Pathogenesis of gallstones in Crohn's disease
Brink, M.A.

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Chapter 6

THE BILIARY CONCANAVALIN A-BINDING FRACTION EXPLAINS INCREASED CHOLESTEROL CRYSTALLIZATION IN BILE FROM PATIENTS WITH CROHN'S DISEASE COMPARED TO PATIENTS WITH ULCERATIVE COLITIS
THE BILIARY CONCANAVALIN A-BINDING FRACTION EXPLAINS INCREASED CHOLESTEROL CRYSTALLIZATION IN BILE FROM PATIENTS WITH CROHN'S DISEASE COMPARED TO PATIENTS WITH ULCERATIVE COLITIS

Department of Surgery and Gastrointestinal and Liver diseases, Academic Medical Center, Amsterdam.
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Background: Crohn's disease (CD) is a risk factor for gallstone formation. In contrast, patients with ulcerative colitis (UC) have an incidence of gallstone formation comparable to the general population. The reason for this difference is not known.

Objective: To elucidate the factors controlling cholesterol crystallization in gallbladder bile of CD and UC patients.

Methods: Gallbladder bile was obtained by aspiration during bowel resections (26 CD patients, 20 UC patients). Biliary lipid composition, crystal detection time (CDT) and the effect of extraction of the concanavalin A-binding fraction (CABF) on crystal formation were determined. Results Cholesterol crystals were present in 7 of the 26 bile samples of CD-patients and 1 of the 20 UC-patients. Four of the bile samples of CD patients were fast nucleating. None of the 20 UC-patients had fast nucleating bile. Lipid composition, total lipid concentration and CSI were not significantly different between the two groups. In CD patients extraction of CABF decreased crystallization in 10 bile samples but accelerated crystallization in one bile sample. In 8 bile samples from UC patients crystallization increased after CABF-extraction.

Conclusions: Compared to UC patients gallbladder bile of CD patients showed increased cholesterol crystallization despite comparable lipid composition and CSI. This difference is caused by increased cholesterol crystallization promoting activity. Bile from UC patients contains a Con A-binding factor which inhibits cholesterol crystallization.
Introduction

The reported incidence of gallstone disease in patients with Crohn’s Disease (CD) affecting the ileum is higher than that in the general population (30-40% and 10-15%, respectively). This increased incidence is thought to be caused by bile salt malabsorption due to ileal dysfunction. Bile salt malabsorption may lead to an increase in biliary cholesterol supersaturation and is thought to also induce enterohepatic cycling of bilirubin. Increased concentrations of bilirubin have indeed been demonstrated in bile from patients with ileal Crohn’s disease. Hence ileal dysfunction might explain an increase in formation of pigment stones as well as cholesterol gallstones. Little information is available about the nature of the stones in CD patients. In most studies screening for gallstones was performed using ultrasound or oral cholecystography. In these studies both radiolucent and radiopaque stones have been reported suggesting that indeed either pigment or cholesterol stones could form in these patients.

It is now generally assumed that cholesterol supersaturation is the primary factor in cholesterol gallstone formation. Whether ileal dysfunction indeed induces an increase in cholesterol saturation is a matter of controversy. Increase, no change and even a decrease in cholesterol saturation have been found. Mok et al. demonstrated that cholesterol saturation of bile was increased by interruption of the enterohepatic circulation of bile salts, particularly in patients with a preexisting defect in biliary lipid secretion. However, this is not sustained in the chronic state, because de novo bile salt synthesis may compensate for bile salt loss. In addition to cholesterol saturation biliary proteins regulate cholesterol crystallisation. Both crystallization promoting and inhibiting proteins have been described in bile. Recently, we have shown that extraction of promoting proteins from bile of cholesterol gallstone patients indeed inhibits crystallization providing the first direct evidence for a regulating role by these proteins. In the present study we have investigated the role of both cholesterol supersaturation and proteins in cholesterol crystallization from bile of CD patients and ulcerative colitis (UC) patients. Since gallstone incidence in UC patients is similar to the general population a comparison between the two groups should reveal whether in addition to the increased risk for pigment gallstone disease there is also an enhanced risk for cholesterol gallstones in CD patients.
Materials and methods

