Pathogen manipulation of B cells: the best defence is a good offence

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Abstract | B cells have long been regarded as simple antibody production units, but are now becoming known as key players in both adaptive and innate immune responses. However, several bacteria, viruses and parasites have evolved the ability to manipulate B cell functions to modulate immune responses. Pathogens can affect B cells indirectly, by attacking innate immune cells and altering the cytokine environment, and can also target B cells directly, impairing B cell-mediated immune responses. In this Review, we provide a summary of recent advances in elucidating direct B cell–pathogen interactions and highlight how targeting this specific cell population benefits different pathogens.

Immunoglobulin
A large, Y-shaped protein that is produced by B cells. Immunoglobulins exist in a membrane-bound form as B cell receptors or are secreted as antibodies.

Plasma cells
Finally differentiated B cells that have the main function of producing and secreting antibodies. Each plasma cell produces antibodies of one specificity. They can be functionally distinguished by the antibody isotype and their half-life.

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B cells are essential components of the adaptive immune system, and they were first defined and distinguished from T cells almost 50 years ago[1]. Both B cells and T cells recognize pathogens with antigen-specific receptors, but they differ in their developmental pathways and functions during infections. T cells differentiate in the thymus and orchestrate immune responses as $CD4^+$ helper or $CD8^+$ cytotoxic T cells. B cell development occurs in the bone marrow with gene recombination in the immunoglobulin locus, which results in the surface expression of a unique B cell receptor (BCR)[2]. Naive B cells exit the bone marrow, seed the bloodstream and peripheral immune organs and, following exposure to antigens, differentiate into plasma cells or memory B cells (FIG. 1). Plasma cells contribute to the recovery from primary infection by secreting antigen-specific immunoglobulins, termed antibodies. Antibodies aid in the clearance of invading pathogens by direct neutralization, by activating the complement cascade or by interacting with other immune cells — which is often mediated by binding to Fc receptors. Memory B cells are long lived and can quickly be reactivated to differentiate into plasma cells following secondary infection. Together, memory B cells and long-lived plasma cells form the basis for life-long B cell-mediated protection against infections. The importance of B cells in ensuring protective immunity to reinfection is highlighted by mass vaccination; the generation of antigen-specific antibodies is the hallmark of most efficient vaccines developed to date[3].

During immune responses, B cells are directly activated by invading microorganisms, either by detecting a specific antigen through their BCR or by detecting pathogen-associated molecular patterns (PAMPs) through general pattern recognition receptors (PRRs)[4–6] (FIG. 1). The activation of several B cell clones (termed polyclonal B cell activation) and their subsequent differentiation into short-lived plasma cells results in the production of low-specificity antibodies, which are generally associated with the beneficial effects of an early weakening of infections[7]. Also, B cell maturation following antigen recognition can take place in organized lymphoid structures called germinal centres (GCs). In GCs, activated B cells integrate several immune signals — including cytokines, such as interleukin-21 (IL-21) and IL-4, which are released by follicular T cells and dendritic cells (DCs) — and the B cells then enter a selection process. Furthermore, B cells present processed antigens on major histocompatibility complex class II (MHC II) molecules, which can be recognized by cognate T cells. Interactions with helper T cells result in the activation of co-receptors on the B cell surface, such as CD40, which binds to its ligand CD40L, which is expressed by T cells. Sustained B cell activation leads to B cell proliferation and the upregulation of the enzyme activation-induced cytidine deaminase (AID), which, in turn, induces somatic hypermutation (SHM) and class-switch recombination (CSR) in the immunoglobulin locus (BOX 1). SHM results in affinity maturation of selected B cell antibodies, and CSR defines their effector functions (BOX 1). Fully differentiated effector B cells circulate in the blood or migrate to effector sites, such as mucosal tissues or the bone marrow. Thus, B cell maturation in GCs results in the generation of specific, long-lived plasma cells and memory B cells that circulate in the blood or migrate to effector sites and thereby confer protective immunity (FIG. 1).

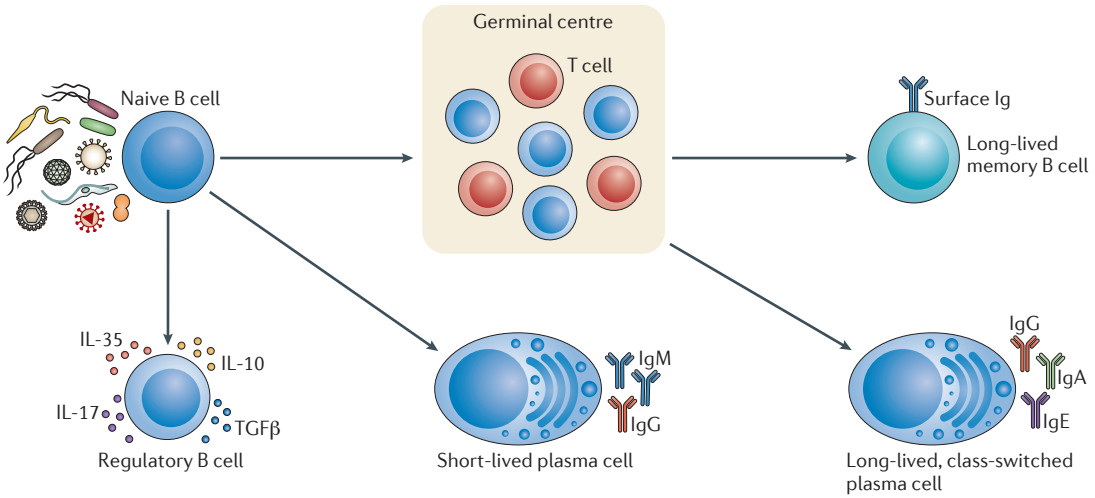

Figure 1 | **B cell responses to infection.** In response to activation signals, naive mature B cells proliferate and differentiate into effector cells. B cell activation results from the integration of several infection-related signals, including binding of specific antigens to the B cell receptor (BCR) and pattern recognition receptor (PRR) ligands[4–6]. In an early polyclonal response, short-lived plasma cells that secrete polyreactive antibodies can be generated[7]. Regulatory B cells can also be induced and exert an immunosuppressive function by secretion of interleukin-10 (IL-10), IL-17, IL-35 and transforming growth factor-β (TGFβ), which modulate T cell responses[12,13]. Sustained B cell activation leads to further differentiation and selection in organized lymphoid structures, called germinal centres (GCs). This occurs through cytokine signalling and the interaction between CD40 on B cells and CD40 ligand on cognate T cells (not shown). The activation of nuclear factor-κB (NF-κB) and upregulation of activation-induced cytidine deaminase (AID) induce affinity maturation of antibodies through somatic hypermutation and class-switch recombination of the antibody heavy chain[99–101]. This ultimately results in the differentiation of specific, long-lived plasma cells and memory B cells, which confer protective immunity. Ig, immunoglobulin.

Memory B cells
Finally differentiated B cells that are long-lived and can be quickly reactivated to differentiate into antibody-secreting plasma cells following secondary exposure to their specific antigen.

Fc receptors
These receptors are expressed by several innate immune cells and bind to the constant region of antibodies. Antibody binding can result in phagocytosis of immune complexes or stimulation of cell-mediated cytotoxicity.

