Phospholipase A$_2$ activating protein and idiopathic inflammatory bowel disease

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Abstract

Background—Crohn's disease and ulcerative colitis are idiopathic inflammatory bowel diseases (IBD) involving synthesis of eicosanoids from arachidonic acid (AA), which is released from membrane phospholipids by phospholipase A$_2$ (PLA$_2$). A potentially important regulator of the production of these mediators is a protein activator of PLA$_2$, referred to as PLA$_2$ activating protein (PLAP).

Aims—The purpose of this investigation was to discover if PLAP values might be increased in the inflamed intestinal tissue of patients with IBD and in intestinal tissue of mice with colitis.

Patients—Biopsy specimens were taken from patients wtih ulcerative colitis and Crohn's disease undergoing diagnostic colonoscopy, and normal colonic mucosa was obtained from patients without IBD after surgical resection.

Methods—Immunocytochemistry with affinity purified antibodies to PLAP synthetic peptides was used to locate PLAP antigen in sections of intestinal biopsy specimens from IBD patients compared with that of normal intestinal tissue. Northern blot analysis with a murine [^{32}P] labelled *plap* cDNA probe was performed on RNA extracted from the colons of mice fed dextran sulphate sodium (DSS) and cultured HT-29 cells exposed to lipopolysaccharide (LPS).

Results—PLAP antigen was localised predominantly within monocytes and granulocytes in intestinal tissue sections from IBD patients, and additional deposition of extracellular PLAP antigen was associated with blood vessels and oedema fluid in the inflamed tissues. In contrast, tissue sections from normal human intestine were devoid of PLAP reactive antigen, except for some weak cytoplasmic reaction of luminal intestinal epithelial cells. Similarly, colonic tissue from DSS treated mice contained an increased amount of PLAP antigen compared with controls. The stroma of the lamina propria of the colonic mucosa from the DSS treated mice reacted intensely with antibodies to PLAP synthetic peptides, while no reaction was observed with control mouse colons. These data were supported by northern analysis which showed that PLAP mRNA was increased in the colons of DSS treated mice and cultured HT-29 cells exposed to LPS.

Conclusions—As PLAP values were increased in the intestinal mucosa of IBD patients and mice with colitis, as well as in LPS treated cultured HT-29 cells, a role was postulated for PLAP in increasing PLA$_2$ activity, which leads to the increased synthesis of eicosanoids in intestinal tissues of patients with these inflammatory diseases.

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Crohn's disease (CD) and ulcerative colitis (UC), collectively known as inflammatory bowel disease (IBD), are chronic idiopathic diseases that primarily affect the gastrointestinal tract. In UC, the intestinal lesions are confined to the colon, while CD may affect mucosal surfaces in any part of the digestive system and is often associated with transmural disease and fistula formation. Although little is known about the pathogenesis of IBD, new information is emerging about the role of cytokines, inflammatory cells, and arachidonic acid metabolites, which collectively produce intense gastrointestinal inflammation.[1]

Prostaglandins and leukotrienes have been implicated in the pathogenesis of a number of inflammatory diseases, including rheumatoid arthritis, asthma, psoriasis, multiple sclerosis, and IBD. Increased release of arachidonic acid (AA) from membrane phospholipids leads to increased synthesis of pro-inflammatory eicosanoids (for example, leukotriene B$_4$ (LTB$_4$)).[2] [3] An increase in synthesis of prostaglandin E$_2$ (PGE$_2$) and thromboxane B$_2$ in mononuclear cells from CD patients during relapse was noted by Rachmilewitz *et al*.[4] In fact, patients with CD have increased PLA$_2$ activity in their intestinal mucosa,[5] and monocytes isolated from the mucosa of patients with IBD spontaneously produced more interleukin 1β (IL1β) than did monocytes from normal intestinal mucosa.[6] Lipopolysacharide (LPS) stimulation of monocytes from patients with active IBD increased IL1β production, while IL1β synthesis by mononuclear cells from normal mucosa was unaffected.[6] In addition, leucocytes in the peritoneal cavity of rats with experimental colitis (induced with acetic acid or immune complexes) were shown to have increased PLA$_2$ activity and eicosanoid levels severalfold higher than that of control animals.[7] However, little is known about the early events in the mechanism responsible for the activation of PLA$_2$ in IBD.

