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From ASCA breakthrough in Crohn's disease and *Candida albicans* research to thirty years of investigations about their meaning in human health

Boualem Sendid [a,b,*], Marjorie Cornu [a,b], Camille Cordier [a,b], Julie Bouckaert [c], Jean Frederic Colombel [d], Daniel Poulain [a,*]

[a] INSERM U1285, CNRS UMR 8576, Glycobiology in Fungal Pathogenesis and Clinical Applications, Université de Lille, F-59000 Lille, France
[b] Pôle de Biologie-Pathologie-Génétique, Institut de Microbiologie, Service de Parasitologie-Mycologie, CHU Lille, F-59000 Lille, France
[c] CNRS UMR 8576, Computational Molecular Systems Biology, Université de Lille, F-59000 Lille, France
[d] Department of Gastroenterology, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA

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ABSTRACT

Anti-*Saccharomyces cerevisiae* antibodies (ASCA) are human antibodies that can be detected using an enzyme-linked immunosorbent assay involving a mannose polymer (mannan) extracted from the cell wall of the yeast *S. cerevisiae*. The ASCA test was developed in 1993 with the aim of differentiating the serological response in two forms of inflammatory bowel disease (IBD), Crohn's disease and ulcerative colitis. The test, which is based on the detection of anti-oligomannosidic antibodies, has been extensively performed worldwide and there have been hundreds of publications on ASCA. The earlier studies concerned the initial diagnostic indications of ASCA and investigations then extended to many human diseases, generally in association with studies on intestinal microorganisms and the interaction of the micro-mycobiome with the immune system. The more information accumulates, the more the mystery of the meaning of ASCA deepens. Many fundamental questions remain unanswered. These questions concern the heterogeneity of ASCA, the mechanisms of their generation and persistence, the existence of self-antigens, and the relationship between ASCA and inflammation and autoimmunity. This review aims to discuss the gray areas concerning the origin of ASCA from an analysis of the literature. Structured around glycobiology and the mannosylated antigens of *S. cerevisiae* and *Candida albicans*, this review will address these questions and will try to clarify some lines of thought. The importance of the questions relating to the pathophysiological significance of ASCA goes far beyond IBD, even though these diseases remain the preferred models for their understanding.

1. Introduction

Among the biological tests that have contributed to the diagnosis of inflammatory bowel diseases (IBD), the enzyme-linked immunosorbent assay (ELISA) developed by us in 1993 [1] and published in 1996 [2], and named ASCA (anti-*Saccharomyces cerevisiae* antibodies) in 1999 [3], was a pioneer. For the past 30 years, it has remained a robust test as a marker of Crohn's disease (CD) in terms of prediction and prognosis.

Historically, this test was derived from pioneering observations made after immunofluorescence studies on different strains of *S. cerevisiae* by Mckenzie et al., and then by us [2,4]. Its transition to an automatable ELISA format, which is adaptable [2] to a large number of patients, has generated significant medical and commercial interest [5–7]. On a fundamental level, the demonstration of the existence of anti-yeast antibodies that are markers of IBD opened up investigations into the role of the mycobiota in human disease [8–11]. Our early studies incriminated the yeast *Candida albicans*, which is a major component of the mycobiota, capable of colonizing all segments of the human digestive tract, as well as a major opportunistic pathogen whose dissemination from the gut is a regular cause of fatal invasive fungal infections [12].

The current review was carried out for three main reasons: (i) ASCA have now been detected in a large number of human diseases; (ii) studies on the mycobiota have increased considerably and have been refined, resulting in them becoming more in line with traditional methods of microbiology; (iii) fundamental studies on the interactions between *C. albicans* and the digestive tract have reached an unparalleled level of scientific quality.

* Corresponding authors at: Faculté de Médecine, Pôle Recherche, 1 Place Verdun, 59045 Lille CEDEX, France.
E-mail addresses: boualem.sendid@univ-lille.fr (B. Sendid), daniel.poulain@univ-lille.fr (D. Poulain).

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In this review, we discuss the main findings of this vast amount of research, which raises the question of the origin of ASCA, the regulation of ASCA synthesis, and ultimately, the meaning of ASCA in human health.

2. ASCA test for Crohn's disease

2.1. Initial contribution to the differential diagnosis of IBD, to patient stratification, and prognosis

This area of research originated from a gastroenterologist community who was interested in ASCA as a biomarker of CD. Thirty years later, ASCA remain the strongest and most studied biomarker in this setting. At the beginning of this review on the meaning of ASCA, we first need to briefly address the clinico-epidemiological framework established about ASCA *i.e.*, the differential diagnosis of IBD, patient stratification, prognosis, and prediction of CD. We do not intend to be exhaustive as this has already been the subject of excellent reviews and meta-analyses [13,14].

ASCA first contributed to IBD management by differentiating CD from ulcerative colitis (UC). Assays for ASCA in CD patients show a prevalence ranging from 50 to 60% depending on the geographic and ethnic origin of the patients, in contrast to 0–6% in the general population [15,16]. The combined detection of ASCA and perinuclear-antineutrophil cytoplasmic antibodies (pANCA) differentiates CD from UC [3,17–19], with a sensitivity ranging from 30 to 64%, a specificity of >90%, and positive predictive value ranging from 77 to 96% [20]. This discrimination was shown to extend to indeterminate colitis [21]. Subsequently, additional fungal biomarkers including antilaminaribioside antibodies (ALCA) and antichitobioside antibodies (ACCA) were shown to be positive in 26% of ASCA-negative CD patients [20].

It was also discovered that ASCA positivity relates to early CD development and concerns ileal forms requiring surgery [3,22–24]. Similarly, single or multiple detection of antimicrobial antibody markers including ASCA, as well as the amplitude of the antibody response, were independently linked to a severe disease phenotype [14,25–30]. This was confirmed by Vasiliauskas et al. using multiple regression analyses, notably for fibro-stenosing and internal penetrating disease behaviors [24–26,31]. Other studies have reported that CD patients with serological positivity for ASCA more frequently present with an ileal or ileocolonic location [29], but these antibodies do not differentiate stricturing and non-stricturing forms [32]. In a meta-analysis, Ricciuto et al. reported that 5/8 studies showed a significant association between ASCA status and surgery. The pooled Odds ratios (OR) for the five studies was 2.31, while the pooled hazard ratio (HR) for four of these studies also showed a significantly increased risk of surgery (HR = 2.59) [33].

In agreement with the association between ASCA and the early development of CD, the sensitivity of ASCA is higher (50–86%) in pediatric patients with suspected IBD, with good specificity (85–95%), making ASCA more useful for the screening of CD in this patient subgroup [17,34–36]. Consistent with their profile in adults, ASCA have been shown to be independently associated with a complicated phenotype, ileal involvement, and the need for surgical resection [27].

With the advent of “biologics” it became obvious that as ASCA were associated with severe forms of CD, the detection of ASCA should initiate their early use. A prospective study in a newly-diagnosed, treatment-naive cohort showed that if ASCA were positive at baseline, CD patients had an almost 9-times higher odds of receiving early TNF blocker treatment compared to those who were ASCA negative, with a probability of 70% (OR = 8.8 [95%CI: 2.0–37.7]; $p < 0.01$) [37]. Another study reported more aggressive features in seropositive patients, such as more extensive involvement and moderate to severe disease [38]; interestingly, these severe ASCA-positive forms had comparatively lower relapse rates than patients with negative ASCA titers when anti-TNF biological therapy was introduced early.

In addition to their contribution to the clinical management of CD, ASCA have contributed to unravelling epidemiological clues about the disease. The first is familial aggregation. In our initial CD family study, ASCA were detected in 35/51 (69%) patients with CD and in 13/66 (20%) of healthy relatives vs. 1/63 controls ($p < 0.001$) [16]. The presence of ASCA in healthy relatives was observed in 12/20 families and was not restricted to a few particular multiplex families [15]. The prevalence of ASCA in relatives did not depend on the ASCA status of affected members. These findings were confirmed by Seibold et al., who found ASCA in 48 (25%) of 193 healthy first-degree relatives [39] as well as in a large series of Belgian families having one or more than two affected members [40]. Moreover, a study focusing on 98 twin pairs with IBD showed a high degree of concordance between ASCA titers in monozygotic twin pairs with CD suggesting that the level of increase is genetically determined [41].

