

The love–hate relationship between bacterial polysaccharides and the host immune system

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Abstract | This article explores the fascinating relationship between the mammalian immune system and the bacteria that are present in the mammalian gut. Every human is an ecosystem that hosts 10^{13} – 10^{14} bacteria. We review the evidence that immunomodulatory molecules produced by commensal bacteria in the gut have a beneficial influence on the development of certain immune responses, through eliciting the clonal expansion of CD4⁺ T-cell populations. This process seems to contribute to the overall health of the host by offering protection against various diseases and might provide supporting evidence at a molecular level for the ‘hygiene hypothesis’ of allergic immune disorders.

The study of pathogenic microorganisms and the host response to infection has been central to the fields of microbiology and immunology for more than a century¹. The research of microbiologists Louis Pasteur and Robert Koch was driven by the goal of identifying the microbial agents that cause infections in humans. One hundred years after Koch received the Nobel Prize for the discovery of the aetiological mediator of tuberculosis, J. Robin Warren and Barry Marshall were awarded the Nobel Prize in Physiology or Medicine 2005 for their work on the pathogenic bacterium *Helicobacter pylori*, the causative agent of many gastric ulcers. Furthermore, ~30 Nobel Prizes have been awarded for work related to immunology. As a result of some of these studies, numerous molecular and cellular inflammatory responses to bacterial virulence factors are now well documented². From this information, it is evident that scientific investigations have been overwhelmingly focused on microbial pathogenesis and on immune responses to pathogens. However, bacterial infections are relatively rare and opportunistic, and most human encounters with bacteria involve benign microorganisms that are found in the environment or commensal microorganisms that live in the gut^{3–6}. These commensal microorganisms are the species with which we have co-evolved. It now seems that commensal microorganisms, unlike pathogens, might have a role in our development, physiological function and health.

All mammals are born sterile and are colonized subsequently by microorganisms⁷. Nearly every surface of mammals that is exposed to the environment is inhabited by commensal bacteria. There is no better

example of such a surface than the lower gastrointestinal tract, which contains an astounding number of bacteria^{8,9}. How — and, more importantly, why — does this immunocompetent environment allow these microorganisms to coexist in such high numbers? And what distinguishes these permanent and benign members of the gut microflora from pathogenic bacteria that induce inflammation? Recently, researchers have proposed that commensal bacteria have evolved in ways that improve the health of their hosts, and several microorganisms are being investigated for their beneficial potential: for example, as probiotics^{10–15}.

It has recently been reported that an immunomodulatory molecule that is expressed by commensal bacteria is involved in directing the development of a normal mammalian immune system¹⁶. *Bacteroides fragilis* produces a zwitterionic polysaccharide (ZPS) that activates CD4⁺ T cells and can correct certain immune defects, such as the reduced proportion of CD4⁺ T cells in the splenic lymphocyte population and the dysregulated systemic cytokine production that are found in the absence of bacterial colonization. So, in contrast to the virulence factors of pathogenic bacteria, which induce disease, the ZPSs of symbiotic bacteria have emerged as the archetypal members of a family of health-promoting microbial molecules known as symbiosis factors. Commensal bacteria have been implicated as crucial mediators of several physiological, metabolic and immunological functions of their mammalian hosts^{17–20}. Furthermore, human and animal studies have indicated that a gut microflora that contains the appropriate bacterial constituents helps to

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Box 1 | Structure and immunological activity of bacterial carbohydrates

Capsular and cell-wall-associated polysaccharides (such as teichoic acids) are composed of carbohydrates. Carbohydrates are also important constituents of lipopolysaccharides (LPSs), lipoteichoic acids and glycoproteins. The cell wall of all bacteria is composed of peptidoglycan, which is a polysaccharide that consists of repeating units of a disaccharide (*N*-acetylmuramic acid linked to *N*-acetylglucosamine) crosslinked by oligopeptides. LPS of Gram-negative bacteria consists of three distinct structural components: species-specific repeating units of a polysaccharide (the O antigen); a conserved core polysaccharide with a glycosidic linkage to the O antigen; and an immunogenic component with a ketosidic linkage to the core polysaccharide (lipid A). Many Gram-positive bacteria synthesize teichoic acids or lipoteichoic acids (which contain glycerol phosphate, ribitol phosphate or other sugar alcohols with phosphodiester linkages), which seem to be important in the attachment of bacteria to foreign surfaces and in their interactions with the innate and the adaptive arms of the immune system¹⁰⁴.

The structural diversity of bacterial polysaccharides is created by several features, including sugar (monosaccharide) composition, variations in glycosidic linkages between the sugars, enantiomeric form of the sugars (D or L form), number of carbon molecules that form a ring structure of the sugar (furanose or pyranose form) and configuration of the anomeric centre of each sugar (C α , in which the hydroxyl group of C1 is below the plane of the ring; or C β , in which the hydroxyl group of C1 is above the plane of the ring). Two distinct capsular polysaccharides from group B *Streptococcus* serotype Ia and serotype Ib provide an example of how minimal structural changes alter recognition by the immune system. Polysaccharides from both serotypes contain the same five sugars in each repeating unit and have the same linkages in all but one case (a galactose-to-*N*-acetylglucosamine linkage, which is a β 1-3 linkage in the polysaccharide from serotype Ib but a β 1-4 linkage in the polysaccharide from serotype Ia), yet these two molecules do not induce cross-protective antibodies¹⁰⁵. Each is recognized by the immune system as being immunologically distinct.

protect against allergy and asthma, a concept known as the 'hygiene hypothesis'^{21,22}. Therefore, microbial molecules such as ZPSs might interact with the host in a manner that has beneficial outcomes. In this article, we review the immunological research on the fundamental biological process of molecular symbiosis, and we discuss the implications of this previously underappreciated process, which is crucial for human health.

Immune responses to polysaccharides

Carbohydrate-containing structures are abundant at the surface of bacteria²³. Carbohydrates are components of several surface molecules — including lipopolysaccharides, teichoic acids and lipoteichoic acids, peptidoglycans and glycoproteins — that are expressed by Gram-negative bacteria and/or Gram-positive bacteria²⁴. Capsular polysaccharides consist of several hundred repeating units, and these repeating units contain one to eight sugars that are usually linked by glycosidic bonds. Variations in sugar composition, ring forms, linkage positions, anomeric-centre configurations, isomer forms and conformation all contribute to differences in the immunological epitopes that are present. These variations result in the generation of the huge diversity of structures that interact specifically with the immune system (BOX 1).

