Published in final edited form as:

Immunol Cell Biol. 2016 November ; 94(10): 918–924. doi:10.1038/icb.2016.78.

Germinal centers and autoimmune disease in humans and mice

Anthony L. DeFranco

Department of Microbiology and Immunology, University of California, San Francisco, CA, USA
94116

Abstract

Antibodies are involved in the pathogenesis of many autoimmune diseases. While the mechanisms underlying the antibody response to infection or vaccination are reasonably well understood, we still have a poor understanding of the nature of autoimmune antibody responses. The most well studied are the anti-nuclear antibody responses characteristic of systemic lupus erythematosus and studies over the past decade or so have demonstrated a critical role for signaling by TLR7 and/or TLR9 in B cells to promote these responses. These TLRs can promote T cell-independent extrafollicular antibody responses with a heavy chain class switch and a low degree of somatic mutation, but they can also strongly boost the germinal center response that gives rise to high affinity antibodies and long-lived plasma cells. TLRs has been shown to enhance affinity maturation in germinal center responses to produce high affinity neutralizing antibodies in several virus infection models of mice. While more data is needed, it appears that anti-nuclear antibodies in mouse models of lupus and in lupus patients can be generated by either pathway, provided there are genetic susceptibility alleles that compromise B cell tolerance at one or another stage. Limited data in other autoimmune diseases suggests that the germinal center response may be the predominant pathway leading to autoantibodies in those diseases. A better understanding of the mechanisms of autoantibody production may ultimately be helpful in the development of targeted therapeutics for lupus or other autoimmune diseases.

Introduction

While antibodies are essential for defense against most viruses and microbes that replicate outside host cells, antibodies against harmless environmental or food antigens can cause allergic symptoms, and in some individuals, immune tolerance to self fails for one or a few self components. Autoantibodies are responsible for disease manifestations in a variety of autoimmune diseases, including systemic lupus erythematosus (lupus or SLE), Graves' disease, myasthenia gravis, autoimmune hemolytic anemia, and pemphigus vulgaris, and additionally may contribute to the severity of disease in other autoimmune diseases such as rheumatoid arthritis. While initial studies suggested that extensive somatic mutation and antigen-based selection for higher affinity in specialized structures called germinal centers (GCs) is primarily responsible for the generation of autoantibodies in people with autoimmune disease, more recent studies have suggested a more complex picture. A better understanding the cellular and molecular basis of autoantibody responses may ultimately lead to improved ability to selectively block such responses. In this review, I first give an overview of current understanding of antibody responses that result from infection or vaccination, briefly describe mechanisms for tolerizing self-reactive B cells, and then

discuss how these pathways may relate to the production of autoantibodies. In this regard, the mechanisms leading to the production of the anti-nuclear antibodies characteristic of SLE may have some unique properties. Evidence for the involvement of the GC component of the antibody response in human autoimmune diseases is described, and finally, how research in this area may inform therapeutic efforts is briefly discussed.

Overview of the Antibody Response

The antibody response unfolds over several stages to produce antibodies of increasing affinity and efficacy. This process has been reviewed in depth elsewhere (1) and is briefly described here. Naïve mice are often found to contain antibodies of weak affinity for antigen, which nonetheless have been shown to contribute to host defense against influenza virus infection and also play homeostatic roles in aiding clearance of debris (2, 3). These antibodies are referred to as “natural antibodies” and their production may not require any overt antigenic stimulation. Upon initial antigen contact and typically requiring either innate stimulation or interactions with helper T cells, B cells are stimulated to proliferate and undergo clonal expansion for several days. Starting at about 4 days after infection or vaccination, there is an induced response that produces antibody of higher but still only moderate affinity for antigen. As this response occurs just outside of the B cell-rich follicles of secondary lymphoid tissues, this rapid, induced antibody production is referred to as the “extrafollicular” response (4). Some of the clonally expanded B cells terminally differentiate into antibody-secreting effector cells, which continue to proliferate for several more days, during which they are called plasmablasts, and then become post-proliferative plasma cells. These short-lived plasma cells typically make moderate affinity antibodies, which may be IgM or may be class-switched to IgG, IgA, or IgE. At the time that some clonally expanding B cells terminally differentiate, other antigen-specific B cells migrate into the follicles of the spleen, lymph node, or Peyer’s patch along with antigen-specific helper T cells, which at this stage are referred to as “T follicular helpers” (T_{FH}) (5), and jointly create GCs. In the GC, B cells rapidly proliferate and induce expression of activation-induced cytidine deaminase (AID), which mutates their Ig heavy and light chain variable domains. Although affinity for antigen is often compromised by these mutations, it is improved in a minority of mutated B cells. Those B cells with better affinity for antigen can preferentially internalize antigen and then load peptides from protein antigens onto their MHC class II molecules. For this reason, high affinity B cells interact more strongly with antigen-specific T_{FH} cells in the GC and receive greater cell-bound and soluble cytokine signals that promote their proliferation and survival. Enhanced BCR signaling may also contribute to the response in the GC, and it certainly does contribute to terminal differentiation to plasma cells (6, 7). In any case, somatic mutation of Ig variable domains is coupled with a stringent selection for B cells that make antibodies of increased affinity for antigen. Therefore, over a prolonged period of weeks to months, the average affinity of secreted antibody and of membrane Ig on the surface of GC B cells steadily increases. In parallel and also mediated by AID, class switch recombination changes the type of Ig heavy chain constant regions to allow production IgG, IgA or IgE in place of IgM. Output of the GC includes two components that protect against reinfection: memory B cells, which respond rapidly upon reappearance of the infectious agent or the antigen months later, and plasma cells secreting high affinity, class-

switched antibodies. Many of these plasma cells migrate to the bone marrow, where they access survival niches and may survive for years and are therefore called long-lived plasma cells. These plasma cells insure a steady supply of high affinity IgG recognizing the antigen for long lasting protection.

Interestingly, memory B cells are generated during both the early extrafollicular phase of the response and throughout the GC response (8) and thus represent a wide array of affinities, whereas long-lived plasma cells are preferentially generated from the higher affinity GC B cells (9). When antigen reappears, memory B cells are capable of rapid terminal differentiation but are also capable of participating in GC responses. In individuals who were infected with a recent epidemic of pandemic influenza virus, it was observed that many of the neutralizing antibodies they made also neutralized the seasonal influenza virus strains that are used in annual vaccinations, strongly suggesting that they were generated from memory B cells that were further diversified in GCs to increase their cross-reactive affinity for the pandemic influenza strain while retaining their affinity for seasonal influenza (10). Thus, the biological purpose of memory B cells may be to promote immune defense against pathogenic infections that are similar but not identical to the original infection. One hypothesis for the origin of autoantibodies is that they are generated as part of an antibody response to an infectious agent, which cross-reacts with an autoantigen. This scenario is referred to as molecular mimicry and it is discussed further below.

