PROTOCOL NAME: THE ROLE OF SITE-SPECIFIC MUCINS RESTORATION AS CANDIDATE MARKER OF MUCOSAL HEALING IN ULCERATIVE COLITIS.

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CENTRE SIGNATURE – PRINCIPAL INVESTIGATOR

I have read this Protocol Amendment relevant to the study entitled "**THE ROLE OF SITE-SPECIFIC MUCINS RESTORATION AS CANDIDATE MARKER OF MUCOSAL HEALING IN ULCERATIVE COLITIS**" and I agree to conduct the study as detailed herein and in compliance with guidelines for Good Clinical Practice and applicable regulatory requirements. I will provide all study personnel under my supervision with all information provided by the Sponsor and I will inform them about their responsibilities and obligations.

Printed name

Role & Department

Address

Signature Date

Glossary of abbreviations

IBD	Inflammatory Bowel Disease
UC	Ulcerative Colitis
MH	Mucosal Healing
H-E	Haematoxylin and Eosin

1 Summary

Title	**THE ROLE OF SITE-SPECIFIC MUCINS RESTORATION AS CANDIDATE MARKER OF MUCOSAL HEALING IN ULCERATIVE COLITIS.**
Investigator sponsor	Italian Group for Inflammatory Bowel Diseases
Study coordinator	Giuseppe Leoncini
Protocol identifying number	
Protocol version date	1.0
Background and rationale	*Both endoscopic and histologic MH are lacking of a globally accepted definition. Since histologic healing cannot be derived from the endoscopic healing, the latter might be confirmed with the microscopic assessment. At a date, there is disagreement about this topic and a reliable histologic marker of MH is lacking. The structural weakening of the mucosal colonic barrier has been recently found to be an early event in the pathogenesis of UC. Thus, the aim of our study project is to evaluate the expression of site-specific mucins expression in patients with active treatment naive (onset) UC, with relapsing disease after ineffective medical treatment, with quiescent disease after treatment (at least 1 year). Such experimental evaluation will be performed through the implementation of immunohistochemical assays on colonic mucosal biopsies.*
Population and patient selection criteria	*Sixty cases of UC will be enrolled in the study from three Centers (Brescia, Desenzano del Garda, Modena) belonging to IG-IBD: every center will provide ten cases both during the onset (treatment-naive)phase of disease and after treatment. Ten cases on histologically normal colonic mucosa will be also enrolled as controls. The selected population will range from 0 to90 years, without racial restrictions. Criteria for inclusion have been identified, such as the diagnosis of UC, the availability of the whole clinical, serologic and endoscopic data, the adherence to the P-IG-IBD guidelines*

	for both the mucosal biopsies handling and processing. Patient with co-morbidities or malignancies, infective disease, previous history of steroid and/or immunosuppressive drugs use will be excluded. The sample size has been estimated to amount to seventy patients.
Study design and study duration	*The study project has been designed to be multi-centric retrospective. The study duration has been estimated to range from 8 to 12 months.*
Objectives	*Our aim is to evaluated the variations in the site-specific mucins expression with immunohistochemical assays, in order 1) to better understand their different pattern of expression during the active, relapsing and remitted phase of disease and 2) to candidate the possible restoration of the site- specific mucins expression profile as a novel histologic marker of MH.*
Statistical methods, data analysis	—
Ethical considerations	—
Study time table	*Project starting date: 01-03-2020* *Project completion of data collection: 01-06-2020* *Project data analysis: 01-10-2020* *Project presentation of scientific report: 01-03-2021*

2 BACKGROUND AND INTRODUCTION

The background should include a clear explanation of the main research question along with a full literature review, a detailed justification for the study and discussion of its feasibility.