Studies that were carried out several decades ago in mice showed that carbohydrates are T-cell-independent antigens^{25–28} (FIG. 1a). Purified polysaccharides induce specific IgM responses, without a detectable IgG response. A failure to induce immunoglobulin class switching from IgM to most IgG isotypes (excluding IgG3) and a lack

of increased antibody production after rechallenge with antigen are hallmarks of a classic T-cell-independent immune response. The conjugation of polysaccharides to proteins seems to allow carbohydrate-specific responses that elicit T-cell help^{29,30}, and this technique has been harnessed to improve the efficacy of vaccines^{31,32}. The generation of glycoconjugate vaccines has been one of the greatest success stories in the biomedical sciences; the outcome is that, in immunized populations, infection with *Haemophilus influenzae* type b, *Streptococcus pneumoniae* (of the vaccine serotypes) and *Neisseria meningitidis* has been almost eliminated^{33,34}. The current view is that the protective responses to glycoconjugate vaccines are based on a mechanistic 'trick' played on the immune system³⁵. Presumably, a B cell that recognizes the carbohydrate takes up the glycoconjugate and presents a peptide from the attached carrier protein to T cells that recognize the peptide (FIG. 1b). Stimulation of the B cell (with consequent production of carbohydrate-specific antibody) and activation of the peptide-recognizing CD4⁺ T cell result in T-cell help, which promotes immunoglobulin class switching to IgG and memory responses. Immunoglobulin class switching and B-cell memory depend on co-stimulation of the B cell through CD80 and/or CD86 interacting with CD28, through CD40 interacting with CD40 ligand (CD40L) and perhaps through other interactions between co-stimulatory molecules.

ZPS-specific T-cell responses

ZPSs constitute a structurally distinct category of carbohydrates and seem to elicit immune responses that are unique among bacterial polysaccharides. As mentioned, unless conjugated to proteins, pure polysaccharides are considered to be classic T-cell-independent antigens (FIG. 1a,b). However, a series of recent investigations has found that ZPSs are immunomodulatory polysaccharides that specifically activate T cells. Seminal observations made ~30 years ago showed that intra-abdominal abscesses in patients recovering from surgery are induced by bacteria that are present in the normal microflora of the colon^{36,37}. Since the advent of modern surgery, clinicians and patients have been burdened with post-surgical complications. The formation of abscesses in the abdomen is one of the most common problems encountered after surgical procedures that involve the peritoneum. The most abundant microorganism isolated from such abscesses after surgery or physical trauma to the intestine is the Gram-negative obligate anaerobe *B. fragilis*, which is present in all mammals^{38,39}.

Immunological analysis has revealed that abscess formation in response to *B. fragilis* is a T-cell-dependent reaction⁴⁰. In the mid-1970s, an animal model for intra-abdominal abscess formation was established, in which the surgical implantation of pure cultures of *B. fragilis* and sterile caecal contents induced the formation of abscesses in laboratory rats^{41,42}. The ability to induce abscess formation was attributed to a high-molecular-weight capsular polysaccharide complex (CPC) at the surface of *B. fragilis*⁴³. However, it was soon shown that, although the CPC and sterile caecal contents together induced pathology, administration of the CPC alone

Immunoglobulin class switching

The somatic-recombination process by which the class of immunoglobulin expressed by naive B cells is switched from IgM to IgG, IgA or IgE on exposure to antigen.

Obligate anaerobe

An anaerobic organism can grow in the absence of oxygen. Obligate anaerobes die when exposed to atmospheric levels of oxygen, unlike facultative anaerobes, which can use oxygen when it is present.

Sterile caecal contents

A preparation of the cell-free material that is present in the caecum of the gastrointestinal tract. The preparation contains all of the soluble materials that are released by commensal bacteria but does not contain viable organisms.

