

Review

Dirk Roggenbuck*, Dirk Reinhold, Peter Schierack, Dimitrios P. Bogdanos, Karsten Conrad and Martin W. Laass

Crohn's disease specific pancreatic antibodies: clinical and pathophysiological challenges

Abstract: Crohn's disease (CrD) and ulcerative colitis (UC) are the main inflammatory bowel diseases (IBD). IBD-specific humoral markers of autoimmunity in the form of autoantibodies have been reported first in the late 1950s by demonstrating the occurrence of autoimmunity in UC, while humoral autoimmunity in CrD can be traced back to the 1970s. Ever since, the pathophysiological role of autoimmune responses in IBDs has remained poorly understood. Notwithstanding, autoreactive responses play a major role in inflammation leading to overt IBD. In CrD, approximately 40% of patients and <20% of patients with UC demonstrate loss of tolerance to antigens of the exocrine pancreas. Glycoprotein 2 (GP2) has been identified as a major autoantigenic target of the so-called pancreatic antibodies. The previously unsolved contradiction of pancreatic autoreactivity and intestinal inflammation in IBD was elucidated by demonstrating the expression of GP2 at the site thereof. Intriguingly, GP2 has been reported to be a receptor on microfold cells of intestinal Peyer's patches, which are believed to represent the origin of CrD inflammation. The development of immunoassays for the detection of antibodies to GP2 has paved the way to investigate the association of such antibodies with the clinical phenotype in CrD. Given the recently discovered immunomodulating role of GP2 in innate and adaptive intestinal immunity, this association can shed further light on the pathophysiology of IBD. In this context, the association of anti-GP2 autoantibodies as novel CrD-specific markers with the clinical phenotype in CrD will be discussed in this review.

Keywords: clinical phenotype; Crohn's disease; glycoprotein 2; inflammatory bowel disease; pancreatic antibody.

***Corresponding author: Prof. Dr. Dirk Roggenbuck, MD, PhD,** Faculty of Sciences, Brandenburg Technical University Cottbus-Senftenberg, Großenhainer Strasse 57, 01968 Senftenberg, Germany, Phone: +49 33708 441716, Fax: +49 33708 441725, E-mail: dirk.roggenbuck@hs-lausitz.de; and GA Generic Assays GmbH, Dahlewitz, Germany

Dirk Reinhold: Institute of Molecular and Clinical Immunology, Otto-von-Guericke University Magdeburg, Magdeburg, Germany

Peter Schierack: Faculty of Sciences, Brandenburg Technical University Cottbus-Senftenberg, Senftenberg, Germany

Dimitrios P. Bogdanos: Division of Transplantation Immunology and Mucosal Biology, Institute of Liver Studies, King's College London School of Medicine at King's College Hospital, Denmark Hill Campus, London, UK

Karsten Conrad: Institute of Immunology, Technical University Dresden, Dresden, Germany

Martin W. Laass: Children University Hospital, Technical University Dresden, Dresden, Germany

Autoimmunity in Crohn's disease

Crohn's disease (CrD) and ulcerative colitis (UC) are the most frequently diagnosed inflammatory bowel diseases (IBDs) with a prevalence ranging from 0.01% up to 0.44% depending on age and demographic factors [1–3]. In a recent systematic review, the highest annual incidence of CrD has been determined at 12.7 per 100,000 person-years in Europe, 5.0 person-years in Asia and the Middle East, and 20.2 per 100,000 person-years in North America [4]. The time-trend analyses of this review revealed an increasing incidence of statistical significance for 75% of CrD studies. Interestingly, a comparable figure (60%) has been found for UC suggesting an overall increasing incidence and prevalence of IBDs with time. The reasons for this rise are currently unclear. Therefore, it is not surprising that both IBDs account for a substantial percentage of the overall direct and indirect costs spent on the healthcare system as recently reported by the Centers for Disease Control and Prevention (CDC) for the US. In fact, as many as 1.4 million persons in the USA suffer from IBDs (<http://www.cdc.gov/ibd/#epidIBD>) and these diseases represent one of the five most prevalent gastrointestinal disease burdens. Altogether, IBD account for more than 700,000 physician visits and 100,000 hospitalizations with an overall healthcare cost of more than \$1.7 billion. Based on the data estimations, up to 75% of patients with CrD and 25% of those with UC will develop complications requiring surgery in the course of disease.

CrD is a lifelong disease characterized by various clinical symptoms including abdominal pain, weight loss, and bloody or non-bloody diarrhea [5]. The transmural inflammation characteristic for patients with CrD affects all layers of the bowel wall and adventitia [1]. In contrast to UC, CrD-related tissue lesions are not confined to the rectum and colon and can be detected in the whole digestive tract [6] often with the involvement of the terminal ileum [1, 7]. Tissue lesions in CrD can manifest as fissures, abscesses, strictures, or fistulas. Histologically, CrD is characterized by a focal (discontinuous) chronic inflammation, focal crypt architectural irregularities, and granulomas in about half of the patients. In UC, the main symptoms are bloody diarrhea and abdominal pain. The inflammation is usually confined to the colon with a continuous expanse ascending from the rectum. Typically histological features of UC are different from CrD and include mononuclear inflammation in the lamina propria, crypt distortion, and goblet cell (mucin) depletion. The relative risk for colorectal cancer is increased in patients with CrD and UC compared to the non-IBD population and is associated with the anatomic expanse of inflammation, duration of disease, and presence of additional risk factors (e.g., primary sclerosing cholangitis) [8, 9]. Patients with CrD have also an increased risk for small bowel cancer [10].

Thus, CrD and UC encompass a multisystem group of global disorders with specific clinical and pathophysiological features [4, 11]. Over the last few years, significant attempts have been made focusing on therapeutic interventions for patient care. Also, research initiatives have been focused on the pathophysiological alterations that characterize these heterogeneous diseases.

Autoimmunity has been assumed to partake in the pathophysiology of IBD in the late 1950s by demonstrating the occurrence of autoreactive antibodies in UC [12]. First reports on humoral autoimmunity in CrD can be traced back to the late 1970s. Walker described a possible diagnostic test for CrD by the use of buccal mucosa as a substrate in indirect immunofluorescence (IIF) for the detection of respective autoantibodies [13]. Loss of immunological tolerance observed in CrD and UC has been reported mainly for exocrine pancreatic, neutrophilic, and intestinal goblet cellular antigens [14–16].

Apart from genetic predisposition and environmental factors, autoimmunity is thought to play an important role in the induction of IBD and particularly in CrD [1, 17]. Regarding genetics, NOD2 gene mutations have been reported to be associated with the risk and site of disease in IBD [18]. By means of genome-wide association studies, IBD5 locus (5q31–33 region) has been demonstrated to be strongly associated with UC, whereas the NOD2 (16q12)

and major histocompatibility complex (MHC) (6p21) locus appear to be linked to CrD [19]. In general, it is believed that a “western” or “westernized” diet on a pre-disposed genetic background seems to lead to an imbalance between innate and acquired immunity which is accompanied or brought about by an impairment of the intestinal barrier, as well as changes in the gut microbiota [20–23]. The intestinal flora is essential to perpetuate the inflammatory process in the pathophysiology of IBD [23].

Association of pancreatic autoimmunity with the Crohn's disease phenotype

Following Walker's report of antibuccal mucosa autoantibodies in CrD, Stöcker et al. noted in 1984 that the presence of pancreatic antibodies (PAB) determined by IIF is a characteristic feature of patients with CrD [24]. Up to 40% of patients suffering from CrD develop loss of tolerance to exocrine pancreatic antigens. The prevalence of humoral autoimmunity largely varies amongst studies investigated (Table 1). This discovery has led to intensive research on the role of pancreatic autoimmunity and the presumed association of these autoantibodies with the clinical phenotypes. Research has also been focused on the pathophysiology of CrD and the mechanisms that lead to the induction of autoreactive responses. According to the Montreal classification, stratification of CrD patients is based on age at onset of disease, disease behavior and location [25]. An extensive Hungarian study including 579 CrD patients associated the presence of PABs with penetrating disease behavior, perianal disease, and extraintestinal manifestations but not with the CrD-characteristic genotype of NOD2/CARD15 or the expression of the innate immunity toll-like receptor 4 (TLR4) [26]. Indeed, 68% of CrD patients with extraintestinal complications such as idiopathic chronic pancreatitis appear to demonstrate PAB [27–30]. Investigating 252 CrD patients, Seibold's group in Switzerland found a negative association of PABs with inflammatory CrD and revealed a trend towards small bowel disease and small bowel surgery. In contrast to the Hungarian study, this latter report did not find a significant correlation with penetrating disease behavior [31]. A multicenter study involving 109 CrD patients from five university hospitals in France and one in Luxemburg, failed to identify an association between PABs and clinical features of the disease except of a relationship between the presence of the autoantibodies and an early disease

Table 1 Occurrence of pancreatic antibodies (PAB) detected by indirect immunofluorescence on human or monkey pancreas sections in patients suffering from inflammatory bowel diseases and controls.

