Activation of B cells by non-canonical helper signals

Andrea Cerutti¹⁻³⁺, Montserrat Cols³ & Irene Puga²

¹Catalan Institute for Research and Advanced Studies (ICREA), and ²Institut de Recerca Hospital del Mar (IMIM), Barcelona Biomedical Research Park, Barcelona, Spain, ³Immunology Institute, Department of Medicine, Mount Sinai School of Medicine, New York, New York, USA

Cognate interaction between T and B lymphocytes of the adaptive immune system is essential for the production of high-affinity antibodies against microbes, and for the establishment of long-term immunological memory. Growing evidence shows that—in addition to presenting antigens to T and B cells—macrophages, dendritic cells and other cells of the innate immune system provide activating signals to B cells, as well as survival signals to antibody-secreting plasma cells. Here, we discuss how these innate immune cells contribute to the induction of highly diversified and temporally sustained antibody responses, both systemically and at mucosal sites of antigen entry.

Keywords: B cells; NKT cells; eosinophils; neutrophils; basophils; immunoglobulins

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See the Glossary for abbreviations used in this article.

Introduction

Invading microbes initiate protective antibody responses by stimulating follicular B cells located in secondary lymphoid organs such as the lymph nodes and the spleen. This stimulation involves the recognition and internalization of native antigen by IgM and IgD receptors expressed on the surface of B cells [1,2]. Large antigens require the interaction of B cells with antigen-sampling macrophages and dendritic cells located in the subcapsular sinus and paracortical areas of the lymph nodes or in the marginal zone of the spleen [3–6]. By contrast, small soluble antigens gain access to B cells after entering the follicle through a specialized transport system known as the follicular conduit network [7–9]. This structure communicates with afferent lymphatic vessels and consists of collagen fibre cores surrounded by myofibroblast-like cells, known as fibroblastic reticular cells [9,10].

After receiving activating signals from surface Ig receptors, B cells process the antigen and migrate to the T-cell zone–follicle boundary, known as the T–B border (Fig 1). There, B cells establish an initial cognate interaction with T follicular helper (T_{FH}) cells—a professional $CD4^+$ T-cell subset induced by dendritic

cells that secrete the cytokine IL-12 [11–15]. The T–B border contains early T_{FH} cells that express the co-stimulatory receptor ICOS, the chemokine receptor CXCR5, the inhibitory receptor PD1 and the transcription factor Bcl6—which help the B cells through the tumour necrosis factor (TNF) family member CD40L—and various cytokines, including IL-21, IL-4 and IFN- γ [15–21]. After interacting with early T_{FH} cells, antigen-specific B cells differentiate along one of two alternative pathways. The extrafollicular pathway generates short-lived plasmablasts that secrete IgM, whereas the follicular pathway generates Bcl6-expressing germinal centre B cells known as centroblasts and centrocytes that mediate antibody diversification, selection and production [22–29].

The germinal centre reaction fosters antibody diversification through somatic hypermutation (SHM) and class switch recombination (CSR), two immunoglobulin-gene-diversifying processes that require the DNA-editing enzyme AID [30–33]. Somatic hypermutation introduces point mutations within the V(D)J genes, which encode the antigen-binding variable region of immunoglobulins, thereby providing the mechanism for the selection of high-affinity antibody mutants by antigen [34]. By contrast, CSR generates antibodies with new effector functions without changing antigen specificity by replacing the $C\mu$ and $C\delta$ genes that encode IgM and IgD with $C\gamma$, $C\alpha$ and $C\epsilon$ genes, which encode IgG, IgA and IgE, respectively [35]. In humans, a non-canonical form of CSR from $C\mu$ to $C\delta$ has also been documented in lymphoid structures of the upper respiratory tract [36].

After increasing the expression of CXCR5, early T_{FH} cells and activated B cells move to the follicle in response to the chemokine CXCL13, a CXCR5 ligand produced by follicular dendritic cells (FDCs; [16,37,38]). Early T_{FH} cells become germinal centre T_{FH} cells by entering a Bcl6-dependent genetic programme, which is induced by signals present in the follicular environment [39,40]. These signals include those elicited after ICOS on T_{FH} cells binds to ICOSL on B cells [18,41]. By expressing high levels of CD40L and IL-21, germinal centre T_{FH} cells sustain the proliferation, differentiation, diversification and selection of centroblasts and centrocytes [22–29]. Centroblasts undergo clonal expansion and extensive SHM in the dark zone of the germinal centre and then exit the cell cycle to become centrocytes, which sample antigens on the surface of FDCs by using their newly hypermutated surface immunoglobulin receptors [23,25–27,42]. After binding to antigen, centrocytes establish a cognate interaction with germinal centre T_{FH} cells, which promote the selection of high-affinity and class-switched centrocytes, as well as their differentiation, into memory B cells and plasma cells [25–28,42–46].

