Expression of phenotypic markers of mast cells, macrophages and dendritic cells in gallbladder mucosa with calculous cholecystitis

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The study aimed at quantitative analysis of expression involving markers of mast cells (tryptase), monocytes/macrophages (CD68 molecule) and dendritic cells (S100 protein) in gallbladder mucosa with acute and chronic calculous cholecystitis.
Routinely prepared tissue material from the patients with acute (ACC) (n = 16) and chronic calculous cholecystitis (CCC) (n = 55) was evaluated. Three cellular markers were localized by immunocytochemistry. Their expression was quantified using spatial visualization technique.
The expression of tryptase was similar in acute and chronic cholecystitis. CD68 expression in ACC was significantly higher than in the CCC group. Expression of S100 protein was significantly higher in CCC as compared to the ACC group. No significant correlations were disclosed between expression of studied markers and grading in the gallbladder wall. A weak negative correlation was noted between expression of CD68 and number of gallstones in the CCC group.
The spatial visualization technique allowed for a credible quantitative evaluation of expression involving markers of mast cells (MCs), monocytes/macrophages (Mo/Ma) and dendritic cells (DCs) in gallbladder mucosa with ACC and CCC. For the first time mucosal expression of S100 protein-positive DCs was evaluated in calculous cholecystitis. The results point to distinct functions of studied cell types in the non-specific immune response in calculous cholecystitis.

Key words: cholelithiasis, cholecystitis, tryptase, CD68, S100 protein.

Introduction

Acute cholecystitis most frequently coexists with cholelithiasis. At the beginning, cells of inflammatory infiltrates include neutrophils, which may lead to a phlegmon. With elapsing time, granulocytes gradually vanish, replaced by lymphocytes, plasma cells and macrophages. Connective tissue proliferates, leading to thickening and stiffening of gallbladder walls [1].

The most frequent morphological alteration in cholelithiasis involves chronic cholecystitis with thickening of the gallbladder wall, steatocholecystitis, muscle hypertrophy and incrustation with gallstones [1, 2]. Microscopically detectable lesions of mucosa in chronic calculous cholecystitis (CCC) comprise inflammatory infiltrates of a variable cellular composition, present in mucosa and in deeper layers of the gallbladder wall [3]. Several morphological varieties of CCC

exist. Cholesterolosis is present in 20% of cholecystectomy specimens with foamy macrophages in *lamina propria* and epithelium [4]. A special form of CCC showing localized accumulation of abundant foamy macrophages intermingled with lymphoplasmacytic cells (mainly T cells) is xanthogranulomatous cholecystitis [5-8]. In development of such lesions participation of bacterial antigens, in particular those of *Escherichia coli*, is suggested [7, 9]. Mucosa of calculous gallbladder also contains infiltrates of mast cells (MCs) [5]. On the other hand, the so-called eosinophilic cholecystitis (≥ 90% eosinophils) most frequently appears in acalculous cholecystitis [10].

Tissue MCs play a role in allergic reactions, angiogenesis, non-specific immunity and at preliminary stages of the specific immune response of the body [11, 12]. Their basic categorization in humans reflects their content of neutral serine proteases (tryptase and chymase). Tryptase seems to participate in pro-inflammatory functions of MCs, while chymase seems to be more involved in inflammatory reactions [13]. Studies have demonstrated that MCs may also be involved in synthesis of chemokines and cytokines [11, 14]. Tryptase-positive and chymase-negative cells in bronchial muscle biopsies were shown to be the source of interleukins (ILs) IL-5 and IL-6, while IL-4 was distributed in both MC phenotypes [14, 15]. Also an increase in the number of MCs staining for tumor necrosis factor α (TNF-α) was demonstrated in asthmatic biopsies [14]. TNF-α mRNA and protein were expressed constitutively in isolated human intestinal MCs [16]. Attempts were made to describe involvement of MCs both in physiology and in several inflammatory diseases and non-inflammatory diseases of the alimentary tract in humans [12, 17, 18], including pathology of gallbladder [19-22]. Higher numbers of MCs were detected in chronic exacerbated and chronic sclerotic cholangitis [21], and in Hirschsprung's disease [18], as compared with those in controls. An increased number of MCs in *lamina propria* was demonstrated in symptomatic cholelithiasis, as compared to patients with biliary dyskinesia [22]. The quoted correlations between number of MCs and intensity of inflammation and/or fibrosis in biliary ducts are not unequivocal [17, 21, 22]. In pathogenesis of gallbladder diseases many pathomechanisms with involvement of MCs are considered [5]. Mast cells containing numerous chemotactic factors may be responsible for accumulation of also numerous eosinophils in inflammatory infiltrates, observed in chronic cholecystitis [23] and in eosinophilic gastroenterocolitis [24].

