Human intestinal epithelial cells express receptors for platelet-activating factor

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Merendino, Nicolo, Michael B. Dwinell, Nissi Varki, Lars Eckmann, and Martin F. Kagnoff. Human intestinal epithelial cells express receptors for platelet-activating factor. *Am. J. Physiol.* 277 (*Gastrointest. Liver Physiol.* 40): G810–G818, 1999.—The intestinal epithelium produces and responds to cytokines and lipid mediators that play a key role in the induction and regulation of mucosal inflammation. The lipid mediator platelet-activating factor (PAF) can be produced and degraded by the human intestinal epithelium and is known to mediate a range of proinflammatory and other biological effects in the intestinal mucosa. In the studies herein, we assessed whether or not human intestinal epithelial cells express cell surface or intracellular PAF receptors (PAF-R), whether expression of these receptors can be regulated, and whether human intestinal epithelial cells respond to PAF. Several human colon epithelial cell lines (HT-29, Caco-2, T84, HCT-8, HCA-7, I407, and LS-174T) were shown by RT-PCR to constitutively express mRNA for PAF-R. In addition, PAF-R expression was demonstrated by immunoblot analysis and PAF-R was shown to be constitutively expressed on the cell surface of several of these cell lines, as assessed by flow cytometry. PAF-R expression by human colon epithelial cells was upregulated by stimulation with retinoic acid but not by stimulation with PAF, proinflammatory agonists (tumor necrosis factor-α, interleukin-1, interferon-γ), or transforming growth factor-α. PAF-R on intestinal epithelial cells were functional, as PAF stimulation of the cells increased tyrosine phosphorylation of several cellular proteins, including proteins of 75 and 125 kDa, and this response was blocked by a PAF-R antagonist. Consistent with the findings using cell lines, PAF-R were also constitutively expressed by normal human colon and small intestinal epithelium in vivo, as shown by immunohistology. The constitutive and regulated expression of functional PAF-R by human intestinal epithelium suggests PAF produced by the intestinal epithelial cells or cells underlying the epithelium has autocrine or paracrine effects on intestinal epithelial cells.

colon; colon epithelial cell lines; platelet-activating factor receptor exon 2; small intestine; tyrosine phosphorylation; retinoic acid

THE SINGLE LAYER of intestinal epithelial cells that lines the human intestinal mucosa produces a regulated array of cytokines, chemokines, and lipid mediators that are important in signaling the onset of mucosal inflammation (13, 14, 22, 24, 52). Intestinal epithelial cells can also respond to proinflammatory cytokines and lipid mediators that play a key role in host innate and acquired immunity (13, 14, 38, 40). Platelet-activating factor (PAF) 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine is a potent phospholipid mediator that increases vascular permeability, causes vasodilatation, and has been implicated as a mediator of diverse pathophysiological events, including allergic and inflammatory processes (21). Epithelial cells in the human colon are thought to contribute to intestinal PAF production under normal and inflammatory conditions (15). In addition, human colon epithelial cells synthesize and secrete PAF acetylhydrolases, enzymes that play an important role in the degradation of PAF (41). PAF has been reported to increase prostaglandin E_2 production and chloride ion secretion by isolated human intestinal mucosa (3). Increased levels of PAF are produced within inflamed mucosa of patients with ulcerative colitis or Crohn's disease (2, 47, 49, 51). In animal models of colitis, increased levels of PAF have been noted (5, 31, 32) and PAF-R antagonists have been reported to decrease mucosal inflammation in these models (33). Furthermore, PAF may play an important role in inducing intestinal damage during the course of ischemia-reperfusion injury and neonatal necrotizing enterocolitis (6, 12).

PAF mediates its effects on cells by signaling through PAF receptors (PAF-R) localized on the cell membrane. The gene encoding the human PAF-R gene has two 5′ noncoding exons, each of which is driven by a different promoter, but each of these exons is spliced to a common acceptor site on a third exon that encodes a single functional PAF-R protein (26, 35, 45). The promoter for exon 1 has three consensus sequences for nuclear factor (NF)-κB and is responsive to stimulation of cells with PAF and proinflammatory cytokines. The resulting transcript is most abundantly expressed in peripheral blood leukocytes and ubiquitously expressed in a broad range of other tissues (35, 45, 46). The promoter for exon 2 lacks NF-κB binding sites but contains activator protein (AP)-1 and AP-2 binding sites, a transforming growth factor (TGF)-α inhibitory element, and a hormone responsive element. The resulting mRNA transcript is more limited in its tissue distribution (lung, heart, spleen, kidney, but not leukocytes), and its expression can be upregulated by retinoic acid and thyroid hormone (35, 46).

PAF-R belongs to the family of seven transmembrane-spanning G protein-coupled receptors. Binding of PAF to its receptor results in tyrosine phosphorylation of cellular proteins in a variety of cell types, including human endometrium, neutrophils, and B cell lines (1, 16, 17) and rat Kupffer cells (7). This leads to the activation of multiple other intracellular signaling path-

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ways (8, 46), which include phospholipases A_2, C, and D, mitogen-activated protein kinase, and phosphatidylinositol 3-kinase, as well as to the inhibition of adenylate cyclase (8, 46). PAF stimulation of cells transfected with PAF-R has also been shown to activate NF-κB (27) and NF-κB target genes [e.g., interleukin (IL)-8 and COX-2] in fibroblasts and epidermal cells (39, 42). In addition, stimulation with PAF upregulated c-*fos* and c-*jun* expression in human lung fibroblasts (42).