¹Catalan Institute for Research and Advanced Studies (ICREA), and ²Institut de Recerca Hospital del Mar (IMIM), Barcelona Biomedical Research Park, Avenida Dr. Aiguader 88, 08003 Barcelona, Spain

³Immunology Institute, Department of Medicine, Mount Sinai School of Medicine, 1425 Madison Avenue, New York, New York 10029, USA

*Corresponding author. Tel: +34 933 160 389; Fax: +34 933 160 410;

E-mails: acerutti@imim.es; andrea.cerutti@mssm.edu

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The helping activity of T_{FH} cells is controlled by negative signals provided by plasma cells and a T_{FH} cell-like subset of regulatory $CD4^+$ T cells known as T follicular regulatory (T_{FR}) cells [47–49]. Similarly to conventional T_{Reg} cells, T_{FR} cells express the transcription factor Foxp3 and optimize antibody affinity maturation by impairing the outgrowth of B cells with low affinity for antigen [48,49]. T_{FR} cells might also regulate the differentiation of centrocytes into memory B cells or plasma cells [48,49]. Memory B cells enter the circulation, but also form IgG-expressing extrafollicular aggregates and IgM-expressing follicle-like structures in lymphoid organs [50]. After a subsequent exposure to recall antigen, IgG-expressing memory B cells rapidly generate antibody-secreting plasmablasts, whereas IgM-expressing memory B cells initiate a secondary germinal centre reaction [50,51].

These memory responses probably require cognate interaction of memory B cells with T_{FH} cells, but some evidence also points to an important role of non-specific microbial signals that stimulate the TLRs expressed by B cells [52]. These pattern recognition receptors induce polyclonal expansion of memory B cells [52], as well as co-stimulating germinal centre B cells, although the impact of this co-stimulation varies with the nature of the immunizing antigen [53,54]. Unlike memory B cells, plasmablasts that emerge from the germinal centre reaction move to special niches in the bone marrow, where they become long-lived plasma cells that continuously release high-affinity antibodies into the circulation [43,45,55].

The innate and adaptive immune systems are functionally linked by mechanisms that were originally predicted by Charles Janeway Jr in his unified model of the immune response [56]. In this model, non-specific innate immune cells instruct T and B cells to initiate highly specific adaptive immune responses after sensing conserved microbial molecular signatures through non-specific, germline-encoded pattern recognition receptors, including TLRs [57]. In addition to presenting antigen to somatically recombined antigen receptors on T and B cells, innate immune cells use cytokines and antibodies produced by effector T and B cells, respectively, to optimize the clearance of invading microbes [56,57]. However, growing evidence points to the presence of extra layers of complexity in the cooperation between the innate and adaptive immune systems.

Whilst providing immune protection and memory, B-cell responses induced by T_{FH} cells have relatively slow kinetics, and need to be integrated with faster B-cell responses that involve multiple components of the innate immune system. Indeed, invariant NKT (iNKT) cells generate early waves of IgM and IgG antibodies to pathogens by interacting with B cells in the absence of T_{FH} cells. Similarly, macrophages, dendritic cells, neutrophils, basophils, mast cells and other cells of the innate immune system initiate rapid B-cell responses at sites exposed continuously to antigen, including the marginal zone of the spleen and mucosal surfaces. In addition to mediating alternative T-cell-independent pathways for antibody production, innate immune cells such as eosinophils sustain canonical T-cell-dependent antibody responses over long periods of time by delivering survival signals to plasma cells in the bone marrow. Here, we discuss the mechanisms by which these non-canonical B helper cells regulate antibody diversification and production.

iNKT cells

The regulation of follicular B-cell responses is not restricted to T_{FH} and T_{FR} cells, but also involves iNKT cells (Fig 1). These innate lymphoid cells express an invariant $V\alpha 14^+$ TCR that recognizes soluble glycolipids—such as α -galactosylceramide—presented by the

Glossary

AID	activation-induced cytidine deaminase
APRIL	a proliferation-inducing ligand
BAFF/BLyS	B-cell-activating factor of the TNF family
Bcl6	B-cell lymphoma 6
BCMA	B-cell maturation antigen
CD40L	CD40 ligand
C4BP	C4B-binding protein
CXCL	CXC chemokine ligand
GM-CSF	granulocyte monocyte colony stimulating factor
ICOS	inducible T-cell costimulator
ICOSL	inducible T-cell costimulator ligand
IFN- $\alpha/\beta/\gamma$	interferon $\alpha/\beta/\gamma$
Ig	immunoglobulin
IL	interleukin
iNOS	inducible nitric oxide synthase
LPS	lipopolysaccharide
MHC	major histocompatibility complex
NKT $_{FH}$	natural killer T follicular helper cell
NET	neutrophil extracellular trap
PD1	programmed death 1
TCR	T-cell receptor
TGF- β	transforming growth factor- β
T_H	T helper cell
TLR	Toll-like receptor
TSLP	thymus stromal lymphopoietin
T_{Reg}	T regulatory cell
$V\alpha 14^+$	variable $\alpha 14$ gene
V(D)J	variable-(diversity)-joining gene

non-polymorphic MHC-I-like molecule CD1d [58–60]. After recognizing α -galactosylceramide on dendritic cells or subcapsular macrophages, iNKT cells upregulate the expression of CD40L and IFN- α , which stimulates the maturation of dendritic cells into efficient antigen-presenting cells [58]. Cognate interaction of these dendritic cells with T_{FH} cells is then followed by a germinal centre reaction that induces moderate IgG production by long-lived plasma cells, affinity maturation through SHM and immune memory [28].

