β for increased ICAM-1 expression by HT29 and T84 cells following infection with invasive bacteria, antibodies to TNFα, IL-1β or both were added to the cultures during infection and throughout the subsequent culture period. As shown in Table II, blocking of TNFα decreased ICAM-1 expression by HT29 cells by ~ 30% after infection with *Y. enterocolitica* or *S. dublin.* In contrast, antibodies against IL-1β or control antibodies had no effect on increased ICAM-1 expression. Anti-TNFα antibodies had no effect on increased ICAM-1 expression by T84 cells following bacterial infection. Anti–IL-1β antibodies were not tested in T84 cells since these cells do not express IL-1 type 1 receptors (M.F. Kagnoff, unpublished data) or respond to IL-1 (39). Of note, IFN-γ, a potent agonist of ICAM-1 expression, is not expressed by the HT29 or T84 cell lines either in response to agonist stimulation or bacterial invasion (8) and, therefore, was not tested. Further, GM-CSF, which can be expressed and secreted by intestinal epithelial cell lines (8), and is known to upregulate ICAM-1 in some cell types (35–37), did not affect ICAM-1 expression by these cells. Taken together, these data indicate that TNFα and IL-1β released by colon epithelial cells in response to bacterial invasion played only a minor role in upregulating ICAM-1 expression on those cells over the time course examined.

Co-localization of invaded bacteria and increased ICAM-1 expression in colon epithelial cells. The finding that cytokine release was of minor importance for increased ICAM-1 ex-