Pathogen-associated molecular patterns
(PAMPs). Molecules that are expressed by groups of pathogens and are recognized by a range of pattern recognition receptors on immune cells.

Pattern recognition receptors
(PRRs). Proteins that are expressed by immune cells and recognize a range of pathogen-associated molecular patterns.

Germinal centres
(GCs). Lymphoid structures that are formed during an immune response and that are dedicated to the maturation and continuous selection of B cells. T cells and dendritic cells cooperate with B cells in GCs to generate protective immunity.

Somatic hypermutation
(SHM). A molecular process that results in the introduction of selected mutations in immunoglobulin variable regions, thereby adapting antibodies to their respective antigen by increasing affinity and specificity.

Class-switch recombination
(CSR). A molecular process used to change the constant region of the immunoglobulin heavy chain, thereby changing antibody effector functions but not affecting the antigen-binding site.

Although the production of antibodies is a central feature of B cells, recent studies have revealed antibody-independent mechanisms of immune regulation mediated by B cells, such as the alteration of cytokine responses or through their involvement in antigen presentation to $CD4^+$ T cells[8–10]. Indeed, B cells are now becoming known as key players in both innate and adaptive immune responses. Recently, particular attention has been given to regulatory B cells, which were initially reported to have an immunosuppressive function by secreting anti-inflammatory cytokines such as IL-10 and transforming growth factor-β (TGFβ)[11,12] (FIG. 1). The secretion of IL-10 results in the suppression of helper T cell responses and innate immune cell responses[13,14]. The importance of regulatory B cells was highlighted in mouse infection models, in which B cell deficiency correlated with enhanced resistance to infection, suggesting that these cells suppress protective immune responses[15,16]. Interestingly, regulatory B cells seem to correspond to several cytokine-secreting plasma cells and are not limited to the secretion of IL-10; B cell-produced IL-17 and IL-35 can contribute to immune-regulation and immune-suppression, respectively[17–19].

Considering the diverse roles of B cells during infection, it is not surprising that manipulation of this lymphocyte population provides a selective advantage for pathogens. Indeed, some pathogens have broad effects on B cell responses in both animal models and humans. For example, by comparing immune responses to malaria infection and tetanus vaccination, it has been shown that *Plasmodium falciparum*-specific memory B cells and antibodies are only acquired gradually, which contrasts with the rapid development of the tetanus-specific B cell response and suggests that the development of B cell-mediated immunity to malaria is impaired[20]. However, gaining mechanistic insight into how B cell responses are skewed by pathogens remains challenging. Nonetheless, reports of direct interactions of pathogens with B cells have become more frequent and are starting to elucidate the mechanisms by which B cell responses are directly diverted by different pathogens. Here, we review different interactions between pathogens and B cells and highlight how bacteria, viruses and parasites directly manipulate B cells to subvert immune responses and promote pathogen survival.

B cells as a pathogen reservoir

Viruses and intracellular bacteria are capable of infecting and persisting within cells, raising the possibility that B cells could be a reservoir for some pathogens. Indeed, B cells are targeted during certain viral infections, a role that was first discovered through the study of B cell lymphomas. Furthermore, B cells can also provide an infection niche for intracellular bacteria.

B cells as viral reservoirs. Infection of B cells by viruses (TABLE 1) has long been associated with the direct suppression of protective B cell responses, which is exemplified by both viruses responsible for persistent infections, such as cytomegalovirus, and viruses that cause acute infections, such as the measles virus[21–23]. However, the

Box 1 | **Immunoglobulin maturation and functions**

Immunoglobulins are composed of two heavy and two light chains, and mediate B cell functions as components of the B cell receptor (BCR) in a membrane-bound state or as secreted antibodies. Functionally, they can be categorized into variable domains that bind antigens and constant domains that define their effector functions.

During development, a unique BCR is generated for each B cell by gene recombination. Only cells recognizing their cognate antigen in the periphery initiate further differentiation and antibody production. Two main mechanisms are involved in antigen-dependent maturation: somatic hypermutation (SHM) and class-switch recombination (CSR). The key enzyme for both processes is activation-induced cytidine deaminase (AID), which is upregulated following sustained activation of B cells[98].

SHM occurs in the variable regions of the immunoglobulin loci. AID initiates deamination of cytidine to uracil on single-stranded DNA and thereby generates a mismatch between the newly formed uracil and guanine on the complementary DNA strand. This mismatch is resolved through various DNA repair mechanisms, often leading to the introduction of base-pair mutations and subsequent replacements of amino acids in the translated immunoglobulin protein. These mutations can have positive or negative effects on antigen recognition and affinity, and they are continuously evaluated by selection in germinal centre (GC) reactions. B cells that cannot recognize antigens undergo apoptosis, whereas B cells with mutations that increase antigen affinity survive and proliferate. SHM in the variable regions is thus associated with antigen-dependent affinity maturation of antibodies[99,100].

AID also initiates the switch of the constant regions of the immunoglobulin heavy chain (IgH), which defines the antibody isotype. The *IGH* locus contains multiple constant-region-encoding genes. In naive mature B cells, variable domains are spliced to *IGHM* (which encodes the constant region of IgM), resulting in the expression of IgM. Upstream of each constant region are switch regions, which contain multiple AID hotspot motifs. Upon deamination of cytidines in these hotspots, two abasic sites on opposite DNA strands can occur in close proximity and result in double-strand breaks. The simultaneous induction of double-strand breaks in different switch regions can lead to DNA recombination. The intervening DNA is excised and the variable regions are coupled to the respective downstream heavy-chain constant region. CSR of the immunoglobulin constant regions thus results in a change of the antibody isotype from IgD or IgM, which are expressed by naive B cells, to IgG, IgA or IgE, which are expressed by antigen-experienced B cells[101]. The different constant regions mediate distinct functions. As the first immunoglobulin expressed during B cell development, IgM displays low antigen affinity but is efficient in opsonizing (coating) antigens for destruction by multimeric interactions. IgG is the main antibody isotype found in serum, and has complement-activating and pathogen-neutralizing abilities. Secretory IgA is crucial at protecting mucosal surfaces by means of direct neutralization of toxins or pathogens, or by preventing their binding and tissue invasion. The constant domain of IgE binds with high affinity to mast cells and neutrophils, and is associated with hypersensitivity and allergic reactions[102].

best-known virus targeting B cells is Epstein–Barr virus (EBV), against which protective antibodies are efficiently generated.

Entry of EBV into B cells is mediated by the viral glycoprotein gp350, which binds to CD21 on the B cell surface, and by gp42, which interacts with surface MHC II molecules, triggering endocytosis and subsequent fusion with the endocytic membrane. By contrast, EBV entry into epithelial cells is mediated by direct fusion with the cell membrane and is impeded by the viral gp42 protein[24]. Notably, the gp42 complexes are degraded in the B cell endoplasmic reticulum, resulting in the generation of viral particles that express low numbers of these molecules and are more infectious for epithelial cells[25]. EBV is thus able to modify its cellular tropism and potentially uses B cells to facilitate dissemination. More importantly, EBV has been reported to transform infected B cells into long-lived resting memory B cells[26,27]. This mechanism occurs in parallel with the evolution of EBV into latency and presumably allows the virus to hide from antibody-mediated immune responses and immune surveillance, while persisting in the host.

