gests that at least a fraction of IFN-γ⁻ cells is subject to the bystander down-regulatory activity of IFN-γ⁺ cells. It would be of interest to evaluate IFN-γRβ expression in both cell populations. Indeed, earlier reports claimed that T_H1, but not T_H2, clones down-regulate IFN-γRβ expression and are resistant to IFN-γ signaling[9,10]. If this were also the case for the subpopulations described by Wu *et al.*[1], an indirect or even IFN-γ–independent mechanism for the elimination of IFN-γ⁺ cells would be implicated.

More recently a popular new concept has emerged, which divides memory T cells into two different subsets: central and effector memory cells[11,12]. This division is based on the tissue localization and function of memory cells: central memory cells reside in lymphoid tissue and produce mainly IL-2, whereas effector memory cells are found in nonlymphoid tissue and produce IL-4 and IFN-γ. Some of the properties of the populations described by Wu *et al.*[1] are reminiscent of these subsets. They found that IFN-γ⁺–secreting effector cells were present in lower numbers than naïve cells in lymphoid tissue and thus did not develop into central memory cells[1]. It remains formally possible that IFN-γ⁺ effector cells preferentially migrate to nonlymphoid tissues, and thus resemble effector memory cells rather than short-lived effectors. Wu *et al.* did examine one nonlymphoid tissue, the lung, and concluded that IFN-γ⁺ cells were not residing outside lymphoid organs and thus were short-lived[1].

However, this conclusion is not definitive for two reasons. First, IFN-γ⁺ cells appeared to survive longer in the lung than in lymphoid tissue and reached numbers similar to naïve cells[1]. This supports the idea that the disappearance of IFN-γ⁺ cells is, at least in part, a reflection of their distinct tissue-homing properties. Second, the lamina propria of the gut was not examined for the presence of IFN-γ⁺ cells[1], although this site has been described as the prime location of effector memory cells[12]. It should also be noted that a higher percentage of IFN-γ⁺ cells was present in the lymph nodes and spleen after 120 days than after 10 days[1]. This suggests that a small population of IFN-γ⁺ cells does survive and finds its way back to lymphoid tissue in due course. It would be interesting to follow expression of the lymph node–homing receptors CCR7 and L-selectin in these cells. A scenario in which a fraction of IFN-γ⁺ cells survives as effector memory cells in nonlymphoid tissue for a limited period of time and then, by re-expressing CCR7 or L-selectin, is slowly drawn back to the lymphoid tissue may fit well with current ideas on memory cell localization.

Finally, the question remains: how are these two T_H1 cell lineages related? Are the IFN-γ⁺ cells a further differentiated form of the IFN-γ⁻ cells or are both lineages different to begin with? The fact that repetitive stimulation with antigen drives all cells into the IFN-γ⁺ population[1] suggests that the IFN-γ⁻ cells are precursors of IFN-γ⁺ cells. This developmental pathway is consistent with the linear model of T cell differentiation in which prolonged TCR stimulation and exposure to cytokines drives T cells progressively towards a final effector stage[13]. Differences in accessibility to antigen and cytokines would then account for the diversity in differentiation status that is found in antigen-stimulated T cell populations. In this model, central memory cells develop from less differentiated nonpolarized T cells, whereas effector memory cells are derived from fully differentiated effectors. A similar way of thinking could be applied to the subsets described by Wu *et al.*[1], with the less differentiated, although clearly polarized, IFN-γ⁻ cells being the source of central memory cells and the further differentiated IFN-γ⁺ effectors developing into effector memory cells.

In the final analysis, the choices between differentiation, death and persistence of antigen-stimulated T cells *in vivo* are likely to be influenced by numerous factors, including cytokine production, susceptibility to apoptosis and anatomic localization. Understanding these choices and how they are controlled will be fundamental to the rational design of vaccines and immunotherapeutic strategies.

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BLySsful interactions between DCs and B cells

Andrew J. Macpherson and Alain Lamarre

T cell help is not obligatory for B cell Ig class switching. Instead, DC expression of BLyS and APRIL can provide the signals to induce T-independent B cell production of IgG and IgA.