Studies show that iNKT cells also establish a cognate interaction with follicular B cells (Fig 1; [61,62]). Indeed, a subpopulation of iNKT cells upregulates CXCR5 after interacting with α -galactosylceramide presented by CD1d-expressing B cells. Subsequent entry into the follicle stimulates iNKT cells to differentiate into NKT $_{FH}$ cells through the activation of a Bcl6-dependent programme that induces the expression of CD40L, IL-21 and other canonical T_{FH} cell-associated molecules, including ICOS and PD1 [61,62]. The ensuing cognate interaction of NKT $_{FH}$ cells with germinal centre B cells induces strong primary IgG production by short-lived follicular plasmablasts but little affinity maturation and no immune memory (Sidebar A).

iNKT cells further enhance antibody production by inducing the formation of short-lived extrafollicular plasmablasts (Fig 1). In this response, iNKT cells establish a cognate interaction with CD1d-expressing B cells that express lipid-specific immunoglobulin receptors, including B cells located in the marginal zone of the spleen [59,63]. The resulting CD1d-instructed iNKT cells upregulate the expression of CD40L and release cytokines such as IFN- γ —two stimuli that trigger the differentiation of lipid-specific B cells into extrafollicular plasmablasts that secrete IgM and IgG [59,63]. Thus, by establishing cognate or non-cognate interactions with follicular

and extrafollicular B cells, iNKT cells activate T-cell-independent-like pathways that elicit rapid waves of high-affinity or low-affinity antibodies to invading microbes.

Eosinophils

Plasmablasts that emerge from the germinal centre transiently enter the circulation and subsequently home to the bone marrow [64]. This highly vascularized lymphoid compartment contains a specialized niche that promotes the differentiation of plasmablasts into long-lived and terminally differentiated plasma cells that continuously release high-affinity antibodies into the circulation [64]. The plasma cell niche contains multiple non-lymphoid cell types, including eosinophils (Fig 2). This granulocyte subset is well known for its ability to

repel helminth infections and trigger allergic reactions, through the release of a broad array of inflammatory and cytotoxic molecules, which are pre-stored in intracellular granules [65]. In addition, eosinophils regulate adaptive immune responses by presenting antigen to T cells and releasing T_H2 -polarizing and B-cell-stimulating cytokines, such as IL-4 and IL-13 [66].

In mice, eosinophils present in bone marrow plasma cell niches release large amounts of APRIL [55]. This TNF family member is structurally and functionally related to CD40L, and stimulates plasma cell survival by interacting with the BCMA receptor [55,67]. Bone marrow eosinophils also secrete IL-6 (Sidebar A), a cytokine with an important role in the survival and maturation of plasma cells [55]. In the presence of antigenic priming, bone marrow eosinophils enhance APRIL and IL-6 secretion, and secrete

other plasma cell survival factors, including the cytokines IL-4, IL-10 and TNF [55,68]. Of note, eosinophils are in close contact with fibroblast-like reticular stromal cells that secrete CXCL12, a chemokine that binds to the CXCR4 receptor, which is highly expressed by both eosinophils and plasma cells [55,69]. Signals from CXCR4 support the migration of both plasma cells and eosinophils towards a common CXCL12-producing stromal niche in the bone marrow, and promote their retention in this niche [55].

However, eosinophils are not the only cell type that control plasma cell survival, as megakaryocytes and osteoclasts are also important. Megakaryocytes are platelet-generating haematopoietic cells and support plasma cell survival by releasing APRIL and IL-6, as do eosinophils [70]. Depletion of either eosinophils or megakaryocytes reduces the number of plasma cells in the bone marrow, although to a different extent [55,70]. Plasma cell survival signals are also provided by osteoclasts, bone-remodelling cells that stimulate plasma cell survival through poorly understood contact-dependent signals [71]. Further survival signals are provided by basophils that express IL-4 and IL-6 after immune stimulation, and by macrophages and dendritic cells that express APRIL and IL-6, at least at a young age [72,73].

Evidence indicates that the plasma cell niche is a multi-component and flexible bone marrow compartment that requires survival signals from both stromal and haematopoietic cell types. The relative impact of each of these 'nurse' cells on plasma cell survival seems to vary depending on age and the presence of active immunization. Different subsets of 'nurse' cells could also release distinct chemotactic factors to unevenly attract plasma cells that express specific classes of antibodies. In agreement with this, plasma cells that secrete IgG, IgA or IgM have distinct chemokine receptors and specific migration patterns [74].

Neutrophils

Unlike follicular B cells—which elicit high-affinity but slow-appearing T-cell-dependent antibody responses to specific epitopes on microbial proteins—splenic marginal zone B cells predominantly promote low-affinity but fast-appearing T-cell-independent antibody responses to highly conserved antigenic determinants present in the circulation under homeostatic, post-immunization or infectious conditions [75,76]. These antigens stimulate homing

Sidebar A | In need of answers

- (i) Why does the cognate NKT_{FH} –B cell interaction induce little or no B-cell memory?
- (ii) How can eosinophils deliver survival signals to plasma cells without discharging the inflammatory and cytotoxic mediators contained in their granules?
- (iii) How can N_{BH} cells infiltrate splenic perimarginal zone areas and deliver B-cell activation signals without inducing inflammation and tissue damage?
- (iv) Can N_{BH} cells enhance post-immune antibody responses? If so, do they regulate the activation of T_{FH} cells, iNKT cells and dendritic cells?
- (v) Which is the receptor for IgD on basophils? How do IgD and IgE induce basophil release of B-cell-stimulating factors without triggering inflammatory discharge of vasoactive factors, such as histamine?

of both dendritic cells and neutrophils to the marginal zone of the spleen [3]. The role of dendritic cells in the activation of marginal zone B cells by microbes is relatively well understood and involves the release of BAFF and APRIL [3,77]. By contrast, the impact of neutrophils on marginal zone B-cell responses remains unclear, partly due to the limitations of commonly used *in vivo* experimental models [78,79]. In particular, neutrophils account for less than 25% of circulating leukocytes in mice, but up to 75% of circulating leukocytes in humans.

