Raised serum activity of phospholipase A₂ immunochemically related to group II enzyme in inflammatory bowel disease: its correlation with disease activity of Crohn's disease and ulcerative colitis

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Abstract

Calcium dependent phospholipase A₂ activity in the mixed micelles of 1-palmitoyl-2-oleoyl-phosphatidylglycerol and cholate was measured in sera of 39 patients with Crohn's disease, 40 patients with ulcerative colitis, and 40 healthy controls. The phospholipase A₂ activity was significantly raised in those sera of the patients with active Crohn's disease and those with moderate and severe ulcerative colitis. The major phospholipase A₂ activity derived from the sera was separated into two peaks by reverse phase high performance liquid chromatography. The phospholipase A₂ active fractions were immunochemically characterised using specific antibody directed against human group II phospholipase A₂ purified from rheumatoid synovial fluid. The results suggest that raised serum phospholipase A₂ activity in patients with Crohn's disease and ulcerative colitis was mainly attributed to the two forms of phospholipase A₂ immunochemically related to group II enzyme. In patients with Crohn's disease, serum phospholipase A₂ activity decreased in parallel with clinical improvement, and correlated with serum C-reactive protein and erythrocyte sedimentation rate. The results suggest that serum phospholipase A₂ activity may serve as an additional indicator of disease activity. Serum phospholipase A₂ activity in patients with ulcerative colitis tends to increase in relation with endoscopic severity, and may be a more sensitive laboratory index than serum C-reactive protein and erythrocyte sedimentation rate to evaluate disease activity.

played by phospholipase A₂ as an inflammatory mediator. A large amount of group II phospholipase A₂ accumulated in the synovial fluid of the patients with rheumatoid arthritis.⁷ Intra articular injection of group II phospholipase A₂ purified from rheumatoid synovial fluid induced dose dependently acute inflammatory changes in synovial tissue.⁸ Furthermore, interleukin 1 increased concentrations of mRNA for synovial fluid group II phospholipase A₂, phospholipase A₂ activity and prostaglandin E₂ synthesis in rabbit chondrocytes.^{9,10} These results support an idea that group II phospholipase A₂ plays a role in the pathogenesis of the inflammatory response through its direct action or indirect action via its metabolites.¹¹

Inflammatory bowel disease – that is, Crohn's disease and ulcerative colitis, is chronic intestinal inflammatory disease of unknown aetiology. Arachidonic acid derived chemical mediators may be involved in the pathogenesis of inflammatory bowel disease. Raised intestinal contents of prostaglandins and leukotriene B₄ have been reported in inflammatory bowel disease.¹²⁻¹⁴ In this context, increased phospholipase A₂ activity of ileal and colonic mucosa of Crohn's disease^{15,16} has been reported. This suggests involvement of phospholipase A₂ in the pathogenesis of intestinal inflammation. To clarify the pathophysiological role of phospholipase A₂ in inflammatory bowel disease, it is important to examine the relationship between the severity of disease and phospholipase A₂ activity levels in serum, and the isozymic nature of phospholipase A₂ present in serum of the patients with inflammatory bowel disease. Increased serum phospholipase A₂ activity has been reported in patients with acute pancreatitis,¹⁷ rheumatoid arthritis,¹⁸ and septic shock.¹⁹ It is, however, unknown what kind of phospholipase A₂ contributes to raised serum phospholipase A₂ activity. In inflammatory bowel disease, serum phospholipase A₂ activity level and the molecular entity of serum phospholipase A₂ remain to be examined in detail.

In the present study, we found raised serum phospholipase A₂ activity in patients with Crohn's disease and ulcerative colitis. To examine the molecular entity of the increased phospholipase A₂, serum phospholipase A₂ was immunochemically characterised, after being separated by reverse phase high performance liquid chromatography, by means of immunoblot analysis using a specific antibody directed against group II phospholipase A₂ purified from

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Phospholipase A₂ hydrolyses the fatty acyl ester bond at the *sn*-2 position of glycerophospholipids and produces free fatty acids and lysophospholipids. Cellular calcium dependent phospholipase A₂s have been thought to participate in the regulation of the phospholipid metabolism in biomembranes as well as eicosanoid biosynthesis.¹ Recent studies on mammalian phospholipase A₂s have shown structural diversity. Cellular calcium dependent phospholipase A₂s can be classified into at least two groups according to their distinct characteristics in primary structure.²⁻⁶ One is of the pancreatic type (group I), and the other of the viperid and crotalid type (group II).

