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Review
# Antigen receptor signaling in the rheumatic diseases

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## Abstract

Antigen receptor signaling in lymphocytes has been clearly implicated in the pathogenesis of the rheumatic diseases. Here, we review evidence from mouse models in which B-cell and T-cell signaling machinery is perturbed as well as data from functional studies of primary human lymphocytes and recent advances in human genetics. B-cell receptor hyper-responsiveness is identified as a nearly universal characteristic of systemic lupus erythematosus in mice and humans. Impaired and enhanced T-cell receptor signaling are both associated with distinct inflammatory diseases in mice. Mechanisms by which these pathways contribute to disease in mouse models and patients are under active investigation.

## Introduction

The classic concept of autoimmune disease rests upon the notion that the adaptive immune system generates inappropriate antigen-specific responses to self epitopes which in turn drive disease. Indeed, the presence of autoantibodies is one of the most characteristic features of the rheumatic diseases. Since the canonical definition of the adaptive immune response relates to the ability of somatic recombination to produce an enormous range of antigen receptors on lymphocytes, it follows that antigen receptor signal transduction ought to play a role in autoimmune diseases. The T-cell antigen receptor (TCR)-beta chain was cloned in 1983, and the subsequent decade saw the discovery of the signal transduction pathway downstream of the TCR [1]. Parallel discoveries for B-cell antigen receptor (BCR) signaling followed. Not only antigen receptors themselves but the complex machinery that elaborates the cellular response to antigen have been implicated in the rheumatic diseases. The past decade has seen evidence confirm this view from a range of sources, including engineered and spontaneous mouse models, primary lymphocytes from patients, as well as human genetics. Here, we provide a selective overview of

some of these advances and propose some general principles that tie these observations together.

## Overview of antigen receptor signal transduction

TCR signal transduction is initiated following interaction of the TCR $\alpha\beta$ chains with peptide antigen bound to major histocompatibility complex (MHC) class I or II molecules. The signal is transmitted to a complex network of kinases, phosphatases, and adaptors (Figure 1). The TCR $\alpha\beta$ chains lack any ability to transmit signals on their own and depend on CD3 ($\epsilon$, $\delta$, and $\gamma$) and $\zeta$ chains that contain varying numbers of immunoreceptor tyrosine-based activating motifs (ITAMs). The dual tyrosines of ITAMs are phosphorylated by the Src family kinases (SFKs), which, in T cells, are Lck and Fyn. Dually phosphorylated ITAMs, in turn, form docking sites for the tandem SH2 domains of Syk family kinases, ZAP-70 and Syk. The Syk kinases are activated upon binding to phospho-ITAMs and phosphorylation by the SFKs. Once activated, the Syk kinases phosphorylate the critical adaptors Slp-76 and Lat, which together form the scaffolds for assembly of further signaling molecules. Among these is the enzyme phospholipase C $\gamma$1 (PLC$\gamma$1), which is responsible for transmission of signals to phosphorylate mitogen-activated protein kinases (MAPKs) and increase cytoplasmic free calcium. Functional consequences of antigen receptor signaling are varied and context-dependent, including cell activation, proliferation, differentiation, and death [2,3].

In addition to antigen binding, there are many levels of regulation in this signaling pathway. The SFKs themselves are tightly regulated by phosphorylation of their inhibitory C-terminal tyrosine residue. Reciprocal regulation of this phosphotyrosine by the receptor-like tyrosine phosphatase CD45 and the cytoplasmic kinase Csk can set thresholds for

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ANA = anti-nuclear antibody; BCR = B-cell antigen receptor; CR2 = complement receptor-2; dKO = double knockout; dsDNA = double-stranded DNA; IL = interleukin; ITAM = immunoreceptor tyrosine-based activating motif; ITIM = immune tyrosine inhibitory motif; Me$^v$ = moth-eaten viable; MHC = major histocompatibility complex; PLC$\gamma$1 = phospholipase C $\gamma$1; RA = rheumatoid arthritis; SFK = Src family kinase; SLE = systemic lupus erythematosus; TCR = T-cell antigen receptor; Tg = transgene; Treg = regulatory T cell.