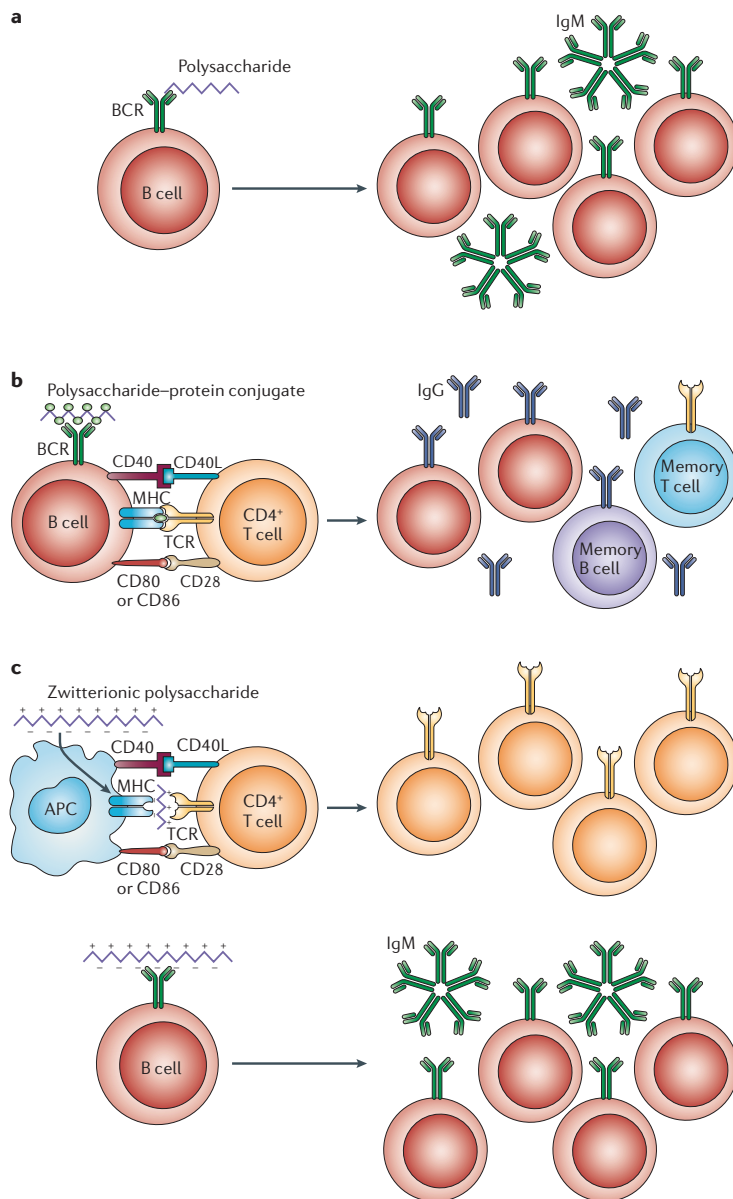


Figure 1 | Immune-cell activation by polysaccharides. **a** | Bacterial polysaccharides are classic antigens for B cells and are recognized by a B-cell receptor (BCR) that has the correct specificity. Interaction between the polysaccharide and the BCR is sufficient to induce the signals that are required to stimulate clonal expansion of B cells and antibody production. However, this pathway by itself does not result in immunological memory. **b** | Polysaccharide–protein conjugates interact with a BCR in a similar manner to pure polysaccharides; however, in addition, they elicit T-cell help, through antigen presentation of the protein component to CD4⁺ T cells, which provide the necessary co-stimulation to induce memory B cells and memory T cells. Therefore, antibody production is achieved, and the consequent immunological memory results in antigen-specific immunity to both the polysaccharide and the protein. This strategy has been exploited to produce pathogen-specific vaccines that target bacterial polysaccharides. **c** | Zwitterionic polysaccharides interact directly with CD4⁺ T cells in a manner similar to protein antigens. These polysaccharides are taken up by antigen-presenting cells (APCs), degraded and presented to T cells, leading to T-cell activation. In addition, zwitterionic polysaccharides elicit B-cell-dependent antibody responses similar to those elicited by conventional polysaccharides. CD40L, CD40 ligand; TCR, T-cell receptor.

protected animals against challenge with *B. fragilis* or the CPC^{44,45}. In addition, treatment with the CPC alone protected animals against challenge with other encapsulated bacteria that are present in the caecum. At the time of these findings, this result might have provided a glimpse into why *B. fragilis* is highly associated with intra-abdominal abscesses, whereas other commensal bacteria are not⁴⁵. However, a molecular understanding of the mechanisms that engendered this protection would be gained only after several more decades had passed.

In an investigation of the mechanism of protection against *B. fragilis* infection, transfer of serum antibodies from rats that had been immunized with *B. fragilis* CPC to unimmunized rats protected the recipients against infection with *B. fragilis* but not against the development of intra-abdominal abscesses^{46,47}. However, transfer of splenocytes from mice that had been immunized with *B. fragilis* CPC to unimmunized mice was sufficient to protect the recipients against abscess formation, indicating that cellular immunity was crucially involved^{48,49}. It was then found that the protective capacity could be transferred to unimmunized animals by the transfer of T cells alone⁵⁰. In addition, a low-molecular-weight soluble factor (now known to be interleukin-10 (IL-10); discussed later) that was produced by the T cells of CPC-immunized animals conferred protection against abscess formation⁵¹. In the early 1980s, some investigators (D.L.K. and colleagues) even suggested that the CPC of *B. fragilis* was inducing the activity of suppressor T cells⁴⁸. The proposed T-cell-mediated anti-inflammatory response is reminiscent of the function that has now been attributed to distinct subsets of regulatory T cells, particularly T regulatory 1 (T_R1) cells.

The most immunodominant constituent of the CPC is a molecule known as polysaccharide A (PSA), which was later shown to contain both a positive charge and a negative charge in each repeating subunit (the hallmark feature of ZPSs). In studies with highly purified PSA, transfer of CD4⁺ T cells from immunized animals to unimmunized animals provided protection against abscess formation³⁰. The CD4⁺ T cells isolated from animals that had been immunized with PSA had upregulated expression of the T-cell-activating cytokine IL-2. Further cellular evidence of T-cell activation came from *in vitro* studies showing that purified PSA induces the proliferation of CD4⁺ T cells cultured in the presence of antigen-presenting cells (APCs)⁵². Depletion of CD4⁺ T cells from a preparation of splenocytes resulted in a loss of PSA-induced proliferation. Furthermore, PSA induced the proliferation and activation of human CD4⁺ T cells but not CD8⁺ T cells, an observation that further indicated that specific T-cell populations were involved⁵³. Finally, the dogma that bacterial polysaccharides are T-cell-independent antigens met its strongest challenge when it was shown that PSA-induced proliferation required the presence of APCs⁵⁴. B cells — which are themselves APCs — can proliferate when appropriately stimulated by conventional polysaccharides. However, for CD4⁺ T cells to be activated by PSA *in vitro*, APCs were required. The crucial finding that APCs were required

for the induction of PSA-mediated CD4⁺ T-cell activation had a profound impact on our understanding of the biology of PSA and ultimately shed light on why a commensal bacterium might have evolved the ability to induce protective T-cell responses.

Is it possible that immunomodulatory molecules of symbiotic bacteria evolved to induce immune responses that are distinct from (or perhaps opposite to) those induced by virulence factors of pathogens? The protection conferred against abscess formation by ZPSs reflects the unique anti-inflammatory properties of these polysaccharides. An investigation into the mechanisms of this protection has recently shown that ZPSs induce CD4⁺ T cells to express the cytokine IL-10 (REF. 55), which has been shown to protect against inflammation in numerous *in vivo* and *in vitro* systems^{56,57}. In response to treatment with a ZPS, IL-10 was produced by a subpopulation of CD4⁺ T cells that are CD45RB^{low}. This heterogeneous subpopulation contains activated T cells, memory T cells and regulatory T cells (naturally occurring (CD4⁺CD25⁺) regulatory T cells). In addition, treatment of animals with a ZPS induced the clonal expansion and/or generation of CD45RB^{low} T cells. Furthermore, the production of IL-10 by these T cells was required for protection against both intra-abdominal abscesses and surgical fibrosis. It has also been shown that exposure to ZPSs induces the expression of MHC class II molecules and the co-stimulatory molecules CD80, CD86 and CD40L at the surface of APCs^{16,58}, and signalling through CD86 has been shown to promote the generation of IL-10-producing T cells^{59,60}. The soluble factor that mediates protection against abscess formation is therefore probably IL-10, and the suggestion, ~20 years ago, that ZPSs (then referred to as CPCs) induce suppressor (regulatory) T cells might ultimately hold true⁵¹. Perhaps ZPSs can inhibit other inflammatory pathologies that have been shown to be prevented by IL-10-producing regulatory T cells, such as inflammatory bowel disease (IBD) and asthma. Further investigations into this possibility will be crucial for determining the unique nature of immune responses to ZPSs and the mechanism of protection against inflammation.

