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# Anti-*Saccharomyces cerevisiae* Autoantibodies in Autoimmune Diseases: from Bread Baking to Autoimmunity

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**Abstract** *Saccharomyces cerevisiae* is best known as the baker's and brewer's yeast, but its residual traces are also frequent excipients in some vaccines. Although anti-*S. cerevisiae* autoantibodies (ASCAs) are considered specific for Crohn's disease, a growing number of studies have detected high levels of ASCAs in patients affected with autoimmune diseases as compared with healthy controls, including antiphospholipid syndrome, systemic lupus erythematosus, type 1 diabetes mellitus, and rheumatoid arthritis. Commensal microorganisms such as *Saccharomyces* are required for nutrition, proper development of Peyer's aggregated lymphoid tissue, and tissue healing. However, even the commensal nonclassically pathogenic microbiota can trigger autoimmunity when fine regulation of immune tolerance does not work properly. For our purposes, the protein database of the National Center for Biotechnology Information (NCBI) was consulted, comparing *Saccharomyces* mannan to several molecules with a pathogenetic role in autoimmune diseases. Thanks to the NCBI bioinformation technology tool, several overlaps in molecular structures (50–100 %) were identified when yeast mannan, and the most common autoantigens were compared. The autoantigen U2 snRNP B ″ was found to conserve a superfamily protein domain that shares 83 % of the *S. cerevisiae* mannan sequence. Furthermore, ASCAs may be present years before the diagnosis of some associated autoimmune diseases as they were retrospectively found in the preserved blood samples of soldiers who became affected by Crohn's disease years later. Our results strongly suggest that ASCAs' role in clinical practice should be better addressed in order to evaluate their predictive or prognostic relevance.

**Keywords** Anti-*Saccharomyces cerevisiae* autoantibodies · Autoimmune diseases · Molecular mimicry · Autoantigenicity · Vaccines

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## Introduction

*Saccharomyces cerevisiae*, also known as the baker's or brewer's yeast, has long been utilized to ferment the sugars of rice, wheat, barley, and corn to produce alcoholic beverages, and in the baking industry to raise dough. Thousands of years ago, yeasts accidentally "contaminated" flour or drinks, and the results were pleasant for the people who tasted them. As a consequence, we are now commonly exposed to yeast [1]. Nonetheless, anti-*S. cerevisiae* antibodies (ASCAs), directed against the phosphopeptidomannan part of the cell wall of the yeast, have been identified as an important and specific serological marker for Crohn's disease (CD) [2]. Furthermore, ASCAs were detected retrospectively as being present years before CD clinical onset in 31.3 % of preserved blood samples of Israeli Defence Force soldiers compared to none of the controls [3].

The pathogenic significance of ASCAs is not yet fully understood, but molecular mimicry of self-antigens remains a possibility. Although ASCAs are considered specific for CD, a growing number of studies have individually identified high levels of ASCAs in other autoimmune diseases such as antiphospholipid syndrome (APS) [2], systemic

lupus erythematosus (SLE) [4], diabetes mellitus type 1 [5], and rheumatoid arthritis (RA) [6].

In recent decades, the pathogenesis of autoimmune diseases has increasingly come to be understood as a multifactorial process which develops through the interaction of the pebbles of genetic, immunological, environmental, and hormonal factors which combine to compose the complex mosaic of autoimmunity [7–10]. Evidence of the association between ASCAs and autoimmune disorders has increased over the past two decades, and to the best of our knowledge, this is the first review aimed at discussing these findings and providing the molecular background for further studies.