Our earlier studies demonstrated eosinophils as a dominating type of cells in the inflammatory infiltrate in a great number (27% of cases) of young patients (up to 25 years old), as compared to a single patient in the older group [25].

The role of macrophages in pathogenesis of calculous cholecystitis is only fragmentarily described. Detection and evaluation of their number have taken advantage of various investigatory techniques [5, 19, 20]. Presence of markers typical of tissue (histiocytic) macrophages, particularly presence of α-1-antitrypsin and α-1-antichymotrypsin, was observed both in normal and non-metaplastic, chronically inflamed gallbladder mucosa [19]. Some investigators demonstrated significantly more numerous MCs and Mo/Ma in calculous gallbladders with minimal inflammation (classified as "normal") than in gallbladders with features of chronic cholecystitis [5].

Dendritic cells (DCs) are effective antigen-presenting cells that can stimulate both primary and secondary T- and B-cell responses. Dense infiltrates of S100 protein-positive DCs were noted in the majority of hyperplastic tonsils [26] and Langerhans cell sarcoma that involved gallbladder [27]. Their distribution in gallbladder mucosa and role in calculous cholecystitis have not been studied to date.

This study aimed at quantitative analysis of expression involving tryptase (mast cells), CD68 (monocytes/macrophages) and S100 protein (dendritic cells) in gallbladder mucosa of patients with acute and chronic types of calculous cholecystitis, in order to elucidate their possible differential distribution and roles.

Material and methods

Patients

All the patients were subjected to cholecystectomy in the T. Chałubiński Municipal Hospital in Ostrów Wielkopolski. The groups with acute calculous cholecystitis (ACC) (n = 16; 7 women and 9 men) and chronic calculous cholecystitis (CCC) (n = 55; 44 women and 11 men) included patients who were diagnosed and subjected to surgery in the period 2003-2007. Duration of cholelithiasis symptoms in the analysed groups of patients most frequently ranged from 6 months to about one year. The available epidemiological data included age, gender and body mass index (BMI) upon admission to the hospital. The other available variables included results of laboratory/biochemical tests. The number of gallstones disclosed following cholecystectomy was estimated using a semi-quantitative 1-4 point scale, as previously described [28]. In both groups of patients mostly mixed, cholesterol/pigment/calcium gallstones were detected. However, their detailed chemical composition was not tested. Written informed consent was obtained from each patient before the operation, and approval for the study was granted by the institution's Ethical Committee (no. 21/09).

Tissue material and microscopy image analysis

The archival material was fixed in buffered 10% formalin and embedded in paraffin using the routine procedure, and ~5 µm paraffin sections were placed on SuperFrost/Plus microscope slides. Studies using paraf-

fin sections included staining with haematoxylin and eosin (HE). Patterns of HE-stained gallbladder histological preparations were examined using an Olympus BH-2 light microscope by two histopathologists (WB, AK). Thickness (width) of the total wall of the gallbladder was measured (in mm). Each tissue specimen was also evaluated based on a simple numerical scoring system for the grade of *lamina propria* inflammation (G1) (0-3), and the grade of muscularis externa/adventitia inflammation (G2) (0-3), in which 3 points denoted an intense and, most frequently, diffuse inflammatory infiltrate, 2 points referred to moderately intense but also diffuse inflammatory infiltrate, 1 point indicated individual, dispersed cells or focally arranged cells of inflammatory infiltrate, and 0 points indicated tissue sections in which no cells of inflammatory infiltrate could be detected [28].