Another virus that infects B cells is mouse mammary tumour retrovirus, which predominantly infects B cells in gut-associated lymphoid structures and can disseminate from these structures[28]. Dissemination is facilitated by the presentation of a viral superantigen to T cells, which results in nonspecific polyclonal T cell activation. Superantigen-activated helper T cells activate infected B cells independently of cognate interaction, inducing B cell proliferation and leading to the establishment of a reservoir of infected lymphocytes in mouse infections[29–31].

Similarly, the polyoma JC virus infects human B cells *in vitro* and *in vivo*, and has been suggested to rely on these cells as a vehicle to disseminate to the brain, possibly by using B cells to cross the blood–brain barrier[32–34].

B cells as bacterial reservoirs. Gram-negative *Brucella* spp., *Moraxella* spp., *Salmonella* spp., *Yersinia* spp. and *Shigella* spp., as well as Gram-positive *Listeria* spp., are examples of facultative intracellular bacteria that cause a variety of diseases, including infections of the respiratory and gastrointestinal tracts. Similarly to viruses, these bacteria can infect B cells (TABLE 1). Interestingly, some bacteria show a preference for particular B cell populations. For example, *Brucella abortus* preferentially targets marginal zone B cells in the mouse spleen[35]. B cells infected with *B. abortus* secrete TGFβ and contribute to bacterial dissemination, as adoptive transfer of these cells into uninfected mice results in the transfer of live bacteria[35]. Intriguingly, brucellosis is more rapidly cleared in B cell-deficient mice owing to a reduction in the levels of IL-10 and an increase in protective T cell responses, suggesting that *B. abortus* infection induces the differentiation of B cells into immunosuppressive regulatory B cells[15].

Salmonella enterica subsp. *enterica* serovar Typhimurium infects mouse B cells at different developmental stages in the bone marrow, which suggests that *S.* Typhimurium might use the bone marrow as a long-term infection niche[36]. Following the *in vitro* infection of human blood lymphocytes with *S.* Typhimurium, bacteria are found inside IgM-producing memory B cells. Infection of these cells results in B cell activation and the induction of specific antibody production, but also leads to increased survival and intracellular persistence of bacteria in infected B cells[37].

Several mechanisms have been described for the internalization of bacteria by B cells. For example, a role for phagocytosis of *B. abortus* mediated by Fc receptors or by receptors of the complement system was proposed, based on the observation that the percentage of infected B cells increases when bacteria are coated with antibodies[35]. The BCR can also be involved in bacterial internalization by B cells. For example, *Moraxella catarrhalis* is readily endocytosed by human naive tonsillar B cells through a process dependent on nonspecific BCR crosslinking by the superantigen *Moraxella* IgD binding protein (MID)[38] (FIG. 2). By contrast, internalization

Regulatory B cells
B cells that exert a regulatory function by the secretion of immunosuppressive cytokines, such as interleukin-10. Regulatory B cells seem to correspond to several cytokine-secreting plasma cells that are able to suppress inflammation and protective T cell responses.

Table 1 | **Pathogen manipulation of B cell function**

Pathogen	Infection of B cells	Diversion of B cell activation	Diversion of antibody production	Induction of regulatory B cells	Induction of B cell death	Induction of B cell survival
Parasites	NR	• *T. cruzi*[19,43,45] • *P. falciparum*[51]	• *T. cruzi*[44,46,47,49] • *P. falciparum*[50]	• *L. major*[52,54]	• *T. cruzi*[49,74,77] • *T. brucei*[75] • *P. chabaudi* [76]	NR
Viruses	• CMV[21] • Measles virus[22,23] • EBV[24] • MMTV[28] • JCV[32–34] • HCV[58] • Influenza virus[82] • Norovirus[90]	• Measles virus[22,23] • EBV[26,27] • MMTV[29–31] • HCV[57] • HIV-1 (REFS 59,62)	• Measles virus[103] • EBV[26,27] • HCV[58] • HIV-1 (REFS 60,61) • Influenza virus[82]	• CMV[63] • HBV[64] • HIV-1 (REF. 65) • Polyoma virus[66]	• Influenza virus[82]	• EBV[78–80] • HCV[81] • HIV-1 (REF. 59)
Bacteria	• *B. abortus*[35] • *M. catarrhalis*[38] • S. Typhimurium[36,37,39,40,104] • *L. monocytogenes*[105] • *S. flexneri*[41]	• *M. catarrhalis*[38] • *S. aureus*[106] • *N. gonorrhoeae*[71] • *Y. pseudo-tuberculosis*[73]	• *E. muris*[67] • *B. burgdorferi*[68] • *M. catarrhalis*[69,70] • *B. anthracis*[72] • *S. aureus*[107]	• *B. abortus*[15] • S. Typhimurium[16,18] • *C. abortus*[8]	• *L. monocyto-genes*[83,84] • *F. tularensis*[85,86] • *S. flexneri*[41] • *H. pylori*[87]	• *H. pylori*[88] • S. Typhimurium[39,89]

B. abortus, *Brucella abortus*; *B. anthracis*, *Bacillus anthracis*; *B. burgdorferi*, *Borrelia burgdorferi*; *C. abortus*, *Chlamydia abortus*; CMV, cytomegalovirus; EBV, Epstein–Barr virus; *E. muris*, *Ehrlichia muris*; *F. tularensis*, *Francisella tularensis*; HBV, hepatitis B virus; HCV, hepatitis C virus; *H. pylori*, *Helicobacter pylori*; JCV, polyoma JC virus; *L. major*, *Leishmania major*; *L. monocytogenes*, *Listeria monocytogenes*; *M. catarrhalis*, *Moraxella catarrhalis*; MMTV, mouse mammary tumour retrovirus; *N. gonorrhoeae*, *Neisseria gonorrhoeae*; NR, no report; *P. chabaudi*, *Plasmodium chabaudi*; *P. falciparum*, *Plasmodium falciparum*; *S. aureus*, *Staphylococcus aureus*; *S. flexneri*, *Shigella flexneri*; S. Typhimurium, *Salmonella enterica* subsp. *enterica* serovar Typhimurium; *T. brucei*, *Trypanosoma brucei*; *T. cruzi*, *Trypanosoma cruzi*; *Y. pseudotuberculosis*, *Yersinia pseudotuberculosis*.

of *S.* Typhimurium into B cells has been reported to involve interaction with an antigen-specific BCR[39]. Interestingly, the type III secretion system (T3SS) of *S.* Typhimurium, which mediates virulence and invasion of host cells, is involved in BCR-dependent internalization of the bacterium, as shown by the use of bacterial mutants with defects in the T3SS SPI-1 and SPI-2 (encoded by *S.* Typhimurium pathogenicity island 1 and 2, respectively); the number of intracellular bacteria found in B cells was substantially reduced when the bacterial T3SS was made non-functional[37]. Similarly, a functional SPI-1 T3SS was shown to be necessary for B cell membrane ruffling and macropinocytosis, demonstrating that *S.* Typhimurium forces its entry into B cells by triggering active invasion processes[40]. More recently, *Shigella flexneri* was also shown to invade human B cells in a process that is dependent on the T3SS but independent of B cell-mediated phagocytosis, as only bacteria carrying a functional T3SS were found inside B cells, both *in vitro* and in an *ex vivo* intestinal infection model[41].