Interest has recently been focused on the role

TABLE I Distribution of disease

	Cases (n)
Crohn's disease	
Small intestine	19
Terminal ileum	4
Large intestine	8
Small and large intestine	8
Ulcerative colitis	
Whole colonic	10
Rectum to mid transverse	16
Distal colonic	14

rheumatoid arthritic synovial fluids. Rat splenic group II phospholipase A₂ could be successfully separated from rat pancreatic phospholipase A₂ by high performance liquid chromatography.⁴ In addition, to evaluate the usefulness of the level of serum phospholipase A₂ as an indicator of disease activity, we have compared serum phospholipase A₂ activity levels with serum C reactive protein levels and erythrocyte sedimentation rate (Westergren) which have been frequently considered to be useful markers of activity of inflammatory bowel disease although not in all cases.²⁰⁻²⁴

Methods

PATIENTS

Thirty nine patients with Crohn's disease (27 men and 12 women) aged 18-64 years (mean age of 31.5 (2.1)), and 40 patients with ulcerative colitis (17 men and 23 women) aged 19-59 years (mean age of 31.3 (1.4)) were studied. Six of the 39 patients with Crohn's disease had been admitted to our hospital. Controls consisted of 40 healthy volunteers (28 men and 12 women) aged 24-71 years (mean age of 33.2 (1.4)).

The diagnosis of Crohn's disease was based on clinical symptoms, radiographic findings, and endoscopic examination. All patients with Crohn's disease were on special diets including elementary and low residual diet. Six of the 39 patients with Crohn's disease were receiving no medicine, 24 were receiving sulphasalazine (1.5-4.0 g/day), two were receiving prednisolone (5-30 mg/day), and seven were receiving prednisolone (5-30 mg/day) in addition to sulphasalazine (2.0-4.0 g/day). The diagnosis of ulcerative colitis was based on clinical symptoms, barium enema study and endoscopic examination. Six of the 40 patients with ulcerative colitis were receiving no medicine, six were receiving sulphasalazine (1.0-4.0 g/day), and 28 were receiving prednisolone (5-30 mg/day) in addition to sulphasalazine (0.5-4.0 g/day). The localisation of diseased areas in the gut is shown in Table I.

EVALUATION OF DISEASE ACTIVITY OF CROHN'S DISEASE AND ULCERATIVE COLITIS

On the basis of the clinical severity evaluated according to simple index described by Harvey and Bradshaw,²⁵ the patients with Crohn's disease were divided into two groups: inactive Crohn's disease (20 patients) when simple index ≤ 3 , and active Crohn's disease (19 patients) when simple index ≥ 4 . No significant difference of simple index was found among Crohn's

disease patients on four types of treatment. In contrast with the fact that Crohn's disease exhibits much clinical variation, ulcerative colitis is primarily a mucosal disease. Degree of inflammation in affected lesion of ulcerative colitis can be relatively easily and more precisely evaluated by endoscopic examinations than evaluated on the basis of clinical criteria. Therefore, disease activity of ulcerative colitis was evaluated on the basis of colonoscopic findings. We used endoscopic grading of the state of disease, Matts' score,²⁶ as a measure of activity of ulcerative colitis. The score was determined separately at the following six segments of the colon: rectum, sigmoid, descending, left transverse, right transverse and ascending colon. The highest score was taken as a measure of disease activity, and the patients with ulcerative colitis were divided into three groups as regards Matts' score: mild (Matts' score 2, 11 patients), moderate (Matts' score 3, 15 patients), and severe (Matts' score 4, 14 patients). There were no patients with Matts' score 1 in this study. There was no significant difference of Matts' score among ulcerative colitis patients on three types of treatment. No difference of age was found among controls, inactive, and active Crohn's disease patients, and among controls, mild, moderate, and severe ulcerative colitis patients.

SAMPLES

Blood samples of patients with Crohn's disease were taken on the same day when the disease activities were evaluated on the basis of simple index, and those of patients with ulcerative colitis were obtained within a week before colonoscopic examinations. Phospholipase A₂ activity, C-reactive protein and erythrocyte sedimentation rate were measured for the same blood sample. The sensitivity of C-reactive protein was 0.2 mg/dl in this study. When C-reactive protein ≥ 0.2 mg/dl, its level was defined as raised. When erythrocyte sedimentation rate > 15 mm/h, its level was defined as raised. Biopsy specimens of histologically normal ileal mucosa and normal colonic mucosa from ascending and descending colons were endoscopically taken from a patient undergoing colonoscopy for a colonic polyp. Informed consent was obtained. These samples were stored at -35°C until use.