**Figure 1**
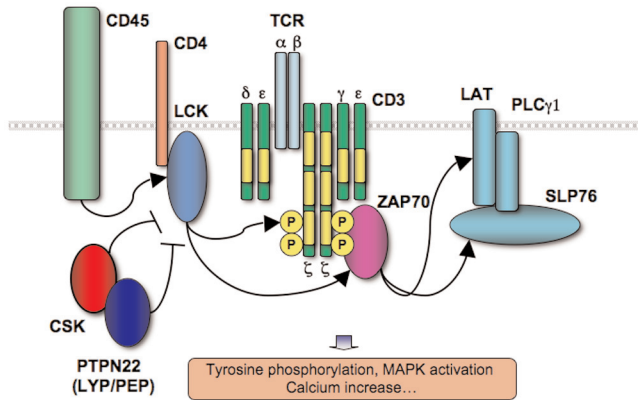


Schematic representation of T-cell receptor signal transduction. CD4-associated Lck is reciprocally regulated by CD45 and Csk/PTPN22 and in turn phosphorylates CD3 chain immunoreceptor tyrosine-based activating motifs (ITAMs) and ZAP-70. ZAP-70 phosphorylates additional downstream effectors, including the adaptors Slp-76 and Lat. Yellow bands represent CD3 chain ITAM domains. Phosphotyrosines are not depicted on all CD3 chain ITAMs. MAPK, mitogen-activated protein kinase; PLCγ1, phospholipase C γ1; TCR, T-cell antigen receptor.

**Figure 2**
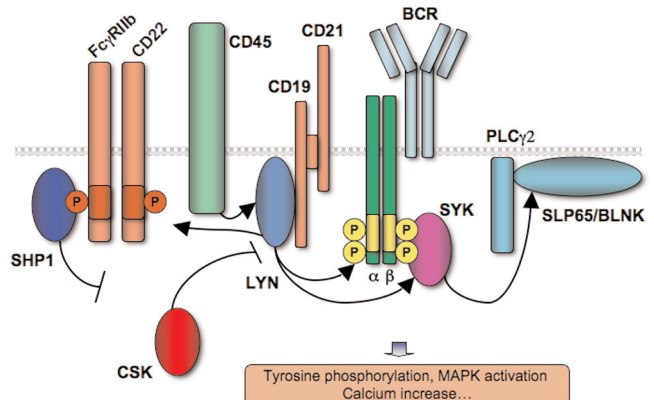


Schematic representation of B-cell receptor signal transduction. Lyn is reciprocally regulated by CD45 and Csk and in turn phosphorylates B-cell antigen receptor (BCR) immunoreceptor tyrosine-based activating motifs (ITAMs) as well as immune tyrosine inhibitory motif (ITIM)-containing immunoreceptors. Positive and negative signals are in turn transmitted by Syk and SHP-1, respectively. Yellow bands on Igα and β chains represent ITAM domains. Orange bands on CD22 and FcγRIIb represent ITIM domains. MAPK, mitogen-activated protein kinase; PLCγ2, phospholipase C γ2.

antigen receptor signal transduction. Added complexity is presented by tight regulation of the activating tyrosine of the SFKs. Negative regulators of TCR signaling, such as the phosphatases Pep and SHP-1, can dephosphorylate this critical residue [4,5].

The BCR immunoglobulin chains are responsible for antigen recognition (Figure 2). BCR signal transduction resembles TCR signaling in many ways, relying upon the ITAMs of the associated Igα and Igβ chains, the B-cell-expressed SFKs Lyn, Fyn, and Blk, and the Syk kinase as well as homologous adaptors (Blnk/Slp-65 instead of Slp-76). CD45 and Csk also regulate SFKs in B cells as they do in T cells [6].

Multiple pathways feed into this network at several proximal signaling nodes, including positive and negative regulators of antigen receptor signaling. In T cells, for instance, the coreceptors CD4 and CD8 play a positive regulatory role not only by facilitating MHC recognition, but also by bringing the SFK Lck into the proximity of the TCR [2]. The complex of CD19/CD81/CD21 (CR2, complement receptor-2) that interacts with the Lyn SFK plays a similar coreceptor role on B cells. These coreceptors are counterbalanced by the action of receptors with negative regulatory function. Cell surface molecules that exert negative regulation often contain a cytoplasmic motif, called an ITIM (immune tyrosine inhibitory motif), which upon phosphorylation by SFKs recruits negative regulators of signaling, such as the protein tyrosine phosphatases SHP-1 and SHP-2 and the lipid phosphatase SHIP. Such ITIM-containing receptors are best characterized