Materials
Concanavalin A (Con A)-Sepharose and Sepharose 4B were obtained from Pharmacia AB, Uppsala, Sweden. Rabbit anti-human IgA (α-chain and secretory component) and peroxidase conjugated rabbit anti-human heavy chain IgA were obtained from DAKO (Copenhagen, Denmark). Standard plasma was purchased from Behring (Marburg, Germany). Mouse anti-human IgA₁, mouse anti-human IgA₂ and mouse anti-human Secretory Component (free and bound) were obtained from Nordic (Tilburg, The Netherlands). Goat anti-human Secretory Component and peroxidase conjugated anti-goat IgG were obtained from Sigma (St. Louis, MO). Dynagard filters with a cutoff of 0.2 μm were from Microgon (Laguna Hills, CA, USA). Microtiter plates with medium binding capacity were derived from Greiner (Frickenhausen, Germany). All other reagents used were of analytical grade.

Patients
Patients with confirmed inflammatory bowel disease (radiological or endoscopic diagnosis) undergoing elective bowel resections or end-to-end ileal pouch-anal anastomosis were included. The diagnosis of Crohn's disease (CD) or ulcerative colitis (UC) was established by histological examination of the resected bowel segments. At entry sex, age, year of diagnosis, previous bowel resections, immunosuppressive treatment in the months before surgery, disease activity (reflected by ESR and white blood cell count) and nutritional status (reflected by serum albumin) were recorded. Localization of disease in CD patients was established considering X-ray examination, perioperative findings and histological evaluation of the resected bowel segment.

Bile collection
Gallbladder bile was collected from 26 patients with Crohn's disease and 20 patients with ulcerative colitis during elective bowel resections. The gallbladder was punctured with a sterile 21 French needle and syringe. Care was taken to aspirate the gallbladder contents completely. The puncture hole was stitched with Vicryl 3.0. Presence of gallstones was investigated by palpation of the gallbladder. Absence of gallstones was confirmed by
Cholesterol Crystallization in Crohn's Disease

To determine the amount of crystals present at the time of isolation, 10 μL of the fresh bile was examined under polarizing light microscopy at 100x magnification for the presence of cholesterol monohydrate crystals. Cholesterol crystals were defined as colorless, transparent thin crystals with parallel edges.

Lipid composition

Cholesterol, phospholipid and bile salt concentrations were determined using standard enzymatic procedures. Total lipid concentration (TLC) was the sum of cholesterol, bile salt and phospholipid concentrations expressed in g/dL. Cholesterol saturation indices (CSI) were calculated according to Carey's critical tables.

Bile salt composition (HPLC)

The pattern of the conjugated bile salts in gallbladder bile was determined with high performance liquid chromatography according to Rossi et al.

Extraction of Concanavalin A-binding glycoproteins from bile

Fresh human gallbladder bile was centrifuged (10 min, 1000 g) and two portions of three ml were taken. The remaining bile was stored at -20°C for determination of lipid and protein composition. From one of the three ml portions, CABF was removed using ConA-Sepharose-4B. The ConA-Sepharose beads were prewashed three times with ConA-buffer containing 0.2 M NaCl, 10 mM Tris (pH 7.4), 1 mM MnCl₂, 1 mM CaCl₂ and 1 mM MgCl₂ and 0.02% NaN₃. The beads were pelleted (5 min, 1,000g) and the supernatant was carefully removed completely. Three ml of bile was added to 1.5 mL packed beads, mixed thoroughly and incubated for two hours on a roller bank at 20°C. As a control the other portion of bile was added to 1.5 mL washed packed Sepharose 4B beads and subjected to the same procedure. After two hours of incubation on a roller bank at room temperature the beads with the bile samples were centrifuged for 15 minutes at 500g. The supernatant from both ConA and control Sepharose beads was collected and 1 ml of this supernatant was passed through a 0.22μm Millipore (Millex-GP) filter into a sterile container. Each filtrate was incubated at
37°C to determine crystal detection time. The remaining supernatant was stored at -20°C for determination of lipid and protein composition.

