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Platelet-activating factor receptor mRNA is localized in eosinophils and epithelial cells in rat small intestine: regulation by dexamethasone and gut flora

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SUMMARY

Platelet-activating factor (PAF) is a potent mediator involved in bowel injury. We investigated PAF receptor transcription and its mRNA localization in the small intestine of normal (conventionally fed) and germ-free rats, by competitive polymerase chain reaction (PCR) and *in situ* hybridization. A dose of PAF (1·5 μg/kg, i.v.) insufficient to cause gross bowel injury was injected into rats. Some rats were pretreated with dexamethasone (1 mg/kg). We found: (1) PAF receptor (PAF-R) mRNA localized predominantly in lamina propria eosinophils and in epithelial cells; (2) PAF increased PAF-receptor signals in the epithelial cells; (3) Dexamethasone depleted eosinophils in the intestine and markedly decreased PAF-receptor transcripts; the response to PAF was also weaker than control rats; (4) Germ-free rats had less PAF-R mRNA than normal rats, and showed a weaker response to PAF than conventionally fed rats. Thus, we conclude: (1) PAF receptor mRNA is constitutively expressed in the epithelium and in lamina propria eosinophils in the intestine. (2) PAF-R transcription is up-regulated by PAF and gut flora, mostly in the epithelium. (3) PAF-R transcription is down-regulated by glucocorticoids, mainly as a result of eosinophil depletion. These results suggest a functional role for PAF receptors both in host defence and the inflammatory response in the small intestine.

INTRODUCTION

Platelet-activating factor (PAF, 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine), a phospholipid mediator, possesses potent and diverse biological effects.[1] It is produced by many types of cells, including macrophages, mast cells, neutrophils, eosinophils, endothelial cells and platelets (reviewed in ref. 1). Its role in inflammation and shock has been well established.[1] Most immune cells, e.g. neutrophils,[2,3] monocytes/macrophages,[2,3] eosinophils,[3,4] activated lymphocytes,[5] and some non-immune cells, such as platelets,[2] endothelial[6] and airway epithelial cells,[7] are known to express PAF receptor (PAF-R) on their surface. PAF-R is a member of the G protein-coupled receptor superfamily characterized by seven transmembrane domains.[3] Binding of PAF to its receptor leads to the activation of several signal transduction pathways,[8] resulting in diverse biological responses.

PAF may be involved in various pathological processes *in vivo*, such as septic shock,[1] gastric ulcer,[9] intestinal inflammation,[10] and injury.[11] Its role in intestinal injury is demonstrated by the findings that administration of PAF into animals causes intestinal necrosis,[11] and that intestinal injury induced by lipopolysaccharide (LPS)[12] or tumour necrosis factor (TNF)[13] could be abrogated by PAF antagonists. We have previously shown that, compared to other visceral organs, PAF-R is preferentially expressed in the small intestine.[14] However, the cellular localization of PAF-R in the small intestine remains unknown. Thus, in the first part of this study, we aimed to identify the cellular source of PAF-R transcripts.

How PAF-R is regulated in physiological and pathological conditions is not completely understood. It has been shown that the expression of PAF-R could be augmented by TNF-α[15] in polymorphonuclear cells (PMNs), by interleukin-5 (IL-5) in eosinophils,[4] and by LPS[16] or PAF[17] itself, in monocytes/macrophages. Our previous study showed that the gene expression of PAF-R in the intestine can also be up-regulated by administration of PAF and LPS *in vivo.*[14] Previous reports demonstrated a protective effect of anti-inflammatory glucocorticoids against PAF-induced mortality[18] and gastrointestinal damage.[19] It was suggested that this protective effect was caused by increased activity of plasma acetylhydrolase,[20] the

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Abbreviations: PAF, platelet-activating factor; PAF-R, platelet-activating factor receptor; LPS, lipopolysaccharide; TNF, tumour necrosis factor-α; DTT, dithiothreitol; Q-PCR, quantitative PCR; cRNA, competitor RNA; PMN, polymorphonuclear leucocyte(s); Hct, haematocrit; WBC, white blood cell(s); NF-κB, nuclear factor-κB.

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enzyme that degrades PAF. However, the mechanism of the protective action is not completely understood. Thus, in this study we also examined whether PAF-R in the small intestine could be modulated by pharmacological agents such as dexamethasone.

Our previous study suggested that PAF-induced intestinal injury is dependent on the presence of gut flora, since no injury develops in germ-free rats.[21] This protection was presumably caused by, at least in part, the absence of LPS and/or bacterial products, which synergize with PAF[11] and cytokines[13] to mediate tissue injury. However, based on our previous observation that LPS induces PAF-R gene expression,[14] it seemed possible that germ-free animals could have less PAF receptors, and thus, be less responsive to PAF. Thus, in the last part of this study we examined the amount of PAF-R transcripts in the intestine in germ-free rats.