## Environment/Infections and Autoimmunity

The immune system is exposed to different antigens from the very first stages of its development and maturation. Positive selection allows weakly self-binding lymphocytes to survive, while negative selection leads to the accomplishment of the death program for cells interacting too tightly with immunogenic molecules, with the aim of maintaining self-tolerance. Only a very small minority of lymphocytes will survive and interact with several microbial antigens that could potentially cause pathology. The burden of infections dealt with from childhood makes every individual's microbial pattern unique [11]. Microbial agents can induce autoimmunity through four main mechanisms [12]: (1) *molecular mimicry* is perhaps the most likely mechanism and occurs when shared epitopes (carbohydrate, protein/peptides, or DNA) in the pathogen and host's molecular structures cross-react in the presence of an active immune response. Cross-reactivity is confirmed by the association between the pathogen and the autoimmune disease, the elicitation of the specific immune response, active immunization or autoantigen-induced disease in animal models, and passive immunization if the disease occurs either after autoreactive T cell or autoantibody administration in animal models. (2) *Epitope spreading* is the distinction from the original shared sequence after an antigen is processed and presented on the cell surface by antigen-presenting cells (APCs) which results in a new autoreactive response directed against the neo-epitope, as occurs for rheumatoid factor (RF) mimicking collagen or laminin in chronic rheumatic heart disease [13]. (3) *Bystander activation* is based on the release of sequestered antigens as a consequence of tissue damage, usually due to viral infections, and involves autoreactive lymphocytes previously not committed. Furthermore, a pro-inflammatory microenvironment can switch on an autoimmune response in a bystander manner, killing adjacent healthy cells too [14]. (4) *Persistent activation of the immune response*, particularly during recurrent viral load increases, can lead to autoimmunity. Indeed, in mixed cryoglobulinemia, chronic HCV infection causes prolonged stimulation of the immune system which can drive the activation of the humoral immune response to produce monoclonal and polyclonal autoantibodies [15]. Toll-like receptors (TLRs), as an important part of the innate immunity and as effector molecules leading to the adaptive immune response, can detect fungal, viral, and bacterial pathogens that expose their conserved pathogen-associated molecular patterns [18]. The lack of TLR3 in mice has been associated with an ineffective immune response to CMV, while rare human mutations in TLR3 or UNC93B and in interleukin-1 receptor-associated kinase 4 or MyD88 result in recessive susceptibility to HSV-1 encephalitis and recurrent bacterial infections, respectively [16]. The role of TLRs has only very recently been considered important in dealing with fungal infections, particularly by yeasts such as *Candida* spp.; TLR1, TLR3, TLR4, and TLR6 are all involved in the MyD88 molecular downstream pathway, transducing fungal "danger signals" [17]. Notably, TLR3 stimulation was linked with the improvement of T1D in models and experimental autoimmune encephalomyelitis before the L412F variant of TLR3 had been found in patients with chronic mucocutaneous candidiasis (in the absence of potentially related AIRE, LYP, dectin 1, or CARD9 mutations) [16]. The mucosal surface is the main localization site of the recently defined $CD4^+$ Th17 lymphocytes which release IL-17, involved in the response to extracellular bacterial and fungal infections. Saprophytic microbial flora is likely to maintain the delicate Th17-T regulatory (Treg) balance in gut-associated lymphoid tissue, one of the most important human barriers to external environmental factors. Nonetheless, dysregulated IL-17 secretion drives immune-mediated pathology in the gut, notably inflammatory bowel disease (IBD) [19]. Thus, even the commensal nonclassically pathogenic microbiota can trigger autoimmunity when fine regulation of immune tolerance does not work properly [20]. Dietary components can also impact on Th17 activity since gliadin-specific Th17 cells have been characterized from biopsies of celiac patients [21], and vitamin D receptor-deficient experimental models were found to develop more severe IBD with IL-17 overexpression [22]. There is a lack of data concerning the effect that dietary intake of "the brewer and baker's yeast" *S. cerevisiae* may induce on Th17 cells. Currently, Th17 cells are deemed to play a role in the development and relapse of many autoimmune diseases, especially multiple sclerosis, RA, CD, and psoriasis [18]. Inflammation and cell stress amplify posttranscriptional regulation mechanisms increasing expression of microRNAs (miRNAs). MicroRNAs are emerging as posttranscriptional regulators of many biological processes including cellular proliferation, differentiation, and apoptosis with clinical implications for cancer and autoimmune diseases [23]. TNF-α, IFN-β, and several TLRs can induce miR155 in macrophages [24], and its

overexpression has also been described in response to the eukaryotic yeast cells of *Candida albicans* [25]. Furthermore, miRNAs, especially miR155, are also involved in maintaining the proper T cell-dependent humoral response, certainly by modulating the release of cytokines and possibly through other mechanisms as well [26]. In a recent study by Iborra et al. increased miRNA expression was found in IBD, and the expression of miR192 and miR21 was also related to ulcerative colitis disease activity compared with healthy controls [23]. A prominent role, among several infective agents associated with autoimmune diseases, has been recognized for viruses and bacteria. Persistent Epstein–Barr virus (EBV) infection is linked with RA, where the EBV DNA load can increase by up to tenfold, and with SLE, where impaired T cell function was reported in addition to latently infected peripheral B cells [27]. Moreover, anti-EBV viral capsid and anti-EBV nuclear antigen 1 antibodies were also detected in almost all patients with multiple sclerosis (MS), and titers become two- to three-fold higher than those of controls during the third decade of life, increasing in line with the MS risk [28]. Concerning bacterial infections, in a prospective study, half of 246 patients with inflammatory arthritis were found positive for RF, and their anti-*Proteus mirabilis* IgM and IgA titers were significantly higher than those in other groups of patients. Currently, there are no studies reviewing the impact of environmental exposure to fungal antigens on autoimmunity. However, Shinohara et al. induced coronary arteritis (a hallmark of Kawasaki arteritis) in mice by intraperitoneal injection of *C. albicans* water-soluble fraction, and the disease has been related to the activation of the complement lectin pathway [29], possibly through mannan-binding lectin (MBL), a pattern recognition molecule [30]. MBL can bind mannan which is a fungal cell wall antigenic element notably exposed on the yeast cell surface of *S. cerevisiae*. The detection of ASCAs typically associated with CD, perhaps more than just an epiphenomenon, may also be implicated in the pathogenesis of IBD [31]. Interestingly, *C. albicans* is able to induce the production of ASCAs (but *S. cerevisiae* does not elicit the reverse), as tested in rabbit experimental models and as shown by immunohistochemical stains from biopsies of patients with diagnosed systemic candidiasis [32].

