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Resolving a paradox between mouse and man: a genetic link between TLR7 and the pathogenesis of human lupus nephritis

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It has been known for over half a century that anti-nuclear antibodies (ANA) are a characteristic feature of systemic lupus erythematosus (SLE). The mechanism for this propensity towards nuclear reactivity was enigmatic until a seminal study linked dysregulated Toll-like receptor (TLR) signaling with ANA production[2]. Toll-like receptors are germline encoded, pattern-recognition receptors evolved to recognize conserved ligands on exogenous pathogens. However, in the context of autoimmunity, nucleic acid-containing particles derived from apoptotic cells can preferentially activate autoreactive B cells via the dual engagement of B cell receptor (BCR) and TLR signaling pathways. Of the several Myeloid differentiation primary response 88 (Myd88)-dependent TLR family members, animal studies have identified TL7 and TLR9 as the relevant receptors in lupus pathogenesis. Specifically, the single stranded RNA sensor TLR7 promotes autoantibodies against RNA and RNA-associated proteins, while autoantibodies targeting double stranded DNA (dsDNA) and chromatin require engagement of the DNA sensor TLR9[3].

Based on this model, one would have anticipated that deletion of TLR7 or TLR9 would each result in partial projection against autoimmunity in murine models of lupus. As predicted, genetic disruption of TLR7 function has been shown to eliminate autoimmunity in multiple independent animal models of SLE, to a similar degree as is achieved by deleting the shared adaptor molecule Myd88[3, 4]. In contrast, TLR9 deletion exacerbates disease development despite the loss of dsDNA autoantibodies. In keeping with a dominant role for TLR7 in

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lupus pathogenesis, independent models of TLR7 over-expression resulted in accelerated murine SLE[4].

Although these opposing pathogenic and protective roles for TLR7 and TLR9 in lupus pathogenesis have been replicated in multiple independent animal studies, whether this paradigm applies to human lupus remains uncertain. Indeed, anti-dsDNA autoantibodies, and not RNA-associated specificities, have been most closely linked to the pathogenesis of lupus nephritis, as evidenced by epidemiological links to lupus nephritis incidence and flare rate, and the observation that adoptive transfer of DNA-reactive monoclonal antibodies can promote proteinuric kidney disease in mice[5, 6]. While these studies suggest a direct pathogenic role for dsDNA autoantibodies, a more comprehensive assessment of immunoglobulin eluted from lupus nephritis glomeruli identified broad autoreactivity, including against several RNA-associated autoantigens[7]. Ultimately, despite extensive murine modeling and circumstantial evidence from human studies, it remains uncertain whether TLR7 engagement and RNA-associated autoantibodies have an actual pathogenic role in human SLE.

What did the study show?

To identify genetic variants linked with early-onset SLE, whole genome sequencing was performed on a 7-year-old child with lupus, characterized by positive ANA, autoimmune thrombocytopenia, neurologic involvement, and lupus nephritis. This revealed a de novo *TLR7* p.Tyr264His (Y264H) missense mutation predicted to be damaging by bioinformatic analysis. While this mutation seemed to be a strong lupus risk candidate, this subject also carried a second heterozygous variant in *RNASH2B*, which is known to cause SLE when homozygous. For this reason, the authors performed a series of mechanistic studies aimed at establishing a causal role for the $TLR7^{Y264H}$ allele in human lupus. They first measured downstream signaling using cell lines overexpressing $TLR7^{Y264H}$ vs. control *TLR7* alleles. These experiments demonstrated that $TLR7^{Y264H}$ exhibited *increased* NF-κB activation following guanosine or single cell RNA (ssRNA) stimulation. In keeping with enhanced signaling in vitro, computational modeling predicted increased binding of guanosine to variant TLR7. While these findings suggested that $TLR7^{Y264H}$ functions as a gain-of-function variant, additional animal modeling was required to confirm that this mutation is sufficient to promote lupus-like autoimmunity. For this reason, the authors used CRISPR-Cas9 gene editing to generate $Tlr7^{Y264H}$ knock-in mice. Notably, $Tlr7^{Y264H}$ mice exhibited spontaneous immune activation, including expansion of activated B and T cell populations observed in human SLE, production of class-switched autoantibodies, and the development of lupus nephritis. Importantly, intercrossing with *Rnaseh2b*-haploinsufficient mice only slightly increased in the lupus-associated type 1 interferon signature, without impacting other disease manifestations, suggesting that $TLR7^{Y264H}$ is the true causative allele.

An available murine model of the human gain-of-function TLR7 allele allowed the investigators to interrogate the immune mechanisms underlying TLR7-driven lupus pathogenesis, unhindered by limited availability of human samples. These yielded several insights likely to be important for our understanding of lupus immune pathogenesis. First,

using mixed bone marrow chimera models, the authors showed that B cell, and not $CD4^+$ T cell, expression of the *Tlr7*^{Y264H} allele promoted adaptive immune activation. Notably, these findings are consistent with earlier animal studies in which B cell-specific deletion of TLR7 or TLR9 was shown to fully recapitulate the phenotype of global *Tlr7*$^{-/-}$ and *Tlr9−/−* lupus prone mice[8], emphasizing the importance of B cells in facilitating breaks in immune tolerance.

