Anti-*Saccharomyces cerevisiae* antibodies in Behçet's disease - a familial study

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ABSTRACT

Objective. *To evaluate the rate and clinical correlations of antibodies against* saccharomyces cerevisiae *(ASCA) among healthy family members of patients with Behçet's disease (BD).*

Methods. *Twenty-one BD patients and 52 healthy family members (HFM) were studied. Data from medical files and from patients' interviews was collected, regarding the entire spectrum of disease manifestations. Each family member was personally interviewed and a questionnaire composed of BD symptoms and their temporal relation was compiled. IgA- and IgG-ASCA levels, determined by ELISA, were studied in all BD patients and their family members, the results were compared to a group of 23 healthy controls (HC).*

Results. *Eight (38.1%) BD patients were ASCA positive, compared to five among HFM (9.6%) and none among healthy unrelated controls (p=0.001). Mean IgG and IgA-ASCA levels were significantly higher in BD patients compared with HFM and HC groups (p = 0.002 and p = 0.03, respectively). No correlation was found between positive ASCA tests and any of BD-related manifestations. Mean IgG-ASCA levels were significantly lower in HFM compared to BD patients (p = 0.03), yet IgA-ASCA levels were similar in HFM and BD. Mean IgG and IgA-ASCA levels were higher in HFM compared with healthy unrelated controls (p=0.09 and p=0.03). No difference was found in ASCA rates between relatives of BD patients who had positive or negative ASCA tests, or between spouses of BD patients and genetically related relatives. In HFM with recurrent oral ulcers there was a positive correlation between titers of IgA-ASCA and the yearly number of oral ulcers episodes (p = 0.01), and mean ulcers healing time (p = 0.01). IgG-ASCA titers correlated with yearly number of aphtae episodes (p = 0.03).*

Conclusion. *The results of this study confirm our previous observation on a high prevalence of ASCA in BD. ASCA levels are also increased in healthy family members of BD patients, and are probably influenced by genetic as well as environmental factors. ASCA in HFM were significantly associated with a more severe oral ulcer disease. The role of ASCA as a marker for predisposition to develop future BD remains to be evaluated.*

Introduction

Saccharomyces cerevisiae is the most common species of the genus *Saccharomyces*, commonly known as baker's or brewer's yeast. In the last years, anti-*Saccharomyces cerevisiae* antibodies (ASCA), directed against oligomannosidic epitopes within the cell wall of the yeast (1), have been identified as an important and specific serological marker for Crohn's disease (CD) (2). At present, ASCA combined with pANCA serve as a valuable tool for the differentiation between CD and ulcerative colitis (UC) in patients with inflammatory bowel diseases (IBD) (2). The pathogenic significance of ASCA is unknown, and their exact origin, as well as the epitope against which they are directed, is unclear. They are not thought to be autoantibodies, although molecular mimicry to self-antigens remains a possibility. In subsequent studies, increased rates of ASCA of about 20-25% were reported among healthy family members of CD patients (3-5). It is not clear, however, whether the increased ASCA rates in CD family members reflect environmental or genetic factors.

While ASCA are considered rather specific for CD, several recent studies suggested a wider panel of autoimmune and rheumatic disorders that may be

associated with high prevalence of ASCA, among them autoimmune liver diseases, celiac disease and ankylosing spondylitis (AS) (6). Behçet's disease (BD) is a multi-systemic disorder, the clinical expression of which may be dominated by mucocutaneous, ocular, articular, neurologic, urogenital, vascular, intestinal or pulmonary manifestations (7). Others and we have recently showed a high prevalence of ASCA in patients with BD (8-10). In a recent study Fresko *et al.* reported on a comparable rate of ASCA positivity in BD and in patients with UC and AS, while patients with BD who had GI involvement had higher levels of ASCA (11). In the current study we evaluated the prevalence and clinical associations of ASCA among BD patients and their healthy family members.

Patients and methods

Fifty-two unaffected family members of twenty-one BD patients were studied. All BD patients fulfilled the International Study Group (ISG) criteria for BD (12). Data from medical files and from patients' interviews was collected, regarding the entire spectrum of disease manifestations. Each family member participating in the study was personally interviewed and a questionnaire composed of BD symptoms and their temporal relation was compiled. None of the BD patients had inflammatory bowel disease (IBD), nor a first-degree family relative with IBD. Furthermore, none of the patients had gastrointestinal manifestation of BD.