Immunoglobulins (Ig) are extraordinarily adaptable. At one end they have a variable antigen-binding site—a heterodimer composed of heavy and light Ig chains—that can be adjusted to bind to almost any other biological molecule and at the other there is the heavy chain tail that tells the rest of the immune system what to do next. Without Igs mothers could not share their immune experience with their offspring and we would be almost defenseless against encapsulated extracellular pathogens. Because the heavy chain tail regions of different isotypes (IgM, IgG, IgE and IgA) determine their oligomeric form (number of binding sites) and downstream signals (to fix complement, enhance phagocytosis, trigger mast cell degranulation or transport across mucous membranes), Igs can be tailored to almost any site and pathogen. To change IgM into another isotype without disrupting the antigen-binding site, B cells use class switch recombination (CSR); in this process they excise DNA to substitute a new heavy chain constant region gene. Most immunologists think of B cell CSR as secondary to help from specific T cells that have already been activated by dendritic cells (DCs). However, a report by Litinskiy *et al.* in this issue of *Nature Immunology*[1] shows that it can actually be much simpler: antigen-presenting cells (APCs) alone can directly trigger CSR

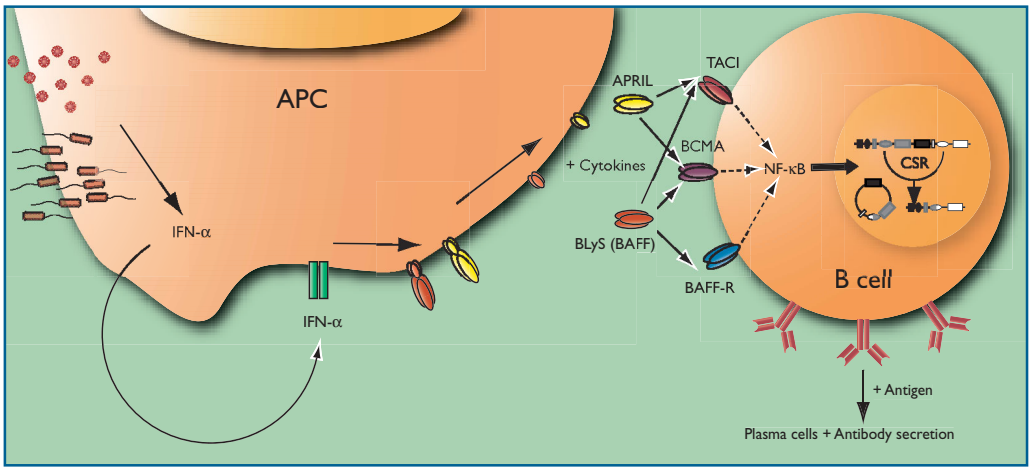

Figure 1. CD40-independent Ig class switching mediated by BLyS and APRIL. According to the model proposed by Litinskiy *et al.*[1], viral or bacterial antigens taken up by DCs or macrophages (APCs) trigger the production of IFN-α that can up-regulate expression of the TNF family members BLyS and APRIL. BLyS binds to three receptors expressed on B cells: BAFF-R, BCMA and TACI. However, APRIL binds with high affinity to BCMA and with lower affinity to TACI but does not interact with BAFF-R. In the appropriate cytokine milieu, BLyS and APRIL interactions with their respective receptors induce CD40-independent CSR through the action of NF-κB. Although not required to initiate CSR, BCR cross-linking by antigen is needed to promote plasma cell differentiation and antibody secretion.

in B cells. Their findings are exciting because they potentially link innate immunity with autoantibody formation and explain the mechanism of T cell–independent isotype switching, which is seen in the intestinal mucosa and during infections.

For classical germinal center CSR in lymphoid tissues, a T cell receptor engages a specific peptide bound to major histocompatibility complex (MHC) class II on the B cell surface, and T cell CD40 ligand (CD40L) binds B cell CD40. The Ig isotype that results is determined by the spectrum of cytokines present, secreted mainly by adjacent—T helper type 1 (T_H1) or T_H2 polarized—T cells: these specifically enhance germline transcription from a promoter upstream of one of the Ig heavy chain genes (and presumably expose this region to the recombination machinery)[2]. A DNA recombination reaction then occurs between one switch region just upstream of the μ and δ (IgM and IgD) gene cluster and another upstream of the new Ig isotype; once the intervening DNA is excised, expression of the new heavy chain constant region gene that has replaced μ and δ ensues.