Taken together, these studies show that the entry of pathogenic bacteria into B cells is not only mediated by phagocytosis, but can also involve active invasion processes mediated by bacterial secretion systems. This process is similar to those used by certain viruses, such as EBV that target specific host cell populations through the expression of viral glycoproteins. Together, these data demonstrate that some pathogens have evolved mechanisms to force their entry into B cells, leading to the establishment of intracellular reservoirs.

Diversion of B cell maturation

Besides using B cells as a reservoir, some pathogens have evolved mechanisms to interfere with immune signalling and B cell differentiation to impair the maturation of B cells into protective memory B cells and plasma cells (TABLE 1).

Diversion of B cell maturation by parasites. During infection, parasites can modulate B cell responses and stimulate the production of low-affinity antibodies, which, in some cases, has been associated with the dilution of specific, long-lived antibodies (FIG. 2; TABLE 1). For example, *Trypanosoma cruzi*, the causative agent of Chagas disease, attacks B cells at different developmental stages, depleting immature B cells during their development in the bone marrow but also inducing polyclonal expansion of mature B cells in the spleen, which is thought to allow the parasite to avoid B cell-mediated responses and to persist in the host[42,43]. Indeed, nonspecific B cell activation can be triggered by a variant antigen of the *Trypanosoma* spp. surface coat and results in an increase in the production of nonspecific antibodies, which is accompanied by a delay in parasite-specific immune responses[43–45]. Additionally, certain cytosolic and secreted proteins, such as *T. cruzi trans*-sialidase and mitochondrial malate dehydrogenase, induce polyclonal B cell proliferation independently of T cell help *in vitro* and result in the differentiation of B cells into short-lived plasma cells that produce non-protective antibodies[46–49]. These T cell-independent antigens activate B cells in a non-specific manner by binding outside the antigen-binding site of the BCR, thereby promoting BCR crosslinking, or by signalling through PRRs. The exact mechanisms of B cell activation by parasitic T cell-independent antigens are often unknown, but the activation of B cells by the *T. cruzi trans*-sialidase depends on Bruton's tyrosine kinase (BTK), a key signalling molecule in B cells that is involved in BCR signalling[46] (FIG. 2). Interestingly,

Superantigen
Antigens that promote nonspecific, polyclonal activation of lymphocytes. T cell superantigens result in massive pro-inflammatory cytokine release and increased helper T cell functions. B cell superantigens often bind to the constant regions of the immunoglobulin and thereby result in B cell maturation into effector cells independent of B cell receptor specificity.

Marginal zone B cells
Mature, non-circulating B cells found in the marginal zone of the spleen that are associated with polyclonal B cell responses and the secretion of antibodies of low affinity and specificity.

The complement system
As part of innate immune responses, the complement system helps to clear pathogens. It requires antigen–antibody complexes for activation and induces a proteolytic cascade that results in membrane lysis of target-cell membranes or in the release of cytokines, which contribute to the recruitment of phagocytic cells.

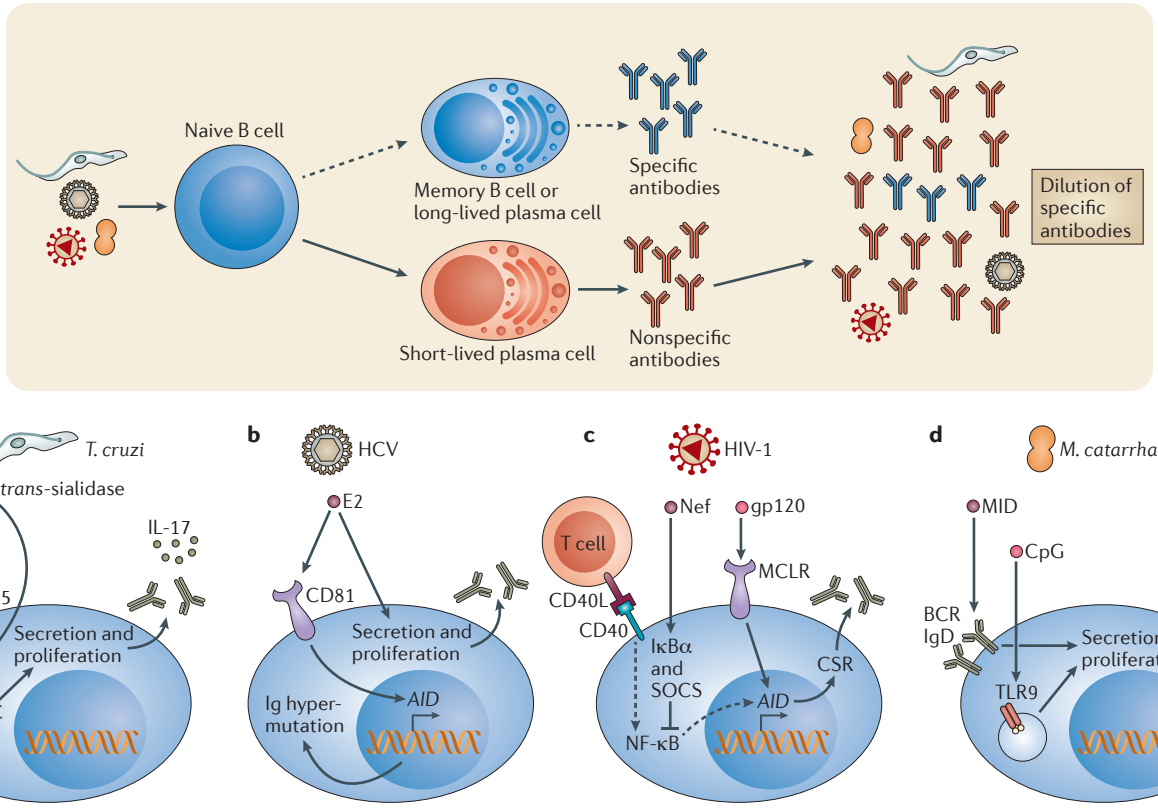

Figure 2 | **Subversion of protective B cell responses by antibody dilution.** Long-lived plasma cells and memory B cells normally constitute the B cell compartment and provide protection from reinfection. However, some pathogens have been reported to deliberately induce short-lived, polyclonal plasma cells in order to dilute long-lived, specific antibody responses. **a** | Several components of the parasite *Trypanosoma cruzi* induce polyclonal B cell activation and proliferation independently of T cell help, ultimately resulting in the dilution of the antibody response by secretion of nonspecific antibodies[43–45]. The activity of the parasite-produced *trans*-sialidase is dependent on Bruton's tyrosine kinase (BTK)[46]. In addition, the secretion of interleukin-17 (IL-17) occurs via CD45-dependent activation of BTK and SRC[19]. **b** |The hepatitis C virus (HCV) glycoprotein E2 binds to CD81 on the B cell surface, leading to the upregulated expression of activation-induced cytidine deaminase (*AID*) and the induction of immunoglobulin (Ig) hypermutation. This results in lower affinity, lower neutralizing activity and lower complement-mediated toxicity of E2-specific antibodies[57,58]. **c** | HIV-1 uses several mechanisms to induce T cell-independent antibody responses. The viral protein negative factor (Nef) inhibits cytokine and CD40 signalling by cognate T cells by inducing upregulation of NF-κB inhibitor-α (IκBα) and suppressor of cytokine signalling (SOCS) proteins, which block nuclear factor-κB (NF-κB) activation[60–62]. Viral gp120 binds to mannose C-type lectin receptors (MCLRs), thereby inducing *AID* upregulation and class-switch recombination (CSR) independently of T cell help[59]. **d** | *Moraxella catarrhalis* induces proliferation and polyclonal antibody production by inducing B cell receptor (BCR) and TLR signalling simultaneously. Bacterial CpG activates TLR9 and *Moraxella* IgD binding protein (MID) crosslinks IgD BCRs on naive B cells[38,69,70]. Dashed arrows indicate normal pathways that are weakened or impaired during infection.