MATERIALS AND METHODS

Materials

PAF (1-*O*-hexadecyl-2-acetyl-*sn*-3-phosphocholine), dexamethasone, and molecular biological reagents were purchased from Sigma Chemical Co. (St Louis, MO). PAF (2 mg/ml) was dissolved in albumin saline (5 mg/ml), aliquoted and stored at −80°. Working solution was prepared fresh immediately before the experiment.

Animal experiments

Young male conventionally fed (Harlan, Indianapolis, IN) and germ-free (Taconic, Germantown, NY) Sprague–Dawley rats (70–120 g) were allowed to acclimate 2 days before the experiments. The animals were anaesthetized, tracheotomized, and catheterized via the carotid artery and jugular vein for continuous blood pressure recording, blood sampling and drug injection. The animals were divided into six groups: (a) sham-operated, conventionally fed (normal); (b) normal rats injected with PAF (1·5 μg/kg, i.v.); (c) normal rats pretreated with dexamethasone 24 hr before PAF injection; (d) sham-operated, germ-free rats; (e) germ-free rats injected with PAF; (f) normal rats pretreated with dexamethasone (1 mg/kg, i.p., 24 hr before the experiment), but without PAF injection. The dose (1·5 μg/kg) used was below that causing prolonged shock and intestinal necrosis in order to minimize the possible loss of RNA via cell necrosis. The rats were euthanized at 0, 30 or 90 min after PAF injection. The distal half of the small intestine was removed and rinsed with ice-cold saline to expel the intestinal contents. (Preliminary experiments showed that PAF-R transcripts are higher in the ileum than in the jejunum). Intestinal tissue was divided, a portion was embedded in OCT compound and frozen in liquid nitrogen for *in situ* hybridization study, and another portion was minced and immediately frozen in liquid nitrogen. In some experiments, intestinal epithelial cells were isolated following published procedure.[22] Animals were handled in accordance with NIH guidelines and the protocol was approved by our Institutional Animal Care and Usage Committee.

Isolation of intestinal epithelial cells

Epithelial cells were isolated from the small intestine following a previously described procedure.[22] Briefly, the freshly collected small intestine was everted, cut into 1 cm segments, and incubated in chelating buffer (Tris–sodium citrate 27 mM, Na_2HPO_4 5 mM, NaCl 96 mM, KH_2PO_4 8 mM, KCl 1·5 mM, d-sorbitol 55 mM, sucrose 44 mM, and dithiothreitol (DTT) 0·5 mM, pH 7·3) at 4° with stirring for 45 min. The suspension was passed through a piece of cheesecloth and centrifuged at 1000 ***g*** at 4° for 5 min. The cell pellet was washed twice with phosphate-buffered saline (PBS) (pH 7·4), frozen in liquid nitrogen and stored at −70°.

Preparation of riboprobes

We constructed a plasmid pGRPR, which includes a cDNA fragment of rat PAF-R (From bp −119 to bp 848) inserted in pGEM-T (Promega, Madison, WI) containing SP6 and T7 RNA polymerase promoters flanking multiple cloning regions.[14] Thus, both antisense and sense (used as control) riboprobes to rat PAF-R could be synthesized by using a riboprobe system (Promega, Madison, WI). With linearized pGRPR DNA templates, *in vitro* transcription reaction was carried out using [^{35}S]-labelled uridine triphosphate (UTP; 40 mCi/ml, Amersham, Arlington Heights, IL) following Promega's instruction manual. The riboprobes were subjected to alkali hydrolysis to give a mean size of 150 bases for efficient hybridization. The specific radioactivity was 10^9 c.p.m./μg RNA.

In situ *hybridization*

Cryostat sections (5 μm) were mounted on polylysine-coated slides, rinsed in PBS (pH 7·4) for 5 min, fixed in freshly prepared 4% paraformaldehyde/PBS for 60 min at 4°, and permeabilized with 0·3% Triton-X-100 for 20 min at room temperature. After treatment with proteinase K (100 μg/ml) in 50 mM Tris (pH 7·5) containing 5 mM EDTA for several minutes and 4% paraformaldehyde/PBS for 10 min, the sections were dehydrated through graded ethanol, dried, and stored at −70° until use.