PAF receptors are expressed by human lung epithelial cells (30), corneal epithelial cells (43), and cultured human keratinocytes (44), but the expression of cell surface and intracellular PAF-R by human intestinal epithelial cells has not been directly demonstrated (3, 4, 26, 50). In the studies reported herein, human intestinal epithelial cells are shown to express PAF-R in vitro and in vivo. Furthermore, PAF-R on these cells are functional and activate intracellular signaling pathways on PAF stimulation. Moreover, the expression of epithelial PAF-R can be upregulated by retinoic acid.

MATERIALS AND METHODS

Reagents. The following cytokines and antibodies were used in these studies: recombinant human tumor necrosis factor (TNF)-α, IL-1α, and TGF-α (R & D Systems, Minneapolis, MN); rabbit polyclonal and murine IgG1 monoclonal antibody (MAb) against human PAF-R (Alexis, San Diego, CA); rabbit anti-PAF-R antibody (COOH-terminal peptide) (20) (gift of D. Predescu); mouse IgG2b anti-phosphotyrosine (p-Tyr, PY 99) MAb (Santa Cruz Biotechnology, Santa Cruz, CA); biotinylated goat anti-mouse IgG (Amersham Life Sciences, Arlington Heights, IL); *R*-phycoerythrin-labeled goat anti-mouse IgG (Southern Biotechnology Associates, Birmingham, AL); and mouse IgG1 anti-CD54 (ICAM-1) MAb (AMAC, Westbrook, ME). Synthetic PAF, the PAF-R antagonist hexanolamino-PAF, and *trans*-retinoic acid were from Calbiochem, La Jolla, CA. Triton X-100, NP-40, rabbit IgG, and bacterial lipopolysaccharide (LPS) (*Escherichia coli* O111:B4) were from Sigma Chemical, St. Louis, MO. Results obtained using synthetic PAF were confirmed using lyophilized PAF prepared from bovine heart lecithin (Sigma Chemical). Streptavidin-horseradish peroxidase conjugate was from Amersham Life Sciences.

Cells. The following human cell lines were obtained from the American Type Culture Collection: HT-29 colon adenocarcinoma cells (CCL 227), I407 embryonic intestinal cells (CCL 6), HCT-8 human ileocecal adenocarcinoma cells (CCL 244), LS-174T human colon adenocarcinoma cells (CCL 188), Caco-2 human ileocecal colon adenocarcinoma cells (HTB 37), and U937 human promonocyte-like cells. T84 human colon carcinoma cells were as described previously (13, 22, 52). The human colon adenocarcinoma cell line HCA-7 colony 29 was provided by S. C. Kirkland (10, 25). HT-29, I407, LS-174T, and HCA-7 cells were grown in DMEM. HCT-8, Caco-2, and U937 cells were grown in RPMI 1640. Culture media were supplemented with 10% heat-inactivated FCS and 2 mM L-glutamine. T84 cells were grown in 50% DMEM-50% Ham's F-12 medium supplemented with 5% newborn calf serum and 2 mM L-glutamine. Cells were maintained in 95% air, 5% CO_2 at 37°C. Caco-2 cells were grown in RPMI 1640 for 18–20 days to obtain differentiated monolayers (29). A differentiated subclone of HT-29, HT-29.18.C1 (19) (provided by M. H. Montrose), was grown in DMEM high-glucose (4.5 g/l) supplemented with 10% heat-inactivated FCS, 2 mM L-glutamine, and 10 μg/ml human apo-transferrin (Sigma Chemical).

RNA extraction and RT-PCR analysis. Total cellular RNA was extracted using TRIzol reagent (GIBCO BRL, Gaithersburg, MD). Total cellular RNA (1 μg) was reverse transcribed at 37°C for 70 min in a 20-μl volume containing 2.5 units Superscript-II reverse transcriptase (GIBCO BRL), 10 mM 1,4-dithiothreitol, 1 mM each of dATP, dTTP, dCTP, and dGTP (dNTP mix), and 5 μg/ml oligo(dT) primer (Pharmacia, Piscataway, NJ). Reactions were stopped by heat inactivation for 10 min at 85°C. Subsequently, sequences were amplified from cDNA by PCR in a total volume of 50 μl containing 5 μl of RT reaction mixture, 1.5 mM $MgCl_2$, 200 μM each of dATP, dTTP, dCTP, and dGTP, 25 pmol of each primer, and 4.0 units *Taq* polymerase (GIBCO BRL) in RNase-free distilled water. Primers for the coding third exon of PAF-R were 5′-TATAACCGCTTCCAGGCAGT-3′ (sense) and 5′-GAAACAGTAGATAACAGGGTC-3′ (antisense) (9, 36, 37, 48). Primers to amplify PAF-R transcript 1 (leukocyte type) and transcript 2 (tissue type) correspond to primers P2 (sense) and P3 (antisense) and primers P1 (sense) and P3 (antisense), respectively, as described by Kotelevets et al. (26). After a hot start, the amplification profile for PAF-R was 34 cycles of 1-min denaturation at 94°C, 2-min annealing at 62°C, and 2-min extension at 72°C. The same amplification profile was applied for 28 cycles for β-actin (22). RNA from U937 cells was used as positive control for PAF-R. In negative controls, RNA was omitted from the RT and subsequent PCR amplification reactions. After amplification, aliquots of the PCR reactions were size separated on a 1% agarose gel containing ethidium bromide and photographed.

