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INSIGHTS

For whom the B(c)ell tolls: CXCL4 AIDs human autoimmunity

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In this issue of *JEM*, Çakan et al. (2023. *J. Exp. Med.* https://doi.org/10.1084/jem.20230944) explore a CXCL4-mediated mechanism by which TLRs cause autoimmunity in human B cells, breaching bone marrow tolerance.

B cells can produce antibodies against almost any antigen. While this is ideal in the context of controlling infectious diseases, production of Abs against self-antigens (Ags) can be pathogenic, causing myriad autoimmune conditions. Numerous mechanisms exist to prevent the generation of autoAbs; however, these processes can also be disrupted. Identifying mechanisms underlying immune dysregulation raises the prospect of developing therapies that target the root cause of these diseases rather than merely treating symptoms. A new study reveals that the chemokine CXCL4, which is often elevated in systemic autoimmune conditions, may contribute to disease pathogenesis by sequestering ligands from TLR9, thereby limiting the ability of these innate stimuli to trigger the B cell intrinsic tolerogenic function of TLR9 which contributes to silencing autoreactive B cells (Çakan et al., 2023).

B cells arise in the bone marrow following the progressive development of hematopoietic stem cells (HSCs) into pro-B cells, pre-B cells, and immature B cells. These stages of B cell development are characterized by the sequential rearrangement of genes encoding the variable (V), diversity (D), and joining (J) elements of the immunoglobulin (Ig) heavy chain, expression of a pre-B cell receptor (BCR) complex, recombination of Ig light chain genes, and expression of a functional BCR on immature B cells. Immature B cells exit the bone marrow as transitional B cells, which mature in the periphery into naïve B cells capable of recognizing an almost infinite number of Ags (Tangye et al., 2023). As V(D)J recombination is essentially a random process, B cells specific for self, rather than foreign, Ag will be generated. Thus, B cell development is stringently regulated to avoid generating self-reactive B cells. Indeed, several mechanisms have been elucidated that restrain autoreactive B cells, including receptor editing, clonal anergy, or deletion, thereby establishing B cell tolerance (Burnett et al., 2019).

A fundamental role of naïve B cells is to differentiate into Ab-secreting cells that recognize and clear foreign Ags, thereby protecting the host from subsequent infectious diseases following infection and/or vaccination. This is achieved by the integration of numerous signals received by B cells activated within microenvironments of secondary lymphoid tissues (Tangye et al., 2023). However, despite best efforts, B cell–specific tolerogenic mechanisms can be disrupted by various factors. Thus, the flipside to Ab-mediated protection from infection is the aberrant generation of self-reactive B cells producing autoAbs against different self-Ags. The inability to inactivate autoreactive B cells underpins the pathophysiology of many human autoimmune diseases, such as systemic erythematosus (SLE), rheumatoid arthritis, Sjogren's syndrome, or systemic sclerosis (SSc), which are characterized by production of high levels of pathogenic autoAbs (Burnett et al., 2019).

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In addition to the BCR, inputs from other receptors play critical roles in maintaining tolerance, inducing immunity and/or driving immune dysregulation. These include the Toll-like receptors TLR7 and TLR9, innate immune sensors recognizing bacterial and viral-derived nucleic acids (single-stranded RNA, unmethylated double-stranded

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DNA; Fillatreau et al., 2021; Rawlings et al., 2012). Internalization of these foreign DNA/RNA species by B cells and delivery into endosomes enables binding to cytosolic TLRs, and activation of signaling pathways downstream of the adaptor proteins IRAK4 and MYD88 (Fillatreau et al., 2021; Rawlings et al., 2012). Thus, coordinated engagement of the BCR and TLRs initiates B cell activation and differentiation in response to microbial DNA and/or RNA. However, in the setting of autoimmune diseases, TLRs can contribute to disease pathogenesis by recognizing mammalian (i.e., self) DNA/RNA released via apoptotic cells, either directly via the BCR or following internalization of immune complexes comprising nuclear proteins and stimulatory nucleic acids (Lau et al., 2005; Leadbetter et al., 2002).

