Adequate functioning of the intestinal barrier is required in order to repel invading pathogens while tolerating commensal microbiota and self-antigens. Inflammatory bowel diseases (IBDs), encompassing Crohn’s disease (CD) and ulcerative colitis (UC), are characterized by disrupted intestinal barrier integrity, resulting in excessive passage of luminal antigens and the activation of aberrant immune responses against otherwise unexposed antigens. A comprehensive overview of the exact antigens associated with IBD is still lacking, but recent innovative antibody profiling technologies have enabled systematic characterization of humoral immunity in health and disease. Here, we review established serological antibodies and novel high-throughput methods, such as protein arrays, phage-display immunoprecipitation sequencing (PhIP-Seq), and B cell receptor sequencing (BCRseq), and provide an outlook on their applications in disease diagnostics, therapeutic interventions, and opportunities for prevention in IBD.

Inflammatoy bowel disease and antibody responses
The human gut microbiota, which includes bacteria, viruses, fungi, and various other microorganisms, closely interact with the intestinal barrier, where a large number of luminal antigens (see Glossary) enter the tissue, and the underlying mucosal immune system continuously samples these antigens as an immune surveillance mechanism [1]. In IBDs, increased exposure of luminal antigens to the mucosal and systemic immune system occurs, which may in turn drive intestinal inflammation [2] (Box 1). Thus, antimicrobial antibody responses against the gut microbiota, but also antibody responses against self-antigens (autoimmunity), are frequently observed in patients with IBD, particularly in CD [3]. However, the exact nature and functional capacity of the antigens targeted is largely unknown, including their potential implications for IBD pathogenesis. A key challenge lies in the systematic characterization of the human antibody epitope repertoire, measurement of which is usually limited to a few hundred to few thousand antigen epitopes [4]. However, recently developed high-throughput, high-resolution antibody profiling technologies have overcome this limitation and may thus become powerful tools for revealing novel immunological targets and improving our understanding of IBD immunopathogenesis. Importantly, these technologies harbor potential for the development of new clinical applications for IBD such as prediction of treatment responses or early detection of disease onset.

In this review, we provide a concise overview of humoral immunological alterations and host–microbe interactions in IBD in concert with loss of immunologival tolerance. We also critically assess the currently known serological indicators for IBD and their clinical utility and discuss both conventional and innovative state-of-the-art antibody profiling techniques. We further discuss the clinical potential of systematic antibody epitope repertoire profiling for IBD. Finally,
**Box 1. Pathophysiology of IBD and serological antibodies**

Inflammatory bowel diseases (IBDs), encompassing Crohn’s disease (CD) and ulcerative colitis (UC), are chronic, immune-mediated inflammatory diseases of the gastrointestinal (GI) tract. These noncommunicable diseases are characterized by a considerable degree of interpatient heterogeneity and pathophysiological complexity [5]. The incidence of IBD is rising globally, especially in Westernized countries, showing varying trends depending on genetic background but similar patterns for men and women [6]. Although the exact cause of IBD is unknown, its pathogenesis is considered to be multifactorial, consisting of an interplay between genetic susceptibility, gut microbiota, host immune system, and environmental triggers such as lifestyle and dietary habits [7,28].

IBD clinically manifests in symptoms such as abdominal pain, diarrhea (with or without blood loss), fatigue, weight loss, and several extraintestinal manifestations (e.g., arthritis, uveitis, and skin abnormalities). Patients typically show a ‘relapse-remitting’ disease course in which periods of remission alternate with disease exacerbations that require urgent medical intervention [9]. However, clinical symptomatology is heterogeneous, which necessitates the use of additional diagnostics. Usually, diagnosis is based on a combination of clinical, biochemical, endoscopic, radiologic, and histological investigations [10]. Upon initial clinical presentation, an ileocolonoscopy (endoscopy) must be performed. Endoscopically, CD is characterized by discontinuous involvement, ulcerative inflammation that may occur in any part of the GI tract (the terminal ileum being most commonly affected), a ‘cobblestone appearance’, and (peri-)anal lesions; by contrast, UC is macroscopically marked by superficial inflammation found exclusively in the colon [11,12]. Most patients with IBD require life-long immunomodulating treatment and/or surgical intervention, necessitating adequate disease biomarkers that may be used to predict disease behavior and therapeutic response [8].

Currently, serologic testing for IBD is not recommended for routine diagnostics because the added value of serological markers is considered only marginal [13]. However, in clinical practice, few well-known serological markers are frequently determined. These include anti*-Saccharomyces cerevisiae* (ASCA, most specific to CD) and perinuclear anti-neutrophil cytoplasmic antibodies (pANCA, most specific to UC). Furthermore, commercially available serum biomarker panels are sometimes applied that contain the antimicrobial antibodies anti-CBir1 or anti-OmpC, among others [14,15]. Based on the notion that specific antibody responses may constitute one of the earliest pathogenic events in IBD, characterization of antibody-based biomarker signatures may facilitate early detection of IBD and/or facilitate the initial disease diagnosis [16]. Most of these serological markers represent microbial antigens, potentially reflecting impaired epithelial barrier function, loss of immunological tolerance, and increased bacterial translocation in IBD.

we provide future perspectives on how to leverage antibody-based biomarker signatures to enable **precision medicine** in IBD.

