MINISTRY OF HEALTH
OF THE CZECH REPUBLIC

Part A

Project Proposal

Tender within the Programme to support medical applied research and development in 2015 to 2022

Panel: **P05**

Component objective of the National priority Healthy Population
2.2.2. - Identification of new therapeutic objectives, new methods and procedures for biological testing
3.3.1. - Screening and prevention of tumour incidence

Registration No.: **15-28064A**

Applicant

Name and surname:	**doc. MUDr. Pavel Drastich Ph.D.**
Institution:	**Institute for the Clinical and Experimental Medicine**
Address:	**Vídeňská 1958/9, Prague**

Co-applicant 1

Name and surname:	**MUDr. Miloslav Kverka Ph.D.**
Institution:	**Institute of Microbiology of the AS CR, v.v.i.**
Address:	**Vídeňská 1083, Prague**

Project title:

Immunological biomarkers for noninvasive diagnosis, outcome prediction and therapy selection in inflammatory bowel disease

Keywords:

Inflammatory bowel disease; colorectal cancer; primary sclerosing cholangitis; biomarkers; microbiota; cytokines; gut barrier function; serology; early diagnistics

Starting date: **2015-05-01**

Duration (years): **4**

Abstract:

Inflammatory bowel diseases (IBD), such as Ulcerative colitis and Chron's disease, are severe chronic inflammatory diseases. Despite the tremendous progress in this field, their pathogenesis remains unclear, their course cannot be predicted and their therapy often fails or is accompanied by severe side-effects. Moreover, IBD is accompanied by colorectal cancer or primary sclerosing cholangitis in some patients. By using cutting-edge immunological and microbiological methods, we will search for biomarkers closely related to key pathogenetic mechanisms. These includes: changes in gut microbial ecology, immune response against microbiota, gut barrier dysfunction and

dysregulation of immune system. By identifying the patients with high risk of unfavorable disease progression will lead to more rational use of preventive and therapeutic measures, thus reduces the morbidity, mortality and the cost of the therapy.

MINISTRY OF HEALTH
OF THE CZECH REPUBLIC

Part D2 - bibliography

Applicant (Institution, legal entity or physical person submitting research proposal)	**Institute for the Clinical and Experimental Medicine**
Principal investigator:	**doc. MUDr. Pavel Drastich Ph.D.**
Registration No.:	**15-28064A**

Full bibliographic data regarding the most important results of scientific and research activity as defined in the currently valid Methodology for Evaluating the Results ofResearch and Development:

	Result	Result type code	Number of citations (without autocitations) according to WOS	Imapact factor of journal or ERIH database category	Number of citations in NRRE fields (fields evaluated prilarily) in the national context	Journal included in SCOPUS database (only to be completed for journals not included in WOS
1	TLASKALOVÁ-HOGENOVÁ, H., STEPÁNKOVÁ, R., HUDCOVIC, T., TUCKOVÁ, L., CUKROWSAK, B., LODINOVÁ-ZÁDNÍKOVÁ, R., KOZÁKOVÁ, H., ROSSMANN, P., BÁRTOVÁ, J., SOKOL, D., FUNDA, DP., BOROVSKÁ, D., REHÁKOVÁ, Z., SINKORA, J., HORMAN, J., DRASTICH, P., KOKESOVÁ, A. Commensal bacteria (normal microflora), mucosal immunity and chronic inflammatory and autoimmune diseases. Immunology Letters. 2004, 93(2-3), 97-108. ISSN 0165-2478.	J imp	233	2,367		
2	TLASKALOVÁ-HOGENOVÁ, H., STĚPÁNKOVÁ, R., KOZÁKOVÁ, H., HUDCOVIC, T., VANNUCCI, L., TUČKOVÁ, L., ROSSMANN, P., HRNČÍŘ, T., KVERKA, M., ZÁKOSTELSKÁ, Z., KLIMEŠOVÁ, K., PŘIBYLOVÁ, J., BÁRTOVÁ, J., SANCHEZ, D., FUNDOVÁ, P., BOROVSKÁ, D., SRŮTKOVÁ, D., ZÍDEK, Z., SCHWARZER, M., DRASTICH, P., FUNDA, DP. The role of gut microbiota (commensal bacteria) and the mucosal barrier in the pathogenesis of inflammatory and autoimmune diseases and cancer: contribution of germ-free and gnotobiotic animal models of human diseases. Cellular and Molecular Immunology. 2011, 8(2), 110-120. ISSN 1672-7681.	J imp	64	4,185		
3	FROLOVA, L., DRASTICH, P., ROSSMANN, P., KLIMESOVA, K., TLASKALOVA-HOGENOVA, H. Expression of Toll-like receptor 2 (TLR2), TLR4, and CD14 in biopsy samples of patients with inflammatory bowel diseases: upregulated expression of TLR2 in terminal ileum of patients with ulcerative colitis. Journal of Histochemistry and Cytochemistry. 2008, 56(3), 267-274. ISSN 0022-1554.	J imp	49	2,403		
4	LUKÁŠ, M., DRASTICH, P., KONEČNÝ, M., GIONCHETTI, P., URBAN, O., CANTONI, F., BORTLÍK, M., DURICOVA, D., BULITTA, M. Exogenous alkaline phosphatase for the treatment of patients with moderate to severe ulcerative colitis. Inflammatory bowel diseases. 2010, 16(7), 1180-1186. ISSN 1078-0998.	J imp	20	5,475		
5	MARTÍNEK, J., BENEŠ, M., HUCL, T., DRASTICH, P., ŠTIRAND, P., ŠPIČÁK, J. Non-erosive and erosive gastroesophageal reflux diseases: No difference with regard to reflux pattern and motility abnormalities. Scandinavian journal of gastroenterology. 2008, 43(7), 794-800. ISSN 0036-5521.	J imp	15	2,329		