In parallel, a large number of studies have revealed a unique characteristic of ASCA positivity in CD, namely their life-long stability. ASCA-positive levels appear to be stable in CD patients irrespective of medical or surgical treatment [3,26,40,42,43]. This characteristic is discussed further below.

In the line with these characteristics, another significant finding was that ASCA pre-existed the development of CD. This fact was established after investigations on serum repositories from conscripts archived before a diagnosis of IBD. They demonstrated that ASCA pre-exist CD for as long as 3–5 years before clinical diagnosis of the disease [44,45].

In conclusion, although the ASCA test is not recognized as a diagnostic test for CD by some gastroenterologists, who point to its non-optimal sensitivity, its contribution to the early diagnosis of CD should not be overlooked insofar as a recent meta-analysis showed that all complications arise from the late diagnosis of CD [46].

3. The basic question of the ASCA epitope(s)

In contrast to the thousands of papers concerning the meaning of ASCA, little attention has been paid to the nature of the epitope(s) recognized by these antibodies, a basic question which, if unsolved, precludes any rational interpretation.

3.1. Preliminary identification of the major ASCA epitope

Following the early development of an ELISA test to detect human antibodies directed against *S. cerevisiae* mannan [2] (later designated the ASCA test [3]), we identified a tetramannoside composed of α-1,2 linked mannose with an α-1,3 mannose at the non-reducing end among the complex *S. cerevisiae* mannan repertoire as the major epitope supporting the human response during CD (see Fig. 1). This identification was confirmed unambiguously by another independent study ascribing the antigenic activity of the original high molecular weight mannan to terminal Man α-1,3 Man α-1,2 [47]. Subsequent studies using synthetic analogues of the tri- and tetramannoside epitopes showed that such constructions were able to detect antibodies in patients with CD [48,49] and conversely to elicit animal antibodies reacting with the ASCA test [50].

3.2. The question is much more complex

At the start of the investigations on anti-yeast antibodies in CD [51], the methods used consisted of the detection of antibodies against whole yeast cells by immunofluorescence, agglutination, or after direct coating on microtiter plates. In a remarkable pioneering study, McKenzie et al. showed that all *S. cerevisiae* and *C. albicans* strains tested varied in their ability to bind patients' antibodies and confirmed this antigenic heterogeneity by cross-absorption experiments [4]. Later molecular investigations on yeast mannan antigens mainly concerned *S. cerevisiae* strains whose cells were selected for being the most reactive against patients' sera (i.e., Su1 strain for our study [2] and Sc500 strain for the study of Barnes et al. [47] in which the ASCA major epitope was over-

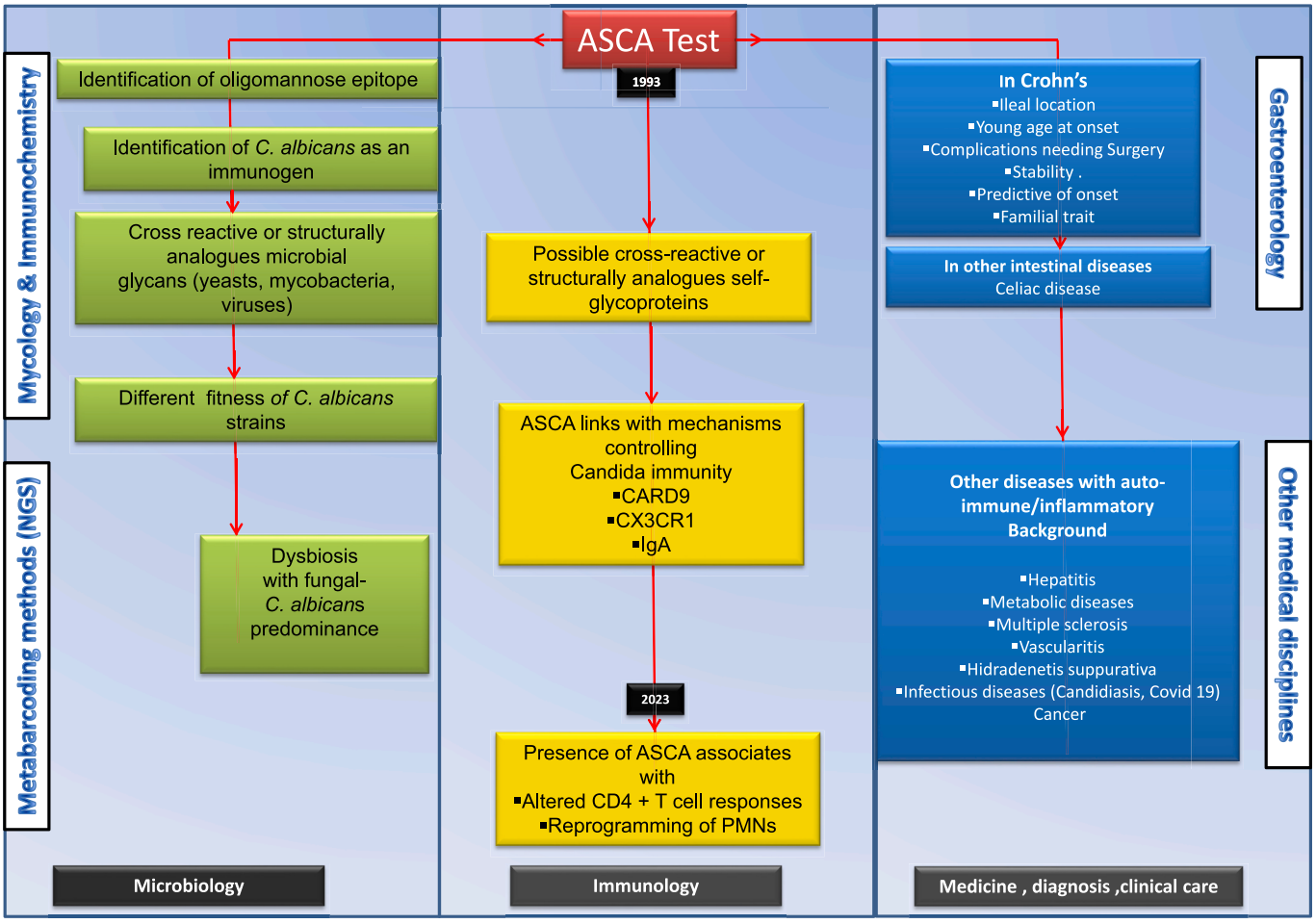

Fig. 1. Graphical abstract: inter-related traits of ASCA discovered over time.
Representation of the evolution of knowledge in the 3 fields involved in scientific and medical research concerning ASCA (Vertical panels). The two major evolution of medical concepts and microbiological methods are shown laterally. In each of these panels, rectangles represent the successive achievements over 30 years with immunology being at the interface.

represented).

Other investigations on *S. cerevisiae* antigens revealed reactivity with the carbohydrate moiety of a 200 kDa mannoprotein of *S. cerevisiae*, but the existence of the major mannan ASCA epitope on this antigen was not determined. This would have made sense bearing in mind the ability of yeasts to express a given epitope on the carbohydrate moieties of different molecules, either glycoproteins or even glycolipids [52–54]. A monoclonal antibody against *S. cerevisiae* GP 200 carbohydrate moiety was raised by the same group [55,56], but it is unknown if it reacts with mannan.

When synthetic ASCA epitopes were used to detect antibodies in a large multicenter study, including 1365 sera, the specificity for CD was similar to the ASCA test [49], although, as could be expected for a non-microbial native product, the sensitivity was lower (38% vs. 55%). Surprisingly, in spite of this lower sensitivity, the synthetic epitope allowed the detection of a substantial number of CD patients (24%), mostly with colonic involvement, who were negative for ASCA and/or any associated serological markers. This agrees with the reactivity of so-called AMCA (anti-mannoside carbohydrate antibodies), a dimannoside corresponding to the non-reducing terminal end of the ASCA epitope which is observed in some CD-negative ASCA patients [57]. Regarding native "natural" yeast antigens, a similar conclusion about complementation was reached when the ASCA responses of CD patients from North Africa were investigated using an in-home test involving mannan from a strain designated W303 and our original ASCA test with Su1 mannan. In this case, the combination of tests resulted in a slight decrease in specificity to 80%, but an impressive sensitivity of 80% for differentiating CD from UC [58].

