Lactoferrin in Gastrointestinal Disease

Tetsuo Hayakawa\textsuperscript{1}, Chun Xiang Jin\textsuperscript{2}, Shigeru B. H. KO\textsuperscript{3}, Motoji Kitagawa\textsuperscript{4} and Hiroshi Ishiguro\textsuperscript{5}

Abstract

Lactoferrin, a major whey protein, is a red iron-binding protein present mainly in external secretions such as breast milk and in polymorphonuclear neutrophils. The presence of lactoferrin in body fluids is proportional to the flux of neutrophils and its assessment can provide a reliable biomarker for inflammation. In gastrointestinal diseases increased fecal lactoferrin is a sensitive and specific surrogate marker for inflammatory bowel diseases in patients with chronic diarrhea and pain, and ascites lactoferrin can also provide a promising and reliable biomarker for bacterial peritonitis. Lactoferrin in pancreatic juice and stone could provide pathophysiological information of protein plug and stone formation in the pancreatic duct. Serum anti-lactoferrin autoantibody might contribute to the clarification of the pathogenetic mechanisms of autoimmune pancreatitis and liver diseases, although its diagnostic and prognostic value appears to be limited. Further studies will be necessary to elucidate the exact details.

Key words: lactoferrin, inflammatory bowel disease, chronic pancreatitis, anti-lactoferrin antibody, autoimmune pancreatitis, autoimmune liver disease

\textsuperscript{(Inter Med 48: 1251-1254, 2009)}
\textsuperscript{(DOI: 10.2169/internalmedicine.48.2199)}

Introduction

Whey proteins are used as common ingredients in various products including infant formulas, specialized enteral and clinical protein supplements, and sports nutrition products with the expectation of the therapeutic potential of whey proteins and peptides. Lactoferrin, one of the major whey proteins, is a red iron-binding protein present mainly in external secretions such as breast milk and in polymorphonuclear neutrophils. This protein probably plays an important role in the defense mechanism of mucosal surfaces, since in an iron-depleted state it has bacteriostatic properties (1). Lactoferrin is released from polymorphonuclear neutrophils on activation of these cells and its presence in body fluids is proportional to the flux of neutrophils (2-5).

A small but important component of body iron is that bound to transferrin, the iron transport protein in plasma, and to lactoferrin. Lactoferrin may also regulate granulopoiesis. \textit{Helicobacter pylori} gastric infection has emerged as a new cause of refractory iron deficiency anemia, unresponsive to iron therapy, and not attributable to the usual causes such as intestinal losses or poor intake, malabsorption or diversion of iron in the reticulo-endothelial system (6-8). Lactoferrin has been found in significant amounts in human stomach resection specimens from patients with superficial or atrophic gastritis (9). The iron uptake of \textit{Helicobacter pylori} via a specific human lactoferrin receptor may play a role in the refractory iron deficiency anemia (10). Although the interaction between infection and iron metabolism is now well consolidated, the pathogenic mechanism underlying the anemia remains unclarified. Lactoferrin has been demonstrated and measured also in inflammatory synovial exudates and it is considered to contribute to a low serum-iron level in chronic inflammatory diseases such as rheumatoid arthritis via complex processes (11).

The measurement of lactoferrin in specimens from patients could provide a reliable biomarker for the presence of polymorphonuclear neutrophils and subsequently provide an important diagnostic clue or pathophysiological information.
for various diseases. The present review is focused on the diagnostic or pathophysiological significance of lactoferrin in feces, ascites, pancreatic juice and pancreatic stone and serum anti-lactoferrin autoantibody in gastrointestinal diseases.

**Lactoferrin in feces**

Diarrhea is common throughout the world posing diagnostic and therapeutic questions for physicians. The etiology of diarrhea varies widely depending on situations. If we can know whether or not the diarrhea in question is inflammatory, using a simple screening test, invasive diagnostic or therapeutic procedures would not be necessary in many patients with diarrhea. The presence of leucocytes in feces suggests an inflammatory process caused by infection or other inflammatory bowel disease. The methylene blue examination for fecal leucocytes requires fresh fecal specimens in a cup not swab or diaper and prompt microscopic examination of stained fecal specimen.

Guer rant et al have developed a simple, sensitive test for the detection of leucocytes in fecal specimens despite the destruction or loss of leucocytes morphology using antilactoferrin antibody. The immunoassay of fecal lactoferrin can be quickly and easily done in the clinic or later in the laboratory. They confirmed increased fecal lactoferrin in 96% (25/26) of samples from patients with shigellosis and concluded that fecal lactoferrin is a useful marker for fecal leucocytes (4).