Flow cytometry. HT-29 and HCT-8 cells were detached with 25 mM EDTA in PBS for 10 min on ice and either incubated for 2 h on ice with 10 μg/ml of anti-human PAF-R MAb in PBS with 1% BSA and 1% goat serum (dilution buffer) or fixed for 10 min at 4°C in 4% paraformaldehyde in PBS prior to incubation with anti-PAF-R antibody in dilution buffer. In some experiments, paraformaldehyde-fixed cells were permeabilized by inclusion of 0.1% saponin in the dilution and washing buffers. Cells were then incubated for 1 h on ice with *R*-phycoerythrin-labeled goat anti-mouse IgG and analyzed by flow cytometry (FACScan, Becton Dickinson, Sunnyvale, CA).

PAF-R and phosphotyrosine immunoblots. For PAF-R immunoblots, confluent monolayers of intestinal epithelial cells in 6-well plates were lysed with ice-cold lysis buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 1% sodium deoxycholate, and 0.1% SDS) containing a 1:200 dilution of protease inhibitor cocktail set III (Calbiochem). Cell lysates were sonicated for 5 s and centrifuged for 20 min at 13,000 *g* at 4°C. Supernatants were removed, and protein concentrations were determined by the Bradford assay (Bio-Rad protein assay kit, Bio-Rad, Hercules, CA). Forty micrograms of total protein per well were electrophoresed on 10% SDS-PAGE gels according to the method of Laemmli (28) and then transferred overnight to nitrocellulose (Hybond ECL, Amersham Life Sciences). Membranes were blocked for 1 h at room temperature in a solution of PBS containing 10% nonfat dry milk, 1% donkey serum, and 0.1% Tween 20. After membranes were washed, they were incubated for 2 h at room temperature in a 1:16,000 dilution of rabbit PAF-R antibody (COOH-terminal peptide) (20) or rabbit IgG as a control in dilution buffer (1% nonfat dry milk, 1% donkey serum, 0.1% Tween 20 in PBS). Blots were washed and incubated for 1 h with biotinylated donkey anti-rabbit Ig followed by streptavidin-horseradish peroxidase conjugate (Amersham). Immunoblots were developed with enhanced chemiluminescence agents according to the manufacturer's instructions (Amersham) and exposed to imaging film (XAR, Kodak).

For phosphotyrosine immunoblots, confluent monolayers of intestinal epithelial cells in 6-well plates were incubated with PAF (100 nM) alone or together with PAF antagonist in serum-free medium supplemented with L-glutamine for times ranging from 30 s to 5 min, after which cells were lysed with ice-cold lysis buffer (20 mM Tris, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1% NP-40, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, 1 mM aprotinin, 1 mM EDTA, and 50 mM NaF). Cell lysates were centrifuged for 20 min at 13,000 *g* at 4°C, after which supernatants were removed, added to 200 µl of sample buffer (9% wt/vol SDS, 6% vol/vol 2-mercaptoethanol, 10% vol/vol glycerol, and 0.04% wt/vol bromophenol blue) and boiled for 5 min. Aliquots (10 µl) were electrophoresed on 10% SDS-PAGE gels, after which proteins were transferred for 3 h to nitrocellulose membranes (Schleicher & Schuell, Keene, NH). Membranes were blocked for 2 h at room temperature with a solution of Tris-buffered saline containing 5% (wt/vol) nonfat dry milk and 0.05% Tween 20. After membranes were washed, they were incubated overnight at 4°C with anti-p-Tyr MAb. Blots were washed and incubated for 1 h with biotinylated goat anti-mouse IgG followed by streptavidin-horseradish peroxidase conjugate. Immunoblots were developed with enhanced chemiluminescence as described above for PAF-R immunoblots.

Immunohistochemistry. Tissue specimens from grossly and microscopically normal-appearing areas of human small intestine and colon obtained at the time of surgery from 4 different adult subjects were embedded in OCT compound and snap-frozen in isopentane/dry ice. Cryostat sections (5 µm) were air dried and fixed with 10% buffered formalin and were blocked for endogenous peroxidase and nonspecific binding. Sections were overlaid overnight with mouse MAb to human PAF-R (Alexis) or an isotype control antibody at the same concentrations, after which endogenous biotin was blocked (avidin/biotin blocking kit, Zymed, South San Francisco, CA). Specific binding was detected using the LSAB 2 kit (DAKO, Carpenteria, CA) according to the manufacturer's instructions and visualized using 3-amino-9-ethyl-carbazole (Vector Laboratories, Burlingame, CA) and H_2O_2. Sections were counterstained with Mayer's hematoxylin (Sigma Chemical) and coverslipped using aqueous mounting medium.

RESULTS

Cultured human intestinal epithelial cells express PAF-R mRNA. To determine if human colon epithelial cell lines express mRNA transcripts for PAF-R, total RNA was isolated from HT-29, T84, HCT-8, undifferentiated Caco-2, I407, HCA-7, and LS-174T cells and was analyzed for expression of PAF-R transcripts (exon 3 coding region) by RT-PCR. As shown in Fig. 1, each of these cell lines constitutively expressed PAF-R mRNA. In contrast, PAF-R mRNA was not detected in HT-29.18.C1 cells or in Caco-2 cells grown for 18–20 days to obtain differentiated monolayers (data not shown).

