

Identification of the *Yaa* lupus gene as a *Tlr-7* gene duplication event

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Evaluation of: Pisitkun P, Deane JA, Difilippantonio MJ, Tarasenko T, Satterthwaite AB, Bolland S: Autoreactive B-cell responses to RNA-related antigens due to *TLR7* gene duplication. *Science* 312, 1669–1672 (2006). Elucidating the pathogenesis of systemic lupus erythematosus (SLE) has been a difficult task, given its polygenic nature, complicated epistatic interactions and various environmental factors. The use of murine models has greatly augmented our understanding of the genetic contributions of various molecules and pathways to SLE. One locus derived from the autoimmune BXSB strain, termed the Y-linked autoimmune accelerator (*Yaa*) locus, has been shown to be a locus capable of exacerbating the severity and kinetics of disease when bred onto a variety of autoimmune-prone backgrounds. Indeed, the introgression of *Yaa* onto the autoimmune-prone *FcyRIIB*^{-/-} mouse strain drives fatal lupus. Pisitkun and colleagues uncovered the identity of the causative gene in the *Yaa* locus to be a member of the toll-like receptor (TLR) family, *TLR-7*, which senses pathogen-derived RNA. They show that the *Yaa* locus is a duplication of the distal end of the X-chromosome, which contains *Tlr-7*, leading to a twofold increase in *TLR-7* expression. The paper demonstrates that B cells from *Yaa*-containing strains proliferate better to a *TLR-7* agonist and have skewed autoantibody specificity to RNA antigens. Finally, the authors show that Bruton's tyrosine kinase (*Btk*) is a molecule downstream of *Yaa* signaling, and that, by deleting *Btk*, autoimmunity is dampened. The identification of *Yaa* as *TLR-7* uncovers a crucial role for the innate immune system in SLE pathogenesis.

Systemic lupus erythematosus (SLE) is a complex autoimmune disorder characterized by the loss of tolerance to ubiquitous self-antigens, which culminates in a variety of end-organ pathologies. The hallmark autoantigens in SLE are nuclear antigens, such as chromatin, ribonucleoproteins and double-stranded DNA. Indeed, one of the most striking features of SLE is the emergence of high-titer antinuclear autoantibodies (ANAs), which accumulate in end organs where they induce disease. The Toll-like receptor (TLR) family of molecules is crucial in detecting pathogen-derived nucleic acids: *TLR3* sense double-stranded RNA, *TLR7* senses single-stranded RNA and *TLR9* senses CpG DNA [1]. Indeed, recent studies have suggested that ligation of various TLRs by self nucleic acids may play an important role in lupus pathogenesis [2,3]. The success of hydroxychloroquine sulfate (Plaquenil®) – a drug that acidifies the endosomes where TLRs function (thereby possibly interfering with TLR function) – in the treatment of human lupus is further demonstration of the potential importance of TLRs in lupus.

Of relevance to the reviewed article is the BXSB-derived Y-linked autoimmune-accelerator (*Yaa*) locus, which potentiates lupus on certain

strain backgrounds, including the lupus-prone *FcyRIIB*^{-/-} mouse model [4]. In the reviewed article, Pisitkun and colleagues demonstrate that the fundamental lesion underlying the *Yaa* locus is a twofold upregulation of *Tlr7* due to a translocation of genes proximal to the pseudo-autosomal region on the distal X-chromosome to the Y-chromosome, resulting in a gene duplication in males carrying *Yaa*. Importantly, their studies highlight TLRs and other molecules situated at the interface between the adaptive and innate immune systems, as key pathogenic players as well as potential therapeutic targets in lupus.

The BXSB mouse model develops an early-onset and highly-penetrant lupus-like disease, characterized by high titers of ANAs and lethal glomerulonephritis [5]. Early studies documented a locus on the Y-chromosome, which accelerated disease in males. It was termed the *Yaa* locus. Subsequent studies demonstrated that the introgression of the BXSB-derived *Yaa* locus onto a number of lupus-prone strains grossly aggravated disease kinetics and severity [6,7], demonstrating that *Yaa* is a potent epistatic modifier of a variety of lupus genes. In the reviewed paper, Pisitkun and colleagues study the effects of *Yaa* on the *FcyRIIB*^{-/-} mouse model (on a C57BL/6 genetic background), which also

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develops spontaneous SLE-like disease [8]. Interestingly, utilizing a bone marrow chimera strategy, they demonstrate that B cells carrying Yaa have preferred antibody specificity to nucleolar antigens, while B cells bearing only the *FcγRII*^{-/-} deletion recognize chromatin predominantly, indicating that Yaa is capable of skewing B-cell specificity in an intrinsic fashion. In an analogous study, Subramanian and colleagues used autoantigen arrays to demonstrate that B6.Yaa sera strongly reacted with RNA-containing antigens [9]. Thus, it appears that the *Yaa* locus bears gene(s) that influence antigenicity of RNA-containing antigens.

Since Yaa was known to be associated with a marginal zone defect and generalized B-cell hyper-reactivity [10], Pisitkun and colleagues reasoned that, by deleting Bruton's tyrosine kinase (Btk), a molecule known to be critical for marginal zone B-cell formation and B-cell hyper-reactivity [11], they would be able to rescue the humoral autoimmune phenotype caused by Yaa. In line with their expectation, they demonstrated that the introduction of Btk deficiency completely rescued both the marginal zone defect and the B-cell hyper-reactivity caused by Yaa. The Btk deficiency also rescued all autoimmune phenotypes in these mice, effectively eliminating glomerulonephritis and ANA production. Taken together, these data demonstrate that Btk signaling is essential for the expression of the Yaa lesion, and suggest that perhaps the manipulation of Btk and Btk-associated pathways may be an attractive therapeutic approach in lupus.