Among naïve B cells, there are several distinct subpopulations that are generated during B cell development before encounter with antigen. In the mouse, there are four B cell subtypes, referred to as B1a cells, B1b cells, marginal zone B cells and follicular B cells. The latter two B cell types are recognized in humans, and recent evidence is consistent with the presence of B1 cells in human as well (11), but their similarity to mouse B1a and B1b cells remains uncertain. In the mouse, the natural antibody is largely made by B1a cells, which are generated during fetal and infant life, prior to the generation of the other three types of B cells. B1 cells in general are most prevalent in the peritoneal and plural cavities, where they make rapid responses of low affinity to infections entering via the intestines or lungs, respectively. Typically the antibody responses of these B cells do not require helper T cells. Infectious agents that reach the bloodstream primarily induce antibody responses in the spleen, which contains moderate numbers of B1a and B1b cells as well as marginal zone B cells and follicular B cells. Marginal zone B cells are so named because they reside primarily in the marginal zone of the spleen (12), which is exposed to the blood as it flows through the splenic red pulp. They contribute to the early antibody response to blood-borne antigens and may respond with or without T cell help. The most abundant B cell population in the spleen and by far the dominant population in the lymph nodes is the follicular B cells, which are named for their preferential localization within the follicles of the white pulp of the spleen, the lymph nodes, and the Peyer's patches of the intestines. Follicular B cells recirculate between these three types of secondary lymphoid organs and they are the predominant population to participate in the GC response.

Mechanisms for Silencing Autoreactive B cells

Autoantibodies are the main cause of pathology in some autoimmune diseases, and may contribute to disease progression in others. The primary repertoire of B cells has a large fraction of autoreactive specificities, based on examination of reactivity with nuclear extracts, insulin and some other autoantigens (13). B cells that encounter self-antigen in the bone marrow use VDJ recombination events to replace their Ig light chain rearrangement with another one, a process called receptor editing (14, 15). If this fails to reduce self-reactivity, then the cells remain in the bone marrow and ultimately die (clonal deletion or negative selection). If self-reactivity is below a threshold level, the immature B cell exits the bone marrow and completes maturation in the spleen. In the spleen, contact with self-antigen can induce cell death or acquisition of a relatively unresponsive state called clonal anergy. Clonal anergy is a spectrum that ranges from a deep anergy, which is accompanied by transcriptional changes that limit responsiveness (16–18) to a milder anergy in which responsiveness is limited primarily by recruitment of inhibitory signaling components to signaling BCRs (19). Reduced responsiveness to self-antigens is in part achieved by reduction in the level of mIgM on the cell surface (20, 21). Anergic B cells can make antibody responses in some circumstances (22, 23), and it is possible therefore that they contribute to autoimmune responses.

In healthy control individuals, the frequencies of B cells specific to nuclear self-antigens or polyreactive antibodies are reduced from a majority to about 5% in two steps, one occurring at the immature B cell stage and a second occurring during the mature B cell stage. Genetic analysis in humans indicates that the first step depends on the integrity of the BCR signaling pathway and therefore very likely represents a cell intrinsic regulation (24, 25), whereas the second step depends on molecules involved in B cell-T cell interactions (26), indicating that T cells are somehow involved in the impaired survival of nuclear antigen-specific B cells. The impaired survival of DNA-reactive B cells also involves TLR9 (27). The pathways that lead to the loss of such B cells, particularly at the second step, appear to be impaired in individuals with a number of autoimmune diseases, including lupus (28) and rheumatoid arthritis (29).

Although the combined efforts of receptor editing, clonal deletion, and anergy reduce the reactivity to self-antigens in mature B cells, they do not eliminate it, and therefore a lack of T cell help is also a key mechanism that limits production of autoantibodies. It is generally believed that tolerance to self of CD4 T cells is more stringent than for B cells, due to deletion of autoreactive T cells during their development in the thymus (negative selection) and the action of natural regulatory T cells (nTreg). Interesting in this regard is the disease autoimmune polyendocrine syndrome type 1 (APS-1), which is caused by either complete or partial loss-of-function mutations of the *AIRE* gene (30, 31). Aire is expressed in medullary thymic epithelial cells and directs the expression of hundreds of proteins that are otherwise only expressed in a selected tissue such as an endocrine gland. Aire-deficient patients or mice develop autoimmune attack on one or several organs due to loss of tolerance of CD4 T cells against one or a few autoantigens (31). Although the disease pathology that results is mostly due to cell-mediated inflammatory responses in the targeted organ expressing one or

a few of these tissue-restricted self-antigens, it is accompanied by autoantibodies against the Aire-regulated organ-specific protein of that tissue (31).

Thus, a failure of tolerance of CD4 T cells can underlie a breakdown of B cell tolerance and secretion of autoantibodies. Similarly, in mice with a mutation in the *sanroquin* gene, autoantibody production and severe autoimmunity occurs resulting from a dysregulation of T_{FH} cells (32). The product of the *sanroquin* gene is an RNA-binding protein that regulates the expression of several genes of importance to T_{FH} cells, including ICOS (33). In addition to an increase of T_{FH} cells in the lymph nodes and spleen of these mice, there is an increase in the peripheral blood of cells with a partial T_{FH} phenotype (34), which may be memory T cells generated from T_{FH}. Interestingly, similar T_{FH}-like CD4 T cells can be detected in human peripheral blood and their numbers are increased in some autoimmune diseases, suggesting that T_{FH} are expanded in these patients (32, 34, 35). The frequency of these T_{FH}-like cells in the blood may have significance as a new biomarker with research and/or clinical usefulness.