in B cells and natural killer cells. Inhibitory phosphatases, once localized to the plasma membrane by phosphorylated ITIMs, are placed in proximity to ITAM-containing receptors and, in turn, negatively regulate their function. CD22 and FcγRIIb are examples of B-cell-specific ITIM-containing surface receptors that are critical modulators of BCR signaling [7,8]. Inhibitory cell surface molecules such as PD-1 and CTLA-4 are expressed on T cells and analogously modulate TCR signal transduction, although only PD-1 contains a canonical ITIM [9]. Despite abundant similarities, wiring differs critically between T and B cells and among distinct stages of lymphocyte development. Most notably, the Lyn SFK in B cells is felt to play a non-redundant negative regulatory role downstream of numerous ITIM-containing receptors [10]. A homologous 'negative' role for Lck or Fyn in T cells has yet to be clearly demonstrated.

## Antigen receptor signaling in lymphocyte development

Studies in mice have revealed that antigen receptor signaling is critical not only in the response of mature lymphocytes to foreign antigen but in the progression of lymphocytes through a series of developmental stages in which both ligand-dependent and ligand-independent signals are required to proceed. Perhaps most significantly, antigen receptor signaling is necessary for 'testing' and refining the antigen receptor repertoire during development. Candidate TCRs are tested in the thymus for 'just right' signal strength by positive and negative selection. Perturbations in TCR signal transduction influence this process [11]. Analogous processes have been

identified in B cells in the bone marrow and the periphery [12]. Thymic lineage decisions have been shown to critically depend upon antigen receptor signal strength, including Foxp3^{+} regulatory T cell (Treg) fate [13]. Antigen receptor signaling in the periphery is also critical in the maintenance of immune homeostasis and tolerance to self. These antigen receptor-dependent events are likely relevant for the interpretation of disease pathogenesis in signaling mutants.

## Mouse models

An extensive mouse model literature can teach us about the signaling requirements for tolerance and autoimmunity. Evidence for the role of antigen receptor signaling in auto-immunity and insight into disease pathogenesis comes from both forward and reverse genetic approaches, involving both engineered and spontaneous mutations. Our approach here is to group mutations with similar functional consequences (hypo- or hyper-responsiveness) in T cells or B cells and to explore links to disease.

### B-cell antigen receptor signaling mutants and murine lupus

Several single-gene mutants develop a lupus-like disease characterized by the production of anti-nuclear antibodies (ANAs) in the context of hyper-responsive BCR signaling. Examples include FcγRIIb^{−/−}, Lyn^{−/−}, Lyn[up/up], CD45 E613R, CD22^{−/−}, CD19 transgenic (Tg), and SHP-1 (Me[v]) mice (see [14] for detailed review). These mutations, in turn, can be grouped into functional pathways. CD22, FcγRIIb, and SHP-1 are exclusively negative regulators of BCR signaling [6]. The moth-eaten viable allele of SHP-1 (Me[v]) is a spontaneously arising hypomorph with reduced phosphatase activity [14]. The SFK Lyn plays a more complex role in BCR signal transduction [10]. A confusing observation has been that two opposing alleles of Lyn (Lyn^{−/−} and Lyn[up/up]) both produce B-cell hyper-responsiveness and ANAs. This strongly suggests that Lyn has both positive and negative regulatory roles. Lyn is critical for BCR signal transduction as well as function of inhibitory coreceptors such as FcγRIIb and CD22. Lyn exerts its negative regulatory role by phosphorylating ITIMs that in turn recruit SHP-1 and SHIP. Lyn is thought to subserve this function in a non-redundant fashion despite expression of two other SFKs in B cells, Fyn and Blk. CD19 is a B-cell-specific cell surface protein that forms the signaling component of the CR2 complement receptor (CD21) in conjunction with CD81 [6]. CD19 contains multiple tyrosines and positively regulates BCR signal transduction. Its overexpression in mice leads to the production of autoantibodies [15]. E613R is a dysregulated allele of CD45, which in turn influences SFK activity. Mice harboring this mutation develop a lympho-proliferative syndrome and a lupus-like disease on permissive genetic backgrounds [16]. The disease is driven by B cells that are extremely hyper-responsive to BCR signaling [17].