The gene encoding the human PAF-R has two 5′ noncoding exons, each of which is spliced to a common acceptor site on the exon 3 coding region (26, 35, 45). Leukocyte-type and tissue-type PAF-R mRNAs correspond to transcription of exon 1 (transcript 1) and exon 2 (transcript 2), respectively. Consistent with the findings of Kotelevets et al. (26), HT-29 and Caco-2 cells expressed PAF-R transcript 2 (252- and 334-bp fragments), whereas control U937 cells expressed PAF-R transcript 1 (166-bp fragment), as assessed by RT-PCR (data not shown).

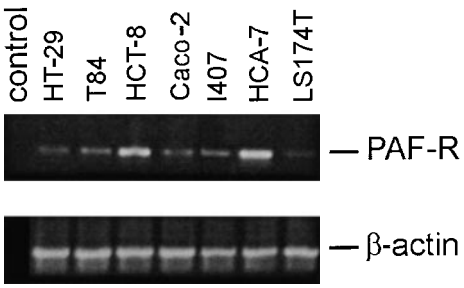

Fig. 1. Constitutive expression of platelet-activating factor receptor (PAF-R) mRNAs by human intestinal epithelial cell lines. Total RNA was extracted from indicated human colon epithelial cell lines and amplified by RT-PCR for PAF-R mRNA or β-actin as described in MATERIALS AND METHODS. As a negative control, RNA was omitted from RT-PCR amplification. Results are representative of 2 or more repeated experiments for each cell line.

PAF-R expression by immunoblot analysis. To determine if the expression of PAF-R mRNA was paralleled by the production of PAF-R protein, HT-29 cell lysates were analyzed for PAF-R protein by immunoblot analysis. As shown in Fig. 2, HT-29 cells expressed PAF-R. Consistent with previous reports of PAF-R expression in U937 and Raji cells (34), PAF-R in HT-29 cells had a molecular mass of ~68 kDa.

Cell surface expression of PAF-R by intestinal epithelial cell lines. To determine if the constitutive expression of PAF-R mRNA and PAF-R protein was paralleled by expression of PAF-R on the cell surface, several of the human colon epithelial cell lines were stained with anti-PAF-R antibodies and examined by flow cytometry. PAF-R was constitutively expressed on the cell membrane of HT-29, HCT-8, I407, and HCA-7 cells

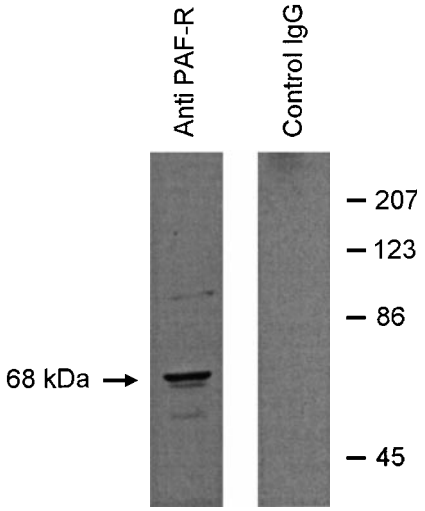

Fig. 2. Immunoblot analysis of PAF-R expression in HT-29 cells. HT-29 cell lysates were size fractionated and blotted onto a nitrocellulose membrane. Blots were probed with rabbit antibody to a COOH-terminal peptide of PAF-R (*left*) or normal rabbit IgG as a control (*right*) and were developed using enhanced chemiluminescence as described in MATERIALS AND METHODS. Size markers in kDa are indicated on *right*. A major band representing PAF-R was seen at ~68 kDa (arrow).

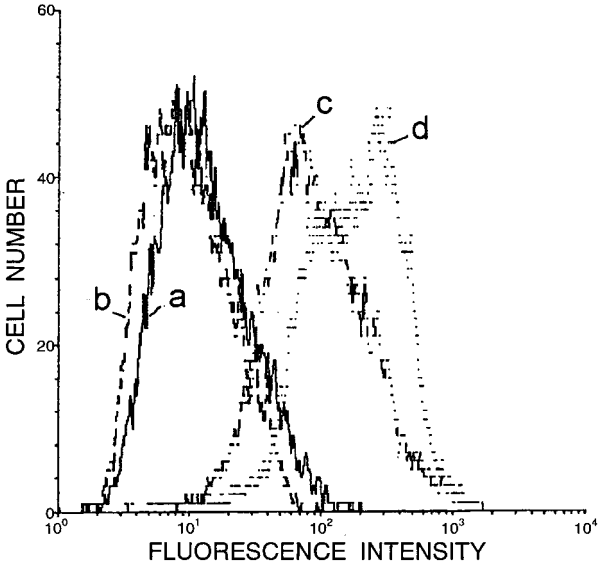

Fig. 3. Flow cytometric analysis of PAF-R on HT-29 human colon epithelial cells. Representative flow cytometric analysis in which HT-29 cells were fixed with 4% paraformaldehyde. Curves *c* and *d* represent cells stained with antibody to PAF-R as primary antibody and curves *a* and *b* represent cells stained with an isotype-matched control primary antibody. In curves *a* and *c*, cells were not permeabilized, whereas in curves *b* and *d* cells were permeabilized with 0.1% saponin during antibody staining and subsequent washing. All cells were stained with *R*-phycoerythrin-conjugated secondary antibody. As shown, PAF-R are constitutively expressed on surface of HT-29 cells (compare curves *a* and *c*) and intracellularly (compare curves *c* and *d*). Median intensity fluorescences for curves *a*, *b*, *c*, and *d* were 11.9, 9.1, 85.8, and 184.3, respectively. Results are representative of 3 repeated experiments.