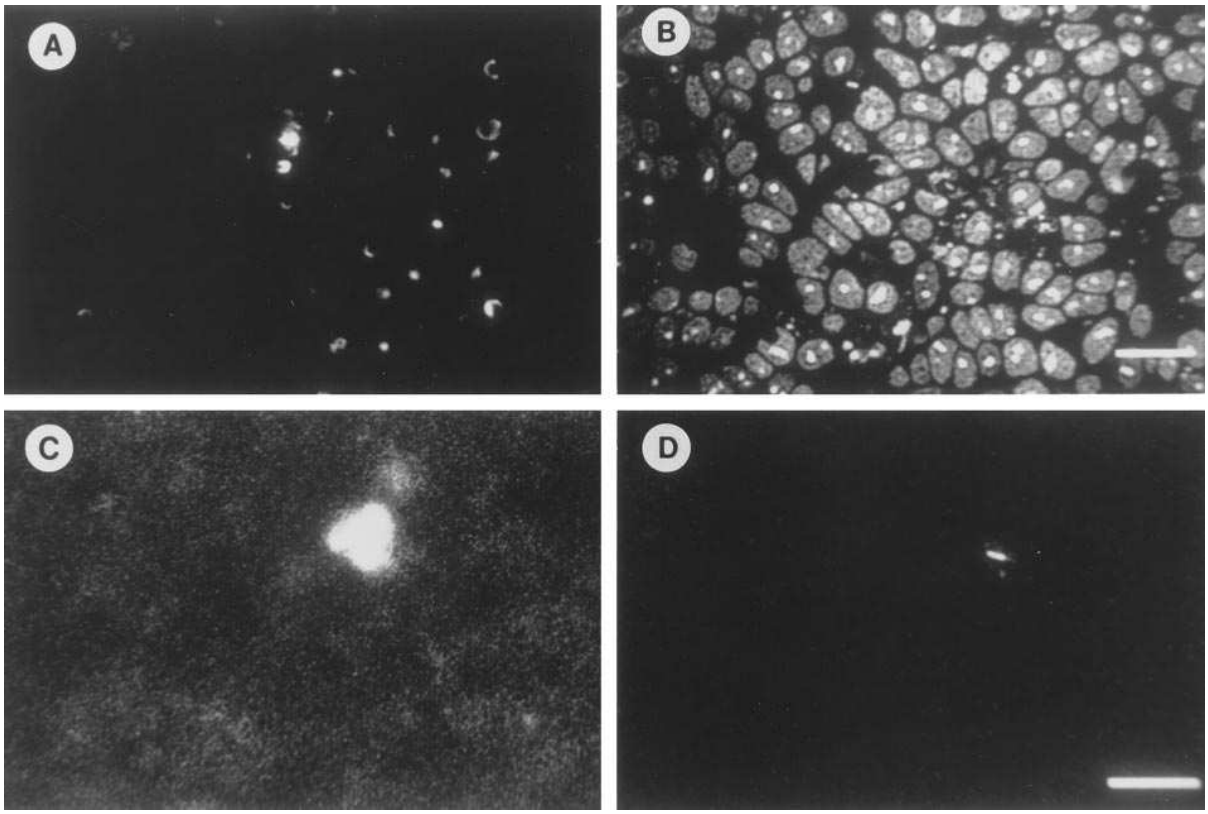

Figure 5. Co-localization of invaded *S. typhi* and ICAM-1 expression in T84 colon epithelial cells. Confluent T84 monolayers grown on collagen-coated coverslips were infected for 1 h with $\sim 1 \times 10^7$ *S. typhi* Ty2 *aro*A *aro*C per well in a 1 ml volume in 24-well plates, washed, and incubated for an additional 7 h with 50 μg/ml gentamicin. Monolayers were fixed with methanol, and stained by indirect immunofluorescence for ICAM-1 expression (*A*) and counterstained with propidium iodide (*B*), or stained by indirect immunofluorescence for ICAM-1 expression (*C*) and expression of *Salmonella* common structural antigen-1 (*D*). *A* and *B* are low-power photomicrographs that were taken from an identical area of the coverslip at the same magnification but with two different wavelength filters of 488 and 514 nm, respectively. Bar (*B*), 50 μm. *A* shows ~ 20–30 cells that stain positive for ICAM-1. Note that some cells show a ring-like staining pattern. *B* shows staining of nuclei (moderately bright, ovoid staining, 10–30 μm in size) and of 1–2 nucleoli per nucleus (bright round staining, 2–6 μm in size). The area displayed in *A* and *B* covers ~ 200 cells. Comparison of panels *A* and *B* shows that only 10–15% of all cells stained positive for ICAM-1 following *S. typhi* infection. *C* and *D* are high-power photomicrographs that also were taken from an identical section of the coverslip at the same magnification but with two different wavelength filters of 488 and 514 nm, respectively. The figures show co-localization of ICAM-1 expression (*C*) and intracellular *Salmonella* (*D*). The latter can be identified by the rod-shaped positive staining for *Salmonella* common structural antigen-1. Bar (*D*), 10 μm.

pression in the first 9 h after bacterial invasion suggested that increased ICAM-1 expression early after infection resulted mainly from a direct interaction of invaded bacteria with host cells. To further characterize that interaction, T84 cells were infected with an invasive, but replication-deficient *S. typhi*, at a dose selected to leave a large proportion of cells uninfected, and two-color immunofluorescence studies were performed to co-localize invaded bacteria and increased ICAM-1 expression. As shown in Fig. 5, *A* and *B*, and consistent with the use of a relatively low infectious dose, only a small fraction of the cells stained positive for ICAM-1. Furthermore, when invaded bacteria were visualized in cells by staining for *Salmonella* common structural antigen-1, bacteria were found to co-localize in epithelial cells expressing ICAM-1 (Fig. 5, *C* and *D*). Uninfected control T84 monolayers did not stain for ICAM-1 or *Salmonella* common structural antigen-1 (data not shown).

ICAM-1 is expressed on the apical side of polarized T84 epithelial cells after infection with invasive bacteria. Intestinal epithelial cells in vivo are structurally polarized into apical and basolateral sides, and many epithelial surface molecules, such as digestive enzymes or specific ion channels, selectively localize to those domains. To address the question whether ICAM-1 is expressed in a polarized manner, T84 cells grown as polarized monolayers were used as a model system. The monolayers were infected with a relatively low dose of an invasive *S. typhi*, stained for ICAM-1 expression, and staining was analyzed by confocal microscopy. As demonstrated in Fig. 6, ICAM-1 expression was confined to the apical side of the polarized T84 cells. Furthermore, the majority of ICAM-1 staining was localized close to the intercellular junctions, as evidenced by a ring-like staining pattern (Fig. 6 *A*). Consistent with the data presented in Table I, polarized control T84 monolayers expressed little, if any, ICAM-1, as determined by confocal microscopy (data not shown).