Hybridization was performed with the antisense, [^{35}S]-labelled PAF-R RNA probe (1×10^6 cpm/section) diluted in hybridization buffer containing 50% formamide, 300 mM NaCl, 20 mM Tris (pH 7·5), 5 mM ethylenediamine tetra-acetic acid (EDTA), 10 mM NaH_2PO_4 (pH 8·0), 10% dextran sulfate, 500 μg/ml yeast RNA, and 1 × Denhardt's solution (Sigma) in a humidified chamber overnight (18–20 hr) at 50°. Following hybridization, sections were washed once in 4 × saline sodium citrate (SSC)/10 mM DTT for 10 min at room temperature and twice in 4 × SSC/10 mM DTT for 15 min at 37°. Unhybridized single-stranded riboprobe was then removed by RNase digestion (RNase A (50 μg/ml) and RNase T1) in 2 × SSC for 45 min at 37°. The sections were washed in 2 × SSC for 60 min at 50°, 0·5 × SSC for 60 min at 55°, and 0·1 × SSC for 2 hr at 55°. After final dehydration through ethanol containing 0·3 M ammonium acetate, dried slides were dipped in Kodak NTB2 emulsion (Eastman Kodak Co., Rochester, NY), air dried, and exposed at 4° in light-tight boxes for 1–2 weeks. The slides were developed in Kodak D-19 developer, fixed in Kodak fixer, washed in distilled water for 30 min and counterstained with eosin or haematoxylin and eosin (H & E). Non-specific hybridization was determined by parallel incubation of adjacent sections with the sense [^{35}S]-labelled PAF receptor RNA probe (which is identical to the coding strand of rat PAF-R mRNA). Each slide was examined and photographed with both light and dark field microscopy.

RNA preparation and quantitative polymerase chain reaction (Q-PCR)

Total RNA was isolated from frozen tissues or isolated epithelial cells, as previously described.[14] The concentration of RNA was determined by measuring A_{260}, and the purity and integrity were checked by the A_{260}/A_{280} ratio (should be $>1{\cdot}8$) and by electrophoresis. PAF-R Q-PCR assay was performed as previously reported.[14] We designed the competitor RNA (cRNA) to have the same length as the wild-type RNA, but with one base difference in the sequence. The adenine at bp 259 was changed to guanine using a site-specific mutagenesis PCR; the resulting cRNA products, but not target RNA products, can be digested with *Eco*RI restriction enzyme. cRNA was synthesized using a RiboMax Large Scale RNA Production System (Promega). The Q-PCR assay was performed as follows: a serially diluted cRNA standard mixed with 0·5 μg of total RNA was added to a reaction mixture (10 μl) containing 1 × r*Tth* (Perkin-Elmer, Norwalk, CT) reverse transcription buffer, 1 mM $MnCl_2$, 200 μM each of dNTP, 2·5 units of r*Tth* DNA polymerase, and 1·5 μM of primer 1 (5′-CTGTGGCCAGGAAG TAGGAT-3′). The reaction mixture was incubated at 70° for 15 min and then quickly chilled on ice. PCR was carried out in a total volume of 50 μl containing 10 μl of reverse transcription (RT) reaction mixture, 1 × chelating buffer, 2·5 mM of $MgCl_2$, and 0·3 μM primer 2 (5′-GCCACAACACAGAGGCTTGA-3′). The reaction mixtures were heated at 95° for 2 min and immediately carried through 30 cycles of PCR with denaturing step of 40 s at 95°, annealing step of 30 s at 68° and extension step of 1·2 min at 72°. In the final cycle, a 5-min extension step at 72° was included. After PCR, the DNA products were digested with *Eco*RI restriction enzyme at 37° for 60 min and were separated by electrophoresis on 1·2% agarose gel containing 1 : 10 000 diluted SYBR Green I. The DNA band generated is from target RNA is 585 bp, and that from cRNA is 380 bp or 205 bp. The density of each band on the gel was evaluated with Storm Phosphorimager (Model 860, Sony, Japan) and analysed by a computer software ImageQuant™ (Molecular Dynamics, Sunnyvale, CA). To correct the error caused by the difference in the molecular weights of the DNA bands generated from target RNA and cRNA, the density of each of the target DNA bands was multiplied by 0·65 (380/585) or 0·35 (205/585). A standard curve was established after determining the ratio of the amplified products of the two DNA bands (i.e. target RNA/cRNA). The log of this ratio is graphed as a function of the log of the known amount of cRNA standard added to the reaction mixture. The concentration of PAF-R mRNA in each sample was calculated by extrapolating from the intersection of its own standard curve (where the amounts of the target and the competitor are equal, or $\log_{10}=0$) to the x-axis $\log_{10}$ cRNA(molecules) (Fig. 2b). The validity of each PCR test was determined with a negative control of RT-reaction mixture without competitor RNA and without sample RNA.

Statistical analysis

Results were expressed as mean ± SEM and were analysed by analysis of variance (ANOVA). *P*-values were assigned by the Dunnett multiple comparison test. Results were considered significant when $P<0{\cdot}05$.