**Humoral immunity and host-microbe crosstalk in IBD**

The microbiota in humans largely resides within the gastrointestinal (GI) tract, requiring the maintenance of a delicate balance with the host immune system. In particular, the large variety of bacterial species that make up the majority of the gut microbiota represent a tremendous antigenic space. Development of the immune system is shaped by gut microbial colonization, which aims to achieve a mutualistic host-microbe environment with immunological tolerance toward nonhazardous microbial antigens [17]. Under physiological conditions, microbial antigenic structures (i.e., pattern-associated molecular patterns (PAMPs)) are recognized by antigen-presenting cells (APCs) that inhabit the intestinal mucosa, mediated by activation of innate immune receptors such as Toll-like receptors or Nod-like receptors. In addition, the gut microbiota is regulated by host immune defense components such as mucins and the mucus layer (glycocalyx), antimicrobial peptides (AMPs, e.g., defensins or C-type lectins), epithelial cytokine production (e.g., IL-33, thymic stromal lymphopoietin (TSLP), or transforming growth factor-β (TGF-β)), and secretory IgA. Conversely, the microbiota may also influence mucosal immunity, for example, by promoting tolerogenic dendritic cells (DCs) and macrophages, steering differentiation of specific subsets of T lymphocytes, or modulating affinity maturation (by somatic hypermutation) of protective B lymphocytes (plasma cells) (Figure 1) [18].

Compared with healthy individuals, patients with IBD display several abnormalities of the gut microbiota, including decreased microbial diversity, lower numbers of commensal bacteria, and increased proportions of potentially pathogenic bacteria [19]. Simultaneously, intestinal barrier...
function is compromised in IBD, which may facilitate the passage of microbial antigens across the mucosa and may elicit aberrant mucosal and systemic immune responses. This occurs in combination with IBD-associated immunological defects such as defective bacterial clearance.

Figure 1. Immune homeostasis and alterations in healthy and inflamed intestinal mucosa. Schematic illustration of the intestinal mucosa showing a gradient of disrupted intestinal barrier integrity with concomitant inflammation and microbial dysbiosis. Under physiological conditions, when the gut microbiota live in homeostasis (eubiosis), intestinal epithelial cells sense the luminal contents for potential antigens and secrete mucins, AMPs, and regulatory cytokines (e.g., TSLP, IL-25, IL-33, TGF-β) upon stimulation, favoring a tolerogenic immunological environment. APCs such as DCs and macrophages stimulate the development of Tregs via secretion of anti-inflammatory cytokines such as TGF-β and IL-10. These cytokines, in turn, promote the activity of secretory IgA-producing plasma cells. In addition, induced Tregs further contribute to a homeostatic mucosal environment by maintaining a state of immunological tolerance. Under pathophysiological circumstances, pathogenic bacteria (dysbiosis), injury, or other stress factors trigger intestinal epithelial cells to produce proinflammatory cytokines such as IL-6 and IL-12, and IL-23 upon antigenic exposure, and these promote the proliferation and differentiation of effector T lymphocyte subsets (Th1, Th2, Th9, and Th17 being the main subtypes). In addition, innate immune cell types including neutrophils, eosinophils, NK cells, and mucosa-residing fibroblasts respond to the proinflammatory cytokine environment by, for example, the production of epithelial barrier-protective IL-22 (which counteracts the disruptive actions of IL-9). Abbreviations: AMPs, antimicrobial peptides; APCs, antigen-presenting cells; DCs, dendritic cells; IEL, intraepithelial lymphocyte; IFN, interferon; sIgA, secretory IgA; NK cell, natural killer cell; TGF-β, transforming growth factor β; Th, helper T lymphocyte; Treg, regulatory T lymphocytes; TSLP, thymic stromal lymphopoietin.

**Glossary**

**Antibody (immunoglobulin/Ig)**
- **Eptope repertoire:** the collective of actual structures recognized by the antibody repertoire of a person.
- **Antibody repertoire:** the collective of all antibodies in a person. Typically characterized by BCRseq, which reveals the DNA sequences encoding the antibodies but not their functional consequences of antigens recognized.
- **Antigen:** a substance, either of foreign or human origin, capable of eliciting an immune response, that is, activating immune cells to exert their immunological functions.
- **B cell receptor (BCR) sequence:** the DNA sequence within a B cell that encodes an antibody. BCR sequences are generated by random VDJ recombination and somatic hypermutation to increase affinity to antigens.
- **Epitope:** a part of an antigen that represents the binding site of that antigen to a specific antigen receptor where binding only occurs when the structures of both are complementary to each other.
- **Humoral immunity:** the parts of the immune system that freely circulate or are present outside of human cells. It deals with (mostly foreign) antigens and is regulated by molecules such as antibodies, complement proteins, and antimicrobial peptides and also known as ‘antibody-mediated immunity’.
- **Precision medicine:** healthcare actions that are tailored to the basis of an individual’s biology (e.g., genetics, immune system), lifestyle (e.g., dietary habits), and environment.
- **Sensitivity:** the probability of a positive result to a given test, conditioned on truly having the condition that is tested for.
- **Specificity:** the probability of a given test correctly identifying individuals who do not have the condition tested for.
impaired autophagy, or diminished production of AMPs, which may originate from genetic variation in IBD risk genes such as NOD2, ATG16L1, or IRGM [20]. Consequently, this defective acute immune response may trigger a compensatory adaptive immune response that is accompanied by the initiation and perpetuation of chronic intestinal inflammation [21]. A dysbiotic and inflammatory intestinal microenvironment is mediated by the production of proinflammatory cytokines [e.g., IL-1β, IL-6, tumor necrosis factor (TNF)-α, or IL-12] by intestinal epithelial cells, DCs, and macrophages, thereby overriding the anti-inflammatory defense components but promoting the development of effector CD4+ Th1-, Th2-, Th9-, and/or Th17-differentiated T lymphocytes and plasma cells to produce the corresponding neutralizing antibodies. Synchronously, other immune cells, such as natural killer (NK) cells, γδ-T lymphocytes, neutrophils, and eosinophils, are recruited and become activated, depending on the nature of the immunological triggers and the cytokine environment [17].