B cell Self-reactivity Resulting from Somatic Mutation

While much attention has been focused on mechanisms that help silence immature and naive B cells with self-reactive membrane Ig, it is also possible for somatic mutation of B cell Ig genes in the GC response to generate autoreactivity *de novo* in a B cell that previously had weak or no reactivity to self. If the resulting anti-self GC B cell retains some binding to the initiating antigen and therefore can continue to present foreign antigen peptides to T_{FH} cells, it may be selected to expand and secrete the autoantibody and thereby lead to or contribute to autoimmune disease. It appears, however, that B cell tolerance to self can be enforced at the GC or immediately post-GC stage to reduce the deleterious potential of this scenario. The tolerization of self-reactive GC B cells was initially tested in a somewhat artificial system in which an antigen was injected into mice undergoing an immune response. This treatment was found to terminate an ongoing GC response (36–38). The way these experiments were designed, the injected antigen lacked the epitopes being recognized by the T_{FH}, so it is possible that the injected mimic of a self-antigen acted by competing with the immunizing antigen for uptake by the antigen-specific B cells, and therefore, the silencing of self-reactive B cells may have resulted from loss of T cell help. Perhaps equally importantly, the injection of the “self antigen” during an ongoing response may not mimic the way that B cells normally encounter self antigens in lower amounts and more uniformly over time. Recently a more elegant and perhaps more physiologically relevant version of this experiment has been conducted in which mice with hen egg lysozyme (HEL)-specific Ig knockin B cells were immunized with a low affinity variant of lysozyme (HEL^{3x}). The affinity maturation that occurred during this response generated many B cells that reacted strongly with HEL^{3x} and also reacted to a further variant (HEL^{4x}) that was not recognized by the original HEL-specific BCR. When HEL^{4x} was expressed in the mice as a ubiquitously expressed transgene and the mice immunized with HEL^{3x}, now the ensuing affinity maturation did not generate antibodies cross-reactive with HEL^{4x} (39). However, if HEL^{4x} was expressed as a tissue-restricted transgene in liver or kidney, then antibodies that crossreacted to HEL^{4x} were generated as in mice with no HEL^{4x} transgene. Thus, this model shows the property of molecular mimicry and demonstrates that tolerance mechanisms exist

to suppress generation of crossreactive autoantibodies, provided the antigen is available within the active GC (39). Interesting, the opposite phenomenon has also been observed, that is, somatic mutation may eliminate cross-reactivity with a self-antigen, allowing those mutant B cells to participate more fully in the GC reaction (40, 41), implying that the self-reactivity does not completely prevent participation in the GC response, but rather attenuates the contribution of self-reactive cells.

Several molecular mechanisms that can silence self-reactive B cells at the GC or post-GC stage have been described. In one model system based on molecular mimicry between a synthetic peptide and DNA, it was observed that RAG1 and RAG2 are re-induced in DNA cross-reactive B cells at the post-GC plasmablast stage and this leads to late stage receptor editing, reducing secretion of anti-DNA antibodies (42). CD84 and Ly108, two adhesion molecules of the SLAM family expressed on the surface of B cells, have also been implicated in a GC stage tolerance checkpoint on the basis of allelic variations found in the *Sle1b* lupus-susceptibility locus from NZW mice. The function of the C57BL/6 lupus-resistant alleles of these molecules appears to support silencing of autoreactive B cells at the GC stage (43).

A third mechanism for promoting tolerance during the GC reaction is inhibition of BCR signaling by the inhibitory Fc receptor for IgG (Fc γ RIIB1) (44). GC B cells from C57BL/6 mice upregulate expression of Fc γ RIIB1, but this upregulation does not occur in autoimmune susceptibility strains, including the NZW strain, due to loss of an AP-1 transcription factor binding site in the promoter (45). A related promoter polymorphism has been observed in human populations and is associated with lupus susceptibility (46). Mice lacking Fc γ RIIB1 due to gene ablation exhibit increased susceptibility to developing lupus-like autoimmunity, which is background dependent (47, 48). Interestingly, although autoreactive *Fcgr2b*^{-/-} B cells are much more prevalent than wild type counterparts among GC B cells, their relative frequency decreases among plasma cells (44), indicating there is likely a post-GC tolerance checkpoint that does not involve Fc γ RIIB1. A similar autoimmune susceptibility is seen when Fc γ RIIB1 is selectively ablated in activated B cells by using *Fcgr2b*^{fl/fl} together with the γ 1-Cre transgene, which is expressed in B cells undergoing class switch recombination to IgG1 (49). This Cre induces deletion well in GC B cells, but may delete to some extent in extrafollicular B cells, but taken together with the other evidence cited above, these results provide additional evidence of a GC tolerance checkpoint dependent upon Fc γ RIIB1. Moreover, in humans, there is a relatively common allelic variant associated with lupus susceptibility that controls human Fc γ RIIB function by altering an amino acid in the transmembrane region. The majority A232 allelic form supports normal Fc γ RIIB inhibitory function from within lipid raft subdomains of the plasma membrane, whereas the minority T232 allelic form causes exclusion from lipid rafts and compromised inhibitory function (50). The T232 allele is present at ~10% frequency in Caucasians and ~22–25% frequency in South-East Asians and African, and may contribute to the higher incidence of lupus in the latter ethnic groups.

Events Leading to Production of Autoantibodies

As infectious agents induce robust antibody and T cell immune responses, a widely held hypothesis is that infections may trigger autoimmune responses in genetically susceptible individuals by virtue of cross-reactivity between antigens of the pathogen and a self component. This idea is referred to as “molecular mimicry”. An alternative hypothesis is that damage to a tissue, as may occur associated with some infections or injuries, triggers an autoimmune reaction in a genetically susceptible individual due to simultaneous release of self antigens together with danger-associated molecular patterns or DAMPs, which can serve as adjuvants by stimulating innate immune receptors. In either case, an individual may have to carry several autoimmune susceptibility alleles for the insult to result in a strong enough autoimmune response to lead to disease manifestations.

A particularly well established case of molecular mimicry involves Guillain-Barre´ syndrome, which is a peripheral autoimmune neuropathy often caused by gastrointestinal infection with certain strains of *Campylobacter jejuni*. These strains have a glycosyltransferase that makes a glycan that is similar in structure to the ganglioside GM1, expressed on peripheral neurons. Antibodies induced by this strain react both with the glycolipid on the microbe and with GM1 on peripheral neurons, inducing damage to these cells and the resulting neuropathy (51). Given the tissue-restricted nature of the GM1 ganglioside, it is possible that B cell maturation and/or GC tolerance mechanisms do not remove specificities directed against this glycolipid. Analysis of six IgM anti-GM1 antibodies from patients showed a considerable level of somatic mutation concentrated in the regions of the IgH and IgL genes encoding the CDR regions, indicative of an antigen-driven extrafollicular or GC response (52). More closely associated with acute forms of Guillain-Barre´ syndrome are IgG anti-GM1 antibodies, which exhibit fine specificity patterns indicating the response is dominated by a single clone (53), suggestive of a GC response.

