Future advances in pharmacogenomics: BAFF, APRIL and plasma cells

Evaluation of: Koarada S, Tada Y, Sohma Y *et al.*: Autoantibody-producing RP105$^-$ B cells, from patients with systemic lupus erythematosus, showed more preferential expression of BCMA compared with BAFF-R than normal subjects. *Rheumatology (Oxford)* 49(4), 662–670 (2010). Systemic lupus erythematosus patients have an expanded population of plasmablasts/plasma cells in the peripheral blood that is negative for the Toll-like receptor-like molecule RP105 (CD180) and expresses the B-cell-activating factor (BAFF)/a proliferation-inducing ligand (APRIL) receptors transmembrane activator and calcium modulator ligand interactor and B-cell maturation antigen. This article discusses the role of BAFF and APRIL in maintaining the survival of plasmablasts and plasma cells and the effects of selective inhibition of BAFF versus dual inhibition of BAFF and APRIL on these specialized B cell subsets.

KEYWORDS: BAFF ■ B cell ■ BCMA ■ CD180 ■ plasma cell ■ RP105 ■ SLE ■ SLE therapies ■ TACI

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Summary of methods & results

In this paper, Koarada *et al.* describe a subset of peripheral blood B cells found in patients with systemic lupus erythematosus (SLE) that is negative for the expression of RP105 (CD180) [1]. The authors have previously shown that RP105$^-$ B cells are increased in patients with SLE and are CD38^{hi}/intracellular IgG^{hi} [2]. Other studies have similarly shown that while RP105 expression is higher on memory B cells than on naive B cells, it is absent from plasma cells [3]. Koarada *et al.* further show that RP105$^-$ B cells express B-cell maturation antigen (BCMA) and transmembrane activator and calcium modulator ligand interactor (TACI) but not B-cell-activating factor (BAFF) receptor (BAFF-R), and their survival *in vitro* is supported by BAFF, consistent with their plasmablast/plasma cell phenotype. Taken together, these studies indicate that RP105 downregulation is a feature of plasmablast/plasma cell differentiation.

Discussion

RP105 is a Toll-like receptor (TLR)-like protein expressed on B cells and myeloid cells. In myeloid cells RP105 is an inhibitor of TLR4 signaling during responses to microbial stimuli. It is also reported to function as an accessory molecule for TLR2 [4]. In B cells, crosslinking of RP105 induces B-cell proliferation and activation. RP105 crosslinking synergizes with CD40L in inducing the proliferation of naive B cells, but has less effect on memory B cells that respond vigorously to CD40L alone [3]. *In vivo*, B cells from RP105 deficient mice respond poorly to lipopolysaccharide and to TLR2 agonists but nevertheless mount normal antibody responses to T-dependent antigens (reviewed in [4]).

Systemic lupus erythematosus patients have several abnormalities of their peripheral blood B-cell populations (reviewed in [5]). They have decreased numbers of naive B cells, with a concomitant increase of class-switched memory B cells. There is expansion of a CD5$^+$ transitional B-cell subset and of a unique population of CD27$^+$/IgD$^-$ cells, which includes somatically mutated memory B cells. A hallmark of active systemic lupus erythematosus (SLE) is the appearance of many plasmablasts in the peripheral blood. These comprise two populations based on their expression of HLA-DR. DR^{bright} cells are newly derived plasmablasts that correlate with SLE disease activity. DR^{low} plasma cells do not correlate with disease activity and may represent recirculating plasma cells [6]. Thus, in the active SLE patients described by Koarada *et al.*, RP105$^-$ cells in the peripheral blood are most likely to be newly derived plasmablasts whereas RP105$^+$ cells are heterogeneous but may include a dominant population of memory B cells.

The role of BAFF and a proliferation-inducing ligand (APRIL) in providing survival signals for various B-cell subsets and in regulating B-cell tolerance is currently of interest owing to the positive preliminary results of Phase III clinical trials of a BAFF inhibitor in SLE. BAFF and APRIL are trimeric TNF-like molecules made by multiple cell types that bind to three receptors BAFF-R, TACI

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and BCMA, found on B cells, T cells (BAFF-R) and activated monocytes (TACI). The effect of BAFF and APRIL on B cells depends both on their individual binding characteristics and on the differential expression of the three receptors that are dynamically regulated throughout B-cell ontogeny. In humans, BAFF binds to all three receptors whereas APRIL binds only to TACI and BCMA (reviewed in [7,8]). The BAFF trimer binds to BAFF-R whereas optimal binding to TACI and BCMA occurs only when BAFF and APRIL are in multimeric form. BAFF can exist naturally as a 60-mer form, whereas APRIL is multimerized on the cell surface via its alternate binding capability to proteoglycans [9].