Immunocytochemical (IHC) studies

Detection and studies on cellular localisation of tryptase, CD68 and S100 protein in gallbladder mucosa took advantage of the new polymer-based IHC method [29]. Mouse anti-human monoclonal antibodies (mAbs) were employed, directed against human mast cell tryptase (clone AA1, isotype IgG1) (in dilution 1 : 400) (Code M 7052), against CD68 (in dilution 1 : 100) and against S100 protein (in dilution 1 : 400) (clone Z0311) (all mAbs from Dako, Glostrup, Denmark). The sections were incubated with these primary mAbs at night at 4°C, and afterwards were incubated with dextran backbone to which peroxidase (HRP) was attached and with secondary biotinylated link anti-rabbit and anti-mouse IgG (Dako REAL™ EnVision™ Detection System peroxidase/DAB+, Rabbit/Mouse, Dako). Following deparaffinization and rehydration the preparations were additionally boiled in 10 mM citrate buffer in a 700 W microwave oven for 18 min (in the case of anti-tryptase and anti-CD68), washed in phosphate buffered saline (PBS), and then subjected to the reaction according to the standard procedure. Every test was accompanied by a negative control, in which specific antibodies were supplemented by a normal serum of a respective species in 0.05 M Tris-HCl, pH ~7.6, supplemented with 0.1% bovine serum albumin (BSA) and 15 mM sodium azide. For comparison of MC, Mo/Ma and DC marker expression in gallbladder tissues, identical studies were performed in the archival tissue material from reactive lymph nodes ($n = 6$) removed during cholecystectomy (positive control). Studies in the control group aimed only at verification of quality of the applied antibodies and were not subjected to statistical analysis.

Histological slides with expression of all tissue markers were examined under the optical Olympus BH-2 microscope coupled to a digital camera. Colour microscopic images were recorded using the 40× objective (at least 10 fields in every microscope slide with an IHC positive reaction) and archived using LUCIA Image 5.0 computer software, documenting them in jpg format on the computer hard disc.

Microscopy image analysis and statistical analysis

The quantitative evaluation of the IHC expression of the proteins was performed using an image processing method based on spatial visualization of markers in microscope images, elaborated and programmed in the A4D computer software C++ language by Kaczmarek and Strzelczyk [30] and described in detail in our previous paper [31]. Results obtained in the two programs for image analysis (LUCIA Image 5.0 and A4D) were exported to the Microsoft Excel program, and were statistically analysed with the Statistica PL v. 8 program (StatSoft, Inc., Tulsa, OK, USA). In this study results related to expression of proteins were presented as area fraction (percentage) of the IHC reactions manifested by gallbladder mucosa (epithelium + *lamina propria*).

At the first stage of the statistical analysis, compatibility of the obtained results with a Gaussian distribution was checked using the Shapiro-Wilk test. Employing the Levene test, equal variances were documented for the first three parameters and, therefore, significance of differences was evaluated using the t test. Subsequently, parameters of descriptive statistics were calculated (arithmetical mean, standard deviation, median value, minimum and maximum values). Results of microscopic image analysis were compared between the ACC and CCC independent groups of patients using the Mann-Whitney U test. The Wilcoxon test was used for non-parametric dependent data. Correlations between data rows were determined employing Spearman's rank correlation index. Differences and relationships were accepted to be statistically significant at the level of $p < 0.05$.

Results

Cellular localisation of human tryptase-positive mast cells in gallbladder mucosa

Expression of tryptase-positive MCs was detected in all patients (100%) of groups ACC and CCC. In all cases it involved mast cells with typical morphology ("fried eggs") (Fig. 1A). Tryptase-positive MCs were dispersed both in gallbladder *lamina propria* and within its epithelium (Fig. 1B).

Cellular localisation of CD68-positive monocytes/macrophages in gallbladder mucosa

Expression of the CD68 molecule was also demonstrated in gallbladders of all patients in both groups. The protein was seen in the cytoplasm, mainly in monocytes and macrophages but also in individual neutrophils