From a fundamental point of view, these studies demonstrate the considerable heterogeneity of the ASCA response in humans, revealed by comparison of various commercially available tests [59,60] but which has never been explored about complementation for diagnostic purposes or, importantly enough, addressed to understand the meaning of the ASCA response. Thus, although the oligomannose sequences composed of α-1,3 Man at the non-reducing end of α-1,2 Man chains is undoubtedly highly reactive with sera from CD patients, the human anti-mannose ASCA response comprises a wide variety of more or less structurally related motifs that remain to be elucidated, as well as their clinical significance.

4. *C. albicans* is undoubtedly an ASCA immunogen

4.1. Experimental and clinical evidence

With regard to the large and increasing number of papers and reviews on the mycobiota, which suggest a role for *C. albicans* in CD [61], very few papers have addressed this question from a molecular point of view.

A number of concordant scientific facts have been established. Experimentally, *C. albicans* was shown to generate ASCA when it was

used to infect rabbits by the intravenous route [62], or when it thrives in the guts of mice with dextran sulphate sodium (DSS)-induced inflammation [63]. In humans suffering from systemic candidiasis caused by *C. albicans*, as demonstrated by the isolation of this species from blood, a strong ASCA response can be observed [64] which resolves after curative treatment, in contrast to the ASCA stability in CD [65]. Conversely, probing of the pathogenic phase of *C. albicans* in tissue sections from biopsies of patients with systemic *C. albicans* infection with ASCA immunopurified from CD patients showed strong reactivity [62]. Thus, there is no doubt that *C. albicans* can be the origin of ASCA [66,67] even though this does not preclude the existence of other microbial immunogens or auto-antigens (see below).

4.2. Possible mechanisms of ASCA generation by C. albicans

4.2.1. On the Candida side

4.2.1.1. S. cerevisiae and C. albicans mannans as structural models (Fig. 1). Understanding the mannosylation process, and thus the building and alteration of sequences of mannose residues acting as epitopes -and as pathogen-associated molecular patterns (PAMPS)- by *C. albicans* requires us to refer to the large number of basic structural glycobiology studies conducted over several decades complemented by the identification of genes responsible for the synthesis of mannosyl transferases (Mnts). These enzymes, located in the Golgi apparatus, establish specific linkages (either α-1,6, α-1,2, or α-1,3) with strong specificities for the acceptor molecule (the pre-existing mannoside sequence). This leads to a highly complex polymer which is more or less species specific, the archetype of which is called mannan (or more exactly, phosphopetidomannan (PPM)).

PPM is a water-soluble polysaccharide of high molecular weight bound non-covalently to the cell wall surface of yeasts. The activity of Mnts was first characterized in the PPM of *S. cerevisiae*, a yeast cell model providing many clues to our understanding of glycosylation in eukaryotic cells and the first fungal organism to be sequenced. As shown in Fig. 2, the mannose residues are branched on a protein chain, either by N-glycosidic linkages on an asparagine [68], or by O-glycosidic linkages on a serine or threonine amino acid [69]. Due to the need for large quantities of material to define the structure of the numerous mannoglycoconjugates synthesized by *C. albicans* by nuclear magnetic resonance (NMR) of native molecules or sequences released after sequential chemical or enzymic depolymerization, most studies on the variability of mannosylation have concerned PPM. Fig. 2 was derived from comprehensive reviews compiling dozens of structural papers on PPM taking in account strains and environmental variations still representing hallmarks in the domain [70,71].

4.2.1.2. Necessary extension of the model from mannan to mannoproteins (Fig. 3). Restriction of structural analysis to PPM to analyze the activity of Mnts left a completely unexplored field of research which concerned the variability of glycosylation of the wide variety of cytoplasmic and cell wall mannoproteins synthesized by *C. albicans*. Fig. 3 shows representative examples of the recognition of *C. albicans* mannoproteins in an attempt to answer this question with detailed explanations gathered from previous publications.

Despite the unquestionable issue of the relevance of anti-protein antibodies in terms of the diagnosis of host invasion by *C. albicans*, the question of their mannosylation has never been addressed, mostly because recombinant proteins generally produced in *Escherichia coli* and thus, not glycosylated, are used for diagnostic purposes. Among these are proteins that have been identified over time as *C. albicans* virulence factors (i.e., the Als family, Hwp1…). The fact that a given variable mannan epitope may be shared by these proteins depending on the growth conditions, including during the pathogenic phase, deserves our attention. The legend to Fig. 3 provides an illustration of this statement. Thus, mannosylation affects both the function of the molecule and its recognition by the immune system, and this revealed the existence of important gaps in our knowledge. In other words, and as an example, it is highly likely that the function and immune reactivity of Als3 [72,73] will depend on the environmental signals perceived by *C. albicans* cells, including under pathogenic conditions. It is anticipated that variations in Mnt activities in relation to the growth conditions (pH, temperature, osmolarity…) inhibit some β-mannosyltransferases and thus unmask α-1,2/α-1,3 linked Mans [74–76] affecting the total machinery of mannoprotein mannosylation. This has never been considered or investigated. A recent paper demonstrated that a significant part of the mannosylation regulatory process was dependent on a complex of mitochondrial proteins whose resultant form could dramatically alter the host response. [77]. Thus, we can say in conclusion that: (i) the majority of *C. albicans* proteins are mannosylated; (ii) the same epitope

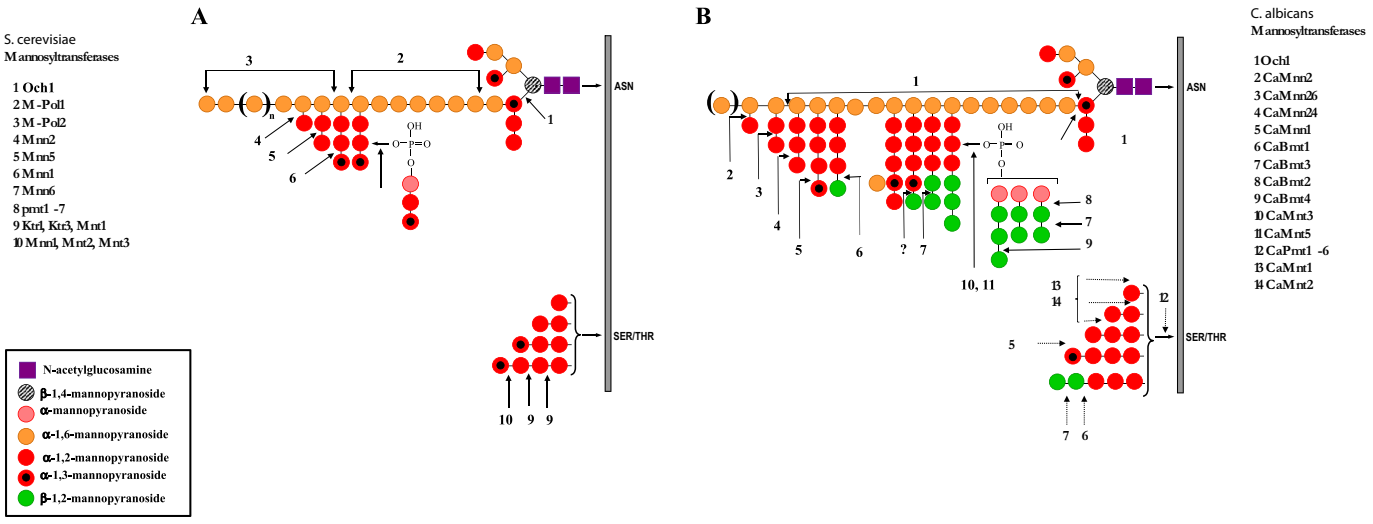

Fig. 2. Schematic representation of *S. cerevisiae* (A) and *C. albicans* (B) mannans.
Mannose residues are represented by different colors according to linkage type and anomery. The arrows indicate the main reported Golgi mannosyltransferases (Mnts) involved in polymerization. The specificities and basic information regarding these Mnts are shown in the *Candida* genome database (http://www.candidagenome.org/). The specificity of the Mnts depends on the length of the oligomannose sequence, the type of linkage, and the anomery of the mannose at the non-reducing end.

