Summary

It is currently accepted that the term "IBD" encompasses a group of diseases, triggered and perpetuated by a variety of diverse genetic, environmental, and immunologic factors that share similar clinical manifestations. They cause life-impaired symptoms, necessitate long-term dependence on powerful drugs, and often result in debilitating surgery and even death, ***(Daryani et al., 2006)***.

IBDs are a group of diseases due to chronic inflammation of the gastrointestinal tract, but without proved etiology. IBD appears to be resulted from a dysregulated immune response with contributions from environmental, genetic, and bacterial factors. The increasing attention given to the ecosystem of the gut may help define the antigens responsible for immune reactivity and provide opportunities toward application of antigen-specific therapeutic interventions such as induction of tolerance. Further investigation into probiotic agents and their mechanisms is especially appealing as a way to provide alternative therapies to decrease the inflammatory response, ***(Wejman et al., 2006)***.

The human gastrointestinal tract possesses a complex ecosystem, the components of which are multifaceted and metabolically diverse. Although the presence of intestinal microflora certainly contributes to the maintenance of human health, intestinal mucosa has the task, among others, of preventing the passage of commensal microflora and occasional pathogens to other compartments. To carry out such a function, the mucosa has to behave as a physical barrier but it also has to play an active role, ***(Macfarlane et al., 2003)***.

Oral tolerance (OT) consists of the oral administration of antigens that could alter the response of the immune system, ***(Strobel & Mowat, 1998)***. This is a form of peripheral immune tolerance in which mature lymphocytes in the peripheral lymphoid tissues are rendered non functional or hyporesponsive by

prior oral administration of antigens. The mechanisms by which OT is mediated include deletion or anergy and active cellular suppression. The primary factor determining which form of tolerance will be developed after oral administration of antigen is its dosage. Thus, it is thought that low doses of antigen can induce the generation of active suppression, via regulatory T cells in the gut-associated lymphoid tissue (GALT), which then migrate to the systemic immune system. These regulatory T cells produce down-regulatory cytokines such as IL-4, IL-10 and TGF-α in a Th2 /Th3 cytokine pattern. Conversely, high dose of antigen favors anergy or clonal deletion. The phenomenon in which regulatory cells, as generated by oral tolerance, are primed in an antigen specific manner, but act in the respective microenvironment in a non-antigen specific manner is called bystander suppression, ***(Barta et al., 2003)***.

Antibodies to an oligomannose epitope of *Saccharomyces cerevisiae* demonstrated in 60-70 % of the patients with Crohn's disease. The antibodies in the sera of the analyzed ASCA positive cases proved a systemic immune response against *Saccharomyces cerevisiae* and suggested the end of the oral tolerance against the yeast's antigens, ***(Hisabe et al., 2003)***. The diet restriction (elemental diet, total parenteral nutrition, and fecal diversion) may ameliorate the status of the patients with Crohn's disease. It can also be speculated that the yeast-free diet as a part of the therapy for the ASCA positive patients can be reasonable, moreover the permanent "forbidding" of the yeast can be an acceptable alternative in case of getting well, ***(Kanauchi et al., 2003)***.

Moreover, enhanced inflammatory activity in the gut is thought to increase the risk of bacterial translocation and endotoxemia. Patients with IBD show increased serum levels of endotoxin-signaling cascade activation, including augmented levels of endotoxin, lipopolysaccharide-binding protein and soluble CD14 receptor, this alteration correlates with disease activity, with normal levels recovered after treatment, although less completely in Crohn's disease, and parallels a rise in proinflammatory cytokines, suggesting a contribution of bacterial products to the inflammatory cascade in these patients, ***(Rojo et al., 2006)***.

Carious studies spotted light on the incorporation of noninvasive diagnostic tests into the initial diagnostic approach to avoid unnecessary invasive procedures and facilitate clinical decision-making when the diagnosis of IBD, ***(Canani et al., 2006)***. Thus, the current study was designed to assess the effectiveness of the use of anti-Saccharomyces cerevisiae antibody (ASCA) and perinuclear staining antineutrophil antibody (pANCA) in the diagnostic work-up of patients with suspected inflammatory bowel disease.

There were 19 samples positive for pANCA with a frequency rate among the studied IBD patients of 61.3%; 18 samples were of patients diagnosed to have UC with a frequency among UC patients of 75% and only one sample among those found to have CD with a frequency of 14.3% among CD patients. Fourteen samples were pANCA+/ASCA- and the other 5 samples were pANCA+/ASCA+ and 4 of them had UC and one had CD. Thus, pANCA+/ASCA- had a sensitivity rate for diagnosis of UC of 56%, specificity rate of 85.7% and accuracy of diagnosis by a rate of 62.5%, while pANCA-/ASCA+ had a sensitivity rate for diagnosis of UC of 22.2%, specificity rate of 16.7% and accuracy of diagnosis by a rate of 20.8%.