(Fig. 3 and data not shown). PAF-R was also present intracellularly, as determined by an up to twofold increase in staining after cell permeabilization of HT-29 cells (Fig. 3).

Cell surface PAF-R expression has been reported to decrease after in vitro stimulation of human platelets and hepatic Kupffer cells with PAF and increase in macrophages stimulated with LPS (reviewed in Ref. 8). Cell surface and intracellular PAF-R levels were not affected by stimulation of HT-29 or HCT-8 cells for 6, 12, or 24 h with titrated doses of PAF (0.1, 1, 2.5, 5, 10, or 100 μM) or with proinflammatory mediators and agonists, including TNF-α or IL-1 (2, 20, or 200 ng/ml), which, in parallel, increased IL-8 secretion by the same cells (data not shown). Similarly, cell surface and intracellular PAF-R levels were not affected by stimulation of HT-29 or HCT-8 cells with LPS (2 μg/ml) or

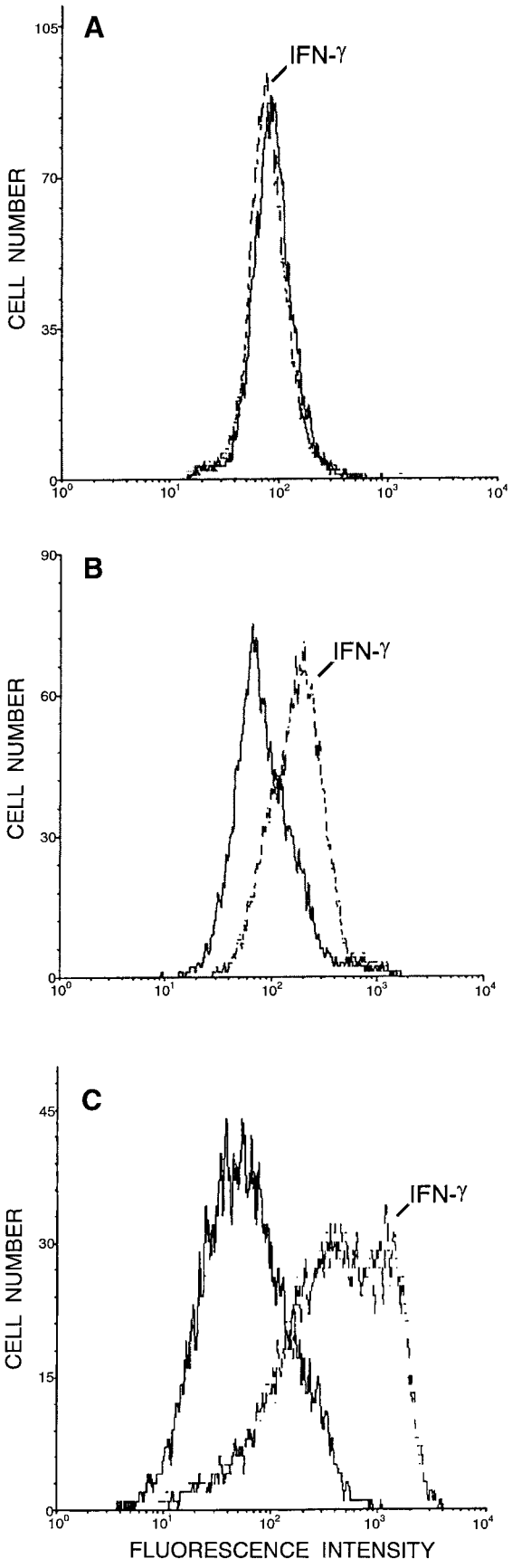

Fig. 4. Flow cytometric analysis of PAF-R expression on interferon (IFN)-γ-stimulated HT-29 and U937 cells. HT-29 epithelial cells or U937 human monocytic cells were incubated in absence or presence of IFN-γ (40 ng/ml) for 24 h before unfixed cells were stained with primary anti PAF-R antibody and *R*-phycoerythrin-labeled secondary antibody as described in MATERIALS AND METHODS. IFN-γ stimulation did not upregulate PAF-R expression on HT-29 cells (*A*) but did upregulate PAF-R expression on U937 cells (B) used as a positive control. As shown in *C*, stimulation of HT-29 cells with IFN-γ resulted in increased surface expression of ICAM-1, indicating these cells responded to stimulation with that agonist. Similar results were obtained when cells were stimulated with IFN-γ for 12 h (not shown). Results are representative of 2 repeated experiments.

TGF-α (2, 20, or 200 ng/ml). In addition, PAF-R expression on HT-29 cells was not upregulated by IFN-γ stimulation (Fig. 4*A*), although IFN-γ stimulation upregulated the expression of another cell surface molecule, ICAM-1, on those cells (Fig. 4*C*), and PAF receptor expression was upregulated, as also noted by others (9), on IFN-γ-stimulated U937 human monocytes used as a further control (Fig. 4*B*).

