Old Herborn University Seminar Monograph 20:
Inflammation and infection. The golden triangle: food - microflora - host defence.
Editors: Peter J. Heidt, Tore Midtvedt, Volker Rusch, and Dirk van der Waaij.
Herborn Litterae, Herborn-Dill, Germany: 25-36 (2007).

GLUTEN AND CHRONIC DISEASES: INFLAMMATORY ACTIVITY OF GLUTEN

HELENA TLASKALOVÁ-HOGENOVÁ, RENÁTA ŠTĚPÁNKOVÁ, LUDMILA TUČKOVÁ, LENKA PALOVÁ-JELÍNKOVÁ, HANA KOZÁKOVÁ, TOMÁŠ HUDCOVIC, DANIEL SANCHEZ, LENKA FROLOVÁ, JANA CINOVÁ, BARBARA PECHAROVÁ

Department of Immunology and Gnotobiology, Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic

SUMMARY

Initial events as well as effector mechanisms involved in most inflammatory and autoimmune diseases remain largely unknown. We suppose that dysfunction of the innate and adaptive immune system associated with mucosae, i.e. with the largest body surfaces representing an interface between the organism and environment could lead to the impairment of mucosal barrier function followed by the development of localised or systemic inflammatory and autoimmune processes.

Celiac disease (CD) is a frequent chronic autoimmune disorder affecting small bowel and developing in genetically susceptible individuals because of intolerance to wheat gluten. Beside gut mucosa celiac disease affects also a number of other organs: extra-intestinal symptoms are pronounced mainly in adult celiac patients. Various immunologically mediated chronic diseases were demonstrated to be associated with celiac disease. Interestingly, in a subset of non-celiac patients suffering from various chronic diseases introduction of gluten free diet was shown to improve the clinical symptoms.

Animal models are helping to elucidate the aetiology and pathogenic mechanisms of human diseases. We have developed a rat model of gluten-induced enteropathy by repeated intragastric application of gliadin starting at birth. Using this model, we demonstrated a protective effect of breast-feeding in the development of gluten-induced enteropathy. Epidermal growth factor (EGF) as one of the important components of the milk was shown to be responsible for the protective effect against the development of severe forms of gluten induced enteropathy.

We have shown that wheat gliadin and its peptic fragments have the unique ability (in contrast to other food proteins) to activate mouse macrophages and human monocytes to the production of pro-inflammatory cytokines through NF kappa B signalling pathway. Human monocyte-derived dendritic cells were demonstrated to upregulate maturation markers and to increase the production of chemokines and cytokines when cultivated with the peptic digest of gliadin (but not with other tested food proteins).

Our results suggest that activation of innate immunity cells by some food proteins (e.g. gliadin) or commensal bacteria components could lead to mucosal inflammation and participate in the impairment of intestinal mucosal barrier, which consequently leads to the development of inflammatory and autoimmune diseases.

THE ROLE OF THE MUCOSAL IMMUNE SYSTEM AND THE MUCOSAL BARRIER IN THE DEVELOPMENT OF INFLAMMATORY AND AUTOIMMUNE DISEASES

Body surfaces covered by epithelial cells are immediately after birth coming into contact with a number of microoganisms and foreign substances. While the surface of the skin (about 2 m^2) is protected mechanically by several epithelial layers, surfaces of the gastrointestinal, respiratory and urogenital tracts (about 300 m^2) are mostly covered with a single-layered epithelium and to resist the invasion of microorganisms they require extensive protection: this is represented by a complex of mechanical and chemical mechanisms responsible for degradation and removal of heterogeneous substances and by highly effective innate and highly specific immune systems. However, the interface between the organism and the outside world is also the site of exchange of nutrients, export of products and waste components; mucosae must therefore be selectively permeable and at the same time, they must constitute a barrier equipped with local defence mechanisms against environmental threats (e.g. invading pathogens). The mucosal immune system evolved mechanisms discriminating between harmless antigens from food and microflora and dangerous antigens. Characteristic features of mucosal immunity distinguishing it from systemic immunity are: strongly developed mechanisms of innate defence, the existence of characteristic populations of unique types of lymphocytes and their products, colonization of the mucosa and exocrine glands by cells originating from the mucosal organized tissues ("common mucosal system"), transport of polymeric immunoglobulins through epithelial cells into secretions (sIgA) and preferential induction of inhibitory mechanisms directed against mucosal non-dangerous antigens ("oral, mucosal tolerance"). Innate mucosal immune system is represented by cells (epithelial cells, macrophages, dendritic cells, mast cells and other cells) and their humoral products (e.g. antimicrobial proteins and peptides). Basic functions of the mucosal immune system are protection against pathogenic microorganisms and prevention of penetration of immunogenic components from mucosal surfaces into the internal environment of the organism (barrier and anti-infectious functions). Another important function is induction of unresponsiveness of the systemic immunity to antigens present on mucosal surfaces ("oral, mucosal tolerance") and maintenance of the homeostasis on mucosal surfaces (immunoregulatory function) (*Mestecky* et al., 1995; *Ogra* et al., 1999; *Tlaskalová-Hogenová* et al., 2002; *Mestecky* et al., 2005).