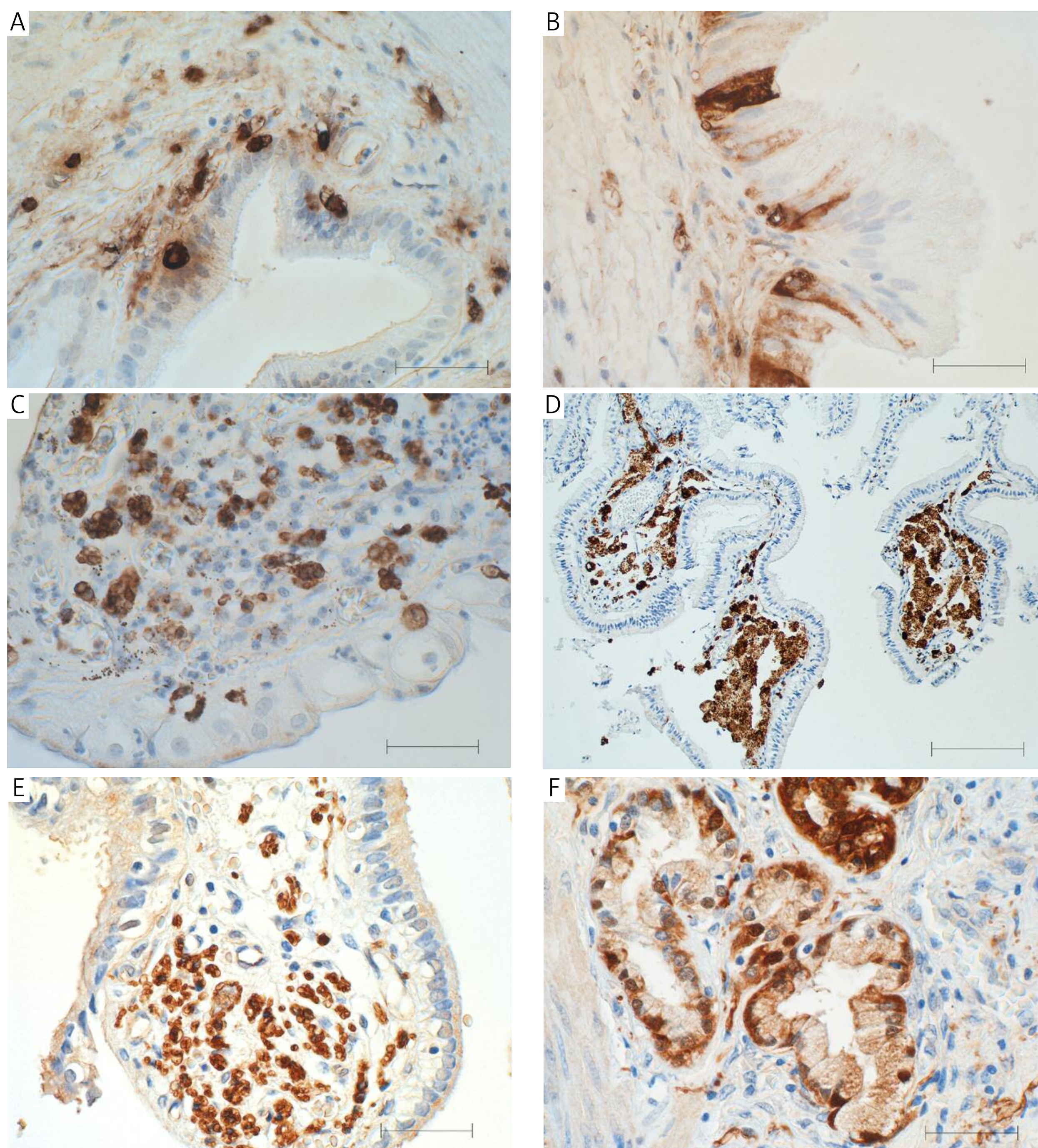

Fig. 1. Immunocytochemical localisation of cellular markers in gallbladder mucosa of patients with cholelithiasis: A) Tryptase-positive mast cells in *lamina propria*. B) Tryptase-positive mast cells located in gallbladder epithelium. C) CD68-positive macrophages among other inflammatory cells in *lamina propria*. D) CD68 expression in vast infiltrates of foamy cells (cholesterolosis). E) S100 protein-positive dendritic cells located in *lamina propria* under epithelium. F) S100 protein expression in tubulo-acinar glands in *lamina propria*. Occasionally nuclear localisation is present. Hematoxylin counterstained. Bar = 50 µm (A, B, C, E, F), 100 µm (D)

(Fig. 1C). A particularly strong reaction was detected in clumps of enlarged foamy cells, occasionally very numerous under epithelium in patients of the CCC group (Fig. 1D). Vast infiltrates of foamy cells (cholesterolosis) were observed in 8/55 (14%) patients from the CCC group.