Because HT-29 cells express mRNA for PAF-R transcript 2 (see Ref. 26 and this study), we tested whether the expression of PAF-R by those cells was upregulated in response to stimulation with *trans*-retinoic acid (0.1 or 1 μM) for 6, 16, or 24 h. PAF-R expression was not upregulated 6 h after retinoic acid stimulation. However, as shown in Fig. 5, PAF-R expression was markedly upregulated at 16 h after retinoic acid stimulation and decreased toward baseline by 24 h (data not shown), as assessed by flow cytometry. Upregulated expression was greatest when cells were stimulated with 0.1 or 1 μM *trans*-retinoic acid, with ~50% of the increase in PAF-R expression being on the cell surface and 50% due to increased intracellular PAF-R.

Stimulation of HT-29 cells with PAF results in increased protein tyrosine phosphorylation. Stimulation of human neutrophils or B cell lines via PAF-R has been shown to result in protein tyrosine phosphorylation (16, 17). To test if PAF-R on human colon epithelial cells could also transduce signals into human colon epithelial cells, HT-29 and HCT-8 cells were stimulated with PAF (100 nM) for 30 s to 5 min and tyrosine phosphorylation of cellular proteins was assessed by phosphotyrosine immunoblots. As shown in Fig. 6, stimulation of HT-29 and HCT-8 cells with PAF resulted in a rapid increase in tyrosine phosphorylation of several cellular proteins, including proteins of 125 kDa and 75 kDa. Tyrosine phosphorylation increased within 30 s after stimulation, peaked by 1 min, and began to return toward control levels by 5 min. Increased tyrosine phosphorylation of those cellular proteins was not seen at any of the above times after addition of PAF to cells in which the PAF-R was blocked using the PAF-R antagonist, hexanolamino-PAF (Fig. 6).

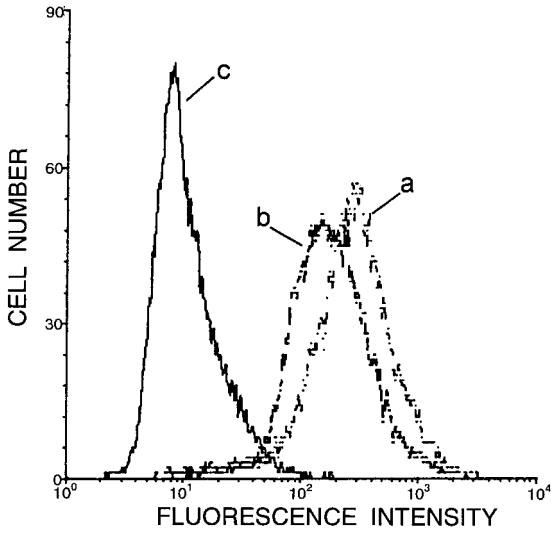

Fig. 5. Retinoic acid upregulates PAF-R expression by HT-29 cells. HT-29 cells maintained in serum-free medium for 2 h before use were left unstimulated or were stimulated with *trans*-retinoic acid (0.1 μM) for 16 h, after which cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% saponin. Curve *a* shows cells stimulated with retinoic acid and stained with primary anti PAF-R antibody and *R*-phycoerythrin-labeled secondary antibody. Curve *b* shows unstimulated cells stained as in *a*. Curve *c* shows cells stained with isotype-matched control primary antibody and *R*-phycoerythrin-labeled secondary antibody. Median intensity fluorescences for curves *a*, *b*, and *c* were 273.8, 170.0, and 9.5, respectively. Median intensity fluorescence after stimulation of cells with 1 μM *trans*-retinoic acid was 248.1 (not shown). Results are representative of 3 repeated experiments.

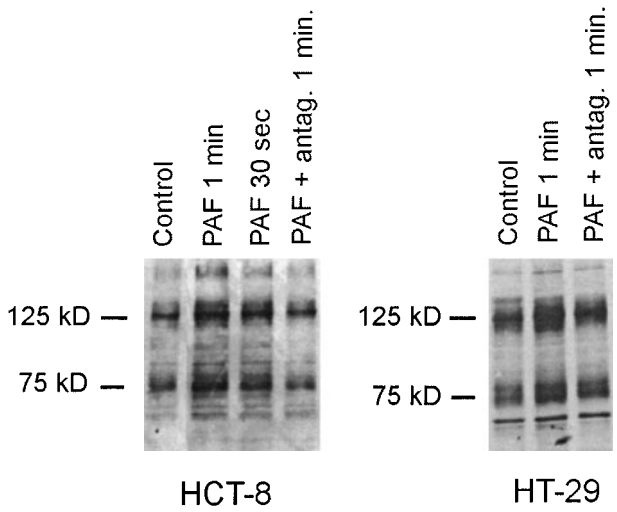

Fig. 6. Increased protein tyrosine phosphorylation in response to PAF stimulation of HT-29 and HCT-8 cells. HCT-8 and HT-29 cells were stimulated with 100 nM PAF for up to 1 min in presence or absence of PAF-R antagonist. Cell lysates were assayed as described in MATERIALS AND METHODS. Increased tyrosine phosphorylation of proteins of ~125 kDa and 75 kDa was seen in PAF-treated cells by 1 min after stimulation. This response was blocked by treating cells with PAF-R antagonist (PAF + antag; 100 nM). Results are representative of 3 or more repeated experiments.

