

Anti-*Saccharomyces cerevisiae* Autoantibodies and Autoimmune Diseases: the Sweet and Sour of Baking Yeast

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S*accharomyces cerevisiae* has long been used both to ferment the sugars of cereals to produce alcoholic drinks and in the baking industry to leaven dough until it became the gold-standard in brewing and baking. Thousands of years ago, humans were exposed to yeast, which accidentally “contaminated” flour or drinks. The flavorsome results have brought it into almost every meal nowadays depending on the culinary culture [1]. The probable physiopathologic mechanism of molecular mimicry of self-antigens behind the induction of anti-*Saccharomyces cerevisiae* autoantibodies (ASCAs) may indicate their pathogenic role in several associated autoimmune diseases. In recent decades, the pathogenesis of autoimmune diseases has increasingly come to be understood as a process involving several different factors. These factors constitute the interesting complexity of the mosaic of autoimmunity whose pieces include genetic, immunologic, hormonal and environmental factors, such as microbial agents among which yeasts are becoming apparent as part of the whole [2]. Evidence of the association between ASCAs and autoimmune disorders has increased over the past two decades.

EXPOSURE TO MICROBIAL AGENTS AND AUTOIMMUNITY

The immune system is exposed to different antigens owing to the burden of microorganisms. Therefore, the microbial pattern of each subject is unique. This physiologic process allows only mildly self-binding lymphocytes to survive according to positive selection, whereas those interacting too tightly with immunogenic molecular structures are negatively selected through a pathway leading to programmed cell death. It is noteworthy that microbial agents can induce autoimmunity through four main mechanisms:

- Molecular mimicry (cross-reactivity due to overlapping molecular sequences)
- Epitope spreading, which is the detection of new epitopes that differ from the original shared sequence after an antigen has been processed and presented on the cell surface by antigen-presenting cells (APCs)
- Bystander activation, which is based on the release of sequestered antigens as a consequence of tissue damage especially by microbial injury
- Persistent activation of the immune response, as may well occur during viral spread [1].

The gut-associated lymphoid tissue (GALT) is the main localization site of the recently defined CD4+ Th17 lymphocytes that release interleukin-17 (IL-17), involved in the response to extracellular bacteria and fungal infections. Saprophytic microbial agents can ordinarily preserve the dynamic Th17 T regulatory (Treg) balance in GALT. Nonetheless, dysregulated IL-17 secretion drives immune mediated pathology, notably inflammatory bowel diseases (IBD) in the gut [4]. Consequently, even the apparently non-pathogenic microbiota could trigger autoimmunity when the finely regulated balance is aberrantly fragile [5]. Critical data on the effect that dietary intake of “the brewer and baker’s yeast” *Saccharomyces cerevisiae* may induce on T helper (Th) 17 cells are still lacking.

IMMUNOLOGICAL ASPECTS AND CLINICAL-LABORATORY ANALYSIS

Yeasts are employed as efficient biological machinery that produces several antigenic components for vaccines needed to elicit protective immune responses. The phagocytosis of the yeast by dendritic cells is activated by the immunogenic cell wall molecules, such as β -1,3-D-glucan and mannan. These proteins can induce critical signals usually associated with microbial infection. In fact, this pair of events constitutes the first step and is followed by antigen degradation and its fragment presentation on the APC surface by major histocompatibility complex (MHC) I and MHC II molecules, interacting with the T cell receptor and prompting

co-stimulatory signals to lead the adaptive T cell (CD8+ or CD4+)-mediated response [1,8]. Subsequently, the development of the humoral immune response leads to the production of antibodies against the yeast by both B lymphocytes and plasma cells, thus it is not yet clear whether this outcome might be an epiphenomenon or could have a direct pathogenic role through a co-stimulatory CD80/86-CD28-mediated effect. By contrast, concerns have been raised regarding the current safety of vaccines due to the presence of adjuvants [3]. Shoenfeld and Agmon-Levin described the autoimmune/inflammatory syndrome induced by adjuvants (ASIA syndrome) in which adjuvants may trigger an autoimmune/inflammatory response in predisposed individuals. In this context, heat-killed *Saccharomyces* can act like common adjuvants, such as aluminium and silicone, when injected together with preventive vaccines [1,3,6]. ASCAs are directed against the cell wall mannan (phosphopeptidomannan) of *S. cerevisiae*. The assessment of ASCAs by enzyme-linked immunosorbent assay (ELISA) resulted in 50–79% sensitivity and 74–93% specificity in Australian patients with Crohn’s disease [7], depending on the commercial kits used such as those giving ASCA IgA/IgG-positive results at 10 U/ml with a detection threshold of 1 U/ml (ORG 545 ASCA IgG/IgA, ORGENTEC® Diagnostika GmbH, Germany).