Structure of ZPSs

It was initially thought that the CPC of *B. fragilis* consists of two high-molecular-weight molecules: PSA and PSB. However, genomic analysis has helped to determine the heterogeneity of the CPC of *B. fragilis*, showing that this bacterial species can express at least eight structurally unique polysaccharides from distinct genomic loci⁶¹⁻⁶³. This number of polysaccharides far exceeds the number that is expressed by any non-*Bacteroides* species that has been studied and is also greater than the number expressed by other *Bacteroides* species. However, biochemical analysis of the purified CPC of *B. fragilis* not only indicated the presence of multiple capsular types but also established that the unusual immune activity of the CPC resulted from its unique structure (compared with polysaccharides from other bacterial species). Subsequently, high-resolution nuclear magnetic resonance (NMR) studies revealed the chemical composition

of PSA and PSB, showing that these molecules have an unprecedented structure: each molecule has both positively and negatively charged motifs in each repeating unit⁶⁴. At that time (and at present), it was unusual for a bacterial polysaccharide to be shown to have any positive charges; most are either neutral or negatively charged. It was proposed that this unique structural feature might be crucial for the T-cell-activating property of PSA. This idea was eventually shown to be correct for both PSA and PSB. Chemical neutralization of either charged group abrogated the ability of PSA to protect animals against intra-abdominal abscess formation⁶⁵⁻⁶⁷. Ultimately, the protective activity was shown to depend on the zwitterionic structure of the polysaccharide, and it was shown that naive animals could be protected by transfer of CD4⁺ T cells that had clonally expanded in response to PSA⁶⁸.

PSA is the immunodominant capsular polysaccharide of *B. fragilis*. PSA consists of several hundred repeating units of a tetrasaccharide (FIG. 2a) and has a molecular weight of ~110 kDa⁶⁴. The three-dimensional structure of another ZPS, the highly related molecule PSA2 (from the clinical isolate *B. fragilis* 638R), shows several interesting features that are unique to this family of molecules and might be associated with their biological activity⁶⁹. The model that was determined for the preferred conformations of PSA2 (REF. 69) (with slight structural differences from PSA based on strain variation) is shown in FIG. 2b. In this study, the structure of PSA2 was described as a right-handed helix with two repeating units per turn and a pitch of 20 Å. The molecule is covered with positive and negative charges, and these charges alternate along the sides of the helical backbone and are exposed on the outermost surface of the molecule in positions that favour interactions with other molecules. This conformational model of PSA2 indicated plausible mechanisms for the interaction of ZPSs with other molecules. The 'groove-binding model' proposed by the authors of this study (D.L.K. and colleagues) provided an explanation for the T-cell-stimulatory activity of PSA⁶⁹. On the basis of computer-modelling studies, they proposed that PSA might form a complex with MHC by docking onto the α-helices that make up the lateral boundaries of the peptide-binding groove of MHC molecules (FIG. 2c). And, on the basis of these data, the idea was put forward that perhaps PSA, a pure polysaccharide, is displayed on MHC molecules and presented to the T-cell receptor (TCR) of CD4⁺ T cells, a mechanism that was previously thought to apply exclusively to protein antigens.

Antigen presentation of PSA

The finding that PSA initiates CD4⁺ T-cell responses in an APC-dependent manner raised an important question: what is the mechanism of antigen presentation in this case? MHC class I molecules are expressed in most tissues, and these molecules constitutively sample peptides that are generated by the normal turnover of proteins in the cytoplasm. MHC class II molecules are expressed by a subpopulation of dedicated (professional) APCs, including dendritic cells, macrophages and

Immunodominance

The result of an antigen(s) within a complex mixture (such as whole virus) or an epitope(s) within a protein being preferentially recognized during an immune response.