Dai et al evaluated the relationship between fecal lactoferrin and intestinal inflammation including ulcerative colitis (UC, 42 active and 17 inactive), Crohn’s disease (CD, 14 active and 5 inactive), 41 infectious bowel disease, 25 irritable bowel syndrome (IBS) and 34 healthy volunteers. Fecal lactoferrin was significantly higher in inflammatory bowel disease including infectious bowel disease than in IBS and healthy volunteers. It provides us with a valid method to discriminate between inflammatory and non-inflammatory bowel disease (12). Schoepfer et al also reported the overall accuracy of fecal lactoferrin for discriminating IBS from inflammatory bowel disease or other forms of colitis including infectious colitis (13). However, Ashraf et al concluded that fecal lactoferrin was not useful in differentiating IBD from non-inflammatory bowel disease or other forms of IBD (15). Differentiation of active IBD from inactive IBD is also possible by fecal lactoferrin both in CD and UC as well as differentiation of IBD from IBS (15-21). Elevation of fecal lactoferrin is a useful triage tool to differentiate organic bowel disorders from functional disorders. Fecal lactoferrin has an additional role in monitoring inflammatory bowel disease activity and predicting relapse.

Total proctocolectomy with ileal pouch-anal anastomosis (IPAA) has become the surgical procedure of choice in patients with ulcerative colitis with either dysplasia or disease refractory to medical therapy. Increased frequency, urgency, and abdominal pain in patients with IPAA may be due to inflammatory conditions, including pouchitis, cuffitis, or CD or noninflammatory conditions such as irritable pouch syndrome (IPS). Fecal lactoferrin can serve as a sensitive and noninvasive initial screening test in an algorithm for evaluation of symptomatic patients with IPAA. If fecal lactoferrin is low, IPS can be diagnosed. If fecal lactoferrin is high, pouch endoscopy and biopsy are warranted to distinguish among the different causes of inflammation (22).

Among the neutrophil-derived proteins in feces lactoferrin, calprotectin, and neutrophil-elastase represent useful markers for differentiation of IBD by disease activity and differentiation of IBD from IBS when compared with other fecal markers (myeloperoxidase, lysozyme, hemoglobin) and serum CRP (13, 16, 17, 19-21).

**Lactoferrin in ascites**

Ascites is the most common complication of cirrhosis in patients with liver disease. Ascitic fluid often becomes infected without any apparent intra-abdominal source of infection called spontaneous bacterial peritonitis (SBP). The prevalence of SBP in unselected in-patients ranges from 10% to 30% (23). The infection resolution is high with antibiotic therapy, although in-hospital mortality is still over 20% mainly in secondary to hepato-renal syndrome (24). The diagnosis of SBP is based on a manual count of ascitic fluid polymorphonuclear cells. This procedure is operator-dependent and lysis of the cells during transport to the laboratory may lead to false negative results. The situations are similar to those in fecal leucocyte count. Furthermore, ascitic fluid culture is insensitive and leads to delays in diagnosis.

Parsi et al assessed the utility of ascitic fluid lactoferrin for the diagnosis of SBP in a total 218 consecutive ascitic samples from 148 patients with cirrhosis. An ascitic fluid polymorphonuclear neutrophil count of 250 cells/mL or greater with or without a positive culture was used for diagnosis of SBP. Twenty-two (10.1%) samples fulfilled the diagnostic criteria for SBP. Samples with SBP had a significantly higher lactoferrin concentration compared with non-SBP samples. The sensitivity and specificity of the assay for diagnosis of SBP were 95.5% and 97%, respectively using a cut-off level of fluid of 242 ng/mL. They concluded that qualitative bedside assays for the measurement of ascitic
fluid lactoferrin can be developed easily and may serve as a rapid and reliable screening tool for SBP in patients with cirrhosis (5).

**Lactoferrin in pancreatic juice and stone**

Chronic pancreatitis is characterized by progressive and irreversible pancreatic damage. A classic feature of the exocrine dysfunction is decreased secretion of bicarbonate and enzymes. Calcium and lactoferrin secretion, however, is increased.