## Immunological Aspects and Assays

Yeasts are known as biological machines capable of producing antigenic components for vaccines needed to elicit protective immune responses. A question arises: is *S. cerevisiae* immunogenic itself? The use of *Saccharomyces*-based therapeutic vaccines is supported by the evidence of their ability to stimulate tumor or viral-specific CD4 and CD8 T cell responses. Heat-killed *S. cerevisiae* seems to be an attractive carrier because it can express different antigens and is cost-effective as it provides danger signals that lead dendritic cells (DCs) to maturation without the need of additional adjuvants. The phagocytosis of the yeast by DCs is triggered by the immunogenic cell wall molecules, such as β-1,3-D-glucan and mannan, which induce critical signals usually associated with microbial infection. This event represents the first step, and it is followed by antigen degradation and the presentation of their fragments on the APC surface by MHC I and MHC II molecules, interacting with the TCR and inducing co-stimulatory signals to lead the adaptive T cell ($CD8^+$ or $CD4^+$)-mediated response [33, 34]. *S. cerevisiae* is an effective inducer of innate and adaptive cellular immunity, and recombinant yeast cells (GI-5005) can lead to a potent antigen-specific proliferative and cytotoxic T cell response associated with Th1-type cytokine secretion [35].

Commensals such as *Saccharomyces* are required for nutrition, proper development of Peyer's aggregated lymphoid tissue, and tissue healing. However, it is possible that in the proinflammatory pathological microenvironment that characterizes many immune-mediated diseases, notably IBD, the alteration of the finely regulated interaction between APCs, the nonclassically pathogenetic microbiota, and Th17 cells in the gut could trigger autoimmunity [20]. Moreover, MyD88, as an adaptor molecule shared by most TLRs, has been proven to play a key role in antifungal defense by several in vivo studies, underlining the relevance of TLRs in the host interaction with fungal microbial antigens [36].

The subsequent development of the humoral immune response leads to the production by B lymphocytes and plasma cells of antibodies against the yeast, so it is not yet clear whether it might simply represent an epiphenomenon or could have a direct pathogenic role through a co-stimulatory CD80/86-CD28-mediated effect. Indeed, it is plausible that in a proinflammatory state, a mechanism of molecular mimicry involving the eukaryotic microorganism and self-antigens takes over. Furthermore, infection by *C. albicans* can induce the production of ASCAs in humans, while *Saccharomyces* does not lead to the production of antibodies to *C. albicans* [29].

On the other hand, concerns have been raised regarding the current safety of vaccines due to the presence of adjuvants. Since heat-killed *Saccharomyces* can act like common adjuvants such as squalene, aluminum, and silicone (Table 1) when injected together with preventive vaccines, there is a risk of inducing an autoimmune disorder by administering *Saccharomyces*-based therapeutic or even preventive vaccines instead of traditional ones [37–39]. It is very difficult to find the ideal compromise between immune system stimulation and modulation. Indeed, procedures used to manufacture vaccines for hepatitis B and/or hepatitis A result in products that contain no more than 1–5 % residual yeast proteins (Recombivax HB, Engerix B; Merck & Co.) [40]. Although the risk/benefit analysis still

**Table 1** Vaccines containing *S. cerevisiae* as an adjuvant

| Vaccines containing *S. cerevisiae* | Extract | Protein |
|---|---|---|
| DTaP-HepB-IPV (Pediatrix) | | ✓ |
| Hip/Hep B (Comvax) | | ✓ |
| Hep B (Engerix-B) | | ✓ |
| Hep B (Recombivax) | | ✓ |
| HepA/HepB (Twinrix) | | ✓ |
| Meningococcal (Menveo) | ✓ | |
| Pneumococcal (Prevnar) | ✓ | |
| Pneumococcal (Prevnar13) | ✓ | |
| Typhoid (oral Ty21a) | ✓ | |
| HPV (Gardasil) | ✓ | |

indicates ensuring that vaccinations are used, we now face the challenge of developing risk-free vaccines.