DATABANK SEARCH METHOD AND RESULTS

The Protein Database of the National Center for Biotechnology Information (NCBI) was consulted, focusing on the most specific and significant results (highest identity/positivity). Table 1 summarizes the main findings from our group. We also evaluated the E value, which represents the number of different alignments with scores equivalent to or better than is expected to occur in a database search by chance. The lower the E value, the more significant the score and the alignment (mean 5.03 ± 4.86) [9].

ASCA POSITIVITY IN AUTOIMMUNE DISEASES

ASCA immunoglobulin A, G and M levels were measured by ELISA in 30 patients affected with RA and in 152 healthy adult controls. ASCA IgA prevalence was significantly higher in patients suffering from RA than in the healthy subjects (40% vs. 5.3%). In RA patients, ASCA IgA levels were also strongly correlated with C-reactive protein (CRP) ($r = 0.695, P < 0.01$) as well as erythrocyte sedimentation rate (ESR) ($r = 0.708, P < 0.01$) [1]. As shown in Table 1, significant similarities were observed between the sequence of autoantigens and mannan expressed by the cell wall of *S. cerevisiae*. In a different supporting study, serum samples taken from 40 patients with SLE and 152 healthy subjects were compared for ASCA IgA, IgG and IgM levels as tested by ELISA. The prevalence of ASCA

Table 1. Original selection of the most significant results from our exhaustive collection

Autoimmune disease	ASCA		Antigen (Homo sapiens)	Comparison to mannan: # EDV13046,1	
	IgG	IgA		Identities	Positives
RA	neg	pos	gp130-RAPS	4/5, 80%	4/5, 80%
SLE	pos	neg	U2snRNP B'	5/6, 83%	5/6, 83%
APS	pos	pos	antiCL/ β2GPI	7/11, 64%	8/11, 73%
ACS/AMI	pos	pos	cardiac myosin	5/8, 63%	7/8, 88%
Behçet’s disease	pos	pos	α-enolase 1	5/8, 63%	7/8, 88%
Crohn’s disease	pos	pos	Calprotectin	3/5, 60%	5/5, 100%

The percentage of sequence identities and/or positive substitutions expresses the extent to which the protein sequences are related

RAPS = rheumatoid arthritis antigenic peptide-bearing soluble form, antiCL/β2GPI (anti-cardiolipin/anti-β2 glycoprotein I, light chain variable region)

IgG, but not IgM and IgA, was significantly raised in active SLE patients (57.5%) compared to healthy controls (8.5%). Conversely, ASCA IgG levels were relatively lower in SLE patients during remission, indicating a possible correlation with disease activity [1]. Several SLE autoantigens have been found to share sequences with yeast mannan, and U2 snRNP B' shows the best match. Considering several autoimmune disorders associated with ASCA positivity [1], we also discovered other autoantigens that might cross-react with antibodies against mannan of *S. cerevisiae* according to the percentages of sequence identities (ID) and/or positive substitutions (PS) such as GAD65 (ID 35%, PS 57%) and zinc-transporter 8 (ID 43%, PS 57%), α-enolase 1 in Behçet’s disease with ocular involvement (uveitis) (ID 63%, PS 88%), thyroid peroxidase (ID and PS 71%) for autoimmune thyroid disease, and calprotectin (ID 60%, PS 100%) for Crohn’s disease [Table 1] [1].

ASCA POSITIVITY AND ATHEROSCLEROSIS

Interestingly, several homologies have been detected by analyzing and matching the molecular sequence of *S. cerevisiae* phosphopeptidomannan with cardiac myosin (ID 63%, PS 88%). Complementary to this, elevated ASCA IgA and IgG levels were found in patients who had suffered an acute coronary syndrome (ACS) or acute myocardial infarction (AMI), suggesting that ASCA positivity in AMI could be identified as a useful marker for atherosclerotic plaque instability [10]. Therefore, since autoantibodies can be considered stable over time, they may be less dependent on the period between plaque rupture and AMI onset than other assessable inflammatory biomarkers [10]. Additionally, the assessment of these autoantibodies could be a valid addition for the careful screening of patients whose anti-cardiac troponin I (anti-cTnI) serum

level may produce false negatives, bringing about a delay in non-ST segment elevation myocardial infarction (NSTEMI) diagnosis [10].

CONCLUSIONS

Our results strongly suggest that the role of ASCAs in clinical practice, as well as the dietary recommendations for specific groups of patients, should be further explored in order to evaluate their predictive or prognostic relevance. New manufacturing challenges may need to be considered for vaccines containing *S. cerevisiae*.

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