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*J Immunol* 2012; 189:2774-2783; Prepublished online 13 August 2012; doi: 10.4049/jimmunol.1103190

http://www.jimmunol.org/content/189/6/2774

Supplementary Material

http://www.jimmunol.org/content/suppl/2012/08/13/jimmunol.1103190.DC1

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Identification of Pancreatic Glycoprotein 2 as an Endogenous Immunomodulator of Innate and Adaptive Immune Responses

Lael Werner,* Daniela Paclík,* Christina Fritz,† Dirk Reinhold,‡ Dirk Roggenbuck,† and Andreas Sturm*†

Pancreatic autoantibodies are Crohn disease-specific serologic markers. The function and immunological role of their recently identified autoantigen, glycoprotein 2 (GP2), are unknown. We therefore investigated the impact of GP2 on modulation of innate and adaptive immune responses to evaluate its potential therapeutic use in mucosal inflammation. Our data indicate a previously unknown function for GP2 as an immunomodulator. GP2 was ubiquitously expressed on cells vital to mucosal immune responses. The expression of GP2 was upregulated on activated human T cells, and it was further influenced by pharmaceutical TNF-α inhibitors. Recombinant GP2 significantly decreased human intestinal epithelial cells, mucosal and peripheral T cell proliferation, apoptosis, and activation, and it distinctly modulated cytokine secretion. Furthermore, intestinal epithelial cells stimulated with GP2 potently attracted T cells. In conclusion, we demonstrate a novel role for GP2 in immune regulation that could provide a platform for new therapeutic interventions in the treatment of Crohn disease. The Journal of Immunology, 2012, 189: 2774–2783.

Inflammatory bowel diseases (IBD), consisting of Crohn disease (CD) and ulcerative colitis (UC), is a chronic condition affecting a steadily rising number of patients worldwide. IBD results in significant social and economic costs, as it is complex, unpredictable, and incompletely understood.

Diagnosis of IBD and the differentiation between UC and CD are established by a combination of clinical, laboratory, radiologic, endoscopic, histopathologic, and serologic markers. The major serologic markers for IBD are antineutrophil cytoplasmic Abs, anti-Saccharomyces cerevisiae Abs, and Abs to outer membrane porin C of Escherichia coli. However, the significance of these autoantibodies for discriminating between UC and CD is still controversial (1), and thus the need for accurate serologic markers in IBD remains essentially.

Novel markers discovered in recent years, such as anti-chitobioside, anti-laminaribioside, and anti-mannobioside, are Abs directed against glycans (2). As such, glycans are being recognized as key compounds in mucosal immunology, and glycans are proposed to bridge innate and adaptive immune recognition and response (3). This realization led to a growing interest in Abs to carbohydrate (glycan)-based Ags, as cellular and humoral immune responses rely heavily on interactions between glycans and specific glycan-binding proteins (4). One such glycan-directed, CD-specific serological marker is pancreatic autoantibody (PAB), first described more than two decades ago (5). However, its antigenic epitope was unknown until recently.

In 2009, Roggenbuck et al. (6) identified glycoprotein 2 (GP2) as the major autoantigenic target of a PAB. Anti-GP2 IgG and IgA have been reported to be novel serologic parameters in CD (7). Their possible association with disease behavior and activity in CD is controversial (8). Why GP2 is an autoantibody target in patients with CD remains also elusive, and whether it plays a pathophysiological role in the development of the disease or is an epiphenomenon needs to be determined.

GP2 is a 78-kDa GPI-anchored protein. Initially, GP2 was proposed to be the major zymogen granule membrane in the pancreas (9), accounting for a large percentage of all of the zymogen (glyco) proteins. Upon stimulation, GP2 is shed into the pancreatic duct during exocrine secretion, and then into the intestine (10, 11), for a possible, yet unknown physiological function.

However, as GP2 knockout mice do not exhibit nutrient malabsorption or predisposition to pancreatitis (12), and GP2 overexpression does not influence secretory processes (13), another, previously unknown functional and physiological relevance for GP2 needs to be discerned. Remarkably, apart from the pancreas, GP2 has been shown to be overexpressed at the site of CD inflammation in the gut, in contrast to UC patients, supporting a pathophysiological role for this protein in the development of the disease (7).