The basic mechanism of mucosal immunity is innate, natural immunity represented by processes that protect the host immediately, within the first minutes and hours, of exposure to infection. It is of interest that these de-

fence mechanisms of vertebrates are implemented by structurally related effector molecules present in plants and insects, which do not possess higher, specialised forms of adaptive immunity. A characteristic, although not yet clearly defined, feature of innate immunity is an ability to distinguish between potentially pathogenic microbial components and harmless antigens by ''Pattern Recognition Receptors (PRRs)''. An example of these molecules is the so-called Toll like receptors (TLRs) enabling mammalian cells to recognise conserved characteristic molecules present on microorganisms and representing so called Pathogen Associated Molecular Patterns (PAMP) (*Medzitov* and *Janeway,* 2000; *Akira,* 2001). As these molecules, e.g. lipopolysaccharides, peptidoglycans and others are present also on commensal bacteria it seems more precise to call them Microbe Associated Molecular Pattern (MAMP). In mammals, PRRs are present on macrophages, neutrophils, dendritic cells and other cells belonging to innate immune system. It was demonstrated that recognition of microbes activates Nf kappa B signalling pathway, triggering in this way cytokine production, and upregulation of co-stimulatory molecules on antigen presenting cells leading to activation of T cells (*Tlaskalová-Hogenová* et al., 2005a).

In addition to well-known humoral components of innate immunity (humoral forms of PRRs) present on mucosal surfaces such as complement, lysozyme, lactoferrin, mannan binding protein and others, recently described factors have been the subject of intensive study. An important component of non-specific mechanisms are antimicrobial peptides widely distributed throughout plant and animal kingdoms. Various antibiotic peptides, defensins, were found in epithelial cells (e.g. in apical granules of Paneth epithelial cells) (*Bevins,* 1999). Innate immunity is closely linked to adaptive, acquired immunity represented by secretory immunoglobulins and epithelial compartment containing intraepithelial lymphocytes and lamina propria lymphocytes.

Starting from first hours after delivery from the sterile uterine environment, microorganisms colonize most of the mucosal surfaces and skin. The number of autochthonous bacteria (10^{14}) exceeds the number of cells forming the human body. The highest numbers of commensal bacteria exhibiting enormous diversity are found in distal parts of the gut; their identification and characterization is however hampered by the fact that many intestinal bacteria are not cultivable. The highly protective colonization of the mucosal surfaces by commensals has an important stimulatory effect on innate and adaptive immunity, metabolic processes (e.g. nutrition) and other host activities. Using gnotobiotic animal models (animals reared in germfree conditions) we and others demonstrated that components of intestinal microflora play a crucial role during early postnatal development of the immune system and cause "physiological inflammation" of the gut (*Tlaskalová-Hogenová* et al., 1971; *Tlaskalová-Hogenová* et al., 1983; *Tlaskalová-Hogenová*, 1997; *Štěpánková* et al., 1998; *Hooper* and *Gordon*, 2001; *Cebra* et al., 2005). However, under specific conditions commensal bacteria could participate in the development of intestinal inflammation (*Singh* et al., 2001; *Tlaskalová-Hogenová* et al., 2004; *Tlaskalová-Hogenová*, 2005b; *Cebra* et al., 2005).

The epithelium of most mucosal surfaces consists of a layer of interconnected, polarised epithelial cells separated by a basal membrane from the

connective and supporting tissue surrounding various types of cells present in the lamina propria. The epithelial layer is reinforced by tight junctions present in paracellular spaces of epithelial cells and forming an interconnected network. Tight junctions were found to act as a dynamic and strictly regulated port of entry that opens and closes in response to various signals (e.g. cytokines) originating in the lumen, lamina propria and epithelium. The molecules forming tight junctions (zonulins, occludins, claudins) are connected to the cytoskeleton of epithelial cells (*Fasano,* 2001). Mucosal barrier function is greatly influenced by the products of the nervous system (neurotransmitters) (*Mestecky* et al., 2005).

Initial events leading to the development of chronic inflammatory and autoimmune disease have not yet been elucidated. We suppose that dysfunction of the immune system associated with the gut and other mucosal surfaces, i.e. with the largest and most critical area of the body, which is in permanent contact with the environment and with large numbers of living bacteria and their cytokine inducing components, is a prerequisite for impairment of physiologically developing, regulatory mechanisms. Numerous chronic diseases may occur as a result of disturbances of mucosal barrier function or of changes in mechanisms regulating mucosal immunity (*Singh* et al., 2001). This may involve infectious diseases, inflammatory diseases and autoimmune diseases developing either in their initial phase or throughout on mucosal surfaces (*Tlaskalová-Hogenová* et al*.,* 1998, 2002).

