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Lipopolysaccharide and silica-stimulated mononuclear cell prostaglandin production in ulcerative colitis

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BASAL, lipopolysaccharide (LPS) and silica-stimulated prostaglandin (PG) production were compared between peripheral blood mononuclear cells (PBMNC) from UC patients and healthy subjects (HS). Basal and LPS-stimulated PBMNC PGI_2, but not PGE_2, production was greater in UC. LPS stimulated both PGE_2 and PGI_2 by PBMNC from HS and UC patients. Silica stimulated production of both PGs by cells from HS but only PGE_2 by cells from UC patients. The differences in responses to silica and LPS may result from differences in activation of NFκB or, alternatively, prior sensitisation to one of these agents. That PBMNC PGE_2 production is not increased in UC, as it is in Crohn's disease, suggests that there are differences in PBMNC behaviour between these two diseases.

Key words: Colitis, Leukocytes, Mononuclear, Prostaglandins, Lipopolysaccharide, Silica, NFκB

Introduction

The accumulation of leukocytes that produce prostaglandins (PG), specifically mononu-clear cells (MNC), within the inflamed mucosa is closely associated with, and responsible for, the elevated levels of PGs reported in luminal contents and mucosal incubations in inflammatory bowel disease (IBD). However, there have been relatively few studies on MNC PG production in IBD, and only one of peripheral blood MNC (PBMNC) PG production in UC.[1] In addition, different stimuli have different effects on PG production and previous studies have either measured unstimulated production or used only lipopolysaccharide (LPS). There have been no previous studies into the differences in MNC cell responses to various stimuli in IBD.

Thus the aim of this study was to investigate whether the production of both stimulated and unstimulated PG production by peripheral blood MN cells (PBMNC) is increased in UC in response to two different stimuli, namely silica and LPS. PG production was measured as PGE_2 and PGI_2, as differential production of cyclo-oxygenase products by different stimuli is well recognised.

Materials and methods

PBMNC were separated from 21 UC patients (14 male, median age 36 years, range 22–84) and 14 healthy subjects (controls; 10 male, median age 30 years, range 21–41).[2] Healthy subjects (HS) had not taken any medication, including non-steroidal agents, for at least five days prior. Of the 21 patients: 13 had inactive disease;[3] two [inactive] were receiving no treatment; 10 were receiving sulphasalazine, and 12 corticosteroids. The study was approved by the St Thomas' Ethical Committee and all patients gave informed written consent.

Heparinised, peripheral venous blood samples (40 ml) were centrifuged at 200 g for 10 min. The plasma was then discarded and the cells resuspended to the original volume in Dulbecco's calcium, magnesium-free phosphate buffered saline and RPMI–1640 tissue culture medium, 1:1 (v/v) mixture with additives.[2] Ten ml was then layered onto 8 ml Ficoll Hypaque and centrifuged at 400 g for 30 min at room temperature.

PBMNC were aspirated, washed three times and resuspended in 15 ml of the same solution. Aliquots of cells were taken for total cell counts, viability (trypan

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Table 1. PG production in response to stimulation with lipopolysaccharide (LPS) and silica by PBMNC from patients with ulcerative colitis (UC), compared with that by cells from healthy subjects (HS), with and without correction for basal production. Data expressed as medians and lower to upper quartiles

Prostaglandin	pg/10^6 PBMNC; median (lower–upper quartiles)		
	Basal or stimulated	HS	UC
PGE_2	Basal	380 (268–458)	439 (324–557)
	LPS	^{e}652 (497–888)	^{e}745 (563–884)
	LPS – basal	260 (182–452)	326 (141–390)
	Silica	^{e}685 (512–793)	^{c}702 (524–849)
	Silica – basal	318 (129–398)	166 (58–278)
PGI_2	Basal	110 (63–132)	**156 (122–183)
	LPS	^{c}137 (106–182)	^{e}183 (148–223)
	LPS – basal	27 (12–38)	44 (16–82)
	Silica	^{c}150 (108–192)	161 (150–183)
	Silica – basal	43 (17–92)	*13 (–22–37)