RESULTS

PAF-R transcripts are localized in epithelial cells and eosinophils of the lamina propria. PAF up-regulates PAF receptor gene expression in epithelial cells

As revealed by *in situ* hybridization studies, PAF-R mRNA is localized predominantly in the lamina propria's eosinophils and in epithelial cells (although less abundantly), of normal, conventionally fed rats (Fig. 1a, b). One and half hours after PAF challenge, PAF-R signals intensified in the epithelial cells (Fig. 1c, d), compared with sham-operated rats. However, PAF-R in the cells within the lamina propria were not perceivably amplified. To confirm that the epithelial cells are responsible for most of the observed response to PAF, we compared the increase of PAF-R transcripts in isolated epithelial cells to that in the whole tissue homogenate by using Q-PCR. As shown in Fig. 2 and Fig. 3, in sham-operated rats PAF-R mRNA remained unchanged throughout the 90-min period of the experiment. In contrast, the amount of PAF-R transcripts were doubled in isolated intestinal epithelial cells as well as in tissue homogenate 90 min after PAF treatment. This observation suggests that PAF up-regulates PAF-R gene expression in intestinal epithelial cells.

Dexamethasone treatment depleted intestinal eosinophils and reduced PAF-R transcripts: effect of PAF

Twenty-four hours after dexamethasone treatment, the small intestine was almost completely depleted of eosinophils, and PAF receptor mRNA was observed only in epithelial cells, being absent from lamina propria cells (Fig. 1e, f).

The numbers of PAF-R gene transcripts in the ileum of dexamethasone-treated rats were quantified by Q-PCR. Figure 3 shows the comparison of ileal PAF receptor mRNA in sham-operated and dexamethasone-treated rats, before and after PAF challenge. Dexamethasone-treated rats have a much lower number of PAF receptor transcripts in their small intestine, only 35·3% of control, sham-operated rats ($P<0{\cdot}05$). Although after PAF challenge steroid-treated animals also showed an increase of PAF receptor transcripts, these did not exceed the level shown by sham-operated rats which were not pretreated with dexamethasone (Fig. 3).

PAF-R transcripts are low in germ-free rats: response to PAF

By Q-PCR we found that the number of PAF receptor transcripts in the ileum of germ-free rats (Fig. 3) is much lower, only 42·9% of the value in normal, conventionally fed rats ($P<0{\cdot}05$). PAF-R transcripts in the small intestine of germ-free rats increased rapidly after PAF injection, approaching the baseline level of conventionally fed rats at 30 min, but still distinctly lower than PAF-injected, conventionally fed rats at the same time point. In contrast to conventionally fed rats which showed a continuous increase at 90 min after PAF, germ-free rats peaked at 30 min, and then plateaued (Fig. 3).

In situ hybridization study showed that the localization of PAF receptor transcripts in the intestine in germ-free rats was similar to that of the normal rat, i.e. mainly in eosinophils in the lamina propria (Fig. 1g). However, unlike conventionally fed rats, whose PAF-R mRNA became intensified in intestinal