The main characteristics of chronic, "idiopathic", inflammatory and autoimmune diseases are tissue destruction and functional impairment as a consequence of immunologically mediated mechanisms, which are principally the same as those functioning against dangerous (pathogenic) infections. One of the most attractive explanations for inflammatory and autoimmune phenomena has always centred on various infections as possible natural events capable of initiating the process in genetically predisposed individuals (*Tlaskalová*, 1997). There are various mechanisms by which infectious components are supposed to trigger inflammatory and autoimmune processes (*Tlaskalová-Hogenová* et al., 2002, 2004). However nutritional components could be involved in pathogenic processes as well. From empiric experience it is known that various kinds of diet could influence the clinical outcome of chronic diseases. Celiac disease belongs to autoimmune disorders caused by dietary component – gluten.

PARTICIPATION OF GLUTEN IN THE PATHOGENETIC MECHANISM OF CELIAC DISEASE AND VARIOUS CHRONIC DISEASES

Celiac disease is a disorder characterized by gluten-dependent enteropathy. Small intestinal mucosal villous atrophy with hyperplasia of the crypts, abnormal surface epithelium and increased inflammatory cell infiltration are regularly found in biopsy specimens taken from jejunum or duodenum of patients with active disease. The features of the mucosal lesions suggest that wheat gluten and other prolamins (secalins in rye and hordeins in barley) lead to aberrant, pathologically increased immune response in geneti-

cally predisposed individuals. Celiac disease is a disorder strongly associated with HLA-DQ2 expressed in more than 90% of patients, a small number of patients express HLA-DQ8. Intolerance to gluten seems to be caused by break down of oral tolerance to dietary gluten leading to the development of autoimmune responses by not yet well characterized mechanism. Increased activity of gliadin specific lamina propria CD4+T cells producing Th0 Th1 cytokines and cytotoxic intraepithelial CD8+ T cells expressing NK receptors are supposed to be involved in the pathogenic mechanism (*Brandtzaeg*, 2006; *Stepniak* and *Koning,* 2006). The increased level of antibodies to gliadin in sera of patients is regularly accompanied by the presence of auto-antibodies. We have shown that auto-antibody to calreticulin present in the sera of patients is the consequence of molecular mimicry between gliadin and this auto-antigen (*Krupičková* et al., 1999). Endomysial auto-antibodies of IgA isotype have for years been used as a specific and sensitive serological marker for celiac disease (*Ascher* et al., 1996; *Dewar* and *Ciclitira*, 2005). Recently the molecular nature of the target of anti-endomysial antibodies was identified as type 2 tissue transglutaminase (*Dieterich* et al., 1997). Serological IgA positivity for human transglutaminase seems to be nowadays the best serological marker for celiac disease. However, the definitive diagnosis of the disease is obtained by performing a small intestinal biopsy looking for characteristic pathological changes (*Dewar* and *Ciclitira*, 2005). In spite of the progress in diagnostic possibilities the diagnosis of celiac disease is still challenging due to the great variability of clinical presentations. The classical symptoms like diarrhoea, abdominal pain and weight loss with nutritional deficiencies (iron, folate, calcium) are seen less often, and are present mainly in infants. Unfortunately, most adult patients have either silent or atypical presentations, thus escaping diagnosis for several years. In patients with an atypical form of celiac disease clinical presentations are characterized by the presence of extra-intestinal manifestations including anaemia, osteopaenia, infertility, psychiatric and neurological abnormalities, hyposplenism and gastrointestinal malignancies, especially lymphomas (*Collin* et al., 1994; *Tlaskalová-Hogenová* et al., 1999; *Schuppan* et al., 2005). This is the reason why celiac disease remains undiagnosed and diagnosed and treated patients represent only the "tip of the iceberg" from the population of celiac patients (prevalence is estimated to be 0.5-1%). Although there are attempts to develop novel therapeutic options based on enzymatic digestion of "toxic" gluten peptides, the life-lasting gluten free diet is still the only treatment (*Gianfrani* et al., 2006).

Diverse inflammatory and autoimmune diseases are frequently associated with celiac disease and untreated celiac patients also carry an increased risk of various complications involving anaemia, infertility, osteoporosis and gastrointestinal malignancies. Disorders frequently associated with celiac disease are: endocrinological diseases (e.g. type 1 diabetes, thyroiditis), connective tissue diseases, liver diseases (e.g. primary biliary cirrhosis), Down syndrome, and disorders of the nervous system encompassing epilepsy, ataxia, peripheral neuropathies and other diseases (*Colin* et al., 1994; *Pynone* et al., 2004; *Szodoray* et al., 2004; *Dewar and Ciclitira*, 2005; *Abenavoli* et al., 2006). Beside dermatitis herpetiformis, which is considered a skin form of celiac disease, the most pronounced association of CD is with autoimmune diabetes, where the incidence of CD

among diabetic patients was described to occur in 5-10 % of various populations (*Barera* et al., 2002; *Ashabani* et al., 2003; *Kučera* et al., 2003). Our finding of beneficial effect of gluten free diet in spontaneously diabetic NOD mice suggests that gluten could be involved in the development of this disease (*Funda* et al., 1999). The frequent association of autoimmune diseases with celiac disease led to recommendation for regular serological screening of celiac disease in the risk groups of patients suffering from type 1 diabetes and other diseases associated with celiac disease (*Barera* et al., 2002).