Cellular localisation of S100-positive dendritic cells in gallbladder mucosa

Expression of the marker was also demonstrated in all patients of both groups. S100 protein-positive cells were manifested first of all in lamina *propria* (Fig. 1E).

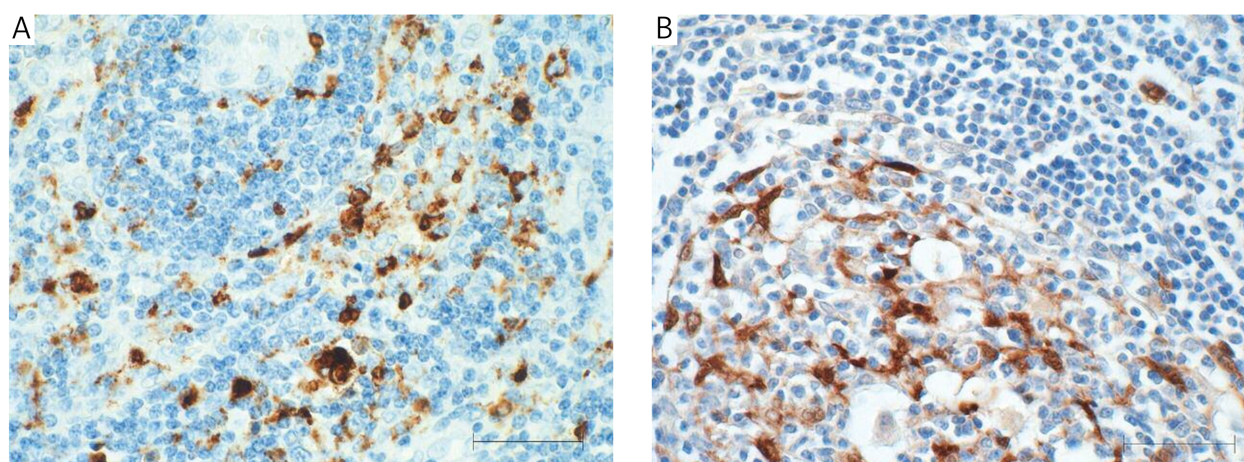

Fig. 2. Immunocytochemical localisation of cellular markers in reactive lymph nodes of patients with cholelithiasis (positive control): A) evident positive expression of CD68 in a great number of monocytes/macrophages; B) localisation of S100 protein in follicular dendritic cells. Hematoxylin counterstained. Bar = 50 μm

A positive reaction (including occasionally nuclear localisation) was also observed in tubulo-acinar glands (Fig. 1F).

In the positive control cellular expression of CD68 and S100 protein involved cells of lymph nodes (monocytes/macrophages and follicular DCs) (Fig. 2A and 2B).

Morphometric evaluation of tryptase, CD68 and S100 protein in gallbladder mucosa

Mean expression of tryptase in the CCC group manifested no significant differences as compared to the ACC group. Mean expression of CD68 in the ACC group was significantly higher than that in the CCC group. On the other hand, expression of S100 protein was significantly higher in the CCC group than in the ACC group (Table I, Fig. 3).

Upon comparison of expression involving the three marker proteins (Wilcoxon's test) within the ACC group, significant differences were disclosed in expression of tryptase (higher expression) and CD68 (higher expression) in comparison to expression of S100 protein.

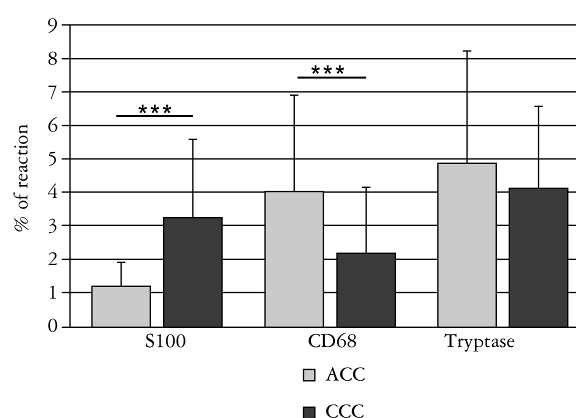

Fig. 3. Comparative expression of S100 protein, CD68 and tryptase between patients with acute and chronic calculous cholecystitis. ***p (level of significance) value < 0.001