In this respect, the role of humoral immunity in the immunopathogenesis of IBD has long been recognized. Compared with healthy individuals, patients with IBD generally exhibit increased activation of humoral immune responses and distinct differences in the production of immunoglobulin subclasses [22]. Antibody responses in IBD generally fall within two broad categories: autoimmune reactivity and antimicrobial reactivity. Although the biological relevance of autoimmune antibodies remains elusive, they are particularly observed in patients with UC. For instance, the frequency of perinuclear anti-neutrophil cytoplasmic antibody (pANCA) seropositivity and associated titers is consistently higher in patients with UC compared with CD patients or healthy individuals [23]. Although the exact antigenic structure targeted by pANCA has never been identified, it has historically been suggested that it may represent cross-reactivity with a bacterial structure directed against the perinuclear membrane of neutrophils [24]. Similarly, an autoimmune response against the microfilament protein tropomyosin (especially the 1 and 5 isoforms) is typically found in the blood and intestinal mucosa of patients with UC, but not in CD [25]. Conversely, antibody formation against various microbial antigens is characteristically observed in CD. Relevant examples include ASCA, anti-flagellin antibodies (e.g., anti-CBir1), or anti-glycan antibodies (summarized in Table 1) [3,26,27]. These observations may support the notion that microbial antigens are relatively more important in the immunopathogenesis of CD, while autoimmunity is more prominently involved in UC pathogenesis. Thus far, the exact pathogenic role of the specific antibody signatures for CD and UC is largely unknown, but many studies have demonstrated potentially useful associations with clinical phenotypes and outcomes in both types of IBD.

Serological antibodies in IBD: clinical utility and phenotypic associations

Disease diagnosis and classification

In clinical practice, some serological antibodies may support the diagnosis and classification of IBD, but their diagnostic accuracy is generally considered too limited for conclusive differentiation between CD and UC [10]. To date, no single serological indicator exists that is useful for diagnosing CD or UC. ASCA and pANCA are the most commonly known and best available serological markers with a relatively high specificity but poor sensitivity. Albeit moderate, their diagnostic utility seems to be higher in pediatric patients with IBD than in adult patients [28,29]. Similarly, the additional value of antimicrobial antibodies, for example, anti-CBir1 and anti-OmpC (outer membrane protein of Escherichia coli), in differentiating CD from UC appears limited [13,30]. Although these serological antibodies could help identify subsets of patients with similar clinical features, they typically do not occur in all patients and, consequently, differential antibody reactivities may also reflect differences in the underlying immunopathogenesis. As a result, serological testing is not currently recommended for routine diagnostic assessment of CD or UC [10]. Nonetheless, multiple studies have attempted to find combinations of serological antibodies, for
Table 1. Established serological antibodies in IBD