Neutrophils are the first immune cells that migrate to sites of infection or inflammation [78,79]. However, work shows that neutrophils also occupy perimarginal zone areas of both human and macaque spleen under homeostatic conditions (Sidebar A), in the absence of overt infection or inflammation (Fig 3; [80]). The spleen of mice also contains perifollicular neutrophils, but about tenfold less than humans and monkeys [80]. These neutrophils interact with perifollicular and marginal zone B cells through a non-inflammatory pathway, which begins during fetal life and accelerates after birth; a time that coincides with the colonization of mucosal surfaces by commensal bacteria [80]. A crosstalk of neutrophils with B cells might seem surprising but it is consistent with studies showing that neutrophils release large amounts of APRIL, and its homologue BAFF (or BlyS), after stimulation by cytokines or microbial products [81–83].

In humans, splenic neutrophils constitutively release large amounts of APRIL, BAFF and IL-21, thereby delivering powerful

◀ **Fig 1** | T_{FH} cells and iNKT cells deliver activation signals to B cells. (1) Cognate interaction of B cells with T_{FH} cells. B cells use IgM and IgD receptors to internalize antigen captured by subcapsular sinus macrophages and paracortical dendritic cells (DCs), and subsequently migrate to the T–B cell border. Here, B cells present a peptide–MHC-II antigenic complex to early T_{FH} cells—which express CD40L and IL-21, and originate from a cognate interaction of T cells with DCs. After this initial activation, B cells can follow one of two alternative pathways: (1A) they become extrafollicular IgM-secreting plasmablasts or (1B) they upregulate CXCR5 expression to enter the follicle in response to CXCL13, a chemokine from FDCs that also attracts T_{FH} cells. In the follicle, B cells differentiate to germinal centre B cells that undergo clonal expansion, and establish a second cognate interaction with T_{FH} cells after recognizing antigen on FDCs with high-affinity immunoglobulin receptors. This interaction leads to a germinal centre reaction characterized by CSR, affinity maturation through SHM, differentiation of high-affinity germinal centre B cells into either long-lived memory B cells or plasma cells and primary IgG production. (2) Non-cognate interaction of B cells with iNKT cells. DC presentation of soluble CD1d-restricted glycolipids to iNKT cells expressing an invariant TCR promotes the cognate interaction of DCs with T cells, followed by formation of T_{FH} cells with B cell helper activity. The ensuing germinal centre reaction generates an antibody response similar to that induced by T_{FH} cells. (3) Cognate interaction of B cells with NKT_{FH} cells. B-cell presentation of soluble CD1d-restricted glycolipids upregulates CD40L and IL-21 expression by iNKT cells, and induces their differentiation into NKT_{FH} cells, which provide cognate help to antigen-specific follicular B cells. The ensuing germinal centre reaction induces CSR, little or no affinity maturation, but strong primary IgG production and differentiation of B cells into short-lived plasmablasts but not into memory B cells. (4) Cognate interaction of B cells with iNKT cells. B-cell presentation of soluble CD1d-restricted glycolipids activates iNKT cells, which provide cognate help to antigen-specific B cells. The ensuing extrafollicular reaction induces some CSR and primary IgM and IgG production by short-lived plasmablasts, but no affinity maturation and no B-cell memory. CD40L, CD40 ligand; CSR, class switch recombination; CXCL13, CXC chemokine ligand 13; CXCR5, CXC chemokine receptor 5; FDC, follicular dendritic cell; IgD/M, immunoglobulin D/M; IL, interleukin; iNKT cell, invariant natural killer T cell; MHC, major histocompatibility complex; NKT_{FH} cell, natural killer T follicular helper cell; SHM, somatic hypermutation; TCR, T-cell receptor; T_{FH} cell, T follicular helper cell.

Fig 2 | Eosinophils, megakaryocytes, osteoclasts and other non-lymphoid cells provide survival signals to bone marrow plasma cells. (1) Pre-immune conditions. Plasmablasts that emerge from the germinal centre reaction upregulate the expression of the chemokine receptor CXCR4, enter the circulation and home to the bone marrow in response to CXCL12—a chemokine released by local stromal cells. In the bone marrow, plasmablasts become long-lived antibody-secreting plasma cells in response to APRIL and IL-6 secreted by eosinophils, which express CXCR4, and are therefore retained in the niche occupied by plasma cells. Further plasma cell survival signals are supplied by megakaryocytes through APRIL, whereas macrophages and dendritic cells (DCs) produce both APRIL and IL-6. The plasma cell survival signals are then generated by osteoclasts in a contact-dependent manner. (2) Post-immune conditions. After immunization, eosinophils enhance their constitutive APRIL and IL-6 secretion and release IL-4, IL-10 and TNF to increase plasma cell survival. Under similar conditions, basophils provide extra plasma cell survival signals through IL-4 and IL-6. APRIL, a proliferation-inducing ligand; BCMA, B-cell maturation antigen; CXCL12, CXC chemokine ligand 12; CXCR4, CXC chemokine receptor 4; Ig, immunoglobulin; IL, interleukin; TNF, tumour necrosis factor.

antibody-inducing signals to marginal zone B cells [80]. Accordingly, splenic neutrophils are known as B cell helper neutrophils (N_{BH} cells). N_{BH} cells differ from conventional neutrophils present in the circulation, in that N_{BH} cells express phenotypic, genetic and functional traits that reflect activation by local micro-environmental signals [80]. Consistent with this, the accumulation of N_{BH} cells in perifollicular areas of the spleen coincides with postnatal deposition of discrete amounts of microbial TLR ligands of mucosal origin, such as LPS [80,84–86]. In addition to activating N_{BH} cells, these microbial products stimulate the recruitment of N_{BH} cells, or their circulating precursors, to the spleen by eliciting the

release of neutrophil-attracting chemokines from perifollicular sinusoidal endothelial cells. Conversely, a lack of TLR signals or mucosal bacteria decreases the number of N_{BH} cells in the spleen [80].