Inflammatory bowel diseases (IBD) are relapsing and remitting diseases reflecting the inflammatory status of the intestinal tract. For decades, treatment goals for IBD had been to achieve clinical response or remission. More recently, the normalization of laboratory parameters and intestinal mucosa, named *mucosal healing* (MH), have been addressed as major target to prevent extra-intestinal complications and obtain sustained clinical remission (**Mazzuoli S** et al. 2013). MH has been found closely related with histological disease activity in both ulcerative colitis (UC) and Crohn's disease (CD) and a good predictor of long term clinical remission (**De Chambrun G** et al 2010). MH is at a date a topic of discussion, since some controversies about the available definitions persist and there is a disagreement about both the endoscopic features to report and the endoscopic score to use, the latter being complex and time-consuming. Moreover, the proposed clinical definition of MH as *the complete absence of all inflammatory and ulcerative lesions* lacks a validation (**William J** et al 2002; **D'Haens GR**, et al 2009) Nowadays, MH has also been defined as the *absence of any abnormality of the intestinal mucosa and normalization of fecal biomarkers such as fecal calprotectin* (FC). (**De Chambrun G** et al 2010; **Fiorino G** et al 2001). Several treatment protocols have been proposed, based on different drugs, between which anti-Tumor Necrosis Factor-α (TNF-α) agents, namely infliximab and adalimumab, have been reported as effective MH promoters (**Rutgeerts P**et al 2007). The optimal treatment goal should be toachieve clinical response or remission, but the histological remission is not mentioned. Since the endoscopic healing does not necessarily imply that the intestinal mucosa is histologically healed, the microscopic assessment of MH should play a key role in the assessment of remission (**Casellas F**et al 2012; **Geboes K** et al 2002). The *histological* MH cannot be derived from *endoscopic* evidence of mucosal healing (**Rosemberg L** et al 2013; **Geboes K** et al 2002). Indeed, microscopic evidence of inflammation has been found to persist in 16-100% of patients with endoscopically quiescent colitis, predicting clinical relapse. (**Bryant RV** et al 2014; **Riley SA** et al 1991). Hence, endoscopic remission might be confirmed by histological assessment and the histological healing could be an endpoint in future clinical trials of new UC drugs.

The pathologist should receive at least 2 oriented biopsies from each endoscopically explored segment, from the terminal ileum to the rectum, comprehensive clinical informations about both the duration of disease and the treatment protocols (**Magro F** et al 2013; **Villanacci V** et al 2011). The histological assessment is usually based on the analysis of at least 4 microscopic sections, stained routinely with haematoxylin and eosin (H-E).

A globally accepted definition of histological MH does not currently exist. Histological healing has been defined as the disappearance of inflammation (**Villanacci V** et al 2013; **Villanacci V** et al 2015). Hence, a healed mucosa has few architectural abnormalities, normally differentiated epithelial cells, no signs of active inflammation (i.e. neutrophils), and a normal density of lymphocytes and plasma cells. Thus, mucosal healing indicates a histological state in which the mucosa becomes free of active inflammation, erosions and crypt abscesses, its surface and glandular epithelial cells are intact, its general architecture is not disturbed, and the lamina propria is free of oedema, fibrosis and the accumulation of lymphocytes and eosinophils. (**Villanacci V** et al 2019).

A PHYSIOPATHOLOGICAL VIEWPOINT OF COLONIC MUCOSA.

The intestine plays an important role in the digestion and absorption of ingested food and the elimination of undigested food, microbes, and microbial products. The functional integrity of the intestinal mucosal epithelial cells depends on the coordinated regulation of the mucus layer, the intercellular tight junction, epithelial cells, and host innate and adaptive immune response. (**Lievin-Le Moal V** 2006; **Dharmani P** 2009). The mucus layer overlying the epithelium secreted by the goblet cells promotes the elimination of gut contents and provides the first line of defense against physical and chemical injury caused by ingested food, microbes and the microbial products. The major component of the mucus is secreted *mucins*, large glycoproteins with highly polymeric structure. (**Hollingsworth MA** 2004; **Andrianifahanana M** 2006). Intestine is the major site of bacterial colonization, with more than 1000 prevalent bacterial species identified. These commensal bacteria are trapped in the mucus layer, failing to reach the epithelial cell surface, and are eliminated by peristaltic movement. The microbes and microbial products are recognized by the sensor system of the intestinal epithelial cells and the immune cells, activating the host innate defense system. Balanced and dynamic interactions among mucus layers, intestinal epithelial cells,

