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Naive and memory B cells in T-cell-dependent and T-independent responses

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Abstract. This review focuses on the properties and roles of distinct subsets among the primary and the memory B lymphocytes regarding their contribution to helper-T-cell-dependent and -independent antibody responses. The naive/memory B cell functions are explained in the context of current concepts on the basic mechanisms of humoral immunity. Differences between murine and human B cells are also discussed.

Continuous B cell production maintains primary B repertoires

New B cells are produced at a high rate from hematopoietic stem cells until advanced age in humans [59]. This maintains primary B cell repertoires for the constitutive production of natural antibodies, and for the generation of T-independent and T-dependent adaptive immune responses to antigens to which the organism becomes exposed. The V(D)J DNA rearrangements which create the primary B cell antigen receptor (BCR) specificity repertoires occur in a context of non-random utilization of variable region (V) genes reflecting the evolutionary pressures to produce antibodies reacting with frequent pathogens [1].

At the immature B cell stage, where a BCR formed of μ heavy and kappa (or lambda) light chains becomes expressed at the cell surface for the first time, self-reactivity generates signals that lead to receptor editing: replacement of unsuitable V regions by re-rearrangement of DNA. This occurs frequently; it is only if such editing fails within an allowed time frame that apoptosis occurs [10]. The bone marrow environment permits editing [61]. The immature B cells then undergo final maturation in the periphery in contact with all the tissues accessible to recirculating cells. Strong self-reactivity causes apoptosis, weaker reactivity causes different degrees of inactivation called anergy. Anergic cells still can be recruited into immune responses by appropriate activation signals. A low level of self-reactivity leads to positive selection of B1 B cells producing the natural antibodies [28]. Differentiation into this B sublineage thus seems to be instructed by BCR signals. However, all naive B cells must express a BCR to survive.

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The set point of the BCR molecular signalosome [20], which determines these outcomes according to spatial density and affinity of self-determinants, as well as to B maturation stage, is influenced by signals from a variety of other receptors that modulate the BCR signal, such as CD5, CD22, FcγR2b, PD-1 receptor, etc. [18, 54]. Reduced capacity for anergy and apoptosis causes increased self-reactivity, usually together with expansion of B1 B cells. Coexpression of IgD together with IgM defines mature B cells. IgD seems to exert subtle effects on B cell activation/tolerance, so subtle that δ knockout is irrelevant for the life of a laboratory mouse.

Symbiosis of B cells with their microenvironments

Distinct B sublineages emigrating from the bone marrow express different chemokine receptors and homing molecules which direct them according to combinatorial codes to different microenvironments [13]. B1 B cells migrate mainly into peritoneal and pleural tissues/cavities. Of the other ("conventional" or B2) B cells, some home to the primary B follicles of either the Peyer's patches of gut-associated lymphoid tissue (GALT) or other mucosa-associated lymphoid tissue (MALT), and/or the spleen or lymph nodes. Other B2 cells home to the splenic marginal zone and microenvironments corresponding functionally to the splenic marginal zone between follicles in human lymph nodes and Peyer's patches [48]. The marginal zone is a macrophage- and dendritic cell-rich microenvironment which favors T-independent B responses. It is located at the white–red pulp junction, close to the sinuses where the circulating antigens arrive. The tyrosine kinase Pyk-2 and the transcription factor Aiolus must be expressed in murine B cells for marginal zone homing [26]. In a symbiotic relationship the B cells actively support the development and maintenance of the microenvironments on which they depend. B cells are required for the development of Peyer's patches including M cells [22]. B cells produce lymphotoxin α2β and tumor necrosis factor α (TNFα) for the follicular stroma cells [3]. Only a minority of the newly generated B cells become relatively long-lived cells that recirculate between blood and their home environments. All primary B cells must express an antigen receptor for survival and for homeostatic expansion to predetermined cell levels. This is also the case for naive T cells. The TCR-proximal tyrosine kinase p56lck is necessary for homeostatic proliferation, but not for survival [64].