**Crystal Detection Time (CDT)**

The bead treated samples were incubated without shaking at 37°C for a maximum of 21 days. A sample of 10 μL was examined daily. The samples were observed under a light microscope with polarized light at a 100x magnification. The presence of cholesterol monohydrate crystals was determined and if so, the number of crystals was counted using a hemocytometer. The crystal detection time (CDT) was defined as day, at which the first cholesterol crystal in a bile sample was observed. Fast nucleating bile was defined as bile that formed crystals within 4 days after incubation as suggested by Jüngst et al.28

**ELISA**

The amount of IgA, IgA<sub>1</sub>, IgA<sub>2</sub> and secretory Component (SC) was measured separately in the different bile fractions. Microtiter plates were coated with rabbit antihuman IgA (1:5,000), mouse anti-human IgA<sub>1</sub>, IgA<sub>2</sub> or SC (1:1,000) respectively, for 16 hr at 4°C. The plates were washed two times with PBS/Tween (0.05%, v/v). Blocking was obtained with 1% (w/v) BSA in PBS/Tween 0.05% for 1 hour at 37°C. After washing two times with PBS/Tween 0.05% the wells were incubated with serially diluted samples (in PBS/Tween with 0.1% BSA) or standard plasma for 2 hours at 37°C. For SC this procedure was followed by incubation with goat anti-human Secretory Component (1:5,000). After washing four times in PBS/Tween, incubation followed for 1 hour at 37°C with peroxidase-conjugated rabbit antihuman IgA (1:3,000 for IgA, 1:2,000 for IgA<sub>1</sub> and IgA<sub>2</sub>), or peroxidase-conjugated rabbit anti-goat IgG (SC) (1:1,000) diluted in PBS/Tween buffer containing 0.1% BSA. Color development was performed with 0.1 mg/mL 3,3',5,5'-tetramethylbenzidine in dimethylsulfoxide, 0.0029% H<sub>2</sub>O<sub>2</sub> and 0.1 mol/L sodium acetate, pH 5.5. The reaction was stopped with a 4N sulfuric acid solution and absorbance was recorded in automatic ELISA plate reader (Medgenics Diagnostics, Belgium).
Statistical analysis

Statistical evaluations were performed with the Mann-Whitney U test (quantitative parameters) and χ² (qualitative parameters) between groups and the Wilcoxon Signed Rank Test within them. Correlations between parameters were measured by the Spearman rank correlation coefficient. Statistical significance was set at P < 0.05. Data are given as mean ± SEM.

The study was approved by the Ethics Committee and Research Committee of the Academic Medical Center, Amsterdam. Each patient gave written informed consent before entry into the study.

Results

Patients

The total group of 46 inflammatory bowel disease (IBD) patients consisted of 26 patients with Crohn’s disease (CD), 12 men and 14 women, with a mean age of 33 ± 2 years and 20 patients with ulcerative colitis (UC), 8 men and 12 women, with a mean age of 37 ± 2 years. None of these patients was on parenteral nutrition. Six of the CD-patients and 9 of the UC-patients previously underwent bowel resections. Duration since diagnosis was 5 ± 1 year in CD patients and 7 ± 1 year in UC-patients. All previous resections had been performed more than 6 months before bile sampling. Immunosuppressive treatment was comparable between both patient groups. Also ESR, leucocyte count and serum albumin determined less than one week before surgery were not significantly different between CD and UC patients (Table 1). Sixteen CD patients had IBD restricted to the ileum, 2 patients had ileocolitis and 8 CD patients had isolated colitis. None of the patients had gallstones at the time of surgery.
Table 1. Characteristics of 46 patients with inflammatory bowel disease: 26 patients with Crohn’s disease (CD) and 20 patients with Ulcerative Colitis (UC).