Expression of ICAM-1 by intestinal epithelial cells in human intestinal xenografts infected with invasive bacteria. We next asked whether ICAM-1 can be expressed by intestinal epithelial cells in vivo. In order to acutely infect human intestinal epithelial cells with invasive bacteria, we used a human fetal intestinal xenograft model where human fetal intestine (gesta-

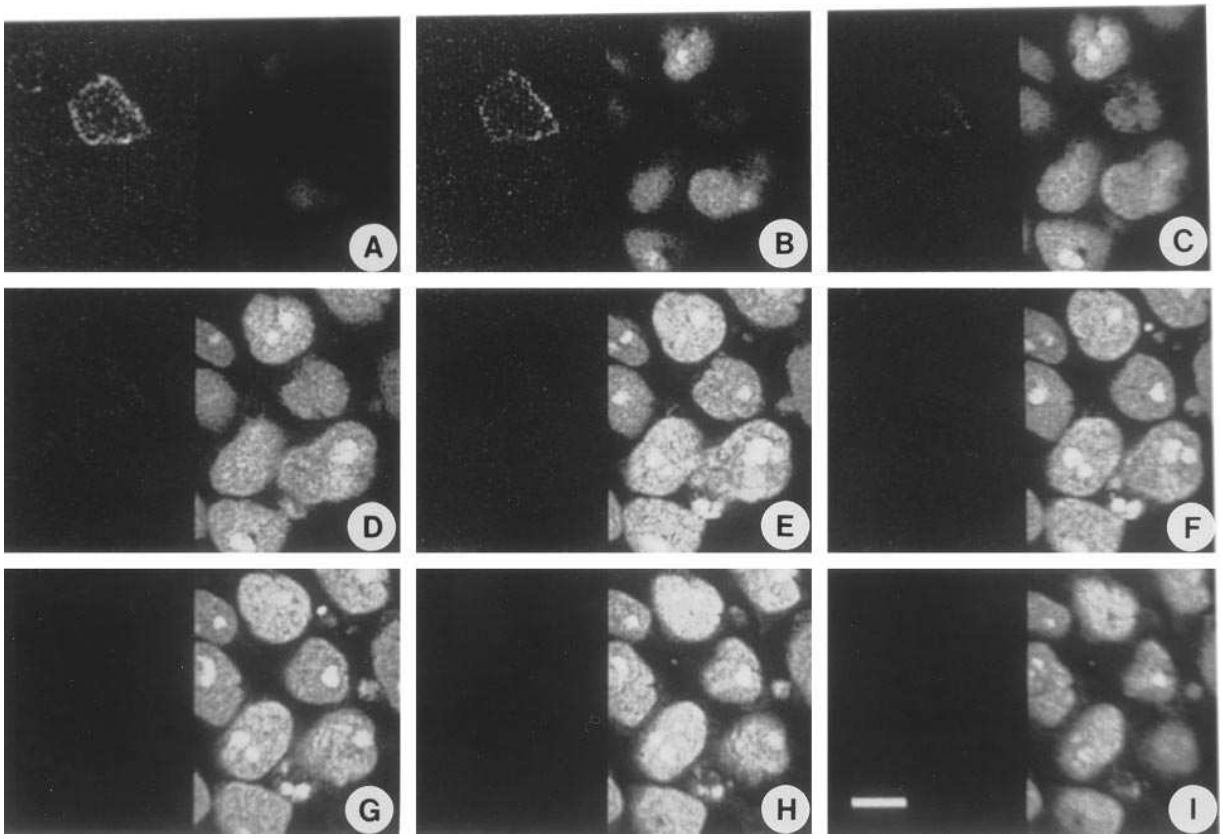

Figure 6. Apical ICAM-1 expression by *S. typhi*-infected T84 colon epithelial cells. T84 cells were grown on collagen-coated filter inserts (Nunc, 10 mm diameter, 0.2 μm pore size) or cover slips in 24-well plates for at least 7 d after reaching confluence to allow full differentiation of the monolayers. Monolayer confluence and formation of tight junctions, as well as morphologic differentiation, were confirmed by electrical resistance measurements and scanning electron microscopy, respectively. Monolayers in 24-well plates were infected on the apical side with 1×10^7 *S. typhi* Ty2 *aro*A *aro*C per well for 1 h, washed, and further incubated for 7 h with gentamicin. Monolayers were fixed with methanol, and stained for ICAM-1 expression by indirect immunofluorescence (left side of each panel), and counterstained with propidium iodide (right side of each panel). Stained monolayers were analyzed by confocal microscopy. An optical section was obtained every 2 μm parallel to the filter support. *A* represents the most apical optical section, and *I* represents the most basolateral optical section. Bar (*I*), 5 μm.

tional age 10–14 wk, $n = 6$) is transplanted subcutaneously into SCID mice. The xenografts develop a fully differentiated epithelial layer of entirely human origin over a 10- to 20-wk period (33). Fully differentiated xenografts were infected intraluminally with invasive *S. typhi*, and tissue sections were prepared and stained for ICAM-1 by indirect immunofluorescence. As depicted in two examples in Fig. 7, a small proportion of intestinal epithelial cells stained positively for ICAM-1 following *S. typhi* infection of the xenograft, whereas no epithelial ICAM-1 staining was observed in uninfected control xenografts (data