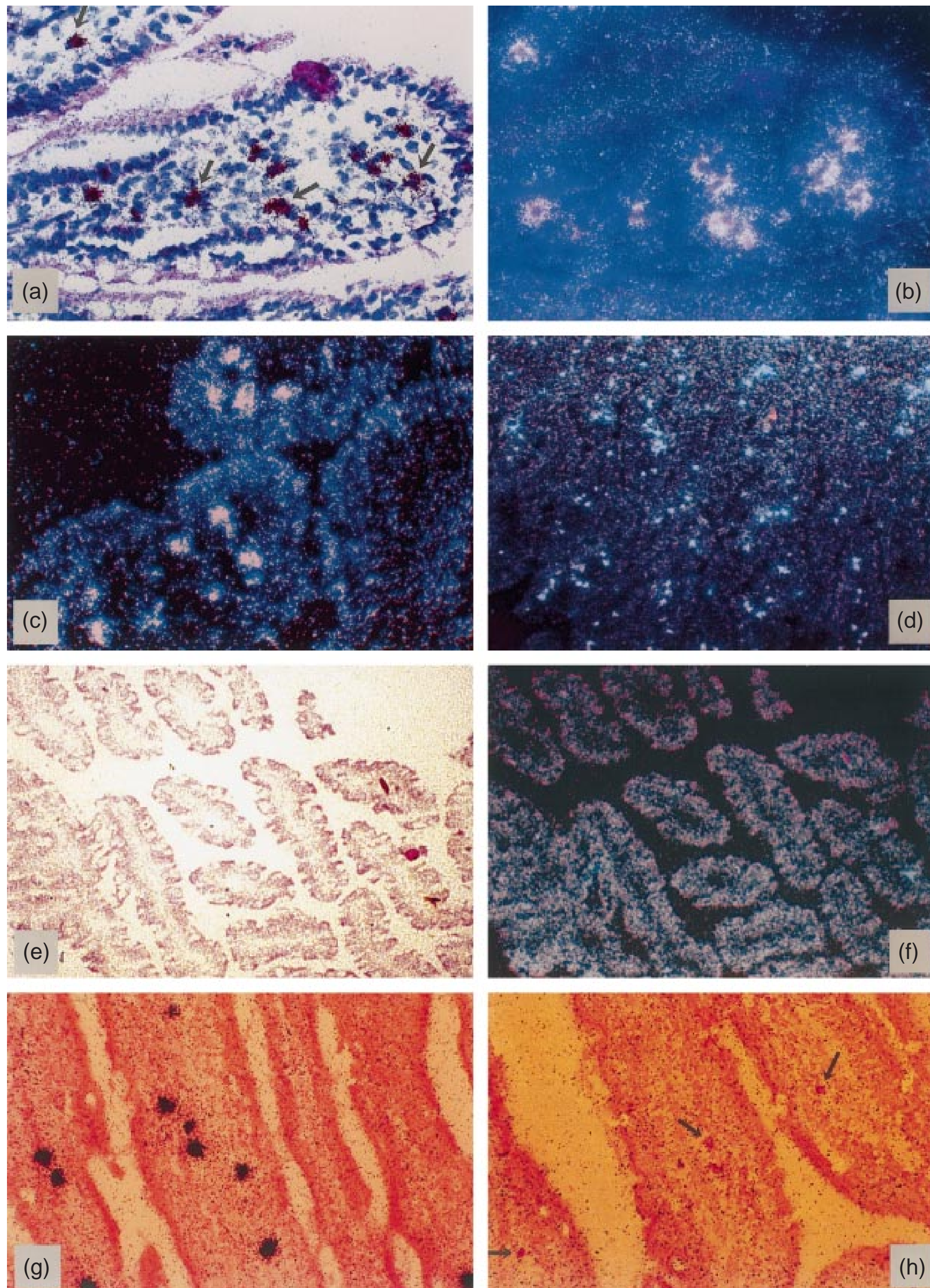

Figure 1. Expression of PAF receptor transcripts in eosinophils of lamina propria and in epithelial cells in rat small intestine. *In situ* hybridization was performed with [^{35}S]-labelled PAF-R antisense RNA probe (a–g) and sense RNA probe control (h). Rats were killed at 90 min (c–f) or 30 min (g, h) after PAF (1·5 μg/kg, i.v.) injection: (a) (light field, H & E) and (b) (dark field) are from normal, conventionally fed rats; (c) (dark field) and (d) (dark field) from PAF-injected rats; (e) (light field) and (f) (dark field) from PAF-injected, dexamethasone (1 mg/kg, i.p.) pretreated (24 hr before PAF) rats; (g) (light field, antisense) and (h) (light field, sense control) from PAF-injected, germ-free rats. Magnification: ×210 (a, b, c, g and h) and ×110 (d, e and f). H & E stain (a, b) or eosin stain (c–h). The H & E stain in (a) highlights the eosinophils (stained red, arrows) within the lamina propria. The same slide is used for both light and dark field microscopy. Note that (a) and (b) show the same area, and the location of the positive cells in (b) matches exactly the eosinophils in (a). The amount of PAF-R transcripts in epithelial cells in control animals is low (a, b). In contrast, rats injected with PAF showed a more intense positivity of PAF-R mRNA, brought out especially by dark field examination (c, d,and f). (e, f) (Depicting the same area on the slide) show that dexamethasone treatment resulted in the disappearance of both eosinophils (e) and PAF-R transcript-containing cells (f) in the lamina propria. However, the PAF-R transcripts are still abundant in the epithelial cells located at the periphery of villi (f). (h) (Sense probe) shows that the background (black grains) in eosinophils (arrows) is very low.