ASCA levels

ASVA levels were determined by ELISA employing commercial kits for IgG- or IgA-ASCA (QUANTA Lite™, INOVA Diagnostics, Inc. San Diego, CA, USA), and following manufacturer's instructions. This kit is routinely used in our center for the determination of ASCA, and was tested against healthy subjects as well as patients with known high ASCA levels. Values of 20 or more units were regarded positive according to the assay manufacturer's perception. IgG- and IgA-ASCA levels were measured in the group of BD patients, healthy family members, and in a control groups of twenty-three healthy volunteers.

Statistical analysis

Mean levels of IgG and IgA in the three groups (BD patients, healthy family members and healthy volunteers) were compared using analysis of variance, employing STATA 9 software. Chi-Square test was performed or Fisher's Exact test if appropriate, to analyze statistical significant differences between categorical variables, and two-tailed student's t-test for mean values. The Pearson product was applied to evaluate the correlation between ASCA titers, demographic and clinical parameters.

Results

Twenty-one BD patients and fifty-two family-members were studied. Among the BD patients there were 7 males (33.3%) and 14 females (66.7%), mean age was 49 ± 13 years. All the patients, per definition, had recurrent aphthous stomatitis, three patients (14.3%) also had scarring of oral ulcers, documented by a physician. The prevalences of other manifestations were as follows: genital ulcers - 59.1%, ocular involvement - 34.8%, typical cutaneous lesions - 56.5%, positive pathergy reaction - 11.1% (tested in 18 patients), articular disease - 80.9%, central nervous system involvement - 9.5%, vascular disease (deep or superficial vein thrombosis or arterial aneurysms) 20.0%. Eleven patients were tested for HLA-typing, nine of them (81.8%) had HLA-B51). In the group of family members there were 23 males (44%) and 29 females (56%), mean age was 30 ± 15 years. There were 5 (9.6%) parents, 9 (17.3%) siblings, 30 (57.7%) children and 8(15.4%) spouses.

ASCA in BD patients

ASCA titers were determined for three groups- BD patients, healthy family members (HFM) and healthy controls (HC) as shown in Fig. 1. Eight (38.1%) BD patients were either IgG- or IgA-ASCA positive, compared to five among HFM and none among healthy unrelated controls ($p = 0.001$). Mean IgG and IgA-ASCA levels were significantly higher in BD patients compared with HFM and HC groups ($p = 0.002$ and $p = 0.03$, respectively). No correlation was found between positive ASCA tests and the presence of genital ulcers, ocular disease, skin lesions, positive pathergy reaction, deep or superficial vein thrombosis, arterial disease, joint manifestations, neurological involvement or the presence of HLA-B5. There was, however, a tendency for a positive correlation between IgA-ASCA levels and a propensity of oral ulcers to heal with scarring ($p = 0.05$).

ASCA in healthy relatives of BD patients

Mean IgG-ASCA levels were significantly lower in HFM compared to BD patients (11.9 ± 8.5 *vs.* 19.0 ± 13.8, $p = 0.03$), yet IgA-ASCA levels were similar in HFM and BD (9.9 ± 4.3 *vs.* 12.1 ± 8.3, NS). Mean IgG and IgA-ASCA levels were higher in HFM compared

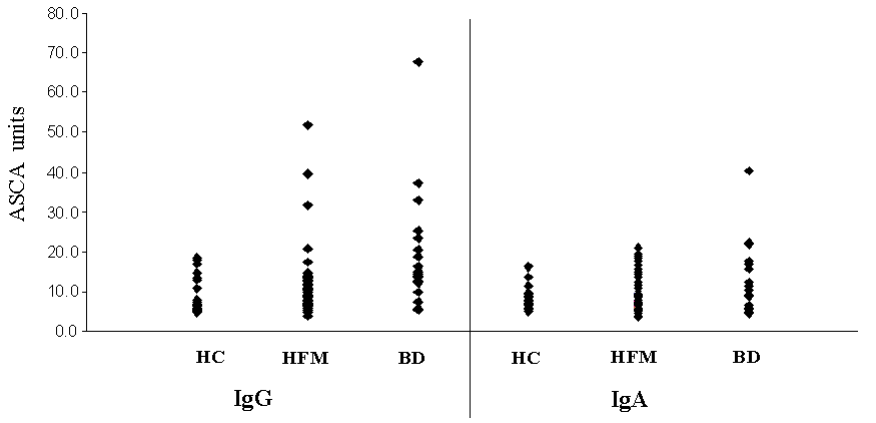

Fig. 1.. Levels of IgG- and IgA-ASCA in patients with Behçet's disease (BD), healthy family members of BD patients (HFM), and healthy unrelated controls (HC).