not shown). Furthermore, consistent with the findings in polarized T84 cells, ICAM-1 staining was confined to the apical side of the epithelial cells (Fig. 7 *C*), and ICAM-1 expression was greatest close to the intercellular junctions (Fig. 7 *A*). Thus, ICAM-1 expression by intestinal epithelial cells is seen in response to in vivo infection with invasive bacteria. In contrast to ICAM-1, the xenograft epithelium did not stain for ICAM-2, ICAM-3, VCAM-1, or PECAM-1 in control tissues or after *S. typhi* infection (data not shown), indicating that these adhesion molecules were not expressed by intestinal epithelial cells and that epithelial ICAM-1 staining was specific and not related to a Fc receptor-like binding activity of these cells.

Increased surface ICAM-1 expression is paralleled by increased neutrophil adhesion to bacteria-infected colon epithelial cells. Neutrophil infiltration at the site of infection is a hallmark of acute bacterial infections. Since bacterial infection of colon epithelial cells upregulates ICAM-1 expression and neutrophils express counter-receptors for ICAM-1, we next tested the possibility that ICAM-1 expressed on the colon epithelial cells enhances the binding of neutrophils to those cells. As shown in Table III, neutrophil adherence to HT29 colon epithelial cells was markedly increased following infection with *Y. enterocolitica* or *S. dublin.* Such was also the case following TNFα stimulated upregulation of epithelial ICAM-1 expression. Furthermore, addition of anti–ICAM-1 antibodies to epithelial cells blocked neutrophil binding by ~ 40–50%, which indicated that enhanced neutrophil adhesion was at least partly mediated by ICAM-1. Of note, this degree of blocking is consistent with that found by others using different cell types (9, 20), and may reflect the inability of the anti–ICAM-1 antibodies to completely interfere with the interaction of ICAM-1 with its counter-receptor on neutrophils. In addition, intestinal epithelial cells are reported to express receptors that may bind neutrophils via carbohydrate ligands (40). In further studies, incubation of neutrophils with anti-CD18 antibodies prior to