The most interesting part of this story is represented by some reports suggesting that in non-celiac patients suffering from various chronic diseases gluten free diet improved clinical symptoms. Skin diseases: part of patients with psoriasis was described to benefit from introduction of gluten free diet: positive response to dietary regimen was observed in patients who had higher concentrations of anti-gliadin antibodies (about 10% of psoriatic patients), and a similar finding was described in patients with urticaria (*Wolters*, 2005). Neurological syndromes: some patients suffering from cryptogenic ataxia and peripheral neuropathies have been reported to respond to gluten restriction (*Hadjivassiliou* et al., 1998, 2003). In a subset of patients suffering from schizophrenia a reduction of schizophrenic symptoms after initiation of gluten withdrawal has been noted in a variety of the studies (*Kalaydijian* et al., 2006). Similarly, in rheumatic arthritis the vegan diet with exclusion of gluten led in a subset of patients with anti-gliadin positive antibodies to clinical improvement (*Kjeldsen-Krag*, 2000; *Paimela* et al., 1995; *Hafstrom* et al., 2001). It seems that higher concentrations of antibodies directed to food antigens could suggest that the gut barrier function is impaired (*Hafstrom* et al., 2001; *Kučera* et al., 2003). Underlying mechanisms of these effects are not yet elucidated. It could be speculated that part of the patients have impaired gut barrier function; gluten could therefore pass into circulation in an incompletely digested form and in this way activate the innate and adaptive immunity (*Drago* et al., 2006). Installation of gluten free diet then represents removal of dietary stimulus activating under specific conditions the immune system.

ANIMAL MODELS OF CELIAC DISEASE

Animal models of human diseases are helping to elucidate the pathogenic mechanisms of the diseases and to develop new preventive and therapeutic approaches. Several groups of researches tried to develop an experimental model for celiac disease and to induce celiac- like lesions by administrating gluten to various strains of mice and rats. No conspicuous changes were observed on intestinal mucosa of adult animals after gliadin application (*Troncone* and *Ferguson,* 1991). We have found that repeated intra-gastric application of gliadin to conventionally reared rats of AVN strain induced profound jejunal changes, provided that gliadin was administered immediately after birth (*Štěpánková* et al., 1989). Components of intestinal microflora induce a major stimulatory effect on mucosal immune system especially during early postnatal development. We have therefore used defined gnotobiotic conditions, which made it possible to differentiate the effects of micro-

flora and dietary antigens (*Štěpánková* et al., 1996). On analyzing the changes induced by gliadin application we found that repeated gliadin application led to shortening of the jejunal villi, crypt hyperplasia, increased number of mitoses in the crypt epithelium, increased number of inflammatory cells in gut mucosa and an increased level of IgA anti-gliadin antibodies in intestinal washings and in the sera of experimental rats. Interestingly, we have found that intra-gastric gliadin application into germfree rat pups increased the number of intra-epithelial lymphocytes and had similar effects on intra-epithelial lymphocyte subpopulation as colonization with microflora (*Štěpánková* et al., 1996). Transfer of intra-epithelial lymphocytes separated from gliadin treated rats into the intestinal loops of untreated germfree rats caused damage in the recipient gut enterocytes - disruption of tight junctions was observed on enterocytes of intestinal loops. Moreover, lymphocytes from gliadin treated germfree rats penetrated through the intestinal wall and were detected in lamina propria, while lymphocytes from control germfree rats remained in the lumen. Control lymphocytes did not cause structural damage to the epithelium of intestinal loops (*Štěpánková* et al., 1996). Together with morphological changes of enterocytes, the brush border enzymatic activities have been changed (*Kozáková* et al., 1998, 2000). Interestingly, when we used various neurological and behavioural tests to assess the changes caused by gliadin treatment, higher emotionality of gliadin treated rats was found in an open field test (*Castany* et al., 1995).

The use of the described rat model of gluten-induced enteropathy demonstrated a protective effect of breast-feeding. Suckling animals given gliadin never displayed the flat mucosa characteristic of destructive celiac disease. A significant relationship is generally assumed to exist between suckling and the occurrence of the celiac disease, which appears after introducing gluten into the diet (*Ivarsson* et al., 2002). The basis of this protective effect of maternal milk against the deleterious influence of gluten remains unclear. Maternal milk contains proteins, saccharides, fat and a number of biologically active components like hormones, immunoglobulins, cytokines and growth factors. In rats´ maternal milk, epidermal growth factor (EGF) plays an important role in the process of regeneration and proliferation of jejunal enterocytes. We studied its potential protective effect on the damage of intestinal mucosa by gliadin in a model system (*Štěpánková* et al., 2003). Enteropathy was induced by repeated intra-gastric gliadin application in inbred rat pups of AVN strain, delivered by Caesarean section, breast fed or hand-fed with a milk formula. All experimental groups were treated with interferon gamma, administered intraperitoneally after birth. One group of hand-fed pups received EGF continuously in the diet. Gliadin in interferon gamma treated formula-fed rat pups showed villous atrophy, increased numbers of inflammatory cells and damage to epithelial tight junctions and enterocyte brush border. Addition of EGF to the diet protected the rats against pathological mucosal changes and prevented celiac crisis. The role of EGF and other regulatory peptides in the development of gluten mucosal impairment is not yet fully understood and needs further study (*Štěpánková* et al., 2003).