Transitional B cells exiting the bone marrow express BAFF-R and their survival and selection is dependent on the interaction of BAFF with BAFF-R, with no role for APRIL (reviewed in [7,10]). B cell activation through the B-cell receptor (BCR) upregulates the expression of BAFF-R in mature naive B cells; however, the role of signaling through BAFF-R in the survival or differentiation of B cell subsets past this stage is not well defined. In antigen-activated B cells, FcRIIB signaling decreases signaling through the BCR and this in turn prevents upregulation of BAFF-R, thus potentially decreasing B-cell viability [11]. This function may be impaired in SLE patients due to their failure to upregulate FcRIIB expression on antigen-activated B cells [12]. Germinal center B cells express low levels of BAFF-R and germinal center size and longevity are decreased when BAFF or BAFF-R are absent, although affinity maturation does not appear to be affected [13] (reviewed in [14]).

Under physiologic circumstances, memory B cells do not depend on BAFF or APRIL for their survival or reactivation, although they express both BAFF-R (in humans) and TACI (in humans and mice) [15,16]. Detailed studies in mice and humans have demonstrated that the effect of BAFF in promoting the survival of antigen-activated B cells is limited to the plasmablast stage with much less effect on class-switched memory B cells or mature plasma cells [15–17]. The same function is carried out by APRIL, suggesting that BAFF-R is not required for this effect [17]. In accordance with these findings, Koarada *et al.* show that RP105- cells (plasmablasts) from active SLE patients are rescued from cell death by either BAFF or APRIL, whereas RP105$^+$ B cells (memory B cells) are not. BAFF independence of memory B cells is further confirmed by the observation that both humans and mice treated with BAFF inhibitors manifest an increase in the percentage of circulating memory B cells despite a marked decrease in the naive B-cell population [15,18]. However, it must be noted that BAFF can collaborate with inflammatory cytokines such as IL-17 and IL-21 in promoting the differentiation of memory cells to plasma cells during active SLE [19,20]. Thus, it is still conceivable that BAFF plays a role in memory B-cell function during inflammatory states.

Transmembrane activator and calcium modulator ligand interactor and BCMA have distinct functions. T-independent B-cell responses are dependent on TACI, which may also be required for class-switch recombination. Class switching to IgA is promoted preferentially by the interaction of APRIL with TACI. Stimulation of B cells via Toll-like receptors amplifies the expression of TACI while at the same time resulting in loss of BAFF-R, further indicating a role for TACI in bridging innate and adaptive immune responses (reviewed in [21]). B cells upregulate expression of TLRs in response to BAFF, completing a positive feedback loop that may be involved in the pathogenesis of lupus [22]. Short-lived plasma cells also express TACI and can therefore be supported by either BAFF or APRIL; however, IgM and IgA producing plasma cells are more dependent on BAFF and APRIL than IgG producing plasma cells [23,24]. Long-lived bone marrow plasma cells upregulate expression of BCMA. Increased expression of BCMA upon B-cell activation and differentiation is coupled with loss of BAFF-R and can be prevented by signals received through the BCR. Conversely, BAFF-R signaling antagonizes plasma cell development [25].

Blockade of both BAFF and APRIL by the receptor fusion proteins BCMA-Ig or TACI-Ig markedly reduces the number of newly formed plasma cells in the bone marrow of normal mice [15,26]. In SLE however, terminally differentiated plasma cells and plasmablasts generated during active inflammation may survive without the need for BAFF or APRIL. In NZB/W SLE-prone mice with established disease, TACI-Ig decreases the frequency of IgG-producing plasma cells in the spleens but has no effect on the survival of bone marrow IgG-producing plasma cells, and serum levels of IgG are unaffected [23]. Importantly, when disease in NZB/W mice is accelerated with IFN-α, TACI-Ig markedly decreases IgM production but no longer affects newly formed splenic IgG-secreting plasma cells [Liu Z, Davidson A, Unpublished Data].

Several mechanistic studies have investigated the effects of selective BAFF blockade and dual BAFF/APRIL blockade on plasma

cells in humans with rheumatoid arthritis and SLE. As expected, selective blockade of BAFF has little effect on the number of circulating plasmablasts and causes only a 10% decrease in serum IgG levels [18]. Blockade of both BAFF and APRIL with TACI-Ig causes only a 20% decrease in total serum IgG levels with little effect on antibody titers to recall antigens such as tetanus toxoid [24,27,28]. This observation is consistent with the idea that fully differentiated bone marrow plasma cells receive adequate local survival signals and are no longer dependent on BAFF and APRIL. As expected, TACI-Ig has a more profound effect on the serum level of IgM than does selective blockade of BAFF alone.