Lipopolysaccharide (LPS) or silica stimulation vs none (basal): c = $p < 0.005$, d = $p < 0.002$, e = $p < 0.001$. Ulcerative colitis (UC) vs healthy subjects (HS): * = $p < 0.05$, ** = $p < 0.005$. PGI_2 measured as 6KF1α.

blue exclusion) and percentage monocyte composition (non-specific esterase staining). Basal (unstimulated), LPS (10 μg/ml) – stimulated or silica (Gasel 23, Crossfields Chemicals, Warrington, Cheshire) – stimulated incubations of 1×10^6 viable PBMNC ml^{-1}, were performed in 50 μmol l^{-1} 2-mercaptoethanol supplemented RPMI–1640 medium, at 37°C, in a humidified atmosphere of 5% CO_2 in air, for 24 h. Incubations were centrifuged at 350 ×*g* for 7 min at 4°C and cell-free supernatants stored at –20°C for less than two months.

Samples were assayed, in duplicate, by radioimmunoassay.[2] Measures of quality control were within previously determined values and complete inhibition of PG production by indomethacin confirmed assay specificity.

The Wilcoxon 2-sample test for paired data was used to test whether stimulation has an effect on PG production, and the Mann-Whitney test for unpaired data used for comparisons between groups.

Results

Basal PGI_2 production by PBMNC from UC patients was greater than that by cells from HS. LPS stimulation increased both PGE_2 and PGI_2 production by PBMNC from patients with UC and HS (Table 1). Stimulation with silica increased PGE_2 production by cells from patients with both UC and HS but only stimulated PGI_2 production by cells from HS. There was no difference in basal PGE_2 production by PBMNC from patients with UC and HS.

Stimulated PG production was independent of basal production, with no correlation between basal and either LPS-, or silica-stimulated production in UC patients or HS. Thus PG production in response to stimulation alone, derived by subtracting basal from stimulated PG levels, was compared, thereby possibly eliminating any effects of stimulation arising from or due to preparation techniques. After correcting for basal production, there was no difference in either LPS- or silica-stimulated PGE_2 production between HS and UC patients, or in LPS-stimulated PGI_2 production. However, PBMNC from UC patients produced significantly less silica-stimulated PGI_2 than did cells from HS.

No effects of disease activity, or either steroid or sulphasalazine therapy, on PG production were discernible. There were no differences in viability, purity or percentage monocyte composition of the PBMNC preparations from UC patients and HS; thus, in agreement with previous studies, any differences do not arise from different numbers of monocytes in the incubations.

Discussion

PBMNC from patients with UC had an increased ability to synthesise PGI_2 but not PGE_2. Unlike cells from HS, PBMNC from UC patients failed to increase PGI_2 production in response to silica. This response appeared to be specific to PGI_2 as cells from both UC patients and HS increased PGE_2 production in response to silica. The previous lack of detection of PGI_2 production by PBMNC in UC,[1] whereas these cells have been widely reported to produce this eicosanoid, may result from differences in incubation or preparation techniques, or different assay sensitivities, all of which have been reported to influence the detection of PGs.

In agreement with previous studies of peripheral blood and intestinal MN cells, PBMNC production of PGE_2 by was not increased in UC.[1,4] Although,

previous studies have identified effects of sulphasalazine on PBMNC PG production *in vitro*, these were not confirmed in patients, possibly owing to confounding factors of disease activity and other treatments.[2]

The different effects of the two stimulants upon the cyclo-oxygenase pathway may arise from the different effects of LPS and silica on PBMNC NFκB activation,[6,7] involved in regulation of cyclooxygenase activity. Increased levels of NFκB activation occur in MN cells in the bowel.[5] Alternatively, silica has been implicated in the pathogenesis of IBD,[8] while there could be increased passage of endotoxins across the inflamed bowel. Moreover, previous studies in animals have shown that prior exposure *in vivo* to such stimulants can effect responses on cells *in vitro*.[9,10] The response of PBMNC to silica is selectively altered in UC, which may indicate changes in cellular activation of MNC, while the differences in PG production between CD and UC may indicate some intrinsic differences in MNC activity in the two IBDs.

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