Microbial products could also stimulate the differentiation of N_{BH} cells from circulating precursors. Indeed, human perifollicular sinusoidal endothelial cells exposed to LPS stimulate the reprogramming of conventional neutrophils into N_{BH} cells, through a mechanism involving IL-10, an anti-inflammatory cytokine that provides regulatory signals to neutrophils [80,87]. When exposed to microbial products, neutrophils acquire regulatory properties and themselves release IL-10, particularly in mice [88–90].

Fig 3 | Neutrophils, dendritic cells and macrophages deliver activation signals to marginal zone B cells. (1) Pre-immune conditions. In humans, marginal zone B cells receive help from N_{BH} cells, which probably arise from the reprogramming of conventional circulating neutrophils by cytokines—including IL-10—released by splenic perifollicular sinusoidal endothelial cells and macrophages. This process is activated by the physiological translocation of small amounts of microbial products from mucosal surfaces, including TLR ligands. N_{BH} cells trigger CSR, SHM and the formation of plasmablasts that secrete IgM, IgG or IgA by activating marginal zone B cells through BAFF, APRIL and IL-21. Microbial products trapped by NET-like projections emanating from N_{BH} cells might enhance the activation of marginal zone B cells by engaging immunoglobulin receptors and TLRs. This pathway leads to the formation of a pre-immune antibody repertoire to conserved microbial antigens. (2) Post-immune conditions. Marginal zone B cells receive activation signals from dendritic cells (DCs) and macrophages that migrate to perifollicular areas of the spleen after capturing blood-borne microbes. These DCs and macrophages not only release BAFF and APRIL, but also present native antigen, which activates immunoglobulin receptors and TLRs in marginal zone B cells. The resulting marginal zone reaction induces some CSR but no SHM (at least in mice) and generates short-lived plasmablasts secreting IgM and IgG antibodies. APRIL, a proliferation-inducing ligand; BAFF, B-cell-activating factor of the TNF family; CD40L, CD40 ligand; CSR, class switch recombination; CXCL13, CXC chemokine ligand 13; CXCR5, CXC chemokine receptor 5; FDC, follicular dendritic cell; IgA/G/M, immunoglobulin A/G/M; IL, interleukin; N_{BH} cell, B cell helper neutrophil; MHC, major histocompatibility complex; NET, neutrophil extracellular trap; SHM, somatic hypermutation; TCR, T-cell receptor; T_{FH}, T follicular helper cell; TLR, Toll-like receptor.

Thus, IL-10 might be instrumental in generating N_{BH} cells able to stimulate antibody production in a non-inflammatory environment. GM-CSF might also have a prominent role in this process, as identified in innate response activator B cells—a plasmablast-like subset of splenic perifollicular B cells that protect against

microbial inflammation [91]. Given that GM-CSF stimulates the survival, activation, chemotaxis and B cell helper reprogramming of neutrophils [78–80], it might cooperate with IL-10 to foster a non-inflammatory crosstalk between N_{BH} cells and marginal zone B cells in perifollicular areas of the spleen.

In humans, N_{BH} cells upregulate the expression of AID and induce CSR from IgM to IgG and IgA by activating marginal zone B cells through BAFF, APRIL and IL-21 [80]. Furthermore, N_{BH} cells enhance marginal zone B-cell survival and trigger their rapid differentiation into antibody-secreting plasmablasts [80]. Consistent with these findings, patients with severe congenital neutropenia have fewer marginal zone B cells and reduced steady-state production of IgM, IgG and IgA to various T-cell-independent antigens, including LPS [80]. By contrast, neutropenic patients show conserved steady-state production of IgM, IgG and IgA to T-cell-dependent antigens [80], highlighting the predominant marginal zone B cell helper function of N_{BH} cells.

Human marginal zone B cells accumulate several mutations in their immunoglobulin genes, possibly through an extrafollicular T-cell-independent pathway that is already active during fetal life [92–95]. This pathway could include N_{BH} cells, as marginal zone B cells strongly upregulate AID expression and accumulate more immunoglobulin gene mutations after exposure to N_{BH} cells [80]. On the other hand, marginal zone B cells from neutropenic patients lacking N_{BH} cells have fewer immunoglobulin gene mutations [80]. The mechanism by which N_{BH} cells trigger SHM is unknown, but could involve the activation of marginal zone B cells by extracellular DNA-containing traps emanating from N_{BH} cells [80,96]. In addition to trapping intact antigen for possible presentation to marginal zone B cells, these post-apoptotic NET-like structures are associated with the release of TLR ligands, which could induce AID expression and SHM, at least in humans [97–101]. In mice, a similar T-cell-independent pathway elicits SHM in some extrafollicular B cells [102–104], but thus far there is no evidence of an involvement of marginal zone B cells.