ACTIVATION OF HUMAN INNATE IMMUNITY CELLS BY GLIADIN

It seems that the unique structure of gliadin and its fragments could be responsible for the involvement of innate immunity mechanisms in the pathogenic mechanism of this disease (*Novák* et al., 2002; *Schuppan* et al., 2003; *Maiuri* et al., 2003; *Zanoni* et al., 2006). In our previous experiments we have demonstrated that, in contrast to other dietary proteins tested, gliadin stimulated IFN gamma treated mouse peritoneal macrophages for NO production and secretion of cytokines (TNF alpha, Il-10, RANTES) (*Tučková* et al., 2002). Recently we found that cultivation of cells of human monocytic cell line THP1 with the peptic digest of gliadin leads to the production of IL8 and TNF alpha and that the production is augmented by pre-treatment of cells with IFN gamma or its addition to the culture. Ovalbumin and soya protein or their peptic digests had no effect on IL-8 and TNF alpha production either when applied alone or in combination with IFN gamma. The participation of nuclear factor- kappa B (NF kappa B) in stimulatory effect of gliadin digest on monocytes was documented by a marked increase of the DNA binding activities of NF kappa B subunits p50 and p65. Moreover, the use of NF kappa B inhibitors sulfasalazine , PDTC and TPCK led to the detection of an inhibition of p65 and p50 subunits binding (*Jelínková* et al., 2004).

The effect of a peptic digest of gliadin on the maturation of human monocyte-derived dendritic cells was studied by Palová-Jelínková et al. (2005). In contrast to other tested food proteins (ovalbumin, soya protein) gliadin led to increased expression of costimulatory molecules - maturation markers (CD 80, CD 83, CD 86 and HLADR molecules) and increased secretion on chemokines and cytokines (IL-06. IL-08, IL-10, ATN ALFA, growth related oncogine, NCP-1, NCP-2, macrophage damaged chemokine and RANTES). Moreover, gliadin-induced phosphorylation of members of three MAPK families ERK1/2, JNK, and p38 MAPK was demonstrated. Gliadin treatment also resulted in increased NF-kappa B/DNA binding activity of p50 and p65 subunits (*Palová-Jelínková* et al., 2005). It seems that gliadin can contribute to phenotypic and functional maturation of dendritic cells, which is followed by efficient processing and presentation of gliadin peptides to specific T lymphocytes and in this way the activation of innate immune system can facilitate the development of the disease.

ACKNOWLEDGEMENTS

This work was supported by grants no. 310/05/2245, 303/04/0849, 303/06/0974, 303/05/2249, 310/03/H147 (Grant Agency of the Czech Republic), IAA5020210, IAA5020205, A5020101 (Grant Agency of Academy of Sciences of the Czech Republic), the project AV0Z 50200510, 1QS500200572 (Academy of Sciences) and the project of Ministry of Agriculture no. 1B53002.

LITERATURE

Abenavoli, L., Proietti, I., Leggio, L., Ferrulli, A., Vonghia, L., Capizzi, R., Rotoli, M., Amerio, P.L., Gasbarrini, G., and Addolorato, G.: Cutaneous manifestations in celiac disease. World J. Gastroenterol. 12, 843-852 (2006).

Akira, S.: Toll-like receptors and innate immunity. Adv. Immunol. 78, 1-56 (2001).

Ashabani, A., Abushofa, U., Abusrewill, S., Abdelazez, M., Tučková, L., and Tlaskalová-Hogenová, H.: The prevalence of coeliac disease in Libyan children with type 1 diabetes mellitus. Diabetes/Metabolism Res. Rev. 19, 69-75 (2003).

Ascher, H., Hahn-Zoric, M., Hanson, L.A., Kilander, A.F., Nilsson, L.A., and Tlaskalová, H.: Value of serologic markers for clinical diagnosis and population studies of coeliac disease. Scand. J. Gastroenterol. 31, 61-67 (1996).

Barera, G., Bonfanti, R., Viscardi, M., Bazzigaluppi, E., Calori, G., Meschi, F., Bianchi, C., and Chiumello, G.: Occurence of celiac disease after onset of type 1 diabetes: a 6-year prospective longitudinal study. Pediatrics 109, 833-838 (2002).