CrD	UC	BD	Disease controls	Country	References
23/59 (39%)	2/46 (4%)	3/100 (3%)		Germany	[14]
31/82 (38%)	0/65 (0%)	0/250 (0%)	0/100 (0%) SARD, ALD, intestinal neoplasia	Germany	[101]
32/77 (42%)	18/73 (25%)	0/100 (0%)	0/31 (0%) NIBD, 1/16 (6%) SARD	Greece	[116]
54/168 (32%)	28/120 (23%)	0/100 (0%)	24/108 (22%) FDR, 1/78 (1%) NIBD	Belgium	[32]
68/222 (31%)	2/51 (4%)	0/65 (0%)	0/133 (0%) NIBD	Germany	[34]
29/76 (38%)	2/61 (3%)	2/56 (4%)	7/106 (7%) FDR	Germany	[117]
43/109 (37%)	6/78 (8%)	1/50 (2%)		France	[30]
9/64 (14%)	5/63 (8%)	0/28 (0%)	4/130 (3%) FDR	Turkey	[114]
238/579 (41%)	25/110 (23%)	8/100 (8%)	3/64 (5%) NIBD, 10/43 (22%) CeD	Hungary	[26]
60/210 (29%)	0/47 (0%)	0/50 (0%)		Germany	[115]
13/43 (30%)	0/28 (0%)	0/41 (0%)		Slovenia	[118]
34/100 (34%)	4/99 (4%)	1/100 (1%)		Australia, China	[119]
72/178 (40%)	21/100 (21%)	0/162 (0%)		Germany	[46]
35/103 (34%)	10/49 (20%)	0/104 (0%)		Hungary ^a	[33]
29/96 (30%)	2/39 (5%)	0/50 (0%)		Germany	[42]
55/252 (22%)	0/53 (0%)	0/43 (0%)		Switzerland	[120]

^aPediatric disease onset only. ALD, autoimmune liver diseases; BD, blood donors; CeD, celiac disease; CrD, Crohn's disease; FDR, first degree relatives of patients with IBD; NIBD, non-IBD inflammatory gastrointestinal disorders; Ref, reference; SARD, systemic autoimmune rheumatic diseases; UC, ulcerative colitis.

onset [30]. An earlier study from Leuven including 169 CrD patients established a significant negative association of stricturing disease behavior and PAB seropositivity [32].

A recent study based on a cohort of 103 Hungarian children with CrD did not demonstrate any association of PAB with clinical features of disease [33]. These data could support the notion that pediatric IBD are probably distinct entities compared to the adult forms of the disease. However, discrepancies amongst studies could be a result of the poor standardization of PAB testing by IIF due to the use of different sources of pancreatic substrates and technical experience with the method. Thus, the search for the autoantigenic targets responsible for the CrD-specific IIF patterns seen on pancreatic tissue could provide the necessary tools for molecularly based assays. Such a progress could assist the efforts made by investigators to study the fine specificity of humoral and cellular autoimmune responses and their role in the development of CrD. However, there has been surprisingly little progress in the identification of the putative targets of PAB and the understanding of their possible impact on the pathophysiology of IBD until recently.

Identification of CrD-specific pancreatic autoantigens

Since its first description in 1984, several groups have tried intensively to identify the autoantigenic targets of

PAB [24, 34–38]. Hence, the simultaneous report of the discovery of glycoprotein 2 (GP2) as a CrD-specific pancreatic autoantigen by two groups – that of Stöcker's and our group in 2008 was a remarkable coincidence [39–42]. Employing two-dimensional electrophoresis and matrix-assisted laser desorption ionization time-of-flight mass spectrometry, we identified GP2 by an interdisciplinary collaboration and demonstrated the specific interaction of PAB with recombinant human GP2 transiently expressed in mammalian HEK293 cells [40].

Pancreatic autoantibodies can reveal two different IIF patterns according to the location of specific IIF signals providing the basis for the differentiation of two PAB types [43]. Type 1 and 2 PABs are characterized by an extracellular drop-like staining of the acinar lumen and a speckled cytoplasmic staining of acinar cells, respectively. These PABs can interact with GP2 after its release, together with digestive enzymes, into the pancreatic ducts (PAB type 1) or with its membrane form of granules in the acinar cells (PAB type 2) [44]. Due to its tendency for self-binding, GP2 forms high molecular complexes in the pancreatic juice after discharge (Figure 1). This may lead to the formation of conformational autoantigenic neoepitopes [44]. This phenomenon is consistent with the documented high molecular weight of the PAB reactive pancreatic juice protein observed by Seibold et al. [34].

Stöcker et al. discovered GP2 only as a PAB type 1-reactive pancreatic autoantigen by a different immunochemical approach [42]. Notably, these authors identified CUB/

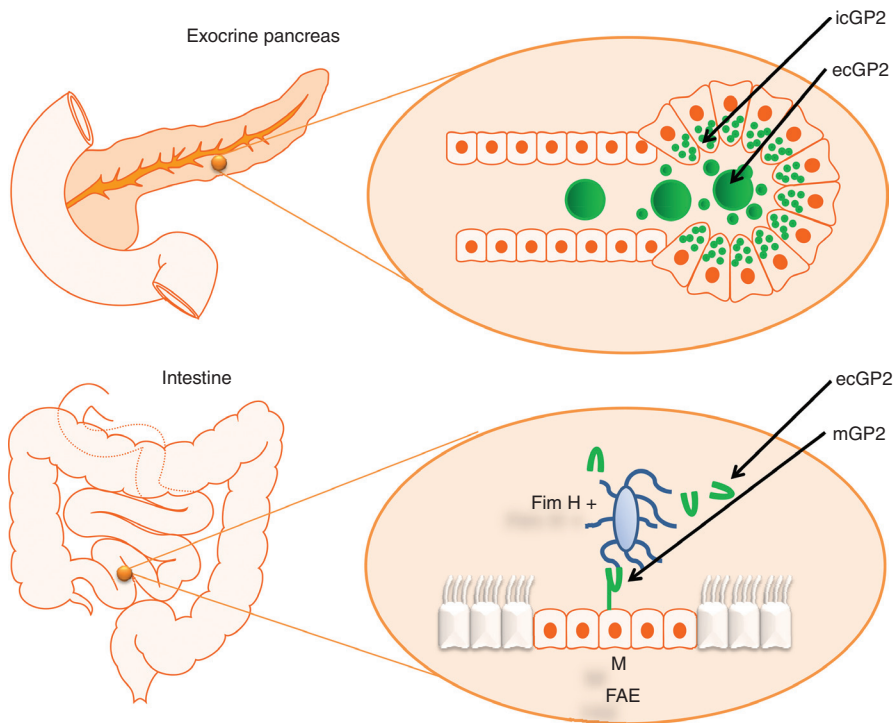


Figure 1 Synthesis and function of human glycoprotein 2 at different gastrointestinal sites.

