

# Abnormal metabolism of arachidonic acid in chronic inflammatory bowel disease: enhanced release of leucotriene B<sub>4</sub> from activated neutrophils

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**SUMMARY** The metabolism of endogenous arachidonic acid P(AA) was investigated in activated neutrophils from 20 patients with Crohn's disease, 20 with ulcerative colitis, and 25 healthy volunteers. 1-<sup>14</sup>C-P(AA) was incorporated into intracellular pools of phospholipids prior to activation of the cells with ionophore A23187 and analyses of released arachidonic acid metabolites by thin layer chromatography. Total release of radioactivity expressing the release of arachidonic acid and its metabolites, was equal in the experimental and control groups, which suggests a normal substrate availability. In contrast, there was a marked increase in the relative release of leucotriene B<sub>4</sub> (LTB<sub>4</sub>) and its ω-oxidation products, 20-hydroxy-LTB<sub>4</sub> (20-OH-LTB<sub>4</sub>) and 20-carboxy-LTB<sub>4</sub> (20-COOH-LTB<sub>4</sub>), with LTB<sub>4</sub> values exceeding the reference interval in seven of 20 patients with Crohn's disease, median 8.7%, and in six of 20 patients with ulcerative colitis, median 7.7%, as compared with a median of 5.3% in healthy volunteers. Furthermore, a decreased release of unmetabolised arachidonic acid, correlating inversely with the release of LTB<sub>4</sub> in all experimental and control groups, and normal values for the production of other metabolites of arachidonic acid – for example, 5-hydroxyeicosatetraenoic acid (5-HETE) and 12-hydroxyheptadecatrienoic acid (HHT), point to an enzymatic abnormality such as increased activity of leucotriene B synthetase. An increased capacity for release of LTB<sub>4</sub>, the major pro-inflammatory metabolite of arachidonic acid lipoxygenation by polymorphonuclear leucocytes, may contribute to perpetuation of the inflammation and to tissue destruction in chronic inflammatory bowel disease. Our findings agree with previous reports of an increased release of LTB<sub>4</sub> by the colonic mucosa in this condition.

Polymorphonuclear leucocytes possibly play a role in the pathogenesis of chronic inflammatory bowel disease. Involvement of complement<sup>1-3</sup> with resulting release of the chemotactic split product C5a<sup>4</sup> thus may account for their vast numbers in the colonic exudate of patients with ulcerative colitis.<sup>5,6</sup> Local accumulation and activation of this cellular element of inflammation with release of toxic oxygen metabolites, lysosomal enzymes, and metabolites of arachidonic acid, such as leucotriene B<sub>4</sub> (LTB<sub>4</sub>), the less potent ω-oxidation products, 20-OH-LTB<sub>4</sub> and 20-COOH-LTB<sub>4</sub>, and 5-hydroxyeicosatetraenoic

acid (5-HETE) accordingly may contribute to perpetuation of inflammation and tissue destruction in chronic inflammatory bowel disease as it does in rheumatoid arthritis.<sup>7</sup>

Biopsy specimens of affected colonic mucosa from patients with chronic inflammatory bowel disease show increased production of the lipoxygenase products of arachidonic acid, and increased release of prostaglandins and leucotrienes has been shown *in vitro*,<sup>8,9</sup> and *in vivo* in patients with ulcerative colitis.<sup>10,11</sup> The cell type and stimulus responsible for this production of inflammatory mediators have not yet been identified.

A recent study showed a marked abnormality in the oxidative metabolism of polymorphonuclear leucocytes in Crohn's disease as assessed by the release of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and superoxide

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Table 1 Clinical data of patients with chronic inflammatory bowel disease

Group	No.	Age* (yr)	Sex		Disease activity		Disease site			Disease* duration (yr)
			♂	♀	Remission	Active stages	Small bowel	Small bowel + colon	Colon	
Crohn's disease	20	48 (18-79)	7	13	11	9	6	8	6	10 (2-24)
Ulcerative colitis	20	52 (20-75)	8	12	13	7	—	—	20	10 (½-30)
Healthy volunteers	25	41 (17-68)	9	16	—	—	—	—	—	—

\*Median, range in brackets.

anions ( $O_2^-$ ) after activation.<sup>12</sup> The aim of the present study was to assess the capacity of neutrophils for activation of arachidonic acid metabolism in untreated chronic inflammatory bowel disease patients.