Tonsillar B cells
The tonsil is the main lymphoid organ of the aerodigestive tract. Naive, tonsillar B cells mature in germinal centre reactions and are able to produce all antibody isotypes, especially locally secreted IgA.

Type III secretion system
(T3SS). A bacterial secretion system that is composed of a basal body, a hollow needle-like structure and a pore that can be inserted in the host cell membrane. T3SSs are used by certain pathogenic bacteria to translocate virulence factors into the host cell, thereby modulating cellular functions.

T cell-independent antigens
Antigens that are able to induce B cell activation and maturation independently of T cell help. T cell-independent antigens include pathogen-associated molecular patterns, which signal through pattern recognition receptors, and superantigens, which induce nonspecific B cell receptor signalling.

trans-sialidase also induces the secretion of the pro-inflammatory cytokine IL-17 by B cells, in a process that involves the activation of BTK and SRC kinases in conjunction with the expression of CD45 by B cells[19]. B cell production of IL-17 was shown to have an immunoregulatory role during *T. cruzi* infections by reducing inflammation and tissue damage, and it was required for efficient clearance of the parasite[19]. Therefore, *trans*-sialidase seems to have a dual role during infection by promoting both the production of non-protective antibodies and the induction of regulatory B cells.

P. falciparum infection is also associated with the polyclonal activation of B cells and an increase in the production of nonspecific antibodies. The cysteine-rich interdomain region 1α (CIDR1α) of the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) does not interfere with components of the BCR signalling pathway, but leads to the phosphorylation of downstream kinases and the upregulation of Toll-like receptor (TLR) signalling components, suggesting that it also functions as a T cell-independent antigen[50]. Interestingly, CIDR1α has been shown to preferentially activate the memory B cell compartment, suggesting that this mechanism may be linked to the lack of specific memory responses observed in children from areas where malaria is endemic[51].

Leishmania major also affects B cell differentiation, which results in the generation of immunosuppressive regulatory B cells. Furthermore, adoptive transfer of

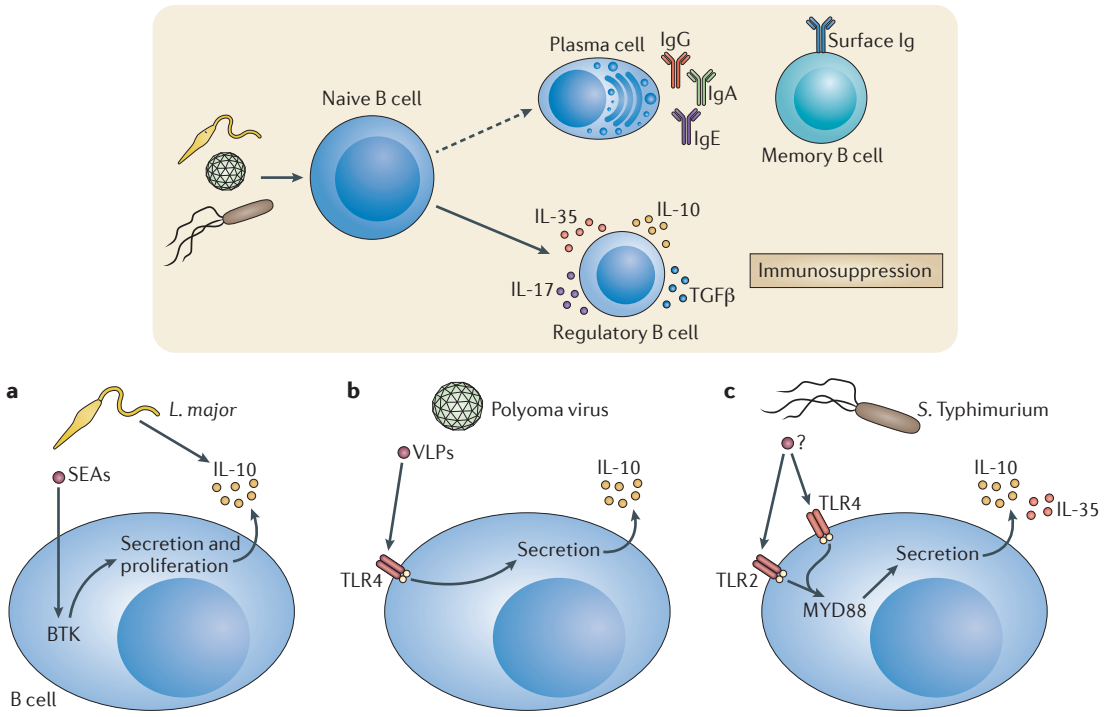

Figure 3 | **Induction of regulatory B cells with immunosuppressive functions.** A number of pathogens have been reported to induce the differentiation of regulatory B cells to suppress protective immune responses. **a** | Several components of the parasite *Leishmania major* induce interleukin-10 (IL-10)-producing B cells, and these cells downregulate T cell and allergen responses[52]. Soluble egg antigens (SEAs) induce proliferation of and IL-10 secretion by normal B cells, but not B cells that carry a Bruton's tyrosine kinase (BTK) mutation[53,54]. **b** | The induction of regulatory B cells as a mechanism of immune escape during viral infections has only recently been shown. IL-10 production by B cells is induced by virus-like particles (VLPs) in response to polyoma virus infection in a Toll-like receptor 4 (TLR4)-dependent manner[66]. **c** | Regulatory B cells make mice more susceptible to *Salmonella enterica* subsp. *enterica* serovar Typhimurium infection. Deletion of TLR2 and TLR4 or the TLR adaptor molecule myeloid differentiation primary response protein 88 (MYD88) in B cells suppresses IL-10 secretion[16]. IL-35 has recently been shown to have a role in B cell regulatory function during S. Typhimurium infection[18]. Dashed arrows indicate normal pathways that are weakened or impaired during infection.

regulatory B cells (induced following *L. major* infection) modulates T cell and allergen responses in mice, suggesting that regulatory B cells might be useful as therapies against allergies and autoimmune disorders[52]. However, only a few studies have addressed how regulatory B cells are induced by direct contact with *Leishmania* spp. or its secreted effectors. For example, antigens that induce IL-10 production by mouse spleen B cells *in vitro* include soluble proteins, such as *Leishmania infantum* tryparedoxin, or sugars, such as lacto-*N*-fucopentaose III, which is found on soluble egg antigens of *L. major*[53,54] (FIG. 3). However, the B cell signalling pathways involved in this process are unknown.