Intracellular glycoprotein 2 (icGP2) has been found in acinar cells of the exocrine pancreas and reported to be shed together with digestive enzymes into the pancreatic duct as extracellular GP2 (ecGP2). Further, ecGP2 is transported via the pancreatic duct into the intestinal lumen. Provided that GP2 is not degraded by activated digestive enzymes, GP2 can opsonize FimH-positive microbes (FimH+) in the gut. Simultaneously, GP2 (mGP2) is synthesized in microfold cells (M) of the follicle-associated epithelium (FAE) and presented as a membrane bound-receptor which can grab FimH-positive bacteria for transcytosis by these cells.

zona pellucida-like domain-containing protein (CUZD1) as an autoantigenic target of type 2 PAB [41, 45]. In this context, it is interesting to note, that almost all type 2 PAB positive sera also show a weak type 1 PAB staining by IIF at least on human pancreas [43]. Furthermore, type 2 PAB does not seem to differentiate CrD patients from UC patients whereas type 1 PAB appears to be able to do so [32].

In fact, anti-GP2 reactivity is not universally found in all PAB-positive patients. Thus, the presence of other autoantigenic PAB targets cannot be ruled out [40, 46, 47]. However, GP2 is the only PAB reactive autoantigen which appears to have a profound link with the intestinal location of disease in CrD patients. In contrast to CUZD1, both elevated transcription of GP2 mRNA and translation of GP2 have been demonstrated in intestinal biopsy samples of CrD patients apart from the main pancreatic GP2 synthesis [17, 40]. CUZD1, also known as UO-44, is expressed in cancerous ovarian tissue and is considered a novel serological biomarker for ovarian cancer [48]. However, Northern blot analysis revealed two differing human pancreatic UO-44 transcripts [49, 50]. To the best of our knowledge,

corresponding data regarding CUZD1 expression in the intestine are still lacking [17, 51].

Glycoprotein 2 and the link to intestinal inflammation

For several years, GP2 has been generally known as the most abundant membrane protein of pancreatic acinar cells not expressed in endocrine pancreas tissue [52]. Noteworthy, autoimmunity to endocrine pancreas is a hallmark of type 1 diabetes [53]. Mainly during digestion, GP2 is discharged along with digestive enzymes by the acinar cells into the pancreatic duct (Figure 1) [44, 54].

Thus, the identification of GP2 as a specific receptor on microfold (M) cells of intestinal Peyer's patches (PP) by Hase et al. ushered in a new age of autoimmunity research in IBD [55–57].

The seminal paper of Hase et al. demonstrated GP2 to be involved in the generation of humoral immune responses to molecules of the intestinal content interacting

specifically with GP2 on M cells [55]. These findings have led to a better understanding of autoimmunity against GP2 in CrD-specific inflammation and to a dramatic change in the understanding of the physiology of GP2. It remains to be elucidated why GP2 is expressed at two different body sites and to what extent the expression of GP2 in pancreas or in the intestine regulates the loss of tolerance to this unique glycoprotein in CrD (Figure 1). Noteworthy, an elevated expression of GP2 at both mRNA and protein levels has been shown in biopsy samples from patients with CrD in contrast to patients with UC hinting at a possible direct involvement of GP2 in the inflammatory processes in CrD [40]. Furthermore, a higher mucosal synthesis of GP2 has been reported in patients with pouchitis, an inflammation of the small bowel developing in up to 60% of UC patients undergoing proctocolectomy and ileal pouch anal anastomosis (IPAA) [58]. In this disease subset, patients with de novo development of CrD demonstrated a loss of tolerance to GP2 [59].

To elucidate the role of GP2 in intestinal inflammation and to understand the loss of tolerance to this unique glycoprotein, its physiological role at both sites and, in particular, in the intestine warrants special attention.

Physiological role of glycoprotein 2

Until the identification of GP2 as an intestinal receptor on M cells, GP2 has been considered a critical constituent which affects pancreatic granule formation by interacting with other zymogen granule membrane proteins or proteoglycans in a submembranous matrix [60–62]. However, much to the surprise of gastroenterologists, this hypothesis could not be supported by the pathophysiological features of a GP2-deficient mouse model [63]. Therefore, research on GP2's urinary homologue, the Tamm-Horsfall protein (THP) or uromodulin, has also been considered important to reveal GP2's physiological functions [64, 65]. THP is the most abundant urinary protein which is secreted by renal tubular epithelial cells of the ascending limb of the loop of Henle in the urinary tract [65, 66]. Interestingly, both glycoproteins share one common ancestor gene which evolved separately during the phylogenesis to acquire tissue specificity in the digestive and urinary systems [67]. It has been tempting to speculate that common features regarding their putative functions could have been preserved in both organ systems. Extensive research revealed an anti-microbial function of THP by its binding to uropathogenic type 1 fimbriated *Escherichia coli* [68]. Defective THP synthesis is associated with

an elevated susceptibility of mice to urinary tract infections [69]. In this context, the elegant study by Hase et al. demonstrated that GP2 selectively binds to a subset of commensal and pathogenic enterobacteria, including *E. coli* and *Salmonella enterica* serovar *Typhimurium* [55]. Akin to THP, this type of binding is mediated by FimH of type I pili on the bacterial outer membrane [70]. Thus, GP2 on M cells can serve as a transcytotic receptor for bacterial antigens and, therefore, partake in the mucosal immune response to these particular bacteria [56]. Thus, the elevated phagocytosis of *E. coli* by monocytes observed after treatment with GP2 supports the notion that GP2 may have a broader pro-phagocytotic ability [71].

Intriguingly, THP has also been shown to modulate innate and adaptive immunity of the urinary tract [72]. Likewise, a putative immunomodulating role of GP2 has been addressed recently [71, 73]. Glycoprotein 2 has been identified as a binding partner of the scavenger receptor expressed on endothelial cells I (SREC-I), which can be also found on dendritic cells [73]. Actually, SREC-I is present on monocyte-derived dendritic cells and may react with GP2 or GP2-bound complexes. The ability of SREC-I expressing dendritic cells to internalize GP2 or GP2-related complexes has also been considered. This ability has profound effects on the understanding of GP2 as dendritic cells play an important role in the generation of innate and adaptive immune responses [74]. Interestingly, the synthesis of GP2 appears to be up-regulated on activated human T cells and to be modulated by pharmaceutical TNF α inhibitors [71]. An intriguing finding was the reduction of human intestinal epithelial cell, mucosal, and peripheral T cell proliferation and apoptosis by GP2. Furthermore, intestinal epithelial cells stimulated with GP2 are potent chemoattractors of T cells.

In this context, it is interesting to note that pro-inflammatory CXCL8 secretion decreased in freshly resected mucosal specimens whereas regulatory TGF β 1 increases in response to GP2. Altogether, these data seem to support an anti-inflammatory role of GP2 in the mucosal immune system. Investigating this putative immunosuppressive effect, Werner et al. obtained data indicating that GP2 modulates such an effect through its interaction with regulatory T cells [71].

In summary, GP2 is located at the epithelial frontier of the intestine on the surface of M cells. It appears to play an important role in keeping the balance of the intestinal immune system by partaking in the enormous task of differentiating between pathogenic and commensal microbiota. Therefore, it seems likely that the loss of tolerance to pancreatic and/or intestinal GP2 modulates the pathophysiology of IBD and in particular that of CrD [17].

Autoimmunity to glycoprotein 2 in the pathophysiology of Crohn's disease

The pathophysiology in CrD remains to be elucidated. The current discourse in this context refers to an imbalance between tolerance to commensal microbiota or food-derived antigens and immune responses to pathogens [1]. Thus, mucosal inflammation observed in CrD is triggered by such dysregulation of the innate as well as adaptive immune responses [1, 75]. A molecule like GP2 interacting with FimH positive microbes and facilitating the phagocytosis thereof could play a major role in triggering and perpetuating inflammatory processes in CrD. In fact, data demonstrating specific pathogenic species to be linked with CrD have been lacking so far. Notwithstanding, high concentrations of mucosal microbes and especially adhesive bacteria which interact with PP have been found in patients with CrD [76–78]. Furthermore, gastrointestinal infections appear to pose a higher risk for triggering CrD inflammation [79]. The de novo development of CrD seen in pouchitis patients suffering from UC initially could be an attractive model to shed light on the induction of CrD-like inflammatory processes [58]. As mentioned before, the IPAA performed after proctocolectomy in these patients brings about a change in the intestinal flora being in contact with the small bowel epithelium [21]. Glycoprotein 2 as a specific M cell receptor in the FAE of the PP is bound to interact with this new microbial environment and could be involved in the immune dysbalance leading to inflammation due to its reported immunomodulating role. The development of autoreactivity to GP2 observed in a part of such patients supports the assumption loss of tolerance to GP2 leads to or partakes in CrD inflammation. Even the more interesting, the fact anti-GP2 levels are in particular elevated in pouchitis patients not receiving probiotics compared to those which do underscores the link of autoimmunity to GP2 with the intestinal microbiota [58].