Bevins, C.K., Martin-Poter, E., and Ganz, T.: Defensins and innate host defence of the gastrointestinal tract. Gut 45, 911-915 (1999).

Brandtzaeg, P.: The changing immunological paradigm in coeliac disease. Immunol. Lett. 105, 127-139 (2006).

Castany, M.A., Štěpánková, R., Tlaskalová, H., Turner, L.F., Liu, Z. and Bureš, J.: Study of behavior of rats with gluten-induced enteropathy. Int. J. Neurosci. 83, 7-15 (1995).

Cebra, J.J., Jiang, H-Q., Boiko, N., and Tlaskalová-Hogenová, H.: The role of mucosal microbiota in the development, maintenance, and pathologies of the mucosal immune system. In: Mucosal Immunology, 3rd Edition (Eds.: Mestecky, J., Bienenstock, J., Lamm, M.E., Mayer, L., McGhee, J., and Strober W.). Elsevier Academic Press, Amsterdam, 335-368 (2005).

Collin, P., Reunala, T., Pukkala, E., Laippala, P., Keyrilainen, O., and Pasternack, A.: Coeliac disease - Associated disorders and survival. Gut 35, 1215-1218 (1994).

Dewar, D.H. and Ciclitira, P.J.: Clinical features and diagnosis of celiac disease. Gastroenterology 128 (Suppl. 1), S19-S24 (2005).

Dieterich, W., Ehnis, T., Bauer, M., Donner., P., Volta, U., Riecken, E.O., and Schuppan, D.: Identification of tissue transglutaminase as the autoantigen of celiac disease. Nat. Med. 3, 797-801 (1997).

Drago, S., El Asmar, R., Di Pierro, M., Grazia Clemente , M., Tripathi, A., Sapone, A., Thakar, M., Iacono, G., Carroccio, A., D´Agate, C., Not, T., Zampini, L., Catassi, C., and Fasano, A.: Gliadin, zonulin and gut permeability: Effects on celiac and non-celiac intestinal mucosa and intestinal cell lines. Scand. J. Gastroenterol. 41, 408-419 (2006).

Fasano, A.: Intestinal zonulin: Open sesame! Gut 49, 159-162 (2001).

Funda, D.P., Kaas, A., Bock, T., Tlaskalová-Hogenová, H., and Buschard, K.: Gluten-free diet prevents diabetes in NOD Mice. Diabetes/Metabolism Research and Reviews 15, 323-327 (1999).

Gianfrani, C., Auricchio, S., and Troncone, R.: Possible drug targets for celiac disease. Expert Opin. Ther. Targets 10, 601-611, (2006).

Hadjivassiliou, M., Grunewald, R.A., Chattopadhyay, A.K., Davies-Jones, G.A., Gibson, A., Jarratt, J.A., Kandler, R.H., Lobo, A., Powell, T., and Smith C.M.: Clinical, radiological, neurophysiological, and neuropathological characteristics of gluten ataxia. Lancet 352, 1583-1585 (1998).

Hadjivassiliou, M., Grunewald, R., Sharrack, B., Sanders, D., Lobo, A., Williamson, C., Woodroofe, N., Wood, N., and Davies-Jones, A.: Gluten ataxia in perspective: epidemiology, genetic susceptibility and clinical characteristics. Brain 126, 685-691

(2003).

Hafstrom, I., Ringertz, B., Spanberg, A., von Zweigbergk, L, Brannemark, S., Nylander, I., Ronnelid, J., Laasonen, L., and Klareskog, L.: A vegan diet free of gluten improves the signs and symptoms of rheumatoid arthritis: The effects on arthritis correlate with a reduction in antibodies to food antigens. Rheumatology (Oxford) 40, 1175-1179 (2001).

Hooper, L.V. and Gordon, J.I.: Commensal host-bacteria relationship in the gut. Science 292, 1115-1118 (2001).

Ivarsson, A., Hernell, O., Stenlund, H , and Persson, L.A.: Breast-feeding protects against celiac disease. Am. J. Clin. Nutr. 75, 914-921 (2002).

Jelínková, L., Tučková, L., Cinová, J., Flegelová, Z., and Tlaskalová-Hogenová, H.: Gliadin stimulates human monocytes to production of IL-8 and TNF-alfa through a mechanism involving NF-kappa B. FEBS Letters 571, 81-85 (2004).

Kalaydjian, A.E., Baton, W., Cascella, N., and Fasano, A.: The gluten connection: The association between schizophrenia and celiac disease. Acta Psychiatr. Scand. 113, 82-90 (2006).

Kjeldsen-Kragh, J.: Rheumatoid arthritis treated with vegetarian diets. Am. J. Clin. Nutr. 71, 1211-1213 (2000).

Kozáková, H., Štěpánková, R., Kolínská, J., Farre, M.A., Funda, D.P., Tučková, L., and Tlaskalová-Hogenová, H.: Brush border enzyme activities in the small intestine after long-term gliadin feeding in animal models of human coeliac disease. Folia Microbiol. (Prague) 43, 497-500 (1998).