Indeed, an additional risk is that of developing the recently identified syndrome, namely, autoimmune/inflammatory syndrome induced by adjuvants [37] that comprises several medical conditions characterized by hyperactive immune responses accompanied by a similar array of signs and symptoms. Since adjuvants are the common ground that could possibly induce autoimmune or autoinflammatory diseases in humans, the use of immunogenic matter as an adjuvant, such as *S. cerevisiae*, should be carefully evaluated.

ASCAs were found in several autoimmune diseases by means of ELISA tests used for the quantitative measurement of IgG- and IgA-class autoantibodies against mannan of *S. cerevisiae* in human sera or plasma. The assessment of ASCAs by ELISA resulted in 50–79 % sensitivity and 74–93 % specificity in Australian CD patients, depending on the commercial kits used [41].

ASCAs are directed against the cell wall mannan (phosphopeptidomannan) of the yeast *S. cerevisiae*. IgG as well as IgA ASCAs are held to be highly specific for CD. However, the determination of ASCAs is also reliable in other autoimmune disorders besides CD [3]. In a normal range study with serum samples from healthy blood donors, ASCA tests for either IgG or IgA subclasses were considered positive at a titer of >10 U/ml.

Nevertheless, positive results should be interpreted in the light of the patient's clinical status. It is recommended that each laboratory establishes its own normal and pathological ranges for serum ASCAs. The lower detection limit for ASCA ELISA was determined at 1 U/ml. The solid phase is coated with mannan from *S. cerevisiae* (ORG 545 ASCA IgG/IgA, ORGENTEC Diagnostika GmbH, Germany). Therefore, the ASCA test kits only recognize autoantibodies specific for this phosphopeptide [3].

**Table 2** Link between rheumatoid arthritis-associated antigens and ASCAs

| Autoimmune disease | ASCA | | Antigens (*Homo sapiens*) | Comparison to mannan; accession no. EDV13046.1 | |
|---|---|---|---|---|---|
| | IgG | IgA | | Identities | Positives |
| Rheumatoid arthritis | Neg | Pos | RF | 5/10, 50 % | 6/10, 60 % |
| | | | Anti-citrullinated collagen type2 | 3/6, 50 % | 6/6, 100 % |
| | | | gp130-RAPS | 4/5, 80 % | 4/5, 80 % |
| | | | EIF4G1 | 6/8, 75 % | 7/8, 88 % |
| | | | Bip/GRP78 | 5/7, 71% | 5/7, 71% |

The main autoantigens in RA with the highest sensitivity and specificity have been considered with the relative percentage of sequence identities and/or positive substitutions. Accession number EDV13046.1 is a code which corresponds to mannan molecular structure in the NCBI database, which was considered the referring element for each comparison

*RF* rheumatoid factor, *RAPS* rheumatoid arthritis antigenic peptide-bearing soluble form, *EIF4G1* eukaryotic translation initiation factor 4 gamma 1, *Bip*/*GRP78* glucose-regulated protein 78

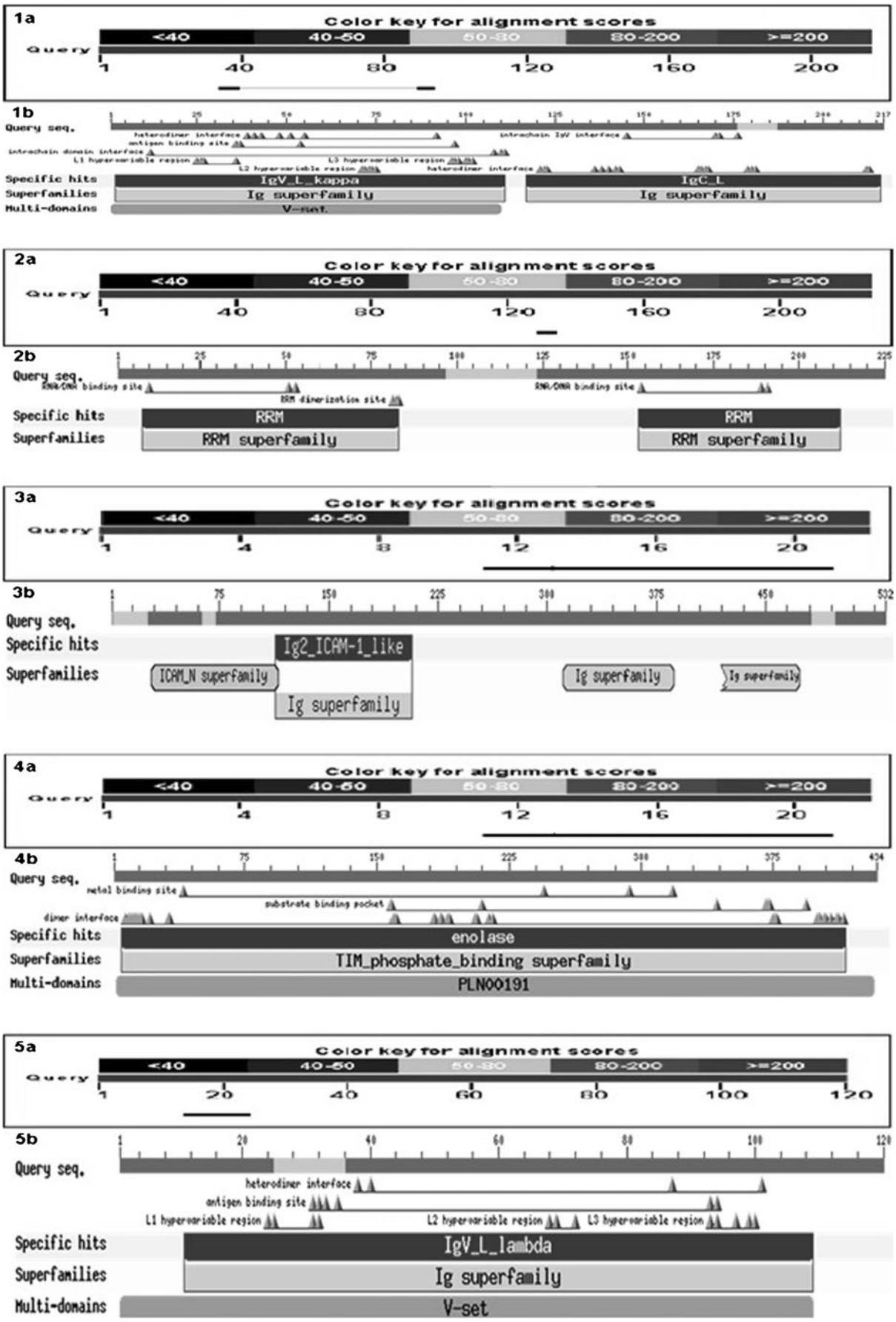
## Database Searching Method and Results