It is not entirely clear why IgM-producing plasma cells are more sensitive to BAFF/APRIL inhibition than IgG-producing plasma cells. One possibility involves signaling through the BCR itself. Preliminary exploration of BCR signaling cascades has demonstrated considerable differences in gene expression and an exaggerated calcium flux in IgG when compared with IgM-bearing cells [29]. Thus, BAFF/APRIL signaling may be required to enhance BCR-mediated survival signals in IgM-bearing plasmablasts but may be dispensable in IgG-bearing plasmablasts that use other strategies to avoid cell death. Alternatively, changes in expression of other B cell surface molecules, or differences in the microenvironment might influence the relative dependence of IgM and IgG plasma cells on BAFF/APRIL for survival. Regardless of the cause, preferential depletion of IgM by BAFF/APRIL inhibition may be counterproductive in SLE because serum IgM helps to clear apoptotic debris that can exacerbate SLE flares. In addition, the compromise of the protective short-lived plasma cell response to microbial antigens may lead to an increase in infectious complications.

Future perspective

Downregulation of RP105 may be part of a program by which plasma cells decrease their proliferative capacity while at the same time they increase their secretory capacity and migrate to specialized survival niches where they can be maintained long-term by local growth factors.

Executive summary

Context of the study

- B-cell-activating factor (BAFF) and a proliferation-inducing ligand (APRIL) are TNF-like molecules that support B-cell survival, selection and differentiation. Systemic lupus erythematosus (SLE) patients express abnormally high levels of BAFF and APRIL in the blood serum.
- Recent preliminary reports of Phase III clinical trials of a BAFF inhibitor have shown efficacy in patients with moderately active SLE.
- BAFF and APRIL bind differently to the receptors BAFF receptor (BAFF-R), transmembrane activator and calcium modulator ligand interactor (TACI) and B cell maturation antigen (BCMA) and it is still not clear whether there will be a clinical advantage of blocking both BAFF and APRIL over blocking BAFF alone.
- While the survival effects of the interaction of BAFF with BAFF-R on transitional and naive B cells is well understood, the effects of BAFF and APRIL on antigen activated B cells are still being explored.
- SLE patients have multiple abnormalities of their B-cell subsets, including decreased numbers of naive B cells, expansion of memory B cells and increased numbers of circulating plasma cells that have downregulated expression of the TLR-like molecule RP105.

Objectives of the study

- To determine whether there is abnormal expression of BAFF and APRIL receptors on B cells of SLE patients.
- To analyze the effect of BAFF on survival of $RP105^+$ and $RP105^-$ B cells from human SLE patients.

Methods

- $RP105^+$ and $RP105^-$ B cells were purified from the blood of active SLE patients by cell sorting.
- Expression of the various BAFF receptors was evaluated by flow cytometry and real time PCR.
- The *in vitro* proliferative response of sorted B cell populations to BAFF was measured by flow cytometry.

Results

- $RP105^+$ cells express TACI and BAFF-R whereas $RP105^-$ cells express TACI and BCMA.
- $RP105^+$ B cells are not responsive to BAFF whereas RP105- B cells are.

Conclusion

- The study confirms that the phenotype of RP105- B cells in SLE patients is that of plasma cells/plasmablasts, similar to what is found in normal individuals.
- As in normal individuals, memory B cells are not responsive to BAFF whereas circulating plasma cells/plasmablasts are.

Future perspective

- Because plasma cells express TACI and BCMA they can be supported by either BAFF or APRIL and they are not depleted by selective inhibition of BAFF. However, their dependence on BAFF and APRIL for survival varies depending on their microenvironment and the presence of other soluble and local growth and survival factors. This may influence the outcome of clinical interventions with selective and dual-specific BAFF/APRIL inhibitors.

Multimerized BAFF and APRIL, via interactions with TACI and BCMA are important growth factors that support plasma cell survival outside these niches, or while these cells are en route to their final destination, but are less important when other growth factors are present. Nevertheless, it is possible that the combination of TACI-Ig with immunosuppressive agents that decrease levels of these growth factors will uncover an important role for BAFF/APRIL in the maintenance of bone marrow plasma cells and may cause unacceptable decreases in serum immunoglobulin or infectious toxicity. The next decade will bring definitive studies that identify the precise effects of BAFF, APRIL and their receptors on B cell and other lymphocyte subsets and a more detailed evaluation of the clinical and immunologic effects of selective BAFF blockade and dual BAFF/APRIL blockade in human autoimmune diseases.

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- ▪▪ of considerable interest

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