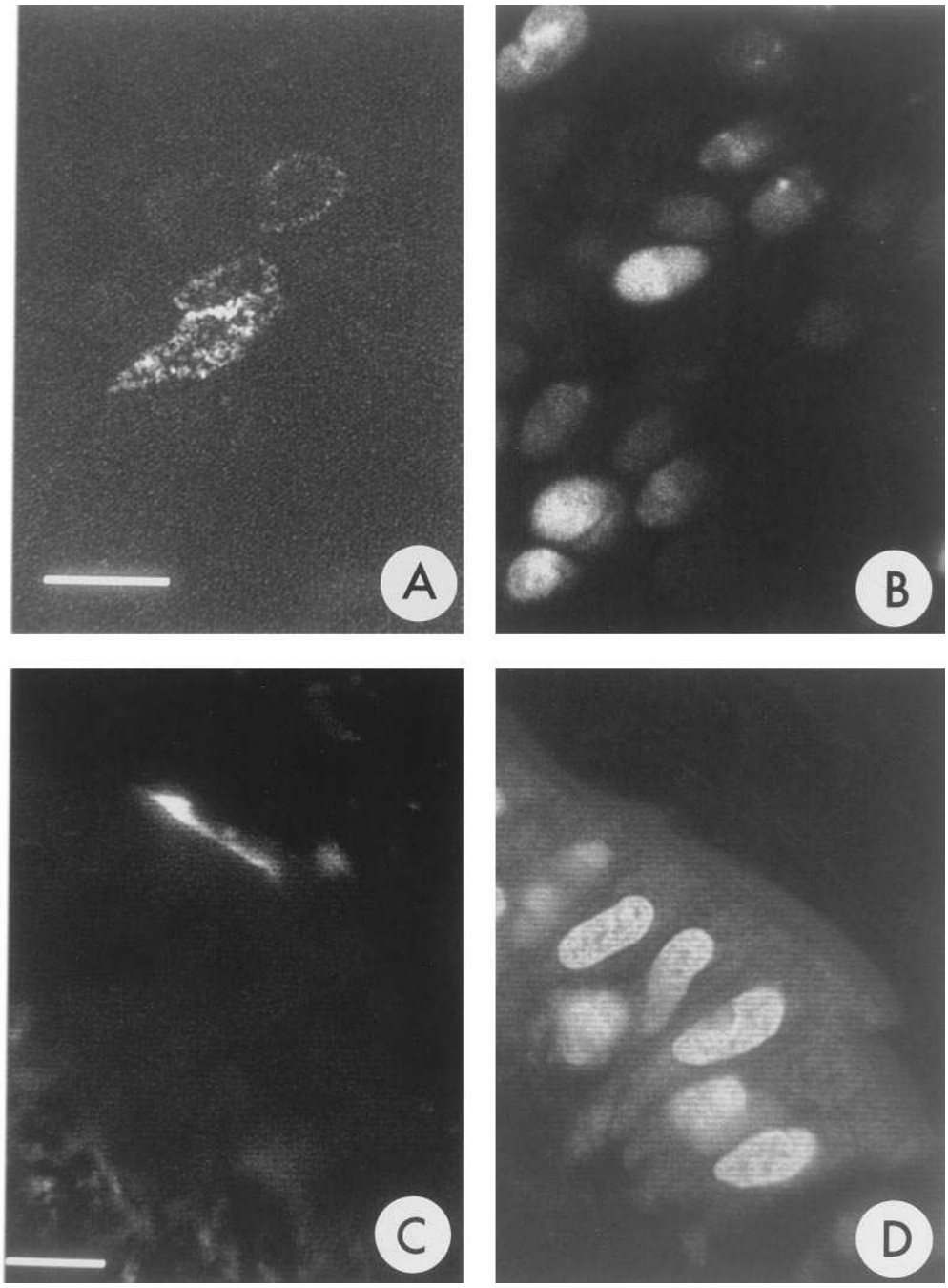

Figure 7. ICAM-1 expression by intestinal epithelial cells in human intestinal xenografts infected with *S. typhi*. Fully differentiated human fetal intestinal xenografts in SCID mice were infected intraluminally with 5×10^7 *S. typhi* Ty2 *aro*A *aro*C, and tissues were removed 6 h after infection. Whole-tissue mounts (*A* and *B*) or frozen sections (*C* and *D*) were prepared, and stained by indirect immunofluorescence for ICAM-1 (*A* and *C*), and counterstained with propidium iodide (*B* and *D*). *A* and *B* are photomicrographs taken from an identical tissue area at the same magnification but with two different wavelength filters of 488 and 514 nm, respectively. The viewing angle is from the lumen onto the epithelium so that all cells seen in these two frames are epithelial cells. *A* shows a small group of epithelial cells positive for ICAM-1, while *B* shows a cross section of ~ 20 epithelial cell nuclei at different angles and locations, suggesting that the displayed area covers at least 20, but more likely 30–60, epithelial cells. *C* and *D* show a second example of epithelial ICAM-1 staining. These photomicrographs are also taken from an identical section area at the same magnification but with two different wavelength filters of 488 and 514 nm, respectively. The sectional plane is approximately perpendicular to the epithelial layer. *C* shows apical ICAM-1 staining of one or two epithelial cells. *D* shows that the displayed area covers approximately 10 epithelial cells. Bars (*A* and *C*), 10 μm.

their addition to the bacteria-infected epithelial monolayers almost completely inhibited neutrophil adherence to the monolayers (Table III), indicating that CD18, probably as part of a complex with CD11, is the key receptor on neutrophils for mediating adhesion to bacteria-infected intestinal epithelial cells.

Discussion

These studies demonstrate in vitro and in vivo that human intestinal epithelial cells can respond to infection with invasive enteric bacteria with increased expression of surface ICAM-1. Moreover, increased ICAM-1 expression was confined to the apical side of polarized epithelial cells, and was accompanied by increased adherence of neutrophils to bacteria-infected epithelial cell monolayers. Together, these findings suggest that the epithelial ICAM-1 response can function to allow neutrophils that have transmigrated through the epithelial