



any antithrombotic approach, the difficulty is finding efficacy without tipping the hemostatic balance to increased bleeding risk. Indeed, as a player in primary hemostasis, the vWF/platelet GP-Ib-IX axis has, to date, been a challenge to target without considerable risk. The findings by Casari et al. suggest an antithrombotic pathway mechanistic approach that has yet to be considered. While antagonizing the ligand/receptor interaction could be justified for interrupting primary hemostasis, a type 2B mimetic has potential to bind to normal platelet GP-Ib-IX and alter signaling pathways. Could such an approach lead to an antithrombotic effect beyond just blocking a ligand/receptor interaction? Could such a strategy lead to inhibition of activation pathways that mirror aspirin and/or be complementary? While exploring this strategy is beyond the scope of the study by Casari et al., the identification of altered signaling as a consequence of mutant vWF binding to platelets suggests such an approach might be feasible. Casari and colleagues are to be commended for providing

important data to explain molecular consequences for a gain-of-function mutation in vWF many years after the recognition of type 2B as a distinct subtype of vWD.

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## BAFF-ling autoantibodies

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**There is emerging evidence that autoantibodies directed against cytokines modulate the severity of autoimmune disease. Identification of cytokine-targeted autoantibodies in patients can be informative for diagnosis and predicting clinical outcome. In this issue of the JCI, Price and colleagues used a multiplex protein microarray to identify autoantibodies in serum from SLE patients. They found autoantibodies directed against the B cell-activating factor (BAFF) were associated with greater disease severity. This study highlights the contribution of cytokine-directed autoantibodies in disease and describes a valuable tool for identifying autoantibodies against serum antigens.**

### Cytokine-targeting autoantibodies in disease

Spontaneous autoantibody production is a hallmark of many autoimmune diseases, and these disease-specific autoantibodies are often useful in affirming a clinical autoimmune diagnosis. For instance, clinicians

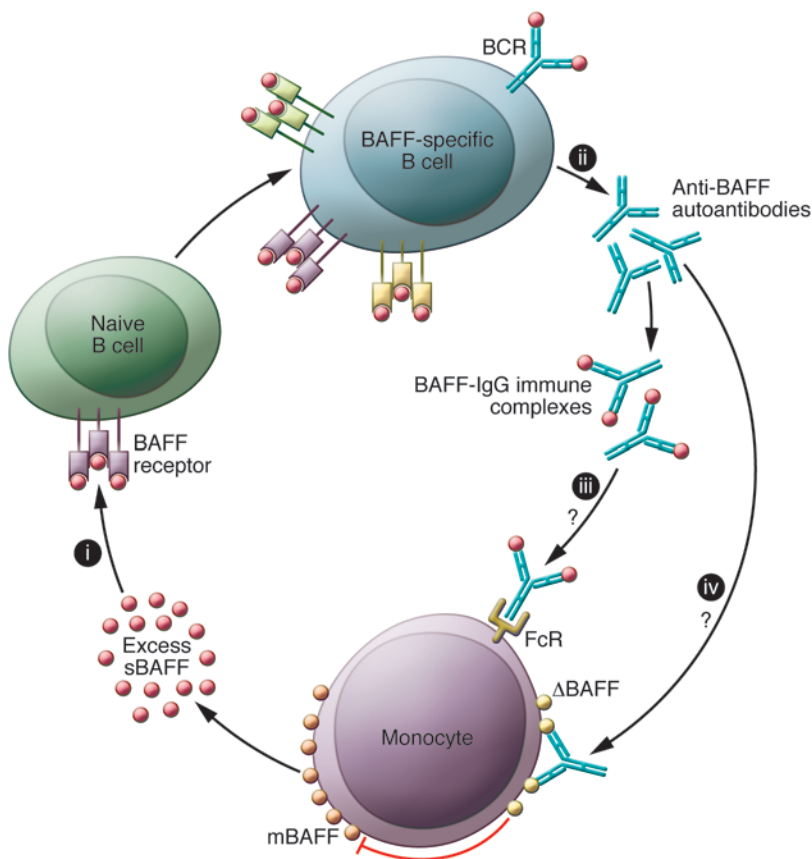
test for autoantibodies against insulin and other pancreas-specific antigens to differentiate autoimmune (type 1) diabetes from other types of diabetes. In addition to serving as markers for autoimmunity, autoantibodies can play a central role in disease pathogenesis. Strong evidence exists that autoantibodies are important in development of SLE, a systemic autoimmune disease characterized by immune-mediated injury that affects a large number of tissues, including brain, blood vessels, and