For our purposes, we consulted the protein database of the National Center for Biotechnology Information (NCBI), comparing mannan of *Saccharomyces* to several molecules with a pathogenetic role in autoimmune diseases. We focused on specific and significant results (highest identity/positivity), displaying them in tables by pathological conditions. The related graphics show the location of the positive match, along sequence bars composed of ranges with different color shades, according to the resulting score.

We also evaluated the expectation or expect value (*E* value); this represents the number of different alignments with scores equivalent to or better than what is expected to occur by chance in a database search. The lower the *E* value, the more significant is the score and the alignment [54]. The *E* values for our results range between 0.17 and 9.90 with a mean of 3.11. This reliability index supports and reinforces the evidence of our findings.

## Autoimmune Diseases Associated with *S. cerevisiae*

The association between infectious and autoimmune diseases has been widely described, and *S. cerevisiae* may represent another dowel in this parquetry puzzle. In one study, ASCA IgA, IgG, and IgM levels were measured with ELISA in 30 patients affected with RA and in 152 healthy adult controls. ASCA IgA prevalence was significantly higher in RA patients (40 %) than in healthy subjects (5.3 %). In RA patients, ASCA IgA levels strongly correlated with C-reactive protein (CRP) ($r$=0.695; $p$< 0.01) and erythrocyte sedimentation rate ($r$=0.708; $p$<0.01) [6]. As shown in Table 2, we observed significant similarities be-


1a
Color key for alignment scores
<40
40-50
50-80
80-200
>=200
Query
1
40
80
120
160
200
1b
Query seq.
Specific hits
IgV_L_kappa
IgC_L
Superfamilies
Ig superfamily
Ig superfamily
Multi-domains
V-set
2a
Color key for alignment scores
<40
40-50
50-80
80-200
>=200
Query
1
40
80
120
160
200
2b
Query seq.
Specific hits
RRM
RRM
Superfamilies
RRM superfamily
RRM superfamily
3a
Color key for alignment scores
<40
40-50
50-80
80-200
>=200
Query
1
4
8
12
16
20
3b
Query seq.
Specific hits
Ig2_ICAM-1_like
Superfamilies
ICAM_N superfamily
Ig superfamily
Ig superfamily
Ig superfamily
4a
Color key for alignment scores
<40
40-50
50-80
80-200
>=200
Query
1
4
8
12
16
20
4b
Query seq.
Specific hits
enolase
Superfamilies
TIM_phosphate_binding superfamily
Multi-domains
PLN00191
5a
Color key for alignment scores
<40
40-50
50-80
80-200
>=200
Query
1
20
40
60
80
100
120
5b
Query seq.
Specific hits
IgV_L_lambda
Superfamilies
Ig superfamily
Multi-domains
V-set