cell layer to adhere to, and stay in close contact with, the luminal side of the epithelium. Without the close adherence of transmigrated neutrophils to the epithelium, neutrophils likely would be removed with the luminal contents. This is particularly the case since bacterial invasion of the intestinal mucosa results in increased fluid secretion into the intestinal lumen and a more rapid transit of luminal contents (41).

The apical expression of ICAM-1 by intestinal epithelial cells suggests that ICAM-1 is not involved in the transmigra-

*Table III. Increased Neutrophil Adherence to Bacteria-infected HT29 Colon Epithelial Cells is Mediated by an Interaction between ICAM-1 and CD18**

	Neutrophil adherence to HT29 cells‡						
	Control	+ Anti-ICAM-1§		+ Anti-CD18§		+ Control Ig (Anti-CD4)§	
Stimulus added	Ratio	Ratio	Percent inhibition‖	Ratio	Percent inhibition‖	Ratio	Percent inhibition‖
Y. enterocolitica	6.6±0.4¶	3.1±0.2	53	1.1±0.2	83	6.7±0.3	None
S. dublin	5.8±0.7	3.3±0.4	43	1.0±0.1	83	6.1±0.6	None
TNFα	7.2±0.5	3.7±0.3	49	1.4±0.1	81	6.7±0.3	7

*HT29 monolayers in 48-well plates were infected with bacteria as described in Methods, and further incubated for 9 h. Parallel cultures were stimulated for 9 h with 25 ng/ml TNFα. Subsequently, monolayers were washed and 10^6 freshly prepared peripheral blood neutrophils were added per well, allowed to adhere for 10 min at 37°C, and nonadherent cells were washed off. Total lysates were analyzed for myeloperoxidase activity as a measure of the number of neutrophils adhering to the monolayers. ‡Neutrophil adherence is expressed as a ratio of myeloperoxidase activity in bacteria-infected or TNFα-stimulated monolayers to that in uninfected and unstimulated control monolayers. §Anti-ICAM-1 and anti-CD18 antibodies (20 μg/ml), as well as an isotype-matched anti-CD4 antibody (20 μg/ml) as a control, were added to epithelial cells or neutrophils, respectively, 30 min before adding neutrophils to HT29 epithelial cell monolayers, and were present throughout the subsequent incubation period. ‖Percentages represent the inhibition of neutrophil adherence in bacteria-infected or stimulated cultures treated with a specific antibody relative to the neutrophil adherence in cultures infected with the same bacterial strain or stimulated with the identical agonist in the absence of added antibody. ¶Numbers represent means±SEM of the results of three independent experiments.