Prostaglandins and leukotrienes are formed from AA, which is released from membrane

phospholipids by the action of PLA_2 or phospholipase C (PLC) acting in concert with diglyceride lipase.[1] The production of eicosanoids is normally limited by the availability of free AA in the cytoplasm of cells, and factors that increase the release of AA from membrane phospholipids (for example, PLA_2 activity) would increase the amount of eicosanoids released into the tissue. We postulated that phospholipase A_2 activating protein (PLAP) could be an important participant in the extensive inflammatory response observed in IBD patients.

The gene encoding PLAP (*plap*) was cloned from a murine smooth muscle-like cell (BC_3H1) cDNA library in $\lambda gt11$, using antisera to melittin, an antigenically related PLA_2 activating peptide present in bee venom.[8 9] From the amino acid sequence of the *plap* gene product, it was apparent that the C-terminus of PLAP (amino acid residues 260–280) was homologous to the 26 amino acid melittin peptide.[8 9] Furthermore, antisera to murine PLAP reacted with human PLAP.[10] The murine *plap* gene consists of 975 bp and encodes a protein with 325 amino acid residues having a predicted molecular size of 36 009 Da.[8] SDS-PAGE analysis of PLAP isolated from synovial fluid of rheumatoid arthritis patients[10] and LTD_4 stimulated BC_3H1 murine fibroblasts[9] revealed the presence of a 28 kDa protein that possessed PLA_2 stimulatory activity.

This report records the presence of PLAP antigen in inflamed colonic mucosa of IBD patients and examines PLAP values in a murine colitis model. Because PLAP may have an important role in regulating eicosanoid synthesis, as well as cytokine production,[11 12] a role for PLAP in the pathogenesis of IBD was investigated.

Methods

DESCRIPTION OF THE PATIENT POPULATION
Intestinal biopsy specimens were collected from five patients with UC and five patients with CD during diagnostic colonoscopy. Additional mucosal specimens of normal colon were obtained from 12 patients undergoing bowel resection for colon cancer or diverticulitis. The biopsy specimens were routinely processed for haematoxylin and eosin staining, while others were frozen in OCT compound (Miles, Elkhart, IN) and stored at –70°C. UC patients were scored clinically as mild, moderate, or severe, and the CD Activity Index (CDAI) was used to assess CD patients.[13] At the time of colonoscopy, biopsy specimens were obtained from areas of inflamed tissue, as well as from normal appearing mucosa (totalling four to six specimens from each patient). All IBD patients were receiving medications, which included glucocorticoids and sulphasalazine or glucocorticoids and mesalamine. The clinical severity of disease in the UC patients varied from mild to moderately severe, and all CD patients had a moderate CDAI (162–212). Informed consent was obtained from all patients before collection

of biopsy specimens, and the study was approved by the UTMB Institutional Review Board. In addition, we examined three more paraffin wax embedded intestinal tissue specimen from UC patients and two more from CD patients.

MURINE COLITIS MODEL
C3H/HeJ mice were purchased from the Jackson Laboratories (Bar Harbor, ME), and Swiss-Webster mice were from Taconic (Germantown, NY). The C3H/HeJ mice were maintained under specific pathogen free (SPF) conditions, while the Swiss-Webster mice were housed under conventional conditions at the UTMB Animal Facility. All animal studies were approved by the UTMB Animal Care and Use Committee. In one experiment, groups of five to six mice were given free access to 5% dextran sulphate sodium (DSS) purchased from ICN (Costa Mesa, CA) in place of water for seven days. For the second experiment, DSS was purchased from TdB Consultancy (Uppsala, Sweden), and groups of five to six mice were fed 5% DSS for two days. Thereafter, DSS was removed and mice were given water and housed for designated periods of time. Total RNA was extracted from colonic tissues taken from randomly selected animals at weekly intervals throughout the experiment.[14] After cervical dislocation, colons from duplicate mice were removed, divided into left and right portions, and processed for total RNA extraction. The remaining animals provided a source of intestinal tissue, which was either frozen for cryostat sectioning or fixed in 10% buffered formalin (Amresco, Solon, OH).