In the group no significant differences were demonstrated between expression of tryptase and CD68. In patients with CCC significantly higher expression of tryptase and S100 protein was demonstrated, as com-

Table I. Comparative expression of tryptase, CD68 molecule, and S100 protein [area fraction (percentage) of the immunocytochemical reaction in gallbladder mucosa] as related to histopathological type of calculous cholecystitis

Cellular marker	Group	n	Mean	SD	Min.	Max.	p
Tryptase	ACC	16	4.87	3.35	0.85	11.02	0.582
	CCC	55	4.13	2.45	0.39	9.94	
CD68	ACC	16	4.04	2.86	1.90	12.31	< 0.001
	CCC	55	2.17	1.98	0.10	10.29	
S100	ACC	16	1.18	0.74	0.13	2.78	< 0.001
	CCC	55	3.25	2.33	0.23	9.05	

ACC – acute calculous cholecystitis, CCC – chronic calculous cholecystitis, n – number of patients, SD – standard deviation, min. – minimum, max. – maximum, p – level of significance

Table II. Comparison of mucosal expression (mean % of immunocytochemical reaction ±SD) involving the three cellular markers within acute and chronic type of calculous cholecystitis

Marker	Group	% of IHC reaction	Marker	% of IHC reaction	p
Tryptase	ACC	4.87 ±3.35	CD68	4.04 ±2.86	0.569
			S100	1.18 ±0.74	< 0.001
	CCC	4.13 ±2.45	CD68	2.17 ±1.98	< 0.001
			S100	3.25 ±2.33	0.069
CD68	ACC	4.04 ±2.86	S100	1.18 ±0.74	< 0.001
	CCC	2.17 ±1.98	S100	3.25 ±2.33	0.014

ACC – acute calculous cholecystitis, CCC – chronic calculous cholecystitis, IHC – immunocytochemical, SD – standard deviation, p – level of significance

Table III. Spearman's rank correlation coefficients (r) between tryptase, CD68 and S100 protein expression (% of reaction) in gallbladder mucosa and selected clinical data in patients with acute and chronic type of calculous cholecystitis

Clinical data	ACC			CCC		
	tryptase	CD68	S100	tryptase	CD68	S100
Age (years)	0.109	–0.024	–0.372	0.238	0.258	–0.015
BMI (kg/m^2)	0.429	–0.072	0.068	0.224	0.198	–0.002
Number of gallstones	0.045	0.436	–0.396	0.110	**–0.357**	0.090
Width of gallbladder wall (mm)	0.155	0.251	0.399	–0.250	0.072	0.086
Grading* (G1)	0.292	0.458	–0.094	0.070	0.021	0.205
Grading* (G2)	0.102	0.249	0.073	–0.016	0.243	0.148
WBC (× 10^9/l)	0.082	0.030	0.464	–0.052	0.156	–0.041

ACC – acute calculous cholecystitis, CCC – chronic calculous cholecystitis, BMI – body mass index, G1 – grade of lamina propria inflammation, G2 – grade of muscularis externa/adventitia inflammation, WBC – white blood cells, bold numbers indicate values of r significant at $p < 0.05$
**score see Material and methods*

pared to expression of CD68. No significant differences were disclosed in intensities of tryptase and protein S100 expression (Table II).

Correlations between expression of tryptase, CD68 and S100 protein and selected laboratory/clinical data of patients with cholelithiasis

In neither patient group could significant correlations be documented between reciprocal expression of the markers (data not shown). Also no significant relationships were noted between expression of individual markers and intensity of inflammatory lesions (grading) in the gallbladder. In patients with CCC a weak negative correlation was disclosed between expression of CD68 and the number of gallstones (Table III).