Furthermore, GP2 secreted by the pancreas into the intestine and not digested by zymogenes can also modulate these inflammatory processes. Currently, they are believed to be triggered and/or perpetuated by an increased leakiness of the epithelial barrier, disturbance of innate epithelial immune mechanisms, and disturbance of antigen recognition as well as processing of professional and atypical antigen-presenting cells [80–82]. Noteworthy, a disturbed regulatory and effector T cell balance appears also to be involved [83, 84]. Thus, the reported regulatory T cell dependent immunomodulating

role of GP2 is another intriguing phenomenon that needs to be taken into account in this context [71]. Further studies are warranted to investigate whether this putative regulatory mechanism plays a role in the pathophysiology of CrD.

Emerging evidence suggests PP which are particularly abundant in the distal part of the ileum to be potential sites of the inflammatory onset in CrD [85–87]. Thus, GP2 expressed on the surface of M cells in the PP appears to be located in the very center of CrD inflammation. Intriguingly, certain pathogenic bacteria such as *S. typhimurium* bind to and induce the transformation of M cells from normal intestinal epithelial cells [77, 78]. Since GP2 expression is elevated in the targeted tissue of patients with CrD compared to patients with UC this would be in line with the previous finding and provide a further hint for the putative role of pathogenic bacteria in triggering CrD inflammatory processes. The association of autoreactivity to GP2 with mainly ileal location of disease seems to support a possible role of autoimmunity in CrD [88, 89].

Intriguingly, loss of tolerance against another receptor in the gastrointestinal tract binding to potential pathogens has been shown for the asialoglycoprotein receptor on hepatocytes. Indeed, this is one of very few organ-specific autoantigenic targets in patients with autoimmune liver diseases and, in particular, in autoimmune hepatitis [90–92]. These findings cumulate in support of the emerging close interplay between infection and autoimmunity. Thus, infectious agents recognized by surface receptors and internalized by epithelial cells may be involved in the breakdown of immunological tolerance to the receptor under investigation.

Provided, GP2 represents a major self-target in CrD inflammation and participates in the triggering events leading to the maintenance of immunological intolerance seen in this disease, a GP2-specific response may play a pathophysiological role in CrD. Thus, humoral loss of tolerance to GP2 would not be considered an epiphenomenon or a bystander effect of unrelated inflammatory phenomena in CrD [17].

Apart from IgG and IgM, PAB of the IgA isotype can be found in patients with CrD [34]. Consequently, anti-GP2 IgA has been demonstrated in PAB-positive CrD sera recently [93, 94]. Remarkably, anti-GP2 IgA appears to be elevated in patients suffering from celiac disease (CeD). This feature is another piece of evidence in support of the hypothesis that loss of tolerance relates closely to the impairment of the epithelial barrier [95] (Roggenbuck, unpublished results). Werner et al. have shown that anti-GP2 IgA can be detected at higher levels in feces of

patients with pouchitis compared with their anti-GP2 IgG levels [58]. Thus, secreted anti-GP2 IgA being a dimeric molecule can theoretically bind to membrane anchored GP2 on M cell and crosslink GP2 opsonized FimH positive pathogens increasing the bacterial uptake of an already inflamed intestinal mucosa.

In summary, the large body of evidence regarding GP2's putative pathophysiological functions reported so far warrants further investigation. In particular anti-GP2 and their association with the disease phenotype in CrD can be helpful elucidating the remaining mysteries of IBD [96].

Serology of Crohn's disease and humoral loss of tolerance to GP2

The role of anti-GP2 in the serological diagnosis of IBD has been reviewed extensively [17, 51]. Due to the introduction of ELISAs for the detection of anti-GP2, quantification of these autoantibodies as a result of the humoral break of tolerance has become available in clinical practice [46]. Studies published so far revealed a prevalence of anti-GP2 by these novel ELISAs ranging from 25% to 30% in patients with CrD. In contrast, patients with UC demonstrated significantly less anti-GP2 (9%–12%) [51]. Patients with, in particular, overt CeD appear also to develop auto-reactivity to GP2 [95] (Roggenbuck, unpublished results). In CeD, anti-GP2 IgA correlated with CeD-specific anti-transglutaminase and antideamidated gliadin IgA giving support to the hypothesis that a leaky gut induces or facilitates this break of tolerance [97]. Excluding patients with CeD and UC, the specificity of anti-GP2 for CrD in comparison with non-intestinal disease controls is about 98%.

Anti-GP2 may be helpful especially in the case of undetermined colitis to predict the course of disease [98]. Indeed, in 10%–15% of IBD cases it is difficult to differentiate between CrD and UC and anti-GP2 antibodies may offer a complementary tool to stratify this specific group of patients [99, 100].

As other antibodies to microbial polypeptides, glycoproteins, and glycans have been reported in patients with CrD, specific antibody profiling could increase the differentiating power of the serological diagnosis of certain IBD entities [101]. Apart from the well-established antibodies to *Saccharomyces cerevisiae* (ASCA), antibodies to the outer membrane porin C (OmpC), I2 protein, CBir1-flagellin, laminaribioside carbohydrate, chitobioside carbohydrate, and mannobioside carbohydrate can

be candidates for CrD-specific markers in the context of antibody profiling [102]. These antimicrobial antibodies have been correlated with the severity of disease and the clinical phenotype in CrD [38, 103–107]. Antibody profiling has been considered a promising new diagnostic tool for other autoimmune disorders characterized by multiple autoantibody specificities such as those of antiphospholipid syndrome or rheumatoid arthritis [108, 109].

Taking into account the above raised link between infection and autoimmunity, patients with stricturing behavior demonstrating fibrostenotic complications have shown a higher prevalence of anti-GP2 IgG [88, 110, 111]. In contrast, CrD patients with penetrating disease seem to have a significantly lower prevalence of anti-GP2 IgG. Such a differential expression of anti-GP2 could not be confirmed for ASCA which are found elevated in both conditions. According to the Montreal classification of CrD, patients with ileocolonic location have a significantly higher prevalence of anti-GP2, whereas CrD patients with colonic location have been shown to demonstrate a significantly diminished prevalence thereof [88, 112]. Furthermore, occurrence of anti-GP2 autoantibodies was significantly more prevalent in CrD patients with young age at onset of disease (<16 years). These findings provide evidence for the assumption loss of tolerance to GP2 is associated with the phenotype in patients with CrD. Anti-GP2 may be a promising candidate for a fibrosis marker in CrD and, thus, support further stratification of CrD patients.

Despite emerging evidence, (auto)antibody-based disease prediction or stratification and correlation of (auto)antibody titers with disease activity or clinical symptoms remains to be elucidated in CrD [94, 113].

Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.

Research funding: None declared.

Employment or leadership: Dirk Roggenbuck is a shareholder of GA Generic Assays GmbH and Medipan GmbH. Both companies are diagnostic manufacturers. All other authors declare that they have no competing financial interests.

Honorarium: None declared.