In humans, marginal zone B cells have also been proposed to undergo SHM through a canonical T-cell-dependent pathway, which might reflect the ability of some marginal zone B cells to deposit antigen in the follicle and activate T_{FH} cells [105,106]. However, in adult humans the spleen contains rare germinal centres and lacks T_{FH} cells [11], which suggests a substantial involvement of T-cell-independent signals in the marginal zone B-cell responses that occur during homeostasis. Marginal zone B cells could also be skewed towards a T-cell-independent pathway due to the ability of N_{BH} cells to suppress the activation of $CD4^+$ T cells in a contact-independent manner [80]. The dual B-cell-activating and T-cell-suppressive functions of N_{BH} cells could allow them to initiate short-lived T-cell-independent antibody responses to circulating commensal antigens, as opposed to long-lived T-cell-dependent antibody responses, which could cause inflammation and unnecessary plasma cell occupation of limited bone marrow niches.

Nevertheless, N_{BH} cells can reverse their T-cell-suppressive activity in the presence of inflammation. Indeed, activated neutrophils acquire dendritic-cell-like antigen-presenting properties, express T-cell co-stimulatory molecules, release T-cell-activating cytokines and enter the lymphoid follicle under inflammatory conditions, possibly to activate T_{FH} cells and enhance T-cell-dependent antibody production [78–80]. In general, the crosstalk of N_{BH} cells with B cells indicates that neutrophils are not only eager users but also efficient inducers of antibodies, and might therefore help to build the large pre-immune, or natural, antibody repertoire that provides early protection against infection [107]. Whether neutrophils also participate in post-immune antibody responses remains to be established.

Basophils

Basophils are the least abundant granulocytes in the circulation and have mostly been studied for their participation in pathological immune responses, including allergic reactions induced by IgE antibodies against allergens [108,109]. These antibodies bind to a high-affinity receptor FcεRI that triggers degranulation and subsequent release of highly inflammatory factors—including histamine—on IgE crosslinking by allergens [108,109]. However, advances have revealed a non-redundant role of basophils in host protection (Fig 4).

In the presence of parasitic infections by helminths, basophils migrate to draining lymph nodes through a process enhanced by IL-3 [110]. At the lymph nodes, basophils release IL-4 to induce the formation of T_H2 cells, which elicit B-cell production of protective IgG1 and IgE antibodies [111–114]. This response would involve the internalization and processing of antigen by basophils, possibly through IgE, as well as cognate interaction of basophils with T_H2 cells. However, the priming of T_H2 cells by basophils in lymph nodes and, more generally, the involvement of basophils in T_H2 responses remains a matter of debate. Indeed, some studies identified prolonged interactions of T_H2 cells with basophils in peripheral tissues but not lymph nodes [115], whereas other studies indicate that T_H2 responses occur in the absence of basophils [116]. Thus, the impact of basophils in T-cell-dependent antibody production probably varies depending on the nature of the immunizing agent.

Remarkably, basophils recognize recall antigens through pre-bound IgE antibodies that are generated during a primary immune response [117]. Antigen recognition by these low-affinity antibodies does not lead to histamine release, but rather to upregulation of CD40L and release of IL-4 and IL-6. These cytokines provide helper signals to memory B cells, both directly and indirectly, through the enhancement of IL-4, IL-6, IL-10 and IL-13 production by T_H2 cells [117]. In this regard, the depletion of basophils decreases memory B-cell responses to pathogens, thereby increasing the susceptibility to sepsis [117]. The involvement of basophils in recall antibody responses is also suggested by studies showing that depletion of basophils reduces the production of autoreactive antibodies and attenuates antibody-mediated inflammation in autoimmune disorders such as lupus [118].

Basophils deliver extra B cell helper signals by interacting with IgD (Sidebar A), an enigmatic antibody isotype released by IgD class-switched plasmablasts that originate in the human upper respiratory mucosa [36,119]. Despite being heavily hypermutated, mucosal IgD is largely polyreactive and would enhance local immune protection by binding to commensals, pathogens and their virulence factors [36,119–121]. In addition to crossing epithelial barriers to reach the mucosal surface, IgD binds to circulating basophils, monocytes, neutrophils and mast cells through an unknown receptor [36]. Crosslinking of pre-bound IgD induces basophil release of BAFF, IL-4 and IL-13, which in turn stimulate B cells to produce IgM and undergo CSR to IgG and IgA in a T-cell-independent manner [36]. IL-4 and IL-13 from IgD-activated basophils could also enhance T-cell-dependent antibody production by inducing the differentiation of T_H2 cells [36,122,123]. Consistent with this scenario, IgD-deficient mice show an impairment of T_H2 -cell-dependent IgG and IgE responses to protein antigens [124]. Thus, basophils might use both pre-bound IgE and IgD antibodies to amplify B-cell responses systemically and at mucosal sites of antigen entry. Whether pre-bound IgE and IgD antibodies also enhance basophil release of plasma cell survival factors such as IL-4 and