The characteristics of the disease(s) in these animals are interesting. All produce autoantibodies but their specificities vary. CD22^{−/−} mice produce anti-cardiolipin antibodies and anti-myeloperoxidase antibodies, whereas CD19 Tg mice produce single-stranded DNA antibodies as well as rheumatoid factor [15,18]. This observation suggests that there may be a common general mechanism for autoantibody production in various autoimmune diseases. It has recently been demonstrated that the innate pattern recognition receptors TLR7 and TLR9 are critical (and sufficient in a B-cell-intrinsic manner) to generate antibodies to DNA/nuclear components broadly and to direct specificities as well [19]. It is likely that BCR signal transduction cooperates with this pathway. Whether other factors cooperate and which ones do so are still unknown. This exciting discovery complicates conventional distinctions between innate and adaptive responses and undercuts assumptions about clonal escape from tolerizing mechanisms.

A general feature of this collection of mouse models is that genetic background effects are very significant. FcγRIIb develops lupus-like disease on the B6 background but not on the Balb/c background [20]. CD45 E613R mice, in contrast, remain healthy with no ANAs on the B6 background, whereas on the Balb/c background 100% of the animals develop ANAs (M Hermiston, V Lam, R Mills, N Oksenberg, N Cresalia, A Tam, M Anderson and A Weiss, manuscript in preparation). Furthermore, a number of these models can produce disease on a 'non-autoimmune' background in the setting of cooperating mutations [20].

When and how is tolerance broken in these mice? The answer to this question is exceedingly complex because many of these models influence cell lineages other than B cells. Indeed, genetic deletion of lymphocytes in Me[v] mice does not fully rescue disease, suggesting that myeloid cell-intrinsic defects can drive the motheaten phenotype [21].

To understand how and where enhanced BCR signal trans-duction produces autoantibodies, we will focus our attention on those mice in which signaling is perturbed *only* in B cells. We are left with the CD22^{−/−}, FcγRIIb^{−/−}, and CD19 Tg models. FcγRIIb^{−/−} is the most extensively studied of these, and crosses to BCR transgenes have revealed that the tolerance break is peripheral and 'late' [22]. Similarly, CD22 is expressed and influences signaling in a relatively narrow pattern upon mature conventional B cells [14]. Lyn^{−/−} (Andrew Gross, personal communication) and CD45 E613R (J Zikherman, M Hermiston, D Steiner, K Hasegawa, A Chan, A Weiss, manuscript in preparation) B cells also exhibit hyper-responsiveness to BCR stimulation predominantly at the follicular mature peripheral B cell stage of development. Taken together, these data suggest that peripheral BCR hyper-responsiveness cooperates with other events (such as TLR signaling) to break tolerance by accelerating differentiation into plasma cells or progression into germinal centers. It may be that sufficient 'escape' of anti-nuclear B cells to the periphery occurs physiologically [23]. A 'central'

tolerance break may not be necessary in these mouse models of lupus.

In other mouse models, both a central and peripheral tolerance break may be required. The NZB/W mouse is a spontaneous polygenic model of lupus which has been studied extensively over the last 20 years. Genetically separable cellular phenotypes resemble those seen in engineered models of lupus. For example, BCR hyper-responsiveness maps to the lupus-prone NZM2410-derived Sle2 genetic locus but cannot independently produce disease [24]. The Sle1 region is associated with the appearance of ANAs [25]. Sle1 recently was mapped to Ly108, a member of the SLAM family of receptors that signal through a non-ITAM/ITIM pathway that relies upon the adaptor SAP and the SFK Fyn [26]. Ly108 is highly expressed in immature B cells and can modulate BCR signal strength. The NZB/W-derived allele of Ly108 produces weaker BCR signaling than the B6 allele in immature B cells. This allele may act early during negative selection of B cells, permitting polyreactive anti-double-stranded DNA (anti-dsDNA) B cells to escape to the periphery.

Thus, opposing signaling phenotypes may be required to breach 'central' and 'peripheral' tolerance mechanisms. The two may even coexist in the same animal as genetically separable phenotypes as demonstrated by the NZB/W lupus model. Whether analogous functional phenotypes characterize human systemic lupus erythematosus (SLE) will be interesting to determine.

## Proximal T-cell antigen receptor signal transduction and autoimmune disease

There are many examples of signaling mutants in which proximal TCR signaling machinery is impaired, and a number of these mutants develop disease. The Skg mouse model of rheumatoid arthritis (RA) is due to a spontaneous mutation that arose in an inbred colony of Balb/c mice [27]. These animals develop a destructive polyarthritis associated with rheumatoid factor and anti-cyclic citrullinated peptide antibody production. The mutation was identified as a single amino acid substitution in ZAP-70 (W613C). This mutation impairs ZAP-70 association with TCRζ-chain ITAMs and results in markedly reduced TCR signal transduction. The mice exhibit impaired positive and negative selection in the thymus as well as a hypo-proliferative phenotype in the periphery. Consistent with a ZAP-70 mutation, the disease is T-cell-mediated; CD4 T cells, but not serum, can transfer the disease, even into RAG$^{-/-}$ hosts lacking endogenous T and B cells [28].