microbiota, and host immune defense is essential for the maintenance of the intestinal mucosal homeostasis. The disruption in the intestinal homeostasis results in the defective mucus barrier with increased permeability that results in inflammation and injury of the intestinal mucosal cells (**McGuckin MA** 2009). The intestinal mucosal epithelium consists of four main cell types - absorptive enterocytes, goblet cells, Paneth cells, and enteroendocrine cells - which undergo continuous cycles of renewal. Notch signaling drives the intestinal epithelial cells differentiation so that Notch pathway inhibition results in a rapid and complete conversion of all epithelial cells to secretory cell lineage cells such as goblet, Paneth, and enteroendocrine cells, whereas the activation of Notch signaling pathway leads to depletion of all secretory cells with the villi lined mainly with absorptive enterocytes concomitant with activation of Hes1 (**van der Flier LG** 2009; **Stanger BZ** 2005). Goblet cells synthesize and secrete bioactive molecules such as secretory and membrane-bound mucins and other components of mucus (**Dharmani P** 2009). Up to 20 different mucin genes have been identified, MUC1 to MUC20 according to order of their discovery. Mucin genes are expressed in tissue and cell type–specific manner and are broadly classified into two types, secretory and membrane-associated. Secretory and membrane mucins have distinct structural features and biosynthetic pathways. MUC2 is the first human secretory mucin to be identified and characterized (**Gum JR** 1989; **Gum JR Jr** 1994). In small and large intestine, MUC2 is the major secretory mucin synthesized and secreted by goblet cells, has structural and physico-chemical properties similar to those of other gel-forming secretory mucins such as MUC5AC, MUC5B, and MUC6, expressed in gastric and respiratory glandular epithelium. Intestinal mucosal epithelial cells also express epithelial membrane-bound mucins, MUC1, MUC3, MUC4, MUC12, MUC13, and MUC17, which have structural similarity. MUC3 is the most abundantly expressed membrane mucin in the small intestine, its expression in the apical membrane of absorptive and goblet cells shows a maturational gradient with increasing expression from crypt to villus. The cysteine-rich EGF-like domains of the mouse MUC3 and human MUC17 mucins have been shown to inhibit apoptosis and stimulate cell migration, implying a bioactive role in maintaining the integrity of the surface epithelial layer (**Ho SB** 2006; **Luu Y** 2010). MUC12 is a protein-coding gene, whose function is involved in the epithelial cell protection (**Yin BWT** 2001). Intestinal mucus layers secreted by goblet cells consist mainly of compact mesh-like network of viscous, permeable, gelforming MUC2 mucin, which provides the frontline host defense against endogenous and

exogenous irritants and microbial attachment and invasion but allows the transport of nutrients (**Lievin-Le Moal V** 2006).

There are two mucus gel layers in the gastrointestinal mucosa, an inner firmly adherent layer and an outer more loosely adherent layer, both consisting largely of MUC2 mucin in the intestine (**Atuma C** 2001; **Johansson ME** 2009). The outer loosely adherent mucus layer formed by proteolytic and glycosidic degradation of highly polymerized gel-like MUC2 mucin was similar in thickness in stomach and jejunum, but markedly increased in ileum, and thickest in colon (**Atuma C** 2001). Microbes are associated mostly in the outer loose mucus layer and absent from the inner firm mucus layer, indicating that the inner firm mucus layer functions as a critical protective barrier against bacterial adhesion and invasion of underlying epithelial cells (**Johansson ME** 2008). The outer loose mucus layer provides a good habitat for microbial colonization, because oligosaccharides of MUC2 mucin provide numerous microbial attachment sites and energy source (**Martens EC** 2009). The thickness of mucus layers is maintained by a balance between synthesis, secretion, and degradation, modulated by the microbial glycosidases and proteases and the mechanical shear forces of peristalsis. The important role of membrane-bound mucins at the apical cell surface and at the interface of the cell surface and the inner mucus layer was demonstrated by Muc1-deficient mice showing increased susceptibility to invasion by Campylobacter jejuni (**McAuley JL** 2007).