Kozáková, H., Štěpánková, R., Tučková, L., Šinkora, J., Jelínková, L., and Tlaskalová-Hogenová, H.: Humoral and cellular immune responses in gluten-treated suckling or hand-fed rats. Physiol. Res. 49, 665-672 (2000).

Krupičková S., Tučková L., Flegelová Z., Michalak M., Walters J.R.F., Whelan A., Harries J., Vencovský J., and Tlaskalová-Hogenová H.: Identification of common epitopes on gliadin, enterocytes, and calreticulin recognised by antigliadin antibodies of patients with celiac disease. Gut 44, 168-173 (1999).

Kučera, P., Nováková, D., Běhanová, M., Novák, J., Tlaskalová-Hogenová, H., and Anděl, M.: Gliadin, endomysial and thyroid antibodies in patients with latent autoimmune diabetes of adults (LADA). Clin. Exp. Immunol. 133, 139-143 (2003).

Maiuri, M.C., De Stefano, D., Mele, G., Fecarotta, S., Greco, L, Troncone, R., and Carnuccio, R.: Nuclear factor kB is activated in small intestinal mucosa of celiac patients. J. Mol. Med. 81, 373-379 (2003).

Medzhitov, J. and Janeway, C.A.: Innate immune recognition: mechanisms and pathways. Immunol. Rev. 173, 89-97 (2000).

Mestecky J., Bienenstock J., Lamm M.E., Mayer L., McGhee J., and Strober W. (Eds.). Mucosal Immunology. Elsevier, Academic Press, Amsterdam (2005).

Mestecky, J., Russel, M.W., Jackson, S., Michalek, S.M., Tlaskalová-Hogenová, H., and Šterzl, J.: Advances in mucosal immunology. Plenum Press, New York and London (1995).

Novák P., Man P., Tučková L., Tlaskalová-Hogenová H., Bezouška K., and Havlíček, V.: Monitoring of *in vitro* deamidation of gliadin peptic gragments by mass spectrometry may reflect one of the molecular mechanisms taking place in celiac disease development. J. Mass Spectrom**.** 37, 507-511 (2002).

Ogra, P.L., Mestecky, J., Lamm, M.E., Strober, W., Bienestock, J., and McGhee, J. (Eds.): Mucosal Immunology. New York, Academic Press (1999).

Paimela, L., Kurki, P., Leirisalo-Repo, M., and Piirainen, H.: Gliadin immune reactivity in patients with rheumatoid arthritis. Clin. Exp. Rheumatol. 13, 603-607 (1995).

Palová-Jelínková, L., Rožková, D., Pecharová, B., Bártová, J., Šedivá, A., and
Tlaskalová-Hogenová, H.: Gliadin fragments induce phenotypic and functional maturation of human dendritic cells. J. Immunol. 175, 7038-7045 (2005).

Pynnone, P.A., Isometsa, E.T., Aronen, E.T.,

Verkasalo, M.A., Savilahti, E., and Aalberg, V.A.: Mental disorders in adolescents with celiac disease. Psychosomatics 45, 325-335 (2004).

Schuppan, D., Dennis, M.D., and Kelly, C. P.: Celiac disease: Epidemiology, pathogenesis, diagnosis, and nutritional management. Nutr. Clin. Care 8, 54-69 (2005).

Schuppan, E., Esslinger, B., and Dietrich, W.: Inante immunity and coeliac disease. Lancet 362, 3-5 (2003).

Singh, B., Read, S., and Asseman, C.: Control of intestinal inflammation by regulatory T cells. Immunol. Rev. 182, 190-200 (2001).

Štěpánková, R., Kofroňová, O., Tučková, L., Kozáková, H., Cebra, J.J., and Tlaskalová, H.: Experimentally induced gluten enteropathy and protective effect of epidermal growth factor in artifically fed neonatal rats. J. Pediatr. Gastroenterol. Nutr. 36, 96-104 (2003).

Štěpánková R., Šinkora J., Hudcovič T., Kozáková H., and Tlaskalová-Hogenová, H.: Differences in development of lymphocyte subpopulations from gut associated lymphatic tissue (GALT) of germfree and conventional rats: Effect of aging. Folia Microbiol. 43, 531-534 (1998).

Štěpánková, R., Tlaskalová-Hogenová H., Frič, P., and Trebichavský, I.: Enteropathy induced in young rats by feeding with gliadin similarity with coeliac disease. Folia Biol. 35, 19-26 (1989).

Štěpánková, R., Tlaskalová-Hogenová, H., Šinkora, J., Jodl, J., and Frič, P.: Changes in jejunal mucosa after long-term feeding of germfree rats with gluten. Scand. J. Gastroenterol. 31, 551-557 (1996).

Stepniak, D. and Koning, F.: Celiac disease – Sandwitched between innate and adaptive immunity. Human Immunol. 67, 460-468 (2006).

