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

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Introduction

The accumulation of leukocytes that produce prostaglandins (PG), specifically mononuclear 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 (PBMC) PG production in UC. 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 MNC cells (PBMC) is increased in UC in response to two different stimuli, namely silica and LPS. PG production was measured as PGE2 and PG12, as differential production of cyclo-oxygenase products by different stimuli is well recognised.

Materials and methods

PBMC 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). 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; 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. Ten ml was then layered onto 8 ml Ficoll Hypaque and centrifuged at 400 g for 30 min at room temperature.

PBMC 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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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 $1 \times 10^6$ viable PBMNC/ml, 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 $\times$ g for 7 min at 4°C and cell-free supernatants stored at $-20\degree$C for less than two months. Samples were assayed, in duplicate, by radioimmunoassay. Measurements 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.

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

<table>
<thead>
<tr>
<th>Prostaglandin</th>
<th>pg/10$^6$ PBMNC; median (lower–upper quartiles)</th>
<th>Basal or stimulated</th>
<th>HS</th>
<th>UC</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGE$_2$</td>
<td>Basal</td>
<td>380 (268–458)</td>
<td>439 (324–557)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LPS</td>
<td>*652 (497–888)</td>
<td>*745 (563–884)</td>
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<tr>
<td></td>
<td>LPS – basal</td>
<td>260 (182–452)</td>
<td>326 (141–390)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Silica</td>
<td>*685 (512–793)</td>
<td>*702 (524–849)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Silica – basal</td>
<td>318 (129–399)</td>
<td>166 (58–278)</td>
<td></td>
</tr>
<tr>
<td>PGI$_2$</td>
<td>Basal</td>
<td>110 (63–132)</td>
<td>**156 (122–183)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LPS</td>
<td>*137 (106–182)</td>
<td>*183 (148–223)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LPS – basal</td>
<td>27 (12–38)</td>
<td>44 (16–82)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Silica</td>
<td>*150 (108–192)</td>
<td>161 (150–183)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Silica – basal</td>
<td>43 (17–92)</td>
<td>*13 (1–22–37)</td>
<td></td>
</tr>
</tbody>
</table>

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.

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, 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. 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. 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, involved in regulation of cyclooxygenase activity. Increased levels of NFκB activation occur in MN cells in the bowel. Alternatively, silica has been implicated in the pathogenesis of IBD, 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. 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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References


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