◀ **Figs. 1–5** *Sequence bars* are displayed as a group of bars in *various shades of gray*, each representing a result sequence that shows the position(s) where the result sequence is similar to the input sequence. *Sequence bars* show their score range by color shade variation, according to the alignment scores color key. The most similar hits are upmost (≥200). **Fig. 1 a** Conserved domains for chain B anti-citrullinated peptide collagen type 2. *Clusters of different arrowheads* which belong to specific hits included in a superfamily protein domain are shown such as the heterodimer interface (polypeptide binding site) on conserved domain IgC_L that compose the conserved feature mapped on the query sequence. **b** Distribution of two hits for chain B anti-citrullinated peptide collagen type 2 compared to mannan. The alignments' result came from the process of matching amino acid residues from both biosequences. In this sequence matching, consistent homology was detected showing the existing degree of similarity between the considered molecular structures. **Fig. 2 a** Conserved domains for U2 snRNP B″. *Clusters of different arrowheads* which belong to specific hits included in a superfamily protein domain are shown, namely, the RNA recognition motif (*RRM*) also known as RNA-binding domain (*RBD*) or ribonucleoprotein (*RNP*) domain, one of the most plenteous protein domains in eukaryotes. Compositionally biased region not used in this domain database search is highlighted on the query sequence. **b** Distribution of the hit for U2 snRNP B″ compared to mannan. In this sequence match, a very high homology was detected showing the existing degree of similarity between the considered molecular structures. **Fig. 3 a** Conserved domains for ICAM 1 which play a central role in intercellular adhesion have been detected; the Ig2_ICAM-1-like-specific hit, included in the immunoglobulin superfamily protein domain, is displayed. Compositionally biased regions not used in this domain database search are highlighted on the query sequence. **b** Distribution of the hit for ICAM 1 compared to mannan. **Fig. 4 a** Conserved domains for α-enolase 1, a glycolytic enzyme, have been detected compared to mannan. *Clusters of different arrowheads* which belong to specific hits included in the triosephosphate isomerase or TIM_phosphate_binding superfamily protein domain are shown such as dimer interface on conserved domain enolase; 37 of 37 of the residues that compose this conserved feature have been mapped to the query sequence. **b** Distribution of five hits for α-enolase 1. In this sequence match, a very high homology was detected showing the existing degree of similarity between the considered molecular structures. **Fig. 5 a** Conserved domains for anti-cardiolipin/beta-2-glycoprotein-I immunoglobulin light chain variable region have been detected compared to mannan. Compositionally biased region not used in this domain database search is highlighted on the query sequence. **b** *Clusters of different arrowheads* which belong to specific hits included are shown. In this sequence match, a very high homology was detected showing the existing degree of similarity between the considered molecular structures such as the antigen binding site on conserved domain IgV_L_lambda where 6/6 residues that compose this conserved feature have been mapped to the query sequence

tween the sequence of autoantigens (Fig. 1a, b) and mannan expressed by the cell wall of *S. cerevisiae*.

In another study, serum samples from healthy volunteers ($n$=152) and patients with SLE ($n$=40) were compared for ASCA IgA, IgG, and IgM levels using ELISA. The prevalence of ASCA IgG, but not IgM and IgA, was significantly raised in active SLE patients (57.5 %) compared to healthy controls (8.5 %) (Fig. 2c). ASCA IgG levels in SLE patients during remission were relatively lower, indicating a possible correlation with disease activity [4]. Several SLE autoantigens have been found to share sequences with yeast mannan, and *U2 snRNP B″* (Fig. 2a, b) shows the best match (Table 3).

**Table 3** Link between systemic lupus erythematosus-associated antigens and ASCAs

| Autoimmune disease | ASCA | | Antigens (*H. sapiens*) | Comparison to mannan; accession no. EDV13046.1 | |
|---|---|---|---|---|---|
| | IgG | IgA | | Identities | Positives |
| Systemic lupus erythematosus | Pos | Neg | SSA (Ro), | 5/10, 50 % | 6/10, 60 % |
| | | | SSB (La) | 6/13, 46 % | 9/13, 69 % |
| | | | snRNP–Sm D3 | 6/11, 55 % | 7/11, 64 % |
| | | | SmN | 5/15, 33 % | 8/15, 53 % |
| | | | U2 snRNP B″ | 5/6, 83 % | 5/6, 83 % |

The percentages of sequence identities and/or positive substitutions can express the extent to which the protein sequences are related