In considering the extent to which these observations may be relevant to other autoimmune diseases, it is worth noting that unlike many autoimmune diseases, Guillain-Barre´ syndrome is self-limiting in most individuals; after the bacteria are cleared, autoantibody production stops and often the nerves heal and function is restored. Thus, it is likely in this disease that T cell tolerance to self is not broken, but rather the antibody responses of GM1-specific B cells are either promoted by T cells recognizing MHC-bound foreign peptides of the bacterium or are independent of T cells and instead rely on robust innate stimulation by bacterial cell wall components. In either case, when the bacterial infection is cleared, the GM1-specific B cells no longer have the additional inputs that they need to continue the antibody response and, at least in most individuals, presumably there are not many long-lived plasma cells secreting anti-GM1 antibodies. In contrast, in autoimmune diseases that have a chronic nature, it is likely that T cell tolerance to autoantigens is broken, either by molecular mimicry or other mechanisms, and/or innate receptors continue to be stimulated by endogenous ligands released from the tissue damage (DAMPs).

An important issue is to what extent the autoantibody response in a given autoimmune disease or patient derives from self-reactive naïve or anergic B cells via the extrafollicular

response or whether it derives from B cells that acquired self-reactivity by somatic mutation in the GC response. This distinction could have implications for which therapeutic approaches might ultimately be most successful. As mentioned above in the case of Guillain-Barre´ syndrome, one informative approach to this problem involves analysis of characteristic autoantibodies from patients. In addition to high-throughput sequencing approaches, it is possible to isolate memory B cells and plasmablasts from the blood, clone their IgH and IgL variable regions and characterize their affinity and specificity. In addition, it is possible to revert somatic mutations to their germ-line form and see if the original BCR had any detectable reactivity to self. While this type of approach is somewhat indirect, in that GC B cells do not circulate outside of the GC and therefore cannot be directly sampled from human subjects in most cases, nonetheless, if the germ-line version of an antibody has no detectable reactivity to the autoantigen, it can be inferred that the autoantibody was derived from a GC response. Studies with three clonally distinct anti-desmoglein 3 antibodies from pemphigus vulgaris patients found that the germ-line versions of these antibodies had no detectable recognition, making it likely that they were derived from the GC response (54).

In the case of lupus, the results provide a more complex picture, with evidence that some anti-nuclear antibodies come from GC responses, but that others derive from self-reactive naïve or anergic B cells that required little somatic mutation, consistent with an extrafollicular response. Using the approach described above, three anti-dsDNA antibodies from lupus patients were studied and all three lost detectable reactivity to dsDNA or even ssDNA after reversion of all somatic mutations (55). Using the same approach, four clonally distinct antibodies reactive to Ro52 or La from memory B cells of a lupus patient were analyzed. Three of the naïve B cell precursors derived by reversion of the somatic mutations lacked detectable reactivity to these ribonucleoprotein antigens, but in one case there was some pre-existing self-reactivity to Ro, although of lower affinity (56). The latter memory B cell therefore may have come from an extrafollicular response, but the first three likely represent products of a GC response.

Complexity in the source of autoantibodies in lupus patients has also been indicated by a recent study in which lupus patients undergoing disease-related flares and previously treated with minimal immunosuppression were analyzed by alternative approaches relying on deep sequencing and identification of clonal variants to gain some insight into the source and extent of clonal diversification in these individuals actively making an autoimmune response (57). This study examined both plasmablasts and a blood B cell population referred to as “activated naïve B cells”, which may represent an extrafollicular response. By focusing on B cells with IgH chains derived from the inherently DNA-reactive V_H4-34 gene segment, they found a highly diverse autoantibody response in these individuals that included multiple auto-reactive clones, some of which did not have somatic mutations, indicating they were derived from naïve or anergic B cells, rather than from somatic mutation in a GC of non-autoreactive B cells. Other clones of autoantibodies were identified from the same patients that had extensive somatic mutation and may have been derived from GCs.

As mentioned above, recently a blood CD4 T cell population has been identified that appears to be derived from T_{FH} cells (32, 34, 35). Interestingly, these cells are clearly elevated in a

subset of lupus patients, suggesting that in these individuals there may be an especially active GC reaction in progress. Combining this analysis with analysis of the autoreactive antibodies obtained from plasmablasts may be informative, especially as lupus patients may be heterogeneous with regard to their disease pathogenesis.

A large number of mouse models of lupus have been studied in some detail. Some of these are genetically inbred strains with multiple natural lupus susceptibility loci, whereas others are derived from genetic ablation of genes that protect from systemic autoimmunity (58). Initial studies of anti-DNA antibodies in the MRL/*lpr* mouse model of lupus and subsequently in other models provided evidence of an antigen-driven somatic mutation process, suggestive of germinal center responses (59, 60). Subsequent studies have indicated that in MRL/*lpr* mice, anti-rheumatoid factor antibody production results from the extrafollicular response that does not require T cells but does require TLRs (61, 62). Similarly, in BAFF-transgenic mice, autoantibody production is independent of T cells but requires TLR signaling (63). In contrast, in many of the other mouse models of lupus, autoantibody production is dependent on T cells and/or seems to depend on GC responses (64–68). For example, in *Fcgr2b*^{-/-} mice, which spontaneously develop lupus-like autoantibodies, GC B cells were shown to include anti-nuclear antigen-specific cells (44). Moreover, in the B6.*nba2* lupus-like mice, AID was shown to be required for generation of anti-nuclear antibodies and they originated from B cells with no detectable self-reactivity (69), strongly suggestive of a GC origin. Thus, there is clearly heterogeneity of murine models of lupus with regard to the source of autoantibodies, and this may be reflected in human lupus as well.