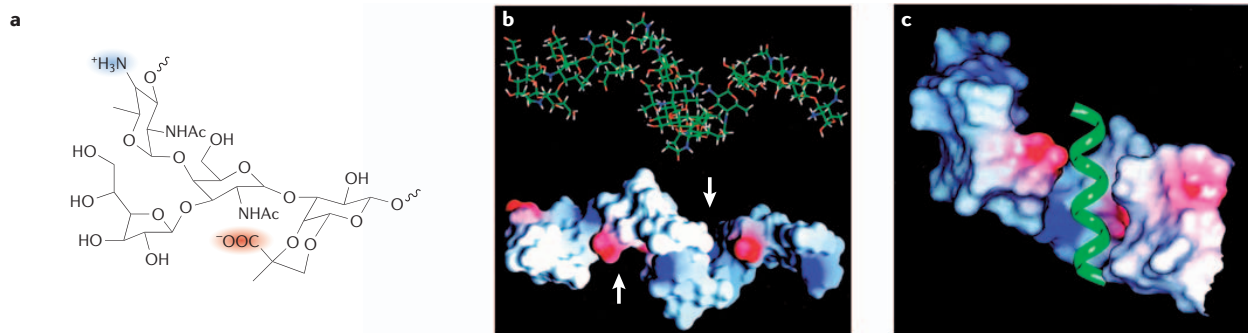


Figure 2 | Structure of polysaccharide A of *Bacteroides fragilis* and proposed interactions of polysaccharide A with MHC class II molecules. **a** | Polysaccharide A (PSA) from *Bacteroides fragilis* (strain NCTC 9343) is composed of several hundred repeating units of a tetrasaccharide. The schematic depicted here is based on chemical analysis and ^1H -nuclear magnetic resonance. Although all bacterial polysaccharides consist of repeating sugars of variable identity, the salient structural feature of PSA — which is a zwitterionic polysaccharide — is the presence of both a positively charged amino group (blue) and a negatively charged carboxyl group (red) in each repeating unit. **b** | A stick model (upper image) and an electrostatic surface representation (lower image) of four repeating units of PSA2 (a molecule that is highly related to PSA and is from *B. fragilis* str. 638R) are shown. Charges displayed at the surface of each repeating unit are shown in each model. In the upper image, carbon atoms are shown in green; oxygen, in red; nitrogen, in blue; and hydrogen, in white. In the lower image, positive charges are shown in blue, and negative charges are shown in red, illustrating the zwitterionic structure of PSA, which is essential for its activity. The two grooves present in each tetrasaccharide are indicated by arrows. **c** | A hypothetical model that shows a protein α -helix (green ribbon) bound to one of the grooves of PSA2 is shown. The protein α -helix could represent the α -helices that are found at the boundary of the peptide-binding groove of MHC class II molecules. The alternating charges at the edges of the groove of PSA2 could help to anchor the protein, forming several salt bridges. Alternatively, the complex could be stabilized by hydrophobic interactions along the inner surface of the groove. The images in part **b** and part **c** are reproduced, with permission, from REF. 69 © (2000) National Academy of Sciences, USA.

B cells⁷⁰. Could PSA be displayed on MHC molecules and recognized by T cells? It has been appreciated for several years that MHC-like molecules (for example, CD1) can present non-protein antigens, such as glycolipids from non-pathogenic or pathogenic bacteria, to effector T-cell-like cells (invariant natural killer T cells)^{71,72}. Therefore, it had been shown that there are forms of antigen presentation other than the display of peptides by MHC molecules. Until recently, however, speculation regarding the presentation of a pure polysaccharide by MHC molecules had been considered heresy. In experiments aimed at understanding the requirement for APCs in ZPS-mediated T-cell activation; however, it was noted that all three professional APCs supported *in vitro* T-cell proliferation induced by PSA⁷³. This activity required a physical association between the APC and the T cell. It was proposed that APC–T-cell interactions were mediated through MHC-class-II–TCR contacts induced by incubation with PSA. Consistent with this, experiments showed that, unlike wild-type B cells, a human B-cell-lymphoma line lacking in MHC class II molecules could not induce T-cell proliferation in response to incubation with PSA⁷³. Furthermore, antibody-mediated neutralization of various MHC haplotypes showed specific involvement of HLA-DR. Moreover, immunoprecipitation of HLA-DR molecules resulted in the isolation of PSA fragments from cells that had been incubated with PSA, showing that there is a physical interaction between a bacterial polysaccharide and an MHC class II molecule⁷³.

In addition to peptide–MHC-mediated T-cell activation, certain intact proteins, known as superantigens, that are produced by pathogenic bacteria can activate CD4⁺ T-cell responses through MHC class II molecules. Superantigens crosslink the TCR and MHC class II molecules by interacting with sites outside the peptide-binding groove of MHC class II molecules⁷⁴. However, a structural analysis of PSA has shown that PSA is not likely to activate CD4⁺ T cells in this manner, because it can be modelled to dock onto the α -helices of MHC class II molecules and into the peptide-binding groove.

So could these unique ZPSs be processed and presented in a manner similar to that documented for conventional protein antigens? Several lines of evidence indicate that this is the case⁷⁵. First, similar to protein antigens, PSA traffics through the endocytic pathway after internalization by APCs, colocalizing with the endosomal markers lysosomal-associated membrane protein 1 (LAMP1) and HLA-DM in a process that requires the polymerization of actin and microtubules and the acidification of endosomes⁷⁶. Second, intact proteins from extracellular pathogens (as well as self proteins) are proteolytically processed into peptides after endocytosis and are subsequently presented by MHC class II molecules⁷⁷. Similarly, the high-molecular-weight molecule PSA is processed into smaller fragments after internalization into endosomes. However, unlike the processing of peptides, the processing of PSA is a chemical reaction that involves oxidation and not proteolysis, as evidenced by *in vitro* cleavage by ozone (O_3) and by the absence

HLA-DM

An MHC-class-II-like molecule that facilitates the formation of high-affinity peptide–MHC complexes through the release of MHC-class-II-associated invariant-chain peptide (CLIP) in endosomes. The release of CLIP allows the binding of peptides derived from phagocytosed particles for presentation to CD4⁺ T cells.

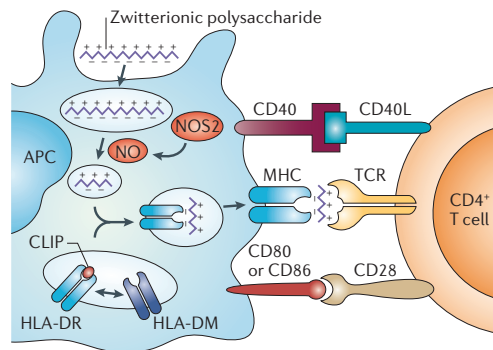


Figure 3 | Antigen presentation of polysaccharide A of *Bacteroides fragilis*. Polysaccharide A (PSA) of *Bacteroides fragilis* is internalized into the endosomes of professional antigen-presenting cells (APCs) as an intact polymer. After PSA enters the cell, it is degraded by an oxidation reaction (unlike proteins, which are processed by proteases in the endosome). This reaction requires the presence of nitric oxide synthase 2 (NOS2) and the generation of nitric oxide (NO), which mediates the oxidative breakdown of PSA into smaller fragments. After removal of the MHC-class-II-associated invariant-chain peptide (CLIP) by HLA-DM, PSA fragments are loaded onto the MHC class II molecule HLA-DR, which is present in the endosome. Endosome fusion and acidification are also required for PSA to be properly presented, in the context of MHC class II molecules, to the T-cell receptor (TCR) of CD4⁺ T cells, leading to the activation of these CD4⁺ T cells. PSA induces upregulation of expression of MHC class II molecules and co-stimulatory molecules (CD40, and CD80 and/or CD86) by the APC. The expression of these molecules and the interactions of CD40 with CD40 ligand (CD40L), and CD80 or CD86 with CD28, are required for the activation of CD4⁺ T cells by PSA.