IMMUNISATION OF RABBITS AND PURIFICATION OF ANTIBODIES FROM SERA
For preparation of specific antibodies to PLAP, two peptides were synthesised, based on prominent hydrophilic domains of the PLAP amino acid sequence.[8] The amino acid sequences of the PLAP synthetic peptides were as follows: F-31 (amino acid residues – NSKDFVTTSEDRSLR) and F-32 (amino acid residues – RVFTESEERTASAEEIK). Likewise, the 26 amino acid melittin peptide that exhibited homology with PLAP amino acid residues 260–280, was purchased from Sigma Chemical Co, St Louis, MO. Adult New Zealand white rabbits were immunised with the PLAP peptides or melittin, after conjugation to bovine serum albumin (BSA) using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide as described by the manufacturer (Pierce Chemical Co, Rockford, IL). For primary immunisation, the peptides were emulsified with Freund's complete adjuvant, while subsequent booster doses were administered in Freund's incomplete adjuvant. Five mg of each PLAP peptide or melittin was conjugated to Epoxy-Sepharose (Pierce Chemical Co) and used to prepare columns for affinity chromatography. High-titred rabbit anti-peptide sera were pumped through the

columns, which then were washed with PBS. PLAP peptide or melittin specific antibodies that bound to the column were eluted with 0·5 M glycine acetate buffer (pH 2·5). The specific antibody preparations were neutralised (pH 7·0), dialysed, concentrated by lyophilisation, and adjusted to a protein concentration of 1 mg/ml. Antibodies to PLAP and melittin peptides reacted specifically with recombinant murine PLAP in crude extracts of *Escherichia coli* expressing a 29 kDa fragment of murine PLAP (kindly provided by R Holtsberg and S Steiner, University of Kentucky, Lexington, KY).

IMMUNOCYTOLOGICAL METHODS FOR PLAP LOCALISATION

Biopsy specimens from IBD patients were available as formalin fixed, paraffin wax embedded blocks and unfixed frozen tissue embedded in OCT compound. Endogenous peroxidase activity of the polymorphonuclear neutrophils (PMNs) in the frozen sections was eliminated by overnight fixation with 10% buffered formalin at room temperature and preincubation in H_2O_2 (3%) and methanol (80%) at room temperature for 30 minutes. Tissue from mouse colon was excised and fixed in 10% buffered formalin immediately after death of the animal as described previously.[15] The formalin fixed tissue was processed using a standard automated system. Deparaffinised and hydrated 5–7 μm thick tissue sections were mounted on glass slides and incubated with droplets of affinity purified rabbit anti-PLAP (1 mg/ml) diluted 1:100–1:200 in 0·1% BSA-PBS ($Na_2HPO_4\cdot7H_2O$, 2·17 g/l; KH_2PO_4, 0·2 g/l; NaCl, 8 g/l; pH 7·4) for one hour at room temperature. After gentle washing of the slides in PBS, a droplet of a 1:300 dilution of affinity purified goat antirabbit Ig-peroxidase conjugate (BioRad, Hercules, CA) was added to the sections for 45 minutes. Then, after washing in PBS, the slides were developed by incubation in 3,3'-diaminobenzidine (DAB)-substrate solution (Sigma Chemical Co, St Louis, MO). The tissue sections were counterstained with haematoxylin solution (Fisher Chemical Co, Fair Lawn, NJ) for two to three minutes to permit cell identification. The slides were dehydrated in graded ethanol solutions, cleared with xylene, and preserved by mounting coverslips with Permount solution (Fisher Chemical Co). The tissue sections were examined for deposition of brown precipitate at specific cellular locations in the mucosal tissue using conventional light microscopy.

CELL CULTURE

HT-29 cells were grown in flasks of McCoy's medium containing 10% fetal bovine serum in the presence of penicillin (100 U/ml), streptomycin (100 μg/ml), and gentamicin (50 μg/ml) at 37°C with 5% CO_2. HT-29 cell monolayers containing approximately 1×10^5 cells in McCoy's medium were exposed to LPS (100 μg/ml) (Difco Laboratories, Detroit, MI) for indicated times.