On the other hand, all cases with CD were missed if diagnosis relied on pANCA alone while the presence of ASCA had a sensitivity rate for diagnosis of CD of 71.4%, specificity rate of 83.3% and accuracy of diagnosis by a rate of 80.6%.

The obtained data point to the high diagnostic yield of pANCA+/ASCA- and the high specificity of pANCA+ for diagnosis of UC and on converse point to high diagnostic yield of pANCA-/ASCA+ and the high specificity of ASCA+ for diagnosis of CD.

These findings go in hand with multiple previous studies; ***Moore et al., (2002)***, found ASCA-/pANCA+ was 100% specific for ulcerative colitis. Also, ***Bartůnková et al., (2002)***, found that ANCA occur more frequently in UC than in CD and control (74, 24, and 10%, respectively), while ASCA are found more often in patients with CD (76% versus 17% in UC). The testing for both ANCA

and ASCA enabled clear-cut differential diagnosis between UC and CD based on the high specificity (ANCA+/ASCA- 92.5% for UC, ANCA-/ASCA+ 93.2% for CD). They concluded that combined testing of ANCA and ASCA represents a valuable tool in the differential diagnosis between UC and CD patients, minimizing invasive diagnostic procedures.

Annese et al., (2004), detected the anti-S. cerevisiae mannan antibodies in 100 of 196 patients with Crohn's disease (51%; $P < 0.0001$ vs. controls), 32 of 197 patients with ulcerative colitis (16%; $P < 0.02$ vs. controls), and six of 100 controls (6%).

Zholudev et al., (2004), identified ASCA antibodies in 44% of CD patients, 0% of UC patients, and 1 control and pANCA antibodies were found in 70% of patients with UC, 18% of CD patients and 3% of controls. The overall sensitivity of the four antibody panel was 65% for CD and 76% for UC, with a specificity of 94%.

Mokrowiecka et al., (2004), observed statistically more often pANCA in patients with UC (58%) than in patients with CD (28%). Both IgA and IgG ASCA occurred more often in patients with CD (57%) than in patients with UC (24%).

Kaila et al., (2005), found ASCA had a sensitivity of 37% and specificity of 97% for diagnosing CD and the likelihood of having an inflammatory disease if ASCA is positive was nearly 40-fold. They concluded that a positive ASCA test using this assay nearly clinches a diagnosis of some form of inflammatory intestinal disease, which is highly likely to be CD and in symptomatic patients, a positive ASCA test should encourage the clinician to pursue further investigations.

Reese et al., (2006), aimed to assess the diagnostic precision of ASCA and pANCA in IBD and evaluate their discriminative ability between UC and CD and found the ASCA+ with pANCA- test offered the best sensitivity for

CD (54.6%) with 92.8% specificity while the sensitivity and specificity of pANCA+ tests for UC were 55.3% and 88.5%, respectively and the sensitivity and specificity of pANCA+ tests were improved to 70.3% and 93.4% when combined with an ASCA- test.

Mokrowiecka et al., (2007), tried to determine the prevalence of pANCA and ASCA in patients with IBD subgroups and found the sensitivity and specificity of pANCA+/ASCA- pattern for UC diagnosis was 36% and 98%; pANCA-/ASCA+ for CD: 35% and 88%.

Desplat-Jégo et al., (2007), aimed to determine the performance of ASCA and anti-nuclear associated anti-neutrophil antibodies (NANA) tests for IBD diagnosis in children and adults and found ASCA+/NANA- profile displayed a positive predictive value of 94.2% for CD and detection of ASCA was correlated with a more severe clinical profile of CD.

Makharia et al., (2007), reported that the sensitivity of anti-Saccharomyces cerevisiae antibody (ASCA) IgG and ASCA IgA in CD is 60%-80%, whereas the specificity is almost 90%.

Papp et al., (2007), reported that ASCA, and atypical pANCA antibodies were present in 59.3% and 13.8% of CD, 13.7% and 48.5% of UC patients and ASCA was associated with increased risk for CD.

Through the current study, serum positivity in CD patients for ASCA showed a negative significant correlation with patients' age, ($r=-0.794$, $p=0.033$) but showed a positive correlation that was significant ($r=-0.798$, $p=0.031$) with endoscopic grading of CD and non-significant with clinical activity scores of patients with CD, ($r=0.586$, $p>0.05$). On contrary, in patients with UC serum positivity for pANCA showed a positive significant correlation with both endoscopic grading, ($r=0.573$, $p=0.003$) and clinical activity scores of patients with UC, ($r=0.483$, $p=0.017$).