Pre-B cells in the bone marrow express the μ heavy chain; the splicing required for δ expression – which experimentally can replace μ for B maturation – is normally suppressed. Mice unable to express μ or δ were found to generate IgA B cells quite abundantly as well as intestinal IgA antibodies [41]. In these mice, bone-marrow-derived cells mature to pre-B and further stages in the GALT, but not in the bone marrow. Conventional B cells capable of generating T-independent adaptive IgA responses as well as IgA-producing B1 cells are apparently generated in the GALT. Possibly this pathway may also be important in normal mice and humans.

Natural antibodies

The natural antibodies, which are mainly IgM and, in the MALT, to a large extent IgA, as found in mice, are important as first-line defense against microorganisms [18, 41, 50]. T-independent as well as γ/δ-T-cell-dependent mechanisms participate

in the differentiation of the B1 into plasma cells [78]. B1 B cells and some γ/δ-T cells can be considered as part of natural immunity. The B1 cells may be positively selected not only by self-antigens but also by determinants of the constitutive commensal bacterial flora in the gut. B1 cells frequently express CD5. This inhibitory receptor [7] is also found on anergic B cells [30] and on B cells recently activated by T cells in vitro [24]. This means that the self-reactivity of the B1 cells is constantly controlled, and that CD5 is not a specific marker for B1cells. The natural antibodies generally show broad cross-reactivity with soluble and cellular self-antigens (low-affinity polyreactive antibodies); they neutralize some pathogens quite efficiently [50]. Most likely, B1 cells can also be recruited into adaptive immune responses.

Antigen recognition in immunological synapses

Chronically persisting antigens are tolerogenic, i.e. as if they were all self-determinants. Chronic BCR ligation leads to diminished calcium signaling. This is sufficient for activation of the transcription factor NFATc, but not for that of NFκB, the key transcription factor for lymphocyte activation. Because the stimulatory effects of NFATc mainly occur by synergism with NFκB, the inhibitory effects of this multifunctional transcription factor predominate [23]. Adaptive immune responses thus occur in response to an acute increase of antigen. The primary responses take place only in the secondary lymphoid tissues [83]. T-dependent and probably most T-independent antigens (at least in humans) are recognized by the B cells on professional antigen-presenting cells (APC), such as migrating dendritic cells (DC) or resident follicular dendritic cells (FDC), by the formation of an immunological synapse.

A synapse is formed by large membrane areas of polarized interacting cells [5, 16, 46]. The antigen receptors and the many other receptors for positive and negative signals are spatially organized, together with their signaling modules, in complex concentric configurations that change over time: first one finds the adhesion molecules and later on the antigen/antigen receptors in the central supramolecular activation cluster (c-SMAC). This requires dynamic cytoskeletal alterations and adaptors for the linkage of the signaling components. WASP, the protein deficient in Wiskott-Aldrich syndrome, is such an adapter [16]. T-independent B cell responses to the polysaccharides of encapsulated bacteria are particularly affected by this condition.

One aspect of the synapses then is that positive and negative signals are organized. Phosphatase CD45, which is required for activation of antigen-receptor-associated tyrosine kinases, is at one time moved to the periphery of the T-cell–APC synapse, but later on retransported into the center by means of endosome traffic [16]. The list of cellular receptors that inhibit B cell activation is rapidly growing [18, 54]. B cell activation signals also favor expression of or signaling from inhibitory receptors [23]. One can assume that ligands on various tissues suppress B cell activation, in analogy to the protective function of CD47 on red blood cells against phagocytosis by macrophages [51]. In particular, synapsing is required for the proper activation of naive lymphocytes.

Another aspect is antigen concentration in the c-SMAC. Rare antigens bound to the APC (e.g. via complement receptors) become concentrated. The BCR become concentrated in the glycolipid-enriched membrane domains called lipid rafts, linked to their signalosomes. B cells selectively internalize the BCR that bind antigen, for processing and presentation of peptides on MHC class II to a potential helper T cell.