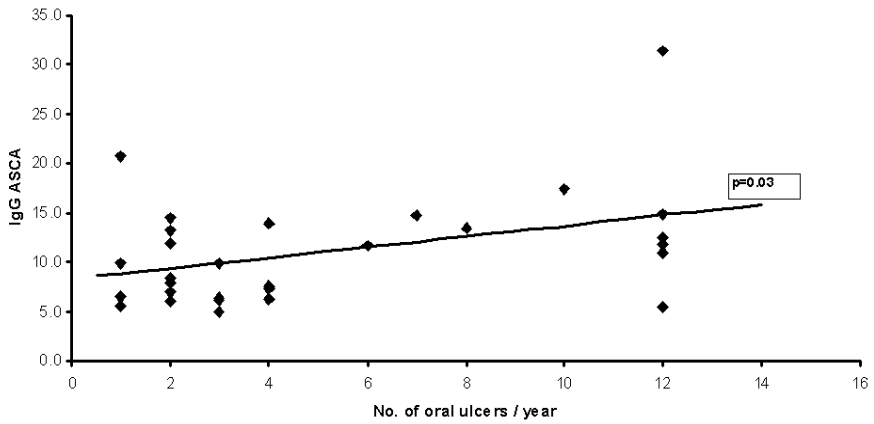

Fig. 2. Correlation between IgG-ASCA levels and yearly number of oral ulcer relapses in healthy family members with recurrent aphthous stomatitis.

with healthy unrelated controls (p = 0.09 and p = 0.03, respectively). No difference was found in ASCA rates between relatives of BD patients who had positive or negative ASCA tests. In comparing spouses of BD patients to genetically related relatives, we found no difference in the levels of IgG or IgA ASCA nor in the absolute number of positive ASCA tests.

ASCA in healthy relatives of BD patients who have recurrent aphthous stomatitis

Thirty-one healthy family members (59.6%) reported on recurrent oral ulcers. The rates of positive ASCA tests, as well as mean IgG and IgA-ASCA levels, were similar in family members with and without oral ulcers. In the group of HFM with recurrent oral ulcers we found a positive correlation between titers of IgA-ASCA and the yearly number of oral ulcers episodes (p = 0.01) (Fig. 2), as well as ulcers healing time (p = 0.01). IgG-ASCA titers were also positively correlated with the number of aphtae episodes in a year (p = 0.03) (Fig. 3).

Discussion

The results of the current study confirm our previous observation on a high prevalence of positive ASCA tests in Israeli BD patients (8). The current study, however, is the first in which healthy family members of BD patients are tested for the presence of ASCA. In order to avoid possible overlap with CD, none of the patients, nor any family relative, had IBD. Furthermore, none of the patients had chronic gastrointestinal manifestations. In accordance with our previous findings, positive ASCA tests were not associated with any of the various BD-related manifestations, hence it appears that the presence of ASCA in BD is an integral part of the disease, not associated with either intestinal or other specific target-organ involvements. Since, however, the absolute number of patients in our study was rather small, it is yet difficult to draw solid conclusions on the prevalence of ASCA in BD patients and their close families. Indeed, as outlined in figure 1, relatively few HFM subjects had significantly higher values of ASCA compared to healthy controls, which accounted for significant higher mean ASCA values, but not for absolute numbers of ASCA positives in HFM compared with HC. Thus, further large-scaled prospective studies are still needed to evaluate the role of ASCA in the pathogenesis and natural history of the BD in both patients and healthy family members.

The etiology of BD is still unknown. Genetic factors, infectious agents, environmental conditions, immunological mechanisms, and endothelial and clothing factors have all been implicated (13). The major involvement of certain ethnic groups and the wide variation of the prevalence of the disease in the same ethnic group in association to the geographic area of residence indicate environmental triggering of a genetically determined disorder (14, 15). Familial occurrence is one of the most commonly reported epidemiological features of BD, however, there are regional differences with familial occurrence being more frequent in Korea (15.4%) than in Japan or China (2.2–2.6%) and in Arab countries, Israel and Turkey (2.0–18.2%) than in Europe (0.0–4.5%) (15). Genetic anticipation in the form of earlier disease onset in children compared with their parents has been identified, corroborating the higher frequency of familial cases in juveniles than in adults and the possibility of a genetic predisposition in BD (16). In order to clarify whether ASCA are predominantly genetically influenced or mainly due to environmental factors we investigated the frequency of ASCA in family members. We found higher mean IgG and IgA-ASCA levels in healthy relatives of BD patients compared with unrelated