MATERIALS

S-Sepharose and *Crotalus adamanteus* venom were obtained from Pharmacia (Upsala, Sweden). Acetonitrile of high performance liquid chromatography grade and trifluoroacetic acid were purchased from Nacalai Tesque Inc (Kyoto, Japan). Other reagents were of analytical grade.

Human pancreatic phospholipase A₂ (active form) was purified from human pancreatic juice as reported by Ono *et al*²⁷ with some modifications. Human group II phospholipase A₂ was purified from rheumatoid arthritic synovial fluids as reported.²⁸ *C. adamanteus* phospholipase A₂ was purified by reverse phase high performance liquid chromatography. The polyclonal

antibodies directed against human pancreatic phospholipase A₂ and synovial fluid phospholipase A₂ were prepared by the same method as reported.²⁹

ASSAY FOR PHOSPHOLIPASE A₂ ACTIVITY

Phospholipase A₂ activity was determined as described previously²⁸ using 0.8 mM 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol (POPG) as a substrate in the presence of 5 mM cholate. Fatty acids released by phospholipase A₂ were labelled with 9-anthryldiazomethane, and the derivatised fatty acids were separated by high performance liquid chromatography on a Superspher RP-18 (4×50 mm, Merk, Darmstadt, FRG) column and each derivatised fatty acid was quantified using margaric acid as an internal standard. The solvent system used was 98% acetonitrile at a flow rate of 1 ml/min. Calcium dependent phospholipase A₂ activity was estimated as the difference between the activity assayed in the presence of 5 mM CaCl₂ and that in the presence of 10 mM ethylenediaminetetra acetic acid (EDTA). Calcium independent phospholipase A₂ activity was calculated as the difference between the activities in the presence of 10 mM EDTA with and without substrate. The phospholipase A₂ activity was expressed as nmoles of oleic acid released/min/ml. The hydrolysis of POPG by phospholipase A₂ in human serum under standard assay conditions proceeded almost linearly during the initial 10% hydrolysis. There was no difference between serum and plasma phospholipase A₂ activities in normal controls, patients with Crohn's disease and ulcerative colitis.

The positional specificity of phospholipase A₂ action can be determined by this method with a mixed acyl phospholipid as a substrate: the time courses of the hydrolysis at the *sn*-1 position and of that at the *sn*-2 position are separately followed and compared with those of the hydrolysis by *C. adamanteus* phospholipase A₂, which is known to exclusively hydrolyse the ester bond at the *sn*-2 position. Phospholipase A action of serum was rather specific for the cleavage of the acyl ester bond at the *sn*-2 position.

The enzyme samples separated by reverse phase high performance liquid chromatography contained acetonitrile. Therefore, we tested the effect of acetonitrile on phospholipase A₂ activity. The inclusion of acetonitrile up to about 50% in the assay mixture did not affect enzyme activity.

ASSAY FOR IMMUNOREACTIVE PANCREATIC PHOSPHOLIPASE A₂

Serum immunoreactive pancreatic phospholipase A₂ (IR-PLA₂) was measured by sensitive and specific radioimmunoassay kit for human pancreatic phospholipase A₂ (S-0932, Shionogi Pharmaceutical Ltd, Osaka, Japan). The sensitivity of this assay was 71 fmol/ml serum.

We tested the crossreactivity of the antibody against human pancreatic phospholipase A₂ used in this kit with purified human group II phospholipase A₂. This antibody proved to have no crossreactivity with human group II phospho-

lipase A₂ purified from rheumatoid synovial fluid.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY ANALYSIS OF PHOSPHOLIPASE A₂ IN SERUM

The phospholipase A₂ in sera of four patients with Crohn's disease and three patients with ulcerative colitis which had the increased phospholipase A₂ activity and that in sera of two healthy controls were characterised by reverse phase high performance liquid chromatography. The serum sample diluted two fold with 20 mM Tris-HCl (pH 7.4) was applied to a short S-Sepharose column (6×10 mm) preequilibrated with 20 mM Tris-HCl (pH 7.4). The column was washed with 20 mM Tris-HCl (pH 7.4) containing 150 mM NaCl. The majority of phospholipase A₂ activity was eluted with 1 M NaCl and 20 mM Tris-HCl (pH 7.4). This fraction was applied to a reverse phase high performance liquid chromatography column (Cosmosil 5C8-300, 2.1×100 mm) preequilibrated with 0.1% trifluoroacetic acid in water. Elution was performed with a linear gradient of acetonitrile in 0.1% trifluoroacetic acid: from 0 to 19% in five minutes, from 19 to 38% in 45 minutes, and then from 38 to 95% in five minutes at a flow rate of 0.1 ml/min. The column was slurry packed in this laboratory. The use of small bore column increased the recovery of phospholipase A₂ activity much more than in the case of conventional column.³