	Result	Result type code	Number of citations (without autocitations) according to WOS	Imapact factor of journal or ERIH database category	Number of citations in NRRE fields (fields evaluated prilarily) in the national context	Journal included in SCOPUS database (only to be completed for journals not included in WOS
6	DRASTICH, P., HONSOVÁ, E., LODERÉROVÁ, A., JAREŠOVÁ, M., PEKARIKOVA, A., HOFFMANOVA, I., TUCKOVA, L., TLASKALOVA-HOGENOVA, H., ŠPIČÁK, J., SANCHEZ, D. Celiac disease markers in patients with liver diseases: A single center large scale screening study. World journal of gastroenterology. 2012, 18(43), 6255-6262. ISSN 1007-9327.	J imp	1	2,433		
7	DRASTICH, P., FROLOVA-BRIZOVA, L., ZANVIT, P., ŠPIČÁK, J., TLASKALOVA-HOGENOVA, H. Spontaneous in vitro IL-6 production in various intestinal segments in patients with inflammatory bowel disease. Folia Microbiologica (Praha). 2011, 56(3), 185-190. ISSN 0015-5632.	J imp	1	1,145		
8	FROL'OVÁ, L., SMETANA, K., BOROVSKÁ, D., KITANOVICOVÁ, A., KLIMESOVÁ, K., JANATKOVÁ, I., MALICKOVÁ, K., LUKÁS, M., DRASTICH, P., BENES, Z., TUCKOVÁ, L., MANNING, JC., ANDRÉ, S., GABIUS, HJ., TLASKALOVÁ-HOGENOVÁ, H. Detection of galectin-3 in patients with inflammatory bowel diseases: new serum marker of active forms of IBD?. Inflammation research. 2009, 58(8), 503-512. ISSN 1023-3830.	J imp	8	2,143		

Total number of results defined in the currently valid Methodology for Evaluating for Results of Research and Development for last 5 years (according to RIV):

1a. Article in professional journal, impacted (type of result: Jimp)	10
1b. Article in professional journal, included in Scopus database (type of result: Jsc)	7
1c. Article in professional journal, non-impacted (type of result: Jneimp)	0
1d. Article in Czech professional journal, peer-reviewed (type of result: Jrec)	8
2a. Scholar book/monograph (type of result: B)	0
2b. Chapter in scholar book/monograph (type of result: C)	0
3. Article in conference proceedings (type of result: D)	0
4. Patent (type of result: P)	0
5. Utility or industrial design (type of result: F)	0
6. Pilot run, verified technology, varienty, breed (type of result: Z)	0
7. Prototype, functional sample (type of result: G)	0
8. Result implemented by funding provider (legislation, norms, strategic documents, policy recommendations, etc.) (type of result: H)	0
9. Specializes map (type of result: L)	0
10. Certified methodology and procedure (type of result: N)	0
11. Software (type of result: R)	0
12. Research report containing information classified pursuant to special legislation (type of result: V)	0

Total number of citations, including autocitations, of all of the scientific work (according to Web o Science)	**468**
H-index according Web of Science	**8**

MINISTRY OF HEALTH
OF THE CZECH REPUBLIC

Part D2 - bibliography

Applicant (Institution, legal entity or physical person submitting research proposal)	**Institute of Microbiology of the AS CR, v.v.i.**
Principal investigator:	**MUDr. Miloslav Kverka Ph.D.**
Registration No.:	**15-28064A**

Full bibliographic data regarding the most important results of scientific and research activity as defined in the currently valid Methodology for Evaluating the Results ofResearch and Development:

	Result	Result type code	Number of citations (without autocitations) according to WOS	Imapact factor of journal or ERIH database category	Number of citations in NRRE fields (fields evaluated prilarily) in the national context	Journal included in SCOPUS database (only to be completed for journals not included in WOS
1	Klimesova K, Kverka M, Zakostelska Z, Hudcovic T, Hrncir T, Stepankova R, Rossmann P, Ridl J, Kostovcik M, Mrazek J, Kopecny J, Kobayashi KS, Tlaskalova-Hogenova H. Altered gut microbiota promotes colitis-associated cancer in IL-1 receptor-associated kinase M-deficient mice. Inflamm Bowel Dis. 2013 May;19(6):1266-77.	J imp	4	5,475		
2	Hansen CH, Nielsen DS, Kverka M, Zakostelska Z, Klimesova K, Hudcovic T, Tlaskalova-Hogenova H, Hansen AK. Patterns of early gut colonization shape future immune responses of the host. PLoS One. 2012;7(3):e34043.	J imp	23	3,534		
3	Zakostelska Z, Kverka M, Klimesova K, Rossmann P, Mrazek J, Kopecny J, Hornova M, Srutkova D, Hudcovic T, Ridl J, Tlaskalova-Hogenova H. Lysate of Probiotic Lactobacillus casei DN-114 001 Ameliorates Colitis by Strengthening the Gut Barrier Function and Changing the Gut Microenvironment. PLoS One 2011 Nov 22; 6(11): e27961	J imp	15	3,534		
4	Tlaskalová-Hogenová H, Stěpánková R, Kozáková H, Hudcovic T, Vannucci L, Tučková L, Rossmann P, Hrnčíř T, Kverka M, Zákostelská Z, Klimešová K, Přibylová J, Bártová J, Sanchez D, Fundová P, Borovská D, Srůtková D, Zídek Z, Schwarzer M, Drastich P, Funda DP.The role of gut microbiota (commensal bacteria) and the mucosal barrier in the pathogenesis of inflammatory and autoimmune diseases and cancer: contribution of germ-free and gnotobiotic animal models of human diseases. Cell Mol Immunol. 2011 Mar;8(2):110-20.	J imp	72	4,185		
5	Kverka M, Klimesova K, Zakostelska Z, Sokol D, Hudcovic T, Hrncir T, Rossmann P, Mrazek J, Jan Kopecny J, Verdu EF, and Tlaskalova-Hogenova H. Oral administration of Parabacteroides distasonis antigens attenuates experimental murine colitis through modulation of immunity and microbiota composition. Clin Exp Immunol. 2011 Feb;163(2):250-9.	J imp	13	3,278		
6	Cinova J, De Palma G, Stepankova R, Kofronova O, Kverka M, Sanz Y, Tuckova L. Role of intestinal bacteria in gliadin-induced changes in intestinal mucosa: study in germ-free rats. PLoS One. 2011 Jan 13;6(1):e16169.	J imp	20	3,534		