Szodoray, P., Barta, Z., Lakos, G., Szakall, S., and Zeher, M.: Coeliac disease in Sjorgren´s syndrome - A study of 111 Hungarian patients. Rheumatol. Int. 24, 278-282 (2004).

Tlaskalová-Hogenová, H.: Gnotobiology as a tool – An introduction. In.: Immunology Methods Manual (Ed: Lefkovits, I.). Academic Press, London, 1524-1559 (1997).

Tlaskalová-Hogenová, H., Kamarytová, V., Mandel, L., Prokešová, L., Kruml, J., Lanc A., and Miler, I.: The immune response of germfree piglets after peroral monocontamination with living *Escherichia coli* strain 086. Folia Biol. 26, 82-91 (1971).

Tlaskalová-Hogenová, H., Štěpánková, R., Farre, M.A., Funda, D.P., Řeháková, Z., Šinkora, J., Tučková, L., Horák, I., Horáková, D., Cukrowska, B., Kozáková, H., and Kolínská, J.: Autoimmune reactions induced by gliadin feeding in germ-free AVN rats and athymic nude mice. Animal models for celiac disease. Ann. N.Y. Acad. Sci. 5, 503-5 (1997).

Tlaskalová-Hogenová, H., Štěpánková, R., Hudcovic, T., Tučková, L., Cukrowska, B., Lodinová-Žádníková, R., Kozáková, H., Rossmann, P., Bártová, J., Sokol, D., Funda, D.P., Borovská, D., Řeháková, Z., Šinkora, J., Hofman, J., Drastich, P., and Kokešová, A.: Commensal bacteria (normal microflora), mucosal immunity and chronic inflammatory and autoimmune diseases. Immunol. Lett. 93, 97-108 (2004).

Tlaskalová-Hogenová, H., Štěpánková, R., Tučková, L., Farre, M.A., Funda, D.P., Verdu, E.F., Šinkora, J., Hudcovic, H., Řeháková, Z., Cukrowska, B., Kozáková, H., and Prokešová, L.: Autoimmunity, immunodeficiency and mucosal infections: Chronic intestinal inflammation as a sensitive indicator of immunoregulatory defects in response to normal luminal microflora. Folia Microbiol. (Prague) 43, 545-550 (1998).

Tlaskalová-Hogenová, H., Tučková, L., Pospíšil, M., and Štěpánková, R.: Malignancy in coeliac disease and dermatitis herpetiformis. Cancer and Autoimmunity. Shoenfeld Y. (Ed.), Elsevier Science B.V., 409-414 (1999).

Tlaskalová-Hogenová, H., Šterzl, L., Štěpánková, R., Dlabač, V., Větvička, V., Rossmann, P., Mandel, L., and Rejnek, J.: Development of immunological capacity

under germfree and conventional conditions. Ann. N.Y. Ac. Sci. 409, 96-113 (1983).

Tlaskalová-Hogenová, H., Tučková, L., Lodinová-Žádníková, R., Štěpánková R., Cukrowska B., Funda D.P., Stříž I., Kozáková H., Trebichavský I., Sokol D., Řeháková Z., Šinkora J., Fundová P., Horáková D., Jelínková L., and Sanchez, D.: Mucosal immunity: its role in defense and allergy. Int. Arch. Allergy Immunol. 128**,** 77-89 (2002).

Tlaskalová-Hogenová, H., Tučková, L., Mestecky, J., Kolínská, J., Rossmann, P., Štěpánková, R., Kozáková, H., Hudcovic, T., Hrnčíř, T., Frolová, L., and Kverka, M.: Interaction of mucosal microbiota with the innate immune system. Scand. J. Immunol. 62, 106-113 (2005a).

Tlaskalová-Hogenová, H., Tučková, L., Štěpánková, R., Hudcovic, T., Palová-Jelínková, L., Kozáková, H., Rossmann, P., Sanchez, D., Cinová, J., Hrnčíř, T., Kverka, M., Frolová, L., Uhlig, H., Powrie, F., and Bland, P.: Involvement of innate immunity in the development of inflammatory and autoimmune diseases. Ann. N.Y. Acad. Sci. 1051, 787-798 (2005b).

Troncone, R. and Ferguson, A.: Animal model of gluten induced enteropathy in mice. Gut 32, 871-875 (1991).

Tučková L., Novotná J., Novák P., Flegelová Z., Květoň T., Jelínková L., Zídek Z., Man P., and Tlaskalová-Hogenová, H.: Activation of macrophages by gliadin fragments: isolation and characterization of active peptide. J. Leukoc. Biol. 71, 625-631 (2002).

Wolters, M.: Diet and psoriasis: experimental data and clinical evidence. Br. J. Dermatol. 153, 706-714 (2005).

Zanoni, G., Navone, R., Lunardi, C., Tridente, G., Bason, C., Sivori, S., Beri, R., Dolcino, M., Valleta, E., Corrocher, R. and Puccetti, A.: In celiac disease, a subset of autoantibodies against transglutaminase binds Toll-like receptor 4 and induces activation of monocytes. PLoS Med. 3, (2006).