<table>
<thead>
<tr>
<th></th>
<th>CD n = 26</th>
<th>UC n = 20</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>33 ± 2</td>
<td>37 ± 2</td>
<td>P = 0.22</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>12/14</td>
<td>8/12</td>
<td>P = 0.68</td>
</tr>
<tr>
<td>Previous surgery (% of patients)</td>
<td>23</td>
<td>45</td>
<td>P = 0.12</td>
</tr>
<tr>
<td>Immunosuppressive treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>11 (42%)</td>
<td>7 (35%)</td>
<td>P = 0.33</td>
</tr>
<tr>
<td>Mesalazine</td>
<td>14 (54%)</td>
<td>9 (45%)</td>
<td>P = 0.23</td>
</tr>
<tr>
<td>Azathioprine</td>
<td>3 (12%)</td>
<td>5 (25%)</td>
<td>P = 0.35</td>
</tr>
<tr>
<td>ESR (mm/hr)</td>
<td>30 ± 5</td>
<td>21 ± 5</td>
<td>P = 0.55</td>
</tr>
<tr>
<td>White blood cell count (10⁹/L)</td>
<td>8.5 ± 0.5</td>
<td>8.6 ± 0.8</td>
<td>P = 0.26</td>
</tr>
<tr>
<td>Serum albumin (g/L)</td>
<td>42 ± 1</td>
<td>46 ± 2</td>
<td>P = 0.09</td>
</tr>
</tbody>
</table>

Bile

Cholesterol crystals were present in 7 of the 26 bile samples of CD-patients and 1 of the UC-patients (Chi Square test; p=0.07, N.S.). Four of the bile samples of CD patients were fast nucleating (CDT<4 days). None of the 20 UC-patients had fast nucleating bile (Chi Square test; P = 0.05).

Total and individual lipid concentrations and cholesterol saturation index (CSI) of the bile samples were not significantly different between the two groups (Table 2). UC patients which underwent previous subtotal or total colectomy tended to have more supersaturated bile (CSI 1.12 vs 0.87) but the difference was not significant. Also the total protein content of the biles was not different between the two groups (Table 2).

Conjugated bile salt composition was determined by HPLC. The percentages of ursodeoxycholate conjugates were significantly different between CD patients and UC patients (0.8 ± 0.4 versus 0.2 ± 0.1, P = 0.01). No significant differences were found for the proportions of the other bile salts (chenodeoxycholate, deoxycholate and cholate). Additionally, the percentage of taurine conjugated bile salts was not significantly different between CD and UC patients (not shown).

The group of CD patients was divided for disease limited to the ileum (n = 16) and disease limited to the colon (n = 8). Total and individual lipid concentrations and cholesterol
saturation index (CSI) of the bile samples were not significantly different between the two groups. The percentage of ursodeoxycholate in the total amount of bile salt was significantly higher in patients with CD located to the ileum compared to patients with CD located in the colon (1.2 ± 0.7 versus 0.2 ± 0.2, \( P = 0.03 \)). For the other bile salts the percentages were not significantly different between the two groups. Also, the percentage of taurine conjugated bile salts was not significantly different between the two groups (not shown).