Received September 23, 2013; accepted October 22, 2013; previously published online November 14, 2013

References

- Baumgart DC, Carding SR. Inflammatory bowel disease: cause and immunobiology. *Lancet* 2007;369:1627–40.
- Shivananda S, Lennard-Jones J, Logan R, Fear N, Price A, Carpenter L, et al. Incidence of inflammatory bowel disease across Europe: is there a difference between north and south? Results of the European collaborative study on inflammatory bowel disease (EC-IBD). *Gut* 1996;39:690–7.
- Logan I, Bowlus CL. The geoepidemiology of autoimmune intestinal diseases. *Autoimmun Rev* 2010;9:A372–8.
- Molodecky NA, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, et al. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology* 2012;142:46–54.
- Schirbel A, Reichert A, Roll S, Baumgart DC, Buning C, Wittig B, et al. Impact of pain on health-related quality of life in patients with inflammatory bowel disease. *World J Gastroenterol* 2010;16:3168–77.
- Lennard-Jones JE. Classification of inflammatory bowel disease. *Scand J Gastroenterol Suppl* 1989;170:2–6.
- Baumgart DC, Sandborn WJ. Inflammatory bowel disease: clinical aspects and established and evolving therapies. *Lancet* 2007;369:1641–57.
- Bernstein CN, Blanchard JF, Kliwer E, Wajda A. Cancer risk in patients with inflammatory bowel disease: a population-based study. *Cancer* 2001;15:854–62.
- Eaden JA, Abrams KR, Mayberry JF. The risk of colorectal cancer in ulcerative colitis: a meta-analysis. *Gut* 2001;48:526–35.
- Laukoetter MG, Mennigen R, Hannig CM, Osada N, Rijcken E, Vowinkel T, et al. Intestinal cancer risk in Crohn's disease: a meta-analysis. *J Gastrointestinal Surg* 2011;15:576–83.
- Engel MA, Khalil M, Neurath MF. Highlights in inflammatory bowel disease – from bench to bedside. *Clin Chem Lab Med* 2012;50:1229–35.
- Broberger O, Perlmann P. Autoantibodies in human ulcerative colitis. *J Exp Med* 1959;110:657–74.
- Walker JE. Possible diagnostic test for Crohn's disease by use of buccal mucosa. *Lancet* 1978;2:759–60.
- Stocker W, Otte M, Ulrich S, Normann D, Finkbeiner H, Stocker K, et al. Autoimmunity to pancreatic juice in Crohn's disease. Results of an autoantibody screening in patients with chronic inflammatory bowel disease. *Scand J Gastroenterol Suppl* 1987;139:41–52.
- Saxon A, Shanahan F, Landers C, Ganz T, Targan S. A distinct subset of antineutrophil cytoplasmic antibodies is associated with inflammatory bowel disease. *J Allergy Clin Immunol* 1990;86:202–10.
- Dobric S, Popovic D, Nikolic M, Andrejevic S, Spuran M, Bonaci-Nikolic B. Anti-neutrophil cytoplasmic antibodies (ANCA) specific for one or several antigens: useful markers for subtypes of ulcerative colitis and associated primary sclerosing cholangitis. *Clin Chem Lab Med* 2012;50:503–9.
- Roggenbuck D, Reinhold D, Werner L, Schierack P, Bogdanos DP, Conrad K. Glycoprotein 2 antibodies in Crohn's disease. *Adv Clin Chem* 2013;60:187–208.
- Cuthbert AP, Sheila SA, Muddassar MM, King K, Hampe J, Croucher PJ, et al. The contribution of NOD2 gene mutations to the risk and site of disease in inflammatory bowel disease. *Gastroenterology* 2002;122:867–74.
- Franke A, McGovern DP, Barrett JC, Wang K, Radford-Smith GL, Ahmad T, et al. Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci. *Nat Genet* 2010;42:1118–25.
- Sanders ME, Guarner F, Guerrant R, Holt PR, Quigley EM, Sartor RB, et al. An update on the use and investigation of probiotics in health and disease. *Gut* 2013;62:787–96.
- Simren M, Barbara G, Flint HJ, Spiegel BM, Spiller RC, Vanner S, et al. Intestinal microbiota in functional bowel disorders: a Rome foundation report. *Gut* 2012;62:159–76.
- Sheil B, Shanahan F, O'Mahony L. Probiotic effects on inflammatory bowel disease. *J Nutr* 2007;137:819S–24S.
- Jostins L, Ripke S, Weersma RK, Duerr RH, McGovern DP, Hui KY, et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature* 2012;491:119–24.
- Stöcker W, Otte M, Ulrich S, Normann D, Stöcker K, Jantschek G. Autoantikörper gegen exokrines pankreas und gegen intestinale becherzellen in der diagnostik des morbus Crohn und der colitis ulcerosa. *Dtsch Med Wochenschr* 1984;109:1963–9.
- Silverberg MS, Satsangi J, Ahmad T, Arnott ID, Bernstein CN, Brant SR, et al. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: Report of a working party of the 2005 Montreal world congress of gastroenterology. *Can J Gastroenterol* 2005;19(Suppl A):5–36.
- Lakatos PL, Altorjay I, Szamosi T, Palatka K, Vitalis Z, Tumpek J, et al. Pancreatic autoantibodies are associated with reactivity to microbial antibodies, penetrating disease behavior, perianal disease, and extraintestinal manifestations, but not with NOD2/CARD15 or TLR4 genotype in a Hungarian IBD cohort. *Inflamm Bowel Dis* 2009;15:365–74.
- Goischke EM, Zilly W. Klinische bedeutung organspezifischer antikörper bei colitis ulcerosa und morbus Crohn. *Z Gastroenterol* 1992;30:319–24.
- Barthet M, Hastier P, Bernard JP, Bordes G, Frederick J, Allio S, et al. Chronic pancreatitis and inflammatory bowel disease: true or coincidental association? *Am J Gastroenterol* 1999;94:2141–8.
- Spiess SE, Braun M, Vogelsang RL, Craig RM. Crohn's disease of the duodenum complicated by pancreatitis and common bile duct obstruction. *Am J Gastroenterol* 1992;87:1033–6.
- Desplat-Jego S, Johanet C, Escande A, Goetz J, Fabien N, Olsson N, et al. Update on anti-saccharomyces cerevisiae antibodies, anti-nuclear associated anti-neutrophil antibodies and antibodies to exocrine pancreas detected by indirect immunofluorescence as biomarkers in chronic inflammatory bowel diseases: results of a multicenter study. *World J Gastroenterol* 2007;13:2312–8.
- Schoepfer AM, Trummer M, Seeholzer P, Seibold-Schmid B, Seibold F. Discriminating IBD from IBS: comparison of the test performance of fecal markers, blood leukocytes, CRP, and IBD antibodies. *Inflamm Bowel Dis* 2008;14:32–9.
- Joossens S, Vermeire S, Van SK, Godefroid G, Claessens G, Pierik M, et al. Pancreatic autoantibodies in inflammatory bowel disease. *Inflamm Bowel Dis* 2004;10:771–7.