The GP2 amino acid sequence exhibits 85% similarity to Tamm–Horsfall protein (THP; uromodulin) (14), suggesting that they might possess homologous functions. THP is the most abundant protein in urine, and autoantibodies to THP have also been reported in urinary tract inflammation (15). Moreover, THP has been implicated in several inflammatory kidney disorders (16), as well as in downregulation of both innate and adaptive immunity (17).
Thus, a similar immunological mechanism may be operative in the mode of action of GP2, and this needs further study.

Elucidation of the role played by GP2 in immunity, as well as the pathophysiological involvement of the GP2 Abs in IBD, could yield great benefit for medical intervention. As defects in T cell expansion and apoptosis are major events in triggering and perpetuating mucosal inflammation in IBD (18), restricting T cell expansion by GP2 might provide a new therapeutic option to restrict inflammation. Moreover, anti-GP2 Abs might serve as a diagnostic tool for recognizing different subtypes of CD.

As an anti-inflammatory function for THP has been described, we conceived a similar immune modulation for GP2. Thus, based on the knowledge currently available and our preliminary data, we hypothesize that anti-GP2 Abs not only differentiate CD from UC and healthy controls, but also that GP2 itself modulates innate and adaptive immune responses.

Materials and Methods

PBMCs from healthy volunteers were isolated using Ficoll-Hypaque (GE Healthcare, Otelfingen, Switzerland) as previously described (19, 20). Cells were cultured in RPMI 1640 (Life Technologies, Darmstadt, Germany) containing 10% FCS (Biochrom, Berlin, Germany) and 2.5% penicillin-streptomycin (Biochrom). Depletion of monocytes and regulatory T cells (Tregs; CD4+CD25+CD127dim–/− cells) from PBMCs was performed by negative selection using magnetic cell sorting kits (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer’s instructions. Approximately 95% of the isolated Tregs were CD4+CD25+. Those, 80–90% were confirmed by flow cytometry to be Foxp3+ (data not shown).

Lamina propria mononuclear cells (LPMCs) were isolated from mucosa of patients undergoing colectomy owing to noninflammatory disorders as previously described (19). Briefly, minced mucosa was digested in DTT (Sigma-Aldrich, Taufkirchen, Germany) plus DNase (Worthington Biochemical, Lakewood, NJ). Forty percent Percoll (GE Healthcare) was used to separate LPMCs. Separation of human intestinal epithelial cells (hI ECs) and intraepithelial lymphocytes (IELs) was performed on supernatant collected after the two dispase cycles, and collagenase (Roche Applied Science, Mannheim, Germany) was applied (Worthington Biochemical, Lakewood, NJ). After centrifugation, the supernatant was removed and then lysed with 100 μl 1 N HCl/isopropanol. Plates were read in a spectrophotometer at 570 nm.

GP2 PCR

Cells were stimulated as mentioned. For RNA isolation, cells were harvested and dissolved in RNA-Butterfly (Tel-Test, Friendswood, TX), with subsequent treatment with chloroform, isopropanol, and ethanol. RNA (1 μg) was taken for cDNA production using a kit from Invitrogen (Karlruhe, Germany). Negative controls were without reverse transcriptase. PCR amplification was performed with Invitrogen Master mix (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Primer sequences were as follows: GPR2, forward, 5′-CAATGTGCCATCCACCTGGGA-3′, reverse, 5′-ATGCCACCCATAAGCARC3′; β-actin, forward, 5′-CTGCATTTCGAAGAGATG-3′, reverse, 5′-TGAAGGATGTCTGGATGGATGC-3′. After predenaturation at 93°C for 5 min, 36 cycles were performed (30 s denaturation at 93°C, 30 s annealing at 62°C, and 30 s elongation at 72°C), followed by final elongation at 72°C for 5 min. Five microliters was loaded onto 2% agarose gel and visualized under UV light.

Phagocytosis assay

Uptake of FITC-labeled E. coli was performed with a commercial kit (Vybrant phagocytosis kit; Molecular Probes, Goettingen, Germany). Briefly, monocytes or T84 cells were incubated with different doses of GP2 for 2 h. Subsequently, GP2 was removed and FITC-labeled E. coli was added to the cells for 2 h. Next, nonphagocytosed E. coli were removed and trypan blue was added for 1 min to quench the extracellular probe. Phagocytosis was measured using a spectrophotometer. Cytochalasin D and nocardazole were purchased from Sigma-Aldrich.