Defects in tolerance at the GC stage have been noted in mouse models of lupus and in human lupus patients. As described above, *Fcgr2b*^{-/-} mice and mice with the *Sle1b* lupus susceptibility locus have defects in GC tolerance mechanisms. Another example comes from mice that have B cells expressing an Ig V_H knockin (called HKIR) that generates a high frequency of B cells that recognize the arsonate hapten and crossreact with a nuclear chromatin antigen. These B cells exhibit an abortive GC response in foreign antigen-arsonate immunized autoimmune resistant C57BL/6 mice, but are present in GCs and produce autoantibodies in C57BL/6 mice containing the *Sle1* lupus susceptibility locus derived from NZW mice (70). Possibly similar observations have been made in human lupus patients by examining B cells with an abundantly expressed Ig heavy chain variable region idiotype (9G4), encoded by the V_H4-34 IgH variable region, which preferentially exhibits DNA reactivity. 9G4⁺ B cells are found in abundance in the GCs of lupus patients, but are largely absent in the GCs of normal individuals, although this V_H is abundantly present in the naïve B cell population of these individuals (71). As apoptotic cells are generated at a high rate in GCs, the antigen is clearly present, suggesting a defect in mechanisms that normally prevent GC responses of self-reactive B cells. Naïve 9G4⁺ B cells exhibit an anergic phenotype (71), so there may be an additional defect in maintenance of tolerance of anergic B cells.

Role of TLRs in Production of Anti-Nuclear Antibodies

A key factor in the breaking of tolerance for production of anti-nuclear antibodies, the hallmark autoantibodies of lupus, is recognition of nucleic acids by TLR7 and TLR9 (72). The autoantigens in this case, including double-stranded (ds) DNA, chromatin, and ribonucleoproteins are exposed on the debris released from apoptotic cells (“apoptotic blebs”) (73) and moreover, contain endogenous ligands for TLR7 and TLR9, which recognize RNA and unmethylated CpG-containing sequence motifs, respectively. The deoxyribonuclease DNASE1L3 reduces the stimulatory potential of apoptotic blebs by digesting the DNA in them in phagosomes (74). The presence of TLR ligands integral to or associated with nuclear self-antigens likely makes the breakdown of tolerance to these self-antigens in lupus distinct from what occurs with organ-specific autoimmunity.

B cells in both mice and humans express TLR7 and TLR9, and moreover in vitro stimulation of B cells through the BCR and either of these TLRs leads to robust activation of the cells (72). A variety of studies have shown that TLR7 and TLR9 and/or the signaling component MyD88, which is required for signaling by both, are required for production of anti-nuclear antibodies in various mouse models of lupus (63, 67, 75–77). Moreover, the y-chromosome autoimmune accelerator locus *yaa* represents a duplication of a short region of the x chromosome onto the y chromosome that includes *Tlr7*. The ability of this locus to promote autoimmunity in the presence of other autoimmune susceptibility loci appears to be due to this mild overexpression of TLR7 (78–80). The expression of TLR7 and TLR9 in B cells has been shown to contribute directly to the production of anti-RNP and anti-DNA antibodies, respectively, since deletion of *Tlr7* results in a considerable decrease in anti-RNP antibodies and a much smaller decrease in anti-DNA antibodies, whereas deletion of *Tlr9* selectively decreases anti-DNA antibodies (75). TLRs expressed on B cells are capable of promoting antibody responses in the absence of T cells help (76, 81, 82), but more recent studies show that these TLRs also can promote GC responses very strongly (82–84).

The ability of TLRs to promote a germinal center is dependent on the degree of oligomerization of the antigen, which is presumed to translate into the degree of BCR signaling (82). Interestingly, virus particles often have a highly oligomeric state of a small number of proteins and of course have RNA or DNA inside, suggesting that the ability of TLR7 or TLR9 to stimulate a GC response may be an adaptive mechanism to promote generation of protective antibodies to fight virus infections. In agreement with this hypothesis, B cell-intrinsic expression of TLR7 and/or MyD88 has been shown to be required for an effective GC response leading to generation of neutralizing antibodies in murine infections with Friend virus (a retrovirus) (85), or with LCMV clone 13 (86, 87). Similarly, TLR7 is required to generate neutralizing antibody against endogenous murine leukemia virus (88). Therefore, it is likely that the ability of TLR7 and TLR9 to promote a robust GC response is an evolved mechanism that provides protection against viral infections, but unfortunately can promote the production of lupus-like autoantibodies in a genetically susceptible background.

Future Prospectives

Antibodies are involved in the pathogenesis of many autoimmune diseases, but treatments for these diseases are generally not satisfactory. Studies with animal models of autoimmune disease, particularly for lupus, have provided some insights into how this disease may develop, but there are many things that are still mysterious. These studies have shown that breaking of tolerance to nuclear antigens in lupus-like autoimmunity requires the action of TLR7 and/or TLR9 in B cells to promoting these responses, and therefore blocking these TLRs could have therapeutic value, although GC responses to viruses also use this pathway, so targeting it could compromise anti-viral immunity. Depending on the mouse model used, anti-nuclear antibodies can be generated via either the extrafollicular response or the GC responses. A key question is to what extent this dichotomy will inform us about heterogeneity of lupus patients, a heterogeneity that could result from different combinations of susceptibility alleles. The cloning of IgH and IgL genes from single B cells or plasmablasts making autoantibodies characteristic of particular autoimmune diseases makes it possible to define the role of somatic mutations in the self-reactivity. Such studies have been informative, and more data of this type may provide additional insights, particularly if coupled with phenotypic or genetic data that may inform about patient heterogeneity. New approaches to quantifying the numbers of T_{FH}-like CD4 T cells in the blood represent another promising tool that may inform us about the strength of GC reactions in the subject. Defining the specificity of these T cells could be a major advance, but is currently a daunting challenge. In select cases, tertiary lymphoid tissue can be obtained from inflamed tissues such as kidneys in lupus (89, 90) or joints in rheumatoid arthritis, and this may be another source of important information. A better understanding of the mechanisms by which those autoantibodies are produced and straightforward clinical tests to aid in that determination could be critical steps toward achieving the goal of having highly targeted therapies that compromise as little of the normal immune response as possible while inhibiting autoantibody production.