The pathogenesis of disease in these animals remains unclear [28]. Impaired negative selection has been observed and may permit autoreactive T cells to escape to the periphery. However, a bona fide autoantigen has not yet been identified. Other potential etiologies of disease include abnormalities in Tregs, which are reduced in number and

impaired in function. Whether Tregs play a critical role in the pathogenesis of Skg arthritis is still uncertain. Cytokine milieu appears to be perturbed in these animals, and dysregulated T-cell differentiation and cytokine production may play an important role. Indeed, crosses to cytokine knockouts have shown that IL-6 and IL-17, but not interferon-gamma, are required to mediate disease. Interestingly, the disease disappeared in a clean specific-pathogen-free facility but could be induced by innate immune stimulation of pattern recognition receptors by dectin, a fungal cell wall component [29]. Thus, the dysregulated immune system in these animals has to be tipped over the edge, so to speak.

An informative allelic series of ZAP-70 hypomorphic mutants was described recently and provided an opportunity to study graded TCR signaling and its role in autoimmunity [30]. The ZAP-70 allelic series revealed a threshold effect in which partial, but neither mild nor severe, T-cell immunodeficiency was sufficient to break tolerance. Partially impaired TCR signal transduction was associated with the appearance of ANAs as well as hyper-IgE and IgG1 antibody production. The latter suggests an unusual Th2 polarization, which we will mention again below in the context of other mutants.

This phenotype did not resemble the ZAP-70 hypomorphic Skg allele. The ZAP-70 allelic series was generated on the B6 genetic background, whereas the Skg ZAP-70 allele leads to arthritis only on the Balb/c background in the context of an innate immune stimulus. A common target molecule with quantitatively or qualitatively impaired TCR signaling may provoke different diseases in different genetic and environmental contexts, as seen with B-cell perturbations. The mouse models discussed above include quantitative and perhaps qualitative impairments in a single critical molecule, ZAP-70, involved in TCR signal transduction. What of perturbations in distinct signaling pathways downstream of the TCR?

The Lat Y136F mutation eliminates binding of PLCγ1 to a critical phospho-tyrosine of the Lat adaptor [31,32]. T cells from Lat Y136F mice exhibit profoundly impaired calcium flux with relatively preserved Erk phosphorylation. Thymic development is perturbed with a partial block at beta selection as well as positive selection. At 2 to 3 weeks of age, the mice develop a lymphoproliferative disorder characterized by CD4 T-cell expansion and overproduction of Th2 cytokines. The mice exhibit associated polyclonal B-cell activation and elevation of IgE and IgG1. An inflammatory disease develops with multiorgan infiltrates and production of ANAs as well as dsDNA antibodies. The phenotype is much more severe than that associated with the ZAP-70 allelic series, but the unusual development of Th2 cytokine overproduction (and hyper-IgE levels) is reminiscent.

Taken together, the pathogenesis of autoimmunity in these models is not obvious [33]. Clearly, impaired signal transduction perturbs thymic selection and influences the T-cell

repertoire. However, as in the Skg mouse, initiating auto-antigens have not been identified and how an autoantigen could stimulate severely signaling-impaired peripheral T cells to produce disease is unclear. Lineage commitment in the thymus is also perturbed; Treg development and function are abnormal. Indeed, a number of mouse models with impaired TCR signaling and autoimmunity are rescued by transfer of wild-type Tregs [33]. However, the ability of transferred Tregs to reverse a disease phenotype does not establish Treg deficiency as a cause of disease.

Another possibility raised in the context of these immuno-deficiency states is that a lymphopenic environment may be critical for homeostatic proliferation/activation of dysregulated T cells. Also, partial immunodeficiency may perturb host defense in such a fashion that the homeostatic burden of gut commensals is abnormal. Stimulation of the innate immune system may interact with abnormal T cells to wreak havoc.

A final hypothesis relates to abnormal homeostasis of peripheral T cells. Impaired TCR signal transduction may alter effector T-cell differentiation and function in multiple ways. It may be that inhibitory feedback loops downstream of TCR triggering are disproportionately impaired in these models such that a weak signal is transmitted but not appropriately downregulated. Alternatively, an appropriate signal to induce anergy is not generated. This group of defects encompasses failure of antigen-specific anergy as well as failure of non-antigen-specific 'self-control'.