Discussion

Mast cells (MCs) participate in the normal function of human mucosae and may play an active role in a group of diverse diseases [12]. In inflammatory infiltrates of the gallbladder, MCs and macrophages have been quantified using histological staining (e.g. toluidine blue), histochemical techniques (e.g. non-specific esterases) or immunohistochemistry (e.g. anti-α-1-antitrypsin) [5, 19, 20]. The pioneer investigations using these techniques were burdened with extensive difficulties of obtaining appropriate quality of histological pictures. Nevertheless, they demonstrated that esterase-positive cells (MCs) and α-1-antitrypsin or α-1-antichymotrypsin positive cells (Mo/Ma) are present in both normal and chronically inflamed gallbladder mucosa [19, 20] and that esterase activity in gallbladder mucosa is significantly decreased in chronic cholecystitis [20]. Other investigators also observed less numerous MCs and macrophages in established chronic cholecystitis than in gallbladders with minimal inflammatory alterations [5].

In our study expression of tryptase was chosen for evaluation of MCs since as an α-protryptase it is constitutively secreted from MCs and is a cell-specific protease. The protease can be measured in the serum, and thus serves as an indicator of the total number of MCs [32]. Its presence represents an exponent of MC degranulation [17]. In gallbladders with acute cholecystitis, mean expression of tryptase was slightly higher than that in the CCC group, but the difference was insignificant. The IHC reaction for the marker was well visible and provided the typical "fried eggs" pattern of MCs [18]. No correlation was detected between number of MCs and inflammatory activity in the gallbladder. Also, no significant relationships were revealed

between the number of MCs in mucosal inflammatory infiltrates and the remaining clinical variables in the patients. We have not been able to confirm the literature data on augmented numbers of MCs in less intense lesions ("normal" tissue) of cholelithiasis in comparison with chronic cholecystitis [5], or their increased numbers as compared to the controls in chronic exacerbated and chronic sclerotic cholangitis in the patients with cholelithiasis [21]. Commenting on the differences, it should be added that for technical reasons we did not examine tryptase expression in "normal" gallbladder mucosa or "normal" choledochal wall, as it was performed in the quoted studies [5, 21]. In the studies of Hudson and Hopwood, other antibodies were applied for detection of MCs (rabbit anti-human α-1-antitrypsin) [5], while Gulubova and Vodenicharov [21] examined cryostat sections while antibodies identical to those used by us were applied in a distinct dilution (1 : 50). In both cases a distinct quantitative evaluation of MCs was used (cells/mm^2) [5, 21]. In our study we focused our attention on evaluation of MCs located exclusively within gallbladder mucosa and tryptase was applied as the basic marker of "mucosal" MCs [12, 15, 21]. It should be stressed that in our patients a tendency was detected toward increased expression of tryptase in the group with more intense inflammatory lesions (ACC), as compared to CCC, even if no correlation could be demonstrated with intensity of inflammatory lesions (grading). Possibly, by using a higher number of patients with ACC or examining tryptase-positive MCs in the entire wall of the inflamed gallbladders we might be able to demonstrate more correlations with grading or fibrosis of the organ. Examination of expression manifested by tryptase- and chymase-positive MCs in intralesional and perilesional skin lesions permitted potential involvement of MCs in pathogenesis of dermal tumours to be described [33]. No such studies are available in the case of gallbladder dysplastic and neoplastic lesions. The recent report demonstrated expression of two other immunohistochemical markers [DNA fragmentation factor-45 (DFF45) and thyroid transcription factor 1 (TTF-1)] in benign and malignant lesions of the gallbladder [34]. On the other hand, definition of the actual role played by MCs both in calculous cholecystitis and fibrosis of the organ as well as gallbladder carcinogenesis remains an open problem and requires continuation of the studies [17, 21, 22].

Our studies have demonstrated significantly more numerous CD68-positive macrophages in gallbladder mucosa in patients with acute cholecystitis, as compared to patients with CCC. On the other hand, only in CCC patients (14% of patients) were typical traits of cholesterolosis demonstrated, with presence of foamy macrophages, manifesting a pronounced positive reaction for CD68. The way in which the cells are generated remains unknown. Some authors suggest that scavenger receptor class A in macrophages is involved in the phagocytosis of *E. coli*, followed by foamy changes [9]. In xanthogranulomatous cholecystitis (XGC), characterized by a particularly pronounced accumulation of foamy cells among other inflammatory cells [35], it was confirmed that the vast majority of foamy cells were derived from monocytes/macrophages because they were positive for CD68 [8].