### Table 2. Lipid composition of 46 gallbladder bile samples of patients with Inflammatory Bowel Disease.

<table>
<thead>
<tr>
<th></th>
<th>CD n = 26</th>
<th>UC n = 20</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phospholipids (mM)</td>
<td>44.7 ± 3.5</td>
<td>49.4 ± 3.7</td>
<td>( P = 0.58 )</td>
</tr>
<tr>
<td>Cholesterol (mM)</td>
<td>16.1 ± 1.5</td>
<td>17.0 ± 1.9</td>
<td>( P = 0.32 )</td>
</tr>
<tr>
<td>Bile salts (mM)</td>
<td>144 ± 9.6</td>
<td>169 ± 13.6</td>
<td>( P = 0.26 )</td>
</tr>
<tr>
<td>Total lipids (g/dL)</td>
<td>10.9 ± 0.8</td>
<td>12.5 ± 1.0</td>
<td>( P = 0.14 )</td>
</tr>
<tr>
<td>CSI</td>
<td>1.10 ± 0.06</td>
<td>0.98 ± 0.06</td>
<td>( P = 0.18 )</td>
</tr>
<tr>
<td>Total protein (g/L)</td>
<td>2.39 ± 0.26</td>
<td>1.83 ± 0.38</td>
<td>( P = 0.07 )</td>
</tr>
</tbody>
</table>

**Extraction of Concanavalin A-binding Fraction (CABF)**

As we have shown previously\textsuperscript{22} due to the internal volume of the Sepharose beads the extraction procedure diluted the bile samples by about 30\% (Table 3) which did not significantly affect the CSI. Among the bile samples of the 26 CD patients, 15 bile samples did not form crystals within 21 days whether or not CABF was extracted. The mean CSI in these bile samples was 0.95 ± 0.06, compared to 1.34 ± 0.16 in the remaining 11 bile samples (\( P = 0.09 \)). The effect of CABF-extraction on CDT in the remaining 11 bile samples is presented in figure 1. In 10 of the 11 bile samples crystal formation was delayed in the bile sample from which CABF was removed compared to the paired sample in which CABF was present. The median difference of CDT between the two samples in these patients was 9 days [range 4-14 days]. In one bile sample crystal formation was accelerated by CABF-extraction. In 5 of the 16 patients with CD located to the ileum crystal formation occurred within 21 days, compared to 5 of the 8 patients with CD located in the colon (N.S.). In all of these bile samples
crystal formation was delayed in the bile sample from which CABF was removed compared to the paired sample in which CABF was present.

In 9 of the 20 bile samples derived from UC patients crystals did not form within 21 days. The mean CSI in these bile samples was $0.93 \pm 0.10$, compared to $1.02 \pm 0.09$ in the remaining biles ($P = 0.67$). Of the remaining 11 bile samples the effect of CABF-extraction on CDT is depicted in figure 2. In one bile sample crystal formation was delayed by extraction of CABF. In 6 of 11 crystal forming UC-patient biles, removal of CABF resulted in earlier appearance of crystals than in the paired sample in which CABF was present. The median acceleration in CDT in these 5 patients was 6 days [range 2-14 days].

Figure 1. Crystal detection time (CDT) was determined in 26 bile samples of patients with Crohn’s disease with and without the concanavalin A-binding fraction (CABF). In 15 bile samples no crystals were formed within 21 days, these biles are left out this figure. Of the remaining 11 bile samples CDT with and without CABF are depicted in the figure connected by a line.

Figure 2. Crystal detection time (CDT) was determined in 20 bile samples of patients with ulcerative colitis with and without the concanavalin A-binding fraction (CABF). In 9 bile samples no crystals were formed within 21 days, results for these biles are not shown. Of the remaining 11 bile samples CDT with and without CABF are depicted in the figure connected by a line.
Table 3. Lipid composition of bile samples before or after incubation with Sepharose or Con A-Sepharose.

<table>
<thead>
<tr>
<th></th>
<th>BS</th>
<th>PL</th>
<th>Chol</th>
<th>CSI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CD-patients (n = 11)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>145 ± 17</td>
<td>50.7 ± 6.3</td>
<td>17.5 ± 2.5</td>
<td>1.05 ± 0.06</td>
</tr>
<tr>
<td>Con A-Seph treated</td>
<td>101 ± 13</td>
<td>40.2 ± 6.0</td>
<td>10.5 ± 7.0</td>
<td>0.83 ± 0.07</td>
</tr>
<tr>
<td>Sepharose treated</td>
<td>104 ± 14</td>
<td>39.7 ± 6.3</td>
<td>11.5 ± 8.1</td>
<td>0.90 ± 0.07</td>
</tr>
<tr>
<td><strong>UC-patients (n = 11)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>158 ± 18</td>
<td>48.9 ± 5.2</td>
<td>17.4 ± 2.6</td>
<td>1.02 ± 0.08</td>
</tr>
<tr>
<td>Con A-Seph treated</td>
<td>99.2 ± 10.7</td>
<td>39.5 ± 4.4</td>
<td>1 ± 1.7</td>
<td>0.95 ± 0.09</td>
</tr>
<tr>
<td>Sepharose treated</td>
<td>97.1 ± 10.1</td>
<td>35.3 ± 3.8</td>
<td>11.5 ± 2.0</td>
<td>1.04 ± 1.02</td>
</tr>
</tbody>
</table>