<table>
<thead>
<tr>
<th>Serological antibody</th>
<th>Description of antigenic target</th>
<th>IBD subtype specificity</th>
<th>Clinical associations</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASCA</td>
<td>Mannan (cell wall) protein of the yeast <em>Saccharomyces cerevisiae</em></td>
<td>CD</td>
<td>Ileal disease involvement, stricturing and penetrating disease, risk of surgery</td>
</tr>
<tr>
<td>ACCA</td>
<td>Chitobioside (cell wall) carbohydrate of yeasts and bacteria</td>
<td>CD</td>
<td>Steroid dependency, fibrostenotic disease, perianal disease</td>
</tr>
<tr>
<td>ALCA</td>
<td>Laminaribioside (cell wall) carbohydrate of yeasts, bacteria, wheat, and algae</td>
<td>CD</td>
<td>Ileal disease involvement, stricturing and penetrating disease, risk of surgery</td>
</tr>
<tr>
<td>AMCA</td>
<td>Mannobioside (cell wall) carbohydrate of several microorganisms</td>
<td>CD</td>
<td>Ileal disease involvement, stricturing and penetrating disease, risk of surgery</td>
</tr>
<tr>
<td>Anti-CBir1</td>
<td>Flagellin, flagella component isolated from bacteria in colitis mice</td>
<td>CD</td>
<td>Ileal disease involvement, stricturing and penetrating disease, long disease duration, early postoperative recurrence</td>
</tr>
<tr>
<td>Anti-Fla-X</td>
<td>Flagellin, Fla-X</td>
<td>CD</td>
<td>Ileal disease involvement, stricturing disease, perianal disease, early postoperative recurrence</td>
</tr>
<tr>
<td>Anti-A4-Fla2</td>
<td>Flagellin, A4 strains, frequently linked to <em>Lachnospiraceae</em> family</td>
<td>CD</td>
<td>Ileal disease involvement, stricturing disease, perianal disease</td>
</tr>
<tr>
<td>Anti-I2</td>
<td>I2 component of <em>Pseudomonas fluorescens</em> in mucosal mononuclear cells</td>
<td>CD</td>
<td>Strictures disease, risk of surgery</td>
</tr>
<tr>
<td>Anti-L</td>
<td>Laminarin</td>
<td>CD</td>
<td>Steroid dependency, penetrating disease, surgery</td>
</tr>
<tr>
<td>Anti-C</td>
<td>Chitin</td>
<td>CD</td>
<td>Penetrating disease, surgery</td>
</tr>
<tr>
<td>Anti-OmpC</td>
<td>Outer membrane protein of <em>Escherichia coli</em></td>
<td>CD</td>
<td>Penetrating disease, risk of surgery</td>
</tr>
<tr>
<td>Anti-MAP</td>
<td>Mycobacterium avium subspecies paratuberculosis</td>
<td>CD</td>
<td>Biological use</td>
</tr>
<tr>
<td>Autoantibodies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pANCA</td>
<td>Lysosomal enzymes present in the cytoplasm of neutrophils and monocytes</td>
<td>UC</td>
<td>Disease activity, colonic disease involvement (in CD)</td>
</tr>
<tr>
<td>Anti-GP2</td>
<td>Glycoprotein 2, membrane receptor located in M cells of (small) intestinal Peyer’s patches</td>
<td>CD</td>
<td>Ileal disease involvement, early disease onset, long disease duration, stricturing phenotype, perianal disease</td>
</tr>
<tr>
<td>Anti-CUZD1</td>
<td>CUB and zona pellucida-like domains 1, regulating immunological tolerance</td>
<td>CD</td>
<td>Ileal disease involvement, perianal disease</td>
</tr>
<tr>
<td>Anti-IF16</td>
<td>IF16 protein, belongs to the PYHIN-200 family of pattern recognition receptors</td>
<td>CD</td>
<td>Nonresponse to TNF-α-antagonists</td>
</tr>
<tr>
<td>Anti-MZGP2</td>
<td>Major zymogen glycoprotein 2</td>
<td>CD</td>
<td>Ileal disease involvement, early disease onset, long disease duration</td>
</tr>
<tr>
<td>Anti-CH3L1</td>
<td>Chitinase-3-like protein 1</td>
<td>CD</td>
<td>Aggressive, complicated disease</td>
</tr>
<tr>
<td>Anti-GM-CSF</td>
<td>Granulocyte macrophage colony-stimulating factor</td>
<td>CD</td>
<td>Ileal disease involvement, aggressive disease</td>
</tr>
<tr>
<td>Anti-ACA</td>
<td>Anti-cardiolipin</td>
<td>CD</td>
<td>Use of TNF-α-antagonists</td>
</tr>
<tr>
<td>Anti-PS/PT</td>
<td>Phosphatidylycerine/prothrombin</td>
<td>CD</td>
<td>Use of TNF-α-antagonists</td>
</tr>
<tr>
<td>Anti-GBA</td>
<td>Goblet cells</td>
<td>UC</td>
<td>–</td>
</tr>
</tbody>
</table>

Abbreviations: ACA, anti-cardiolipin; ACCA, anti-chitobioside carbohydrate antibodies; ALCA, anti-laminaribioside carbohydrate antibodies; AMCA, anti-mannobioside carbohydrate antibodies; anti-C, anti-chitin antibodies; anti-CBir1, anti-flagellin CBir1 antibodies; anti-I2, antimicrobial sequence I2 antibodies; anti-L, anti-laminarin antibodies; ASCA, anti-*Saccharomyces cerevisiae* antibodies; GBA, goblet cell antibodies; GM-CSF, granulocyte/macrophage colony-stimulating factor; CH3L1, chitinase 3-like protein 1; CUZD1, CUB and zona pellucida-like domains 1; GP2, pancreatic glycoprotein 2; MAP, Mycobacterium avium subspecies paratuberculosis; MZGP2, major zymogen glycoprotein 2; OmpC, outer membrane protein of *Escherichia coli*; pANCA, perinuclear anti-neutrophil cytoplasmic antibodies; PS, phosphatidylycerine; PT, prothrombin; UC, ulcerative colitis.

Example, parallel ASCA and pANCA measurements, that could be used to predict whether an indeterminate colitis would progress to either CD or UC [31–33]. Table 1 presents a detailed – but not exhaustive – overview of known serological antibodies in the context of IBD, with a description of their antigenic targets, IBD subtype specificity, and associations to clinical phenotypes and outcomes (for details see the extensive reviews in [15,34,35]).
Identification of at-risk individuals for IBD

Using serological antibodies to identify at-risk individuals and predict future occurrence of IBD could be of great clinical benefit. To this end, previous studies demonstrated that ASCA, pANCA, and selected antimicrobial antibodies, or combinations of them, may serve as predictors of the future onset of IBD [14,16,26,36,37]. More recently, studies have emerged addressing the potential utility of anti-flagellin antibody signatures to define CD or predict the risk of developing CD years before the actual diagnosis [3,16,38]. For example, increased anti-flagellin antibody responses have been strongly associated with the future onset of CD and are independent of signs of subclinical inflammation, gut permeability, and genetic risk [16]. This observation led to the speculation that serological antibody formation may reflect one of the earliest pathogenic events in IBD. Importantly, as serological antibody profiling may help identify individuals at risk for IBD (prediagnostics), it opens up opportunities for primary disease prevention by identifying individuals for disease surveillance or early initiation of therapeutic intervention. The value of such efforts has previously been observed for other immune-mediated inflammatory diseases such as rheumatoid arthritis or type 1 diabetes [39,40].