33. Kovacs M, Lakatos PL, Papp M, Jacobsen S, Nemes E, Polgar M, et al. Pancreatic autoantibodies and autoantibodies against goblet cells in pediatric patients with inflammatory bowel disease (IBD). *J Pediatr Gastroenterol Nutr* 2012;55:429–35.
34. Seibold F, Weber P, Jenss H, Wiedmann KH. Antibodies to a trypsin sensitive pancreatic antigen in chronic inflammatory bowel disease: specific markers for a subgroup of patients with Crohn's disease. *Gut* 1991;32:1192–7.
35. Fricke H, Birkhofer A, Folwaczny C, Meister W, Scriba PC. Characterization of antigens from the human exocrine pancreatic tissue (Pag) relevant as target antigens for autoantibodies in Crohn's disease. *Eur J Clin Invest* 1999;29:41–5.
36. Koutsoumpas A, Polymeros D, Tsiamoulos Z, Smyk D, Karamanolis G, Triantafyllou K, et al. Peculiar antibody reactivity to human connexin 37 and its microbial mimics in patients with Crohn's disease. *J Crohns Colitis* 2011;5:101–9.
37. Vermeulen N, Op de Beeck K, Vermeire S, Van SK, Michiels G, Ballet V, et al. Identification of a novel autoantigen in inflammatory bowel disease by protein microarray. *Inflamm Bowel Dis* 2011;17:1291–300.
38. Saito H, Fukuda Y, Katsuragi K, Tanaka M, Satomi M, Shimoyama T, et al. Isolation of peptides useful for differential diagnosis of Crohn's disease and ulcerative colitis. *Gut* 2003;52:535–40.
39. Conrad K, Hausdorf G, Feist E, Reinhold D, Jungblut PR, Porstmann T, et al. Identification of GP2 as the major autoantigen of pancreatic autoantibodies. Report on the 6th congress on autoimmunity, Porto, Portugal, A1356, 2008.
40. Roggenbuck D, Hausdorf G, Martinez-Gamboa L, Reinhold D, Buttner T, Jungblut PR, et al. Identification of GP2, the major zymogen granule membrane glycoprotein, as the autoantigen of pancreatic antibodies in Crohn's disease. *Gut* 2009;58:1620–8.
41. Stöcker W, Glocker MO, Probst C, Teegen B, Friedrich A, Sokolowski S, et al. Identification of two different proteoglycans from exocrine pancreas as the long sought after autoantigens in Crohn's disease: CUZD1 and GP2. Report on the 6th congress on autoimmunity, Porto, Portugal, A1177, 2008.
42. Komorowski L, Teegen B, Probst C, Aulinger-Stocker K, Sina C, Fellermann K, et al. Autoantibodies against exocrine pancreas in Crohn's disease are directed against two antigens: the glycoproteins CUZD1 and GP2. *J Crohns Colitis* 2013;7:780–90.
43. Seibold F, Mörk H, Tanza S, Müller A, Holzhüter C, Weber P, et al. Pancreatic autoantibodies in Crohn's disease: a family study. *Gut* 1997;40:481–4.
44. Rindler MJ, Hoops TC. The pancreatic membrane protein GP-2 localizes specifically to secretory granules and is shed into the pancreatic juice as a protein aggregate. *Eur J Cell Biol* 1990;53:154–63.
45. Liaskos C, Rigopoulou E, Orfanidou T, Bogdanos DP, Papandreou CN. CUZD1 and anti-CUZD1 antibodies as markers of cancer and inflammatory bowel diseases. *Clin Dev Immunol* 2013;2013:968041.
46. Roggenbuck D, Reinhold D, Wex T, Gohl A, von Arnim U, Malfertheiner P, et al. Autoantibodies to GP2, the major zymogen granule membrane glycoprotein, are new markers in Crohn's disease. *Clin Chim Acta* 2011;412:718–24.
47. Roggenbuck D, Bogdanos D, Conrad K. Loss of tolerance to one or two major targets in Crohn's disease or just cross-reactivity? *J Crohns Colitis* 2013;7:e273–4.
48. Leung F, Soosaipillai A, Kulasingam V, Diamandis EP. CUB and zona pellucida-like domain-containing protein 1 (CUZD1): a novel serological biomarker for ovarian cancer. *Clin Biochem* 2012;45:1543–6.
49. Leong CT, Ong CK, Tay SK, Huynh H. Silencing expression of UO-44 (CUZD1) using small interfering RNA sensitizes human ovarian cancer cells to cisplatin in vitro. *Oncogene* 2007;26:870–80.
50. Leong CT, Ng CY, Ong CK, Ng CP, Ma ZS, Nguyen TH, et al. Molecular cloning, characterization and isolation of novel spliced variants of the human ortholog of a rat estrogen-regulated membrane-associated protein, UO-44. *Oncogene* 2004;23:5707–18.
51. Bogdanos DP, Rigopoulou EI, Smyk DS, Roggenbuck D, Reinhold D, Forbes A, et al. Diagnostic value, clinical utility and pathogenic significance of reactivity to the molecular targets of Crohn's disease specific-pancreatic autoantibodies. *Autoimmun Rev* 2011;11:143–8.
52. Hoops TC, Ivanov I, Cui Z, Colomer-Gould V, Rindler MJ. Incorporation of the pancreatic membrane protein GP-2 into secretory granules in exocrine but not endocrine cells. *J Biol Chem* 1993;268:25694–705.
53. Bonifacio E, Christie MR. Islet cell antigens in the prediction and prevention of insulin-dependent diabetes mellitus. *Ann Med* 1997;29:405–12.
54. Fukuoka S, Freedman SD, Scheele GA. A single gene encodes membrane-bound and free forms of GP-2, the major glycoprotein in pancreatic secretory (zymogen) granule membranes. *Proc Natl Acad Sci USA* 1991;88:2898–902.
55. Hase K, Kawano K, Nochi T, Pontes GS, Fukuda S, Ebisawa M, et al. Uptake through glycoprotein 2 of FimH(+) bacteria by M cells initiates mucosal immune response. *Nature* 2009;462:226–30.
56. Ohno H, Hase K. Glycoprotein 2 (GP2): grabbing the FimH bacteria into M cells for mucosal immunity. *Gut Microbes* 2010;1:407–10.
57. Terahara K, Yoshida M, Igarashi O, Nochi T, Pontes GS, Hase K, et al. Comprehensive gene expression profiling of Peyer's patch M cells, villous M-like cells, and intestinal epithelial cells. *J Immunol* 2008;180:7840–6.
58. Werner L, Sturm A, Roggenbuck D, Yahav L, Zion T, Meiriowitz E, et al. Antibodies against glycoprotein 2 are novel markers of intestinal inflammation in patients with an ileal pouch. *J Crohns Colitis* 2013;7:e522–32.
59. Werner L, Roggenbuck D, Dotan I. Reply to Dr. Pavlidis et al's letter. *J Crohns Colitis* 2013;7:e604–5.
60. Schmidt K, Dartsch H, Linder D, Kern HF, Kleene R. A submembranous matrix of proteoglycans on zymogen granule membranes is involved in granule formation in rat pancreatic acinar cells. *J Cell Sci* 2000;113:2233–42.
61. Kalus I, Hodel A, Koch A, Kleene R, Edwardson JM, Schrader M. Interaction of syncollin with GP-2, the major membrane protein of pancreatic zymogen granules, and association with lipid microdomains. *Biochem J* 2002;362:433–42.
62. Colomer V, Lal K, Hoops TC, Rindler MJ. Exocrine granule specific packaging signals are present in the polypeptide moiety of the pancreatic granule membrane protein GP2 and in amylase: implications for protein targeting to secretory granules. *EMBO J* 1994;13:3711–9.