This is not the whole story because we know that Ig isotype switching can occur in the absence of T cells and organized lymphoid structures, for example, during induction of IgA in the intestinal mucosa in response to nonpathogenic commensal intestinal bacteria[3]. Exactly how this happens is unclear: direct interactions between DCs and CD40L-preactivated B cells enhance CSR to IgA *in vitro*, but whether there are signaling molecules that can substitute for CD40L is unknown[4]. CD40L-deficient mice[5] and humans with inactivating CD40L mutations[6] show reduced expression of non-IgM isotypes, which confirms that this molecule signals the Ig isotype-switch program *in vivo*. Yet even CD40-CD40L interactions are not obligatory, as some Ig isotype switching (particularly to IgG3 and IgA) is seen in CD40L-deficient animals[5]. The new data from Litinskiy *et al.* are significant because they show that B lymphocyte stimulator protein (BLyS, also called BAFF, TALL-1 and zTNF4) and a proliferation-inducing ligand (APRIL) expressed on the surface of DCs can directly stimulate a B cell CSR in the presence of cytokines and independently of CD40L.

Like CD40L, BLyS and APRIL are tumor necrosis factor (TNF) ligand–like molecules of myeloid cells bound by receptors—BAFF receptor (BAFF-R), B cell maturation antigen (BCMA) and transmembrane activator and calcium modulator and cyclophylin ligand interactor (TACI)—on the surface of B cells (**Fig. 1**). Experiments in which soluble ligands or decoy receptors are injected into mice as well as studies in strains that have deletions of BLyS or its receptors show that BLyS is critical *in vivo* for maturation of B cells within the spleen after they have left the bone marrow[7]. BLyS-transgenic animals also have elevated serum concentrations of IgM, IgG, IgA and IgE, although this might be secondary to the increased proportion of mature B cells rather than a primary effect on CSR[7].

Litinskiy *et al.* have shown that naïve mature human B cells (IgD^+IgM^+) incubated with low concentrations of BLyS or APRIL undergo CSR to IgG and IgA in the presence of interleukin 10 (IL-10) or to IgA only in the presence of transforming growth factor-β (TGF-β)[1]. This activation is associated with nuclear translocation of NF-κB, which is a transcription factor that controls B cell proliferation, differentiation and CSR in response to CD40 ligation. If the B cell receptor (BCR) is cross-linked, DCs in culture that have been activated with interferon-α (IFN-α), IFN-γ or CD40L will cause B cells to switch to IgA and have the characteristics of differentiating plasma cells[1]. Litinskiy *et al.* found that these effects were dependent on BLyS but not CD40L, as they were inhibited by soluble BLyS receptors or a BLyS-neutralizing antibody but not CD40-Ig[1].

The usual view of B cell activation for CSR involves a trio of cell types: APCs that activate T cells that, in turn, provide help for B cells; CD40L-CD40 interactions are also essential to the process. Litinskiy *et al.* suggest that we may be underestimating the importance of an APC–B cell duo in which BLyS and APRIL signal CSR[1]. Their data show that up-regulation of BLyS and APRIL on the APC surface by IFN-α and IFN-γ connects innate immune responses with CSR[1], as both can be produced by APCs themselves in response to viral double-stranded RNA (dsRNA) or bacterial (CpG) products[8]. This may explain the mechanism of direct CSR to IgA that occurs within the intestinal mucosa[9], which could follow sampling of commensal bacteria by DCs that protrude through the epithelial surface[10]. It may also underlie the CSR to neutralizing IgG, which is dependent on IFN-γ but not conventional T cell help by B cells in the marginal zones of the spleen after viral infection[11].

Sadly, although the mechanism gives B cells greater flexibility to produce class-switched Igs, regulation of BLyS by IFNs may have an autoimmune downside. Patients with chronic active hepatitis secondary to persistent infection with hepatitis virus B or C are treated with IFN-α: a well known side-effect is the development of autoantibodies and autoimmune disease. The

mechanism is unknown, but we can now postulate that increased B cell survival and Ig isotype switching occurs as a result of BLyS and APRIL up-regulation. There is already some evidence for BLyS involvement in autoantibody production in systemic lupus erythematosus (SLE). BLyS-transgenic mice expand their B cell populations and develop an SLE-like disease with IgG anti-dsDNA, which is the hallmark of human disease[12]. It is even possible to slow the progression of disease in NZBWF1 mice (a murine SLE model which has increased serum concentrations of BLyS) with a TACI decoy receptor, although this does not affect preformed anti-dsDNA concentrations[12]. Intriguingly, IFN-γ deletion in NZBWF1 mice abolishes dsDNA autoantibody formation and disease severity, but, because the IFN-γ–independent isotype IgG1 is affected, it is not explained by a direct effect of the cytokine on B cells[13]. IFNs are very likely to trigger immunopathological B cell responses through BLyS, but the relative importance of B cell maturation (escaping negative selection) and CSR for autoantibody formation remain uncertain. The paper by Litinskiy *et al.* will certainly initiate a closer look at the underlying mechanisms by researchers[1].