Most recently, extensive characterization of the Lat Y136F mouse model has produced unexpected results. The transfer of Lat Y136F CD4 T cells into an MHC II$^{-/-}$ host produces disease [34]. This raises the possibility that participation of Lat in non-TCR signals (in a lymphopenic environment) or ligand-independent tonic TCR signals (in the absence of functional antigen-presenting cells) plays a role. Significantly, proliferating Th2 polarized effector CD4 T cells drive ANA production in wild-type B cells upon adoptive transfer in the absence of a bona fide initiating autoantigen and certainly cannot do so in a cognate manner (in the absence of MHC II molecules).

Most rheumatologists would classify ANA production as 'autoimmune' in nature. It may, however, be important to reassess old assumptions about the antigen-driven etiology of 'autoimmunity' in animal models characterized by ANAs. We have learned in recent years that innate receptors such as the TLRs are required to direct this specificity and now discover that non-specific T-cell help is sufficient to produce ANAs in otherwise normal B cells.

Diseases that develop in the Lat model and in the ZAP-70 allelic series are characterized by IgE and Th2 cytokine over-production. Autoimmunity that sometimes arises in the context of partial human T-cell immunodeficiency is often characterized by IgE production and 'allergic' Th2 diseases. The ZAP-70 allelic series and Lat mouse models bear a much closer resemblance to these relatively rare clinical entities than to common polygenic rheumatic diseases such as RA and SLE. (The reader is referred to an excellent recent review [33].) Nevertheless, this phenomenon raises the possibility that dysregulation of T-cell-intrinsic effector pathways may contribute to disease in some 'classic' rheumatic diseases.

**Hyper-responsive T-cell antigen receptor signaling**
We have observed that B-cell hyper-responsiveness appears to be an overwhelming characteristic of murine lupus mouse models. A large number of mice with impaired TCR signaling, with either proximal or distal transduction perturbations, develop dysregulated lymphoid homeostasis and inflammatory diseases [33]. Only a handful of these have been reviewed here, selected because they feature proximal and T-cell-specific perturbations that are more easily interpretable and simplify the cellular mechanisms of disease.

The clearest model to demonstrate that T-cell hyper-responsiveness can also break tolerance may be the Cbl/Cbl-b double-deficient mice. Cbl and Cbl-b are widely expressed E3 ubiquitin ligases that target their substrates for proteo-somal degradation [35]. By targeting multiple components of the antigen receptor signal transduction machinery for degradation, Cbl and Cbl-b serve as negative regulators of antigen receptor signaling. Both single and double knockouts (dKOs) have been generated, revealing overlapping as well as developmentally distinct roles in antigen receptor signaling [35]. The T-cell-specific dKO develops a severe systemic disease characterized by arteritis and dsDNA production [36]. T cells are hyper-proliferative and produce large quantities of cytokines in response to TCR stimulation. Yet proximal TCR signaling machinery is differentially affected, with enhanced ZAP-70 phosphorylation but impaired PLCγ1 phosphorylation leading to impaired inducible calcium increase. Most interestingly, impaired ligand-induced TCR downmodulation and prolonged Erk phosphorylation characterize dKO T cells. The TCR signaling phenotype is not simply amplified but is qualitatively and kinetically perturbed. Whether the associated disease represents an antigen-specific breach of tolerance or a dysregulated polyclonal response akin to the Lat Y136F mice remains unclear.

In contrast to impaired TCR signaling, relatively few mouse models with a 'pure' defect leading to hyper-responsive T cells develop autoimmune disease. One explanation relates to the wiring of TCR signaling machinery and another to the etiology of rheumatic disease. One can a priori engineer hyper-responsive TCR signaling by generating either a hypermorphic allele of a positive regulator or a knockout/hypomorph of a negative regulator. Although knockouts are easier to generate, negative regulators appear in general to display more functional redundancy than positive regulators of TCR signaling (the opposite may be true in B cells).