IMMUNOBLOT ANALYSIS

Biopsy specimens of histologically normal ileal mucosa and colonic mucosa were homogenised in 3 mM Tris-HCl (pH 7.4) containing 0.25 M sucrose, 1 mM EDTA and 2 mM MgCl₂. Immunoblot analysis was performed on a 14% sodium dodecyl sulphate polyacrylamide gel as previously reported.³⁰

STATISTICAL ANALYSIS

Results are presented as mean (1) SEM. Data of serum C-reactive protein levels in patients with Crohn's disease and ulcerative colitis were analysed by Kruskal-Wallis' test. Data of erythrocyte sedimentation rate values in patients with Crohn's disease were analysed by unpaired Student's *t* test. Other data were analysed by one way analysis of variance and Scheffe's multiple comparison tests.^{31,32} Correlation between serum phospholipase A₂ activity levels and serum C-reactive protein levels were analysed by Spearman's correlation test (by ranks). Regression analysis was used to determine the relationship of serum phospholipase A₂ activity levels to erythrocyte sedimentation rate values. When *p*<0.05, it was considered significant.

Results

PHOSPHOLIPASE A₂ ACTIVITY IN SERA OF PATIENTS WITH CROHN'S DISEASE AND ULCERATIVE COLITIS
The calcium dependent phospholipase A₂ activities in sera of patients with Crohn's disease and

Figure 1: Serum phospholipase A₂ activity in inflammatory bowel disease. The patients were subgrouped as described in 'Methods'. —: mean (1) SEM.: upper limit of standard range (mean +2 SD of controls). (a) $p < 0.001$ compared with inactive Crohn's disease (CD) and controls. (b) $p < 0.01$ compared with mild ulcerative colitis (UC) and $p < 0.001$ compared with controls. (c) $p < 0.001$ compared with mild UC and controls.

ulcerative colitis are summarised in Figure 1. Those of controls and patients with inactive and active Crohn's disease were 2.0 (0.1), 3.6 (0.7), and 14.2 (2.1) nmol/min/ml, respectively. As serum phospholipase A₂ activity levels in controls apparently obeyed the normal distribution, the mean +2 SD of controls (2.8 nmol/min/ml) was defined as the upper limit of standard range. The activities of the active Crohn's disease patients were significantly higher than those of the inactive Crohn's disease patients ($p < 0.001$) as well as those of the controls ($p < 0.001$). Moreover, each activity of all the active Crohn's disease patients was higher than the standard range. No significant difference was found between those of the inactive Crohn's disease patients and those of the controls. No significant difference of serum phospholipase A₂ activity levels was found among patients with Crohn's disease treated with no medicine, sulphasalazine, prednisolone, and prednisolone in addition to sulphasalazine.

The phospholipase A₂ activities of patients with mild, moderate, and severe ulcerative colitis were 2.0 (0.2), 4.5 (0.6), and 6.1 (0.8) nmol/min/ml, respectively. Those of severe ulcerative colitis patients were significantly higher than those of the mild ulcerative colitis patients ($p < 0.001$) and those of the controls ($p < 0.001$). The differences between those of the moderate ulcerative colitis patients and those of the mild ulcerative colitis patients as well as between those of the moderate ulcerative colitis patients and those of the controls were also

significant ($p < 0.01$ and $p < 0.001$, respectively). A significant difference was not found either between those of the moderate ulcerative colitis patients and those of the severe ulcerative colitis patients, or between those of the mild ulcerative colitis patients and those of the controls. No significant difference of serum phospholipase A₂ activity levels was found among patients with ulcerative colitis treated with no medicine, sulphasalazine, and prednisolone in addition to sulphasalazine. There was no significant difference of serum phospholipase A₂ activity levels among ulcerative colitis patients with whole colonic, left sided colonic, and distal colonic types.

In six patients with Crohn's disease admitted to our hospital, serum phospholipase A₂ activity levels on admission decreased at the time of discharge, as the simple index on admission improved on discharge (Table II).

Calcium independent phospholipase A₂ activity towards mixed micelles of POPG and cholate was found to be very low ($< \sim 0.02$ nmol/min/ml) in sera of healthy controls and patients with Crohn's disease and ulcerative colitis, and there was no difference in level of this activity among the sera.