	Result	Result type code	Number of citations (without autocitations) according to WOS	Imapact factor of journal or ERIH database category	Number of citations in NRRE fields (fields evaluated prilarily) in the national context	Journal included in SCOPUS database (only to be completed for journals not included in WOS
7	Kverka M, Burianova J, Lodinova-Zadnikova R, Kocourkova I, Cinova J, Tuckova L, Tlaskalova-Hogenova H. Cytokine profiling in human colostrum and milk by protein array. Clin Chem. 2007;53:955-62.	J imp	25	7,768		
8	Vrabelova Z, Kolouskova S, Bohmova K, Faresjo MK, Sumnik Z, Pechova M, Kverka M, Chudoba D, Zacharovova K, Stadlerova G, Pithova P, Hladikova M, Stechova K. Protein microarray analysis as a tool for monitoring cellular autoreactivity in type 1 diabetes patients and their relatives. Pediatr Diabetes. 2007;8: 252-60.	J imp	6	2,129		

Total number of results defined in the currently valid Methodology for Evaluating for Results of Research and Development for last 5 years (according to RIV):

1a. Article in professional journal, impacted (type of result: Jimp)	15
1b. Article in professional journal, included in Scopus database (type of result: Jsc)	0
1c. Article in professional journal, non-impacted (type of result: Jneimp)	0
1d. Article in Czech professional journal, peer-reviewed (type of result: Jrec)	0
2a. Scholar book/monograph (type of result: B)	0
2b. Chapter in scholar book/monograph (type of result: C)	0
3. Article in conference proceedings (type of result: D)	1
4. Patent (type of result: P)	0
5. Utility or industrial design (type of result: F)	0
6. Pilot run, verified technology, varienty, breed (type of result: Z)	0
7. Prototype, functional sample (type of result: G)	0
8. Result implemented by funding provider (legislation, norms, strategic documents, policy recommendations, etc.) (type of result: H)	0
9. Specializes map (type of result: L)	0
10. Certified methodology and procedure (type of result: N)	0
11. Software (type of result: R)	0
12. Research report containing information classified pursuant to special legislation (type of result: V)	0

Total number of citations, including autocitations, of all of the scientific work (according to Web o Science)	**297**
H-index according Web of Science	**9**

CURRICULUM VITAE

APPLICANT

First Name, Surname: **Ass. Prof. PAVEL DRASTICH, M.D., Ph.D.**

Investigational Site Address: IKEM, Clinic of Hepatogastroenterology
Videnska 1958/9, 140 21, Prague 4
Czech Republic

Date of Birth: 22.05.1963
Languages: English – written, spoken

Education at University/Medical School Level:
Medical Faculty Charles University, Prague from 1981 to 1987
Qualification: M.D.
Details of Medical License, Certifications:
Clinical Gastroenterology, Endoscopy, Advanced Therapeutic Endoscopy

Examinations for Specialties:
Board Examination in Internal Medicine 1st Degree 1991
Board Examination in Gastroenterology 1994
Board Examination in Internal Medicine 2nd Degree 1997

Post Graduate Positions: Resident, registrar, lecturer, consultant

2009 finished Ph.D. study program (Participation of some mechanisms of mucosal immunity in the pathogenesis of Crohn´s disease and ulcerative colitis) with qualification: Ph.D.

Current position: Head of outpatient department

Associate professor in First Faculty of Medicine, Charles University, Prague 2012

Membership : Czech Gastroentrology Society- IBD group, ECCO
Czech Hepatology Society
Intestinal Transplant Association
The European Crohns and Colitis Organization

Scientific interest in the past 5 years

The scientific interest over the past 5 years consists of a variety of topics in gastroenterology and hepatology including inflammatory bowel disease, portal hypertension, celiac disease and liver transplantation. In inflammatory bowel disease I have been actively involved in experimental projects focused on etiopathogenesis on mucosal level, in portal hypertension on primary prevention of first variceal bleeding, in celiac disease on relationship with various liver diseases and in liver transplantation on primary sclerosing cholangitis and issue of related ulcerative colitis.

Date: 25.Jul.2014

TEAM MEMBER **CURRICULUM VITAE**

First Name, Surname: **JULIUS ŠPIČÁK, MD, CSc., Prof.**

Born: 10. 6. 1952, in Prague, Czech Republic

Education
Medical Faculty, Charles University, Prague, 1970-1976

Postgraduate Education, Training and Certifications
1976-1980: Residency in Internal Medicine
1980-1983: Fellowship in Internal Medicine, Gastroenterology
1980: Specialist in Internal Medicine, Ist degree
1983: Specialist in Internal Medicine, IInd degree
1992: Specialist in Gastroenterology

Academic and Scientific Degrees
1976 MD
1983 Ass. Prof., Internal Medicine 1992 CSc (PhD)
1992 (Endoscopic Sclerotherapy in the Treatment of Esophageal Variceal Bleeding),
1994 Habilitation (Docent of Internal Medicine), (Endoscopic Treatment of Difficult Choledocholithiasis – Results and Complications of Various Techniques)
2007 Professor of Internal Medicine

Present Position: Head of the Clinic of Hepatogastroenterology, Institute of Clinical and Experimental Medicine, Vídeňská 1958/9, 140 21, PRAHA 4, Czech Republic

Membership
Czech Society of Gastroenterology: President
European Society of the Gastrointestinal Endoscopy – Governing Board: Councilor 2000-2004
OMED-OMGE: CRC screening committee,
American Society of Gastrointestinal Endoscopy International Member,
American Gastroenterological Association
German Society of Gastroenterology: Corresponding Member
Slovak Society of Gastroenterology: Honorary member
Hungarian Society of Gastroenterology: Honorary member
Endoscopy: International editorial board
Gastrointestinal endoscopy: International editorial board

Bibliography
Approximately 250 papers on: digestive endoscopy, pancreatology, liver transplantation, colorectal cancer screening, chapters in 14 monographies, textbooks and manuals.

Scientific interest in the past 5 years
The scientific interest over the past 5 years consists of a variety of topics in gastroenterology and hepatology including colorectal cancer, natural orifice transluminal endoscopic surgery, experimental endoscopy, acute and chronic pancreatitis, variceal bleeding and liver transplantation. In colorectal cancer I have been actively involved in numerous clinical scientific projects dealing with cancer screening, polyp detection and therapy, diagnosis and management of colorectal cancer and in experimental projects on molecular biology of colorectal polyps/cancer.