Associations with distinct clinical phenotypes and outcomes in IBD

Looking at the serological antibodies listed in Table 1, there is a notably higher number of CD-associated serological indicators. Many of these antibodies are intimately associated with distinct clinical phenotypes characteristic for CD. Multiple studies have indicated that the combined presence and magnitude of titers of multiple positive antimicrobial antibodies is associated with disease severity and progression in CD [16,29]. For instance, ASCA, anti-flagellin antibodies (like anti-CBir-1 and anti-Fla-X), anti-OmpC, and anti-I2, among others, are particularly associated with small bowel or ileal disease involvement, an increased risk of developing strictureting or penetrating disease complications, the presence of perianal disease, and the risk of requiring IBD-related surgery [16,29]. Furthermore, exocrine pancreatic autoantibodies have been associated with higher frequencies of penetrating and perianal disease, extraintestinal manifestations, and antimicrobial antibody reactivity [41]. Such associations, however, have also been reported for UC, for example, pANCA antibodies are associated with a higher rate of complications and surgery in patients with postoperative ileal pouchitis [42].

Prediction of therapeutic response in IBD

In the therapeutic management of IBD, it is notoriously challenging to predict which patients will benefit most from which type of medical treatment, a challenge mainly due to the heterogeneity of the disease and, consequently, patients’ therapeutic responsiveness, particularly to biologicals. For instance, up to 30–40% of patients demonstrate nonresponse or loss of response after induction therapy with TNF-α-antagonists (e.g., infliximab) and approximately 40–50% of patients fail to respond to induction therapy with the α4β7 integrin inhibitor vedolizumab [43,44]. Serological antibodies have therefore also been evaluated for their capacity to predict response to treatment in IBD. For instance, pANCA-positive but ASCA-negative patients with CD demonstrated little benefit from treatment with TNF-α-antagonists [45,46]. Likewise, pANCA-positive/ASCA-negative patients with UC previously showed decreased response rates to TNF-α-antagonists [47]. Another study found that patients with CD who were positive for anti-OmpC or anti-I2 antibodies responded better to antibiotic treatment [13]. Anti-GP2 and anti-IF16 antibodies have been associated with nonresponsiveness to infliximab and the need for surgical intervention in CD [48,49]. Currently, however, the evidence is still insufficient to recommend the use of serological antibodies to predict patient responses to particular medical treatments or surgical interventions in IBD [35].

Antibody profiling techniques: the state of the art

While most of the serological antibodies in IBD listed in Table 1 were identified using conventional enzyme-linked immunosorbent assays (ELISAs), several more advanced antibody profiling
technologies have emerged [50] that have provided unprecedented insights into antibody repertoires in IBD (Figure 2). ELISAs, similar to radioimmunoassays (RIAs), are typically based on the immobilization of antigens on surfaces and subsequent detection of antibody binding by enzymatic (in ELISA) or radiolabeled (in RIA) signal amplification. These methods yield high sensitivity/specificity and can be used for absolute quantifications in combination with calibration curves. ELISAs can be run on thousands of samples. Typically, only the antibody binding against a single antigen is assessed, making parallel measurements against multiple antigens challenging and limiting ELISAs to the analysis of a few antigens in diagnostic settings.

However, in IBD, a large number of antigens with diagnostic potential have already been reported (Table 1). Furthermore, given the large number of different bacterial species making up the human microbiome [51] and increased bacterial translocation in IBD, many additional microbial antigens could be targeted by antibody responses. Alexander et al. [3] applied a protein array (Figure 2) to tap into this uncharacterized space of potential IBD biomarkers. In contrast to ELISAs, array-based technologies allow spotting of hundreds to thousands of antigens on carrier surfaces.

| Molecular methods for the detection of microbial antibody responses in IBD |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| ELISA                      | Peptide Array               | PhIP-Seq                    | IgA-Seq                     | BCR-Seq                     |
| Principle                   | Detection of antibody binding against a single antigen by enzymatic signal amplification | Spotting of antigens on a glass slide and parallel detection of antibody binding by fluorescence | Detection of antibody binding against phage displayed DNA encoded protein antigens, NGS detection | Mixtures of bacterial cells and antibodies are sorted by FACS into bound and unbound fractions, detection by NGS | DNA isolation from B cells, PCR amplification, and NGS of BCR genes |
| Antigens detectable         | Dozens to hundreds          | Thousands to tens of thousands | Hundred thousands of peptides | N/A                         | N/A                         |
| Advantages                  | Easily applicable standard laboratory test, yielding an absolute readout if using a standard curve | Possible to test any type of antigen, ranging from peptides to full-length proteins, and also non-protein antigens | Rational selection of antigens of interest, high-throughput detection of Ig response against antigens previously encountered | Testing of binding against natural antigens, closely mimicking in vivo conditions | Only method providing insights on VDJ usage, SHM, clonality and diversity of B cells |
| Disadvantages               | Relatively low throughput and laborious production of antigens, difficult to multiplex | Similar to ELISAs, the production of antigens can be a limiting step | Complex workflow, NGS-based readout, only antigens present in the library are detectable, technical limitations (only peptide antigens, no PTMs) | Exact antigens bound unknown, low-throughput due to sorting, immunological memory difficult to assess if performed on human samples | No functional readout of the actual antigens bound |
| Findings in IBD             | Conventional markers listed in Table 1 | Lechnaeusiscococcus flagellins in CD patients | Fine mapping of flagellin epitopes and parallel detection of other epitopes | Ig coating of specific microbiota species in CD and UC | Likely IgA binding to microbial antigens, CD and SLE |