Fig 4 | Basophils deliver activation signals to mucosal and lymph node B cells. (1) IgE-mediated signals. In mice, basophils bind to IgE generated during a primary immune response through a high-affinity receptor (FcεRI). In a secondary immune response, binding of recall antigen to IgE triggers basophil release of IL-4 and IL-6, which enhances the differentiation of T_H2 cells secreting IL-4, IL-6, IL-10 and IL-13 (1A). With CD40L, these cytokines enhance the activation and expansion of class-switched memory B cells in the context of a cognate interaction that leads to the generation of IgG-secreting plasma cells. Antigen-activated basophils might also stimulate memory B cells by delivering helper signals through IL-4, IL-6 and CD40L in a T-cell-independent manner (1B). This pathway might also enhance IgE production in mucosal B cells. (2) IgD-mediated signals. In humans, B cells from the upper respiratory mucosa undergo non-canonical CSR from IgM to IgD by either T-cell-dependent or -independent pathways. This process leads to the formation of mucosal and circulating plasmablasts that secrete IgD antibodies to respiratory bacteria. IgD enhances local and systemic immune responses by binding to mucosal and circulating basophils through an unknown IgD receptor (IgDR). In the presence of IgD crosslinking by antigen, basophils enhance CSR and antibody production by activating B cells through various stimuli, including CD40L, BAFF, APRIL and IL-4. Whether IgD-activated basophils also induce T_H2 polarization remains unknown, although some data suggest this possibility. APRIL, a proliferation-inducing ligand; BAFF, B-cell-activating factor of the TNF family; CD40L, CD40 ligand; CSR, class switch recombination; IgD/DR/E/G/M, immunoglobulin D/DR/E/G/M; IL, interleukin; MHC, major histocompatibility complex; TCR, T-cell receptor; TD, T-cell-dependent; TI, T-cell-independent; T_H2 , T helper 2 cell.

IL-6 in the bone marrow remains unclear, although some data suggest this possibility [73,117,122,125].

Mast cells

Similarly to eosinophils and basophils, mast cells express the high-affinity IgE receptor FcεRI, and mediate allergic reactions and protective immune responses against parasites by undergoing degranulation in response to IgE crosslinking by antigen [126]. Growing evidence also implicates mast cells in the regulation of adaptive immune responses, including antibody production by B cells [127]. Mast cells can induce IgA and IgE production by

stimulating B cells through CD40L and the cytokines IL-4, IL-6, IL-10 and IL-13 [128–131]. Mast cells also enhance mucosal IgA responses through a non-inflammatory pathway that might involve their interaction with Foxp3-positive T_{Reg} cells [131,132]. Mucosal T_{Reg} cells express CD40L and release IL-10, IL-21 and TGF-β, which on the one hand stimulate protective IgA production by B cells, and on the other hand, inhibit inflammatory IFN-γ production by T cells [133,134]. This dual activity enables T_{Reg} cells to generate mucosal immunity without causing inflammatory disruption of the epithelial barrier that separates trillions of potentially harmful commensal bacteria from the sterile milieu of the body.

Dendritic cells, FDCs and epithelial cells

In addition to promoting T-cell-dependent IgG responses in lymphoid follicles and T-cell-independent IgM and IgG responses in the marginal zone of the spleen, dendritic cells are important for the induction of IgA responses at the mucosal interface. Mucosal dendritic cells that express the integrin CD103 induce the formation of Foxp3-expressing T_{Reg} cells by releasing retinoic acid, a key immune regulator derived from the vitamin A present in the diet [135–137]. This induction also involves mucosal epithelial cells, which—as dendritic cells—release retinoic acid after sensing microbial products [138]. Although their plasticity is still debated, T_{Reg} cells from mucosa-associated lymphoid follicles, such as Peyer's patches, acquire phenotypic and functional traits that resemble those of canonical antibody-inducing T_{FH} cells, including the expression of ICOS, PD1, CXCR5, CD40L and IL-21 [133,134,139,140].

However, mucosal T_{Reg} cells also express large amounts of TGF- β , a canonical anti-inflammatory cytokine required for optimal induction of IgA in B cells [133,134,141]. In the presence of TGF- β , signals from CD40L and IL-21 induce B cells to produce non-inflammatory IgA instead of inflammatory IgG antibodies [142]. Mucosal T_{Reg} cells could also inhibit IgG production by delivering negative signals to T_{FH} cells with IgG-inducing function. In this regard, the relationship between mucosal T_{Reg} cells and systemic T_{FR} cells remains poorly defined, but evidence shows that mucosal T_{Reg} cells—similarly to systemic T_{FR} cells—optimize antibody affinity maturation [48,49,133,143].

In Peyer's patches, IgA responses are also supported by FDCs. These mesenchyme-derived cells are ontogenetically and phenotypically distinct from dendritic cells, provide a structural scaffold to the lymphoid follicle and trap antigen in the form of periodically arrayed immunocomplexes that contain antibodies and complement fractions, including a C4BP fraction with CD40L-stimulating function [144,145]. In addition to eliciting immunocomplex-mediated activation of immunoglobulin receptors, complement receptors and CD40 on follicular B cells [144,145], FDCs release BAFF, APRIL and TGF- β on receiving imprinting signals from commensal bacteria and retinoic acid. FDCs subsequently enhance IgA CSR and production, possibly by stimulating follicular B cells in a T-cell-independent manner [146,147]. FDCs show similar B cell helper activity also in systemic lymphoid follicles, in which they induce early formation of IgM-secreting plasmablasts in a T-cell-independent manner [145].

B cells from Peyer's patches could receive extra help from TNF and iNOS-producing dendritic cells (TipDCs; [148]). Indeed, TipDCs increase the responsiveness of follicular B cells to IgA-inducing signals from TGF- β by releasing nitric oxide, which in turn increases the expression of the type II subunit of the TGF- β receptor on B cells [148]. However, data show that IgA-secreting plasma cells acquire TipDC-like traits in the intestinal microenvironment, including the expression of antimicrobial mediators such as TNF and iNOS [149]. Thus, some of the functions previously ascribed to intestinal TipDCs could actually be mediated by IgA-secreting plasma cells.