The normal intestinal mucosal epithelium has tolerance to commensal microbiota because of its ability to distinguish commensal microbiota from pathogenic microorganisms by their molecular patterns, such as microbe-associated molecular patterns and pathogen-associated molecular patterns, through pattern recognition receptors (PRRs) such as cell surface Toll-like receptors (TLRs) and cytoplasmic nucleotide-binding oligomerization domain (NOD) proteins (**Moncada DM** 2003; **Fukata M** 2009). Intestinal commensal microbiota depends on mucus and undigested dietary carbohydrates for binding sites and energy source and affects intestinal epithelial functions, including those of goblet cells and mucus layers, by a “cross talk” feedback mechanism (**Van der Sluis M** 2006). Microbiota and microbial products can modulate mucin synthesis and secretion, either by direct activation of diverse signaling cascades or through bioactive factors generated by epithelial and lamina propria cells. Enteric pathogens circumvent the protective function of mucus layer by developing motility, mucolytic activity, and other virulence factors, causing the degradation and penetration of mucus layers and subsequent attachment and invasion of epithelial cells.

IBD is thought to be caused by continuous pathological immune responses to altered and/or dysbiosis of commensal microbes and microbial products. Accumulating evidence indicates that complex interactions of the following events are involved in the pathogenesis of IBD:

1) dysbiosis of commensal microbiota;

2) defective bacterial killing and processing;

3) defective mucosal barrier function;

4) defective host immune response.

Alterations of mucin production and glycosylation occur in IBD, but whether they contribute to initiation of inflammation or are the result of inflammation is unknown. Goblet cells are reduced in number and size in ulcerative colitis. Recent studies in mouse models of colitis highlight the importance of the role of mucin in maintaining the integrity of protective mucus barriers whose breakdown can result in colitis. ***(Sartor RB 2008)***

Since a generally accepted definition of histological MH does not exist, because of the lacking of a standardized approach, several histopathological scores have been proposed, but they are not routinely used because they are more complex and subjective.

Abnormalities of the mucus system, including emptying of upper crypt goblet cells, visible mucus in stool and bacterial penetration of the inner mucus layer, have previously beendescribed in active UC. The present study aims to highlight the variations in site-specific mucins profile expression in the active UC and its possible restoration during the quiescent phase of disease, through immunohistochemical analysis and biochemical, clinical, endoscopic and histopathological correlations.

Such evaluation will encompass:

- a pre-analytical phase, during which the whole clinical, serological and endoscopic data are collected;
- an analitycal phase, in which the prevalence of histological signs of inflammation is evaluated in patients with 1) active and treatment-naive (onset) UC; 2) relapsing disease after treatment; 3) deep remission after at least 1 year of treatment;
- an experimental phase, to assess which pattern of mucins is expressed in the different phases of UC (active, relapsing and quiescent) through immunohistochemical assays.

Methodology

- The study is designed as a multicenter retrospective analysis, with the participation of three centers (Brescia, Desenzano del Garda, Modena) belonging to IG-IBD;
- Each center should provide 10 cases of UC before (onset) as well as after medical treatment, along with comprehensive clinical-endoscopic data;
- 10 cases of health colonic mucosa, as controls will be studied.
- The histopathological samples should be collected according to the P-IG-IBD guidelines: at least 2 oriented biopsies from each endoscopically explored segment, from the terminal ileum to the rectum, aligned on a proper filter;
- The UC samples should be grouped according to the medical treatment (mesalazine group; steroid group; …);
- The UC samples should be grouped according to the MAYO score;
- The prevalence of the inflammatory modifications found in the colonic mucosa should be graded according to the currently accepted guidelines (**Magro F. et al 2013**);
- The histologic evaluation of the mucosal healing should be performed in every explored segment and graded according to the simplified scheme, compared with Geboes simplified scheme, Robarts and Nancy schemes (**Villanacci V et al 2017**);
- Provided data will be analyzed in a referral center;
- Immunohistochemical tests for MUC-1; MUC-2, MUC-4, MUC-5B, MUC-12, MUC-13, MUC-17 will be performed on mucosal samples in a referral center, in order to avoid technical bias. The mucins panels will be evaluated by two experienced pathologists, to minimize any subjectivity;
- Data collection and statistical analysis will be performed in a referral center.