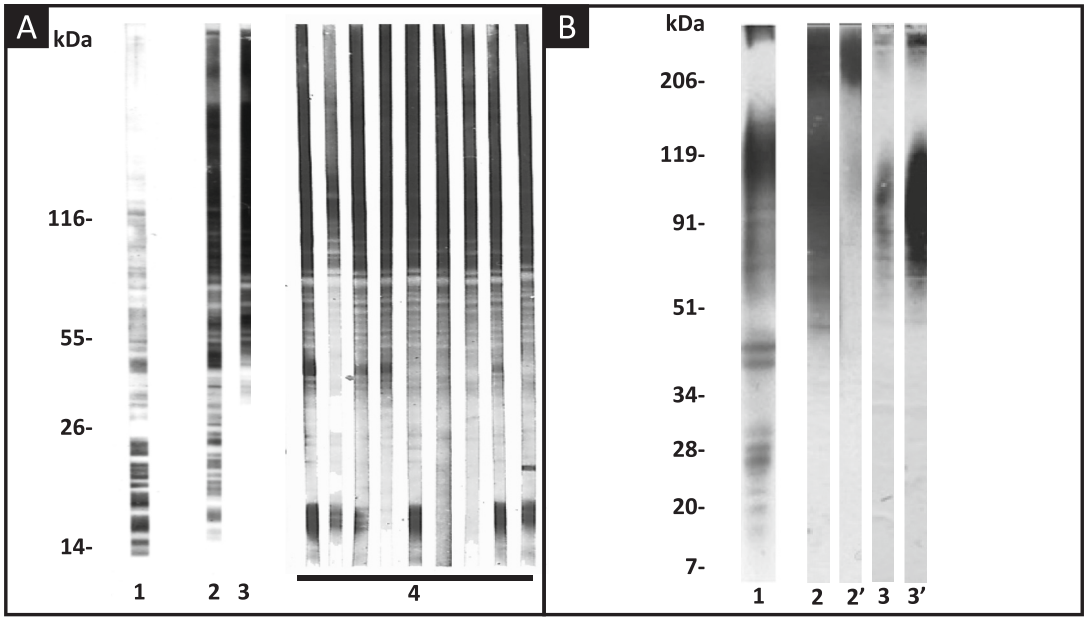

Fig. 3. Western blots of *C. albicans* whole cell extracts illustrating the question of mannosyl epitope expression on various mannoproteins and their variability depending on the growth conditions. (Figures collated from previous publications, with permissions.)
Nature of the staining. Panel A: 1 - protein staining, 2 Con A staining, 3 - mAb anti-α-Man staining (mAB EBCA1), 4 - patients' sera staining. Panel B: 1 - Staining with immunopurified ASCA, 2,3 - staining with an anti-β-Man mAb (mAb 5B2) and GNL (*Galanthus nivalis* lectin*) at neutral pH., 2′,3′ - probed with an anti-β-Man mAb and GNL at acidic pH. * GNL binds to terminal α-1,3 Mans like ASCA does.
Description of the profiles. A1. Protein staining of polyacrylamide gels of whole *C. albicans* cell extracts revealed numerous well-defined bands corresponding to proteins. A2. When these molecules are transferred onto nitrocellulose for Western blotting and probed with Con A reacting with α-mannosides, larger bands appear that correspond to the mannan moieties coupled to proteins. The width of these bands increases as a function of molecular weight leading to polydispersed material (smears) accounting for heterogeneity of the mannose moiety. A3 Mapping of an α-linked oligmannose epitope recognized by a single monoclonal antibody (EBCA1) clearly shows that a single epitope may be shared by many different mannoproteins. A4. Probing of the same blot with sera from patients infected with *C. albicans* shows that a large number of mannoproteins are targets of the human antibody response.
B1 Lane 1. Probing with ASCA generated by rabbit immunization and subsequently immunopurified on *S. cerevisiae* mannan shows that the ASCA epitope is shared by many *C. albicans* mannoproteins. B. Lanes 2–3' Probing of extracts from *C. albicans* grown at neutral and acidic pH clearly shows a reduction in β-Man expression (2 to 3) by lowering the pH and a concomitant increase in the GNL signal (2′ to 3′). This demonstrate that the balance between α and β mannose expression occurring at the mannan level depending on the growth conditions (comments of Fig. 2) also concerns the mannose moiety of mannoproteins.

can be shared by several mannoproteins; (iii) the expression of the epitope can be regulated according to the growth conditions of *C. albicans*; and (iv) the ASCA epitope can be unmasked according to an expression balanced with β-mannosides [78].

Regarding the similarities between *S. cerevisiae* and *C. albicans* mannans, and the ability of the latter species to express ASCA epitopes, it is interesting to refer to the pioneering work of McKenzie et al., who showed that pre-adsorption with *C. albicans* serotype B strains removed anti-*S. cerevisiae* antibodies from CD patients' sera in contrast to serotype A strains [4]. It is worth noting that serotype A specificity is conferred by the expression of β-Mans at the non-reducing end of α-Man acid-stable linked chains [71].

Thus, it seems that combining observations from antibody analysis, structural chemistry of mannans and mannosides, and yeast mannose biosynthetic pathways reinforces our understanding of why *C. albicans* could be at the origin of ASCA [65]. The considerable bulk of knowledge gathered on *Candida* and the mycobiota obtained over the last decade led to consider this hypothesis seriously [79].

To conclude this section about possible *C. albicans* involvement in CD, a striking "coincidence" is noticed when cross-referencing research on *C. albicans* and CD. One resides in the unexpected observation by Marr et al., who reported >20 years ago that prevention of systemic *C. albicans* infection with an antifungal (fluconazole) during an immunosuppressive regimen for hematopoietic stem cell transplantation resulted in a unexpected decrease in graft-versus-host disease (GvHD) [80]. Of note, hematopoietic stem cell transplantation is a condition where patients may develop de novo IBD or an IBD flare [81]. As these patients are at high risk for invasive candidiasis, fluconazole, an antifungal developed to prevent *C. albicans* growth, might also have a preventive effect on this secondary cause of CD because of its activity on *C. albicans* [82].

4.2.2. On the host side

As far as *C. albicans* is concerned, what are the mechanisms of host (human) innate and adaptive immunity?

The question of the unique relationships between *C. albicans* and inflammation was addressed in a review, but no mention was made about which antigen(s) could be relevant or determinant in this process [9]. The discovery of CARD-9 in the genome of patients with the rare condition of chronic mucocutaneous candidiasis (CMC) [83–85] represented a hallmark in the analysis of genetic susceptibility to *C. albicans*. More recently, a single study reported the influence of the same mutation, CARD-9, together with the determinant role of CX3-CR1 on the antibody response in CD, including ASCA generation [86,87]. However, although these papers are important from the host side, they did not take into account the antigenic complexity of *C. albicans* as an indissociable partner. All molecules from the complex and variable antigenic mosaic of *C. albicans* are not equally important, as demonstrated by the strong antibody response reported in patients with CMC using older less sensitive methods such as gel precipitation, revealing multiple precipitin lines. Thus, much remains to be discovered at the molecular level about the complex mechanisms of regulation in *C. albicans* and the host response. In other words, it is difficult to anticipate that the myriad of variable Candida epitopes could be equally affected by the host regulatory response. Considerable progress has recently been made in our understanding of how *C. albicans* is sensed by innate immunity receptors

and how this sensing directs the nature of the immune response upstream towards an inflammatory or anti-inflammatory process. Sophisticated mechanisms involving host membrane, cellular, or soluble receptors were identified to respond to all major *C. albicans* cell wall components (i.e., mannans, glucans and chitin). These mechanisms and their consequences have been summarized in several comprehensive reviews [88–91]. While some therapeutic clues would hopefully be derived from this research, current investigations on adaptive immunity shows that the mechanisms involving host receptors to sense the external and symbiotic microbial communities are probably not sufficiently elaborated to lead to protection against *C. albicans*, which finds its infective niche during host immunosuppression. Thus, research on Candida faces the challenge of identifying determinant targets for adaptive immunity in a complex variable interplay, which depends on both the host's genetic background [92] and yeast commensal/infecting species/strains [93].