kidneys. Autoantibodies against nuclear antigens are important in disease initiation, directly mediating organ injury via complement-mediated cascades and other inflammatory mechanisms.

Interestingly, cytokines have now been described as autoantibody targets in a number of disease settings, resulting in a range of clinical manifestations (1). In many of these diseases, autoantibodies are produced against cytokines that are important in host defense and thus lead to functional immunodeficiency. Autoimmune polyendocrinopathy syndrome type 1 (APS-1), for example, is characterized by multiorgan autoimmunity and mucocutaneous candidiasis (2). For many years, the predisposition to candidiasis, which seemed to reflect a state of immunodeficiency, appeared to be an inconsistent finding among the multiple autoimmune manifestations in APS-1. A possible explanation for this seemingly irregular finding was

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**Figure 1**

Model for how autoantibodies against BAFF may exacerbate SLE. Excess sBAFF promotes outgrowth of autoreactive B cells, which include BAFF-specific B cells (i). sBAFF (red) may then bind to BAFF-specific B cell receptors (BCR) on B cells and promote production of autoantibodies against BAFF (ii). BAFF-IgG immune complexes may be bound by monocyte Fc receptors (FcR), which stimulate cleavage of membrane BAFF (mBAFF; orange) and further production of sBAFF (iii). In addition, autoantibodies against BAFF may neutralize  $\Delta$ BAFF (yellow), a natural regulator of BAFF (iv). Excess sBAFF may then promote autoimmunity and provide further antigen targets for BAFF-specific B cells.

recently revealed by reports of neutralizing autoantibodies against IL-17A and related cytokines in APS-1 patients (3, 4). The activity of IL-17 is essential for host defense against candidal infections (5, 6); therefore, autoantibodies that neutralize IL-17 function may predispose APS-1 patients to mucocutaneous candidiasis (5, 6). These data indicate that mucocutaneous candidiasis in APS-1 may not reflect primary immunodeficiency, but rather may reflect a primary autoimmunity that secondarily results in immunodeficiency. Other immunodeficient states in which neutralizing autoantibodies against cytokines predispose to infection have been described. These include opportunistic infections with nontuberculous mycobacteria as a result of autoantibodies against IFN- $\gamma$  (7, 8)

and staphylococcal skin infections associated with autoantibodies against IL-6 (9). Cytokine-targeting autoantibodies are proving to be a recurring cause of immunodeficiency in various disease settings.

#### **Cytokine-specific autoantibodies modulate disease severity**

In addition to causing immunodeficiency, autoantibodies against cytokines may modulate the severity of autoimmune disease. Approximately 25% of SLE patients produce autoantibodies against IFN- $\alpha$  (10), a cytokine that is increased in SLE patients and implicated in pathogenesis. IFN- $\alpha$ -targeted autoantibodies neutralize IFN- $\alpha$  activity and appear to be associated with milder clinical SLE symptoms. These findings suggest that autoantibody

neutralization of IFN- $\alpha$  may alleviate SLE severity, although further work is required to establish causation. In this issue, Price et al. identified autoantibodies against the unique TNF family member B cell-activating factor (BAFF; also called BLys) in SLE patient serum and demonstrated that these autoantibodies neutralize BAFF activity in cell culture systems (11). Furthermore, the authors reported a positive correlation between the presence of neutralizing autoantibodies against BAFF and features associated with a more severe SLE disease profile (11).