Wound healing assay

T84 cells or rat IEC6 epithelial cells were grown to confluence on 60 mm tissue culture plates (BD Pharmingen). On the day of the experiment, cells were cultured with starvation medium (0.1% FCS) for 6 h. Afterward, a scratch was made as previously described (22) and 25 or 50 μg/ml GP2 was added for 16 h. Images were taken with a Nikon D70 camera. Cells migrating over the scratch were counted manually.

Transwell assay

In vitro migration of cells was assessed using Costar Transwells (Costar/Corning, Corning, NY). Filters were precoated with fibronectin (Milli-
pore, Schwabach, Germany) for 1 h at 37°C. Subsequently, cells (2–5 × 10^6) in 100 μl PBS0.1% BSA were placed in the upper chamber. The lower chamber contained 600 μl RPMI 1640 alone, RPMI 1640 with GP2, or T84 cells pretreated with GP2. Cells were allowed to migrate for 3 h at 37°C. Afterward, cells that transmigrated to the lower well were collected and counted. The percentage of migrating cells was calculated by flow cytometry or a counting grid. For flow cytometry, cells were acquired by FACS for a fixed period of time (90 s). Percentage migration was calculated as the number of migrating cells divided by the total number of cells placed in the upper wells, counted in the same time period, and multiplied by 100.

ELISA

ELISA kits for anti-GP2 (Generic Assays, Dahlewitz, Germany) were performed on sera. Sera were obtained from healthy volunteers or patients suffering from CD, UC, irritable bowel syndrome (IBS), or pancreatitis. Patients' demographics, as well as medications taken, are detailed in Supplemental Table I. In mentioned experiments, supernatants were collected and ELISAs for β-defensin-2 (PeproTech, Hamburg, Germany), CXCL8, IL6, TSLP, TNF-α (R&D, Minneapolis, MN), IL-17, and IFN-γ (both from eBioscience) were performed according to the manufacturers' instructions. For TGF-β1 ELISA (BD Pharmingen) supernatants were activated for 1 h with 1 N HCl and then neutralized with 1 N NaOH.

Statistical analysis

Data are means ± SEM. Statistical analysis for significant differences was performed using ANOVA, with the Student t test for parametric samples (GraphPad Prism version 4; GraphPad Software, San Diego, CA).

Results

Anti-GP2 levels are significantly elevated in CD

Initially, we confirmed GP2 as an autoantigenic target by assessing anti-GP2 IgG and IgA levels in IBD patients and different control groups. As shown (Fig. 1), Abs to GP2, of both IgG and IgA isotypes, are significantly elevated in CD compared with healthy controls, UC, and patients with pancreatitis or IBS. We were unable to observe any correlations of anti-GP2 levels with medications taken (Supplemental Table I).

GP2 is distinctly expressed on various cell types and is regulated on T cells

Because anti-GP2 Abs are elevated in CD, we next investigated expression profiles of the respective Ag GP2 on key cells involved in mucosal inflammation (epithelial, monocytes, T cells, B cells) using a commercial Ab. The specificity of the Ab was verified by preincubating cells with serum of rabbits hyperimmunized with recombinant GP2 (Supplemental Fig. 1). These sera contain Abs against GP2 and thus block GP2 epitopes reactive with the commercial anti-GP2. Preincubation of cells with these hyperimmune sera dose-dependently reduced the binding of anti-GP2, thus proving the specificity of the commercial Ab.

Further evidence for the specificity of the Ab was deduced by preincubation of the Ab with recombinant GP2 itself for 24 h at 4°C. Staining of cells (T cells, monocytes, and epithelial cells; Supplemental Fig. 2) with the preincubated protein-Ab mixture negated expression of surface GP2. Additionally, a further negative control was conducted using isotype-matched IgG control, which confirmed no unspecific binding as observed by lack of staining (data not shown).

As shown, GP2 expression was detected to varying degrees on all cells investigated (Fig. 2A). Approximately a third of peripheral blood and lamina propria T cells expressed GP2. A high percentage of B cells (80.5 ± 4.2%) and monocytes (92.9 ± 7.1%) also expressed GP2. Also, about three quarters of primary hIECs expressed GP2, in contrast to two epithelial cell lines (Caco-2 and T84) that exhibited only modest GP2 expression (12.5 ± 7.2 and 18.8 ± 2.1%, respectively). Our control groups also exhibited variable GP2 expressions. Percentage of pancreatic (PANC1, ASPC1, and DANG), esophageal (KYSE180), and neuronal cell lines (Sy5y) expressing GP2 ranged from 8 to 46% (Supplemental Fig. 1). As the aim of our study was to investigate the biological relevance of GP2, we next investigated whether GP2 expression can be regulated on T cells. As depicted in Fig. 2B and 2C, GP2 expression was significantly upregulated after 48 h T cell activation via the TCR, both at the RNA as well as the protein expression levels.