Co-applicant: MUDr. Miloslav Kverka, Ph.D.

Date and place of birth: September 13, 1978, Prague, Czech Republic

Education and Employment:

1997 – 2003 3rd Faculty of Medicine, Charles University in Prague

2003 – 2011 Institute of Microbiology of the AS CR, v.v.i. Department of Immunology and Gnotobiology, Prague. *Topic: Interaction of commensal bacteria with mucosal immunity and its regulation. Supervisor: Prof. Helena Tlaskalová-Hogenová, MD, DSc.*

since 2011 Staff Scientist, Laboratory of Cellular and Molecular Immunology, Department of Immunology and Gnotobiology, Institute of Microbiology of the AS CR, v.v.i. Prague

Memberships, Honors and Committees:

Member of Czech Immunological Society (2003), Czech Society of Gastroenterology (2003), Czech Society of Allergology and Clinical Immunology (since 2003), Society for Mucosal Immunology (2005), European Academy of Allergology and Clinical Immunology (2005), Czech Society for Probiotics and Prebiotics (2006) and Associate Member of the Faculty 1000 Medicine (2007).

Prize awarded by the Czech Immunological Society (2008), Prize for the best poster at the 6th European Mucosal Immunology Group Meeting (2008), Prize for the best Dissertation in 2011 at the Institute of Microbiology AS CR (2012), Jaroslav Šterzl's prize awarded by the Czech Immunological Society (2012 and 2013)

Member of The Technical Committee for Animal Care and Use at Institute of Microbiology, AS CR, v.v.i. (2009)

Stays:

Department of Cell Biology, University Medical Center Groningen, Groningen, The Netherlands (2006 and 2007, 2 months). Department of Pharmaceutics and Pharmaceutical Chemistry, University of Utah, Salt Lake City, USA (2013, 3 months)

Activities during the last 5 years:

Author and co-author of 15 papers in impacted journals, principal investigator of 3 projects researching mainly in the host-microbe interactions in pathogenesis of chronic inflammatory diseases

Education activities:

Basic course in Clinical Immunology organized for Faculty of Science, Charles University in Prague (Lecturer since 2004 and organizer since 2012).

Supervisor of 1 successful MSc student, currently supervising 2 PhD and 2 MSc students.

Publication activity:

26 publications in Jimp, 2 international patents, 1 chapter in foreign monography, 1 publication in Jrec. Sum of citations (incl. self-citations): 283; H-index: 9 (WoS 29.7.2014)

Team member: Prof. MUDr. Helena Tlaskalová-Hogenová, DrSc.

Date and place of birth: December 29, 1938, Prague, Czech Republic

Education and Employment:

1957 - 1962 Faculty of General Medicine, Charles University, Prague
1962 - 1964 Clinician, Dept. of Hematology, Hospital in Ústí n. Labem
1964 - 1968 PhD student, Institute of Microbiology AS CR (IMIC), Dept. of Immunology
Thesis: Development of immune reactions to antigens of gram-negative microflora, Inst. of Microbiol., Czech Acad. Sci., Prague, Supervisor: Prof. Jaroslav Šterzl, MD, DSc.
1968 - 1991 Staff Scientist, Dept. of Immunology and Gnotobiology, IMIC
1991 - 2004 Head of the Dept. of Immunology and Gnotobiology, IMIC
1994 - DSc - Academy of Sciences of the Czech Republic, Prague
1995 - Associate Professor (1st Medical Faculty, Charles University, Prague)
2001 - Professor (1st Medical Faculty, Charles University, Prague)
since 2014 Staff Scientist, Laboratory of Cellular and Molecular Immunology, Department of Immunology and Gnotobiology, Institute of Microbiology of the AS CR, v.v.i. Prague

Memberships, Honors and Committees:

Awards of the Czechoslovak Academy of Sciences, Prague 1981,1982; Membership of the Council of International Union of Immunological Societies 1996-2003;Nihon University Medal, Tokyo 1999; Membership of the External Advisory Group of European Committee (Life Science – Infectious diseases) 2005-present; Member of the Learned Society of the Czech Republic 2001-present; Honorary Member of the Czech and the Slovak Immunological Society 2002; Membership of the International Study Group on New Antimicrobial Strategies (Herborn University Foundation) 2005-present; J.E. Purkyne Medal of Academy of Sciences of the Czech Republic 2006; Medal "Garnet Immunoglobulin" of the Czech Immunological Society 2006; Member of the Royal Society of Arts and Science in Göteborg 2005-present; Patocka Medal of the Czechoslovak Microbiological Society 2006-present

Member of Editorial Boards:

Faculty 1000 Medicine, Mucosal Immunology, Open Allergy Journal; International Journal of Probiotics and Prebiotics, Folia Biologica, Alergie

Activities during the last 5 years:

Author and co-author of 55 papers in impacted journals, mainly in the field of immunology, host-microbe interactions and biomarker research

Publication activity:

With over 237 of published papers, 55 chapters in Czech and English books, over 4093 citations (incl. self-citations) and h-index of 34 (WoS 29.7.2014), is world leading expert in a field of mucosal immunology.