There remain some caveats. These experiments are based on cell culture, so we do not know the importance of BLyS signaling for direct APC stimulation of B cells *in vivo*. Is the antigen-specificity of this mechanism good enough to deliver Igs that neutralize pathogens or limit penetration of environmental antigens? Also, because the experiments used cross-linking to show the involvement of the BCR, it is unclear whether the form of antigen (repetitive or monomeric) makes a difference. Nonetheless, this "new" (but probably evolutionarily old) pathway neatly simplifies the requirements for CSR and shows how the functional benefits of switching heavy chain constant regions can potentially be achieved without T cell help or organized secondary lymphoid structures.

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Does AID need another aid?

AID burst onto the scene just a couple of years ago. For all of the progress, its actual mechanism for generating changes in DNA in B cells remains elusive.

TASUKU HONJO

Somatic hypermutation (SHM) introduces point mutations almost exclusively in the immunoglobulin (Ig) gene variable diversity joining (V(D)J) exon and its flanking regions at an extraordinarily high frequency of 10^{-3}–10^{-4} base pairs (bp) per generation. This rate of mutation is about one million times higher than the usual background rate of genomic mutation. SHM normally takes place in antigen-stimulated B cells; these B cells, which express high-affinity Igs and proliferate vigorously in the germinal center, are selected by limited amounts of antigen. The combined outcome of SHM and antigen selection of Ig-bearing B cells generates antibodies of increased affinities in a process termed "affinity maturation". Although the mechanism of SHM remains a mystery, excitement in this field was triggered by the discovery of a requirement for activation-induced cytidine deaminase (AID), which has homology with the RNA-editing cytidine deaminase APOBEC-1[1,2]. In gene-targeted mice as well as humans with hyper-IgM type II syndrome, AID deficiency abolishes both Ig class switch recombination (CSR) and SHM. In addition, AID expression alone is sufficient to induce SHM as well as CSR in cotransfected target genes in B cell hybridomas, T cells and fibroblasts[3–5]. Ectopic expression of AID in NIH3T3 fibroblasts induces high-frequency mutations (4×10^{-3} bp per generation) in a green fluorescence protein gene that carries an artificial stop codon in the middle of the coding sequence[5]. These data confirm the previous finding that the target of SHM is not restricted to the V region sequence. In addition, transcription of the target gene is required for SHM and correlates quantitatively with its efficiency[6]. Strikingly, all these SHM properties are shared with CSR. Several models for the molecular mechanism of SHM and the role played by AID have been proposed (**Fig. 1a**); AID may either edit the mRNA precursor for DNA nickase or directly deaminate cytosine bases in DNA. In this issue of *Nature Immunology*, Faili *et al.* describe astonishing observations that may reveal yet another level of SHM regulation[7]. Using a human Burkitt's lymphoma cell line, BL2, that was induced to enhance SHM but not CSR by coculture with T helper cells[8], Faili *et al.* have made several observations[7] that may force us to re-evaluate previous data and their interpretation.

Their first observation was that very brief (90-min), but strong, cross-linking of the B cell receptor (BCR) with a mixture of anti-IgM, anti-CD19 and anti-CD21 was sufficient to induce SHM in BL2 cells, even in the absence of T cells[7]. Within 90 min, this stimulation induced ~5×10^{-4} mutations per bp, as assessed by sequencing of the endogenous V_H gene[7]. Because AID is constitutively expressed and is an absolute requirement for SHM in BL2 cells, these findings suggested that a factor other than AID may be limiting in the BL2 cell line. It should be noted that BL2 cells continuously accumulate a low amount of SHM during long-term culture. Thus, these data showed that in BL2 cells, AID expression alone induced some hypermutation, but strong stimulation through the BCR increased the efficiency of SHM, probably through activation of AID or another factor[7].

The second observation made by Faili *et al.* was that SHM occurs during the G1 phase but not S phase[7]; these results differed from those in a previous study[9], in which in was reported that SHM took place mostly during the S→G2 transition, and it was proposed that SHM involves homologous recombination between sister chromatids (**Fig. 1a, C**). Faili *et al.* also showed that the mutations were found on only one of the DNA strands[7], which suggested that mismatched bases were probably not repaired