GP2 regulates epithelial cell phenotype and function

Having shown that GP2 is expressed and regulated on cells critically involved in mucosal immunity, we went on to evaluate the effect of GP2 itself on central mechanisms of innate and adaptive immunity. Epithelial cells are the first cells to encounter pancreas-originating GP2 in the gastrointestinal lumen (10). Moreover, they represent the first line of defense and are central elements of innate immunity (23). To elucidate the influence of GP2 on epithelial cells, we incubated hIECs or epithelial cell line T84 with recombinant GP2 for 24 and 48 h, after which cells and supernatant were acquired for the different experiments. As the pattern by which GP2 influenced epithelial function was similar at these two time points, results shown are after 48 h GP2 stimulation.

Examination of expression of key receptors in IEC function using flow cytometry showed, to our knowledge, for the first time that GP2 differentially modulates key molecules involved in epithelial cell function (Fig. 3A). A decreased percentage of cells expressed E-cadherin and an increased percentage expressed α4 integrin (CD49d). GP2 also increased the percentage of cells expressing molecules used in Ag presentation (HLA-DR and CD40). Moreover, MICA, which presents Ag to γδ T cells, was also upregulated.

**FIGURE 1.** Abs against GP2 are increased in CD. Sera were collected from healthy volunteers (n = 10) and CD (n = 16), UC (n = 13), pancreatitis (n = 5), and IBS (n = 5) patients. Anti-GP2 IgG (A) and IgA (B) ELISAs confirmed elevated levels in CD, in comparison with all other groups examined. *p ≤ 0.002.
by GP2, suggesting that GP2 has a function in bridging epithelial-to-T cell responses. In contrast to the demonstrated capability of GP2 to modulate other epithelial cell receptors, TLR2 and TLR4 expression were not altered by GP2 (data not shown).

When we examined modulation of apoptosis and proliferation by GP2 on epithelial cells using annexin V/PI staining and MTT incorporation, respectively, we observed that GP2 dose-dependently decreased apoptosis and proliferation of both hIECs and T84 (Fig. 3B, 3C). We also determined the influence of GP2 on epithelial migration over a lesion using the wound-healing assay with two different epithelial cell lines. Interestingly, but confirming the differences between cell migration and cell proliferation (24), cell migration over the wound edge was not influenced by GP2 coculture (Fig. 3D). In inflammatory responses, such as IBD, CXCL8 is upregulated and serves as a potent chemoattractant. Using ELISA, we also revealed that GP2 dose-dependently decreases CXCL8 secretion from epithelial cells (Fig. 3E). However, β-defensin-2 secretion, which is dysregulated in CD (25), and TSLP were unaffected by GP2. IL-6 was not detected in T84 cells (data not shown).

Chemoattractant potential of GP2

As epithelial cells attract cells to sites of inflammation, and as Ag presentation receptors were upregulated in response to GP2, we examined the ability of GP2 to chemoattract T cells, a critical step in mucosal inflammation. To do so we performed Transwell migration assays and determined how many PBMCs migrated toward GP2-stimulated epithelial cells. Migration was determined using flow cytometry for a fixed time of 90 s, and only gated mononuclear cells on forward versus side scatter were counted. As depicted in Fig. 4B, GP2 significantly increased mononuclear cell migration toward GP2-treated T84 cells. CXCL8-treated T84 cells served as positive control. Importantly, PBMCs did not migrate toward GP2 alone (data not shown).