## Methods

### PATIENTS

Twenty consecutive outpatients with well established diagnoses of Crohn's disease<sup>13</sup> and ulcerative colitis<sup>13</sup> were included in the study. Essential clinical parameters are given in Table 1. Disease activity in Crohn's disease was scored according to Harvey and Bradshaw,<sup>14</sup> with remission defined as less than five points, and in ulcerative colitis according to Tvede *et al.*<sup>15</sup> None of the patients had received specific drug treatment within the preceding four weeks, and none of the ulcerative colitis patients were colectomised or had rectal involvement only.

Informed consent was obtained from all patients and healthy volunteers, after verbal and written information, and the study was approved by the Scientific Ethical Committee of the Copenhagen County.

### NEUTROPHIL FUNCTION TEST

Neutrophils were isolated from EDTA-blood (0.2 mmol/l), with a recovery of 45% and a purity of more than 95%, by (1) methyl-cellulose (0.8%) sedimentation of erythrocytes, (2) washing and gradient centrifugation of 'buffy coat' leucocytes according to Böyum,<sup>16</sup> and finally (3) hypotonic lysis of residual erythrocytes. Incorporation of  $1-^{14}C$  arachidonic acid

( $37 \times 10^3$  Becquerel (Bq)/ml,  $2.2 \times 10^6$  Bq/mmol) (Amersham International, UK) with labelling of intracellular pools of phospholipids<sup>17</sup> proceeded for five hours at 37°C under 5% carbon dioxide and 95% atmospheric air in RPMI 1640 ( $0.5 \times 10^7$  cells/ml) (5% autologous serum). After removal of excess extracellular arachidonic acid by washing, the cells were challenged with calcium ionophore A23187 (Calbiochem, La Jolla, California, USA) (10  $\mu$ M, 15 min). Released eicosanoids were extracted with dichloromethane: methanol; 2:1, separated by thin layer chromatography (developing solvents I: chloroform: methanol: acetic acid: water; 90:9:1:0.65, II: ethylacetate: iso-octane: acetic acid: water; 55:25:10:50) and quantified by autoradiography and laser densitometry.<sup>17</sup> All analyses were done singly, and the intra-assay coefficient of variation for release of arachidonic acid metabolites was approximately 15%.<sup>17</sup>

The specific activity of the arachidonic acid metabolites, LTB<sub>4</sub> and 5-HETE, was determined by quantitative high pressure liquid chromatography<sup>17</sup> of samples from patients with Crohn's disease, which had the highest release of activity, and healthy volunteers.

### STATISTICAL ANALYSIS

All values are given as medians and ranges. The data were analysed by a rank sum test for unpaired variables, and a p value of less than 0.05 was considered significant. Linear correlation between released  $^{14}C$ -AA and  $^{14}C$ -LTB<sub>4</sub> was evaluated by the Pearson correlation procedure.

## Results

Total radioactivity released, representing arachidonic acid and its metabolites, was equal in patients and control groups (Table 2).

The relative distribution of arachidonic acid and biologically active metabolites was abnormal in the patients. The 5-lipoxygenase product, LTB<sub>4</sub>, was increased in seven of 20 patients with Crohn's disease ( $p < 0.01$ ) and in six of 20 patients with ulcerative

Table 2 Total radioactivity released by  $1-^{14}C$ -AA labelled PMNs during activation. Medians are given with ranges in brackets

Group	No.	Radioactivity in $10^2$ Bq/ $5 \times 10^6$ PMNs
Crohn's disease	20	7.3 (3.9-11.1)
Ulcerative colitis	20	6.5 (2.8-11.1)
Healthy volunteers	25	6.7 (2.6-9.9)

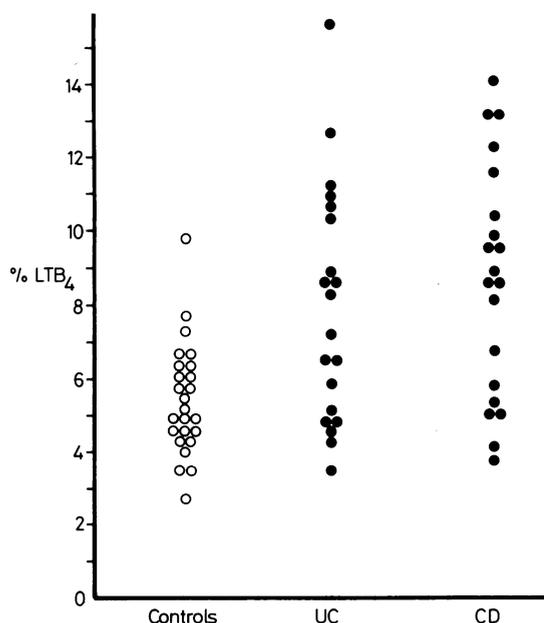


Fig 1 Relative release of the 5-lipoxygenase product,  $1\text{-}^{14}\text{C}$   $\text{LTB}_4$ , expressed in per cent of total radioactivity released by activated neutrophils from healthy volunteers (controls) and patients with ulcerative colitis (UC) and Crohn's disease (CD).