Diversion of B cell maturation by viruses. The induction of B cell activation leading to polyclonal antibody responses that dilute the production of specific antibodies has also been reported as a strategy used by several viruses to skew protective immune responses (FIG. 2; TABLE 1). Whereas the early immune response to some viruses, such as influenza virus, mediates protection, antibodies generated in response to hepatitis C virus (HCV) infection fail to clear the virus in patients with persistent infections and lymphoproliferative disorders such as B cell lymphomas[55,56]. The HCV glycoprotein E2 binds to CD81 on the B cell surface and induces the activation and proliferation of naive B cells[57]. This was shown by incubating B cells with HCV E2 protein *in vitro*, but was also directly linked to the observation that B cells infected *in vivo* show higher expression of activation markers[57]. Additionally, E2 binding and subsequent viral infection of B cells induces the upregulation of AID and SHM of the immunoglobulin heavy chain in hybridoma cell lines that produce E2-specific antibodies, resulting in the production of antibodies with lower affinity, lower neutralizing capacity and lower complement-mediated toxicity, and this could explain why, in patients, serum HCV-specific antibodies fail to neutralize the virus[58]. Therefore, HCV is an intriguing example of how normal B cell maturation can be 'hijacked' by viruses to induce diluted antibody responses (FIG. 2).

HIV-1 infection is also associated with B cell dysregulation and exhaustion of the B cell compartment. The effect of HIV-1 on $CD4^+$ T cells accounts for most of the B cell defects observed during infection, but recent studies

have investigated the direct interaction of HIV-1 with B cells[59–61]. Although HIV-1 is unable to infect B cells, binding of the HIV-1 envelope protein gp120, through mannose C-type lectin receptors, to a subset of tonsillar B cells leads to the upregulation of AID and induces CSR from IgM to IgG and IgA[59] (FIG. 2). This interaction, in conjunction with activation of B cell activating factor (BAFF) signalling, induces the production of polyclonal antibodies independently of T cell help[62]. Furthermore, the viral protein negative factor (Nef) penetrates B cells *in vitro* and *in vivo*, and suppresses CD40-dependent CSR by inducing the expression of NF-κB inhibitor-α (IκBα) and suppressor of cytokine signalling (SOCS) proteins, which block CD40L and cytokine signalling[61] (FIG. 2). Interestingly, Nef shuttles from infected macrophages to B cells by hijacking long-range intercellular conduits, such as nanotubules, which allows HIV-1 to inhibit CSR in lymphoid follicles *in vivo*[60]. Taken together, these studies highlight how direct interaction between HIV-1 and B cells induces a shift from the production of T cell-dependent specific antibodies to the production of nonspecific antibodies in a T cell-independent manner, thereby promoting viral immune escape (FIG. 2).

The induction of regulatory B cells also contributes to immune escape during viral infections, as reported for cytomegalovirus, hepatitis B virus and HIV-1 (REFS 63–65) (TABLE 1). However, mechanistic insight into the induction of regulatory B cells by these viruses is limited. Interestingly, following infection with polyoma virus, IL-10 production by B cells is induced by virus-like particles in a TLR4-dependent manner, suggesting that this pathway might be involved in the generation of regulatory B cells[66] (FIG. 3).

Diversion of B cell maturation by bacteria. Similarly to parasites and viruses, bacteria also trigger polyclonal activation of B cells to impair protective immune responses mediated by the production of specific antibodies (FIG. 2; TABLE 1). For example, mouse models of infection with *Ehrlichia muris* and *Borrelia burgdorferi* are characterized by T cell- and GC-independent expansions of non-switched, IgM-secreting plasma cells, which impairs the development of a protective antibody response[67,68]. Similarly, binding of the *M. catarrhalis* superantigen MID to the BCR, in conjunction with TLR9 signalling, induces strong proliferation of human tonsillar B cells[38]. Interestingly, the shedding of outer membrane vesicles containing MID and CpG DNA has been described as a decoy strategy that is used by *M. catarrhalis* to induce polyclonal B cell activation and nonspecific antibody production[69,70] (FIG. 2). TLR9 signalling is also involved in the proliferative and IgM-producing response of human polyclonal IgD memory B cells during *Neisseria gonorrhoeae* infection *in vitro*. Notably, this response is specific to *N. gonorrhoeae* and is not due to the general presence of bacterial PAMPs, as shown by comparing infection with *N. gonorrhoeae* to infection with non-pathogenic *Escherichia coli*[71].

In addition to the dilution of the specific antibody response, which results from polyclonal B cell activation, bacteria can produce virulence effectors that directly manipulate B cell signalling pathways. Anthrax lethal toxin from the Gram-positive *Bacillus anthracis* directly binds to B cells by the anthrax protective antigen and is able to cleave mitogen-activated protein kinase kinases (MAPKKs) through the lethal factor protease, which results in the inhibition of B cell proliferation and immunoglobulin production, both *in vitro* and *in vivo*[72]. Similarly, several Gram-negative bacteria use T3SSs to deliver virulence effectors into the host cell cytoplasm and manipulate B cell functions. For example, following infection with *Yersinia pseudotuberculosis*, primary B cells isolated from the spleens of hen egg lysozyme (HEL)-specific immunoglobulin-transgenic mice showed reduced activation upon stimulation with their cognate antigen[73]. Through the use of bacterial mutants, the authors showed that the impairment of B cell activation was T3SS-dependent and identified the tyrosine phosphatase YopH as the bacterial virulence effector responsible for this phenomenon. YopH inhibits phosphorylation of the BCR signalling complex, and subsequent antigen presentation on MHC II molecules[73].

Intracellular bacteria such as *Chlamydia abortus*, *B. abortus* and *S.* Typhimurium can also affect ongoing immune responses by favouring the generation of immunosuppressive regulatory B cells[8,15,16] (FIG. 3; TABLE 1). Deletion of the TLR adaptor molecule myeloid differentiation primary response protein 88 (MYD88) or deletion of TLR2 and TLR4 exclusively in B cells leads to decreased secretion of IL-10 by B cells and makes mice more resistant to *S.* Typhimurium infection, suggesting that these signalling pathways are directly activated by the bacterium, repressing protective innate immune responses[16] (FIG. 3). Additionally, IL-35 has recently been shown to contribute to B cell regulatory function during *S.* Typhimurium infection[18].

Collectively, these studies show that pathogens use two main strategies to divert B cell maturation and impair protective immune responses: the induction of short-lived plasma cells (which secrete antibodies of low affinity, leading to the dilution of specific, long-lived antibody responses (FIG. 2)) and the induction of regulatory B cells (which have an immunosuppressive role during infection (FIG. 3)).

Manipulation of B cell survival

In addition to living inside B cells and manipulating B cell maturation, pathogens can influence B cell responses by modulating the intricate balance of pathways that determines whether a B cell lives or dies (FIG. 4; TABLE 1).

Manipulation of B cell survival by parasites. Both *Trypanosoma brucei* and *T. cruzi* infections have severe effects on the B cell compartment, including the induction of B cell death (TABLE 1). In mice, *T. cruzi* induces the upregulation of the death receptor FAS (also known as TNFRSF6) and its ligand, FASL (also known as TNFSF6), on B cells, which makes the cells more susceptible to killing through B cell–B cell interactions[49,74]. Interestingly, B cell death mediated by FAS–FASL occurs predominantly in isotype-switched, parasite-specific IgG B cells in the mouse model[74]. *T. brucei* induces apoptosis of

CpG
CpG sites are DNA sites that are composed of a cytosine (C) coupled to a guanine (G) with only one intervening phosphate. These sites are mostly methylated in mammals, and unmethylated CpG, which occurs in viruses and bacteria, results in the activation of Toll-like receptor 9 in mammalian cells.