3 RATIONALE OF THE STUDY

The mucosal healing (MH), have been addressed as major target to prevent extra-intestinal complications and obtain sustained clinical remission. Both endoscopic and histologic MH are lacking of a globally accepted definition. Since histologic healing cannot be derived from the endoscopic healing, the latter might be confirmed with the microscopic assessment.
At a date, there is disagreement about this topic and a reliable histologic marker of MH is lacking. The structural weakening of the mucosal colonic barrier has been recently found to be an early event in the pathogenesis of UC.

4 OBJECTIVES OF THE STUDY

4.1 General objectives

The aim of our study project is to evaluate the expression of site-specific mucins expression in patients with active treatment naive UC, with relapsing disease after ineffective medical treatment, with quiescent disease after treatment (at least 1 year). Such experimental evaluation will be performed through the implementation of immunohistochemical assays on colonic mucosal biopsies.

4.2 End-points

4.2.1 Primary endpoint

1 – To evaluate the site-specific mucins expression profile in treatment-naive (onset) and treated (after medical treatment) UC; 2 – to candidate the restoration of mucins expression as a novel histological marker of MH.

4.2.2 Secondary endpoint

To better understand the different patterns of expression of the site-specific mucins during 1) the onset (active and treatment-naive phase), the relapsing phase (active disease after a previous drug-induced quiescence) and the stable remitted phase of disease.

5 PATIENT SELECTION CRITERIA

Sixty cases and ten controls of both sexes will be enrolled in the study both in the adult and in the pediatric population, ranging from 0 to 90 years, without racial restrictions. Criteria for inclusion, such as the diagnosis of UC, the availability of the whole clinical, serologic and endoscopic data, the adherence to the P-IG-IBD guidelines for both the mucosal biopsies handling and processing have been identified. Patients with infective disease or with other co-morbidities or malignancies, with previous history of steroid and/or immunosuppressive drugs use will be excluded. The sample size has been estimated to amount to seventy patients.

5.1 Inclusion criteria

- ❖ Patients of either sex, aged 0-90 years (inclusive), without racial restriction.
- ❖ Patients undergoing colonoscopy with mucosal sampling.
- ❖ Full clinical and serological data available
- ❖ Diagnosis of UC according to the worldwide accepted criteria and guidelines
- ❖ Adherence to the P-IG-IBD guidelines for both the mucosal biopsies handling and processing
- ❖ Absence of known malignity.

5.2 Exclusion criteria

- ❖ Presence of significant co-morbidities (infective, others...)
- ❖ Primary immunodeficiencies.
- ❖ Pregnancy.
- ❖ Known malignancy.

- ❖ Previous abdominal surgery.
- ❖ Previous haematopoietic stem cell transplantation.

6 STUDY DESIGN

The present study has been designed as multicentric retrospective analysis of the site-specific mucins expression profile through immunohistochemical assays in patients with treatment-naive UC (onset phase) as well as after treatment.

7 ATTACHED DOCUMENTS

List of the requested materials to perform the immunohistochemical assays described in the study project (ALL.1)