At first glance, this finding is somewhat surprising, since studies in mice and humans have shown that BAFF is crucial for SLE pathogenesis. BAFF is a B cell survival factor that is primarily produced by innate immune cells such as monocytes, dendritic cells, neutrophils, and stromal cells, including central nervous system astrocytes and airway epithelia (12). BAFF is produced as a membrane-bound protein that is released following protease cleavage. SLE patients have high circulating levels of BAFF, which correlate with increased autoantibody titers and SLE activity (13, 14). Additionally, the amount of available soluble BAFF (sBAFF) is a pivotal determinant of B cell homeostasis, and BAFF overexpression in mice leads to an autoimmune disease reminiscent of SLE (15, 16). Together, these findings predict that neutralization of BAFF by autoantibodies would be associated with milder disease, rather than severe disease, as observed in this study. One caveat to consider is that Price et al. found this correlation in a small cohort of SLE patients (11); therefore, these results will require validation in larger, independent SLE cohorts. It is possible that these BAFF-targeted antibodies are merely a reflection of an autoimmune response to high amounts of a protein in the circulation, and not involved in disease pathogenesis. If this association between disease severity and the presence of anti-BAFF autoantibodies is validated in larger studies, then the question of how a neutralizing autoantibody against a pathogenic cytokine worsens clinical disease will need to be addressed, and the answer will affect current understanding of SLE disease mechanisms.

One possible explanation is that autoantibodies against BAFF paradoxically stimulate sBAFF production (Figure 1). The amount of sBAFF dictates the threshold for negative selection of autoreactive B



cell clones (17), and excess sBAFF in SLE patients may promote outgrowth of auto-reactive B cell clones, including B cells reactive against BAFF itself. Since release of sBAFF results from immune complex activation of monocytes (18), perhaps the formation of BAFF immune complexes induces a positive feedback loop that further increases the amount of available sBAFF. Alternatively, if the anti-BAFF antibodies preferentially bind to a dominant-negative form of BAFF, called  $\Delta$ BAFF (19), the pathologic effects of sBAFF may be unleashed (Figure 1).  $\Delta$ BAFF is the only known natural regulator of BAFF and is a plausible autoantibody target, since it is a conserved alternate splice isoform.

### Implications and future directions

The presence of endogenous BAFF-specific autoantibodies in patients has potential clinical implications, since a therapeutic antibody against BAFF, belimumab, has recently been FDA approved for treatment of SLE (20). While belimumab has shown efficacy in autoimmune diseases, responses are incomplete, and its effect on the peripheral B cell pool in these patients is unclear. The complexity of BAFF biology and pathology will require further study if we are to effectively implement agents that target this cytokine.

Given the emerging number of anti-cytokine antibodies being identified, other “immune-modifying” autoantibodies likely exist. Price et al. describe an elegant methodology for screening patient sera using a multiplex protein microarray that will enable discovery of pathophysiologically relevant autoantibodies (11). They validated this platform in patient cohorts of 2 diseases in which autoantibodies against cytokines are a well-described feature: APS-1 and chronic mycobacterial infection (11). Although other multiplexed detection systems are available (21),

the approach used by Price et al. provides the benefit of minimizing serum sample volume and increasing the number of cytokine targets that can be screened in parallel (151 antigen targets in this study; ref. 11). Notably, this approach is not limited to detection of autoantibodies against cytokines. Autoantibodies against growth factors, chemokines, and other serum proteins can also be screened on this platform. Low-level reactivity against human growth hormone that was noted in SLE patients using this method (11) may represent one such tantalizing potential autoantibody-based mechanism that underlies SLE clinical findings. Thus, this platform represents a valuable new tool by which to identify autoantibodies against serum antigens and promises to shed light on a wide array of disease mechanisms.

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