In support of the obtained correlation with disease activity, ***Zholudev et al., (2004)***, found patients who were ASCA-positive were more likely to have disease of the ileum or ileum and right colon than patients who were ASCA-negative (58%vs 18%, $p < 0.001$) and patients with ASCA-positive were also more likely to require ileocecal resection (36%vs 13%, $p < 0.05$).

These findings agreed with ***Montanelli et al., (2005)***, who observed good agreement between histopathological examination and laboratory determination of anti-Saccharomyces Cerevisiae antibodies (ASCA) and anti-neutrophil cytoplasmic antibodies (ANCA).

Also, ***Vergara et al., (2006),*** reported a significant correlation between the presence of ANCA and duration of the UC (<5 years 50%, 5-10 years 42.9%, 15 years 30%) and the number of crises (one crises 31%, 2-5 crises 51.9% and >5 crises 87.5). The proportion of colectomized patients with positive ANCA was higher (57.1%).

Moreover, ***Mokrowiecka et al., (2007)***, found a significant positive correlation between antibodies profiles: pANCA+/ASCA- and active disease; pANCA-/ASCA+ and number of operations, as well as the negative correlation between pANCA-/ASCA- and patient's age has been found.

Müller et al., (2005), investigated ASCA production over time in CD and murine colitis in order to understand their etiology and found ASCA IgG and IgA titers are stable over time in CD and non-CD patients. Fistular disease was associated with a higher rate of ASCA IgA positivity. Ileal disease was found to have a significant influence on the Delta IgG of ASCA. In mice, neither healthy animals nor animals with induced or spontaneous colitis exhibited a marked increase in ASCA titers after high-dose yeast exposure. On the other hand, mice immunized intraperitoneally with mannan plus adjuvant showed a marked and significant increase in ASCA titers compared to adjuvant-only immunized controls. They concluded that the propensity to produce ASCA in a subgroup of CD patients is largely genetically predetermined as evidenced by their stability. Furthermore, in animal models of

colitis, mere oral exposure of mice to yeast does not lead to the induction of marked ASCA titers irrespective of concomitant colonic inflammation. Hence, environment may play only a minor role in inducing ASCA.

Halfvarson et al., (2005), assessed the genetic influence of ASCA in 98 twin pairs with inflammatory bowel disease and found ASCA were more common in Crohn's disease than in ulcerative colitis (57% versus 12%) and associations with ileal Crohn's disease, stricturing/penetrating behaviour, and young age were confirmed. These findings question the concept of ASCA as a marker of genetic susceptibility for Crohn's disease. The agreement in ASCA titres within concordant monozygotic twin pairs with Crohn's disease, suggests that the level of increase is genetically determined. ASCA are proposed as a marker of a response to an environmental antigen and that a specific gene(s) determines the level of response and perhaps also specific phenotypic characteristics.

Basu et al., (2005), evaluated the impact of race and ethnicity on serologic markers, including perinuclear antineutrophilic cytoplasmic antibody (p-ANCA) and anti-Saccharomyces cerevisiae antibody (ASCA) in UC and CD in USA and found that African Americans and whites predominantly had CD, whereas Mexican Americans predominantly had UC. p-ANCA served as a sensitive marker for UC among Mexican Americans. All the Mexican Americans with UC tested had positive p-ANCA compared to only 40% of whites. They concluded that there are significant differences in IBD subtypes and serologic markers among racial/ ethnic groups with IBD in the United States.

Mallant-Hent et al., (2006), tried to evaluate the impact of continuous exposure to S. cerevisiae as source of ASCA through investigating the correlation between ASCA and presence of mucosal S. cerevisiae DNA in a population of CD, UC patients and controls using S. cerevisiae-specific primers and a fluorescent probe and found ASCA (IgA or IgG) were positive in 19 (61%) patients with CD, 12 (27%) with UC and none of controls. PCR amplification detected S. cerevisiae DNA in 7 (29%) CD, 7 (19%) UC and one

(6%) control and in only 4 CD and 4 UC patients ASCA and mucosal S. cerevisiae were positive. They concluded that since the presence of S. cerevisiae in colonic mucosal biopsy specimens is very rare, ASCA is unlikely to be explained by continuous exposure to S. cerevisiae in the mucosa. Therefore, ASCA formation must occur earlier in life and levels remain relatively stable thereafter in immunological susceptible persons.