CHARACTERISATION OF SERUM PHOSPHOLIPASE A₂ OF INFLAMMATORY BOWEL DISEASE PATIENTS

To examine which isozyme of phospholipase A₂ is responsible for raised serum phospholipase A₂ activity of inflammatory bowel disease patients, we immunochemically characterised the enzyme. We first determined immunoreactive pancreatic phospholipase A₂ level in serum by means of a specific radioimmunoassay. No significant difference was found in serum immunoreactive pancreatic phospholipase A₂ levels among the healthy controls and the patients with Crohn's disease and ulcerative colitis, and immunoreactive pancreatic phospholipase A₂ levels seemed to have no correlation with the disease activity of Crohn's disease and ulcerative colitis (Table III). Serum immunoreactive pancreatic phospholipase A₂ level should not be directly proportional to phospholipase A₂ activity, because the antibody used for the present radioimmunoassay system recognises pancreatic prophospholipase A₂ as well as pancreatic phospholipase A₂. To obtain more direct information on the isozymic nature of serum phospholipase A₂, we characterised its activity after separation by reverse phase high performance liquid chromatography as des-

TABLE II Serum phospholipase A₂ activity levels and simple index in six patients with Crohn's disease on admission and discharge

Patient no	Serum phospholipase A ₂ activity (nmol/min/ml)		Simple index	
	A	D	A	D
1	3.6	1.8	2	0
2	23.2	2.8	5	0
3	27.9	3.5	8	0
4	5.3	3.4	4	1
5	15.2	3.2	5	0
6	14.6	2.3	6	0

A: on admission, D: on discharge.

TABLE III Serum immunoreactive pancreatic phospholipase A₂ levels in inflammatory bowel disease

Diagnosis	Serum IR-PLA ₂ (fmol/ml)
Controls	195 (9)
Crohn's disease	
Inactive	181 (12)
Active	214 (20)
Ulcerative colitis	
Mild	171 (17)
Moderate	168 (19)
Severe	210 (18)

Values are represented as mean (1) SEM.

cribed in 'Methods'. Table IV shows a typical result of fractionation of phospholipase A₂ activity in serum. The major part (92.6 (7.0%)) of phospholipase A₂ activity was bound to a S-Sepharose column and eluted from the column

TABLE IV Typical fractionation of phospholipase A₂ activity in serum of inflammatory bowel disease

Step	Total activity (nmol/min)	Yield (%)
Crohn's disease patient		
Serum	2.0	100
S-Sepharose		
Flow through	0.4*	20
Bound	1.7	85
Reverse phases HPLC	1.5†	75
Ulcerative colitis patient		
Serum	1.4	100
S-Sepharose		
Flow through	0.1*	7
Bound	1.3	93
Reverse phase high performance liquid chromatography	0.9†	64

The serum volume applied to a S-Sepharose column was as follows: 0.1 ml for a Crohn's disease patient and 0.14 ml for a ulcerative colitis patient. *Sum of phospholipase A₂ activity in flow-through fractions and that in 150 mM NaCl effluent. †Sum of phospholipase A₂ activity in peak A and peak B (see Fig 2 for details).

Figure 2: Elution profiles of serum phospholipase A₂ activity in patients with Crohn's disease (A) and ulcerative colitis (B) on reverse phase high performance liquid chromatography. (I) the elution position of human pancreatic PLA₂ purified from pancreatic juice; (II) the elution position of human group II phospholipase A₂ purified from synovial fluid. The recoveries of phospholipase A₂ activity on reverse phase high performance liquid chromatography were 88 and 69% in patients with Crohn's disease and ulcerative colitis, respectively.

Figure 3: Immunochemical relationship between serum and intestinal mucosa phospholipase A₂s and group II phospholipase A₂ purified from rheumatoid synovial fluid. (A) Immunoblot analysis of peak A and peak B materials with antihuman group II phospholipase A₂ antibody. Lane 1, concentrates of a serum sample with a S-Sepharose column; lane 2, purified human pancreatic phospholipase A₂; lane 3, peak B material; lane 4, peak A material; lane 5, human group II phospholipase A₂ purified from rheumatoid synovial fluid. (B) Immunoblot analysis of the normal ileal and colonic mucosa homogenates with the antibody. Lane 1, normal ileal mucosa; lanes 2 and 3, normal colonic mucosa from descending colon and ascending colon, respectively; lane 4, human synovial fluid group II phospholipase A₂.