GP2 binds directly to epithelial and T cells

Because our results so far suggest that GP2 modulates key cell functions, we sought to delineate whether GP2 can bind directly to these cells, suggesting a possible mode of action for GP2. To investigate this important question, we incubated PBMCs with biotinylated GP2 for 40 min at 4°C, followed by secondary SA-FITC incubation. As shown (Fig. 4C), GP2 bound to unstimulated T cells to some extent, but CD3 stimulation of the cells greatly increased this attachment. Furthermore, GP2 was also shown to bind potently to the T84 IEC cell line.
GP2 modulates T cell activation, proliferation, apoptosis, and cytokine secretion

Having discovered the effect of GP2 on critical cells of innate immunity, we went on to explore the influence of GP2 on the adaptive immune system. We isolated PBMCs from healthy volunteers, as well as LPMCs and IELs, and stimulated with anti-CD3 or anti-CD2, respectively. Flow cytometric analysis revealed that in all T cell populations examined, GP2 significantly and dose-dependently decreased activation as determined by CD25 expression (Fig. 5A). Of note, CD69 expression was not influenced by GP2. Moreover, annexin V/PI staining uncovered that apoptosis is decreased by as much as 25–50% upon GP2 stimulation in PBMCs, LPMCs, and IELs in comparison with CD3-stimulated cells (Fig. 5B). Further exploring underlying apoptosis pathways, we found that GP2 decreased activation of caspase-3 and -8, but not caspase-9 (Fig. 5C).

Proliferation of CD3-activated PBMCs was also significantly inhibited by GP2 as verified by PI staining (Fig. 5D). However, in LPMCs and IELs we were unable to induce or observe any proliferation, using both PI staining and MTT incorporation.

Similar to epithelial cells, GP2 coculture of activated PBMCs did not influence TLR2 and TLR4 expression on CD3+ cells, compared with cells stimulated in the absence of GP2 (data not shown). Importantly, as outlined in Materials and Methods, all experiments were performed with two different sources of recombinant GP2, showing identical patterns of effects.

We also examined the influence of GP2 on cytokine secretion from the different cell populations. Using ELISA, we observed that GP2 decreased secretion of the proinflammatory TNF-α and IL-17 (Fig. 6A, left panel) from CD3-stimulated PBMCs, but increased regulatory IL-6 (Fig. 6A, left panel) and TGF-β1 release (Fig. 6A, right panel on a different scale). A similar pattern was observed in CD2-stimulated IELs and LPMCs (Fig. 6B, 6C); that is, GP2 coculture decreased proinflammatory (TNF-α and IL-17) and increased regulatory cytokine (TGF-β1) release compared with cells cultured in the absence of GP2. In contrast to PBMCs, however, IL-6 was not detected in the mucosal cells, and concentrations of both TNF-α and IL-17 were considerably lower than in peripheral cells.

Finally, to gain an insight into the influence of GP2 in the more complex mucosal milieu, we examined cytokine secretion in response to GP2 from freshly resected mucosal specimens, using organ culture assay. A slightly different representation of cytokine secretion emerged (Fig. 6D). Proinflammatory CXCL8 secretion decreased and regulatory TGF-β1 increased in response to GP2. However, in contrast to isolated cells, IL-6 secretion in response to GP2 was decreased, and the cytokines TNF-α, IL-17, and β-defensin-2 were not observed. All the data mentioned above regarding the influence of GP2 on T cell function and phenotype were examined both 24 and 48 h after stimulation. As there were no differences in the pattern by which GP2 influenced T cell function, data shown are for 48 h after GP2 stimulation.

Tregs mediate the immunosuppressive effect of GP2

Our results so far assign a previously unknown, immunosuppressive effect of GP2 on T cell function. We were now intrigued to...
elucidate the role of specific cell populations in the response to GP2. Tregs are a specialized subpopulation of T cells that suppress the activation of the immune system and might be responsible for GP2’s effect. Therefore, we used the same experimental conditions as described above and examined the response of isolated CD3+ cells, Tregs, or monocytes to GP2. Interestingly, we detected no influence by GP2 on activation, proliferation, apoptosis, or cytokine secretion on either isolated T cells, Tregs, or monocytes (data not shown). Moreover, when we repeated these experiments on the PBMC fraction depleted from Tregs, GP2 was also unable to influence activation, proliferation, or apoptosis of the T cells (Fig. 7A, gray columns). However, when we then stimulated Treg-depleted PBMCs for 3 h in the presence of GP2, subsequently washed away the GP2, and then returned the autologous Tregs for a further 48 h, the effect of GP2 on T cell activation, proliferation, or apoptosis was restored as shown above (Fig. 7A, black columns).