Given its continuous exposure to dietary and commensal antigens, the intestine has developed alternative T-cell-independent pathways for IgA production. In mesenteric lymph nodes, plasmacytoid dendritic cells stimulate follicular B cells to undergo IgA CSR and production by releasing BAFF and APRIL, but not TGF- β , on exposure to IFN- β from local stromal cells imprinted by gut commensal bacteria [150]. A complex interaction between commensals, lymphoid

tissue-inducer cells and stromal cells generates IgA-inducing dendritic cells in isolated lymphoid follicles—an intestinal IgA-inducing site distinct from Peyer's patches and mesenteric lymph nodes [151]. These dendritic cells stimulate IgA production by secreting BAFF, APRIL and TGF- β . In the diffuse lymphoid tissue of the intestinal lamina propria, IgA production is also supported by TipDCs that secrete BAFF and APRIL, and by flagellin-responsive TLR5-expressing dendritic cells that secrete retinoic acid and IL-6 [148,152].

In intestinal T-cell-independent pathways of IgA production, dendritic cells would not only supply activating factors, but also present antigen to B cells [153,154]. In Peyer's patches, dendritic cells sample large antigens present in the intestinal lumen by extending dendrites through tight junctions connecting conventional epithelial cells, or through transcellular pores formed by specialized antigen-sampling epithelial cells, known as M cells [155–157]. In the lamina propria, dendritic cells capture small antigens across passages generated by mucous-secreting goblet cells [158]. After sampling and internalization of antigen through non-degradative uptake pathways, dendritic cells can recycle unprocessed antigen to the cell surface for presentation to immunoglobulins on B cells [159,160]. Epithelial cells would also contribute to IgA production by releasing BAFF and APRIL, and increasing dendritic cell production of these B-cell stimulation factors through the IL-7-like cytokine TSLP [161,162].

Macrophages

Macrophages that are strategically located in the subcapsular sinus of lymph nodes and in the marginal zone of the spleen recognize, internalize and retain microbial antigens through a variety of germline-encoded pattern recognition receptors, including TLRs, C-type lectin receptors and scavenger receptors [163,164]. In lymph nodes, subcapsular macrophages are important for the presentation of particulate antigens and immunocomplexes to B cells, which then migrate to the T–B border of the follicle to initiate T-cell-dependent antibody production [4–6,165]. Of note, subcapsular macrophages also present CD1d-restricted glycolipid antigens to iNKT cells, which subsequently induce rapid antibody production by activating extrafollicular or follicular B cells [59,60,62,166]. In the bone marrow, macrophages and their precursors could further support T-cell-dependent antibody production by enhancing the survival of plasma cells through APRIL and IL-6 [55,72,167].

Macrophages also promote T-cell-independent CSR and antibody production by releasing BAFF and APRIL [168–171]. This release increases in response to innate signals, such as those provided by microbial TLR ligands [172]. T-cell signals from IFN- γ and CD40L also enhance BAFF and APRIL release by macrophages [168,169]. Although sufficient to elicit CSR, macrophage-derived BAFF and APRIL only induce B-cell proliferation and antibody secretion in the presence of co-stimulatory signals from immunoglobulin receptor ligands, TLR ligands and cytokines, including IL-6, IL-10 and TGF- β [169–171].

Remarkably, the crosstalk between macrophages and B cells also enhances immunity through an antibody-independent mechanism: B cells promote the maintenance of subcapsular sinus macrophages by releasing lymphotoxin [173,174]. This TNF family member induces subcapsular sinus macrophages and splenic stromal cells to secrete powerful antiviral cytokines—such as IFN- α/β —as well as chemokines that attract professional IFN- α/β -producing cells—such as plasmacytoid dendritic cells [173–175].

Concluding remarks

Although T cells have traditionally been considered as the main source of help for follicular B cells, it is clear that innate lymphoid cells such as iNKT cells also have a pivotal role in follicular antibody responses. They sustain the germinal centre reaction and induce T-cell-dependent antibody diversification, selection and production. In addition, iNKT cells could cooperate with T_{FH} cells, T_{FR} cells and FDCs to support the survival and maturation of germinal centre B cells, and orchestrate their differentiation to plasma cell or memory B-cell pathways. Remarkably, plasma cells and memory B cells that emerge from the germinal centre reaction optimize their function and increase their lifespan after receiving helper signals from eosinophils, basophils, megakaryocytes, macrophages, dendritic cells and osteoclasts. Finally, helper signals from dendritic cells, macrophages, neutrophils, eosinophils, basophils, mast cells and epithelial cells support T-cell-independent antibody responses by providing help to B cells present at mucosal surfaces or the marginal zone of the spleen.

The identification of new lymphoid and non-lymphoid helping partners for B cells and plasma cells opens potential avenues for therapeutic intervention. For example, adjuvants that target T_{FH} cells, T_{FR} cells, iNKT cells, granulocytes, dendritic cells, macrophages, mast cells and epithelial cells might enhance the quantity, quality and lifespan of protective antibodies produced by systemic and mucosal B cells, in response to T-cell-dependent or -independent immunogens. Conversely, depleting eosinophils could be useful to dampen the production of pathogenic antibodies by long-lived plasma cells in the context of autoimmune disorders.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Andrea Cerutti

Montserrat Cols

Irene Puga