Remarkably, although TLR7 and TLR9 activate similar pathways, they appear to have distinct effects on autoAb production and disease pathogenesis. Thus, single nucleotide polymorphisms in *TLR7* have been associated with greater risk of developing SLE in humans (Fillatreau et al., 2021), while deletion of *Tlr7* (Christensen et al., 2006; Jackson et al., 2014) or *Myd88* (Lau et al., 2005) ameliorates disease severity in lupus-prone strains of mice. Similarly, some activating mutations in *TLR7* may contribute to SLE is some individuals (Brown et al., 2022). In contrast, deletion of *Tlr9* exacerbates disease in mouse lupus (Christensen et al., 2006; Jackson et al., 2014). Furthermore, combined deletion of *Tlr7* and *Tlr9* prevents autoimmune pathology in mice (Santiago-Raber et al., 2010), establishing that TLR9 prevents immune dysregulation by antagonizing the pro-inflammatory function of TLR7. Importantly, B cell–specific deletion of *Tlr7* or *Tlr9* largely recapitulates the phenotype of *Tlr7*- or *Tlr9*-deficient mice, highlighting the respective B cell intrinsic pathogenic and regulatory roles of TLR7 and TLR9 in mouse models of lupus (Cosgrove et al., 2023; Tilstra et al., 2020).

Inborn errors of immunity (IEIs) result from monogenic germline mutations that disrupt human immune cell development and/or function and present clinically as distinct immune dysregulatory conditions (Tangye et al., 2022). The study of IEI has revealed critical and non-redundant roles for specific genes, pathways, and cell types in fundamental immune processes such as lymphocyte differentiation, host defense, inflammation, allergy, and immune tolerance (Tangye et al., 2022, 2023). Previous studies found that, compared to healthy donors, individuals with mutations in *IRAK4* or *MYD88* accumulated increased frequencies of circulating autoreactive B cells (Isnardi et al., 2008). This suggested that signaling likely via TLR7/9 is required to counter-select autoreactive B cells during development and thus establish B cell tolerance in humans. However, as few if any IRAK4- or MYD88-deficient individuals develop autoimmunity (Isnardi et al., 2008; Tangye et al., 2022), TLR/MYD88/IRAK pathways are also likely required for the subsequent evolution of Ab-mediated autoimmune conditions. Thus, in both humans and mice, TLR signaling can restrain or promote the generation of self-reactive B cells. While several key insights into these opposing roles have been gleaned from elegant mouse models (Fillatreau et al., 2021; Leibler et al., 2022), the mechanisms by which TLRs paradoxically prevent and cause autoimmunity remain incompletely determined.

Çakan et al. (2023) have now explored this by examining the roles of TLRs in human B cell tolerance. First, immunodeficient NSG (NOD/SCID/γc) mice were reconstituted with human HSCs transduced with shRNA specific for *TLR7*, *TLR9*, or *MYD88*. Increased proportions of poly- and autoreactive B cells were generated in mice reconstituted with *TLR9*- or *MYD88*-targeted HSC compared to mice receiving unmanipulated or *TLR7*-targeted HSC. Second, analysis of the specificity of Abs produced by B cells from individuals with hemizygous *TLR7* mutations confirmed that TLR7 is not required for B cell tolerance. Third, in vitro experiments suggested that B cells from SSc patients are hyporesponsive to the TLR9 agonist CpG, but not to TLR7 agonists, consistent with previous studies of B cells from SLE patients (Gies et al., 2018). Thus, consistent with data from mice, signaling via TLR9/MYD88, but not TLR7, appears to play a dominant role in establishing B cell tolerance, and impaired responsiveness to TLR9 ligands may contribute to the escape of autoreactive B cells from tolerogenic mechanisms in human autoimmunity (Çakan et al., 2023).

Next, the authors aimed to determine how or why TLR9 function was reduced in B cells from individuals with autoimmune conditions. The chemokine CXCL4 has been associated with several inflammatory disorders and can form macromolecular structures with mammalian and bacterial DNA to amplify responses of plasmacytoid dendritic cells (pDCs) to the stimulatory effects of TLR9 ligands (Lande et al., 2019). In contrast to pDCs, CXCL4 impeded responses of B cells from healthy individuals to CpG/TLR9 stimulation. Notably, B cells stimulated in vitro with CpG in the presence of CXCL4 exhibited a transcriptomic signature similar to unstimulated or only weakly stimulated B cells, suggesting CXCL4 blocked activation by limiting availability of TLR9 ligands rather than by initiating an inhibitory program (Çakan et al., 2023). Indeed, although CXCL4 enhanced uptake of CpG by human B cells, CXCL4 disrupted intracellular trafficking of CpG to the TLR9-rich late endosome compartments within the B cells, thereby preventing TLR9-mediated sensing of these innate immune stimuli.