RNA EXTRACTION AND NORTHERN BLOT ANALYSIS

Total RNA was extracted from HT-29 cells or 1–2 cm segments of mouse colons after indicated times.[16] Cells or intestinal homogenates were suspended in a solution, containing 4 M guanidine isothiocyanate, 25 mM sodium citrate, 0·5% sodium sarcosyl, and 0·1 M 2-mercaptoethanol at room temperature for 15–20 minutes, before adding a mixture of sodium acetate-phenol-chloroform-isoamyl alcohol to extract the RNA.[17] After centrifugation at 10 000×g for 20 minutes, the RNA was collected from the top aqueous layer. Subsequently, RNA was precipitated with isopropanol and washed with 80% ethanol, before storing in 0·2% diethylpyrocarbonate treated water at –70°C. Aliquots (10–15 μg) of total RNA were loaded onto a 1·2% formaldehyde agarose gel and electrophoresed (4 volts/cm). After transfer to a nitrocellulose membrane, the RNA was hybridised with a [^{32}P] labelled *plap* cDNA probe (1×10^7 cpm/ml), labelled using a random primer kit (Gibco BRL, Gaithersburg, MD). The filters were prehybridised with Quikhyb solution (Stratagene, LaJolla, CA) for one hour at 68°C and then hybridised for two hours at 68°C. Subsequently, the filters were washed with 2×SSC (sodium chloride, sodium citrate, pH 7·0)+0·1% SDS at 68°C for one hour and then in 1×SSC+0·1% SDS at 68°C for another one hour.[17] The filters were dried and exposed to x ray film at –70°C for five hours to three days. A β-actin cDNA probe was used as an internal control to quantitate the RNA load in each lane. All blots were scanned with a densitometer (Applied Biosystems, Pittsburgh, PA), and the relative amount of PLAP message in each lane was normalised to the internal control.

STATISTICAL ANALYSIS

The ratio of the densitometric signal strength for PLAP mRNA to that of β-actin was analysed by Dunnett's procedure and comparisons were made with control preparations. Significance was evaluated at the 0·05 level.

Results

IMMUNOCYTOCHEMICAL LOCALISATION OF PLAP ANTIGEN IN MUCOSAL BIOPSY SPECIMENS

As Figure 1A shows, PLAP antigen could not be detected in sections of normal colon from 12 control patients, whose colons were removed for reasons other than IBD. Some light staining of the luminal epithelial cells in normal colon was observed; however, crypt epithelium was devoid of staining for the PLAP antigen in 12 normal colons examined. In contrast, cells positive for the PLAP antigen were readily seen in colonic tissue from patients with CD (Fig 1B) and UC (Figs 1C and 1D). Upon examining these and other IBD patient specimens, we determined that the PLAP antigen was localised to monocytes and granulocytes (predominantly eosinophils and to a lesser extent PMNs) in the inflamed tissue. In IBD patient specimens, some extracellular deposition of
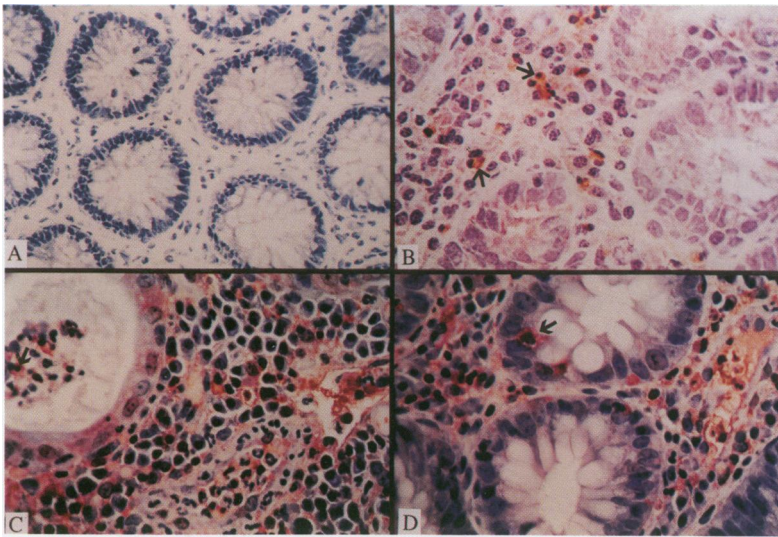
Figure 1: Immunoperoxidase localisation of PLAP antigen in mucosal tissues from UC and CD patients. The sections shown in each panel (A–D) were prepared from human colonic tissue specimens fixed in 10% buffered formalin and embedded in paraffin wax. Endogenous peroxidase was blocked by preincubation in 3% H_2O_2 and 80% methanol. All sections were incubated with affinity purified rabbit antibodies to PLAP synthetic peptide (F-31). The sections then were reacted with affinity purified peroxidase labelled antibodies to rabbit IgG (BioRad), before development in DAB substrate. The nuclei of cells were counterstained with haematoxylin. (A) Depicts a representative section of normal colon from a patient who had a partial colectomy for deverticulitis (original magnification ×216). (B) Shows a section of a biopsy specimen from the colon of a patient with CD (original magnification ×346). The arrows point to mononuclear cells with positive staining for PLAP. (C) and (D) Depict PLAP antigen localisation in sections of mucosal biopsy specimens from two UC patients (original magnification ×346). The arrow in (C) points to a mononuclear cell with positive staining for PLAP in a crypt abscess, while the arrow in (D) points to an intraepithelial lymphocyte staining positively for PLAP.