Immunological biomarkers for noninvasive diagnosis, outcome prediction and therapy selection in inflammatory bowel disease

Current knowledge

Inflammatory bowel diseases (IBD), i.e. Crohn's disease (CD) and ulcerative colitis (UC), are severe chronic inflammatory illnesses of the gastrointestinal tract, affecting approximately 0.2 % of the population. Although their etiology and pathogenesis is not fully understood, it is generally accepted that the inflammation results from an aberrant immune response to antigens of resident gut microbiota in genetically susceptible individuals.(*1*) With 10 times more cells than human body, microbiota represents major stimulus to development of immune system and many other physiological functions.(*2-4*) This is most striking in the gut, where microbiota reaches the highest numbers. The human gut microbiome is composed of at least 1000 distinct bacterial species, out of which almost 50% cannot be cultivated *ex vivo* and therefore properly studied.(*5*) It has been proposed, that either imbalances in intestinal microbiota (dysbiosis), or presence of commensal bacteria with increased virulence (e.g. Adherent-invasive *Escherichia coli*, *Mycobacterium avium* s. paratuberculosis) in IBD patients, could both cause excessive immune response to microbiota, by disrupting the microbiota-mucosa interaction, penetrating through the mucosal barrier and stimulate local and systemic immunity.(*6-9*)

Antigens in the gut lumen are separated from the host's tissue by very sophisticated barrier system made of thick mucus layer and tightly connected line of epithelial cells. This barrier prevents the excessive interaction of these potent antigens with mucosal immune system. Furthermore, the mucus barrier function is strengthened by biologically active substances produced by other epithelial (defensin) or immune (secretory IgA) cells. Apical part of the epithelial cells is sealed by a dynamic complex of proteins regulating cellular connectivity – Tight junctions. Disruption of the barrier (defects of the epithelial continuity) increases permeability and contact with resident microbiota antigens, which is one of the key steps in pathogenesis of IBD and also many other diseases, as we recently reviewed.(*10*)

The impairment of host-microbe interaction was corroborated by genome-wide association studies, by finding that IBD patients have numerous mutations in genes encoding recognition, processing and killing of microorganisms, autophagy, and regulation of immune processes.(*11, 12*) Disruption of T lymphocyte regulatory functions and impairment of the mucosal immune response to normal microbiota play a crucial role in the pathogenesis of chronic intestinal inflammation.(*13*). Although a role of Th1 and Th17 and their effector functions (cytokine production) in the pathogenesis of CD has been proved, the presence of increased antibody levels against some microbial constituents in sera of patients suggest the possible biological role of B cell products.(*14*) The higher levels of certain antibodies against microbial antigens (e.g. ASCA, Ompc, CBir1 and against saccharide epitopes) or other biomarkers (e.g. fecal calprotectin) in IBD patients could be used for diagnostic purposes, and relapse prediction. However, the utility of these serological markers in daily clinical practice is still rather low.(*15, 16*) The diagnosis of IBD and its clinical staging is still based mainly on patient's history and medical examination, where endoscopy plays major part.

There are several well-established approaches (both surgical and conservative) to manage IBD, including classical anti-inflammatory drugs and biologicals, and the ideal therapeutic strategy depends on the type and extent of the disease.(*17*) But despite the well-established headlines in IBD therapy, discontinuation of pharmacological intervention due to the inefficiency or adverse events is very common in IBD for all types of therapy.(*17-19*) Ability to predict the disease relapses and complications or suggest the ideal therapy for particular patient during the first patient's admission to the hospital is a "Holy Grail" of IBD diagnostics.

The most dreaded complication of the IBD is colorectal cancer. With age-standardized incidence rates of 91.2 per 100000 in males and 44.3 per 100000 in females, colorectal cancer (CRC) is one of the most common malignant neoplasia in the Czech Republic, reaching the top numbers in Europe.(*20*) The development of this malignancy is tightly linked with inflammatory changes,(*21*) and its risk is almost 40 times higher in patients with UC than in general population.(*22*) New biomarkers, capable to predict the development of this complication, could therefore lead to early diagnosis in this particular risk group. Primary sclerosing cholangitis (PSC) is another serious complication of UC,

which occurs even in 3 – 7.5% of UC patients. Moreover, PSC is a risk factor for development of both cholangiocarcinoma and colorectal carcinoma, in which the risk factor is even higher than in isolated UC.(*23*) Based on current knowledge the inflammatory bowel disease in patients with PSC could be considered as a distinct phenotype of IBD, in which colitis has a phenotype different from this of conventional IBD.(*24*) The pathogenesis of gut and liver damage in this complex clinical unit is deeply interconnected. While, the portal bacteremia causes pathological activation of the NF-κB pathway in the biliary epithelial cells via an aberrant bacterial translocation through the inflamed intestine (gut barrier failure),(*25*) the alteration of gut microbiota may lead to liver damage by changing the metabolism of bile acids.(*26*)

Biomarkers Definitions Working Group defined biomarker as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological response to a therapeutic intervention.(*27*) Only by predicting the unfavorable disease progression, therapy failure or even the serious therapy-related adverse events, one may be able to prevent them without useless, unnecessarily aggressive and expensive therapeutic options. The best biomarkers are molecules tightly connected to disease pathogenesis, moreover, some of them could became good target for biological therapy in the future. The ideal biomarkers for IBD should, therefore be molecules related to immune response to commensal microbiota, or even some member of gut microbial community, markers of gut barrier failure and molecules with immuno-modulatory properties such as cytokines.

Hypothesis

Recent studies and our previous results imply that development of certain disease-specific and therapy-specific complications could be predicted by use of suitable biomarkers. With reliable biomarkers, clinicians may be able to tailor-made the IBD therapy or follow up for each patient, thus preventing serious disease- or therapy-related complications. This approach will then not only improve the patient's morbidity, but it will also reduce the cost of the therapy. For the best results, these biomarkers should be recruited from known crucial steps in IBD pathogenesis, dysbiosis, gut barrier failure and dysbalance in immune system regulation. We hypothesize that some gut bacteria penetrate into the deeper layers of the mucosa, and elicit an increased immune response which can be detected both on humoral and cellular level. These changes are preceded or followed (vicious circle) by signs of gut barrier failure and immune system dysregulation. Some of these signs may be used to predict the course of the disease, select the best therapy and assign the particular patient to the high risk group, suitable either for aggressive therapy or closer follow up.