Even antigen presented as a transmembrane molecule on an APC can be internalized, by membrane pinching [5].

Synapses and properties of T-independent antigens

For many microbial antigens, the DC and / or B cells are equipped with receptors of innate immunity, such as the Toll-like receptors for *Escherichia coli* lipopolysaccharide (TLR4), bacterial CpG DNA (TLR9), etc. [53]. T-independent type 1 (TI-1) antigens react with such receptors. Human B cells express some toll-like receptors (TLR) [49]; TLR2 (a receptor for peptidoglycans form *Staphylococcus aureus*) is found on GC B cells, but not on peripheral blood B cells [19]. However, in contrast to murine B cells, human B cells do not respond to lipopolysaccharide. Costimulatory molecules leading to B cell activation occur on DC and FDC, e.g. DC-8 on human FDC [37]. Thus, these cells can transfer their activation state onto the B cells.

TI-2 antigens cause extensive BCR cross-linking; they are formed by arrays of repetitive determinants in a rigid form, such as occur on many bacteria and viruses [76, 83]. In contrast to naive murine B cells, in vitro stimulation of naive human B cells by extensive BCR cross-linking [anti-Ig antibodies on beads or coated plates, or protein-A-rich *S. aureus* Cowan I (SAC)], alone or together with many cytokines, including BAFF/BlyS [63], does not lead to Ig secretion, only some proliferation. Even CD40 ligation together with B-differentiation-inducing cytokines – in the absence of intact T cells – does not induce Ig secretion in naive B cells [31]. Memory B cells can be readily induced by SAC to proliferate and secrete Ig [44, 80]. Therefore it appears that the activation of naive B cells in particular is more tightly controlled in humans than in mice, probably because organisms with a longer generation time must exercise a better control over autoimmunity. This may require synapse formation.

How B cell activation by TI-2 relates to synapse formation remains to be elucidated. Manifestly, some antigens induce TI-2 responses better than others, independent of the antigen dose / antigen concentration in the c-SMAC. Affinity for the BCR is an independent variable [34]. Antigens can combine TI-2 and TI-1 properties. Bacterial flagellin is an example, flagellin is recognized by TLR5 [29]. Activated complement (C3d) present on many TI-2 antigens in vivo reacts with the complement receptor CD21 (on B or DC), which on B cells is linked to the activator CD19. In addition, the exact two-dimensional spacing of the repetitive B epitopes on a rigid TI-2 antigen most likely plays a role even in synapses, possibly by maximizing the effects of BCR-associated tyrosine kinases on the immunoreceptor tyrosine-based activation motifs (ITAM) of neighboring BCR [14].

T-dependent immune response

The T-dependent immune responses in the B follicles that lead to the formation of secondary follicles with germinal centers (GC), generate the repertoire of long-lived memory B cells [45, 84]. Like adaptive immunity in general, memory increases the probability of survival in a world in which repeated assaults by opportunistic pathogens are the rule. The GC is a sophisticated incubator for B cell expansion. For the typical generation of 10^3–10^4 progeny from one B cell, multiple rounds of proliferation induced by FDC–B and B–T synapses are required. Somatic V(D)J hypermuta-

tion and Ig isotype switching are particularly frequent during the GC reaction. Affinity maturation, which involves hypermutation together with cell selection, seems to exclusively occur in the GC under normal conditions.

Induction of the GC response

The T-dependent response requires "cognate" T–B interaction. For the initiation of the response, B cells must see, on a DC (or FDC), an antigen which, after internalization and enzymatic processing, generates a peptide (T epitope) presentable on MHC class II. Helper T cells must see antigen first on a DC, and a T cell must then recognize on a B cell the same peptide together with the same MHC class II that it has seen on a DC. The chemokine receptor CXCR5 participates in the homing of recirculating B cells to follicles; the ligand CXCL 13 is elaborated by FDC [13, 62]. Some studies in mice indicate that the B cell encounters the antigen on a DC for the first time when it travels from the high endothelial venules to the follicle through the T zone of the lymph node. The B cell then also encounters a specific helper T cell during its journey. Other data indicate that B cells first recognize the antigen on an FDC in the follicle [21]. FDC efficiently capture antigens coated with antibody (natural antibody or cross-reactive memory antibody) and/or complement C3d, which arrive through lymphatics. In this case, the B cell must encounter a T cell at the edge of (or within) the follicle. Both scenarios may occur depending on the site of the first encounter with antigen.