Bile from UC and CD patients was incubated with Sepharose or Con A-Sepharose as described in Material & Methods. Biliary lipids were determined in the Con A-Sepharose fractions and the corresponding native bile samples. Data are presented as means ± SD. Biliary lipids are given in mmol/L.

**Immunoglobulin A and secretory component**

Biliary IgA concentration in gallbladder bile of CD patients was not different from that in UC patients (Table 4). However, in patients with CD confined to the ileum IgA was significantly higher compared to CD patients with only colitis (Table 5). Interestingly in these patients a decrease in IgA was found whereas the content of secretory component tended to be higher (Table 5).

Biles that did not form crystals within 21 days contained significantly higher concentrations of IgA compared to biles that formed crystals (460 ± 94 mg/L versus 310 ± 46 mg/L, respectively, P = 0.04). Percentage IgA and concentration of secretory component were not significantly different between the two groups.

The biliary content of IgA, percentage IgA and secretory component in bile samples from UC and CD patients in which CABF extraction resulted in a decrease of crystal formation was not significantly different from biles in which CABF-extraction resulted in faster crystal formation (not shown).
Table 4. Content of total IgA, percentage IgA, and secretory component in native bile samples of 25 patients with Inflammatory Bowel Disease.

<table>
<thead>
<tr>
<th></th>
<th>CD n = 13</th>
<th>UC n = 12</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total IgA (mg/L)</td>
<td>474 ± 87</td>
<td>278 ± 50</td>
<td>P = 0.10</td>
</tr>
<tr>
<td>IgA (% of total protein)</td>
<td>29 ± 3</td>
<td>24 ± 4</td>
<td>P = 0.15</td>
</tr>
<tr>
<td>IgA₁ (% in total IgA)</td>
<td>72 ± 3</td>
<td>81 ± 2</td>
<td>P = 0.06</td>
</tr>
<tr>
<td>Secretory component (mg/L)</td>
<td>252 ± 50</td>
<td>208 ± 31</td>
<td>P = 0.62</td>
</tr>
</tbody>
</table>

Table 5. Content of total IgA, percentage IgA, and secretory component in native bile samples of 13 patients with Crohn’s disease.

<table>
<thead>
<tr>
<th></th>
<th>CD-Ileum n = 9</th>
<th>CD-colon n = 4</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total IgA (mg/L)</td>
<td>594 ± 96</td>
<td>205 ± 83</td>
<td>P = 0.03*</td>
</tr>
<tr>
<td>IgA₁ (% in total IgA)</td>
<td>67 ± 4</td>
<td>83 ± 3</td>
<td>P = 0.03*</td>
</tr>
<tr>
<td>Secretory component (mg/L)</td>
<td>298 ± 64</td>
<td>150 ± 53</td>
<td>P = 0.12</td>
</tr>
</tbody>
</table>