Figure 2. Conventional and state-of-the-art antibody profiling technologies. Schematic overview of the key experimental principles underlying the respective methodologies. More details on their general use and application in IBD can be found in Table 1 in the main text (ELISA) and elsewhere (peptide array [3], PhIP-Seq [4,38,52–55], IgA-Seq [56–59], and BCRseq [60–65]). Abbreviations: BCRseq, B cell receptor sequencing; CD, Crohn’s disease; ELISA, enzyme-linked immunosorbent assay; FACS, fluorescence-activated cell sorting; IBD, inflammatory bowel disease; IgA-Seq, immunoglobulin A sequencing; NGS, next-generation sequencing; PhIP-Seq, phage-display immunoprecipitation sequencing; PTMs, post-translational modifications; SHM, somatic hypermutation; SLE, systemic lupus erythematosus; UC, ulcerative colitis.
Antibody binding against all antigens can be assessed in parallel using optical fluorescence readouts. Using such a protein array, Alexander et al. detected significantly increased antibody responses against *Lachnospiraceae* flagellins in patients with CD compared with patients with UC or healthy controls. While anti-flagellin Ig responses in IBD were already known (Table 1), this protein array study enabled the researchers to narrow down the exact bacterial species targeted. Despite accelerating detection by parallelized readouts, protein arrays still require the laborious production of every antigen to be tested, typically making studies on more than a few thousand antigens unfeasible.

PhIP-Seq is a methodology that circumvents this lengthy step of antigen production [52]. PhIP-Seq antigens are encoded by synthetic DNA oligos released from microarray slides, allowing the generation of hundreds of thousands of variants in parallel. The sequences of the DNA oligos can be selected from virtually any source, such as databases or metagenomic sequencing. To date, PhIP-Seq libraries have been generated for the human proteome [53], viruses [54,55], and the gut microbiota [4]. The synthetic DNA oligos are cloned into the genome of T7 phages and translated into peptide antigens that are subsequently displayed as fusion to a phage surface protein. Antibody binding is assessed by next-generation sequencing (NGS), as every phage contains the DNA encoding the protein on its surface, DNA reads of bound antigens are increased after washing away unbound phages in an immunoprecipitation step [52]. For studying *Ig epitope repertoires* against the gut microbiota, various strategies [4] have been applied to enrich for potential antigens that are bound by antibodies (as the coding capacity of bacterial microbiota is enormous [51] and would exceed current library sizes achievable by PhIP-Seq). Existing microbiota antigen libraries have focused on secreted-, membrane-, and surface-exposed proteins and virulence factors, relying on bioinformatic strategies to annotate protein functions [4]. This rational selection of potential antigens to be presented by the phages is a key advantage compared with ‘traditional’ phage display, where antigens are randomly cloned from metagenomic DNA libraries. Cloning of metagenomic DNA by restriction digestion also leads to truncated gene sequences, frameshifts, genes inserted in the wrong orientation, as well as inclusion of noncoding DNA. By contrast, the synthetic DNA cloned in PhIP-Seq is highly uniform resolving these issues.

When applied in IBD, preliminary evidence suggests that PhIP-Seq may hold promise for identifying the exact epitopes of antibody-bound proteins (such as *Lachnospiraceae* flagellins) and for increasing statistical power by processing larger sample numbers [38]. Yet PhIP-Seq is limited to protein antigens, with lipids, glycans, and post-translational modifications typically not covered. The length of displayed peptides is currently constrained to ca. 90 amino acids (aa); therefore, conformational antigens formed by aa stretches further apart would be missed.

An alternative approach based on fluorescence-activated cell sorting (FACS) in combination with NGS (termed IgA-SEQ [56,57] or BugFACS [58]) enables the detection of bacterial antigens in a setting closely matching natural conditions. Fecal material, containing both antibody-bound and unbound bacteria, is stained with secondary antibodies, and the bound bacteria are FACS sorted and subsequently identified via 16S or metagenomic sequencing. This methodology can also be run on panels of precultivated bacteria to test binding of antibodies in other body fluids, for example, blood [59]. While this approach provides the closest resemblance to bacterial antigens in their natural context, it does not inform users about the exact antigens bound, just the bacterial species they originate from. Moreover, when working with panels of precultivated bacteria, not all species of interest may grow under laboratory conditions and the proteins expressed may differ from the proteins expressed upon growth inside of the human gut.
Beyond these antigen-focused approaches, a DNA sequence-based methodology, B cell receptor sequencing (BCRseq), has been applied to analyze antibody repertoires in IBD [60]. BCRseq provides information about the clonality and diversity [61,62] of antibody-producing B cells by investigating VDJ gene usage and mutations occurring in the variable regions [63]. IgA binding to microbial antigens inferred from VDJ gene usage have revealed surprising similarities between CD and systemic lupus erythematosus [60]. Recently, a single-cell-based IgH gene sequencing approach was employed to map the clonotypic landscape of circulating and mucosal B cells in patients with UC [64]. However, since BCRseq only provides information about DNA sequences it is difficult to determine the exact antigens bound and the functional antibody recognition by these BCR repertoires [65].