PLAP reactive antigen was observed in the lamina propria and seemed to be associated with oedema fluid and capillaries. In no case did we observe positive PLAP staining in normal tissue specimens, while in inflamed tissues, the staining reaction was evident mostly in inflammatory cells. In areas that were less inflamed in IBD patients, less PLAP staining was observed. IBD tissue sections stained with haematoxylin and eosin had crypt abscesses, crypt branching, and architectural distortion as evidence of active and chronic disease.

IMMUNOPEROXIDASE LOCALISATION OF PLAP IN COLONIC TISSUE FROM DSS TREATED MICE

Several laboratories have used DSS to induce colitis in mice, which in some aspects, mimics
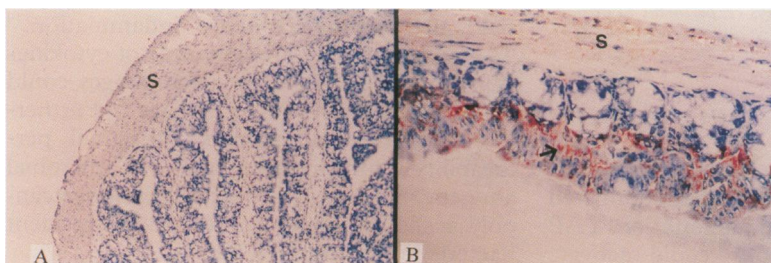

Figure 2: Immunoperoxidase stained sections of colon from Swiss-Webster mice treated with DSS. Swiss-Webster mice were fed DSS (ICN) for seven days followed by 21 days of water and housed under conventional conditions. Tissues were collected, frozen, and sectioned. After fixation in 10% buffered formalin, endogenous peroxidase was blocked by preincubation in 3% H_2O_2 and 80% methanol. The sections were reacted with affinity purified rabbit antibodies to PLAP synthetic peptide (F-31); the second antibody was affinity purified antibodies to rabbit IgG conjugated with peroxidase (BioRad). The sections were counterstained with haematoxylin. (A) Shows a section of normal mouse colon (original magnification ×54) and (B) illustrates a section of mouse colon after 28 days (original magnification ×216). The arrow points to the reaction product depicting the presence of PLAP antigen, and the 'S' indicates the serosal surface.

human IBD.[14][18] We tried initially to use a murine colitis model with SPF maintained C3H/HeJ mice fed 5% DSS (ICN), as described earlier.[18] DSS (ICN) caused extensive desquamation of the colonic mucosa with loss of crypts and mortality approaching 70% within seven to 10 days. After the report by Cooper et al,[14] we repeated our experiments with conventionally housed Swiss-Webster mice. Lower mortality (53% over a four week period) was observed with Swiss-Webster mice treated for seven days with DSS from ICN. We observed extensive damage to the colonic mucosa with disappearance of crypts, and mild inflammatory infiltrates were present 21 days after cessation of seven days of DSS (ICN) exposure. Figure 2B illustrates the immunoperoxidase location of PLAP antigen in a typical section of colonic tissue from a DSS treated Swiss-Webster mouse. Sections of colon from normal mice were devoid of PLAP reactive antigen (Fig 2A), although PLAP antigen appeared to be distributed in the stroma of the lamina propria of the DSS treated mice. Colonic tissue from the DSS treated mice had extensive tissue destruction, although there was some regeneration of the epithelium and crypts after removal of DSS. Minimal influx of inflammatory cells was evident seven to 28 days after initial exposure to DSS.