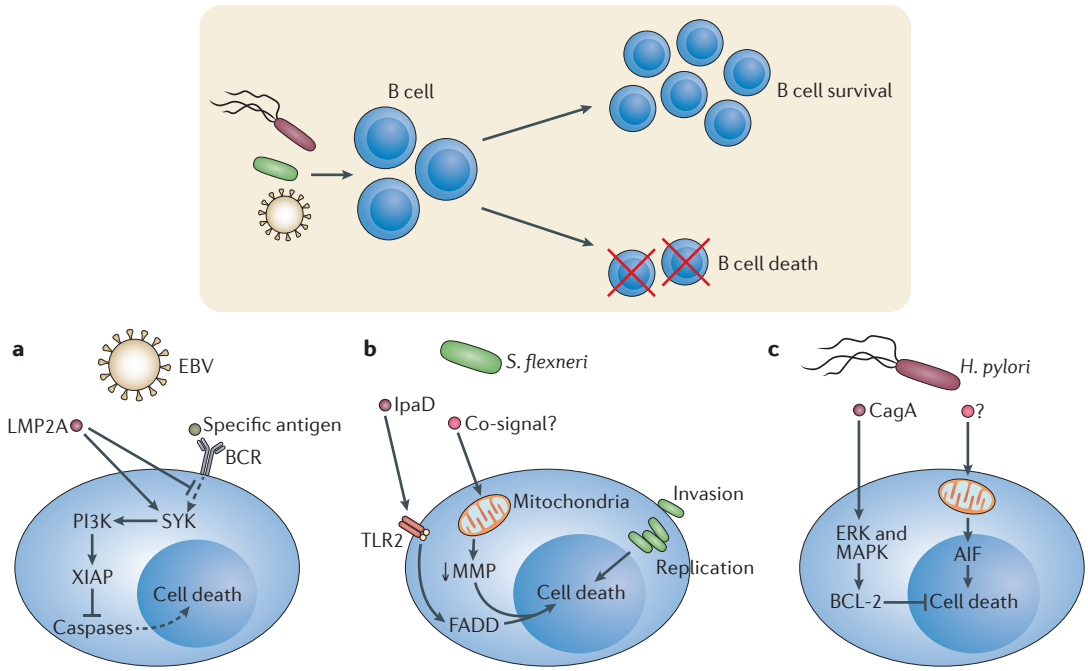

Figure 4 | **Manipulation of B cell survival and death pathways by pathogens.** Several pathogens have been reported to directly interfere with B cell survival and death pathways. **a** | The Epstein–Barr virus (EBV) protein latent membrane protein 2A (LMP2A) triggers the kinases spleen tyrosine kinase (SYK) and phosphoinositide 3-kinase (PI3K) downstream of the B cell receptor (BCR), thereby preventing the loss of X-linked inhibitor of apoptosis protein (XIAP) and caspase-induced cell death[80]. **b** | *Shigella flexneri* invasion of B cells and intracellular proliferation ultimately results in B cell death[41]. Additionally, cell death is induced by two signals in combination: bacterial co-signals (which induce the loss of mitochondrial membrane potential (MMP)), and the type III secretion system (T3SS) virulence factor IpaD, which binds to Toll-like receptor 2 (TLR2) and upregulates FAS-associated death domain protein (FADD). **c** | *Helicobacter pylori* can induce B cell death by releasing apotosis-inducing factor (AIF) from mitochondria. By contrast, translocation of the virulence factor CagA leads to extracellular signal-regulated kinase (ERK) and mitogen-activated protein kinase (MAPK) phosphorylation and induction of the anti-apoptotic protein B cell lymphoma 2 (BCL-2), thereby preventing B cell death[87,88]. Dashed arrows indicate normal pathways that are weakened or impaired during infection.

transitional B cells, which prevents the replenishment of the mature B cell compartment in the spleen[75]. Similarly to *Trypanosoma* spp., *Plasmodium chabaudi* infection disrupts B cell generation in the bone marrow and induces apoptosis of transitional and marginal zone B cells[76]. However, whether B cell death occurs owing to direct contact with *Trypanosoma* spp. or *Plasmodium* spp., or results from a global increase in inflammation is unknown[75–77]. Interestingly, *T. brucei*-induced apoptosis in transitional B cells also occurs *in vitro* and is dependent on contact between the B cells and the parasite surface coat, suggesting that specific virulence factors modulate this B cell response[75]. These parasites also induce the dilution of antibody responses, and their effect on B cells seems to be dependent on the B cell subpopulation that is targeted. Therefore, *Trypanosoma* spp. and *Plasmodium* spp. use several mechanisms to avoid B cell responses at the different developmental stages of B cell maturation.

Manipulation of B cell survival by viruses. Viruses that cause the development of B cell lymphomas often have the capacity to directly increase B cell survival[59,78,79] (TABLE 1). A mechanistic insight into how viral proteins interfere with cell death signalling pathways is given by the EBV protein latent membrane protein 2A (LMP2A), which activates SRC kinases downstream of the BCR, resulting in continuous B cell activation. Activation of spleen tyrosine kinase (SYK) in particular has been linked to activation of phosphoinositide 3-kinase (PI3K) and protein kinase B (PKB; also known as AKT), which prevents loss of the endogenous caspase inhibitor X-linked inhibitor of apoptosis protein (XIAP), in a process that is dependent on the mitochondrial protease HTRA2 (REF. 80) (FIG. 4). Similarly, HCV induces B cell survival through the E2 envelope protein, which engages CD81 expressed at the surface of B cells. This leads to the activation of the nuclear factor-κB (NF-κB) pathway and the enhancement of expression of the anti-apoptotic protein B cell lymphoma 2 (BCL-2) in human B cells, protecting B cells from FAS-mediated cell death[81]. Whereas EBV persists intracellularly in B cells, where it hides from antibody responses, HCV can induce non-protective antibody responses and lymphoproliferative disorders. These two viruses provide an intriguing example of how the induction of B cell survival can facilitate infectious processes.

In contrast to viruses that induce B cell survival, influenza A virus leads to the induction of B cell death. Mouse B cells carrying a BCR specific for influenza

Transitional B cells
A developmental stage of B cells. Transitional B cells have completed development in the bone marrow and are the first B cells to migrate into the blood, but they have not yet fully matured into naive antigen-responsive B cells.

haemagglutinin were found to be infected *in vitro* and *in vivo* in the lungs, failed to produce antibodies and ultimately died[82]. These data suggest that targeting of antigen-specific B cells at the infectious site could be an efficient mechanism to impair or delay the adaptive immune response to infection.

Manipulation of B cell survival by bacteria. Similarly to viruses and parasites, bacterial pathogens can manipulate the survival and cell death pathways of B cells (TABLE 1). For example, *Listeria monocytogenes* infection results in high cytotoxicity for B cells. Interestingly, *L. monocytogenes*-induced B cell apoptosis is dependent on the production of virulence factors by the bacterium, but it is independent of bacterial invasion of B cells[83]. *L. monocytogenes*-induced cell death of a mouse B cell line has been shown to be dependent on the expression of listeriolysin O (LLO), which is a virulence factor that induces membrane damage by its general pore forming activity, thereby leading to apoptosis[84]. Apoptosis of B cells *in vitro* has also been described following infection with *Francisella tularensis*[85]. *F. tularensis* directly infects B cells, but nuclear fragmentation and membrane blebbing (two hallmarks of apoptosis) are also observed in uninfected bystander B cells[85]. *F. tularensis*-induced cell death involves the activation of caspase 3, caspase 8, caspase 9 and BH3 interacting-domain death agonist (BID), which leads to the release of cytochrome *c* and apoptosis-inducing factor (AIF) from mitochondria[86].