Perhaps these mutants, when generated, have too subtle a phenotype (for example, Pep$^{-/-}$, to be discussed later) to produce disease on non-autoimmune genetic backgrounds. Another argument relates to the developmental consequences of strong TCR signaling. Examples of dramatically enhanced TCR signaling, including the Lck Y505F mutant and the Csk$^{-/-}$ mutant, exist [5]. Both have such enhanced TCR signaling that T-cell development in the thymus cannot occur normally due to suppression of RAG expression. In fact, rather than autoimmunity, these types of perturbations cause malignant transformation. In this way, hyper-responsive peripheral T cells may just be hard to generate with reverse genetics in mice. Alternatively, it may be that only impaired TCR signaling can breach T-cell tolerance without help. Indeed, as we will argue below, hyper-responsive T cells may be a feature of specific autoimmune diseases that require an additional and independent B-cell phenotype. Hyper-responsive T cells, in other words, may not be able to act alone. Perhaps this tells us that our immune system is biased toward protecting us from the ravages of overactive T cells but has fewer built-in defenses against impaired TCR signaling. This might make teleogical sense since the overwhelming evolutionary pressure on the immune system has been infection, not autoimmunity, driving the system to over-reaction, not under-reaction.

## Translational data: signaling in B and T cells from patients with rheumatic disease

Do these mouse models have relevance for human disease? Indeed, perturbations in antigen receptor signal transduction have been identified in lymphocytes from patients with rheumatic diseases.

### B cells in human systemic lupus erythematosus

Stimulation of the BCR on peripheral blood B cells from SLE patients has been reported to cause exaggerated calcium increases, recapitulating functional cellular phenotypes seen in mouse mutants with SLE (for example, Lyn$^{-/-}$, Fc$\gamma$RIIb$^{-/-}$, and CD22$^{-/-}$) [37]. Significantly, these functional alterations did not correlate with disease activity or with treatment, consistent with a primary pathogenic role. The mechanistic and genetic basis of this phenotype in primary human B cells remains uncertain. Expression of key BCR signaling molecules in SLE B cells has been studied and reduced levels of the negative regulators Lyn and SHIP have been described, reminiscent of SLE mouse models [38]. The convergence of human and mouse data strongly suggests that exaggerated BCR signal transduction, at least in peripheral B cells, may be a fundamental pathogenic feature of human SLE.

### T cells in human systemic lupus erythematosus

Analogous functional studies of T cells from SLE patients have been undertaken. Exaggerated calcium increases in SLE T cells upon TCR stimulation have been reported [39]. Oddly, SLE T cells generally produce reduced quantities of IL-2 [40].

This has been interpreted as an 'anergic' phenotype and might suggest that some of the observed signaling phenomena reflect the influence of a characteristic inflammatory milieu rather than a cell-intrinsic genetic program.

Interestingly, expression levels of TCR$\zeta$ chain were found to be reduced in SLE T cells [39]. The mechanism for this deranged expression is apparently both transcriptional and post-translational [40,41]. Increased expression of the alternative ITAM-bearing receptor FcR$\gamma$ has been observed in those cells, and TCR stimulation results in enhanced FcR$\gamma$ phosphorylation. An alternative TCR complex composed of FcR$\gamma$-Syk (replacing $\zeta$-Zap70) has been proposed to account for the altered functional signaling phenotype observed in these cells. Similar $\zeta$ chain downregulation in RA T cells from synovial fluid and in memory T cells has been reported [40].

### T cells in human rheumatoid arthritis

Interestingly, distinct functional phenotypes have been reported in peripheral blood T cells from patients with RA, including impaired calcium responses and proliferation to TCR stimulation [42]. This is a provocative observation given the T-cell-signaling-impaired Skg mouse, which develops an RA-like clinical phenotype on a susceptible genetic background.

In the end, whether these changes observed in SLE and RA reflect a cell-intrinsic and disease-specific abnormality in T cells or a general change to activated/effector status is less clear, and whether in turn this represents a cause or effect of the inflammatory disease is unknown.

## Human genetics

Functional studies conducted with primary human cells are suggestive but remain correlative. To address cause and effect, human genetics offers some clues. Indeed, numerous human candidate gene association studies have implicated antigen receptor signaling pathways in the pathogenesis of rheumatic diseases. A hypomorphic allele of Fc$\gamma$RIIb (Ile232Thr) has been associated with SLE in an Asian population [20]. Studies in recent years have also identified disease-associated polymorphisms in CTLA-4, a T-cell inhibitory coreceptor, and mutations that influence splicing and function of CD45 [43,44]. Human genetics has seen an explosion in data with the advent of whole genome association studies in the last two years. Unbiased identification of new genetic risk factors for human autoimmune diseases has implicated antigen receptor signaling machinery as well [45]. The B cell SFK Blk and the BCR signaling adaptor BANK1 were identified in recent whole genome scans for lupus [46,47]. A single missense polymorphism in PTPN22, a negative regulator of the SFKs, is the second strongest common polymorphism associated with RA outside of the MHC [48,49]. Yet the functional consequences of many of these polymorphisms remain unclear. The subtlety of the risk alleles coupled with developmental and network

complexity of antigen receptor signal transduction make interpretation of phenotypes challenging. How then can polygenic susceptibility loci be studied? How do we move from genetics to pathogenesis?