PLAP mRNA VALUES AFTER DSS INDUCED COLITIS IN MICE

When total RNA, extracted from the colons of Swiss-Webster mice fed DSS, was probed with the plap gene probe by northern analysis, it was apparent that the amount of PLAP mRNA had increased (Fig 3). In one experiment, mice were fed 5% DSS (ICN) in water for seven days followed by water for 21 days. In a second experiment, mice were fed 5% DSS purchased from TdB Consultancy for two days followed by water for 26 days. In contrast with the first experiment, we noted that the modified procedure caused little or no mortality and minimal damage to the colonic tissue of the mice. Figure 3 shows a northern blot of total RNA extracted from the colons of control mice, in addition to RNA extracted from the colons of mice at various periods of time after termination of either DSS treatment. In both experiments, the PLAP mRNA signal was increased at 28 days (Fig 3, lanes 5 and 6), which corresponds to the time at which modest tissue inflammation could be detected by microscopy.[14]

EXPRESSSION OF THE PLAP GENE BY HT-29 CELLS

Northern blot analysis was performed on total RNA extracted from HT-29 cells to find out if this human intestinal epithelial cell line could synthesise PLAP in response to an external stimulus. The data in Figure 4 illustrate that HT-29 cells synthesise PLAP constitutively (Fig 4, lane 1); however, the mRNA for PLAP was increased after LPS treatment (Fig 4, lanes
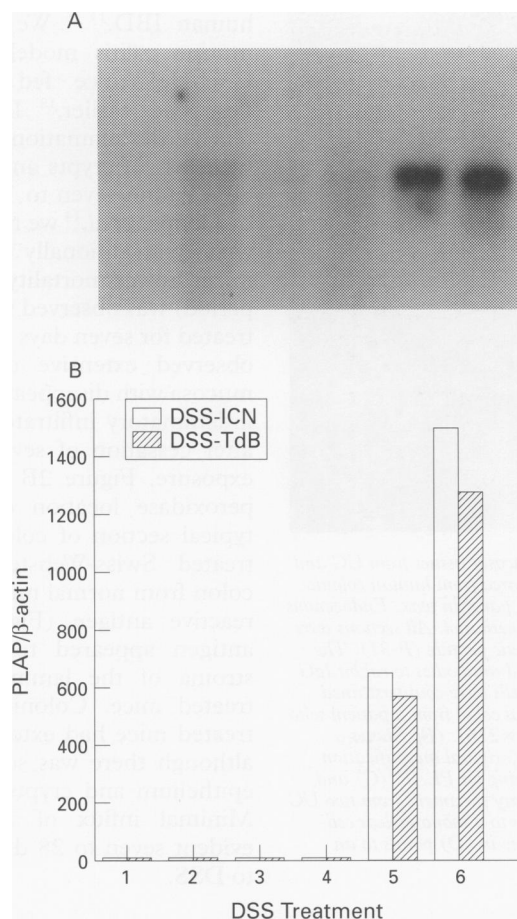
Figure 3: Northern blot analysis of total RNA from mouse colons at various times after exposure to DSS. Pooled RNA from the left and right colons of duplicate mice, which were selected at random from groups of five to six mice fed 5% DSS (ICN) ad libidum, instead of water for seven days, was subjected to northern blot analysis with the murine PLAP gene probe. In the second experiment, mice were fed DSS (TdB Consultancy) for two days. Thereafter, DSS was removed and mice were given water and housed for the indicated periods of time. Approximately 15 μg of RNA was loaded into each lane, and the RNA was blotted onto nitrocellulose membranes. The blot was hybridised with a [³²P] labelled plap gene probe, and the blot was subsequently stripped and reprobed with a β-actin gene probe to compare the amount of RNA in each lane. (A): Photographs of the intensity of the PLAP mRNA signal from control colonic tissue and colonic tissue from mice seven and 21 days after DSS (ICN) treatment are shown from experiment one. Lane 1 – control left colon; lane 2 – control right colon; lane 3 – left colon seven days on water after seven days of DSS; lane 4 – right colon seven days on water after seven days of DSS; lane 5 – left colon 21 days on water after seven days of DSS; and lane 6 – right colon 21 days on water after seven days of DSS. (B): The ratio of PLAP mRNA to β-actin is shown for the left and right colons of mice fed either DSS from ICN for seven days (experiment 1) or DSS from TdB Consultancy for two days (experiment 2). An increase in PLAP mRNA appeared after 28 days in both experiments.
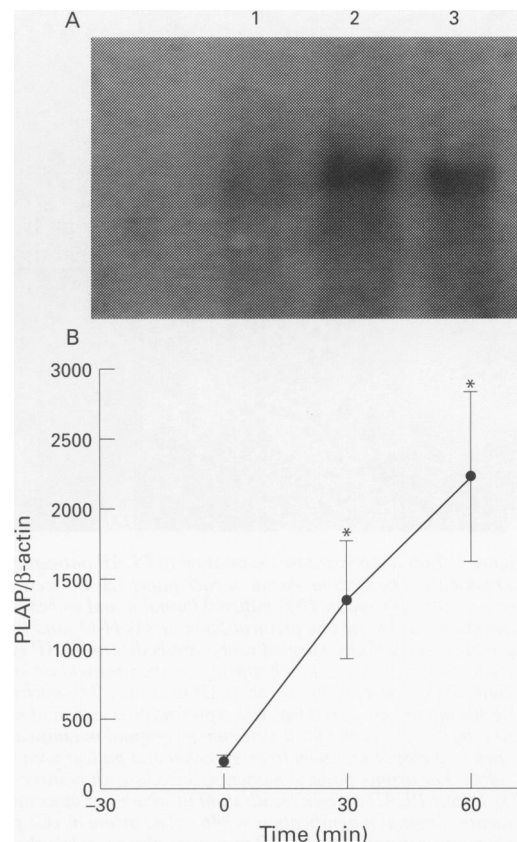
Figure 4: Northern blot analysis of the RNA isolated from LPS treated HT-29 intestinal epithelial cells. (A): Fifteen micrograms of total RNA, extracted from flasks of HT-29 cells, were loaded into each lane and the filters were hybridised with the plap gene probe – Lanes 1–3 contain RNA isolated from LPS-treated HT-29 cells at the following time points: lane 1 – control; lane 2 – 30 minutes; lane 3 – one hour. (B): The average ratio of PLAP mRNA to β-actin responses from three separate experiments are shown. The vertical bars reflect the standard error above and below the mean. The asterisks indicate significant differences compared with the control (p<0·05).