5. Why an ASCA auto-antigen should be considered

An important characteristic of ASCA in CD is their stability over time. Once ASCA levels have increased, sometimes long before disease onset [44,45], they remain remarkably stable during the lifetime of a patient, independently of acute or remission phase of CD [94] and medical or surgical treatments [30]. Considering a microbial hypothesis alone for ASCA generation (*C. albicans* or any other potential immunogenic microbe) does not fit with fluctuations in the microbiota classically observed in long-term studies. Regarding the half-life of immunoglobulins, a decrease in a given microbial immunogen would result in a decrease in ASCA. In a recent unpowered but informative study about the effect of antifungal treatment on the evolution of biological parameters of *C. albicans* pathogenic development and CD activity, a decrease in these latter parameters was observed over a 6-month period. In contrast, despite a decrease in *C. albicans* colonization, ASCA remained stable [82].

Thus, it would make sense to consider that once the antibody response has been triggered by exogenous microbial antigens analogous self-antigen motifs, against which a response is normally down-regulated, escape this control and maintain the stability of the ASCA response. The repertoire of oligomannose motifs express on human glycoproteins or glycolipids is extremely vast and the possibility that the ASCA epitope is expressed is not unrealistic. Furthermore, the Mnt responsible for the transfer of α-1,3 mannose, reported as preponderant in the ASCA epitope, also exists in humans. To date, several human molecules likely to support the ASCA response may be suggested from a literature analysis.

5.1. Why should glycoprotein-2 (GP2) be an ASCA auto-antigen?

The first human antigen against which an antibody response was reported to present a correlation with ASCA during CD is GP2 (zymogen granule membrane) [95]. Antibodies against both GP2 and *S. cerevisiae* mannan are associated with disease severity [96,97]. However, a dissociation between ASCA and anti-GP2 antibody responses was observed in Behcet's disease compared to CD [98]. Kurashima et al. [99] showed that GP2 was a line of defense against adhesive and invasive commensal bacteria during intestinal inflammation. GP2 expressed in Brunner glands was recently described as a putative auto-immune target in CD and celiac disease (CeD) [100]. The presence of ASCA in CeD will be discussed later.

5.2. Why should CEACAM-6 be an ASCA auto-antigen?

Among the microbes that have been identified as involved in CD pathophysiology is an *E. coli* pathotype designated adherent-invasive *E. coli* (AIEC), expressing a mannose binding adhesin [101,102] at the tip of its pili. Oligomannose glycans exposed on early apoptotic cells were identified as the preferred binding targets of AIEC, and apoptotic cells were identified as potential entry points for bacteria into the epithelial cell layer, after which the bacteria propagate laterally into the epithelial intercellular spaces [103]. The AIEC pili bind to terminal mannose of host glycoproteins, including carcinoembryonic antigen-related cell adhesion molecule 6 (CEACAM6 or CD66c) [102], GP2 (see above) [104], Lamp-2 [105], and TLR4 [106]. Each of these processes is deleterious to the host by promoting bacterial invasion via M cells, or the induction of proinflammatory cytokines TNFα and IFNγ. The *CEACAM6* gene is overexpressed in most carcinomas, including those of the gastrointestinal, respiratory, and genitourinary tracts [107]. Increased serum levels of CEACAM6 serve as prognostic indicators of chronic inflammation in CD patients, given that no CEACAM6 production and mannosylation are observed in the healthy ileal mucosa [108]. In >35% of CD patients with ileal involvement, the abundance of mannosidic structures at the ileal mucosa is elevated due to overexpression of *ceacam6* by ileal epithelial cells, which favors AIEC colonization. Oligomannosylation was demonstrated at two distinct sites of CEACAM6 [103]. Strategies to saturate the carbohydrate recognition domain of FimH were developed in an attempt to prevent AIEC adhesion. A recent paper reported that TAK-018, a specific FimH blocker, successfully inhibited bacterial adhesion, preserved mucosal integrity, and prevented inflammation [109]. Some experiments involved *S. cerevisiae* strains or cell walls [110,111], these were also efficient for FimH adhesion blockade in experimental models [112]. As for the molecular receptors for FimH, CEACAM6 and GP2 receptors may be mimicked functionally by *S. cerevisiae* and its mannan and we can question whether the host molecules CEACAM6 and GP2 are able to support the ASCA response.

Direct binding of *C. albicans* to CEACAM6 has also be demonstrated [113]. A further study from the same group recently demonstrated that ligation of CEACAM6 prevented *C. albicans* binding to human neutrophils and induced an altered response of these cells [114]. In these studies, where binding was prevented by anti-CEACAM6 antibodies, it is reasonable to speculate that anti-*C. albicans* antibodies, including ASCA directed against the invasive form *in vivo,* could also prevent ligation.

5.3. The paucimannose track

Regarding the existence of α-1,3 linkages in human molecules and human immunological cross-reactivity with *S. cerevisiae*, it is interesting to note that the human gene encoding the enzyme responsible for α-1,3 Man transfer was discovered after research on *S. cerevisiae*. The *S. cerevisiae* sexual cycle with haploid mating phases has been an important model for conventional genetics by screening for mutants. This particularly concerned the early stages of protein N-glycosylation in the Golgi apparatus, which has been shown to be conserved from fungi to mammals. Glycans with short mannosidic chains corresponding to early stages of human glycoprotein synthesis are expressed in the cellular cytoplasmic compartments (endoplasmic reticulum and Golgi) before being processed in the latter compartment by mannosidases and a wide range of glycosyltransferases, leading to the highly complex repertoire of glycans found in human cells and tissues [115]. In contrast, paucimannosidic glycans, restricted to the core structure of N-glycans, are rare but may be upregulated in pathogenic conditions. It was elegantly documented that during pathogen-based activation polymorphonuclear neutrophils (PMNs) produced bioactive paucimannose-carrying proteins in their azurophilic granules [116]. The atypical glycosylation of one of these proteins, myeloperoxidase (MPO) isolated from human blood neutrophils, was fully characterized in a crystal structure [117]. It has been suggested that paucimannosylation might contribute to its presentation as a self-antigen by antigen-presenting cells and neutrophil-mediated immunity [118]. Interestingly, regarding the ASCA epitope, basic and clinical studies on paucimannose detection in humans have involved a unique monoclonal antibody, designed as mannitou IgM; binding of this monoclonal antibody was

shown to require a non-substituted α-1,3-linked mannose branch [119], a configuration defined as of high importance for ASCA binding. Considering these data together suggest that PMNs, known to be important cells for auto-antibody detection in UC with ANCA (Proteinase 3 P-R3- and MPO), could also contain some hidden auto-antigens relevant for CD. Much remains to be revealed regarding the complexity of the variation in human glycosylation patterns during health and disease [120,121]. The IgG Fc glycosylation pattern associated with the shift from pre- to inflammatory immune conditions [122] is probably worthwhile exploring for ASCA. Ultimately, tissue destruction by inflammation massively exposes normally non-accessible early stages of human glycosylation to an immune response, as well to cancer [123,124] (see below).