**PTPN22 R620W polymorphism**

An elegant example is the PTPN22 R620W polymorphism, which is associated with multiple autoimmune diseases, including SLE, RA, and type I diabetes [50,51]. The PTPN22 gene product Lyp, the murine ortholog of which is Pep, encodes a hematopoietic cytoplasmic phosphatase. Pep/Lyp negatively regulates TCR signaling by dephosphorylating the activating tyrosine of Lck [52,53]. One quarter to one half of Pep is found associated with Csk, a potent negative regulator of TCR signaling which dephosphorylates the inhibitory tyrosine of Lck [54]. Pep cooperatively inhibits TCR signaling by binding Csk and this association in turn is mediated by a proline-rich sequence in the C-terminal region of Pep (PRS1) [52,54].

The R620W polymorphism is located in the critical PRS1 domain of Pep, and impairs the interaction of Pep with Csk [50,55]. The risk allele was therefore initially postulated to represent a loss-of-function in which TCR signaling was less effectively inhibited. However, overexpression of the Lyp risk allele in Jurkat cells suggested the opposite (that is, that the risk allele is a gain-of-function, impairing TCR signaling) [55]. A handful of studies of primary human cells from healthy donors as well as patients harboring the risk allele have been published [55-58]. Several appear to confirm the gain-of-function hypothesis but not all are in agreement. In our laboratory, we revisited the question of the functional significance of the R620W risk allele. Functional studies of the wild-type and R619W (murine homolog) Pep alleles in the context of Csk unmasked Pep R619W as a hypomorphic allele (J Zikherman, M Hermiston, D Steiner, K Hasegawa, A Chan, A Weiss, manuscript in preparation).

The Pep$^{-/-}$ mouse confirms Pep as a negative regulator of TCR signaling but no disease phenotype is discernible [59]. Indeed, the Pep null allele appears to require a cooperating mutation to develop disease, just as the R620W polymorphism in humans does not act alone. By crossing the Pep$^{-/-}$ mice onto a background in which hyper-responsive B cells (characteristic of lupus-prone mice and humans) are active, we have been able to generate a mouse model in which a bona fide human genetic risk factor produces a lupus-like disease. In Pep$^{-/-}$/CD45 E613R double-mutant animals, hyper-responsive Pep$^{-/-}$ T cells and hyper-responsive CD45 E613R B cells cooperate to break tolerance (J Zikherman, M Hermiston, D Steiner, K Hasegawa, A Chan, A Weiss, manuscript in preparation). Definitive functional conclusions about the R620W allele will depend on future studies of a knock-in mouse. Even those will then have to be pursued in the context of cooperating mutations in order to recapitulate human disease pathogenesis.

## Conclusion

Conventional antigen-driven autoimmune disease with pathogenic clones is most clearly observed in the organ-specific autoimmune endocrinopathies, including insulin-dependent diabetes mellitus, autoimmune ovarian failure, and others. Certainly, polyendocrinopathy syndromes, and possibly sporadic variants of these diseases, appear to be characterized by failure of central tolerance [60]. However, more and more questions have been raised regarding the 'autoimmune' nature of diseases arising in the setting of partial immunodeficiency. The common systemic rheumatic diseases, particularly SLE and RA, are under scrutiny as well. Conventional models of disease pathogenesis are being revised as the complex interplay of innate and adaptive pathways is being dissected and appreciated. Are alterations in antigen receptor signaling pathways significant in disease pathogenesis? Overwhelming data suggest that they are. Are specific auto-antigens required to break tolerance and drive disease? This is less clear. We have tried in this review to demonstrate the power of combining genetic and functional studies in mice and humans and to point out the limits of our current understanding. Much remains to be understood in the pathogenesis of rheumatic diseases, and new targets for therapy will no doubt emerge.

## Competing interests

The authors declare that they have no competing interests.

## Acknowledgments

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