of *in vivo* processing in mice that lack nitric-oxide synthase 2 (NOS2; also known as iNOS)⁷⁶. Furthermore, NOS2-deficient animals do not form abscesses when challenged with PSA plus sterile caecal contents unless the PSA is degraded *in vitro* before administration⁷⁶. Third, confocal-microscopy analysis revealed the presence of PSA at the immunological synapse between APCs and T cells⁷⁶; that is, at the site of engagement of the MHC class II molecule by the $\alpha\beta$ -TCR. Although biochemical and immunological analyses are still required, the finding that the TCR is required for responses to PSA (because $\alpha\beta$ -TCR-deficient mice do not develop adhesions in response to surgery) indicates that a tertiary complex between PSA, an MHC class II molecule and a TCR might mediate the activity of PSA⁷⁸. In MHC-class-II-deficient cells, PSA remains in intracellular compartments and cannot traffic to the cell surface⁷⁶. Last, PSA binds purified MHC class II molecules in a manner that requires the peptide-exchange factor HLA-DM. Taken together, these results indicate an alternative to the paradigm of protein-antigen processing and presentation, and they illustrate how polysaccharides can be presented by MHC class II molecules to activate CD4⁺ T-cell responses (FIG. 3). Therefore, the mechanism by which ZPSs mediate their effect on T cells is as unique as their structure.

PSA directs host immune-system development

Colonization of the gastrointestinal tract by commensal bacteria results in an increase in many of the biological processes of the host, including acquisition of nutrients and absorption of otherwise indigestible compounds from ingested food⁷⁹. In addition, development of the gut-associated lymphoid tissue is defective in the absence of bacterial colonization⁸⁰. Could PSA of *B. fragilis* have evolved to mediate a beneficial effect on the host? Initially, to investigate immune responses to symbiotic bacteria, the development of various aspects of the immune system in germ-free mice and in mice that had been colonized conventionally with commensal bacteria was compared⁸¹. Analysis of splenocytes from these mice showed that the proportion of CD4⁺ T cells was lower in germ-free mice than in conventionally colonized mice^{16,82}. The proportions of other lymphocyte populations — CD8⁺ T cells and B cells — were similar regardless of the bacterial microflora of the laboratory animals⁸³. Therefore, in mice, the absence of colonization with commensal bacteria results in cellular defects in extra-intestinal lymphoid tissues. Furthermore, the ultrastructural development of the lymphoid organs was affected in germ-free mice, which had smaller and more fragmented splenic lymphoid follicles than did conventionally colonized animals¹⁶.

In an assessment of the contribution of a symbiotic microorganism to the development of the host immune system, *B. fragilis* was introduced into germ-free mice, and various immunological parameters were measured. Remarkably, colonization with this single bacterium, in the absence of the hundreds of other microorganisms of the gut microflora (including bacteria, viruses, fungi and protozoa), was sufficient to correct the CD4⁺ T-cell defect in the spleens of germ-free animals¹⁶. However, a *B. fragilis* mutant that lacked the ability to produce PSA was unable to correct either the defect in the proportion of splenic CD4⁺ T cells or the defect in lymphoid-follicle development. Furthermore, administration of purified PSA to germ-free mice increased the proportion of splenic CD4⁺ T cells to the proportion found in conventionally colonized animals¹⁶. As had been predicted, CD4⁺ T cells from germ-free animals mounted responses that were skewed towards the T helper 2 (T_H2)-cell lineage. These CD4⁺ T cells produced more of the prototypical T_H2 cytokine IL-4 than did CD4⁺ T cells from mice that had been conventionally colonized^{84,85}. Colonization with *B. fragilis* that expressed PSA restored a normal balance of cytokines, whereas colonization with a mutant strain that was defective in production of PSA did not¹⁶. It seems, therefore, that PSA expressed by bacteria during colonization stimulates the expression of the T_H1 cytokines interferon- γ (IFN γ) and IL-2 by host CD4⁺ T cells. In addition, purified PSA induces the expression of IFN γ *in vitro*, through induction of the specific T_H1-cell-lineage-determining cytokine IL-12 and through the transcription factor signal transducer and activator of transcription 4 (STAT4)⁸⁶. Taken together, these results show that PSA of *B. fragilis*, a ubiquitous symbiotic bacterium in all mammals, is necessary and sufficient to mediate the generation of a normal mature immune system (FIG. 4).

Immunological synapse
A region that can form between two cells of the immune system that are in close contact. This region was named the immunological synapse because of similarities to the synapses that occur in the nervous system; it originally referred only to the interaction between a T cell and an antigen-presenting cell. The immunological synapse involves adhesion molecules, as well as antigen receptors and cytokine receptors.

Gut-associated lymphoid tissue
(GALT). The tissues and cells that constitute the immune system associated with the gastrointestinal tract. This system includes structures such as the Peyer's patches, inducible lymphoid follicles, cryptopatches and the mesenteric lymph nodes, as well as circulating and non-circulating immune cells of the lamina propria, and intra-epithelial lymphocytes.

Germ-free mice
Animals that are born and raised in sterile isolator chambers and are devoid of colonization by any foreign microorganisms, including bacteria, viruses, fungi and protozoa. The experimental colonization of animals with known microorganisms (an approach that is known as gnotobiology) allows the effect of a specific microorganism on the biological functions of an animal to be investigated.

Lymphoid follicles
Anatomical zones in the primary or secondary lymphoid tissues that contain aggregates of lymphocytes, mainly B cells, which are surrounded by T cells. Follicles might also contain structures known as germinal centres, which are areas of proliferating and differentiating B cells.

We have reviewed the evidence indicating that *B. fragilis* is a symbiotic microorganism that has evolved the ability to promote maturation of the immune system of its mammalian host. In return, the host not only tolerates colonization by this microorganism but also seems to ‘welcome’ it, providing the bacteria with an ecological niche for colonization and growth. From this viewpoint, there is a mutually beneficial relationship between *B. fragilis* and its host. However, the beneficial effect of *B. fragilis* on the host immune system was initially found and investigated as a consequence of its pathogenic effects. So how can the beneficial and abscess-inducing (that is, pathogenic) effects of *B. fragilis* and PSA (plus sterile caecal contents) be reconciled?