**Discussion**

The major zymogen granule membrane glycoprotein, GP2, is secreted by pancreatic cells and is suggested to be involved in the “opsonization” of FimH+ in the intestine. Furthermore, GP2 was demonstrated to be a specific receptor on M cells facilitating translocation of FimH+ bacteria across the intestinal epithelium. Interestingly, GP2 has recently been identified as the main autoantigenic target recognized by the CD-specific PABs. IBD patients have a higher prevalence of PABs and consequently anti-GP2 Abs compared with controls (27–30), assuming a potential role for GP2 in mucosal immunology. An immunosuppressive effect has been reported in the mid-1980s for the urinary homolog of GP2, THP (31, 32). Thus, as the role(s) of GP2 in central mechanisms of the mucosal immune system are not defined, we hypothesized a similar mode of influence by GP2.
Indeed, two different recombinant GP2s elicited a largely downregulated immune response, revealing a novel immunomodulatory role for GP2 in modulating both innate and adaptive immune responses.

**FIGURE 5.** GP2 inhibits activation, apoptosis, and proliferation of T cells. CD3-stimulated PBMCs (n = 6) or CD2-stimulated LPMCs (n = 3) and IELs (n = 3) were incubated for 48 h at the mentioned GP2 concentration. Afterward, cells were acquired for activation (A) (gated on CD3+CD25+ cells from total PBMCs), apoptosis (B) (annexin V/PI staining), or proliferation (C) (PI staining). (D) Caspase activation was determined using intracellular staining as described in Materials and Methods. *p ≤ 0.05, **p ≤ 0.01 versus 0 μg/ml GP2.

**FIGURE 6.** GP2 downregulates inflammatory and upregulates regulatory cytokine secretion. PBMCs (A) (n = 6), LPMCs (B) (n = 4), IELs (C) (n = 3), and organ cultures (D) (n = 3) were stimulated as described in Materials and Methods and incubated with the mentioned doses of GP2. After 48 h, supernatants were collected and examined for cytokine secretion using ELISA. *p ≤ 0.05, **p ≤ 0.01 versus 0 μg/ml GP2.
Our findings showing that Abs to GP2 are significantly elevated in CD, but not in UC, confirm its previously reported potential for the differential serological diagnosis in IBD (27–30). Furthermore, since pancreatitis and IBS patients appear to lack Abs to GP2, our data seem to support the assumption that anti-GP2 autoantibodies do not pertain to inflammation in general, but to CD specifically.

As we speculated that GP2 not only serves as an autoantigen but is also a constituent of immune cells, we investigated whether GP2 is expressed on key cells of the innate and adaptive immune systems. Indeed, GP2 expression was detected on human epithelial cells as well as T cells and monocytes. Moreover, GP2 expression was regulated by T cell stimulation, upon TCR ligation, at the RNA as well as at protein expression levels. Our findings appear to be in contrast to a previous report that GP2 is solely expressed on M cells (35). In contrast to its potent effect on CD3-stimulated PBMCs, GP2 did not modulate cell restituion and it decreased epithelial cell proliferation and apoptosis. This suggests an arresting influence by GP2, revealing that the function of GP2 is beyond its previously known actions. Even more, as we provide evidence that GP2 dose-dependently decreased the proinflammatory role for GP2 in the mucosal immune system.

GP2 binds to E. coli and appears to support defense mechanisms against potentially pathogenic bacteria (33). It also serves as an uptake receptor for commensal and pathogenic bacteria by M cells, but not other epithelial cells (34). In our experiments, we confirmed the inability of GP2 to promote phagocytosis by IECs, which are not M cells. Moreover, we showed that GP2 promoted E. coli phagocytosis by monocytes, indicating that GP2 has broader prophagocytic ability than previously assumed. Note that we were unable to inhibit GP2-mediated phagocytosis by monocytes either with cytochalasin D or with nocodazole, implying that this mechanism is independent of a disruption of microfilament function (35).

THP, the renal homolog of GP2, was recently reported to increase transepithelial migration of neutrophils (36). We show that GP2 increased mononuclear cell migration toward GP2-treated epithelial cells. However, as mononuclear cells did not migrate toward GP2 alone, GP2 is not in itself a chemoattractant but induces this feature in epithelial cells.

In the lamina propria, the adaptive immune system initiates and fosters inflammation, especially IBD. In CD, T cells exhibit increased activation, proliferation, and cell cycling compared with healthy controls (18). Therefore, limiting T cell activation is an effective therapeutic approach to limit mucosal inflammation (37). By showing that GP2 potently restricted T cell activation and proliferation, our data suggest its potential therapeutic use to limit mucosal inflammation. Moreover, as GP2 also decreased the secretion of the proinflammatory chemokine CXCL8, we suggest an anti-inflammatory role for GP2 in the mucosal immune system.