6. Other microbial candidates as ASCA immunogens

6.1. Yeasts

6.1.1. Saccharomyces cerevisiae complex

It is logical to start this section with the organism that remains the best producer of antigens to diagnose CD (i.e., *S. cerevisiae*). These antigens have been involved in millions of diagnostic tests over the past 30 years, made by many manufacturers across the world. Our group was at the origin of the acronym ASCA (anti-*S. cerevisiae* antibodies) created for the original ELISA test since it nicely complemented the differential diagnosis of IBD with a similar test ANCA [3]. In retrospect, this was not a good idea since it shed suspicion on a largely innocuous yeast used by humans for millennia to produce bread, wine, and beer. This unfortunate denomination for a good diagnostic test (the ASCA-ANCA paper has been cited 400 times) led to several reductionist studies aimed at proving that *S. cerevisiae* was a dreadful pathogen, without considering experience from medical mycologists. In daily practice, *S. cerevisiae* was very rarely isolated from stools and mouth swabs from the thousands of hospital patients examined each year in a university hospital, including IBD patients (records from the Mycology Department of Lille University Hospital, France). Such observations led several generations of medical mycologists to consider that *S. cerevisiae* was not adapted to thrive in the human gut, or to be an endogenous threat to human health. In contrast to conventional microbiological methods of isolation and identification, the refinement of next generation sequencing (NGS) methods over the years unambiguously confirmed that the mycobiota of IBD patients was characterized by a decreasing presence of *S. cerevisiae* DNA on the one hand and a preponderance of *C. albicans* DNA on the other [125–128].

With regard to *S. cerevisiae*, as discussed in the chapter on antigenic variability, this Linnean binominal denomination corresponds to an extremely vast repertoire of strains with specific biological [129] properties selected for food production including organoleptic properties (i.e., those selected over centuries to produce great vintage wines). Numerous species are now considered to be co-specific, such as *Saccharomyces uvarum* used for beer production and the first reported ASCA antigen [2]. However, it is indisputable that in CD patients with a triggered ASCA response, dietary *S. cerevisiae* strains will interfere with this immune response depending on their oligomannoside repertoire. This huge variablity is probably to consider since *S. cerevisiae* co-specific species *Saccharomyces boulardii*, may display both anti- *C.albicans* and anti-inflammatory properties [130,131] and a *S. cerevisiae* strain designated CNCM I-3856 prevents AIEC induced colitis in a transgenic mouse model mimicking CD [110].

6.1.2. Candida species

In addition to *C. albicans*, which has a role in ASCA generation and CD, as discussed previously, the involvement of other species of the genus *Candida* has also been investigated. Unsurprisingly, this has concerned the species most commonly isolated in clinical mycology laboratories after *C. albicans* (i.e., *Candida tropicalis* and *Candida glabrata*). These three species share a pathogenic potential that allows them to invade the mucosae, resulting in *Candida* vulvovaginitis and 90% of systemic *Candida* infections spreading from the gut.

The involvement of *C. tropicalis* (which has a mannan oligomannosidic repertoire similar to that of *C. albicans*) was suggested from the initial studies on the mycobiota associated with CD [132], as well as its correlation with ASCA levels. With the evolution of NGS methods, further studies failed to demonstrate such a preponderance of *C. tropicalis*, contradicting the results obtained by conventional mycological methods on the same patients [133]. Similarly, a study published 1 year later claimed as "the first demonstration of the existence of an altered fungal microbiota in CD patients" did not isolate *C. albicans*, but showed a preponderance of *C. glabrata* [134]. No relationship was established with ASCA levels, which probably makes sense from an immunochemical point of view since *C. glabrata* constitutively expresses the major ASCA epitope, Man α-1,3, when grown *in vitro* and identified as antigen 34 in the yeast serological classification [71]. In contrast to *C. tropicalis*, a mainly saprophytic yeast common in fruit juices, in transit, or surviving in the gut, *C. glabrata* is a truly endosaprophytic species adapted to colonize the human gut. Experimental models have clearly demonstrated its pathogenic potential in an inflammatory setting as a player able to modify bacterial communities [135,136].

Attention has recently focused on *Candida famata*, a species rarely isolated in the clinical mycology laboratory, the anamorph (asexual stage) of the species *Debaryomyces hansenii.* An elegant experimental and clinical study demonstrated that the abundance of this species in wounds and inflamed tissues was linked to its ability to dysregulate mucosal healing [137] through a specific mechanism involving myeloid cells. Interestingly, *C. famata* is among the yeast species expressing the presumptive ASCA epitope [71].

To conclude this section on yeasts, it is clear that a survey of the extensive literature on the analysis of the microbiota in IBD shows that initial studies failed to demonstrate the importance of yeast species considered by medical mycologists to be the most pathogenic and generated doubt on their possible involvement. The progressive refinement of methods and analyses clarified these points in favor of conventional mycology conclusions. A remarkable short review published recently provided very clear explanations for this evolution and explained that extreme caution and scientific humility in sampling, at the bench, or in front of the computer is of crucial importance regarding the power of methods to draw conclusions [138].

Second, it is highly probable that due to their biological richness and the models represented, namely for mannosylation, *Saccharomyces* and *Candida* yeasts have not yet reached their full potential for research on the mechanisms of ASCA generation.

6.1.3. Malassezia species

The recent incrimination of species of this complex in CD [139,140] is representative of the discrepancies between the results of metagenomic investigations based on DNA sequencing and knowledge gained on these species by mycologists 100 years ago in human and animal samples, studied by direct microscopy and culture on various specific media [141].

The classification of yeasts belonging to the *Malassezia* complex has been clarified considerably by genetic analysis. This complex is composed of 18 species including the species previously named *Pityrosporum* [142,143]. These yeasts are commensals of the human skin, thriving in the lipophilic environment of sebaceous secretions. They were also identified as opportunistic pathogens, capable of changing morphology to be associated with different clinical skin conditions, such as dandruff, seborrheic dermatitis, atopic dermatitis (where their involvement is suspected), or *Pityriasis versicolor* where the yeast invades the tissues [144,145]. With regard to the inflammatory states of atopic dermatitis or dandruff, it is not yet known whether the proliferation of *Malassezia* is the cause or the consequence. However, antifungal treatment does lead to clinical improvement. The only possible involvements reported outside the skin sphere were sepsis observed following the

unfortunate combination of two favoring circumstances, namely deep immunosuppression of premature neonates and skin contamination of lipid infusions [146].

Reports of its presence in the digestive tract as a commensal using conventional methods are scarce or non-existent. Examination of stools samples by direct microscopy does not indicate their presence. Limited data exist on the isolation of *Malassezia* from stool cultures. This yeast cannot be isolated in culture using conventional media and requires the use of lipid-enriched media [147].

Mycobiome characterization by NGS methods has highlighted the presence of *Malassezia* spp. in the stools of CD patients [126]. *Malassezia restricta* was identified in CD patients carrying a polymorphism in the CARD9 gene, involved in antifungal defense and shown experimentally to exacerbate colitis [140]. Pediatric patients with CeD were found to exhibit a 2-fold increase in *Malassezia* spp. in their intestinal mycobiome compared to a control group [148]. Of note, the discordant results in metagenomic detection of *Malassezia* spp. relates to different ribosomal RNA regions selected for high-throughput sequencing [149]. The Human Microbiome Project cohort of healthy patients revealed an unexpectedly high prevalence of *Malassezia* spp. and the presence of *M. restricta* Operational Taxonomic Unit (OTU) in up to 88.3% of samples [150]. However, these findings have not been challenged by culturomic [151], to assess yeast viability. To achieve this goal, Blachowicz et al. [152] proposed the pre-treatment of samples with propidium monoazide, intercalating the DNA of dead cells, to restrict the viable yeast metagenome. The discrepancies between metagenomic and culturomic need to be addressed to determine whether the presence of *Malassezia* spp. reflects contamination of the digestive tract by the skin microbiota where proliferation of *Malassezia* is exacerbated by systemic inflammatory disorders [145]. One mycobiome study of oral samples highlighted the high prevalence and abundance of the *Malassezia* genus among the salivary microbiota [153], in contrast to the low prevalence of *Malassezia* species in stool samples.

Regarding the immunogenicity of *Malassezia* mannan, cross-reactivity with *C. albicans* mannan has been clearly demonstrated regarding IgE, the isotype predominant in patients with atopic dermatitis [154]. Specifically, *S. cerevisiae* gp 200, to which patients with CD exhibit high reactivity [55], supports cross-antibody reactivity during atopic dermatitis [155], an inflammatory disorder in which *Malassezia* is suspected to play a role.