tion of neutrophils, or other cells which express counter-receptors for ICAM-1, through the intestinal epithelium. This is supported by the finding that blocking of ICAM-1 did not affect neutrophil transmigration through polarized intestinal epithelial monolayers in response to IFN-γ (42). Thus, the function of intestinal epithelial ICAM-1 expression likely differs from that of endothelial ICAM-1 expression, which is important for neutrophil transmigration from the vascular to the extravascular space (13, 43).

The presence of neutrophils on the luminal surface of the intestinal epithelium, particularly within colon crypts during acute bacterial infection (26–30), could serve to prevent further invasion of the epithelium, and the underlying mucosa, by pathogenic bacteria in the gut lumen. In particular, the predominant expression of ICAM-1 close to the intercellular junctions between epithelial cells suggests that the junctions, which can be an important route of entry for some invasive bacteria (44, 45), are particularly protected by luminal neutrophils adhering to the epithelium. Thus, this hypothesis envisions that neutrophils which adhere to the luminal surface of intestinal epithelial cells constitute an "extracorporal" defense barrier between the intestinal lumen and the mucosa. In this regard, extracorporal neutrophils are known to be functional, as indicated by studies of neutrophils in milk (46). As an alternative or additional interpretation, it is possible that neutrophils which adhere to bacteria-infected epithelial cells contribute to the destruction of these cells, a notion previously put forward for bacteria-infected hepatocytes (47). However, such an interpretation seems unlikely in light of the finding that ICAM-1 expression is largely limited to the apical side on epithelial cells, since neutrophil-mediated destruction of epithelial cells would not be efficient if the neutrophil/epithelial cell interaction mediated by ICAM-1 was limited to the luminal side of the epithelium.

Infection of human intestinal epithelial cell lines with a high dose of invasive bacteria, or stimulation with IFN-γ or TNFα, caused increased ICAM-1 expression by a large proportion of cells in vitro. In contrast, increased epithelial ICAM-1 expression in vivo was confined to only a small proportion of cells in a human intestinal xenograft model. In this regard, pathogenic bacteria in the intestinal lumen likely invade only a fraction of the epithelial cells, and we note, from our in vitro studies, that the proportion of ICAM-1 positive cells was strictly dependent on the bacterial inoculum. In support of our findings, studies of bone marrow recipients with acute graft versus host disease in the intestine, and studies of crypt abscesses and mucosa adjacent to ulcers in patients with inflammatory bowel disease have also shown epithelial ICAM-1 expression (48, 49). In addition, recent studies by C.A. Parkos and J.L. Madara (personal communication) using immunostaining and confocal microscopy found focal apical membrane positivity for ICAM-1, analogous to that reported herein, in intestinal epithelia of adult humans with inflammatory bowel disease at sites of active inflammation. However, in other studies, ICAM-1 expression could not be detected by immunohistology on the intestinal epithelium of healthy individuals or patients with chronic inflammatory conditions such as ulcerative colitis and celiac disease (22, 23, 50). Taken together, these findings indicate that enterocytes have the capacity to express ICAM-1 under specific conditions, which may depend on the nature and duration of the stimulus.