PAF-R expression by human colon and small intestinal epithelial cells in vivo. To test if expression of PAF-R by human colon epithelial cell lines is representative of normal intestinal epithelial cells in vivo, frozen sections of four different samples of normal human colon or small intestine were immunostained with a MAb to PAF-R. As shown in Fig. 7, PAF-R were expressed by normal colon and small intestinal epithelial cells. Epithelial cells within the crypts and villi of the small intestine and crypt and surface epithelial cells in the colon expressed PAF-R, whereas the underlying muscularis layer did not. In addition to intestinal epithelium immunostaining, macrophages and other cells within the lamina propria of the colon and small intestine also expressed PAF-R (Fig. 7).

DISCUSSION

Human intestinal epithelial cells produce PAF (15), but whether these cells express surface or intracellular receptors for PAF has not been clear (3, 26). As shown herein, seven different human colon epithelial cell lines expressed PAF-R mRNA. Moreover, PAF-R was detected in human colon epithelial cell lysates by immuno-

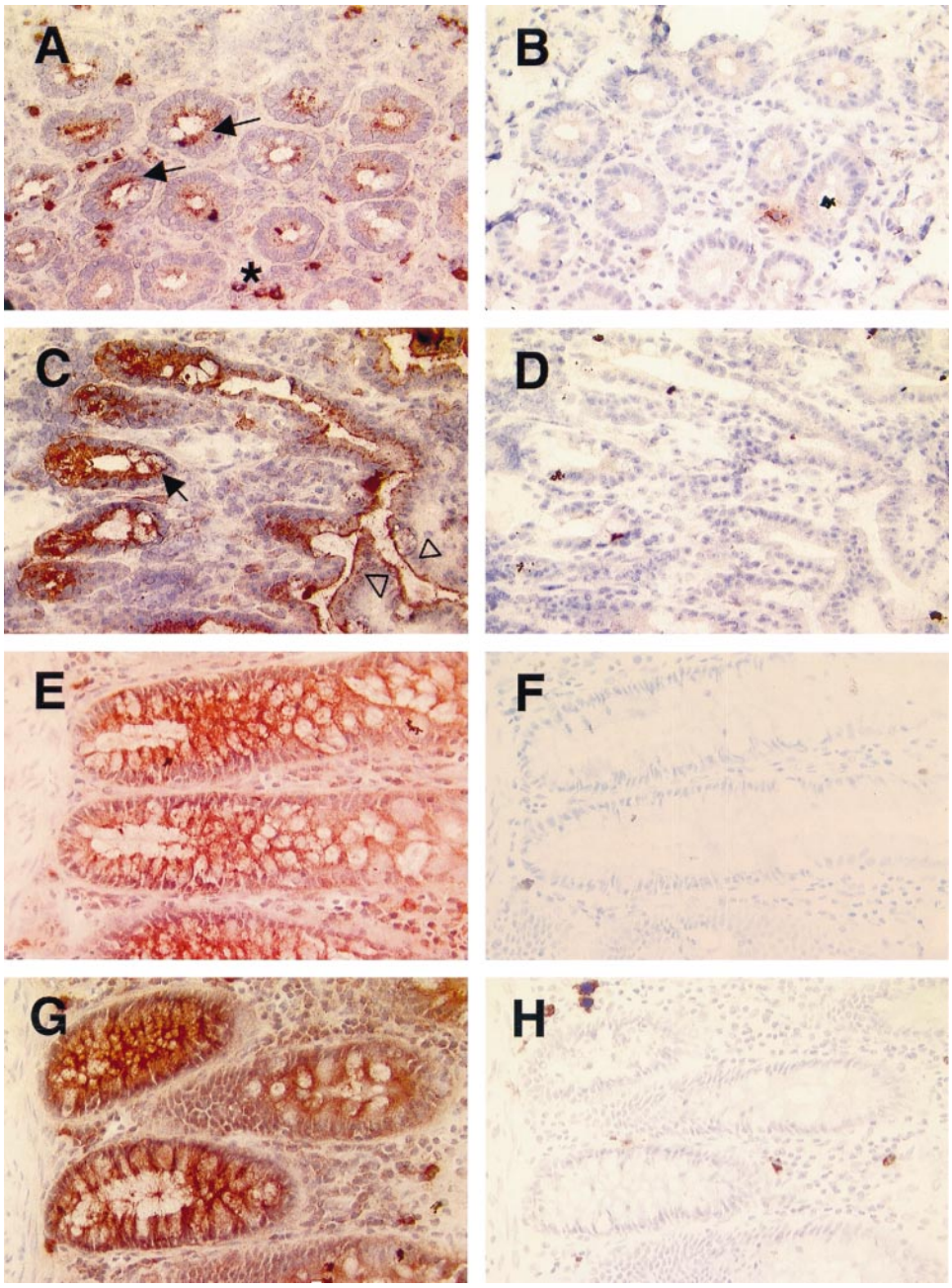

Fig. 7. Immunohistochemical localization of PAF-R in human small intestine and colon. *A–D* show frozen sections of normal human small intestine from 2 subjects and *E–H* show frozen sections of normal human colon from 2 different subjects. Sections in *A*, *C*, *E*, and *G* were immunostained for PAF-R, and the adjacent sections shown in *B*, *D*, *F*, and *H* were stained with an isotype-control antibody as described in MATERIALS AND METHODS. *A*: arrows indicate PAF-R staining of crypt epithelial cells. Macrophages and other cells in lamina propria also stain for PAF-R, as indicated by asterisk. *C*: arrow indicates PAF-R staining of epithelial cells in crypts, and arrowheads indicate staining of epithelial cells lining small intestinal villi. *E* and *G* show PAF-R staining of crypt epithelial cells in colon as well as several positively staining cells within lamina propria. Colon surface cells also stained positively for PAF-R (not shown). Magnification, ×400.