6.2. Mycobacteria

Among the thousands of bacterial species present in humans, many of which have been explored for their relationship with CD, the only species that has so far shown cross-reactivity with ASCA is *Mycobacterium avium subspecies paratuberculosis* [156] the etiologic agent of a severe gastroenteritis in ruminants known as Johne's disease. Two epitopes incriminate *Mycobacteria* as elicitors of antibodies in humans with CD, a terminal α-1.3 mannose [157] and a peptide sequence [158]. At the genetic level, an impressive study has demonstrated considerable overlap between susceptibility loci for IBD and mycobacterial infection [159].

6.3. Viruses

The gut virome consists of eukaryotic viruses, bacteriophages, archeal viruses, and plant viruses originating from food and environmental exposure. With roughly 108–1010 virus-like particles per gram of intestinal content, viruses make up a hefty sum of the gut microbiome [160,161]. Bacteriophages have been described as modifying the bacterial environment in a way that supports IBD development [162], whereas some strategies have been proposed to use them to target bacteria identified as playing a detrimental role [163].

Eukaryotic virome dysbiosis has been associated with IBD pathogenesis, because eukaryotic-targeting viruses integrated into the human genome may play a role in shaping mucosal immunity [161,164]. Regarding glycosylation, which is a central question in our understanding of ASCA, eukaryotic viruses take advantage of the host cells' endoplasmic reticulum and Golgi apparatus to produce complex glycans such as high-mannose and complex elongated N-glycan structures. From an evolutionary point of view, the capacity of viruses to replicate and modify their own N-glycosylation sites brings advantages for host colonization through glycan-mediated molecular mimicry. This glycan-dependent viral adaptation masks viral proteins from host neutralizing antibodies; human immunodeficiency virus, influenza virus, and severe acute respiratory syndrome related Coronavirus 2SARS-CoV-2 are major examples of this process. Some viral proteins have been implicated in the host immune response, triggering the production of anti-glycan antibodies, soluble lectins, and complement activation [165]. Among the viruses suspected to play a role in IBD pathogenesis, the Epstein-Barr virus (EBV) has been proposed as a trigger for IBD [166,167]. EBV can be considered in this setting for three main reasons: (i) host glycoproteins: EBV has a lipid envelope derived from the membranes of infected cells and bristling with host glycoprotein spicules [168]; (ii) a link with MBL deficiency: in a pediatric cohort study, analysis of mannose-binding lectin (MBL-2) genotypes and EBV antibody levels showed that EBV seropositivity was significantly lower and time to seroconversion increased in MBL-insufficient compared to MBL-sufficient children, indicating that MBL may be involved in primary EBV infection in infancy [169]. Of note, low MBL levels are also associated with pediatric IBD and ileal involvement in CD [170], as well as a high ASCA response [66,171]; (iii) persistence of antibodies: EBV infects germinal center (GC) B-cells and establishes persistent infection in memory B-cells. EBV-encoded latent membrane protein 2 A mimics B-cell antigen receptor signaling in murine GC B-cells and has also been shown to cause an altered humoral immune response and autoimmune diseases by inducing a reduction of the stringency of GC B-cell selection. It may also contribute to persistent EBV infection and pathogenesis by providing GC B-cells with excessive pro-survival effects [172].

7. ASCA and other diseases

Reaching the goal of understanding the mechanism of ASCA generation cannot be achieved without considering the panel of human diseases in which their presence has been reported. The availability of the non-invasive ASCA test incited many researchers to explore the presence of ASCA in the diseases they studied and for which they had available many sera from different patient cohorts. Over many years, the incidental observation of the presence of ASCA was confirmed by large studies. Table 1 lists the human diseases in which an increased prevalence of ASCA has been reported. This is an impressive and diverse list and it is not objective of this review to embrace the topic. Deciphering the pathophysiological mechanisms involved to explain the presence of ASCA is the domain of specialists in each of the relevant disciplines. However, when data were found and in coherence with the theme of this review, we attempted to report the changes in the gut bacteriome/mycobiome which are described in these other diseases. Instead of considering the whole and probably still incomplete panel of diseases, we first focused on diseases of the digestive tract and its appendages since some of these are important models to address some basic issues.

(i) Regarding the question of ASCA stability, a character of ASCA associated with CD discussed previously, it must be mentioned that this stability has not been documented/investigated for any other disease. Interestingly, concerning CeD, for which a triggering role for *C. albicans* has been suspected through molecular mimicry between the hyphal protein Hwp1 and gliadin (both substrates of transglutaminase) [173,174], it has been established that ASCA are not stable. Indeed, ASCA decrease under a gluten-free diet [175]. This suggests that CD and CeD differ in the genetic mechanisms leading to ASCA stability.

(ii) Regarding the question of ASCA and *C. albicans* overgrowth, studies on patients with alcoholic hepatitis probably provide the most

Table 1

Left panel. Non-exhaustive list of human diseases in which an increased prevalence of ASCA has been reported to date. The huge amount of available information led us to select a limited number of studies in an attempt at clarity. The methods of ASCA determination, usually commercially available, vary from one study to another [59,60]. The results are expressed as a % of positive tests regarding the cut-off proposed by the manufacturer for differentiating CD from UC, which is not particularly appropriate. We did not discriminate between IgG and IgA, and have reported a global range. For studies in which only statistical comparisons of groups were performed, we reported the results as an "increase". *Right panel.* Results from gut mycobiota analyses in the corresponding diseases reporting fungal dysbiosis or *Candida* overgrowth established using metabarcoding (NGS) and/or conventional methods of mycological isolation/identification (M).

	Presence of anti-*S. cerevisiae* antibodies (ASCA)					Mycobiota		References	
	Prevalence (% or increased)	Familial trait (%)	Stability	Predictive	Diagnostic usefulness / Comments	Fungal dysbiosis	*C. albicans* overgrowth	ASCA	Mycobiota
Control population	6	YES	Unknown			Low fungal diversity compared to bacterial diversity		[2,3,15]	[150]
Gut diseases									
IBDs (Inflammatory Bowel Diseases)					In association with ANCA				
Crohn's disease	50–60	20–30	YES	YES		YES	YES (NGS & culture)	[2,3]	[126,133]
Small Bowel	50–60							[192]	
Large bowel	8							[49]	
Healthy First Degree Relatives	20–30		Unknown	?			YES (culture)	[16,133]	[133]
Ulcerative colitis	11.9		Unknown			YES	YES (NGS)	[3]	[126,178]
Pouchitis	5–12.5		Increase?		Evolution close to CD	YES	YES (NGS), in patients with starch consumption	[177,193]	[181]
Celiac Disease	60–70		NO			Rather No	*Candida* genus (PCR)	[194]	[195–197]
Celiac Disease under gluten diet	8-Jun		?		Resistance to GFD?			[175]	
Candidiasis									
Systemic	72.2		NO	YES	YES	YES	YES (NGS)	[64]	[198]
Vaginal	29		NO	YES	YES	YES	YES (culture)	[199]	[200]
Hepato-biliary diseases									
NAFLD (Non Alcoholic Fatty Liver Disease)	ND					YES	YES (NGS & culture)		[201]
Primary sclerosing cholangitis	6-30					YES	Discordant studies (NGS & culture)	[202]	[203,204]
Alcoholic hepatitis	Up to 24.3		High levels pejorative		YES (linked to prognosis)	YES	YES (NGS)	[205]	[176,206]
Skin diseases									
Atopic dermatitis	ND				*S. cerevisiae* GP 200	YES	Discordant studies (NGS & culture)	[154,155]	[207]
Hidradenitis suppurativa, with chronic inflammatory intestinal disorders	Increased					Unknown	Unknown	[208,209]	[210]
Pulmonary diseases									
Cystic fibrosis	3.7–55.6		Increase in prevalence over time			YES	YES (NGS & culture) prevalence 35–93%	[211–213]	[213–215]
Autoimmune diseases									
Multiple sclerosis	3.5–15					Maybe	Discordant studies (NGS)	[216,217,219,217]	[218,219]
Behçet disease (vasculitis)	4–48.1					Unknown	Unknown	[220–222]	
Intestinal Behçet disease	12.7–25.4							[98,223,224]	