Second, the authors investigated the B cell activation pathway responsible for pathogenic plasma cell generation. It has long been appreciated that, during a humoral immune response, B cells activation can occur within germinal centers or via a parallel extra-follicular activation program. Using an elegant genetic strategy in which animals were rendered unable to form germinal centers, the authors showed that RNA-associated antibodies were still generated. Thus, in keeping with recent human immunophenotyping studies[9], dysregulated TLR7 signaling appears to predominantly drive pathogenic B cell activation via an extra-follicular activation pathway.

Why is it important?

In summary, the study by Brown et al. provides an important link between the immunology insights gained from mouse models and the pathogenesis of the human SLE. Rather than being a unique feature of the mouse immune system, the identification of a rare patient with a gain-of-function *TLR7* variant confirms the critical role for this receptor in human lupus pathogenesis. Moreover, this study affirmed the important role for extra-follicular B cell activation in the formation of autoantibody-producing plasma cells in SLE. Given that extra-follicular B cell activation predominantly generates short-lived plasma cells, in contrast with germinal center-derived long-lived plasma cells, this suggests that effective B cell depletion might be able to “reset” the autoimmune repertoire by eliminating the bulk of pre-formed pathogenic plasma cells. This may be particularly relevant for lupus nephritis given that B cells and T follicular helper (T_{FH}) cells have been shown to interact in the tubulointerstitial compartment of the lupus kidney, coupled with evidence that a B cell targeted antibody exhibiting increased depletion of tissue-resident B cells successfully treated lupus nephritis.[10,11] This genetic discovery also provides support for novel drugs like enpatoran, a dual TLR7/8 small molecule antagonist currently undergoing phase 2 evaluation in SLE (NCT05162586), and encourages the development of drugs like DS-7011a, a TLR7 blocking antibody now in a phase 1 study of healthy volunteers (NCT05203692). Like the remarkable success of PCSK9 inhibition for hypercholesterolemia, target identification informed by human genetics holds the promise of informing new treatments for patients with SLE, including those with lupus nephritis.

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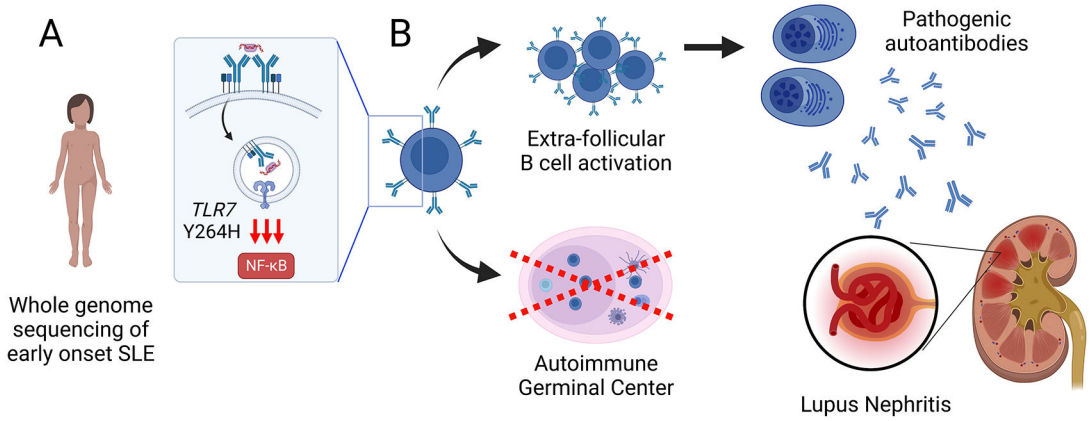

Figure 1: Identification and functional characterization of a pathogenic *TLR7* mutation in early-onset SLE.

(A) Using whole genome sequencing, Brown et al. identified a de novo $TLR7^{Y264H}$ missense mutation in a pediatric patient with early onset lupus nephritis. After binding self-antigens derived from apoptotic cells, autoreactive B cells traffic nucleic acid-containing autoantigens to the endosomal receptors TLR7 and TLR9, resulting in dual BCR/TLR-mediated B cell activation. By increasing activation by guanosine-containing self-ligands, $TLR7^{Y264H}$ promotes pathogenic B cell activation and the development of SLE. (B) Parallel extra-follicular and germinal center-dependent B cell activation pathways contribute to the production of autoantibody-producing plasma cells during humoral autoimmunity. Surprisingly, autoantibodies persisted in $Tlr7^{Y264H}$ mice genetically incapable of forming germinal centers, indicating that extra-follicular B cell activation is the predominant pathway leading to autoantibody formation and the development of immune complex-mediated lupus nephritis. Created with BioRender.com.