Depending on the nature of the antigen, a T-independent B response can be initiated in the GC [75]. Sooner or later a few activated helper T cells that also express CXCR5 - a species of T cell not fitting into the Th1/Th2 paradigm – migrate into the follicle [40, 62]. Initial costimulation of the T cell by the DC involves B7–1/2 interaction with CD28 constitutively expressed on the T cells. Activated T cells then express the inducible costimulator ICOS. The B cell has the ligand, B7RP-1. The B cell has to stimulate the T cell to elicit helper functions mediated by CD40L and other cell-bound or secreted T molecules [70]. The B cell also stimulates the T cell with OX40L; many TNF-family molecules (CD40L, CD27L, CD30L, OX40L, 4–1BBL, TNF, FasL, BAFF) and their receptors are involved in T-B collaboration [38]. Eventually, about two or three B cells per GC generate expanded clones. The cells proliferate as large centroblasts in the dark zone of the GC. After a few rapid divisions, one every 6–8 h, they become smaller centrocytes and localize to the FDC network in the apical light zone where the GC T cells also reside. Here reactivation takes place. Obviously the antibody response should start early after infection by a pathogen. Thus, B cells leave the GC after various numbers of proliferation cycles, some B cells stay in the GC for 2 weeks or longer.

Switching and hypermutation

Hypermutation and Ig class switching occur in the proliferating cells. Both processes are mechanistically transcription-coupled. Hypermutation involves DNA double-strand breaks during transcription of V regions, followed by repair by components of DNA, "mismatch repair", in conjunction with several error-prone, lesion-bypass DNA polymerases [52, 69]. Polymerase η is an A–T mutator in this process, as

found in patients with variant xeroderma pigmentosum with a deficiency of this polymerase [81]. The specific signals inducing hypermutation are not yet known; CD40L is not essential [79].

Class switching occurs as a result of recombination between certain DNA regions located upstream of each Ig heavy-chain constant region. The essential regions remain to be discovered; the classical tandem-repeat μ switch region is important but not essential for switching starting from IgM [39]. Cytokine signals select target regions for switching, by inducing chromatin modification and generation of germline transcripts from the targeted regions [68]. DNA recombination then occurs, linking VDJ to a new constant region located further downstream than the one initially expressed. Various components of non-homologous DNA end-joining that also participate in the V(D)J recombination are utilized (DNA end-binding proteins, DNA-dependent kinase, but RAG1/2 are not involved in the DNA cleavage). In humans with a "non-leaky" CD40L defect mutation, virtually no switch occurs; this is the X-linked hyper-IgM syndrome. Activation-induced cytidine deaminase (AIC) deficiency gives an autosomal hyper-IgM syndrome as well as lack of hypermutation [58]. AIC could be an RNA-editing enzyme. Thus, related proteins resulting from this RNA editing may participate in both processes. In humans with severe combined immunodeficiency with normal B cells (i.e. T cell deficiency), it is also almost exclusively IgM that is produced.

Various cytokines target switching to distinct IgG subclass patterns; switching to IgE is well known to be dependent on interleukin-4 (IL-4) or IL-13. In mice with a conditional (Cre/loxP-mediated) transforming growth factor β (TGFβ) receptor deletion, which allows testing of this receptor function in adult animals, virtually no IgA switch occurs [11]. In naive or memory human B cells from peripheral blood, TGFβ1 strongly induces IgA1 and IgA2 germline transcripts, but IgA switching did not occur in the presence of DC [4], oligomeric CD40L or mouse EL-4 thymoma cells [80] in the presence of many cytokines tested (C. Werner-Favre, F. Bovia, R. Zubler, unpublished). It is possible that IgA switching depends on MALT-type microenvironmental components [41].