Aims and objectives

The general theme of the project is finding the biomarkers in UC and CD patients, that correlate with disease progression (i.e. relapse prediction) and pharmacotherapy outcomes. The general aim of this project is to use these molecules, closely related to IBD pathogenesis, as biomarkers suitable for fast, reliable and non-invasive diagnosis and disease/therapy outcome prediction. This general aim could be divided into several specific aims, following the key moments of IBD pathogenesis. Each parameter will be correlated with the disease course and therapy outcome:

1) To screen the cytokines, chemokines and growth factors in the sera of IBD patients with broad-spectrum protein microarray
2) To measure the serum levels of specific antibodies (B cell response) directed against bacteria with ELISA.
3) To analyze the phenotype and *in vitro* immune response of peripheral blood lymphocytes against microbial antigens in patients with IBD and compare it to healthy controls (T cell response).
4) To characterize the intestinal barrier function by measuring specific serum markers of epithelium damage (e.g. i-FABP or caspase-cleaved cytokeratine 18) and antibodies against commensal microbiota.
5) To analyze the gut microbiota composition as a biomarker
6) To validate new biomarkers by correlations with already used biomarkers (ASCA, ANCA antibodies in serum or calprotectin in stool)

7) By long-term monitoring and retrospective analysis of samples from IBD patients (taken from the IBD serum bank at the IKEM) verify the predictive value of selected serum biomarkers in the development of the disease or its complications

Methods

Patient recruitment and sample collection

Clinical samples - stools, blood for serum and blood cells - will be collected from patients with CD, UC and inflammation-related colon cancer during routine outpatient visits. State of the disease in patients will be evaluated with standard clinical, endoscopic and histopathological criteria (e.g. HBI, CDEIS, Mayo score for UC) as a part of the routine examination.(*28-30*) The control subjects (without intestinal pathology) will be recruited during the colonoscopy performed as a part of the preventive screening for colorectal cancer. Due to the considerable inter-individual variability in the microbiota composition and in immune response to the microbiota, we will need approximately 70 of each CD patients, UC patients, patients with IBD-related colon neoplasia (including all stages of carcinogenesis such as dysplasia, adenoma and carcinoma), PSC-UC patients and control subjects. We will also collect patients in the different stages of the disease (flare-up vs. remission) and those with concurrent PSC. Informed consent and ethical committee statement is attached below. The stool sample will be collected during scheduled examination at the outpatient unit of the clinic, together with other scheduled sample collection. The patients scheduled for the examination will be contacted and instructed by their physician by phone and if they agree to the enrolment in the study, the standard collection tube for stool sample and basic instructions for the collection will be mailed in advance. The stool sample will be frozen within 5h of collection and stored at -80°C until the DNA extraction.

Extraction of bacterial DNA and microbiota analysis

Bacterial DNA for microbiota taxonomy will be extracted using repeated beat beating method described by Yu and Morrison (*31*). DNA will be further amplified using the primers from study by Milani et al. (*22, 32*) and Q5® Hot Start High-Fidelity DNA Polymerase (New England Biolabs). The product consisting of relatively wide region of bacterial 16S rRNA gene will be then sequenced using the Ion Torrent PGM platform (Life Technologies). This should allow us to assess the classification to quite low taxonomic rank and analyze the differences between each groups of patients (PSC, UC, "PSC-UC," controls). Selected groups of microbes will be then quantified by qPCR, using standard protocols.(*33*)

Serum cytokine analysis

Serum levels of cytokines will be measured by state-of-the art broad-spectrum protein microarrays (Ray Biotech), and by well - established ELISA and Luminex. With this approach, we can get information about many potential biomarkers (up to 507 in one analysis), and quantify selected ones. Results will be compared between IBD and control group, and in same disease in different time-points (e.g. during both relapse and remission periods in the same patient), and especially in case of therapy failure or if unexpected complication occurs during the study. The biomarkers will be validated and compared with those already known (e.g. high sensitivity CRP, ASCA, ANCA in sera, and calprotectin and lactoferrin in stool) and with disease activity indexes

Analysis of gut barrier function

There are several markers of gut barrier failure that can be measured from serum, and since gut barrier damage is an important step in IBD pathogenesis, these are obvious candidates for biomarker research. These includes intestinal fatty acid binding protein (i-FABP) that is released from the gut epithelium when it is damaged.(*34*) The ratio of caspase - cleaved fragments of keratin 18 (m30) and its uncleaved form (m65) can be used to measure the damage to epithelium and distinguish between apoptosis and necrosis. This approach has been used to monitor damage to liver epithelium, making it especially interesting to PSC-UC group.(*35*) All these markers are commercially available as an ELISA kit suitable for human serum, plasma or even urine. Since the immune system is strongly primed against own microbiota during barrier failure, serum titers of IgA and IgG antibodies against the bacterial antigens can give good information on the condition of gut barrier. The anti-microbiota

antibodies will be analyzed by in-house ELISA, which was previously developed in our laboratory.(*36*)

Phenotyping of blood cells
Since the cellular response against microbiota may be more appropriate biomarker, we will analyze the response of peripheral blood mononuclear cells (PBMCs) to microbiota *in vitro*. The blood will be collected into the tubes containing anticoagulant EDTA and transported at room temperature to the IMIC. Here, the PBMCs will be isolated using discontinuous Percoll gradient and cultivated with the selected bacterial antigens, including those suspected from driving intestinal inflammation (e.g. adherent-invasive *E. coli*).(*6, 37*) The cytokine response (e.g. IFN-γ, IL-17 and TNF-α) and the T cell phenotype will be measured by ELISA and flow cytometry, respectively.(*38*)

Statistical analyses and bioinformatics
For the data mining and bioinformatic analyses of the complex data (e.g. microarray or gut microbiota sequencing), we will use state-of-the art approaches, currently used in our laboratory. Typical cytokine pattern that can best discriminate between our groups of subject, will be searched by a semi-supervised prediction analysis using the Prediction analysis of microarray (PAM) method as described previously (*39*) By calculating the false discovery rate (FDR) in our predicted biomarker set, the power of the prediction will be analyzed with the significance analysis of microarrays (SAM) method as previously described.(*40*) To identify the biological pathways enriched in our datasets, we will perform pathway analysis using free bioinformatic tools (e.g. The Database for Annotation, Visualization and Integrated Discovery - DAVID, KEGG pathway database or COPE cytokine database). The microbiota bioinformatics will be performed using well established pipelines and the identification of microbiota will be dune using available databases (e.g. SILVA database od 16S rRNA available at http://www.arb-silva.de/) and related web-based toolkit.(*41, 42*)
The statistical analysis of the data will be done using standard approaches, including both parametric and non-parametric tests comparing quantities between experimental groups, followed by suitable post-hoc tests if appropriate. For the measurements using different methods (e.g. validation of the results found by microarray by ELISA) we will use unbiased (e.g. Passing and Bablok) regression analysis.