colitis ( $p < 0.01$ ) as compared with the healthy volunteers (Fig. 1 and Table 3). Furthermore, the two  $\omega$ -oxidation products, 20-OH- $\text{LTB}_4$  and 20-COOH- $\text{LTB}_4$ , which were not resolved in the TLC-system I, were raised in parallel with  $\text{LTB}_4$  (Table 3). In additional experiments using TLC-system II, which separates 20-OH- $\text{LTB}_4$  and 20-COOH- $\text{LTB}_4$ , the ratio between these two compounds was found to be median 3.4:1 (1.9–5.5,  $n = 10$ ). In contrast, the values for the less potent lipoxygenase metabolite, 5-HETE, and for the cyclo-oxygenase metabolite, HHT, did not differ between patients and controls (Table 3) indicating an increased total capacity for metabolising endogenously released arachidonic acid (Fig. 2).

An inverse correlation between released  $^{14}\text{C}$ -AA and  $^{14}\text{C}$ - $\text{LTB}_4$  expressed in per cent of total released radioactivity could be established in each of the three groups, ulcerative colitis patients ( $r = -0.815$ ;  $p < 0.01$ ), Crohn's disease patients ( $r = -0.875$ ;  $p < 0.01$ ), and healthy volunteers ( $r = -0.806$ ;  $p < 0.01$ ).

The specific activity of  $\text{LTB}_4$  was equal in the two groups tested,  $4.1 \times 10^8$  Bq/mmol (range 2.9–5.5) and  $5.4 \times 10^8$  Bq/mmol (range 3.4–6.9) in patients with Crohn's disease and in healthy volunteers, respectively.

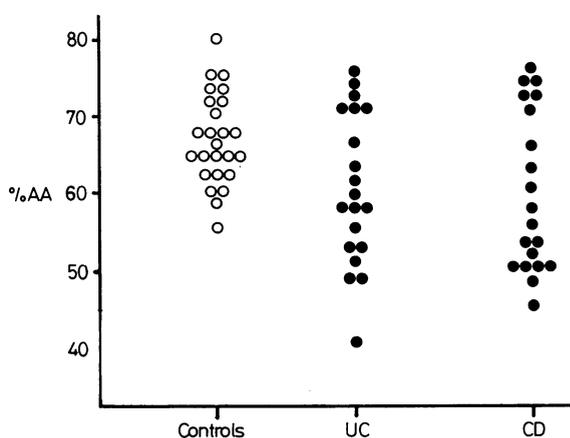


Fig 2 Relative release of unmetabolised  $1\text{-}^{14}\text{C}$  arachidonic acid (AA) expressed in per cent of total radioactivity released by activated neutrophils from healthy volunteers (controls) and patients with ulcerative colitis (UC) and Crohn's disease (CD).

None of the data correlated with clinical scores of disease activity, with duration of the disease, or with the site of intestinal involvement.

## Discussion

Arachidonic acid is metabolised by oxygenation through separate pathways<sup>18</sup> of which the 5-lipoxygenase pathway leading to the inflammatory mediators  $\text{LTB}_4$  and 5-HETE seems to be the major route in human polymorphonuclear leucocytes.<sup>19</sup> Increased release of these metabolites by the colonic mucosa in ulcerative colitis<sup>8,9,11,20</sup> may reflect release from inflammatory polymorphonuclear leucocytes, at least as judging by animal experiments.<sup>21</sup> Our data suggest an underlying enzymatic defect of the circulating neutrophil. As the enzyme responsible for leucotriene synthesis, 5-lipoxygenase, has been shown to exhibit an absolute requirement for calcium ions,<sup>22</sup> we have chosen to study the stimulation induced by calcium ionophore A23187. Although this is an unphysiologic agent it is assumed to produce the maximal synthesis of leucotrienes in response to calcium influx. The procedure therefore reflects the total capacity for leucotriene synthesis in human polymorphonuclear leucocytes. Activated polymorphonuclear leucocytes revealed an increased capacity for metabolising endogenously released arachidonic acid into  $\text{LTB}_4$  and the less potent  $\omega$ -oxidation products – 20-OH- $\text{LTB}_4$  and 20-COOH- $\text{LTB}_4$ ,<sup>23</sup> whereas the values for 5-HETE and the cyclo-oxygenase metabolites were normal. This finding does not reflect increased labelling of membrane phospholipids with  $1\text{-}^{14}\text{C}$ -AA as high pressure liquid