Another factor which may affect epithelial ICAM-1 expression, and determine the proportion of ICAM-1 expressing cells in vivo, is the differentiation state of the epithelial cells. Thus, differentiation state is known to affect the expression of many genes in intestinal epithelial cells, including those for sucrase/isomaltase (51), components of the cytochrome P-450-dependent monooxygenase system (52), members of the VLA family of integrins (53), and keratins (54). Similarly, in keratinocytes, less differentiated cells express higher ICAM-1 levels than more differentiated cells in response to IFN-γ stimulation (19), and we found that undifferentiated Caco-2 colon epithelial cells express higher levels of ICAM-1 than more differentiated Caco-2 cells (T.C. Savidge, unpublished observation). These findings may explain why intestinal epithelial cell lines generally express higher levels of ICAM-1 than their nontransformed counterparts in vivo, since the transformation process is often accompanied by the loss of some differentiated

cell functions. Furthermore, increased ICAM-1 expression by tumor cells may impart an increased potential to these cells to invade surrounding tissues (55, 56), which could select for higher ICAM-1 expression during tumorigenesis. Regardless of these latter considerations, it may be important for the host to better protect the less differentiated intestinal epithelial cells, since these cells are more easily invaded by some pathogenic bacteria (57), and are required for the renewal of the epithelium.

Increased ICAM-1 expression by epithelial cells in the intestinal mucosa in vivo may result from direct bacterial invasion, stimulation of intestinal epithelial cells with proinflammatory cytokines, or a combination of both. Our results with colon epithelial cell lines indicate that direct bacterial invasion is the major stimulus for increased ICAM-1 expression, at least in the initial several hours after infection. This is likely to be the case also in the early period after infection in vivo. However, with prolonged infection, we envision that cytokines released by epithelial cells themselves (7, 8, 58) or cytokines produced by other cells in the intestinal lamina propria in response to bacterial infection may amplify epithelial cell ICAM-1 expression.

Infection of colon epithelial cells with invasive bacteria, but not non-invasive bacteria, upregulated ICAM-1 expression in vitro. Furthermore, infection with both gram negative and gram positive invasive bacteria resulted in upregulation of ICAM-1 expression whereas killed invasive bacteria, or bacterial LPS, did not. These findings are consistent with prior studies which showed that only co-culture with invasive bacteria efficiently induced the secretion of several proinflammatory cytokines by T84 and HT29 cells, whereas non-invasive bacteria or LPS had little effect on cytokine secretion (8). Moreover, the time course of increased ICAM-1 mRNA expression, e.g., a relatively rapid but transient induction, is similar to that found for IL-8 after bacterial invasion (7). This suggests that ICAM-1, like IL-8 and several other cytokines (7, 8), is part of an epithelial "program" which is induced by bacterial invasion, and possibly other stimuli such as IL-1 or TNFα. Many of these genes are regulated at the transcriptional level, and the ICAM-1 promoter shares important transcriptional elements with, for example, IL-8, most notably functionally important regions for the NFκB transcription factor complex (59–61). It appears likely, therefore, that invasion of epithelial cells with different bacteria activates a common set of transcription factors which upregulate transcription of several epithelial cell genes, including ICAM-1 and IL-8. In support of this, infection of HeLa epithelial cells with the invasive bacteria *S. flexneri* was shown to activate the NFκB complex (62).

The current study, together with prior studies (7, 8, 58), suggests that intestinal epithelial cells are involved in several steps of the inflammatory process following bacterial invasion. Thus, in an early step, and possibly the first step, of the inflammatory process, intestinal epithelial cells may be important for imprinting an initial gradient for the chemoattraction of neutrophils from the vascular space into the underlying mucosa (63). In this regard, we and others have demonstrated that bacterial invasion of colon epithelial cells induces the secretion of several chemoattractant cytokines (7, 8, 58). In addition, other cytokines, such as TNFα, IL-1, or GM-CSF released by epithelial cells (8, 64), and other cells in the mucosa, can activate neutrophils and other inflammatory cells that are important for host defense against invading pathogens. Intestinal epithelial cells are also involved in the transmigration of neutrophils from the mucosa to the intestinal lumen (42, 58, 65), and this function, like the secretion of cytokines, is upregulated in response to infection with invasive bacteria such as *Salmonella* (58). In a further step, as shown herein, intestinal epithelial cells may facilitate the establishment of a neutrophil barrier on the epithelial surface.

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