It must be appreciated that *B. fragilis*, unlike many other commensal microorganisms, resides exclusively in mammalian hosts and has no other ecological niche⁸⁷. If the physical trauma that follows surgery and leads to a breach in the colon and the introduction of commensal microorganisms into the peritoneum is viewed as an affront not only to the host but also to the life cycle of these microorganisms, then perhaps an alternative view of abscess formation can be considered. *B. fragilis* clearly influences the immune response that is aimed at neutralizing most, if not all, other colonic microorganisms and therefore at preventing serious or even life-threatening bacteraemia after intestinal trauma. It has recently been shown that other *Bacteroides* species induce the expression of antimicrobial molecules that directly bind and eliminate potentially pathogenic bacteria⁸⁸. It is conceivable that *B. fragilis* evolved the ability to induce abscesses not to harm the host but to protect it from these other microbial insults. Our clinical point of view might be that abscesses are harmful, but from the perspective of the bacterium, the induction of abscesses might be a way to protect its habitat. The potential of *B. fragilis* to function as an abscess-inducing pathogen might only be realized following anatomical displacement of the bacterium from the intestinal lumen and breach of the mucosal barrier. So the position of *B. fragilis* in the pathogen–commensal–bacterium continuum might depend on which side of the mucosal barrier it interacts with the host immune system. However, the evolutionary reason for the induction of abscesses by this microorganism remains to be determined. Therefore, the millennia of co-evolution of *B. fragilis* and mammals, together with the possible capacity of this symbiotic bacterium to preserve its habitat, should be considered in our re-evaluation of the view that bacteria are, in essence, harmful.

We therefore propose that the intricate and intimate relationship between *B. fragilis* and the host immune system has evolved in ways that we are just beginning to understand and that what seems to be a harmful consequence of a bacterium–host association (that is, abscesses) might prove to be essential for our preservation and long-term health. Moreover, the view that all of the genetic programming that is required for immunological fitness is intrinsic to humans must be reconsidered in light of the apparent evolution of humans to require simple microorganisms for development of the immune system.

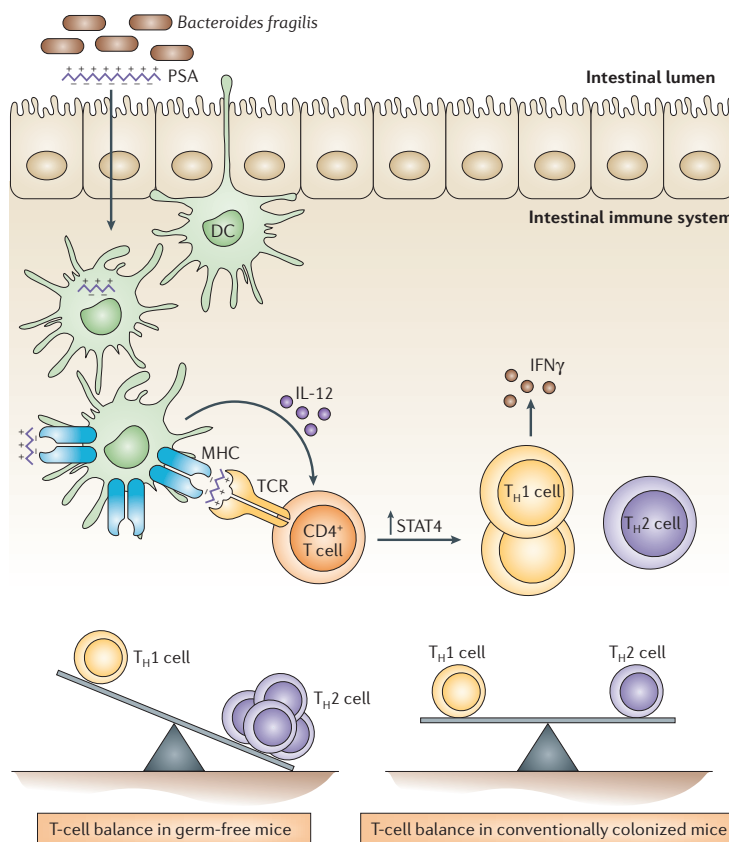


Figure 4 | Impact of polysaccharide A of *Bacteroides fragilis* on development of a mature mammalian immune system. *Bacteroides fragilis*, an abundant member of the colonic microflora of all mammals, produces the immunomodulatory polysaccharide PSA (polysaccharide A). PSA seems to be sampled by dendritic cells (DCs) of the gut-associated lymphoid tissue, and these DCs then migrate to the mesenteric lymph nodes and initiate T-cell responses. PSA is processed in the DCs and is presented to the T-cell receptor (TCR) of CD4⁺ T cells in the context of MHC molecules. The immune system of germ-free mice is highly skewed towards a T helper 2 (T_H2)-cell phenotype, and colonization with PSA-producing *B. fragilis* corrects this immune defect. This correction is achieved through the PSA-induced secretion of interleukin-12 (IL-12) by DCs, leading to activation of the T_H1-cell differentiation factor STAT4 (signal transducer and activator of transcription 4) in CD4⁺ T cells. In response to IL-12 and STAT4, T_H1 cells are generated and produce interferon- γ (IFN γ), and this process corrects the cytokine imbalance that is found in the absence of bacterial colonization.

Future directions: ZPSs and human health

The mammalian immune system has evolved elaborate mechanisms to prevent or suppress inflammation in response to self antigens, and these mechanisms have been extensively studied for decades⁸⁹. By contrast, the mechanisms by which a host controls responses to foreign non-pathogenic particles and molecules — such as commensal bacteria, food and inhaled antigens — remain less well understood^{90,91}. Commensal bacteria might provide instruction for the development of the host immune system. If this is the case, then the developmental signals provided might be crucial for establishing

Box 2 | Explaining the hygiene hypothesis?

Epidemiological and clinical data show that there has been a large increase in the incidence of allergic and atopic disorders in the past few decades, most markedly in developed countries. David Strachan²¹ explained this phenomenon by proposing an idea that is now known as the hygiene hypothesis: this hypothesis states that decreased exposure to infectious agents early in life (owing to antibiotic use, vaccination and improved sanitation) results in aberrant immune responses to otherwise innocuous antigens later in life. A modified view of this hypothesis — the counter-regulation model — was subsequently proposed: this model states that lack of exposure to immunomodulatory molecules of commensal microorganisms contributes to altered immune-system development⁹⁵. Several recent reports have implicated differences in the composition of the gut microflora between individuals who suffer from allergies and those who do not. This observation prompted Gary Huffnagle and Mairi Noverr²² to put forth the microflora hypothesis: this hypothesis states that a 'balanced' microflora contributes positively to human health, whereas perturbations in the numbers and the species of gut bacteria, owing to lifestyle changes in recent decades, predispose certain individuals to allergic disorders.