References

1. Goodnow CC, Vinuesa CG, Randall KL, Mackay F, Brink R. Control systems and decision making for antibody production. *Nat Immunol.* 2010; 11:681–688. [PubMed: 20644574]
2. Baumgarth N, et al. Innate and acquired humoral immunities to influenza virus are mediated by distinct arms of the immune system. *Proc Natl Acad Sci U S A.* 1999; 96:2250–2255. [PubMed: 10051627]
3. Notley CA, Brown MA, Wright GP, Ehrenstein MR. Natural IgM is required for suppression of inflammatory arthritis by apoptotic cells. *J Immunol.* 2011; 186:4967–4972. [PubMed: 21383247]
4. MacLennan ICM, et al. Extrafollicular antibody responses. *Immunol Rev.* 2003; 194:8–18. [PubMed: 12846803]
5. Crotty S. Follicular helper CD4 T cells (TFH). *Annu Rev Immunol.* 2011; 29:621–663. [PubMed: 21314428]
6. Chan TD, et al. Antigen affinity controls rapid T-dependent antibody production by driving the expansion rather than the differentiation or extrafollicular migration of early plasmablasts. *J Immunol.* 2009; 183:3139–3149. [PubMed: 19666691]
7. Wheeler ML, Dong MB, Brink R, Zhong XP, DeFranco AL. Critical role of diacylglycerol kinase α in limiting B cell antigen receptor-induced ERK signaling and controlling the magnitude of the extrafollicular plasmablast. *Sci Signal.* 2013; 6(297):ra91. [PubMed: 24129701]

8. Weisel FJ, Zuccarino-Catania GV, Chikina M, Shlomchik MJ. A Temporal Switch in the Germinal Center Determines Differential Output of Memory B and Plasma Cells. *Immunity*. 2016; 44:116–130. [PubMed: 26795247]
9. Smith KG, et al. bcl-2 transgene expression inhibits apoptosis in the germinal center and reveals differences in the selection of memory B cells and bone marrow antibody-forming cells. *J Exp Med*. 2000; 191:475–484. [PubMed: 10662793]
10. Wrammert J, et al. Broadly cross-reactive antibodies dominate the human B cell response against 2009 pandemic H1N1 influenza virus infection. *J Exp Med*. 2011; 208:181–193. [PubMed: 21220454]
11. Griffin DO, Holodick NE, Rothstein TL. Human B1 cells in umbilical cord and adult peripheral blood express the novel phenotype CD20+ CD27+ CD43+ CD70–. *J Exp Med*. 2011; 208:67–80. [PubMed: 21220451]
12. Pillai S, Cariappa A, Moran ST. Marginal zone B cells. *Annu Rev Immunol*. 2005; 23:161–196. [PubMed: 15771569]
13. Wardemann H, et al. Predominant autoantibody production by early human B cell precursors. *Science*. 2003; 301:1374–1377. [PubMed: 12920303]
14. Nemazee D. Receptor editing in lymphocyte development and central tolerance. *Nat Rev Immunol*. 2006; 6:728–740. [PubMed: 16998507]
15. Gay D, Saunders T, Camper S, Weigert M. Receptor editing: an approach by autoreactive B cells to escape tolerance. *J Exp Med*. 1993; 177:999–1008. [PubMed: 8459227]
16. Goodnow CC, Sprent J, Fazekas de St Groth B, Vinuesa CG. Cellular and genetic mechanisms of self tolerance and autoimmunity. *Nature*. 2005; 435(7042):590–597. [PubMed: 15931211]
17. Browne CD, Del Nagro CJ, Cato MH, Dengler HS, Rickert RC. Suppression of phosphatidylinositol 3,4,5-trisphosphate production is a key determinant of B cell anergy. *Immunity*. 2009; 31(5):749–760. [PubMed: 19896393]
18. Mandik-Nayak L, et al. Functional consequences of the developmental arrest and follicular exclusion of anti-double-stranded DNA B cells. *J Immunol*. 2000; 164:1161–1168. [PubMed: 10640726]
19. Getahun A, Beavers NA, Larson SR, Shlomchik MJ, Cambier JC. Continuous inhibitory signaling by both SHP-1 and SHIP-1 pathways is required to maintain unresponsiveness of anergic B cells. *J Exp Med*. 2016; 213:751–769.
20. Zikherman J, Parameswaran R, Weiss A. Endogenous antigen tunes the responsiveness of naive B cells but not T cells. *Nature*. 2012; 489:160–164. [PubMed: 22902503]
21. Quách TD, et al. Anergic responses characterize a large fraction of human autoreactive naive B cells expressing low levels of surface IgM. *J Immunol*. 2011; 186:4640–4648. [PubMed: 21398610]
22. Alabyev B, Rahman ZS, Manser T. Quantitatively reduced participation of anti-nuclear antigen B cells that down-regulate B cell receptor during primary development in the germinal center/memory B cell response to foreign antigen. *J Immunol*. 2007; 178:5623–5634. [PubMed: 17442945]
23. Moisini I, et al. The Yaa locus and IFN- α fine-tune germinal center B cell selection in murine systemic lupus erythematosus. *J Immunol*. 2012; 189:4305–4312. [PubMed: 23024275]
24. Ng YS, Wardemann H, Chelnis J, Cunningham-Rundles C, Meffre E. Bruton's tyrosine kinase is essential for human B cell tolerance. *J Exp Med*. 2004; 200:927–934. [PubMed: 15466623]
25. Meffre E. The establishment of early B cell tolerance in humans: lessons from primary immunodeficiency diseases. *Ann N Y Acad Sci*. 2011; 1246:1–10. [PubMed: 22236425]
26. Hervé M, et al. CD40 ligand and MHC class II expression are essential for human peripheral B cell tolerance. *J Exp Med*. 2007; 204:1583–1593. [PubMed: 17562816]
27. Nickerson KM, et al. TLR9 promotes tolerance by restricting survival of anergic anti-DNA B cells, yet is also required for their activation. *J Immunol*. 2013; 190:1447–1456. [PubMed: 23296704]
28. Yurasov S, et al. Defective B cell tolerance checkpoints in systemic lupus erythematosus. *J Exp Med*. 2005; 201:703–711. [PubMed: 15738055]
29. Samuels J, Ng YS, Coupillaud C, Paget D, Meffre E. Impaired early B cell tolerance in patients with rheumatoid arthritis. *J Exp Med*. 2005; 201:1659–1667. [PubMed: 15897279]