**Clinical potential of antibody repertoire profiling in IBD**

Over the past few decades, a variety of antibody responses have been profiled in IBD (Table 1), and novel technologies (Figure 2) have deepened our understanding of the exact antigens [3,38], bacterial species [56], and BCR sequence characteristics [60] involved. Despite these advances, several aspects of antibody repertoires in IBD remain incompletely characterized and, from a clinical perspective, recent findings have not been fully translated into direct benefits to patients.

While most biomarker discovery efforts in IBD have focused on antibody responses in blood [3,38] or systemic BCR repertoires [60], fewer studies have compared mucosal [56] versus systemic antibody binding. Comparing the exact microbial antigens bound in blood and fecal material from IBD patients with those in healthy individuals using high-throughput methods such as peptide arrays or PhIP-Seq could thus shed light on immune crosstalk beyond the gut barrier. In this respect, it might also be useful to analyze antibody classes and subclasses [66] separately in order to investigate potential differences in the antigens recognized. For example, while IgA dominates mucosal secretions, IgG is also detectable in the human intestines, with the IgG1 and IgG3 subclasses associated with inflammation compared with primarily IgG2 and IgG4 in healthy individuals [66]. Similarly, DNA sequence-based studies on BCR repertoires in CD have relied on peripheral blood mononuclear cells from the systemic circulation. Taking intestinal mucosal biopsies to obtain the resident B lymphocytes for BCRseq could thus provide novel insights. Ultimately, combining DNA sequence-based methods like BCRseq with functional antibody repertoire profiling methods such as peptide arrays or PhIP-Seq to detect the actual bound antigens could increase our understanding of the sequence–function relationship of antibodies, as well as improve diagnostics.

Overall, the novel experimental directions outlined earlier have substantial potential to advance the field toward clinical implementations (Figure 3; see Clinician’s corner). For example, these methods may expose novel immunological targets in IBD that, by combining the optimal set of antigens, could outperform currently existing diagnostic tools (e.g., commercially available serological antibody panels). Such panels of biomarkers could, in turn, improve our current disease classification by shifting from traditional consensus- or symptom-based approaches toward molecular-data-driven patient stratification. For each clinical outcome, specific tailor-made combinations of serological antibodies could be established by examining them in close association with specific patient traits.

In a similar fashion, in-depth characterization of serological antibodies could also be performed with the goal of predicting patient response to medical and surgical therapies in IBD. As most medical treatments modulate certain immune pathways (e.g., TNF-α-antagonists, anti-integrin inhibitors, JAK inhibitors) [67], combinations of specific antibody reactivities may confer predictive

**Clinician’s corner**

Inflammatory bowel diseases (IBDs), encompassing Crohn’s disease (CD) and ulcerative colitis (UC), are chronic immune-mediated inflammatory diseases of the gastrointestinal tract that are characterized by aberrant immune responses compared with healthy individuals.

Inadequate intestinal barrier function in IBD leads to uncontrolled passage of luminal antigens that would otherwise not be recognized by the immune system. This phenomenon largely explains the existence of serological antibody signatures in patients with IBD, in particular the formation of antimicrobial antibodies.

Well-established serological antibodies for IBD include anti-Saccharomyces cerevisiae antibodies (ASCA, mainly in CD) and perinuclear anti-neutrophil cytoplasmic antibodies (pANCA, mainly in UC), among others. Although these antibodies show strong associations to relevant clinical phenotypes and outcomes, their sensitivity is relatively low compared with their specificity, rendering their clinical utility rather limited. Serological testing is therefore currently not recommended for routine diagnostic assessment of IBD.

Recently, novel innovative antibody profiling technologies have been emerging that may expose novel immunological markers for IBD in a high-resolution, high-throughput fashion. Compared with conventional ELISAs, which can test dozens to hundreds of antigens, high-throughput methods such as peptide arrays, and PhIP-Seq can test thousands to hundreds of thousands of antigens in parallel.

These emerging antibody profiling technologies have the potential to transform into useful clinical applications, which would help to achieve a large number of clinically relevant endpoints in IBD. For example, selected combinations of serological antibodies could help improve disease subtyping and classification and the prediction of disease flares, disease progression, and therapeutic response (to both medical and surgical interventions). Furthermore, antibody signatures may be targets for (immuno)therapeutic
potential for this purpose. Likewise, the risk of the need for surgical interventions in IBD, the responsiveness to surgery, and/or the risk of post-surgical complications might be predicted in an early stage using data-driven and validated selections of serological antibodies. Simultaneously, identification of aberrant antibody responses in IBD may also open up opportunities for immunotherapy. As alluded to previously, patients with CD are marked by distinct antibody responses against bacterial flagellins, which have emerged as potential therapeutic targets. For instance, a