Time schedule
2015
During 2015, we will collect first set of clinical samples for the training part of the analysis. The sample collection will continue throughout the project to collect samples for the validation part of the study and follow up. We will also perform first set of peripheral blood cells stimulation *in vitro*. These experiments will also continue throughout the proposed project since they are dependent on the collection of fresh blood. First set of biomarker profiling by protein microarrays will be performed once the sufficient amount of samples will be collected (probably late 2015 or early 2016)
2016
In 2016, we will continue with the experiments outlined during 2015 as well as with with the sample collection. We will perform advanced bioinformatic analysis of microarray results and perform and analyze the gut microbiota composition. We will also perform the analysis of markers of gut barrier function, and select the best candidate microbial antigen for the detection of serum antibodies against bacteria and its components by ELISA.
2017
In 2017, we will continue with the gut barrier function analysis, increase of the studied cohort and start validation of the selected panel of biomarkers and transfer the analyses from more complex methods (microarray and sequencing) to the methods more usable in the clinical laboratory settings (ELISA, PCR and bead assays).
2018
In 2018, we will finish the experimental part, perform a final evaluation, analysis of the adverse events and publish the results. We will set up for the longer follow-up and apply for the next project.

Collaboration of participants

There are two institutions, with long and successful history of collaboration, directly involved in the current project: 1) IKEM, which will take part in the project coordination and where the patients will be recruited, examined and clinical samples will be collected. Here also will be performed Luminex analysis 2) Institute of Microbiology ASCR, v.v.i. (IMIC), which will be performing the immunological and microbiological analyses. The long history of collaboration and proximity of both institutes will greatly simplify the project management.

International collaboration

Both participants, IKEM and IMIC, are is internationally recognized scientific institutes connected to the broad network of experts that stretches worldwide. Members of the research team have long history of international cooperation, as documented by their publications, and their participation in international projects (e.g. Framework 5th, 6th and 7th of the EU, cooperative projects of NIH, USA). These collaborations included colleagues from the Netherlands (prof. Chris Mulder, prof. Jerry Wells), Denmark (Prof. Axel Hansen), Italy (Dr. Andrea Tringali), USA (Prof. Koichi Kobayashi), Canada (Dr. Elena Verdu) to name few recent ones.

Importance of the proposed project

As briefly outlined in the introduction, IBD results from dysregulated immune response to gut commensal microbiota and it is accompanied by gut barrier failure and dysbiosis. Therefore, we will study these pathogenetic mechanisms by serum protein profiling, analysis of the gut microbiota, and analysis of the humoral and cellular immune response. Recent advances in science and technology now allow to study these mechanisms in more details than ever before. Practical use of serological tests in non-invasive diagnostics of IBD is not widely used because of the lack of reasonable specificity and sensitivity of available tests. It is supposed that finding of the new markers (cytokine panels or new specificities of antibodies directed against intestinal microbiota components) will increase the specificity and sensitivity these non-invasive tests and expediting the diagnostic process in the IBD patients. Moreover, by profiling the biomarkers, we can also unravel new part of IBD pathogenesis or even find suitable targets for future biological therapy. Therefore, this project connects three long-term research interests of laboratory at the IMIC and Hepatogastroenterology clinic of IKEM: microbe interactions with host immune system, intestinal inflammation, cytokine profiling and searching biomarkers suitable for early diagnostics.

Qualification of the research team and preparedness of the laboratories

Patients and healthy individuals' material (blood and fecal samples) will be provided by experienced gastroenterologist Assoc. prof. Pavel Drastich and two of his PhD students (dr. Bajer and dr. Brezina) from the Clinic of Hepatogastroenterology at the IKEM, which is one of the most important health care institutions in the Czech Republic. This is where the examination of all the patients involved will be carried out as well.

Assoc. prof. Drastich will be responsible for general management of the project (including the publication and presentation of the results) and, as a highly experienced physician, will also supervise the clinical evaluation of the patients.

Prof. Spicak, head of the department and president of Czech Gastroenterological Society, will supervise the selection of inclusive patients and evaluation of the results in order to introduce them into clinical practice. He, as a highly respected expert in the field, will also present the results at various international events.

Dr. Bajer and Dr. Brezina are both postgraduate students at IKEM. They will coordinate the collection of the samples from the selected patients. They will also perform and assess the Luminex analyses and PCR and qPCR and correlate the results with clinical condition of the patients, analyses, respectively.

Two technicians (10 % of work capacity) be responsible for samples preparation and storage and related administrative tasks.

Laboratories at IKEM are equipped with all equipment required for the successful participation on the project, including Luminex analyzer and both conventional and RT-PCR cyclers.

Research in the Laboratory of cellular and molecular immunology of the IMIC is focused on the developmental and regulatory mechanisms of mucosal and systemic immunity and their use in biomarker research. Several commercially available tests were developed in the laboratory in the past.

Members of the team are highly experienced in immunological methods. Dr. Kverka will mainly coordinate the work at IMIC, mentoring the student involved in the project, analyze and interpret the data from the experiments and participate on the result publication and presentation. Prof. Tlaskalová-Hogenová will interpret the experimental data and participate on presenting and publishing the results. Postgraduate student MSc. Dusilová will perform the experiments in vitro and flow cytometry analyses and perform the molecular microbiology techniques. PhD student will be hired for the proposed project and will perform the immunological analyses. Technician (20 % of work capacity) will perform routine laboratory work (e.g. image analysis) advanced bioinformatics and administrative activities. Laboratories at IMIC AS CR are well equipped and the members of the team have full access to all laboratory equipment needed for the project (including LASER scanner with high resolution, flow cytometer and sequencing analyzers. The last steps in the bacteria sequencing will be performed at the Institute of animal physiology and genetics AS CR, v.v.i. (located on the same campus) using Ion Torrent sequencer by Dr. Mrazek.