8 REFERENCES

1) **Mazzuoli S,** Guglielmi F.W., Antonelli E, Salemme M, Bassotti G, Villanacci V. Definition and evaluation of mucosal healing in clinical practice. Digestive and Liver Disease 45 (2013) 969– 977.
2) **De Chambrun G,** Peryrin Biroulet L, Lemman M, Colombel JF. Clinical Impli-cations of mucosal healing for the management of IBD. Nature ReviewsGastroenterology & Hepatology 2010;7:15–29.
3) **William J**, Sandborn WJ, Brian G, et al. A review of activity indexes and efficacy end points for clinical trials of medical therapy in adults with Crohn's disease. Gastroenterology 2002;122:512–30.
4) **D'Haens GR**, Fedorak R, Lemann M, et al. Endpoints for clinical trials evaluating disease modification and structural damage in adults with Crohn's disease.Inflammatory Bowel Diseases 2009;15:1599–604.
5) **Fiorino G**, Cesarini M, Indriolo A, Malesci A. Mucosal healing in ulcerative colitis: where do we stand? Current Drug Targets 2011;12:1417–23.
6) **Rutgeerts P**, Vermiere S, Assche GV. Mucosal healing in inflammatory boweldisease:

impossible ideal or therapeutic target. Gut 2007;56:453–5.

7) **Casellas F**, Barreiro de Acosta M, Robles V, et al. Mucosal healing restores normal health and quality of life in patients with inflammatory bowel disease. European Journal of Gastroenterology & Hepatology 2012;24:762–9.
8) **Geboes K**, Dalle I. Influence of treatment on morphological features of mucosal inflammation. Gut 2002; 50 Suppl 3: III37-III42.
9) **Bryant RV**, Winer S, Travis SPL, Riddell RH. Systematic review: histological remission in inflammatory bowel disease. Is "complete" remission the new treatment paradigm? An IOIBD initiative. J Crohns Colitis 2014; 8: 1582-97.
10) **Riley SA**, Mani V, Goodman MJ, Dutt S, Herd ME. Microscopic activity in ulcerative colitis: what does it mean? Gut 1991; 32: 174-8.
11) **Magro F**, Langner C, Driessen A, Ensari A, Geboes K, Mantzaris G.J, Villanacci V, Becheanu G, Borralho Nunes P, Cathomas G, Fries W, Jouret-Mourin A, Mescoli C, De Petris G, Rubio C.A, Shepherd N.A, Vieth M, Eliakim R, on behalf of the European Society of Pathology (ESP) and the European Crohn's and Colitis Organisation (ECCO). European consensus on the histopathology of inflammatory bowel disease. Journal of Crohn's and Colitis (2013) 7, 827–851.
12) **Villanacci V**, Manenti S, Antonelli E, Chiudinelli M, Giuliano V, Bassotti G. Non-IBD colitides: clinically useful histopathological clues. Rev Esp Enferm Dig 2011; 103: 366-372.
13) **Villanacci V**, Antonelli E, Geboes K et al. Histological healing in inflammatory bowel disease: a still unfullfilled promise. World J Gastroenterol 2013; 19: 968–978
14) **Villanacci V**, Antonelli E, Salemme M, Bassotti G. Assessing mucosal healing in ulcerative colitis: the simpler, the better. Endoscopy 2015; 47:759
15) **Sartor RB.** Microbial influences in inflammatory bowel diseases. Gastroenterology 2008, 134:577–94.
16) **Lievin-Le Moal V**, Servin AL: The front line of enteric host defense against unwelcome intrusion of harmful microorganisms: mucins, antimicrobial peptides, and microbiota. Clin Microbiol Rev 2006, 19:315–337.
17) **Dharmani P**, Srivastava V, Kissoon-Singh V, et al.: Role of intestinal mucins in innate host defense mechanisms against pathogens. J Innate Immun 2009, 1:123–135.
18) **Hollingsworth MA**, Swanson BJ: Mucins in cancer: protection and control of the cell

surface. Nat Rev Cancer 2004, 4:45–60.

19) **Andrianifahanana M**, Moniaux N, Batra SK: Regulation of mucin expression: mechanistic aspects and implications for cancer and inflammatory diseases. Biochim Biophys Acta 2006, 1765:189–222.

20) **McGuckin MA**, Eri R, Simms LA, et al.: Intestinal barrier dysfunction in inflammatory bowel diseases. Inflamm Bowel Dis 2009, 15:100–113.

21) **van der Flier LG**, Clevers H: Stem cells, self-renewal, and differentiation in the intestinal epithelium. Annu Rev Physiol 2009, 71:241–260.