63. Yu S, Michie SA, Lowe AW. Absence of the major zymogen granule membrane protein, GP2, does not affect pancreatic morphology or secretion. *J Biol Chem* 2004;279: 50274–9.
64. Hoops TC, Rindler MJ. Isolation of the cDNA encoding glycoprotein-2 (GP-2), the major zymogen granule membrane protein. Homology to uromodulin/Tamm-Horsfall protein. *J Biol Chem* 1991;266:4257–63.
65. Fukuoka S, Freedman SD, Yu H, Sukhatme VP, Scheele GA. GP-2/THP gene family encodes self-binding glycosylphosphatidylinositol-anchored proteins in apical secretory compartments of pancreas and kidney. *Proc Natl Acad Sci USA* 1992;89: 1189–93.
66. Rindler MJ, Naik SS, Naik L, Hoops TC, Peraldi MN. Uromodulin (Tamm-Horsfall glycoprotein/uromucoid) is a phosphatidylinositol-linked membrane protein. *J Biol Chem* 1990;265:20784–9.
67. Kobayashi K, Yanagihara K, Ishiguro K, Fukuoka S. GP2/THP gene family of self-binding, GPI-anchored proteins forms a cluster at chromosome 7F1 region in mouse genome. *Biochem Biophys Res Commun* 2004;322:659–64.
68. Devuyst O, Dahan K, Pirson Y. Tamm-Horsfall protein or uromodulin: new ideas about an old molecule. *Nephrol Dial Transplant* 2005;20:1290–4.
69. Dou W, Thompson-Jaeger S, Laulederkind SJ, Becker JW, Montgomery J, Ruiz-Bustos E, et al. Defective expression of Tamm-Horsfall protein/uromodulin in COX-2-deficient mice increases their susceptibility to urinary tract infections. *Am J Physiol Renal Physiol* 2005;289:F49–60.
70. Yu S, Lowe AW. The pancreatic zymogen granule membrane protein, GP2, binds *Escherichia coli* type 1 fimbriae. *BMC Gastroenterol* 2009;9:58.
71. Werner L, Paclik D, Fritz C, Reinhold D, Roggenbuck D, Sturm A. Identification of pancreatic glycoprotein 2 as an endogenous immunomodulator of innate and adaptive immune responses. *J Immunol* 2012;189:2774–83.
72. Säemann MD, Weichhart T, Zeyda M, Staffler G, Schunn M, Stuhlmeier KM, et al. Tamm-Horsfall glycoprotein links innate immune cell activation with adaptive immunity via a toll-like receptor-4-dependent mechanism. *J Clin Invest* 2005;115: 468–75.
73. Holzl MA, Hofer J, Kovarik JJ, Roggenbuck D, Reinhold D, Goihl A, et al. The zymogen granule protein 2 (GP2) binds to scavenger receptor expressed on endothelial cells I (SREC-I). *Cell Immunol* 2011;267:88–93.
74. Abraham C, Medzhitov R. Interactions between the host innate immune system and microbes in inflammatory bowel disease. *Gastroenterology* 2011;140:1729–37.
75. Baumgart DC, Metzke D, Schmitz J, Scheffold A, Sturm A, Wiedenmann B, et al. Patients with active inflammatory bowel disease lack immature peripheral blood plasmacytoid and myeloid dendritic cells. *Gut* 2005;54:228–36.
76. Darfeuille-Michaud A, Boudeau J, Bulois P, Neut C, Glasser AL, Barnich N, et al. High prevalence of adherent-invasive *Escherichia coli* associated with ileal mucosa in Crohn's disease. *Gastroenterology* 2004;127:412–21.
77. Chassaing B, Rolhion N, Vallee A, Salim SY, Prorok-Hamon M, Neut C, et al. Crohn disease-associated adherent-invasive *E. coli* bacteria target mouse and human Peyer's patches via long polar fimbriae. *J Clin Invest* 2011;121:966–75.
78. Dreux N, Denizot J, Martinez-Medina M, Mellmann A, Billig M, Kisiela D, et al. Point mutations in FimH adhesin of Crohn's disease-associated adherent-invasive *Escherichia coli* enhance intestinal inflammatory response. *PLoS Pathog* 2013;9:e1003141.
79. Garcia Rodriguez LA, Ruigomez A, Panes J. Acute gastroenteritis is followed by an increased risk of inflammatory bowel disease. *Gastroenterology* 2006;130:1588–94.
80. Soderholm JD, Olaison G, Lindberg E, Hannestad U, Vindels A, Tysk C, et al. Different intestinal permeability patterns in relatives and spouses of patients with Crohn's disease: an inherited defect in mucosal defence? *Gut* 1999;44:96–100.
81. Cario E, Podolsky DK. Differential alteration in intestinal epithelial cell expression of toll-like receptor 3 (TLR3) and TLR4 in inflammatory bowel disease. *Infect Immun* 2000;68:7010–7.
82. Franchimont D, Vermeire S, El HH, Pierik M, Van SK, Gustot T, et al. Deficient host-bacteria interactions in inflammatory bowel disease? The toll-like receptor (TLR)-4 Asp299gly polymorphism is associated with Crohn's disease and ulcerative colitis. *Gut* 2004;53:987–92.
83. Josefowicz SZ, Niec RE, Kim HY, Treuting P, Chinen T, Zheng Y, et al. Extrathymically generated regulatory T cells control mucosal TH2 inflammation. *Nature* 2012;482:395–9.
84. Ina K, Itoh J, Fukushima K, Kusugami K, Yamaguchi T, Kyokane K, et al. Resistance of Crohn's disease T cells to multiple apoptotic signals is associated with a Bcl-2/Bax mucosal imbalance. *J Immunol* 1999;163:1081–90.
85. Gullberg E, Soderholm JD. Peyer's patches and M cells as potential sites of the inflammatory onset in Crohn's disease. *Ann N Y Acad Sci* 2006;1072:218–32.
86. Olaison G, Smedh K, Sjobahl R. Recurrence of Crohn's disease in the neo-terminal ileum and colonic factors. *Lancet* 1991;338:1401.
87. Van Kruiningen HJ, West AB, Freda BJ, Holmes KA. Distribution of Peyer's patches in the distal ileum. *Inflamm Bowel Dis* 2002;8:180–5.
88. Bogdanos DP, Roggenbuck D, Reinhold D, Wex T, Pavlidis P, von Arnim U, et al. Pancreatic-specific autoantibodies to glycoprotein 2 mirror disease location and behaviour in younger patients with Crohn's disease. *BMC Gastroenterol* 2012;12:102.
89. Pavlidis P, Romanidou O, Roggenbuck D, Mytilinaiou M, Al-Sulttan F, Liaskos C, et al. Ileal inflammation may trigger the development of GP2-specific pancreatic autoantibodies in patients with Crohn's disease. *Clin Dev Immunol* 2012;2012:640835.
90. Hausdorf G, Roggenbuck D, Feist E, Buttner T, Jungblut PR, Conrad K, et al. Autoantibodies to asialoglycoprotein receptor (ASGPR) measured by a novel ELISA – revival of a disease-activity marker in autoimmune hepatitis. *Clin Chim Acta* 2009;408:19–24.
91. Rigopoulou EI, Roggenbuck D, Smyk DS, Liaskos C, Mytilinaiou MG, Feist E, et al. Asialoglycoprotein receptor (ASGPR) as target autoantigen in liver autoimmunity: lost and found. *Autoimmun Rev* 2012;11:143–8.
92. Roggenbuck D, Mytilinaiou MG, Lapin SV, Reinhold D, Conrad K. Asialoglycoprotein receptor (ASGPR): a peculiar target of liver-specific autoimmunity. *Autoimmun Highlights* 2012;3:119–25.
93. Pavlidis P, Forbes A, Bogdanos DP. Antibodies to glycoprotein 2 (GP2) in patients with inflammatory bowel diseases from UK. *Clin Chim Acta* 2011;412:1163–4.