30. Oftedal BE, et al. Dominant Mutations in the Autoimmune Regulator AIRE Are Associated with Common Organ-Specific Autoimmune Diseases. *Immunity*. 2015; 42(6):1185–1196. [PubMed: 26084028]
31. Chang MH, Anderson MS. Mongenic autoimmunity. *Annu Rev Immunol*. 2012; 30:393–427. [PubMed: 22224765]
32. Ueno H, Banchereau J, Vinuesa CG. Pathophysiology of T follicular helper cells in humans and mice. *Nat Immunol*. 2015; 16:142–152. [PubMed: 25594465]
33. Yu D, et al. Roquin represses autoimmunity by limiting inducible T-cell co-stimulator messenger RNA. *Nature*. 2007; 450:299–303. [PubMed: 18172933]
34. Simpson N, et al. Expansion of circulating T cells resembling follicular helper T cells is a fixed phenotype that identifies a subset of severe systemic lupus erythematosus. *Arthritis Rheum*. 2010; 62:234–244. [PubMed: 20039395]
35. Tangye SG, Ma CS, Brink R, Deenick EK. The good, the bad and the ugly - TFH cells in human health and disease. *Nat Rev Immunol*. 2013; 13:412–426. [PubMed: 23681096]
36. Shokat KM, Goodnow CC. Antigen-induced B-cell death and elimination during germinal-centre immune responses. *Nature*. 1995; 375:334–338. [PubMed: 7753200]
37. Pulendran B, Kannourakis G, Nouri S, Smith KG, Nossal GJ. Soluble antigen can cause enhanced apoptosis of germinal-centre B cells. *Nature*. 1995; 375:331–334. [PubMed: 7753199]
38. Han S, Zheng B, Dal Porto J, Kelsoe G. In situ studies of the primary immune response to (4-hydroxy-3-nitrophenyl)acetyl. IV. Affinity-dependent, antigen-driven B cell apoptosis in germinal centers as a mechanism for maintaining self-tolerance. *J Exp Med*. 1995; 182:1635–1644. [PubMed: 7500008]
39. Chan TD, et al. Elimination of germinal-center-derived self-reactive B cells is governed by the location and concentration of self-antigen. *Immunity*. 2012; 37:893–904. [PubMed: 23142780]
40. Sabouri Z, et al. Redemption of autoantibodies on anergic B cells by variable-region glycosylation and mutation away from self-reactivity. *Proc Natl Acad Sci U S A*. 2014; 111:E2567–2575. [PubMed: 24821781]
41. Reed JH, Jackson J, Christ D, Goodnow CC. Clonal redemption of autoantibodies by somatic hypermutation away from self-reactivity during human immunization. *J Exp Med*. 2016; 213:1255–1265. [PubMed: 27298445]
42. Rice JS, Newman J, Wang C, Michael DJ, Diamond B. Receptor editing in peripheral B cell tolerance. *Proc Natl Acad Sci U S A*. 2005; 102:1608–1613. [PubMed: 15659547]
43. Wong EB, et al. B cell-intrinsic CD84 and Ly108 maintain germinal center B cell tolerance. *J Immunol*. 2015; 194:4130–4143. [PubMed: 25801429]
44. Tiller T, et al. Development of self-reactive germinal center B cells and plasma cells in autoimmune Fc gammaRIIB-deficient mice. *J Exp Med*. 2010; 207:2767–2778. [PubMed: 21078890]
45. Espéli M, et al. Analysis of a wild mouse promoter variant reveals a novel role for FcγRIIb in the control of the germinal center and autoimmunity. *J Exp Med*. 2012; 209:2307–2319. [PubMed: 23109709]
46. Blank MC, et al. Decreased transcription of the human FCGR2B gene mediated by the -343 G/C promoter polymorphism and association with systemic lupus erythematosus. *Hum Genet*. 2005; 117:220–227. [PubMed: 15895258]
47. Boross P, et al. The inhibiting Fc receptor for IgG, FcγRIIB, is a modifier of autoimmune susceptibility. *J Immunol*. 2011; 187:1304–1313. [PubMed: 21724994]
48. Soni C, et al. Distinct and synergistic roles of FcγRIIB deficiency and 129 strain-derived SLAM family proteins in the development of spontaneous germinal centers and autoimmunity. *J Autoimmun*. 2015; 63:31–46. [PubMed: 26162758]
49. Li F, Smith P, Ravetch JV. Inhibitory Fcγ receptor is required for the maintenance of tolerance through distinct mechanisms. *J Immunol*. 2014; 192:3021–3028. [PubMed: 24563255]
50. Willcocks LC, et al. A defunctioning polymorphism in FCGR2B is associated with protection against malaria but susceptibility to systemic lupus erythematosus. *Proc Natl Acad Sci U S A*. 2010; 107:7881–7885. [PubMed: 20385827]