To identify the candidate gene(s) for the Yaa locus, Pisitkun and colleagues performed microarray studies on B-cell RNA, and noted an approximately twofold upregulation in 26 genes including *Tlr7*, *Rab9*, *Tmsb4x* and *Msl131*, four genes positioned in tandem on the X-chromosome. The twofold upregulation was confirmed by quantitative real-time PCR and western blot. Since only *Yaa* males exhibited severe lupus and since the upregulation of TLR-7 and the linked molecules was twofold, the authors hypothesized that there might be a translocation of *Tlr-7*, normally expressed on the distal end of the X-chromosome, onto the Y-chromosome, in effect resulting in duplicate copies of *Tlr-7* in Yaa males. To test this hypothesis, Pitsukin and colleagues constructed BAC probes that spanned the *Yaa* locus and used Fluorescence *In situ* Hybridization (FISH) to visualize the location and copy number of the *Yaa* genes. Their data

clearly demonstrate a genomic duplication of a 4-MB region derived from the X-chromosome spanning *Tlr-7*.

Of the duplicated genes, *Tlr-7* was an attractive candidate because of its known role in recognizing pathogens and mammalian-derived RNAs, its expression in B-cells and its known binding to Btk [12]. To test whether *Tlr-7* was the causative gene for lupus, Pitsukin and colleagues demonstrated that *in vivo* treatment of B6.*FcγRIIb*^{-/-} mice with the TLR-7 agonist, imiquimod, was sufficient to change serum antibody specificity from chromatin to nucleolar specificity. *In vitro* stimulation of whole splenic lymphocytes with imiquimod resulted in increased phosphorylation of the downstream transcriptional regulator, IκBα and increased proliferation in the Yaa-containing strain compared with wild-type controls. Consistent with their *in vivo* studies, deficiency in Btk significantly diminished cellular responses to the TLR7 agonist, confirming its role downstream of TLR7.

In a parallel study, Subramanian and colleagues also demonstrate Yaa as a translocation of the distal X-chromosome containing *Tlr-7* leading to the duplication of *Tlr-7* and linked genes in Yaa-bearing mice [9]. In addition, they show that there are no polymorphic changes in *Tlr-7*, or tissue-expression differences in the Yaa-derived *Tlr-7* [9]. Together, both these reports suggest that a twofold increase in gene transcription of *Tlr-7* may be sufficient to dysregulate the immune system of lupus-prone animals. This pivotal discovery may open doors to many future studies.

Future experiments must first demonstrate definitively that *Tlr-7* is the causative gene in the Yaa lesion, and illuminate the pathogenic potential of other duplicated genes in the Yaa lesion. For example, breeding strategies wherein *Tlr-7*-null animals are crossed onto the B6.*FcγRIIb*^{-/-} (or other) Yaa-bearing backgrounds and, conversely, wherein a *Tlr-7* transgene is introduced onto the B6.*FcγRIIb*^{-/-} or other prone backgrounds, would help substantiate *Tlr-7* as the culprit gene. Such *in vivo* studies would also demonstrate whether other duplicated genes in the Yaa lesion contribute to Yaa-mediated disease.

Understanding how genes that are important in adaptive immunity, such as *FcγRIIb* and *Sle1*, interact with innate immune response genes, such as *Tlr-7*, to produce pathogenic autoimmunity is crucial. Indeed, the studies by Pitsukin and

Subramanian and their respective colleagues demonstrate the necessity of carefully detailing the cross-talk between innate and adaptive immunity [13]. Moreover, the possibility that dysregulations of the X-chromosome may lead to inappropriate expression of *Tlr-7* must be considered. Defects in X-inactivation, or defective genomic regulation of X-linked genes, may potentially lead to increased *Tlr-7* expression in the absence of polymorphic differences in *Tlr-7*. Given the high female-to-male ratio in human lupus, this possibility merits consideration. In addition to *Tlr-7*, TLR-associated and TLR-triggered molecules become

prime candidates for further study in both murine and human lupus. This is particularly relevant in light of the purported role of interferon (IFN)- α in human SLE [14] and the known secretion of IFN- α in response to Tlr-7 stimulation [15]. Finally, the extent to which Tlr-7 and Tlr-7-associated molecules may confer lupus susceptibility in humans must be ascertained. Hence, the finding by Pisitkun and colleagues that toll-like receptor-7 may potentiate lupus pathogenesis looks certain to initiate several novel avenues of research in the coming years.

Executive summary

- Pisitkun and colleagues identified Toll-like receptor (TLR)-7 as the causative gene underlying the *Yaa* lesion, a potent epistatic modifier of lupus susceptibility.
- Dysregulation of TLR7 expression, even by twofold, may be sufficient to alter B-cell antigen specificity from chromatin to RNA-containing antigens, increase B-cell proliferation in response to TLR-7 stimulation and drive fatal lupus on autoimmune-prone genetic backgrounds.
- The *Yaa* lesion occurred due to translocation of the distal arm of the X-chromosome containing *TLR-7* to the Y-chromosome, resulting in the duplication of *TLR-7* and linked genes within the translocated segment in *Yaa* males.
- Bruton's tyrosine kinase (Btk) is an important downstream molecule in TLR7 signaling, as Btk deficiency restores wild-type phenotype completely.

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