## ASCAs, Autoimmunity, and Atherosclerosis

The role of local and general inflammation in atherosclerosis is a major area of interest. In a case–control study, the ASCA IgG and IgA levels of patients with acute myocardial infarction (AMI) were compared with those of controls to investigate the possible role of ASCAs in atherosclerosis. AMI was diagnosed by electrocardiography and serial enzymes. Elevated ASCA IgA and IgG levels were found, suggesting that ASCA positivity in AMI could represent a useful marker for atherosclerotic plaque instability. It might also provide a link between inflammatory processes and increased cardiovascular risk. Interestingly, one of the most significant sequence matches with mannan is ICAM-1 (Fig. 3a, b). ICAM-1 and P-selectin (Table 4) are crucial molecules for transendothelial migration of leukocytes, playing an important role in the process of atherogenesis, especially in patients with chronic systemic inflammatory autoimmune diseases [42]. The role of macrophages expressing the LOX1 receptor for oxidized LDL is well-known in the pathogenesis of atherosclerosis, but more recently, a distinction has been proposed for the cells involved in the atherogenic process, especially when it is accelerated by systemic inflammatory

**Table 4** Link between acute myocardial infarction-associated antigens and ASCAs

| Autoimmune disease | ASCA | | Antigens (*H. sapiens*) | Comparison to mannan; accession no. EDV13046.1 | |
|---|---|---|---|---|---|
| | IgG | IgA | | Identities | Positives |
| Cardiovascular diseases, acute myocardial infarction | Pos | Pos | P-selectin | 8/15, 53 % | 11/15, 80 % |
| | | | Intercellular adhesion molecule-1 | 4/4, 100 % | 4/4, 100 % |
| | | | Myosin | 5/8, 63 % | 7/8, 88 % |

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

**Table 5** Link between Behçet's disease, immunologic ocular disease-associated antigens, and ASCAs

| Autoimmune disease | ASCA | | Antigens (*H. sapiens*) | Comparison to mannan; accession no. EDV13046.1 | |
|---|---|---|---|---|---|
| | IgG | IgA | | Identities | Positives |
| Behçet's disease | Pos | Pos | α-Enolase 1 | 6/8, 75 % | 6/8, 75 % |
| | | | Granulysin NKG5 | 4/7, 57 % | 4/7, 57 % |
| Immunologic ocular disease | Pos | Pos | Rhodopsin | 4/8, 50 % | 5/8, 63 % |
| | | | Glycosamino Glycan Xylose kinase | 5/10, 50 % | 7/10, 70 % |
| | | | α-Enolase 1 | 6/8, 75 % | 6/8, 75 % |
| | | | Retinal S-Ag | 6/12, 50 % | 8/12, 67 % |

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

disease: type 1 (M1) macrophage cells with a proinflammatory effect (expressing high levels of CD80–86, IL23, and MHC II) and type 2 $CD163^{+}$ (M2) cells displaying a Th2-like phenotype (widely represented in atherosclerotic lesions of the vascular intimal layer). The M2a macrophage subtype is activated by signaling molecules such as IL-4 and IL-13 with anti-inflammatory and pro-B cell growth and activation properties. They are also characterized by the expression of mannose receptors and the production of TGF-β, dectin 1, MBL, and IL-10 (among other secretory products), thus promoting immunoregulation, tissue remodeling, and fibrosis induction. Moreover, the dominance of the M1 over the M2 phenotype has been associated with progression of atherosclerotic disease [43].

In a randomized trial, *Saccharomyces boulardii* oral administration in patients with heart failure (NYHA I–II) ameliorated gastrointestinal symptoms (constipation, postprandial fullness, flatulence, and dyspepsia), lowering CRP, leukocyte count, and echocardiographic left atrial diameter compared to placebo. Increased ASCA levels were also reported in several studies on Behçet's disease in which a prevalence of 44 % was shown in patients with intestinal involvement, compared with 3 % in patients without gastrointestinal symptoms [44]. Alpha-enolase 1 (Fig. 4a, b) is known as an autoantigen in Behçet's disease with ocular involvement (uveitis). As reported in Table 5, the similarity of sequences suggests the presence of shared epitopes in different kinds of rheumatic conditions with immunologic ocular involvement. Cross-reactive epitopes on β2-glycoprotein-I (β2GPI) and *S. cerevisiae* (Fig. 5a, b; Table 6) were also found by our group in patients with APS. Anti-β2GPI antibodies were affinity purified from ASCA-positive APS patients and were proven to bind mannan in a dose-dependent manner [2]. Encompassing different autoimmune disorders associated with ASCA positivity [5, 45–53], we discovered other autoantigens that might cross-react with antibodies against mannan of *S. cerevisiae* (Table 7); the percentages of sequence identities (ID) and/or positive substitutions (PS) were pointed out for transglutaminase (ID and PS 60 %) in celiac disease, GAD65 (ID 35 %, PS 57 %) and zinc transporter 8 (ID 43 %, PS 57 %) for diabetes mellitus type 1, proteinase3 (ID 57 %, PS 86 %) and myeloperoxidase (ID 71 %, PS 86 %) for vasculitis, soluble liver/pancreas antigen (ID 40 %, PS 80 %) for autoimmune hepatitis, calprotectin or protein S100-A8 for Crohn's disease (ID 60 % PS 100 %), and thyroglobulin (ID 35 %, PS 52 %) and thyroid peroxidase (ID and PS 71 %) for autoimmune thyroid disease.