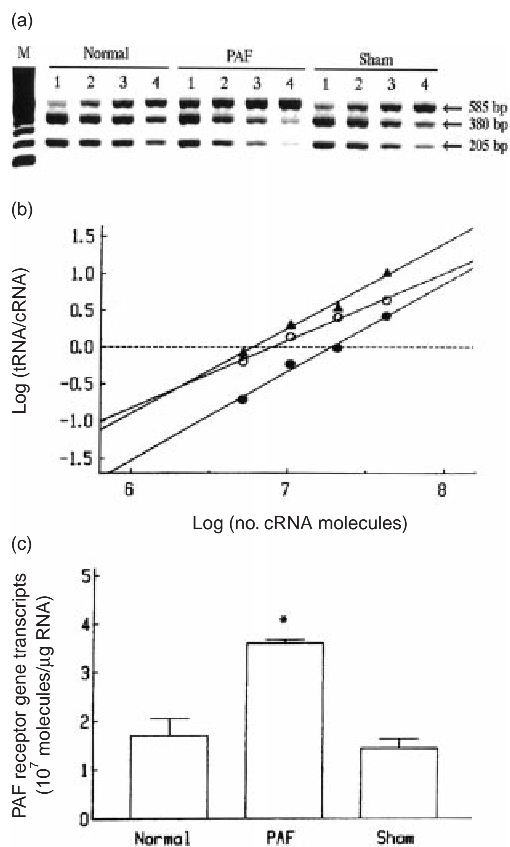

Figure 2. PAF up-regulates PAF-R transcripts in intestinal epithelial cells. (a) Total RNA (0·5 μg) extracted from isolated small intestinal epithelial cells was coamplified by RT-PCR in the presence of $4{\cdot}16\times10^7$ (lane 1), $2{\cdot}08\times10^7$ (lane 2), $1{\cdot}04\times10^7$ (lane 3) and $0{\cdot}52\times10^7$ (lane 4) molecules of competitor RNA. After *Eco*RI digestion, the competitor PCR products were cut into two fragments (380 bp and 205 bp), whereas the target PCR products remain to be 585 bp. Both competitor and target DNA were analyzed by 1·2% agarose-gel electrophoresis followed by staining with SYBR Green I. Lane M, marker of 100 bp ladder. Normal: normal conventionally fed rats, no treatment. PAF: 90 min after PAF (1·5 μg/kg, i.v.) treatment; Sham: sham-operated, 90 min. (b) Standard curve plotting log of (product of target RNA/product of cRNA) ratio against log of known cRNA standard (added to the reaction mixture). The concentration of PAF-R mRNA in each sample was calculated by extrapolating from the intersection of its own standard curve (where the amounts of the target and the competitor are equal, or $\log_{10}=0$) to the *x*-axis ($\log_{10}$ cRNA(molecules).). (▲) normal; (○) sham 90 min; (●) PAF 90 min. (c) PAF-R mRNA in isolated epithelial cells. Results are mean ± SEM ($n=3$).

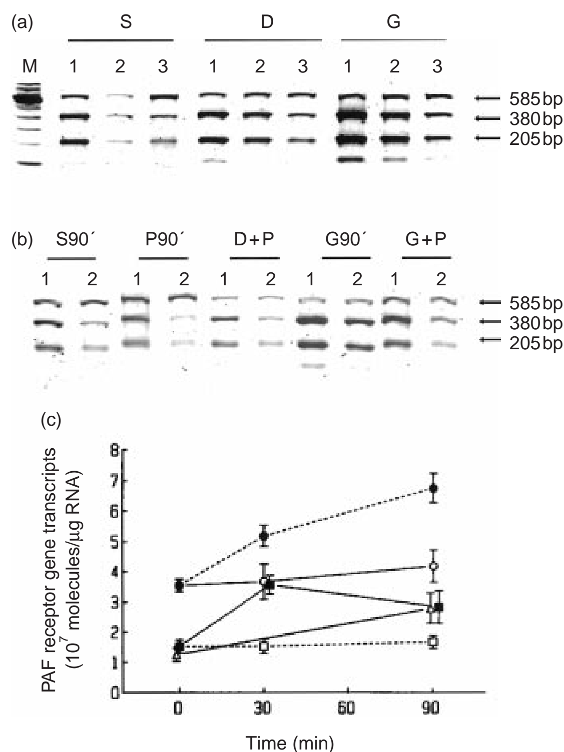

Figure 3. Up-regulation of PAF-R transcripts in rat small intestine following PAF injection. (a) animals killed at time 0. (b) animals killed 90 min after PAF (1·5 μg/kg, i.v.). Lane M, marker of 100 bp ladder. S, sham-operated; P, PAF only; D, rats pretreated with dexamethasone (1 mg/kg, i.p., 24 hr before the experiment); D + P, dexamethasone-pretreated, injected with PAF; G, sham-operated, germ-free rats; G + P, germ-free rats injected with PAF. See Fig. 2(a) legend for methods and symbols. (c) PAF (1·5 μg/kg, i.v.) was injected at time 0. Animals were killed at different time points for PAF-R mRNA quantification. (●) PAF only. (△) Dexamethasone (1 mg/kg, i.p., at −24 hr) followed by PAF injection. (■) Germ-free rats injected with PAF. (□) Sham-operated, germ-free rats. (○) Sham-operated, conventionally fed rats. $n=4$ or 5 for each point.

epithelial cells after PAF challenge (Fig. 1c, d), germ-free rats did not express PAF-R in the epithelium (Fig. 1g).