blot analysis and demonstrated to be located on the cell surface and intracellularly by flow cytometry. Consistent with those findings, we further demonstrated the expression of PAF-R by normal human colon and small intestinal epithelium in vivo. Others failed to detect PAF receptors on two human colon epithelial cell lines that were also studied herein (HT-29 and T84) (50), which may reflect the loss of PAF-R expression by the cell lines used in those studies or the use of less sensitive detection systems ([^{3}H]PAF radioligand binding for PAF-R expression). Consistent with our mRNA results, Kotelevets et al. (26) detected PAF-R mRNA transcripts in HT-29 and Caco-2 human colon epithelial cells, although those studies did not assess PAF-R protein expression. Together, the preponderance of evidence indicates that human intestinal epithelial cells express PAF-R.

PAF can mediate a pleiotropic array of proinflammatory and other biological activities in vitro and in vivo by signaling through its specific seven transmembrane-spanning G protein-coupled receptor. The studies herein demonstrate that PAF-R expressed by intestinal epithelial cells are functional, as determined by increased tyrosine phosphorylation of cellular proteins in response to PAF stimulation and the blocking of that response by PAF-R antagonists. Nonetheless, the physiological consequences of activating PAF-R on intestinal epithelial cells are not known. PAF can increase chloride secretion by isolated segments or biopsies of human intestinal mucosa, but that activity appeared to be

indirect and mediated by downstream effects of PAF on cyclooxygenase and prostaglandin E_2, and there was no evidence indicating a direct role of the intestinal epithelium in this response (3, 50). However, PAF stimulation increases intracellular calcium levels in other cell types, and calcium is an important regulator of chloride secretion (23). Thus one could speculate that PAF produced by or in close proximity to the epithelium might act directly on the epithelium and alter epithelial cell secretory properties, although that remains to be shown.

Transcription of the PAF-R gene can result in the production of two different mRNA transcripts, termed PAF-R transcript 1 and PAF-R transcript 2 (26, 35, 45), that are preferentially expressed in different tissues. PAF-R transcript 1 is widely expressed in peripheral blood lymphocytes and a broad range of other tissues, and its expression is upregulated by proinflammatory cytokines and by PAF stimulation (35, 45, 46). In contrast, PAF-R transcript 2 is more limited in its tissue distribution (lung, heart, spleen, kidney, but not leukocytes), and its expression has been reported to be upregulated by retinoic acid and thyroid hormone and downregulated by TGF-α (35, 46). As demonstrated herein, PAF-R expression was upregulated in colon epithelial cells by retinoic acid but not by IL-1, TNF-α, IFN-γ, or TGF-α, a finding consistent with our data and that of an earlier study (26) demonstrating that Caco-2 and HT-29 cells mainly express mRNA for PAF-R transcript 2.

PAF stimulation of human epidermal cells transfected with the PAF-R or human lung fibroblasts results in upregulated expression of several NF-κB target genes, including IL-8 (39). Moreover, PAF stimulation of murine macrophages, or Chinese hamster ovary cells expressing PAF-R, induced NF-κB binding activity (27). Furthermore, NF-κB was activated in rat intestinal epithelium in vivo after intravenous injection with PAF (11). However, it is not known whether activation of NF-κB, in that case, resulted from a direct effect of PAF on the intestinal epithelium or was due to the effects of other proinflammatory mediators, produced as a result of intestinal inflammation, acting on the epithelium. In the studies herein, signaling HT-29 human colon epithelial cells through the PAF-R did not result in the activation of the NF-κB target genes IL-8 and ICAM-1 (data not shown), although these NF-κB target genes were activated in HT-29 cells by TNF-α or IL-1 stimulation or infection with enteroinvasive bacteria (Refs. 13 and 18 and data not shown). This suggests that the signal transduction pathways in human colon epithelial cells stimulated through the PAF-R, which are downstream of tyrosine phosphorylation, function independently of NF-κB.

In summary, the studies herein demonstrate the expression of PAF-R by human intestinal epithelial cells in vitro and in vivo. The ability of PAF to transduce signals into these cells suggests a cognate role for PAF and PAF-R on the intestinal epithelium in mediating autocrine and/or paracrine effects within the intestinal mucosa.

We thank John Leopard and Jennifer Smith for expert technical assistance and Roslyn Lara for final preparation of the manuscript.

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases grant DK-35108. N. Merendino was supported, in part, by a fellowship from the Nestlé Italia, Italy, and M. B. Dwinell was supported by National Institutes of Health Training Grant 5-T32-DK-07202.

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Received 14 April 1999; accepted in final form 29 July 1999.

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