To extend these studies, human *CXCL4*-transduced HSCs were introduced into the NSG mouse model. CXCL4-expressing B cells had lower responses to CpG stimulation compared to control B cells lacking CXCL4. Furthermore, $CXCL4^+$ B cells gave rise to significantly more poly/autoreactive B cells than corresponding $CXCL4^-$ B cells, indicating that intrinsic CXCL4 expression is permissive to the generation of autoreactive B cells. This is achieved by sequestering TLR9 ligands, thereby limiting the tolerogenic function of TLR9 induced by exposure to self-DNA Ags.

This study reveals a novel CXCL4-mediated mechanism that putatively breaches B cell tolerance. An inference of these findings is that CXCL4 may be an attractive molecular target for treating some autoAb-mediated conditions. CXCL4 blockade may not only restore access of DNA/RNA ligands to TLR9 in endosomes, thereby enabling delivery of B cell intrinsic TLR9-mediated tolerogenic signals, but also suppress the stimulatory effects of CXCL4/DNA complexes on activation of pDCs, which results in heightened production of type I IFNs and is associated with human SLE (Lande et al., 2019). Despite this possibility, many questions remain. For instance, the cell type(s) responsible for CXCL4 production, as well as

the mechanism by which CXCL4 signals—and whether it requires interaction with a specific receptor—are unknown. Similarly, as CXCL4 appears to function within B cells, it may be necessary to engineer specific approaches to target intracellular, rather than circulating, CXCL4. Lastly, while B cells from patients with SLE or SSc exhibit significantly reduced responses to TLR9 ligands in vitro, the magnitude of this reduction is incredibly variable, ranging from >90% inhibition to no effect to even enhanced responses (Çakan et al., 2023; Gies et al., 2018). This no doubt reflects the heterogeneity of SLE and SSc, and the likelihood of there being many different "types" of these conditions arising from myriad disease mechanisms. Thus, it may be beneficial to stratify autoimmune patients according to responsiveness to TLR9 ligands to identify those who may benefit most from CXCL4 blockade. Furthermore, it would be valuable to determine whether serum levels of CXCL4 inversely correlate with TLR9-induced B cell responses in SLE and SSc. Although these questions remain unanswered, the findings by Çakan et al. (2023) certainly provide a foundation to further explore the complexities of humoral autoimmune diseases in humans, with the prospect of tailoring therapies based on mechanisms of disease pathogenesis to enable greater therapeutic outcomes for individuals affected by these conditions.

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References

Brown, G.J., et al. 2022. *Nature*. https://doi.org/10.1038/s41586-022-04642-z

Burnett, D.L., et al. 2019. *Immunol. Rev.* https://doi.org/10.1111/imr.12808

Çakan, E.A., et al. 2023. *J. Exp. Med*. https://doi.org/10.1084/jem.20230944

Christensen, S.R., et al. 2006. *Immunity*. https://doi.org/10.1016/j.immuni.2006.07.013

Cosgrove, H.A., et al. 2023. *JCI Insight*. https://doi.org/10.1172/jci.insight.172219

Fillatreau, S., et al. 2021. *Nat. Rev. Rheumatol.* https://doi.org/10.1038/s41584-020-00544-4

Gies, V., et al. 2018. *JCI Insight*. https://doi.org/10.1172/jci.insight.96795

Isnardi, I., et al. 2008. *Immunity*. https://doi.org/10.1016/j.immuni.2008.09.015

Jackson, S.W., et al. 2014. *J. Immunol.* https://doi.org/10.4049/jimmunol.1400098

Lande, R., et al. 2019. *Nat. Commun.* https://doi.org/10.1038/s41467-019-09683-z

Lau, C.M., et al. 2005. *J. Exp. Med.* https://doi.org/10.1084/jem.20050630

Leadbetter, E.A., et al. 2002. *Nature*. https://doi.org/10.1038/416603a

Leibler, C., et al. 2022. *Nat. Immunol.* https://doi.org/10.1038/s41590-022-01310-2

Rawlings, D.J., et al. 2012. *Nat. Rev. Immunol.* https://doi.org/10.1038/nri3190

Santiago-Raber, M.L., et al. 2010. *J. Autoimmun.* https://doi.org/10.1016/j.jaut.2009.11.001

Tangye, S.G., et al. 2022. *J. Clin. Immunol.* https://doi.org/10.1007/s10875-022-01289-3

Tangye, S.G., et al. 2023. *J. Exp. Med.* https://doi.org/10.1084/jem.20221105

Tilstra, J.S., et al. 2020. *J. Clin. Invest.* https://doi.org/10.1172/JCI132328