In our tissue material the CD68-positive cells included monocytes and macrophages of various size and individual neutrophils as well as markedly enlarged foamy cells (mainly in the CCC group). However, mean expression of CD68 antigen (and thus the number of macrophages) was higher in the ACC group, which seems to point to significant involvement of the cells in the first line of defence against microbes. Moreover, the cells may remove by phagocytosis the locally accumulated inflammatory cells. Thus, the role of macrophages is critical not only in the initiation of the inflammatory response, but also in the resolution of this process [36]. The increase in number of CD68-positive cells as compared to normal mucosa was also described in other active phases of inflammatory diseases in the alimentary tract (active Crohn's disease and ulcerative colitis) [37].

In the local inflammatory lesions of cholelithiasis both macrophages and dendritic cells (DCs) can function as antigen-presenting cells to propagate a local adaptive (antigen-specific) immune response [36].

Presence of S100 protein-positive DCs was demonstrated in 100% of gallbladders in both groups of patients. Using the spatial visualization technique higher expression of S100 protein was demonstrated in the CCC group than in the ACC group. No literature references are available with which the data could be compared. The descriptions related to gallbladder diseases are scarce and related to demonstration of S100 protein-positive Langerhans cells in a tumour of the organ [27]. Comparing expression of the studied cell markers, differences were also found within groups of patients. In mucosa of patients with ACC expression of tryptase (MCs) and CD68 (Mo/Ma) was similar and significantly higher than expression of S100 protein (DCs). This may point to active cooperation of MCs and macrophages in acute cholecystitis, involving mainly recruitment of neutrophils. In the mouse model the neutrophil chemoattractants, CXCL1/CXCL2, produced by both MCs and macrophages, were demonstrated to attract neutrophils to the site of acute inflammation [38]. The experiments permitted the authors to list a sequence of events, involving in the early phase of acute inflammation first the action of MCs located close to blood vessels, followed by neutrophil penetration of the tissue in a macrophage-dependent manner [38]. Other authors have also reported that mast cell-macrophage interactions alter inflammatory cytokine production and may contribute to chronic inflammation [11]. In the

group with CCC expression of tryptase was higher than activity of CD68-positive macrophages, which might suggest other functions of the two cell populations in this phase of inflammation. Literature data include the suggestion of potential involvement of MCs, together with mediators produced by them, also in mucous hypersecretion in the gallbladder, which would promote lithogenesis [5]. In our patients with chronic cholecystitis expression of tryptase and S100 protein was similar and the two proteins manifested higher expression than that of CD68. As compared to ACC, expression of S100 protein was significantly higher in CCC. This might suggest more intense involvement of the cells in maintenance of the chronic stage of inflammation due to *de novo* formation of local lymphoid tissue [39]. Numerous lymphoid follicles were seen in almost 30% of the patients with CCC described in our earlier paper [25]. In addition, interstitial DCs (S100 protein-positive) are involved in stimulation of lymphocytes (mainly T lymphocytes) by constitutive production of the chemokines [26, 39]. Dendritic cells are functionally linked to several aspects of immune tolerance and autoimmunity but our results do not permit us to comment on such literature data [39]. In order to better recognise the role of locally accumulated DCs studies should continue, aimed first of all at definition of the mechanisms due to which DCs accumulate in mucosa of gallbladders with calculous cholecystitis and at evaluation of the quantity of their precursors, *e.g.*, CD34-positive dendritic interstitial cells [26].

Summary and conclusions

The new element in this study involves evaluation of immunocytochemical expression using the modern technique of spatial visualization applied to typical markers of cells which play key roles in inflammatory reactions, the acute (ACC) and chronic type of cholecystitis (CCC) in patients of various age. In gallbladder mucosa of patients with ACC or CCC, similar numbers of mast cells were observed. Expression of CD68 prevailed in ACC and expression of S100 in CCC, which points to distinct functions of the studied cell types in the non-specific immune response accompanying calculous cholecystitis.

The spatial visualization technique allowed for a credible quantitative appraisal of marker expression, typical of MCs, Mo/Ma and DCs in gallbladder mucosa of patients with ACC or CCC. For the first time mucosal expression of S100 protein-positive DCs was characterized in calculous cholecystitis. The results point to distinct functions of studied cell types in the non-specific immune response associated with ACC and CCC.

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