The immune system is responsible for mounting responses that are appropriate to the potential for 'danger' of any given molecule. This issue is particularly important at mucosal surfaces, where the immune system must constantly control responses to environmental antigens. Given that significant increases in the incidence of allergic disorders (such as inflammatory bowel disease, asthma, dermatitis and food allergies), but not autoimmune diseases, have been reported, the immune deviations that are imposed by the lifestyle changes of certain societies in recent decades seem to have affected tolerance at mucosal sites. We therefore propose that immunomodulatory molecules of commensal bacteria, such as polysaccharide A of *Bacteroides fragilis*, promote the development of immunological tolerance to foreign antigens and that perhaps a lack of exposure to these natural (non-pathogenic) molecules subsequently results in allergic disorders. From this viewpoint, there must be divergent mechanisms to control responses to self and non-self antigens. The positive and negative selection of T cells, including naturally occurring (CD4⁺CD25⁺) regulatory T cells, in the thymus explains the absence of autoreactivity to self antigens. The mechanisms that mediate tolerance to acquired (non-self) antigens remain less well defined.

immunological health in mammals^{22,92}. Indeed, a recent report has shown that *Bacteroides thetaiotaomicron*, a species that is related to *B. fragilis*, protects against intestinal inflammation in animals, although the bacterial molecules that mediate this process are unknown⁹³. In addition, the commensal bacterium *Lactobacillus plantarum* induces production of the protective cytokine IL-10 by cells isolated from patients with IBD⁹⁴. These and many other examples of beneficial microbial activities have gained attention for the possibility that probiotic approaches might remedy human diseases. However, it is not known whether the absence of specific commensal bacterial species from the microflora predisposes an individual to health problems.

In the past two decades, it has been suggested that a reduction in exposure to infectious bacterial agents early in life, as a result of improved sanitation and antibiotic use, explains the increased incidence of allergy among residents of developed countries, a concept known as the hygiene hypothesis^{21,95} (BOX 2). Substantial evidence accumulated in the past 40 years indicates that the incidence of asthma, atopy (predisposition to allergy) and IBD has increased markedly in developed countries but not in developing countries. Several epidemiological studies have indicated that the composition of the gut microflora differs between atopic individuals and non-atopic individuals^{96,97}. Analyses of intestinal bacterial speciation showed that children with allergies who live

in a developed nation (Sweden) or a developing nation (Estonia) had lower rates of colonization by *Bacteroides* species and higher rates of colonization by aerobic bacteria than did children from either region who did not have allergies. Furthermore, anthroposophical children (who refrain from antibiotic use) developed atopy at significantly lower rates than did control children, even in the same schools⁹⁸. The composition of the gut microflora differed between these groups of children and correlated strongly with the onset of disease. Therefore, deviations in composition of the gut microflora as a result of modern lifestyles could be the determining environmental factor that underlies the development of atopy and asthma in genetically predisposed individuals.

In the past year, the Global Allergy and Asthma European Network (GA²LEN) has declared hay fever to be present on an epidemic scale in some regions of Europe, including Great Britain, where more than one-third of all residents report symptoms⁹⁹. Asthma and allergies such as hay fever are immune disorders that are mediated by the overproduction of T_H2 cytokines and IgE, as well as by T_H2-cell responses to the allergen¹⁰⁰. Medical expenditure for the treatment of asthma has risen substantially in the past two decades¹⁰¹. In this Review, we have discussed the evidence indicating that colonization with the ubiquitous commensal bacterium *B. fragilis* corrects the systemic T_H2-cell bias that is found in the absence of bacterial colonization of mice, through inducing the specific production of T_H1 cytokines. Because germ-free mice, similar to atopic patients with an abnormal composition of gut microflora, have a cytokine profile that is highly skewed towards T_H2 cytokines¹⁰², it is possible that the widespread antibiotic use and vaccination of children that occur in developed countries, together with improved sanitation, lead to the clearance of symbiotic bacteria such as *B. fragilis* at an essential time in immune development, resulting in the absence of molecules such as PSA. The aberrant development of the immune system that occurs without specific direction by this class of immunomodulatory molecule might lead to the overproduction of T_H2 cytokines and to the onset of atopic and asthmatic disorders. However, further studies are required to reconcile the role of PSA-induced IL-10 production in determining the balance of T_H1 and T_H2 cytokines and protection against disease. One possibility is that PSA induces the differentiation of CD4⁺ T cells that produce both IFN γ and IL-10, similar to those T cells described to protect against experimental asthma¹⁰³.

Various distinct immune mechanisms have evolved to control aberrant and unwanted immune responses to 'innocuous' antigens. The source of the antigen (whether self or non-self) might be a key determinant of the response that is generated. The association between microorganisms and the marked increase in the incidence of IBD, asthma and atopy in the past few decades indicates that there is a connection between changes in the environment and immune responses to foreign (but not self) antigens. IBD results from an inflammatory response to antigens from commensal bacteria in the gut; asthma results from inflammation in response

to inhaled antigens. The immunological basis for the hygiene hypothesis might be that there is a breakdown in self-tolerance in the mucosae. It is conceivable that the development of tolerance to environmental antigens might require immune mechanisms that are mediated by symbiotic bacteria at mucosal surfaces. Perhaps identifying the precise molecular interactions that occur between ZPSs and the immune system during immune-system development will lay the foundation for a comprehensive mechanistic understanding of these two distinct arms of

immunological tolerance: that is, tolerance to self antigens and tolerance to non-self antigens. Similar to the way in which the study of bacterial pathogenesis has resulted in great medical advances in the control of infections, research into symbiotic bacteria promises to improve our understanding of the basic biological functions of humans. Furthermore, understanding the beneficial relationships between symbiotic bacteria and the host immune system might lead to new therapies for non-infectious diseases, including autoimmune diseases and cancers.

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Competing interests statement

The authors declare no competing financial interests.

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