with 1 M NaCl and 20 mM Tris-HCl (pH 7.4) in all cases examined, while a remaining part was mainly recovered in a flow through fraction. As the former fraction comprised the major phospholipase A₂ activity and had purity suitable for further high performance liquid chromatography analysis, the phospholipase A₂ in this fraction was analysed by reverse phase high performance liquid chromatography. Typical elution profiles, on a Cosmosil 5C8-300 column, of phospholipase A₂ activity derived from sera of patients with Crohn's disease and ulcerative colitis are shown in Figures 2A and B, respectively. Purified synovial fluid group II phospholipase A₂, pancreatic phospholipase A₂, and pancreatic prophospholipase A₂ were eluted in this order at the retention times of 29.5, 31.8, and 36.0 minutes, respectively. The phospholipase A₂ activities in both of the cases of Crohn's disease and ulcerative colitis were eluted in the fractions at a retention time of 29.5 minutes (peak A) and at a retention time of 58.5 minutes (peak B). Peak A was eluted at the same retention time as group II phospholipase A₂ purified from rheumatoid arthritic synovial fluid, whereas no appreciable phospholipase A₂ activity was eluted at the retention time corresponding to pancreatic phospholipase A₂. Peak B was eluted significantly later than the standard purified phospholipase A₂s. Similar elution patterns were obtained in all cases examined – that is, four patients with Crohn's disease and three patients with ulcerative colitis, but the ratios of phospholipase A₂ activity in peak A to that in peak B varied from one case to another. Therefore, the recovery of phospholipase A₂ activity was presented as a sum of phospholipase A₂ activities in peak A and peak B: 66.2 (5.2)% (four) for patients with Crohn's disease and 63.1 (2.6)% (three) for patients with ulcerative colitis. Elution profiles of phospholipase A₂ activity derived from serum of two healthy controls were similar to those in patients with Crohn's disease and ulcerative colitis. When peak A and peak B materials were rechromatographed on the Cosmosil 5C8-300 column under the same elution condition, the phospholipase A₂ activities were eluted again at the same positions as peak A and peak B, respectively, indicating that no exchange between peak A and peak B materials occurred during chromatography.

Pancreatic phospholipase A₂ immunoreactivity was not detectable in peaks A and B. In two of nine cases, a small amount of immunoreactive pancreatic phospholipase A₂ was observed in the fraction eluted at the retention time corresponding to pancreatic phospholipase A₂.

On immunoblot analysis (Fig 3A), the enzymes in peaks A and B were immunochemically crossreactive with antibody raised against human group II phospholipase A₂ from rheumatoid synovial fluid, but not with antihuman pancreatic phospholipase A₂ antibody (data not shown). Antisynovial fluid group II phospholipase A₂ antibody did not recognise human pancreatic phospholipase A₂ (Fig 3A, lane 2). All samples gave bands having the same mobility as the purified synovial fluid phospholipase A₂, indicating that the two forms of phospholipase A₂ in serum have the same apparent molecular

weight as synovial fluid phospholipase A₂. These results showed that raised serum phospholipase A₂ activity in inflammatory bowel disease is mainly attributed to group II-like phospholipase A₂.

For comparison, phospholipase A₂ in normal ileal and colonic mucosa obtained by endoscopic biopsy was immunochemically characterised. As in the case of serum phospholipase A₂, the intestinal mucosa contained phospholipase A₂ immunocrossreactive with antisynovial fluid phospholipase A₂ antibody (Fig 3B), but not with antipancreatic phospholipase A₂ antibody.

COMPARISON OF SERUM PHOSPHOLIPASE A₂ ACTIVITY WITH SERUM C-REACTIVE PROTEIN AND ERYTHROCYTE SEDIMENTATION RATE
Of 19 patients with active Crohn's disease, serum phospholipase A₂ activity of all patients showed higher levels than the standard range, and serum C-reactive protein levels of 18 patients and erythrocyte sedimentation rate values of 16 patients were raised. In patients with Crohn's disease, correlation was observed between serum phospholipase A₂ activity and serum C-reactive protein levels ($p<0.005$), and between serum phospholipase A₂ activity levels and erythrocyte sedimentation rate values ($r=0.72$, $p<0.001$) (Fig 4A, B). Serum C-reactive protein levels in patients with active Crohn's disease were significantly higher than those in patients with inactive Crohn's disease ($p<0.001$) as well as erythrocyte sedimentation rate values ($p<0.005$). Of 29 patients with moderate and severe ulcerative colitis, serum phospholipase A₂ activity of 24 patients revealed higher levels than the standard range, whereas serum C-reactive protein levels of nine patients and erythrocyte sedimentation rate values of eight patients were