2 and 3). Thus, the data support the hypothesis that colonic epithelial cells, when stimulated with LPS, have an increased amount of PLAP mRNA, which subsequently could activate PLA$_2$ and result in increased production of AA metabolites.

Discussion

We are investigating the hypothesis that the intestinal inflammatory response to idiopathic IBD is, in part, controlled by changes in a regulatory protein that normally stimulates PLA$_2$ activity. Pro-inflammatory eicosanoids (for example, LTB$_4$) are synthesised from AA hydrolysed from membrane phospholipids.[2] [3] Indeed, patients with CD have increased PLA$_2$ activity in their colonic mucosa, which would increase the AA cascade in intestinal tissues of these patients.[5] Some of the AA metabolites (for example, LTB$_4$), released into the lamina propria, are potent chemoattractants for inflammatory cells, which, in turn, produce other inflammatory mediators (for example, IL8) that promote intense inflammation.[19] The release of excessive amounts of cytokines (for example, TNFα) by macrophages could result in extensive tissue damage.[20] Furthermore, TNF could increase intestinal permeability[21] and promote uptake of microbial antigens, such as LPS and peptidoglycan-polysaccharide, which are released by resident bacteria in the intestinal lumen.[22–24]

In this study, we prepared polyclonal antisera to the melittin peptide and synthetic peptides, representing two hydrophilic domains of the PLAP amino acid sequence, because purified PLAP was not generally available. The specificity of the antipeptide antibodies was based on the following information: (a) the amino acid sequence of the two synthetic peptide immunogens was derived from hydrophilic

domains of murine PLAP, and (b) anti-peptide antibodies reacted specifically with recombinant murine PLAP by western blot analysis. Immunocytochemical examination of IBD patient biopsy specimens (Fig 1B–1D) showed localisation of PLAP antigen to monocytes and granulocytes in the lamina propria. Eosinophils were more often positive for PLAP antigen than PMNs in most sections of colonic tissue. At this time we do not know the function of PLAP in these cells, but it could be involved in their activation and promote the release of eicosanoids.