Cell selection in the GC

In conjunction with cell selection, hypermutation leads to antibody affinity maturation. Hypermutation carries the risk of producing autoantibodies. The general principles of the cell selection for affinity maturation are well known. GC B cells are in an apoptosis-sensitive state; e.g. the anti-apoptotic protein Bcl-2 is down-regulated. The B cells need survival signals from antigen, FDC and T cells. The B cells that are crowded in the GC compete for antigen. Conditions leading to reduced apoptosis relax the cell selection and affinity maturation; they also increase autoantibody generation and the risk of follicular lymphomagenesis.

FasL/Fas-mediated apoptosis is important. Activated T and B cells express FasL and Fas. Because CD40L is a potent inducer of Fas, autoimmunity can even be found in CD40L deficiency [23]. FLIP protein competes with caspase-8 for the formation of the death-inducing signaling complex at the Fas death receptor. BCR cross-linking increases FLIP activity (in addition to various other anti-apoptotic effects, such as up-regulation of Bcl-xL). Murine B cells are protected from Fas death by FLIP hyperexpression [74]. Using lentiviral vectors [60], we found that human B cells trans-

duced with viral FLIPs are completely protected from the effect of oligomeric Fas ligand (F. Bovia, P. Salmon, C. Werner-Favre, et al., studies in progress). In contrast to B cells, DC express FLIP constitutively and are in fact activated by FasL [57]. B cell activation by FasL has not been found.

What normally happens when a BCR mutates to autoreactivity is less clear. If a B cell no longer presents those peptides that are recognized by the T cells in the GC, it should die out. T cells have a key control function. In mice expressing the influenza virus hemagglutinin (HA) as self-antigen, in which naive anti-HA B cells are negatively selected, a strong anti-HA GC response and anti-HA B memory cell generation can be induced in the presence of virus-specific helper T cells [55]. B cells cross-reacting with self and the original antigen pose a problem. Possibly, HLA-DO, a modulator of peptide presentation on MHC II in B cells, is important in the control of autoimmunity [27].

Long-lived plasma cells and memory B cells

Ig secretion does not occur in the GC; it would interfere with the cell selection for affinity maturation. Generally, proliferation signals inhibit differentiation in B cells. In proliferating B cells the transcription factor BSAP/Pax5 is a potent suppressor of other transcription factors required for the terminal differentiation of B cells into antibody-secreting plasma cells, such as XBP-1 and Blimp-1 [56, 73]. The gene repressor Bcl-6 acts on various genes for cell-cycle control [Bcl-6 can become oncogenic in follicular lymphoma by the V(D)J hypermutation mechanism] as well as genes for plasmocytic differentiation [65].

B cells that have been activated in an immune response (T-dependent or T-independent) permanently acquire CD27 expression. CD27 cross-linking in conjunction with IL-10 and IL-2 leads to B cell differentiation [2]. Since some T as well as B cells in the GC express CD27L, a certain proportion of centrocytes can leave the GC already committed for differentiation, equipped with homing molecules for the medullary chords of lymph nodes, the bone marrow, the mucosa and other sites to which the plasma cell precursors disseminate. Since recirculating $CD27^+$ B cells can also be readily induced in vitro to become plasma cells by BCR cross-linking with SAC [44] and cytokines, such as IL-2 and IL-10, which they produce themselves [32, 84], other post-GC B cells could undergo plasmocytic differentiation in various tissues in the presence of antigen. Syndecan-1 is a marker for plasma cells.

Some plasma cells have a long life span [45, 84]. In humans, several months of systemic treatment with anti-B-cell (anti-CD20) antibody for lymphoma does not significantly reduce the serum Ig levels [42]. Other plasma cells are short-lived (days to weeks), in particular those that appear first during the T-dependent response and home to the medullary chords in the local lymph node. The life span could depend on the microenvironment. It is also possible that repeated restimulation in synapses favors longevity of B cells, as has been proposed for T cells [36]. This would explain why the GC reaction generates plasma and memory cells with a long life span.