Figure 3. Potential clinical applications of antibody profiling in IBD. Serological antibody profiling could aid in the development of data-driven biomarker combinations for a variety of clinical outcomes in patients with IBD. Adding novel serological antibodies with proven discriminative capacity for IBD subtype (i.e., differentiating between CD and UC), disease behavior (e.g., differentiating between a fibrostenotic or penetrating disease phenotype), disease location or extent (e.g., differentiating between ileal-localized vs. colonic disease involvement in CD or left-sided colitis vs. pancolitis in UC), or the presence of perianal disease would be of great clinical value compared with current clinically driven classification systems such as the Montreal classification (1 and 2). Furthermore, novel serological antibodies could be investigated for their potential to discriminate between quiescent and active IBD or even their ability to forecast fluctuations in the ‘relapse–remitting’ disease course typically observed in IBD (3). Serological antibodies could also help predict individual responses to medical and surgical interventions in IBD (4 and 5). For this purpose, it is likely that combinations of specific antibody reactivities may confer predictive potential in the foreseeable future. Similarly, the risk of surgical interventions and/or the risk of post-surgical complications could be predicted in early phases using selections of serological antibodies. Finally, in-depth profiling of serological antibody responses may improve early detection of disease onset (prediagnostics), which may open up possibilities for early intervention or disease prevention (primary and secondary prevention strategies) (6). Abbreviations: CD, Crohn’s disease; IBD, inflammatory bowel disease; UC, ulcerative colitis.
recent study demonstrated that a combined treatment of cell activation and metabolic checkpoint inhibition (CAMCI) and a bioengineered flagellin peptide (multiple flagellin T cell epitopes, MEP1) resulted in ablation (cell death and anergy) of flagellin-reactive memory T lymphocytes [68]. Furthermore, this enhanced the corresponding regulatory subset of T lymphocytes in mouse models of colitis. Subsequently, this treatment was able to induce scavenging of pathogenic T lymphocytes in blood from patients with CD. Such an ingenious approach could be adopted to design and implement selective targeting of flagellin-reactive effecter T lymphocytes in patients with CD with high flagellin reactivity. This example illustrates that the identification of previously unknown antibody responses in patients with IBD could lay the foundation for targeted, personalized immunotherapy for specific subgroups of patients [69].

Exposure of novel immunological targets would not only improve our understanding of IBD immunopathogenesis, it could also facilitate the early detection of IBD (prediagnostics, Figure 3). To this end, earlier studies [14,16,26,36,37] could only use a selection of known serological antibodies, whereas emerging antibody profiling technologies have considerable potential to improve accuracy in predicting new-onset IBD. The detection of IBD in an early, preclinical stage would support the implementation of primary and secondary prevention strategies, for example, lifestyle modifications or dietary interventions, as well as early initiation of (intensive) medical treatment so long as aspects such as cost-effectiveness, feasibility, and risk/benefit ratios are favorable.

Concluding remarks
A combination of disrupted intestinal barrier integrity and loss of immunological tolerance is believed to underlie IBD pathophysiology, resulting in the formation of aberrant mucosal and systemic immune responses. These immune responses may be responsible for the initiation and perpetuation of intestinal inflammation in patients with IBD. Serological indicators of these responses may be utilized for clinical purposes by developing or improving diagnostic, therapeutic, and prognostic approaches. The recent advances reviewed in this article could improve our current understanding of disease pathophysiology while also providing a valuable resource of novel, previously unexposed immunological targets that may ultimately represent potent biomarkers in the context of IBD.

Given the recent technological advances in high-throughput human antibody profiling, serological characterization of antibody epitope repertoires in patients with IBD deserves further investigation, preferably in large prospective longitudinal cohort studies (see Outstanding questions). For instance, serological antibody-based signatures could be integrated into machine-learning algorithms while relying on detailed patient phenotypes to infer serological antibody signatures that are associated with a particular disease outcome. However, the main future challenge for such efforts will lie in the current complexity and impracticality of this approach in clinical practice. We should therefore strive to stimulate the parallel development of easy-to-use, robust, cost-effective clinical applications that incorporate these data-driven predictive signatures. For example, the development of arrays capable of assessing sets of key antibodies could become promising decision-support tools to aid treating physicians in disease management.

Antibody repertoire profiling represents a promising approach to improve our understanding of IBD immunopathogenesis and expose novel targets for disease diagnostics and management, potentially paving the way for preventive opportunities. To advance the field, we should prioritize large and carefully designed studies that take relevant patient characteristics and other layers of biological data into account (i.e., adopting a multi-omics strategy). This would enable the identification of personalized ‘immunological fingerprints’ for patients while also including relevant determinants, culminating in an immunology-driven precision medicine approach for IBD.

Outstanding questions
What is the exact pathogenic role of aberrant antibody responses in the pathogenesis of IBD?
What are the exact microbial or human proteins targeted by antibodies and to which functional consequences do these aberrant immune responses translate in patients with IBD?
How do systemic antibody responses in blood relate to mucosal antibody binding in the gut?
How do alterations on the DNA level of BCR sequences observed in IBD translate to functional differences in the antigens recognized?
What are the clinical implications of these antibody responses for patients with IBD?
Which of the state-of-the-art antibody profiling techniques discussed holds the most promise for translation into clinical implementations that might benefit patients with IBD?
What methods should be employed for the rational selection of antibody signatures tailored to predict the occurrence of a specific clinical outcome?
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Declaration of interests

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