(*continued on next page*)

Table 1 (*continued*)

Presence of anti-*S. cerevisiae* antibodies (ASCA)				Mycobiota		References	
Kawazaki disease (vasculitis)	Increased		Increased anti-*Candida* cell wall beta-glucan antibodies	Unknown	Unknown	[225]	
Systemic Lupus Erythematosus	4.5–31.9			YES	(*C. glabrata* culture)	[226,227]	[228]
Thyroiditis	0.8–16.6			Unknown	Unknown	[229,230]	
Sjögren Syndrome	4.8			Unknown	YES oral candidiasis (culture)	[231]	[232]
Spondyloarthritis	18–25	Unknown		YES	YES (NGS)	[233,234]	[235]
Metabolic diseases							
Diabetes	6.2–21			YES	YES (NGS & culture)	[236,237]	[201,238]
Obesity	2.1–22			YES	YES (NGS & culture)	[239,240]	[201]
Myocardial infarction	Increased			Unknown	Unknown	[241]	
Neurologic diseases							
Parkinson disease	Increased			YES	Unknown	[242]	[242,243]
Psychiatric diseases							
Autism	ND		Anti-*C. albicans* IgG antibodies in 36.5% of patients	YES	Discordant studies (cultures & NGS)	[244]	[245–247]
Depression	Increased			YES	Unknown	[248]	[249]
Schizophrenia	6–44.4			YES	Unclear (IgG anti-*C. albicans*)	[248,250–252]	[253,254]
Bipolarity	Increased			Unknown	Unknown	[255]	
Infectious diseases							
Covid-19	13.7–25			YES	YES	[256,257]	[258]
Cancer	ND		Glycosylation modification of epithelial and immune cells*	YES	Unknown	*[123,124,259] [260]	[188,261]

clear-cut model. This acute-on-chronic liver disease occurs suddenly after years of heavy alcohol consumption, for unknown reasons, and is characterized by prominent cholestasis and high mortality rates (20–40% within 6 months). Stunning of the immune system is associated with an increase in ASCA, which are associated with *Candida* overgrowth, a process clearly independent of increased intestinal permeability. Kaplan Meier curves show that ASCA levels are not only predictive, as in other diseases, but also strikingly associated with death [176]. The relation between ASCA/*Candida* overgrowth and this acute auto-immune process probably deserves attention from the scientific and medical communities.

(iii) The mystery of ASCA and UC. Although the term ASCA was proposed initially for a test differentiating the serological response in CD versus UC (with a specificity close to 100% using the ASCA-ANCA combination), an exception exists concerning pouchitis, a complication from ileo-anal anastomosis surgery. The presence of ASCA, but also anti-glycan antibodies, has led some gastroenterologists to consider this complication of UC as CD-like [177]. The relationship between ASCA and *Candida*, and the absence of ASCA in UC, raises some questions. ASCA prevalence is low in colonic forms of CD, whereas ASCA and ANCA co-exist in UC-like CD [21]; the absence of both markers corresponds to a clinico-serological entity representing >40% of cases of indeterminate colitis. The question of the relationships between *C. albicans* and ASCA extends to recent studies demonstrating that clinical improvement of UC after fecal microbiota transplant is associated with a decrease in *C. albicans* load [178,179] or improvement of clinical, histologic scores and calprotectin levels in UC patients colonized by *C. albicans* and receiving oral fluconazole therapy [180]. This suggests that the UC-based micro-mycobiota from the colon to the ileon (associated with low ASCA levels - including in CD) affects ASCA production, and that the site of *C. albicans* growth in IBD matters for ASCA generation [181].

Returning to Table 1, it is clear that during initial studies on ASCA as a marker of CD it was difficult to comprehend why so many diseases could have an increased prevalence of this marker. It should be pointed out that these prevalences are relative and that different studies used commercial tests of different origins. However, although ASCA levels often fell below those considered to be specific to CD their increase in prevalence is obvious. The fact that neurological diseases are part of this panel has been recognized over the past few decades and studies have found that the gut-brain axis as an unexpected player in human health and well-being [182]. The presence of ASCA (a response to microbial antigens) suggests that rather than an axis a triangle may exist involving the immune system, as suggested recently in premature neonates [183]. Metagenomics, allowing easy analysis of the microbiota (and technical progress in assessing the importance of the mycobiota), showed that for most of these diseases with an auto-immune background the presence of ASCA was associated with fungal dysbiosis and *Candida* overgrowth [174,184]. It is only recently that investigations into ASCA and/or *C. albicans* have extended to cancer and the literature in this field has grown. A general review from 2020 about *Candida* immunoreactivity and human diseases in different parts of the body, making reference to ASCA, proposed a framework for the human anti-fungal deterioration of colitis to cancer, but without linking ASCA to the process [10]. Overall,

two major articles addressing the topic of cancer and the mycobiota were published in Cell in 2022. One [185] identified a 20-fungus signature potentially able to distinguish pan-cancer from healthy individuals [185]. The study by Dohlman et al. [186] revealed that *Candida* is correlated with worse survival outcomes, pro-inflammatory gene expression, and metastasis, and that identification of fungal DNA at the tumor site may provide a predictive biomarker for gastrointestinal cancers. A study finely analyzing cross talk between *Candida* and immune cells uncovered regulation mechanisms promoting tumorogenesis [187]. Although impressive information has been obtained from these high-tech studies, the relation between *C. albicans* and ASCA, and ASCA and cancer, has not been addressed. Interestingly, no information can be found in the literature concerning a very simple but important question: "is there a different risk of CD evolution towards cancer in ASCA positive versus ASCA negative patients?"

Finally, convincing evidence has been found in some diseases, but the multiplicity of human pathologic circumstances where *C. albicans* overgrowth has been described makes interpretation of some of these findings in other diseases challenging. Thus, the question about *C. albicans* proliferation as a cause or consequence is legitimate. Inverse reasoning fits with the old medical adage of *C. albicans* as a sensor of human health [188]. The only response to this question would reside in clinical trials using antifungals (or probiotics?) to assess whether they improve the evolution of a patient's primary disease. Although this would probably improve a patient's clinical condition by decreasing the adverse effects of *Candida* proliferation, ethical, legal, and economic considerations render such trials challenging. It is therefore up to medical research to obtain more evidence of the impact of *C. albicans* on diseases in one way or another.

Just at the time this long-lasting review was completed, two important advances were made about ASCA and *C. albicans*. One established a clear link between ASCA and altered CD4+ T cell responses [189]. The second [190], demonstrated that severe infection led to reprograming of granulocytes. These provide new angles to revisit or answer the large number of questions still raised about ASCA meaning in human health.

8. Conclusion

This analytic review was prompted by a recent paper showing that ASCA are probably the most potent markers of CD [45], and that understanding the mechanisms of ASCA generation and persistence would help to decipher the pathophysiology of CD. Regarding the possible role of *C. albicans* in CD, we carried out early investigations on the mechanism of ASCA generation and revisit the contributions to this subject published over the past three decades. We have deliberately opted for a broad analysis of this subject by including papers on basic yeast immuno-glycobiology, medical mycology achievements, gastroenterology, and auto-immune diseases. Over many years, where numerous papers have been published in top ranked scientific journals exploring the question of *C. albicans* gut saprophytic/pathogenic adaptation in relation to the hosts' antibody response [72,73], we hope that some answers finally "emerge from the shadows" [79,191].

Ethics

Not applicable.

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Author contributions

All authors contributed to the study conception and design. Articles retrieval, data extraction, and the first draft of the manuscript were performed by Daniel Poulain and Boualem Sendid.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Boualem Sendid reports administrative support, article publishing charges, and writing assistance had no specific funding and was supported by the endowments of University of Lille, Inserm and CNRS, France. Boualem Sendid reports a relationship with University of Lille that includes: employment, funding grants, speaking and lecture fees, and travel reimbursement. Other authorsdeclare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

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