As with memory T cells, persisting antigen is not required for survival of memory B cells [43]. Recirculating human memory B cells maintain a high expression of Bcl-xL anti-apoptotic protein, in contrast to naive B cells [8]. When such cells are put in culture without stimulation, the Bcl-xL mRNA decreases rapidly, indicating that a high level is maintained by exogenous signals. For memory T cells in mice,

IL-15, but not IL-2, provides an important survival signal [35]. Memory cells (B or T) undergo antigen-independent cell division from time to time; they show a capacity for self-renewal and homeostatic proliferation.

Switched and non-switched CD27+ B cells

The human recirculating CD27+ B cells, but not the naive B cells induced to express CD27 by SAC in culture, have acquired the capacity for plasmocytic differentiation in assays in vitro [44, 80]. This reveals some functional maturity, which may require T–B or DC–B synapse formation and/or still unknown B cell activators. About 45% of recirculating B cells in adults are CD27+. Among these cells, Ig-switched cells, IgM+ cells that lack IgD (IgM-only cells), and IgM+IgD+ cells occur in similar proportions, and more than 90% of all these cells carry V(D)J mutations [33]. Thus, a high proportion of non-switched, mutated B cells occur in humans.

In patients with complete CD40L functional deficiency, both the switched and the IgM-only cells are lacking, whereas some V(D)J mutated IgM+IgD+ CD27+ cells are produced, although in most cases at low levels (1%–2% of peripheral blood B cells in six of eight patients, 4% and, for unknown reasons, 60% in the two others) compared to normal controls (7%–10%) [79]. Follicles are severely disorganized in CD40L deficiency; CD40L is also required to maintain FDC. Thus, some hypermutation could either still occur in such tissue or take place elsewhere, i.e. in the splenic marginal zone [79]. In some species, like sheep, diversification by V(D)J mutation occurs during B cell ontogeny, mainly in the GALT. The marginal zones in spleen, Peyer's patches and lymph nodes are indeed an important reservoir of V(D)J-mutated B cells in adults [15, 71, 72]. However, in hyper-IgM syndrome one expects a compensatory increase of the T-independent marginal zone B cell responses to infectious agents. Thus, the low levels of recirculating mutated B cells in this condition indicate that these normally are mostly GC-derived. Therefore, in normal individuals, when they are not just undergoing an acute immune response (T-dependent or T-independent) with high blood levels of rapidly tissue-homing CD27+ plasma cell precursors, the recirculating CD27+ cells are essentially long-lived post-GC memory B cells. The high proportion of memory B cells may be due to the longer life span of humans compared to mice.

Ig switch capacity of CD27+ B cell subsets

Recently we investigated the Ig switch capacity of the three CD27+ B subsets [80]. IgM+IgD+ cells switch to all IgG subclasses to the same extent as naive cells. In contrast, IgM-only cells have a very low switch activity. This suggests a switch defect, such as could occur when attempted switching leads to loss of certain μ DNA switch regions. Lack of this subset in CD40L deficiency [79] accords with some switch-related mechanism. The CD27+ cells that had already switched to IgG in vivo showed no increase of IgG4 in response to IL-4. Thus, although it is known that secondary switching to further-downstream constant regions can occur [68], at least some switch options are strongly reduced in cells that have already switched.

The generation of these three post-GC subsets may reflect evolutionary pressure for optimization of immune responses. A tendency for "isotype stabilization" in

switched cells would ensure that a secondary response to the same pathogen generates those isotypes which have been instructed by signals of natural immunity during the primary response [82]. The GC also produces V(D)J-mutated B cells that can cross-react with novel pathogens. Fully conserved switch capacity is an advantage when the cells respond to novel pathogens.