raised. In patients with ulcerative colitis, weak correlation ($r=0.56$, $p<0.001$) was found between serum phospholipase A₂ activity levels and erythrocyte sedimentation rate values (Fig 5A, B). No significant difference was found in serum C-reactive protein levels among three groups of ulcerative colitis. Erythrocyte sedimentation rate values in patients with mild, moderate, and severe ulcerative colitis were 4.2 (1.0), 11.5 (2.1), and 14.8 (2.5) mm/h, respectively. Significant difference was found in erythrocyte sedimentation rate values between patients with severe and mild ulcerative colitis ($p<0.01$), but not between those with moderate and mild ulcerative colitis.

Discussion

The present study shows that serum calcium dependent phospholipase A₂ activity towards the

Figure 4: Relations of serum phospholipase A₂ activity with serum C-reactive protein levels (A) and with erythrocyte sedimentation rate (B) in patients with Crohn's disease. Serum phospholipase A₂ activity, serum C-reactive protein levels, and erythrocyte sedimentation rate values are raised in most of active Crohn's disease patients. Serum phospholipase A₂ activity levels correlate with serum C-reactive protein levels ($p<0.005$) and erythrocyte sedimentation rate values ($r=0.72$, $p<0.001$). Closed circle, inactive Crohn's disease; open circle, active Crohn's disease.

Figure 5: Relation between serum phospholipase A₂ activity and serum C-reactive protein levels (A) and erythrocyte sedimentation rate (B) in patients with ulcerative colitis. There is weak correlation of serum phospholipase A₂ activity levels with erythrocyte sedimentation rate values ($r=0.56$, $p<0.001$). Of 29 patients with moderate and severe ulcerative colitis, serum phospholipase A₂ activity levels are raised in 24 patients, whereas serum C-reactive protein levels of nine patients and erythrocyte sedimentation rate values of eight patients are elevated. Raised serum phospholipase A₂ activity levels are found in the majority of the moderate and severe ulcerative colitis patients whose serum C-reactive protein levels and erythrocyte sedimentation rate values are within the normal range. Closed circle, mild ulcerative colitis; open circle, moderate ulcerative colitis; open square, severe ulcerative colitis.

mixed micelles of POPG and cholate was significantly raised in patients with active Crohn's disease and in those with moderate and severe ulcerative colitis, and that raised phospholipase A₂ activity in serum was mainly attributed to phospholipase A₂ immunochemically related to group II enzyme. Although raised serum phospholipase A₂ activity has been reported in other inflammatory diseases including acute pancreatitis,¹⁷ rheumatoid arthritis,¹⁸ and septic shock,¹⁹ to our knowledge, this study provides the first direct evidence for raised group II-like phospholipase A₂ activity in serum in inflammatory disorders.

Serum group II like phospholipase A₂ activity could be separated into two peaks by reverse phase high performance liquid chromatography (Fig 2). The enzyme eluted earlier was similar to rheumatoid synovial fluid phospholipase A₂ as regards molecular weight, retention time, and immunoreactivity with antisynovial fluid phospholipase A₂ antibody, suggesting that this form of serum phospholipase A₂ may be identical, or closely related, to group II phospholipase A₂ purified from rheumatoid synovial fluid. On the other hand, the group II like phospholipase A₂ eluted later seemed to have greater molecular surface hydrophobicity than the one eluted earlier, in spite of having the same apparent molecular weight as the latter (Fig 3A). The detailed structural differences between the two forms of group II like phospholipase A₂ in serum remains to be clarified. In this connection, separation of multiple forms of group II phospholipase A₂ has been reported in the case of rheumatoid synovial fluid phospholipase A₂,³³ and human splenic phospholipase A₂,³⁴ and recent studies showed structural diversity in mammalian group II phospholipase A₂ even within a given species.³⁵⁻³⁷