Similarly to *F. tularensis*, *S. flexneri* also induces cell death in infected and uninfected B cells[41] (FIG. 4). Interestingly, induction of apoptosis in uninfected B cells requires a functional T3SS, but is independent of the translocation of T3SS-dependent virulence effectors. Instead, the virulence effector IpaD — the needle-tip protein of the *Shigella* spp. T3SS — induces apoptosis by binding to TLR2 and induces upregulation of FAS-associated death domain (FADD) protein levels. The presence of an as yet unidentified bacterial co-signal (or multiple co-signals) is necessary for the triggering of IpaD-mediated cell death, as apoptotic B cells were only detected when cells were co-incubated with IpaD and non-pathogenic *S. flexneri* or *E. coli*. Notably, the co-incubation with non-pathogenic bacteria results in the loss of both mitochondrial membrane potential and the upregulation of mRNA encoding TLR2. *Shigella* spp. thus provide an intriguing example of pathogens that use multiple mechanisms to directly induce B cell death (FIG. 4).

Helicobacter pylori infection has also been shown to lead to translocation of AIF and induction of apoptosis in a B cell line, which has been associated with the persistence of *H. pylori*[87] (FIG. 4). By contrast, translocation of the *H. pylori* CagA effector by the bacterial T4SS leads to increased survival of B cells *in vitro*. Translocation of CagA induces extracellular signal-regulated kinase (ERK) and MAPK phosphorylation and upregulation of the anti-apoptotic proteins BCL-2 and BCL-XL[88] (FIG. 4). Whereas the induction of apoptosis has been suggested to facilitate persistence by deletion of protective B cells, the increased survival of B cells has been associated with *H. pylori*-induced lymphoma formation[87,88]. Whether one or both of these mechanisms occur *in vivo* in infections with *H. pylori* remains to be investigated.

In contrast to bacteria that induce B cell death, *S.* Typhimurium induces B cell survival, which has been suggested to benefit the bacterium as it uses B cells as a survival and dissemination niche[39]. Notably, *S.* Typhimurium infection induces cell death in macrophages, in a process dependent on the activation of the NLRC4 (NOD-, LRR- and CARD-containing 4) inflammasome. However, expression of NLRC4 is downregulated in B cells during *S.* Typhimurium infection, which prevents activation of the inflammasome and the induction of cell death[89]. Interestingly, inhibition of the inflammasome occurs in both infected and uninfected cells and requires the *S.* Typhimurium T3SS SPI-1, as shown by the use of a mutant strain with a non-functional T3SS[89].

Together, these studies highlight that pathogens can interfere with both survival and cell death pathways in B cells. Interestingly, pathogens that use B cells as a niche for survival or dissemination or that divert B cell maturation often increase B cell survival, presumably to facilitate their persistence in the host. Acute, recurrent infections, however, are often accompanied by B cell death and impaired protective immune responses, suggesting that reinfection is facilitated by the deletion of the cell population that confers protective immunity.

Outlook

Increasing evidence is emerging that several pathogenic parasites, viruses and bacteria interact directly with and manipulate B cells. Such direct targeting, in addition to the indirect effect of the infection-induced local microenvironment, illustrates the diversity of mechanisms used by pathogens to evade host protective immunity. Pathogens manipulate B cells using three main strategies: the use of B cells as a reservoir, the diversion of B cell maturation (either by the induction of short-lived plasma cells that secrete antibodies of low specificity or by the induction of immunosuppressive regulatory B cells), and the modulation of B cell survival.

Interestingly, some pathogens use multiple mechanisms simultaneously to ensure their survival. For example, several viruses that cause persistent infections induce B cell survival, which can result in lymphoma formation. Although it seems detrimental to the viruses to induce the survival of B cells, these viruses have often found ways to hide from or subvert the antibody response in order to persist within the host. By contrast, in the case of acute infections or host-restricted pathogens, pathogens have evolved mechanisms to facilitate reinfection. For instance, by inducing B cell death, *S. flexneri* directly targets the cells required to confer protection during infection[41]. *S.* Typhimurium suppresses immune responses by a different mechanism involving the induction of regulatory B cells, which modulate protective responses mediated by T cells and other innate immune cells[16,18]. Regulatory B cells have received increasing attention and are also induced in several viral and parasitic infections. Although these cells show therapeutic potential in the treatment of

Inflammasome
A signalling complex of the innate immune system, which triggers the release of cytokines interleukin-1β (IL-1β) and IL-18. The inflammasome can induce pyroptosis, a pro-inflammatory cell death.

Fluorescence resonance energy transfer
(FRET). A mechanism describing energy transfer between two fluorophores that are in close proximity to each other. Measurements of FRET efficiency between donor and acceptor fluorophores can be used as a biochemical tool.

autoimmune diseases, further insight into the mechanisms by which regulatory functions are triggered is needed to provide information on how to prevent their detrimental effects following infections.

To elucidate cellular mechanisms of B cell manipulation by pathogens, a combination of *in vitro* and *in vivo* studies seems particularly promising. For instance, a recent study using human and mouse norovirus strains elegantly shows that B cells provide a cellular target for the virus *in vitro* and *in vivo*, and that infection is promoted by enteric bacteria expressing histo-blood group antigen[90]. Notably, pathogens are often used as a simple tool for deciphering the generation of immune cell functions, but recent evidence highlights their ability to divert immune responses by expressing key virulence factors. New approaches are thus needed to gain insights into the role of such weapons in infections. For instance, a fluorescence resonance energy transfer (FRET)-based assay to directly monitor the delivery of virulence effectors into host cells was recently used to investigate whether B cells are deliberate targets of T3SS-bearing bacteria *in vitro* and *in vivo*[91–94]. The identification of key virulence factors diverting host responses could also affect vaccine design, especially for live attenuated vaccine candidates, which involve the identification and deletion of virulence factors that have a negative effect on the host-protective immune responses. For example, the *S. flexneri* IpaD protein induces protective antibodies *in vivo* and has been suggested as a promising antigen for the development of a subunit vaccine[95,96]. The recent demonstration that IpaD induces B cell death, but only in the presence of bacterial cofactors[41], suggests that IpaD-specific antibodies elicited upon immunization would not only prevent cell invasion but also the induction of B cell death triggered during infection. Therefore, an IpaD-based subunit vaccine seems particularly promising in the fight against *S. flexneri* infections.

Additionally, systems biology approaches targeted at detecting infection and vaccination signatures in people may help us to gain insights into how protective immune responses are established. For example, systems analysis and bioinformatics integration of various 'omics' approaches, in combination with traditional experimental approaches, have contributed to a better characterization of the host immune response against West Nile virus infection[97]. To combine such an analysis with insights into manipulation strategies used by pathogens would substantially increase our knowledge of how protective B cell responses are elicited and diverted during particular infections, which may lead to novel therapeutic and vaccination approaches in the future.

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The authors declare no competing interests.