Histopathological examination of colonic tissue from mice fed 5% DSS from ICN for seven days showed minimal infiltration of inflammatory cells, although the tissues had epithelial desquamation and loss of crypts. These findings were similar to those described in a recent report by Dieleman et al.[25] We observed extensive deposition of DAB reaction product indicating accumulation of PLAP antigen in the DSS damaged tissue 21 days after cessation of DSS exposure (Fig 2B), while little or no PLAP antigen was seen in colonic tissue sections from control mice (Fig 2A). These data signified that dynamic changes in PLAP synthesis had occurred, which could lead to increased eicosanoid synthesis. These PLAP antigen localisation data (Fig 2B) are supported by positive northern blot signals with the plap gene probe in RNA isolated from the colons of DSS treated mice (Fig 3). We tried two procedures in treating the mice with DSS. In the first experiment, we fed the mice 5% DSS, purchased from ICN, for seven days followed by water for 21 days. In contrast, we repeated the experiment by feeding mice 5% DSS from TdB Consultancy for two days followed by water for 26 days. We observed that RNA extracted from the colons of duplicate mice showed similar increases in PLAP mRNA, regardless of the time of exposure and source of DSS used in the treatment (Fig 3). In neither experiment did we see significant infiltration of inflammatory cells into the lamina propria. Whether DSS impaired inflammatory cell infiltration in the mouse colon was not clear, but PLAP synthesis occurred even without a perceptible inflammatory cell response. Thus, PLAP activation of PLA_2 activity, and subsequent eicosanoid synthesis, would seem to be separable from the inflammatory cellular response. Alternatively, PLAP also may be induced by tissue injury, thereby initiating an inflammatory cell response.

We observed in tissue sections of both normal human intestine and normal mouse colon that PLAP antigen was in detectable concentrations only in the mature luminal epithelial cells but not in the crypt epithelial cells. Furthermore, in normal mouse small intestine, the villar epithelial cells were stained lightly with PLAP antibodies, but crypt epithelial cells did not react (data not shown). Because of the innate differences in maturation and differentiation of these epithelial cells, it is plausible that PLAP and PLA_2 activity normally might participate in regulating epithelial cell metabolism or be needed for increased synthesis of digestive enzymes in the more mature epithelial cells. Luminal epithelial cells in the colon and villar epithelial cells in the small intestine are often stimulated by close contact with LPS and other bacterial substances present in the luminal contents; whereas, the rapidly regenerated crypt epithelium might be cleansed continuously by the upward flow of mucus and fluid. We observed that PLAP mRNA values were increased in HT-29 cells after in vitro stimulation with LPS (Fig 4). Thus, the intestinal villar epithelial cells that stained with antibodies to PLAP peptides could be responding to microbial substances such as LPS in the intestinal lumen. No information is available about the relation of PLAP to AA metabolism and cytokine synthesis in HT-29 cells exposed to LPS; however, current models of intestinal inflammation show that the microbial flora and their products seem to be important in stimulating inflammation in the intestinal mucosa.[24]

PLAP may have an important role in regulating both eicosanoid synthesis and cytokine production.[11][12] IL1 and PLAP both have been shown to induce IL2 synthesis in a murine T helper cell line (EL-4)[11] and IL1 and $TNF\alpha$ production in human monocytes.[12] The finding that PLAP synthesis occurred rapidly after activation of some cells,[8][9] along with the paracrine and autocrine activities of PLAP, makes it a potentially important regulator of the inflammatory response or the cytokine cascade, or both, as shown in the pathogenesis of rheumatoid arthritis.[10] Lastly, IL1 was shown to stimulate electrolyte transport in rabbit colonic mucosa by a PLAP mediated mechanism leading to PGE_2 synthesis.[26]

Activation of both the cyclooxygenase and lipoxygenase pathways in IBD suggests that excessive PLA_2 mediated AA mobilisation is responsible for increased mucosal eicosanoids. Indeed, increased PLA_2 immunoreactivity and enzymatic activity are found in mucosal tissue and serum of IBD patients and in animal models of colitis.[5][7] Although increased mucosal PLA_2 activity is a recurring finding in IBD, the molecular mechanisms responsible for its up regulation could involve PLAP activity. Presently, little is known about the existence, production, regulation, or function of PLAP in normal or IBD intestinal tissues, although expression of plap is increased in inflamed colonic tissue. The intense inflammatory response with subsequent intestinal tissue destruction seen in IBD may be a result of overactivation or faulty regulation of PLA_2 activity. Thus, we have postulated that PLAP may have some role in the pathogenic sequence of IBD, and control of mucosal plap expression PLAP activation of PLA_2 might be a useful strategy to modulate inflammatory diseases.

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