51. Koga M, Takahashi M, Masuda M, Hirata K, Yuki N. Campylobacter gene polymorphism as a determinant of clinical features of Guillain-Barré syndrome. *Neurology*. 2005; 65:1376–1381. [PubMed: 16162859]
52. Paterson G, Wilson G, Kennedy PG, Willison HJ. Analysis of anti-GM1 ganglioside IgM antibodies cloned from motor neuropathy patients demonstrates diverse V region gene usage with extensive somatic mutation. *J Immunol*. 1995; 155:3049–3059. [PubMed: 7673721]
53. Lardone RD, Yuki N, Irazoqui FJ, Nores GA. Individual Restriction Of Fine Specificity Variability In Anti-GM1 IgG Antibodies Associated With Guillain-Barré Syndrome. *Sci Rep*. 2016; 6:19901. [PubMed: 26818965]
54. Di Zenzo G, et al. Pemphigus autoantibodies generated through somatic mutations target the desmoglein-3 cis-interface. *J Clin Invest*. 2012; 122:3781–3790. [PubMed: 22996451]
55. Wellmann U, et al. The evolution of human anti-double-stranded DNA autoantibodies. *Proc Natl Acad Sci U S A*. 2005; 102:9258–9263. [PubMed: 15968001]
56. Mietzner B, et al. Autoreactive IgG memory antibodies in patients with systemic lupus erythematosus arise from nonreactive and polyreactive precursors. *Proc Natl Acad Sci U S A*. 2008; 105:9727–9732. [PubMed: 18621685]
57. Tipton CM, et al. Diversity, cellular origin and autoreactivity of antibody-secreting cell population expansions in acute systemic lupus erythematosus. *Nat Immunol*. 2015; 16:755–765. [PubMed: 26006014]
58. Mohan C, Putterman C. Genetics and pathogenesis of systemic lupus erythematosus and lupus nephritis. *Nat Rev Nephrol*. 2015; 11(6):329–341. [PubMed: 25825084]
59. Shlomchik M, et al. Anti-DNA antibodies from autoimmune mice arise by clonal expansion and somatic mutation. *J Exp Med*. 1990; 171:265–292. [PubMed: 2104919]
60. Schroeder K, Herrmann M, Winkler TH. The role of somatic hypermutation in the generation of pathogenic antibodies in SLE. *Autoimmunity*. 2013; 46:121–127. [PubMed: 23181829]
61. Sweet RA, et al. A new site-directed transgenic rheumatoid factor mouse model demonstrates extrafollicular class switch and plasmablast formation. *Autoimmunity*. 2010; 43:607–618. [PubMed: 20370572]
62. Herlands RA, Christensen SR, Sweet RA, Hershberg U, Shlomchik MJ. T cell-independent and Toll-like receptor-dependent antigen-driven activation of autoreactive B cells. *Immunity*. 2008; 29:249–260. [PubMed: 18691914]
63. Groom JR, et al. BAFF and MyD88 signals promote a lupuslike disease independent of T cells. *J Exp Med*. 2007; 204:1959–1971. [PubMed: 17664289]
64. Wofsy D. Treatment of murine lupus with anti-CD4 monoclonal antibodies. *Immunol Ser*. 1993; 59:221–236. [PubMed: 8096400]
65. Mohan C, Shi Y, Laman JD, Datta SK. Interaction between CD40 and its ligand gp39 in the development of murine lupus nephritis. *J Immunol*. 1995; 154:1470–1480. [PubMed: 7529804]
66. Shlomchik MJ. Activating systemic autoimmunity: B's, T's, and tolls. *Curr Opin Immunol*. 2009; 21(6):626–633. [PubMed: 19800208]
67. Hua Z, et al. Requirement for MyD88 signaling in B cells and dendritic cells for germinal center anti-nuclear antibody production in Lyn-deficient mice. *J Immunol*. 2014; 192:875–885. [PubMed: 24379120]
68. Woods M, Zou Y-R, Davidson A. Defects in germinal center selection in SLE. *Front Immunol*. 2015; 6:425. [PubMed: 26322049]
69. Guo W, et al. Somatic hypermutation as a generator of antinuclear antibodies in a murine model of systemic autoimmunity. *J Exp Med*. 2010; 207:2225–2237. [PubMed: 20805563]
70. Vuyyuru R, Mohan C, Manser T, Rahman ZS. The lupus susceptibility locus Sle1 breaches peripheral B cell tolerance at the antibody-forming cell and germinal center checkpoints. *J Immunol*. 2009; 183:5716–5727. [PubMed: 19828626]
71. Cappione, Ar, et al. Germinal center exclusion of autoreactive B cells is defective in human systemic lupus erythematosus. *J Clin Invest*. 2005; 115:3205–3216. [PubMed: 16211091]
72. Green NM, Marshak-Rothstein A. Toll-like receptor driven B cell activation in the induction of systemic autoimmunity. *Semin Immunol*. 2011; 23:106–112. [PubMed: 21306913]

73. Cline AM, Radic MZ. Murine lupus autoantibodies identify distinct subsets of apoptotic bodies. *Autoimmunity*. 2004; 37(2):85–93. [PubMed: 15293878]
74. Sisirak V, et al. Digestion of Chromatin in Apoptotic Cell Microparticles Prevents Autoimmunity. *Cell*. 2016; 166:88–101. [PubMed: 27293190]
75. Christensen SR, et al. Toll-like receptor 7 and TLR9 dictate autoantibody specificity and have opposing inflammatory and regulatory roles in a murine model of lupus. *Immunity*. 2006; 25:417–428. [PubMed: 16973389]
76. Han J-H, et al. Class switch recombination and somatic hypermutation in early mouse B cells are mediated by B cell and Toll-like receptor. *Immunity*. 2007; 27:64–75. [PubMed: 17658280]
77. Kolhatkar NS, et al. Altered BCR and TLR signals promote enhanced positive selection of autoreactive transitional B cells in Wiskott-Aldrich syndrome. *J Exp Med*. 2015; 212:1663–1677. [PubMed: 26371186]
78. Pisitkun P, et al. Autoreactive B cell responses to RNA-related antigens due to TLR7 gene duplication. *Science*. 2006; 312:1669–1672. [PubMed: 16709748]
79. Subramanian S, et al. A Tlr7 translocation accelerates systemic autoimmunity in murine lupus. *Proc Natl Acad Sci USA*. 2006; 103:9970–9975. [PubMed: 16777955]
80. Deane JA, et al. Control of toll-like receptor 7 expression is essential to restrict autoimmunity and dendritic cell proliferation. *Immunity*. 2007; 27
81. Ecki-Doma J, Batista FD. BCR-mediated uptake of antigen linked to TLR9 ligand stimulates B-cell proliferation and antigen-specific plasma cell formation. *Blood*. 2009; 113:3969–3977. [PubMed: 19144984]
82. Hou B, et al. Selective utilization of Toll-like receptor and MyD88 signaling in B cells for enhancement of the antiviral germinal center response. *Immunity*. 2011; 34(3):375–384. [PubMed: 21353603]
83. Kasturi SP, et al. Programming the magnitude and persistence of antibody responses with innate immunity. *Nature*. 2011; 470:543–547. [PubMed: 21350488]
84. Rookhuizen DC, DeFranco AL. Toll-like receptor 9 signaling acts on multiple elements of the germinal center to enhance antibody responses. *Proc Natl Acad Sci U S A*. 2014; 111:E3224–3233. [PubMed: 25053813]
85. Browne EP. Toll-like receptor 7 controls the anti-retroviral germinal center response. *PLoS Pathog*. 2011; 7:e1002293. [PubMed: 21998589]
86. Walsh KB, et al. Toll-like receptor 7 is required for effective adaptive immune responses that prevent persistent virus infection. *Cell Host Microbe*. 2012; 11:643–653. [PubMed: 22704624]
87. Clingan JM, Matloubian M. B cell-intrinsic TLR7 signaling is required for optimal B cell responses during chronic viral infection. *J Immunol*. 2013; 191:810–818. [PubMed: 23761632]
88. Yu P, et al. Nucleic acid-sensing Toll-like receptors are essential for the control of endogenous retrovirus viremia and ERV-induced tumors. *Immunity*. 2012; 37:867–879. [PubMed: 23142781]
89. Kinloch AJ, et al. Vimentin is a dominant target of in situ humoral immunity in human lupus tubulointerstitial nephritis. *Arthritis Rheumatol*. 2014; 66:3359–3370. [PubMed: 25306868]
90. Liarski VM, et al. Cell distance mapping identifies functional T follicular helper cells in inflamed human renal tissue. *Sci Transl Med*. 2014; 6:230ra246.