We also present data that GP2 decreased T cell apoptosis upon stimulation via caspase-3- and caspase-8-dependent pathways. In CD, T cell apoptosis is already decreased (38), indicating that this process is perhaps not desired in this type of IBD. However, in UC, T cell apoptosis is elevated (39), implying that these patients might benefit from this effect.

T cells are the backbones of the mucosal adaptive immune system, and they consist of several phenotypically distinct cell types. Regulatory CD44CD25+ T cells are effective in the prevention and downregulation of experimental colitis and mediate mucosal inflammation (40). In contrast to its potent effect on CD3-stimulated PBMCs, GP2 had no effect on isolated Tregs, regardless of their stimulation status. Also, GP2 was ineffective on Tregs-depleted PMBCs. However, indicating the important role of GP2 in Treg function, GP2 was able to downmodulate activation, proliferation, apoptosis, and cytokine secretion when the autologous Treg fraction was reinstated.
Importantly, although apoptosis and proliferation are considered to be inversely regulated, we observed their simultaneous down-modulation by GP2. This phenomenon of coupled cell death and cycling is a hallmark of activation-induced cell death (41, 42), comparable to our in vitro anti-CD3 stimulations. Moreover, as caspase-8 is implicated in this regulation of apoptosis and proliferation (41, 43), our data are further supported, as we observed involvement of caspase-8 in GP2-mediated responses.

Having identified a novel role for GP2 in immunity, we were finally wondering whether and how IBD therapeutics influence GP2 expression. TNF-α inhibitors, such as IFX or ADA, inhibit T cell and epithelial proliferation by inducing apoptosis and thus limit mucosal inflammation. Interestingly, both TNF-α inhibitors increased GP2 gene transcription in T cells but decreased its surface expression. It can be speculated that this seemingly opposite effect indicates an increased endocytosis of GP2. However, blocking endocytosis did not prevent GP2 downregulation by TNF-α inhibitors (data not shown).

Thus, the different regulation of GP2 at the mRNA level in comparison with the membrane level suggests either an intracellular role for GP2, or more probable secretion of GP2 from the T cells, as it acts as a secreted protein. These ideas need to be investigated in further studies, and ongoing studies in our laboratory will elaborate this observation.

Indicating a specificity of this process, in cells of the innate immune system, such as epithelial cells, addition of IFX or ADA increased both gene and surface GP2 expression.

Our novel data showing additive value of TNF-α inhibitors and GP2 in downmodulating immune response hint to the fact that GP2 could be used for future therapeutic medications.

Although it has been known for >20 y that in CD PAB levels are increased (5), it was not assumed until recently that their main autoantigenic target GP2 might have a biological function in the intestine. To our knowledge, our study revealed for the first time that this autoantigen has a physiological role in the intestinal immune system. Furthermore, it was recently shown that GP2 binds to scavenger receptor expressed on endothelial cells (44). Despite our data regarding the binding of GP2 to epithelial and activated T cells, whether GP2 exerts its biological function in the innate and adaptive immune systems via those receptors needs to be further investigated.

THP was reported to influence chemotaxis of polymorphonuclear leukocytes (45), as well as neutrophils (36), and it fosters bacterial phagocytosis (46, 47). In this study we provide clear evidence that THP and GP2 share functional and immunological resemblances. To explore whether GP2 and THP share structural similarities as well, we generated theoretically calculated three-dimensional structures of GP2 and THP based on their amino acid sequences (Supplemental Fig. 3). Although both proteins exhibit homology of 53% in protein–protein alignment (using amino acid sequences of P55259 = GP2_Human, and P07911 = Urom_Human, with BLASTP 2.2.25), and they share some distinctive structural segments, such as a single α helix and a protruding C terminus, it was difficult to deduce other homologies. However, whether these structural differences indicate an unknown physi-
ological or functional difference remains to be elucidated further using protein crystallization and isoform-dependent studies.

Disclosures
D.R. has a management role and is a shareholder of GA Generic Assays GmbH and Medipan GmbH. C.F. is an employee of GA Generic Assays GmbH and Medipan GmbH. The other authors have no financial conflicts of interest. None of the sponsors had an influence on any experimental setting or preparation of the manuscript.

References