Discussion

It is well known that patients with Crohn’s Disease (CD) are at risk for gallstone formation. Whether the gallstones formed are of the cholesterol or the pigment type is a matter of controversy and it cannot be excluded whether CD patients are at risk for both types of gallstones. Indeed Brink et al. have recently shown that bile of CD patients with ileal dysfunction is enriched in unconjugated bilirubin, a known risk factor for pigment gallstone disease. In the present study we investigated whether bile of CD patients possibly also has increased propensity for cholesterol crystal formation. Seven of the CD patients had cholesterol crystals in their bile which is considered the best predictor of cholesterol gallstone formation. Four of the 26 samples of CD patients showed rapid crystallization (CDT < 4 days). In the group of patients with ulcerative colitis (UC) only one sample contained crystals and none of the biles showed rapid nucleation. The degree of cholesterol saturation is
Currently considered to be the primary factor in cholesterol crystallization. We observed no difference in lipid composition between the CD and the UC groups, nor was there a significant difference in bile salt composition. However, the size of the groups may have caused a type two error. It has been suggested that particularly CD patients with ileal disease are at risk for gallstone formation.\textsuperscript{1,2,9} We indeed observed an increase in CSI in the CD patients with ileal disease. However, this increase was not significant nor was there a difference in crystallization behavior compared to biles from CD patients with the disease confined to the colon. Also here the group may have been too small to pick up these relatively small differences.

Recently we demonstrated that in fast nucleating bile of cholesterol gallstone patients, crystallization can be inhibited by extraction of the biliary ConA-binding fraction.\textsuperscript{22} In the present study we observed a similar effect in 10 of the 11 samples from CD patients. In the 15 samples which did not form crystals in 21 days CABF extraction had no effect indicating that in these samples no ConA-binding inhibitor was present which prevented crystallization. In 10 of these samples the CSI was <1, hence probably they were sufficiently undersaturated to preclude crystallization. The other 5 samples (CSI 1.1-1.32) perhaps contained non-Con A binding crystallization inhibitors. Unexpectedly, in one crystallizing sample CABF extraction induced a decrease in CDT from 17 to 14 days suggesting that in this sample some inhibiting activity could be bound to the ConA-Sepharose beads. Interestingly, a similar phenomenon was observed in 5 of the 11 crystallizing samples of UC patients. In 4 samples no significant effect was observed and one sample showed an increase in CDT from 10 to 21 days. In the 9 remaining samples no crystals formed in 21 days; in 8 of these samples the CSI was lower than 1 hence they were probably too undersaturated with cholesterol or contained a non-ConA-binding inhibiting activity.\textsuperscript{30}

The question arises which factor is responsible for the ConA-binding inhibiting activity. The only characterized glycoprotein that has a crystallization inhibiting effect and in addition binds to Concanavalin A is Immunoglobulin A (IgA). Busch et al. demonstrated that cholesterol crystal binding proteins which could be isolated with the lectin Helix Pomatia (HP) were parts of the immunoglobulin A.\textsuperscript{29,30} To establish if the effect of CABF on crystal formation in UC patients as compared to CD patients could be explained by an increased amount of IgA in UC patient biles, the amount of IgA was determined in both CD and UC bile
samples. The IgA concentration appeared to be significantly higher in CD patients compared to UC patients indicating that at least total IgA could not account for the differences in crystallization behaviour. Biliary IgA concentration was not significantly different between biles in which CABF had crystallization promoting activity compared to biles in which CABF had crystallization inhibiting activity. Busch et al. showed binding of IgA to the surface of cholesterol crystals. It cannot be excluded that these IgA's represent a small specialized subgroup. Since the inhibiting IgA's have not been characterized in detail we have not been able to assess the existence of such subclasses in this study.

In conclusion, we found that patients with Crohn's disease have an increased tendency to form cholesterol crystals compared to patients with ulcerative colitis. As the incidence of gallstones is higher in the former group, we speculate that this demonstrated increased tendency in crystal formation may lead to cholesterol gallstone formation. The retardation of crystallization in CD biles after removal of CABF indicates that crystallization promoters in the CABF, which bind to Con A, contribute to the augmented crystallization in CD patients. In this study we also demonstrate for the first time direct evidence for the activity of crystallization inhibiting activity in native human bile. Particularly, in samples derived from UC patients extraction of CABF accelerated crystallization.

References