22) **Stanger BZ**, Datar R, Murtaugh LC, et al.: Direct regulation of intestinal fate by Notch. Proc Natl Acad Sci U S A 2005, 102:12443–12448.

23) **Hollingsworth MA**, Swanson BJ: Mucins in cancer: protection and control of the cell surface. Nat Rev Cancer 2004, 4:45–60.

24) **Andrianifahanana M**, Moniaux N, Batra SK: Regulation of mucin expression: mechanistic aspects and implications for cancer and inflammatory diseases. Biochim Biophys Acta 2006, 1765:189–222.

25) **Gum JR**, Byrd JC, Hicks JW, et al.: Molecular cloning of human intestinal mucin cDNAs. Sequence analysis and evidence for genetic polymorphism. J Biol Chem 1989, 264:6480–6487.

26) **Gum JR**, Byrd JC, Hicks JW, et al.: Molecular cloning of human intestinal mucin cDNAs. Sequence analysis and evidence for genetic polymorphism. J Biol Chem 1989, 264:6480–6487.

27) **Gum JR Jr**, Hicks JW, Toribara NW, et al.: Molecular cloning of human intestinal mucin (MUC2) cDNA. Identification of the amino terminus and overall sequence similarity to prepro-von Willebrand factor. J Biol Chem 1994, 269:2440–2446.

28) **Ho SB**, Dvorak LA, Moor RE, et al.: Cysteine-rich domains of muc3 intestinal mucin promote cell migration, inhibit apoptosis, and accelerate wound healing. Gastroenterology 2006, 131:1501– 1517; 23.

29) **Luu Y,** Junker W, Rachagani S, et al.: Human intestinal MUC17 mucin augments intestinal cell restitution and enhances healing of experimental colitis. Int J Biochem Cell Biol 2010, 42:996–1006.

30) **Yin B.W.T**., Lloyd K.O. “Molecular cloning of the CA125 ovarian cancer antigen:

identification as a newmucin, MUC16," *The Journal of Biological Chemistry*, vol. 276, no. 29, pp. 27371– 27375, 2001.

31) **Atuma C**, Strugala V, Allen A, et al.: The adherent gastrointestinal mucus gel layer: thickness and physical state in vivo. Am J Physiol Gastrointest Liver Physiol 2001, 280:G922–G929. 37.

32) **Johansson ME**, Thomsson KA, Hansson GC: Proteomic analyses of the two mucus layers of the colon barrier reveal that their main component, the Muc2 mucin, is strongly bound to the Fcgbp protein. J Proteome Res 2009, 8:3549–3557.

33) **Ho SB**, Takamura K, Anway R, et al.: The adherent gastric mucous layer is composed of alternating layers of MUC5AC and MUC6 mucin proteins. Dig Dis Sci 2004, 49:1598–1606.

34) **Johansson ME**, Phillipson M, Petersson J, et al.: The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. Proc Natl Acad Sci U S A 2008, 105:15064–15069.

35) **Martens EC**, Roth R, Heuser JE, et al.: Coordinate regulation of glycan degradation and polysaccharide capsule biosynthesis by a prominent human gut symbiont. J Biol Chem 2009, 284:18445–57.

36) **McAuley JL**, Linden SK, Png CW, et al.: MUC1 cell surface mucin is a critical element of the mucosal barrier to infection. J Clin Invest 2007, 117:2313–2324.

37) **Moncada DM**, Kammanadiminti SJ, Chadee K: Mucin and Tolllike receptors in host defense against intestinal parasites. Trends Parasitol 2003, 19:305–311.

38) **Fukata M**, Abreu MT: Pathogen recognition receptors, cancer and inflammation in the gut. Curr Opin Pharmacol 2009, 9:680– 687.

39) **Van der Sluis M**, De Koning BA, De Bruijn AC, et al.: Muc2- deficient mice spontaneously develop colitis, indicating that MUC2 is critical for colonic protection. Gastroenterology 2006, 131:117–129.