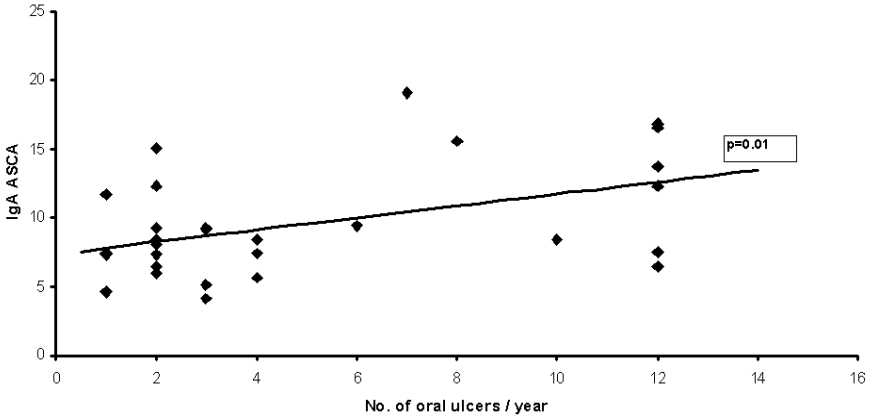

Fig. 3. Correlation between IgA-ASCA levels and yearly number of oral ulcer relapses in healthy family members with recurrent aphthous stomatitis.

healthy controls. The absolute number of positive ASCA essays among family members was not significantly higher compared to unrelated controls (9.6% *vs*. 0%), yet larger study groups are probably required to evaluate true prevalence of ASCA in BD families. When dividing BD-relatives into genetically related *vs*. spouses, we found similar rates and levels of ASCA. Our results, therefore, point to a significant environmental factor contributing to the expression of ASCA in BD. Indeed, relatives of ASCA-positive patients were not more frequently ASCA-positive than relatives of ASCA-negative patients, which would be expected if the generation of ASCA is exclusively due to genetic factors. Nonetheless, in view of the low number of spouses in our study (8 (15.4%)), the role of environmental factors in the development of ASCA still awaits further studies.

Our results further strengthen the association of ASCA to BD expression, though it is yet unclear whether ASCA has a true pathogenic role in the development of the disease, or are merely a phenotypic marker. Notwithstanding, a possible role of ASCA in the pathogenesis of BD my be proposed in view of recent findings on mannose-binding lectin (MBL) status in BD (17, 18). MBL, a C-type serum lectin secreted by the liver, is an important constituent of the innate immune system. It binds to mannose and N-acetyl-glucosamine oligosaccharides on the surfaces of yeasts, bacteria and viruses and serves as the initiator of the third pathway of the complement system independent of antibodies (19). Recent studies showed that MBL gene mutations are additive risk factors for susceptibility to systemic lupus erythematosus, Sjogren's syndrome and rheumatic diseases (20, 21). Recently, Inanc *et al.* studied MBL in sera of 130 BD patients and 64 patients with recurrent oral ulcerations (ROU) (18). Patients with BD had significantly lower median serum MBL levels while no significant difference was observed in median serum MBL levels between BD and ROU. A severe disease course was more frequently observed in BD patients with very low serum MBL levels. It was concluded that MBL deficiency might contribute to the pathogenesis of BD and affect its clinical course (18). ASCA, which bind mannose and N-acetyl-glucosamine oligosaccharides, may possibly cross-react with MBL, which may then contribute to the expression and severity of BD, and perhaps of recurrent oral ulcers. Indeed, we found in our study an association between ASCA and the severity of oral ulcers, since ASCA in healthy family members was significantly correlated with both the higher yearly number of oral ulcers episodes, as well as prolonged ulcers healing time. The results of our study also raise a possibility of ASCA being a marker for future development of BD in family members. It was recently proposed that the presence of ASCA in a high risk healthy individual might be a marker for future development of CD and may even predict the clinical course (22). The presence of ASCA in sera of healthy relatives of BD patients, as found in our study, may indicate that a subclinical phase of BD process is occurring in these subjects, which may or may not eventuate in clinical disease. The potential predictive value of ASCA in these subjects can be determined in a prospective follow up of these individuals.

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