Table 3 Release of arachidonic acid (AA) and its main metabolites by activated neutrophils in healthy volunteers and patients with ulcerative colitis and Crohn's disease. Values are given in per cent of total radioactivity released and expressed in median values with ranges in brackets

Group	No.	20-OH-LTB <sub>4</sub> / 20-COOH-LTB <sub>4</sub>	LTB <sub>4</sub>	5-HETE	HHT	AA
Crohn's disease	20	4.3* (2.1-9.1)	8.7* (3.9-14.2)	16.6 (10.5-19.6)	2.3 (0.9-5.2)	56.8* (45.3-75.7)
Ulcerative colitis	20	4.2† (1.8-13.2)	7.7* (3.5-15.8)	16.3 (10.8-21.3)	2.0 (0.8-5.0)	59.8‡ (41.5-73.5)
Healthy volunteers	25	2.7 (1.1-6.0)	5.3 (2.8-9.6)	14.9 (9.5-19.4)	2.2 (1.0-5.3)	67.0 (56.2-80.4)

\*p<0.01. †p<0.02. ‡p<0.03.

chromatography analyses of the 5-lipoxygenase products revealed that the specific activation was equal in the groups tested. Increased activity of LTB<sub>4</sub> in the gut mucosa may hypothetically lead to accumulation of more neutrophils and thus to perpetuation of the chronic inflammation, LTB<sub>4</sub> being a well established activator of oxygen metabolism, adherence, aggregation, and chemotaxis in neutrophils.<sup>24</sup> The two catabolites of LTB<sub>4</sub>, 20-OH-LTB<sub>4</sub> and 20-COOH-LTB<sub>4</sub> were also found to be raised in chronic inflammatory bowel disease, which indicates that the ω-hydroxylase activity was not defective.<sup>25</sup>

The combined finding of an increased relative release of LTB<sub>4</sub>, and a decreased contribution by arachidonic acid to total radioactivity released is highly suggestive of an intracellular, enzymatic abnormality in chronic inflammatory bowel disease such as an increased leucotriene B synthetase activity. On the other hand, it cannot be excluded that a defective hydrogen peroxide production<sup>12</sup> may contribute to the enhanced LTB<sub>4</sub> level seen in Crohn's disease patients, as hydrogen peroxide degrades leucotrienes unspecifically.<sup>26</sup> Other cell types playing a role in chronic inflammation, for instance tissue macrophages, may show similar changes, but have not so far been investigated.<sup>27</sup>

The increased release of LTB<sub>4</sub> does not suggest an initiating pathophysiologic role of arachidonic acid metabolites. LTB<sub>4</sub> is a soluble mediator of inflammation that may amplify and modulate an inflammatory response already present, essentially by its potential for activating cellular elements by inflammation,<sup>24</sup> but this is not likely to be a primary event.<sup>28</sup>

The described defective metabolism of arachidonic acid may be normalised by steroids, sulfasalazine, and 5-aminosalicylic acid all of which inhibit various steps of the arachidonic acid metabolism and are effective in the treatment of chronic inflammatory bowel disease. Thus, steroids induce the synthesis of phospholipase inhibitors (lipocortin) *in vivo*,<sup>29</sup> whereas sulfasalazine and 5-aminosalicylic acid inhibit the 5-lipoxygenase activity *in vitro*.<sup>30</sup>

The data presented may cause reflections of a possible similarity between chronic inflammatory

bowel disease and chronic granulomatous disease, since in both conditions polymorphonuclear leucocytes show an enhanced capacity for releasing leucotrienes.<sup>26</sup> The intestinal granulomas in Crohn's disease and chronic granulomatous disease cannot be differentiated from each other on morphological criteria.<sup>31</sup>

More information about the complex way in which arachidonic acid is metabolised in the diseased and normal intestine may provide a base for further rational approaches in the treatment of chronic inflammatory bowel disease.

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