94. Op de Beeck K, Vermeire S, Rutgeerts P, Bossuyt X. Antibodies to GP2, the major zymogen granule membrane glycoprotein, in inflammatory bowel diseases. *Gut* 2010;61:162–4.
95. Bonaci-Nikolic B, Spuran M, Andrejevic S, Nikolic M. Autoantibodies to GP2, the major zymogen granule membrane glycoprotein, in patients with gluten-sensitive enteropathy: a possible serological trap. *Clin Chim Acta* 2012;413:822–3.
96. Colombel JF, Watson AJ, Neurath MF. The 10 remaining mysteries of inflammatory bowel disease. *Gut* 2008;57:429–33.
97. Fasano A. Leaky gut and autoimmune diseases. *Clin Rev Allergy Immunol* 2012;42:71–8.
98. Price A. Overlap in the spectrum of non-specific inflammatory bowel disease – ‘colitis indeterminate’. *J Biol Chem* 1978;265:567–77.
99. Guindi M, Riddell RH. Indeterminate colitis. *J Clin Pathol* 2004;57:1233–44.
100. Wells AD, McMillan I, Price AB, Ritchie JK, Nicholls RJ. Natural history of indeterminate colitis. *Br J Surg* 1991;78:179–81.
101. Conrad K, Schmechta H, Klafki A, Lobeck G, Uhlig HH, Gerdi S, et al. Serological differentiation of inflammatory bowel diseases. *Eur J Gastroenterol Hepatol* 2002;14:129–35.
102. Basso D, Zambon CF, Plebani M. Inflammatory bowel diseases: from pathogenesis to laboratory testing. *Clin Chem Lab Med* 2014;52:471–81.
103. Vermeire S, Vermeulen N, Van AG, Bossuyt X, Rutgeerts P. (Auto)antibodies in inflammatory bowel diseases. *Gastroenterol Clin North Am* 2008;37:429–38, vii.
104. Rieder F, Schleider S, Wolf A, Dirmeier A, Strauch U, Obermeier F, et al. Association of the novel serologic anti-glycan antibodies anti-laminarin and anti-chitin with complicated Crohn's disease behavior. *Inflamm Bowel Dis* 2010;16:263–74.
105. Dotan I. New serologic markers for inflammatory bowel disease diagnosis. *Dig Dis* 2010;28:418–23.
106. Papp M, Altorjay I, Dotan N, Palatka K, Foldi I, Tumpek J, et al. New serological markers for inflammatory bowel disease are associated with earlier age at onset, complicated disease behavior, risk for surgery, and NOD2/CARD15 genotype in a Hungarian IBD cohort. *Am J Gastroenterol* 2008;103:665–81.
107. Papp M, Foldi I, Altorjay I, Palyu E, Udvardy M, Tumpek J, et al. Anti-microbial antibodies in celiac disease: trick or treat? *World J Gastroenterol* 2009;15:3891–900.
108. Conrad K, Roggenbuck D, Reinhold D, Dorner T. Profiling of rheumatoid arthritis associated autoantibodies. *Autoimmun Rev* 2009;9:431–5.
109. Roggenbuck D, Egerer K, Feist E, Burmester GR, Dorner T. Antiphospholipid antibody profiling: association with the clinical phenotype of antiphospholipid syndrome? Comment on the article by Otomo et al. *Arthritis Rheum* 2012;64:2807–8.
110. Rieder F, Franke A, Dirmeier A, Lopez R, Lang S, Roggenbuck D, et al. Mo1247 serologic anti-GP2 antibodies are associated with strictures and need for surgical resection in Crohn's disease. *Gastroenterology* 2013;144:S617.
111. Roggenbuck D, Humbel RL, Reinhold D, Bogdanos DP, Conrad K, Laass MW. Glycoprotein 2 antibodies in inflammatory bowel disease – no association with disease phenotype? *J Pediatr Gastroenterol Nutr* 2012;56:e5.
112. Somma V, Ababneh H, Ababneh A, Gatti S, Romagnoli V, Bendia E, et al. The novel Crohn's disease marker anti-GP2 antibody is associated with ileocolonic location of disease. *Gastroenterol Res Pract* 2013;2013:683824.
113. Roggenbuck D, Reinhold D, Wex T, von Arnim U, Malfertheiner P, Sturm A, et al. Authors' response: antibodies to GP2, the major zymogen granule membrane glycoprotein, are specific for Crohn's disease and may reflect treatment response. *Gut* 2012;61:164–5.
114. Demirsoy H, Ozdil K, Ersoy O, Kesici B, Karaca C, Alkim C, et al. Anti-pancreatic antibody in Turkish patients with inflammatory bowel disease and first-degree relatives. *World J Gastroenterol* 2010;16:5732–8.
115. Klebl FH, Bataille F, Huy C, Hofstädter F, Schölmerich J, Rogler G. Association of antibodies to exocrine pancreas with subtypes of Crohn's disease. *Eur J Gastroenterol Hepatol* 2005;17:73–7.
116. Koutroubakis IE, Drygiannakis D, Karmiris K, Drygiannakis I, Makreas S, Kouroumalis EA. Pancreatic autoantibodies in Greek patients with inflammatory bowel disease. *Digest Dis Sci* 2005;50:2339–4.
117. Folwaczny C, Noehl N, Endres SP, Loeschke K, Fricke H. Antineutrophil and pancreatic autoantibodies in first degree relatives of patients with inflammatory bowel disease. *Scand J Gastroenterol* 1998;33:523–8.
118. Homsak E, Micetic-Turk D, Bozic B. Autoantibodies pANCA, GAB and PAB in inflammatory bowel disease: prevalence, characteristic and diagnostic value. *Wien Klin Wochenschr* 2010;122:19–25.
119. Lawrance IC, Hall A, Leong R, Pearce C, Murray K. A comparative study of goblet cell and pancreatic exocrine autoantibodies combined with ASCA and pANCA in Chinese and Caucasian patients with IBD. *Inflamm Bowel Dis* 2005;11:890–7.
120. Schoepfer AM, Schaffer T, Mueller S, Flogerzi B, Vassella E, Seibold-Schmidt B, et al. Phenotypic associations of Crohn's disease with antibodies to flagellins A4-Fla2 and Fla-X, ASCA, p-ANCA, PAB, and NOD2 mutations in a Swiss cohort. *Inflamm Bowel Dis* 2009;15:1358–67.



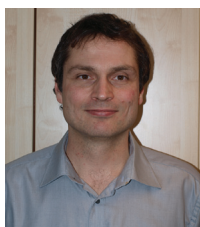
Dirk Roggenbuck was born in Gotha, Germany, in 1965. He graduated in biochemistry from the Russian State Medical University

in 1992. In the same year he received the license to practice medicine. In 1994 he obtained a PhD degree in Immunology at the Humboldt-University Berlin, Universitätsmedizin Charité Berlin. After postgraduate studies in molecular biology at the Institute of Biochemistry, Universitätsmedizin Charité Berlin, he worked as a senior scientist at Dr. Fooke Laboratorien GmbH, Germany. In 1997, he joined the MEDIPAN DIAGNOSTICA GmbH, Germany, as R/D Manager. Currently, he works as managing director of GA Generic Assays GmbH, Germany and MEDIPAN. In 2011, he became a senior Lecturer at Lausitz University of Applied Sciences, Senftenberg, Germany. In 2012 he was appointed Professor of Molecular Diagnostics and Quality Management at the same university, now

Brandenburg Technical University Cottbus-Senftenberg. He is a member of the German Society of Clinical Chemistry and Laboratory Medicine and a member of the editorial boards of *Clinical Immunology* (Russian) and *Eurasian Journal of Rheumatology*.



Dirk Reinhold was trained as a MD (1987, Moscow, Russia). Following this, he moved to Magdeburg, Germany and specialized in Immunology. His scientific interest includes autoimmunity, cytokines and proteolytic enzymes. He is currently research group leader and head of the laboratory for autoimmune diagnostic at the Institute of Molecular and Clinical Immunology at the Otto-von-Guericke University Magdeburg.



Peter Schierack graduated in Veterinary Medicine in 1999 at the Freie Universität Berlin (Germany). He was a PhD student at the Department of Molecular Parasitology at the Humboldt-Universität in Berlin and obtained his PhD degree in 2002. As a Postdoc he worked at the Institute of Microbiology and Epizootics at the Freie Universität Berlin (2002–2007) specializing in bacterial pathogens and commensals and at the University of Applied Sciences Senftenberg (Germany, 2007–2013) developing technologies for medical diagnostic. He habilitated in microbiology in 2010, has been responsible for the Microbiology Department at the hospital in Senftenberg since 2010 and is now a Professor of Multiparametric Diagnostics at the Brandenburg University of Technology Cottbus-Senftenberg, Germany.



Dimitrios P. Bogdanos, MD, PhD, head of Cellular Immunotherapy and Molecular Immunodiagnostics, Institute for Research and Technology. Professor of Immunopathology, Division of Transplantation Immunology and Mucosal Biology, King's College London School of Medicine. He is a recipient of the CLS award, Higher Education

Council for England (HEFCE) and the Dame Sheila Sherlock Medal. From September 2011, he is also a member of the Academic Faculty of the School of Health Sciences, University of Thessaly. He is an editor, associate editor or member of the editorial board of 12 Journals. His research focus is mainly on liver immunopathology, autoimmune rheumatic diseases and inflammatory bowel diseases. He has published more than 150 papers in the field of autoimmunity, with an h-index of 35 and more than 3500 citations.



Karsten Conrad, MD, studied Human Medicine from 1976 to 1982 at the Charité University Medical School, Berlin and at the Medical Academy of Dresden, Germany. In 1982 he received his doctorate and license to practice medicine. In 1987 he was Board certified in Immunology and Director of laboratory at the Institute of Immunology of the Medical Academy in Dresden (since 1993 of the Medical Faculty of the Technical University). Since 1991 he has been the founder and chairman of the international "Dresden Symposia on Autoantibodies". In 2007 he was certified as "specialized immunologist" by the German Society for Immunology, his specialization is Clinical Immunology. Since 2011 he has been chairman of the Section Immunodiagnostics of the German Society for Clinical Chemistry and Laboratory Medicine.



Martin W. Laass is head of the Division for Pediatric Gastroenterology, Hepatology and Nutrition at the Children's University Hospital of the Technical University Dresden, Germany. He studied medicine at the Free University Berlin and completed his undergraduate medical degree at the Charité University Hospital of the Humboldt University Berlin, Germany. For his doctoral thesis he localized the gene for the Papillon-Lefevre syndrome by homozygosity mapping. He started his clinical work at the Children's University Hospital Dresden, where he completed his residency in pediatrics, neonatology and pediatric gastroenterology. His research interests include pathogenesis, diagnosis and therapy of gastrointestinal autoimmune diseases, particularly pediatric inflammatory bowel disease, celiac disease and primary sclerosing cholangitis.