Moreover, affinity maturation of IgM antibodies seems to be very important, although the IgM are pentameric antibodies. Many IgM antibodies to the polysaccharides of encapsulated bacteria in humans carry V(D)J mutations and, at least for some of these antigens, depending on the germline V repertoire, mutations increase the antibody affinity [1]. A cellular receptor for polymeric IgA and IgM (Fcα/μR) mediating phagocytosis of antibody-coated bacteria has recently been found [66], extending the effector functions of IgM beyond antigen neutralization and complement activation. Also, deficiency of serum IgM in mice predisposes them to development of IgG autoantibodies; maintenance of a certain IgM/IgG ratio seems to be important [17]. IgM has a short half-life in vivo but, compared to monomeric IgG, it takes five times more plasma cells to produce an equal number of IgM molecules. Therefore, the immune system might utilize active switch suppression in the GC, and not just rely on the probabilistic nature of switching. GC T cells expressing CD30L can suppress switching in human B cells [12]. Interestingly, this preferentially affects non-antigen-selected cells, i.e. possibly B cells that have mutated and no longer recognize the GC-response-inducing antigen, but which can serve as functionally experienced cells reacting with novel pathogens. Loss of switching capacity in the IgM-only cells may also be a means of isotype stabilization to maintain a certain IgM level.

Memory B cells for T-independent responses

The capacity of the immune system to generate T-independent B responses is important in the case of antigens that can not induce a classical T–B collaboration, such as polysaccharides or phospholipids. Such responses can be enhanced by pathogen receptors of natural immunity. Potentially, γ/δ-T cells recognizing non-peptide antigens on non-classical MHC molecules can also participate [76]. However, it is important to realize that not only naive but also the functionally experienced memory B cells can participate in T-independent responses (Fig. 1). As mentioned above, compared to naive cells, human memory B cells show a much enhanced response to T-independent stimulation. The memory B cell pool is quantitatively important in adults. Depending on the pathogen and the primary BCR specificity repertoire, it is more the functional maturity, the novel cross-reactivities or the affinity maturation of the memory B pool that leads to enhanced T-independent responses. Even pathogens that induce an almost exclusively T-independent primary response can, after repeated exposure, cause an accumulation of specific memory B cells. This occurs when a small proportion of the T-independent antigen forms covalent or non-covalent associations with proteins that allow for a T-dependent GC response with pathogen-specific or cross-reactive T helper cells. This is natural immunization corresponding to a low-dose polysaccharide-protein conjugate vaccine. The clinically relevant immaturity of the T-independent response to the polysaccharides of encapsulated bacteria in infants aged less than 2 years [1] most likely reflects the time required to generate memory B cells. Progressively, the marginal zones in spleen and lymph nodes become enriched in post-GC cells [15, 71, 72].