Performing our database research of shared epitopes, we found very interesting results with regard to systemic sclerosis (SSc) (Table 8); high percentages of sequence

**Table 6** Link between antiphospholipid syndrome-associated antigens and ASCAs

| Autoimmune disease | ASCA | | Antigens (*H. sapiens*) | Comparison to mannan; accession no. EDV13046.1 | |
|---|---|---|---|---|---|
| | IgG | IgA | | Identities | Positives |
| Antiphospholipid syndrome | Pos | Pos | β2-Glycoprotein-1 precursor | 7/18, 39 % | 11/18, 56 % |
| | | | Annexin A5 | 5/8, 63 % | 5/8, 63 % |
| | | | Anti-CL/β-2GPI Ig light chain variable region | 7/11, 64 % | 8/11, 73 % |

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

**Table 7** Link between AID-associated antigens and ASCAs

| Autoimmune disease | ASCA | | Antigens (H. sapiens) | Comparison to mannan; accession no. EDV13046.1 | |
|---|---|---|---|---|---|
| | IgG | IgA | | Identities | Positives |
| Celiac disease | Neg | Pos | Transglutaminase | 6/10, 60 % | 6/10, 60 % |
| Diabetes mellitus type 1 | Pos | Pos | GAD65 | 8/23, 35 % | 13/23, 57 % |
| | | | Zinc transporter 8 | 6/14, 43 % | 8/14, 57 % |
| Vasculitis | Pos | Pos | Soluble liver/ pancreas antigen | 4/10, 40 % | 8/10, 80 % |
| Crohn's disease | Pos | Pos | Calprotectin (protein S100-A8) | 3/5, 60 % | 5/5, 100 % |
| Autoimmune thyroid disease | Neg | Pos | Thyroglobulin | 8/23, 35 % | 12/23, 52 % |
| | | | Thyroid peroxidase | 5/7, 71 % | 5/7, 71 % |

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

**Table 8** Link between systemic sclerosis-associated antigens and ASCAs

| Autoimmune disease | ASCA | | Antigens (H. sapiens) | Comparison to mannan; accession no. EDV13046.1 | |
|---|---|---|---|---|---|
| | IgG | IgA | | Identities | Positives |
| Systemic sclerosis | Missing | | RNA polymerase III | 6/12, 50 % | 8/12, 67 % |
| | | | U3-snRNP fibrillarin | 6/8, 75 % | 6/8, 75 % |
| | | | U3-snRNP MPP10 | 6/8, 75 % | 6/8, 75 % |
| | | | hU3-55kDA | 5/7, 71 % | 6/7, 86 % |
| | | | Nucleophosmin B23 | 4/8, 50 % | 7/8, 88 % |
| | | | Major centromere autoantigen B | 4/7, 57 % | 4/7, 57 % |

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

identities and/or positive substitutions were pointed out for RNA polymerase III (ID 50 %, PS 67 %), U3-snRNP or fibrillarin (ID and PS 75 %), U3sn-RNP or matrix metalloproteinase-10 (ID and PS 75 %), hU3-55 kDa (ID 71 %, PS 86 %), nucleophosmin B23 or nucleolar phosphoprotein B23 or numatrin (ID 50 %, PS 88 %), and major centromere autoantigen B (ID and PS 57 %). Indeed, data suggest seeking out ASCAs in SSc (mean *E* value=3.77).

## Conclusion and Perspectives

This review provides the background and the evidence needed for further studies on the ability of ASCAs to bind autoantigens in several associated diseases, at least for the highest significant results (high identity and positivity with low *E* values). To the best of our knowledge, this is the first review focusing on the relationships between ASCAs and autoimmune diseases, including the molecular aspects. Since *S. cerevisiae* is characterized by the ability to elicit an adaptive immune response, even inducing autoreactive antibody production against mannan, we wonder about the potential limitations of administering vaccines containing *S. cerevisiae* plus adjuvants. It is important to consider new challenges and new vaccination issues in preventive medicine.

ASCAs may be present years before the diagnosis of some related autoimmune diseases as they have been retrospectively detected in the preserved blood samples of soldiers who became affected with CD years later. Even bearing in mind the fact that *S. cerevisiae* is a common baker's and brewer's yeast, we cannot suggest a preventive yeast-free diet, but perhaps, some clinical conditions might benefit from a reduction in yeast exposure.

The importance of improving and better defining the clinical use and reliability of ASCA tests in clinical practice for autoimmune diseases should certainly be underlined. ASCA positivity should be addressed in patients with different clinical courses who may deserve closer management of comorbidities, especially concerning increased cardiovascular risk.

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