The origin of group II like phospholipase A₂s of which activity increased in sera of the patients with inflammatory bowel disease is unknown. In laboratory animals, group II phospholipase A₂s are relatively enriched in pig ileal mucosa,^{38,39} in rat intestinal mucosal cell, especially Paneth cells,⁴⁰ and in various inflammatory cells including rat and rabbit platelets,^{41,42} rat splenic macrophages,⁴³ rat Küpffer cells,⁴³ and rabbit polymorphonuclear leucocytes.³⁷ In human, platelets and spleen cells contain group II phospholipase A₂.^{7,34} The results of immunoblot analysis showed that phospholipase A₂ immunochemically related to group II phospholipase A₂ was also present in human ileal and colonic mucosa (Fig 3B). Platelets have been confirmed to secrete group II phospholipase A₂ upon stimulation,⁴⁴ and various inflammatory cells have been known to release phospholipase A₂s upon stimulation.¹¹ Hence, all these cells may contribute to raised serum phospholipase A₂ activity in inflammatory bowel disease. Wright *et al*³⁷ have recently reported that the rabbit polymorphonuclear leucocyte phospholipase A₂ and serum group II phospholipase A₂ are structurally distinct, because the NH₂-terminal amino acid sequence of the polymorphonuclear leucocyte enzyme differed from that of the serum enzyme at the NH₂-terminus. Structural studies on the two forms of group II like phospholipase A₂ in sera of

patients with inflammatory bowel disease and group II phospholipase A₂s in various tissues may therefore provide insight into the source of serum phospholipase A₂s.

This study showed evidence that serum phospholipase A₂ activity level was a measure of disease activity of Crohn's disease in addition to serum C-reactive protein level and erythrocyte sedimentation rate. This is further supported by the fact that in patients with Crohn's disease admitted to our hospital, serum phospholipase A₂ activity levels decreased in parallel with clinical improvement by antiinflammatory treatment (Table II). On the other hand, Gomes *et al*⁴⁵ described that there was no correlation between macroscopic appearance of the colon and laboratory parameters in patients with ulcerative colitis. Serum C-reactive protein levels did not always reflect endoscopic severity of ulcerative colitis in the present study. Although there was significant difference in erythrocyte sedimentation rate values between patients with mild and severe ulcerative colitis, erythrocyte sedimentation rate values were found to be above the normal range only in four of 14 patients with severe disease. Serum phospholipase A₂ activity levels were significantly raised in the majority (73.7%) of the moderate and severe ulcerative colitis patients whose serum C-reactive protein levels and erythrocyte sedimentation rate values were within the normal range. The phospholipase A₂ levels tended to increase in relation with the index of endoscopic severity in patients with ulcerative colitis. These results suggest that serum phospholipase A₂ activity level would be a more sensitive parameter than serum C-reactive protein level and erythrocyte sedimentation rate value for evaluating disease activity of ulcerative colitis.

Serum phospholipase A₂ activity, of which molecular entity was not known, has been reported to be raised in other inflammatory disease as described above. Therefore, rises in serum phospholipase A₂ activity does not seem to be specific to a defined disorder, but rather to be the consequence of intestinal and systemic inflammation. Recent studies on the roles of various chemical mediators in inflammatory bowel disease suggested that interleukin 1 may be a primary initiator of bowel inflammation.⁴⁶ It has been reported that peripheral blood mononuclear cells from patients with inflammatory bowel disease had the ability of enhanced production of interleukin 1.⁴⁷ Moreover, because interleukin 1 can induce phospholipase A₂ in chondrocytes, mesangial cells, and fibroblasts,⁴⁸ phospholipase A₂ may be involved in the promotion of inflammation rather than a primary cause of inflammation. Hence, there is a possibility that increased phospholipase A₂ activity in serum would damage other organs as well as the gastrointestinal tract as in the case of lung complications in acute pancreatitis.¹⁷ The patients with higher serum phospholipase A₂ activity levels, however, were not accompanied by such complications as far as we examined in this study. This problem could be solved by studies on larger populations of patients with inflammatory bowel disease.

Olaisson *et al*¹⁵ reported that increased phos-

phospholipase A₂ activity in ileal mucosa was associated with recurrence of the disease in patients with Crohn's disease. Increased phospholipase A₂ activity has also been reported in colonic mucosa of active Crohn's colitis.¹⁶ In these studies, the data for phospholipase A₂ activity were reported at rather acidic pH of 6.0 and in the presence of 2 mM CaCl₂. Under these conditions alkaline active calcium dependent and calcium independent phospholipase A₂s, and lysosomal phospholipase A₂s with acidic pH optimum may contribute to the enzyme activity. A large amount of calcium independent phospholipase A₂ has been reported to be particularly present in ileal mucosa.^{49,50} This complicated the interpretation of the relationship between raised phospholipase A₂ activity in the intestinal mucosa reported^{15,16} and raised serum group II like phospholipase A₂ activity as shown in this study. To clarify the pathophysiological relation between phospholipase A₂ activities in serum and intestinal mucosa in inflammatory bowel disease, differential determination of various phospholipase A₂ isozymes should be required.

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