Expected results and significance of the project

The proposed project will have significant impact on both basic science and medical practice, because the search for new biomarkers will not only bring new knowledge about IBD pathogenesis, which will be used to prepare a ground for new noninvasive diagnostic and predictive approaches. These approaches might provide the methodological basis enabling swift and reliable determination of diagnosis, possible disease prognosis and definition of patients resistant to the conventional therapy, ultimately improving disease management with positive medical as well as socio-economic output. Moreover, some of the new biomarkers could be considered as targets of new biological therapies. The project will bring important results which will be published in three impacted scientific journals and presented at international and national conferences. The deliverables are quantified in the section C2 of this proposal (3 articles in international impacted journals, 2 articles in Czech peer-reviewed journals and 3 articles in conference proceedings).

Benefits of the project

We presume that new biomarkers will extend the spectrum of noninvasive tests important not only for diagnostics or differential diagnosis of CD and UC, but also in the prediction of these grave diseases. The proposed project is very relevant to the present and future needs of the society. The Czech Republic belongs among the countries with the highest incidence of colorectal cancer as well as IBD. The panel of biomarkers revealed with this study could extend the spectrum of noninvasive tests used in IBD diagnostics, outcome prediction and simplifying the physicians' therapy decision making (applied research), ultimately leading to improvement of quality of life of IBD patients and savings for the health care delivery system. Important part of the project is education of 4 PhD students of biomedicine. The project's aims are in agreement with the international focus on biomarker discovery and early diagnosis, international cooperation between Czech medical research and world science and support of young scientists.

References

1. R. B. Sartor, *Gastroenterology* **134**, 577 (Feb, 2008).
2. J. J. Cebra, *Am J Clin Nutr* **69**, 1046S (May, 1999).
3. H. Kozakova *et al.*, *Microbes Infect* **8**, 2629 (Sep, 2006).
4. H. Tlaskalova *et al.*, *Folia Biol (Praha)* **16**, 177 (Jun, 1970).
5. L. V. Hooper, J. I. Gordon, *Science* **292**, 1115 (May 11, 2001).
6. A. Darfeuille-Michaud *et al.*, *Gastroenterology* **115**, 1405 (Dec, 1998).
7. R. W. Bentley *et al.*, *Am J Gastroenterol* **103**, 1168 (May, 2008).
8. G. W. Tannock, *Mucosal Immunol* **1 Suppl 1**, S15 (Nov, 2008).
9. D. N. Frank *et al.*, *Proc Natl Acad Sci U S A* **104**, 13780 (Aug 21, 2007).
10. H. Tlaskalova-Hogenova *et al.*, *Cell Mol Immunol* **8**, 110 (Mar, 2011).
11. C. G. Mathew, *Nat Rev Genet* **9**, 9 (Jan, 2008).
12. D. P. McGovern *et al.*, *Nat Genet* **42**, 332 (Apr, 2010).
13. R. S. Blumberg, *Dig Dis* **27**, 455 (2009).
14. R. J. Adams *et al.*, *Am J Gastroenterol* **103**, 386 (Feb, 2008).

15. M. Papp, G. L. Norman, I. Altorjay, P. L. Lakatos, *World J Gastroenterol* **13**, 2028 (Apr 14, 2007).
16. J. D. Lewis, *Gastroenterology* **140**, 1817 (May, 2011).
17. D. C. Baumgart, W. J. Sandborn, *Lancet* **369**, 1641 (May 12, 2007).
18. R. B. Stein, S. B. Hanauer, *Drug Saf* **23**, 429 (Nov, 2000).
19. J. M. Swoger, D. G. Binion, *Dig Dis* **28**, 452 (2010).
20. J. Ferlay, D. M. Parkin, E. Steliarova-Foucher, *Eur J Cancer* **46**, 765 (Mar, 2010).
21. M. Lukas, *Dig Dis* **28**, 619 (2010).
22. L. Beaugerie, H. Sokol, P. Seksik, *Dig Dis* **27**, 375 (2009).
23. U. Broome, R. Lofberg, B. Veress, L. S. Eriksson, *Hepatology* **22**, 1404 (Nov, 1995).
24. K. Boonstra *et al.*, *Inflamm Bowel Dis* **18**, 2270 (Dec, 2012).
25. K. Harada *et al.*, *Lab Invest* **83**, 1657 (Nov, 2003).
26. G. Kakiyama *et al.*, *J Hepatol* **58**, 949 (May, 2013).
27. G. BDW, *Clin Pharmacol Ther* **69**, 89 (Mar, 2001).
28. K. W. Schroeder, W. J. Tremaine, D. M. Ilstrup, *N Engl J Med* **317**, 1625 (Dec 24, 1987).
29. J. Y. Mary, R. Modigliani, *Gut* **30**, 983 (Jul, 1989).
30. R. F. Harvey, J. M. Bradshaw, *Lancet* **1**, 514 (Mar 8, 1980).
31. Z. Yu, M. Morrison, *Biotechniques* **36**, 808 (May, 2004).
32. C. Milani *et al.*, *PLoS One* **8**, e68739 (2013).
33. T. Matsuki, K. Watanabe, R. Tanaka, H. Oyaizu, *FEMS Microbiol Lett* **167**, 113 (Oct 15, 1998).
34. G. Gollin, C. Marks, W. H. Marks, *Surgery* **113**, 545 (May, 1993).
35. H. Bantel *et al.*, *Hepatology* **40**, 1078 (Nov, 2004).
36. B. Cukrowska *et al.*, *Scand J Immunol* **55**, 204 (Feb, 2002).
37. A. M. Petersen *et al.*, *BMC Microbiol* **9**, 171 (2009).
38. M. Pai, A. Zwerling, D. Menzies, *Ann Intern Med* **149**, 177 (Aug 5, 2008).
39. R. Tibshirani, T. Hastie, B. Narasimhan, G. Chu, *Proc Natl Acad Sci U S A* **99**, 6567 (May 14, 2002).
40. V. G. Tusher, R. Tibshirani, G. Chu, *Proc Natl Acad Sci U S A* **98**, 5116 (Apr 24, 2001).
41. A. L. Hartman, S. Riddle, T. McPhillips, B. Ludascher, J. A. Eisen, *BMC Bioinformatics* **11**, 317 (2010).
42. C. Quast *et al.*, *Nucleic Acids Res* **41**, D590 (Jan, 2013).