Effects of dexamethasone and germ-free environment on PAF-induced systemic changes

Systemic physiological changes are presented in Fig. 4. PAF induced a transient hypotension in conventionally fed rats, which returned to near normal value at 60 min (Fig. 4). It also induced mild leucocytosis and haemoconcentration. Dexamethasone-treated and germ-free rats also showed an initial hypotension after PAF injection. However, in contrast to conventionally fed rats, the blood pressure in germ-free rats rapidly returned to normal value within 15 min (Fig. 4). The blood pressure in dexamethasone-treated rats receiving PAF also had a faster recovery, compared with rats without pretreatment. (Fig. 4). Germ-free rats had a low baseline (before PAF) white blood cell (WBC) count (3680 ± 233), compared with normal rats (6608 ± 290) ($P<0{\cdot}05$). Unlike normal and dexamethasone-treated rats, germ-free rats only developed mild transient leucocytosis (Fig. 4, middle) and haemoconcentration (Fig. 4, bottom) after PAF injection; the values returned to the baseline at 90 min. No gross intestinal injury was observed after the administration of this dose of PAF (1·5 μg/kg, i.v.) in all groups.

DISCUSSION

PAF is an important lipid mediator, whose role in bowel injury and inflammation has been well established.[11–13] Recent studies demonstrated that PAF, at doses insufficient to cause bowel injury, may have other important pathophysiological functions in the gastrointestinal tract, such as inducing the

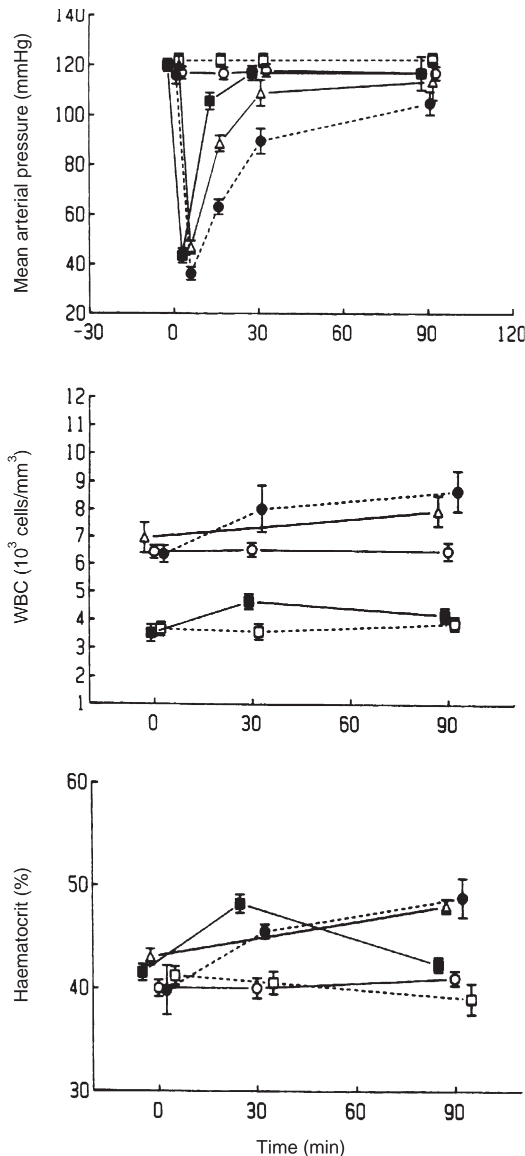

Figure 4. Change of mean arterial pressure (top panel), peripheral WBC count (middle panel) and haematocrit (lower panel) after administration of PAF. PAF (1·5 μg/kg, i.v.) was injected at time 0. See Fig. 3(c) legend for symbols. Results are mean ± SEM ($n = 4$–6 for each point). *Post hoc* tests of analysis of variance results is performed between sham and PAF groups and between the conventionally fed group receiving PAF and the groups of germ-free and dexamethasone-pretreated rats receiving PAF. Significant differences ($P < 0{\cdot}05$) are found between the following groups. Upper panel (blood pressure): between sham and PAF groups at 5, 15, and 30 min; between conventionally fed rats receiving PAF and germ-free rats receiving PAF at 15 and 30 min; and between conventionally fed rats receiving PAF and dexamethasone-pretreated groups at 15 and 30 min. Middle panel (WBC count): between germ-free control and germ-free receiving PAF groups at 30 min. Lower panel (Hct): between control and PAF groups at 30 and 90 min; between conventionally fed rats receiving PAF and germ-free rats receiving PAF at 30 and 90 min.