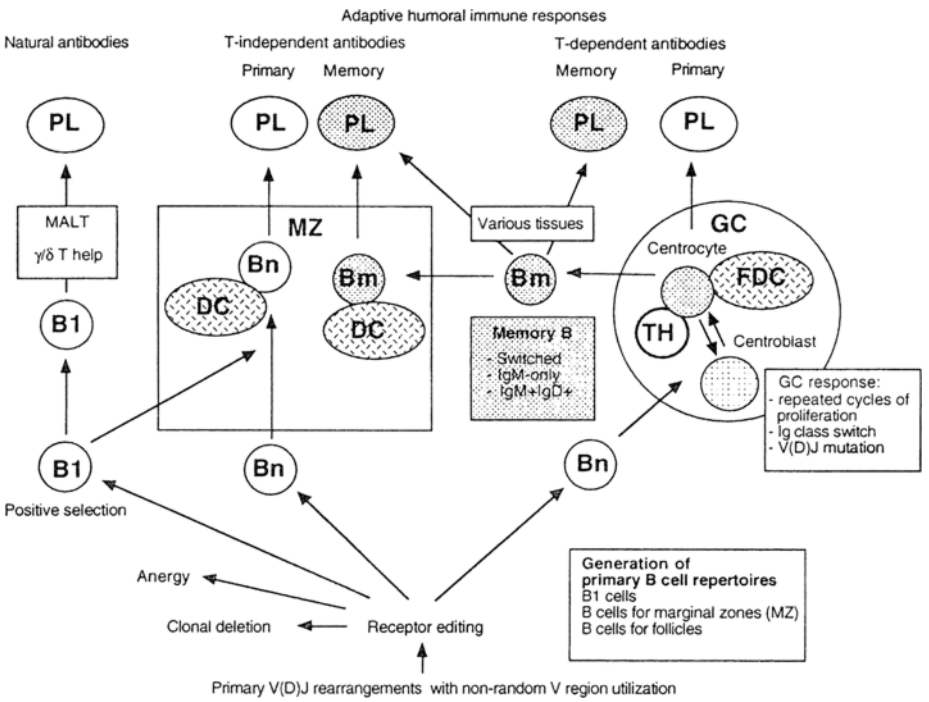

Fig. 1. Participation of primary and memory B cell subsets in T-dependent and T-independent humoral immune response. *Bn* naive B cells, *Bm* memory B cells; *PL* plasma cells, *DC* dendritic cells; *FDC* follicular dendritic cells; *TH* helper T cells; *MZ* marginal zones; *GC* germinal centers

A cytokine receptor for T-independent B responses

The B lymphocyte activator of the TNF family (BAFF), also called B lymphocyte stimulator (BLyS), THANK, TALL-1 or zTNF4, has recently been discovered in humans and mice [47, 63, 67, 77]. This cytokine acts on two known receptors, BCMA, which seems to be B-specific, and TACI found on B and T cells [67]. One function of BAFF is to act as a survival factor for immature B cells emigrating from the bone marrow to the spleen, i.e. for the cells in which self-tolerance has to be established [6]. BAFF hyperexpression in transgenic mice causes B cell hyperplasia and autoimmunity. TACI-Ig fusion protein, used as a decoy receptor, decreases autoantibody production and autoimmune disease in NZBWF1 mice [25]. APRIL, a related TNF-family factor, is produced by various tumor cells and acts on both BAFF receptors, revealing new links between cancer and autoimmunity [77]. Antibodies to APRIL have also been found to reduce T cell activation in mice [67].

Regarding the T-independent B cell response, BAFF is secreted by interferon-γ-activated monocytes and DC [47]. TACI-knockout mice have a severe defect of TI-2 B responses [9]. This is the first example of an important cytokine receptor for the T-independent response. Surprisingly, TACI-knockout mice also have twice as many B cells as control mice [9]. The different BAFF receptors may have opposite regulatory functions during B cell development.

Conclusions

Continuous production of new B cells maintains primary BCR specificity repertoires. B1 B cells are positively selected by weak self-reactivity. Their differentiation into plasma cells producing natural antibodies of mainly IgM and IgA class in mice occurs in the MALT and spleen, with participation of γ/δ-T cells. Conventional naive B cell subsets home to either the marginal zones or the B follicles of secondary lymphoid organs, the preferential microenvironments for T-independent and T-dependent primary B responses respectively. The T-dependent responses in the follicular germinal centers progressively build up the repertoire of long-lived, functionally experienced and V(D)J-mutated memory B cells, which eventually represent about half of all B cells in peripheral blood and the marginal zones in adult humans. These cells can undergo T-dependent and T-independent secondary responses as well as cross-react with novel pathogens. They express CD27 and comprise three subsets in similar proportions: (1) the Ig-class-switched cells, which exhibit a low secondary-switching capacity to at least some isotypes and thus conserve isotype patterns for secondary responses, (2) the IgM-only cells, which have lost switching capacity and assure high-affinity IgM production for complement- and Fcα/μR-mediated defenses, and (3) the IgM^+IgD^+ cells, which conserve full switching capacity and may therefore be optimal for cross-reactive responses to novel pathogens. Because of their functional maturity and long life span, the post-GC memory B cells are important for the T-independent B responses in humans.

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