Protein plugs formed within the interlobular and intralobular ducts are one of the earliest findings in chronic pancreatitis and the plugs subsequently perpetuate inflammation of the pancreas through recurrent obstruction of the pancreatic duct system. Lactoferrin may play a role in the formation of the protein plugs frequently seen in chronic pancreatitis because of its ability to produce an aggregation of a large acidophilic protein, such as albumin (25). Lactoferrin hypersecretion from the pancreas was initially postulated by Figarella and Sarles to be a congenital secretory defect, possibly worsening the damaging defects of alcohol or malnutrition on the pancreas (26). However, their subsequent study failed to reveal a significant correlation between chronic alcohol consumption and lactoferrin concentration in pancreatic secretion, though they confirmed hyperconcentration of lactoferrin in both noncalcified and calcified chronic pancreatitis (27).

Jin et al measured protein, pancreatic stone protein (PSP) and lactoferrin in pancreatic stones obtained from 13 patients with chronic calcified pancreatitis. The PSP was determined in pancreatic stones in all 13 patients, but did not differ significantly between alcoholic (n=6) and nonalcoholic (n=7) pancreatitis. Lactoferrin was detectable in 5 of 13 patients. Recurrent inflammation in a cystic lesion or dilated ducts in 3 of the 5 patients could be a common factor attributable to high contents of lactoferrin (28).

Nagai and Ohtsubo reported calcified stones in the pancreas of elderly people autopsied at a geriatric hospital. The small calculi found in 22 patients over age of 70 were 1-3 mm in diameter and 2-100 in number and distributed throughout the pancreas. All of the small calculi were composed of calcium carbonate and located in the peripheral branch of the pancreatic duct. None of the cases had a history of pancreatitis or abdominal pain suggestive of pancreatic abnormality. Lactoferrin was observed in acinar cells to some extent, but it was present in much greater concentration in the protein plugs and in the cytoplasm of squamous cells of dilated ducts encasing the plugs. They suggested that lactoferrin may have a role in an initial stage of protein plug formation in the pancreatic duct (29).

It is still inconclusive whether lactoferrin is from polymorphonuclear leucocytes or the product in the pancreatic tissue itself and which protein is more important for the precipitate and stone formation.

**Anti-lactoferrin autoantibody (anti-Lf) in serum**

Serum anti-Lf has been reported in patients with several autoimmune diseases, including autoimmune pancreatitis (AIP), hepatitis (AIH) and cholangitis (AIC), primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), type I diabetes mellitus. Anti-Lf was found significantly more often in AIH (25%), PBC (25%), PSC (29%) and AIC (35%) than in HCV-positive chronic hepatitis (3.5%). Among the different forms of autoimmune liver diseases anti-Lf was similarly detected (30). Similar results were also reported in the study of Ohana et al (31). The presence of anti-Lf was not associated with a particular clinical or biochemical profile of an underlying liver disease such as age, gender, ongoing liver injury, cholestasis and liver function.

AIP is a relatively uncommon, nonalcoholic-related form of chronic pancreatitis that has received increasing attention in recent years. AIP is characterized by an increase in serum IgG, particularly IgG4, the presence of serum autoantibodies such as anti-Lf, pancreatic fibrosis with lymphocytic infiltration, association with other autoimmune diseases, and response to steroid therapy (32, 33).

Okazaki detected anti-Lf in 75% of 30 AIP patients (33). Hardt et al found anti-Lf in 21% of 48 patients with nonalcoholic chronic pancreatitis and in 8.3% of 48 patients with type I diabetes (34). Taniguchi et al (35) also observed anti-Lf in 67% of 43 type I diabetes. The diagnostic and prognostic value of anti-Lf appears to be limited because of relatively low sensitivity, specificity and correlation to autoimmune related diseases mentioned above.

From the pathogenetic standpoint, the coincidental appearance of AIP with extrapancreatic autoimmune diseases and association of anti-Lf with autoimmune pancreatitis and liver diseases suggest common target antigens in pancreas, liver and other organs. Lactoferrin is distributed in the various tissues, including the pancreas, liver, biliary tract, and neutrophils leucocytes. The origin of anti-Lf is unknown. It is also unclear whether anti-Lf is simply an epiphenomenon of the autoimmune process or whether it can play a pathogenetic role in the initiation and perpetuation of the autoimmune disease.

**Conclusion**

Fecal lactoferrin is a sensitive and specific marker for the differentiation of IBD from IBS and for the evaluation of disease activity of IBD. Lactoferrin in pancreatic secretion may be a precipitate protein in stone formation in chronic pancreatitis. Serum anti-Lf might contribute to clarifying a pathogenetic mechanism of autoimmune pancreatitis and liver diseases, although its diagnostic and prognostic value appears to be limited. Further studies are required for confirmation.
References


© 2009 The Japanese Society of Internal Medicine
http://www.naika.or.jp/imindex.html