gene expression of nuclear factor κB (NF-κB),[23] TNF-α,[24] and type II phospholipase A_2.[25] Our previous studies[14] showed that PAF-R is constitutively present in normal rat small intestine. Furthermore, we recently showed that PAF-R level is elevated in the small intestine of the rat after PAF injection.[14] However, the detailed mechanism by which PAF mediates its effect and the specific cells which are its target in the intestine are unknown. PAF receptors have been demonstrated on platelets,[2] PMNs, macrophages/monocytes,[2,3] eosinophils,[3] endothelial cells,[6] and airway epithelial cells.[7] Although there have been no studies directly proving the expression of PAF-R in the intestinal epithelial cells, PAF has been shown to cause chloride secretion and electrolyte transport in the small intestine.[26] Endothelial cells and many immune cells are normal residents in the intestinal tissue, except for PMNs and platelets, which are rarely present outside the vasculature. In the present study, we demonstrated a predominant localization of the PAF-R in eosinophils and epithelial cells, and a surprisingly low, almost nondetectable amount of PAF-R in other lamina propria cells: e.g. macrophages, lymphocytes and endothelial cells.

The presence of the abundant PAF-R mRNA in the lamina propria eosinophils should be of no surprise. It has long been known that PAF-R is present on eosinophils.[3,4] PAF may be one of the most important biological modulators of eosinophils, since it induces their chemotaxis,[27] generation of superoxide anions,[28] adherence and degranulation,[29] as well as transmigration through endothelial cells.[30] Although there has been no information appertaining PAF and intestinal eosinophils, a recent *in vitro* study demonstrated that activated eosinophils migrate across cultured intestinal epithelial cells in response to PAF.[31] The mechanism of activation of eosinophils in the intestine *in vivo* is unclear. The high PAF-R expression in lamina propria eosinophils implies that these cells may become activated when endogenous PAF production is triggered during pathological conditions. It remains unclear how PAF-R of intestinal eosinophil is regulated. The observation that intestinal eosinophils of germ-free animals still express large amounts of PAF-R transcripts suggests that the regulation mechanism may not depend on gut flora and its products such as LPS.

It has been reported that glucocorticoid pretreatment prevents PAF-induced bowel injury.[19] The anti-inflammatory effect of glucocorticoids is incompletely understood. Glucocorticoids have been shown to inhibit the production of pro-inflammatory cytokines and arachidonic acid metabolites,[32] and up-regulates PAF–acetylhydrolase,[20] the enzyme degrading PAF. The present study demonstrated an additional mechanism by which glucocorticoids protect against PAF-induced bowel injury: Dexamethasone almost completely depleted eosinophils in the lamina propria, thus markedly reducing the number of PAF-R in the intestine.

A large quantity of PAF-R transcripts was also detected in epithelial cells. The intestinal epithelium functions as a selective barrier that permits the absorption of nutrients, electrolytes and water but prevents the entry of toxins, antigens, proteinase, and microorganisms from the lumen into the systemic circulation. The function of PAF-R in the epithelial cells is not clear. It seems likely that one of the physiological functions of PAF in the small intestine is to regulate mucosal permeability. This hypothesis is supported by previous reports showing that infusion of a low dose of PAF into the splanchnic circulation increased the intestinal mucosal permeability;[33] also, PAF antagonists reduced the early rise in epithelial permeability induced by nitric oxide synthase inhibitor.[34]

Furthermore, PAF has been shown to stimulate the change of shape in endothelial cells by rearranging their cytoskeleton,[35] and endogenous PAF has been implicated to mediate the cholera toxin-induced cytoskeleton change and secretory response of the intestinal epithelium.[36] Our recent work has shown that endogenous PAF regulates macromolecular movement across the intestinal mucosal barrier, probably via tyrosine phosphorylation of E-cadherin and cytoskeletal alteration of enterocytes (unpublished data). Our previous[14] and present studies demonstrated that the gene transcription of PAF-R in the intestine can be up-regulated by PAF itself. Our data suggest that the target cells of this regulation are probably epithelial cells.

We have previously shown that germ-free rats are protected from PAF-induced bowel injury.[21] This protection may be partly a result of the absence, in these animals, of LPS and/or other bacterial products, known to synergize with PAF to induce inflammation and tissue injury. Our present study suggests an additional protective mechanism, i.e. a markedly reduced expression of PAF-R in the small intestine of these animals. Interestingly, the number of eosinophils in the intestine did not appear to be different from normal, conventionally fed rats. Thus, the number of PAF-R in the epithelial cells is most likely down-regulated in the germ-free environment. Although the expression of PAF-R in germfree rats also rises after PAF administration, the response is much weaker than that of the conventionally fed animals. Thus, our studies suggest that the gene transcription of PAF-R in the intestine is tightly regulated by several pathophysiologcal factors: up-regulation by PAF and gut flora in the epithelium, and down-regulation by glucocorticoids via eosinophil depletion.

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