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INSIGHTS

Traffic on the TLR expressway

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Genetic variation in *UNC93B1*, a key component in TLR trafficking, can lead to autoinflammation caused by increased TLR activity. Analysis of seven patient variants combined with a comprehensive alanine screen revealed that different regions of UNC93B1 selectively regulate different TLRs (Rael et al. https://doi.org/10.1084/jem.20232005; David et al. https://doi.org/10.1084/jem.20232066).

Toll-like receptors (TLRs) are a front-line security system against infection and damage. They scan the cell's environment for signs of viruses, bacteria, and injury, activating innate immune responses upon detection. However, there is enough similarity between the molecular patterns associated with viral and bacterial infection and the nucleic acids of steady-state cellular processes that TLRs risk mistaking one for the other if TLR activity is not properly regulated. That regulation is carried out through gene expression control and physical compartmentalization of certain TLRs to the endolysosome. When dysregulated, the consequences can manifest in autoinflammatory conditions including various forms of lupus and arthritis, or as hypersusceptibility to infection depending on whether the dysregulation leads to excessive or insufficient activity.

A handful of the nucleic acid–sensing TLRs are escorted to the endosome by uncoordinated 93 homolog 1 (UNC93B1), a heavily conserved, 12-pass, membrane-bound protein expressed in the endoplasmic reticulum (ER). How exactly UNC93B1 does this remains somewhat nebulous, but we know from mice and humans with nonfunctional variants that certain TLRs get stuck in the ER without it (Brinkmann et al., 2007; Lee et al., 2013; Pelka et al., 2018). We also know that mutations in specific regions of *UNC93B1* can increase the activity of one TLR without affecting the activity level of another (Fukui et al., 2009, 2011), a phenomenon that highlights the complexities underlying the role UNC93B1 plays in balancing TLR activity. However, until now, it was not known whether mutations that impact the interactions between UNC93B1 and the TLRs it chaperones can drive autoinflammation.

In theory, autoinflammation mediated by TLR dysregulation occurs via two avenues. In the first, conditions increase sensitivity to nucleic acid to the point that small amounts of activating material trigger a disproportionate response. In the second, the receptor fails to distinguish self from pathogen. Either can manifest from an excess of TLR protein or from incorrect compartmentalization of the TLR, but it is easy to imagine how changes in the interaction between the TLR and UNC93B1 might drive these scenarios.

Two new studies from the *Journal of Experimental Medicine* address this hypothesis through functional genetic evaluation of seven families with various autoinflammatory conditions ranging from systemic lupus erythematosus (SLE) to juvenile idiopathic arthritis driven by missense mutation in *UNC93B1*. Remarkably, their functional analysis begins to unearth specific regulatory mechanisms employed by UNC93B1 in trafficking different TLRs. Or, put another way, these reports begin to reveal that the way UNC93B1 regulates one TLR is potentially very different from another.

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David et al. (2024) found that different *UNC93B1* variants had an obvious preference toward one TLR over the others, and which TLR was most impacted correlated with the patient's disease. They found that TLR7 and, to a lesser extent, TLR8 (the two single-strand RNA sensors) were hyperactive in response to stimulation in SLE, while the *UNC93B1* mutations in patients with chilblain lupus elevated TLR8 activity and had no impact on TLR7. Meanwhile, Rael et al. (2024) reported autosomal dominant *UNC93B1* mutations in four individuals with tumid lupus and one with juvenile arthritis that also preferentially increased TLR7 and 8 activity. The tumid lupus variant, T93I, was particularly notable because, in addition to producing TLR activity levels higher than the positive control, it also showed signs that it can facilitate baseline TLR signaling. Though this was only through the ERK pathway, which is one of several downstream networks acted on by these TLRs, it brings back the question of whether or not these mutations in *UNC93B1* only drive

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hypersensitivity to TLR ligands or if they blur the line between self and pathogen as well.

To understand what changes in TLR trafficking when these *UNC93B1* variants are expressed, both groups turned to SYNTENIN-1, a known interacting partner of UNC93B1 with a suspected role in TLR negative regulation. SYNTENIN-1 binds UNC93B1 on the cytoplasmic side of the ER and sorts cargo, including TLR7, into exosomes for sequestration and degradation in a process that terminates signaling (Majer et al., 2019). Though a reasonable place to look, only one of the variants across the seven families evaluated between the two studies presented evidence to implicate the interaction between UNC93B1 and SYNTENIN-1 as a way to explain elevated TLR activity. Curiously, the one that did alter UNC93B1 binding to SYNTENIN-1, R336C from the patient with juvenile arthritis, enhanced the interaction in a FLAG-tagged co-immunoprecipitation, which was a completely unexpected result.

So, how does a tighter interaction with a negative regulator enhance function? And, if the mechanisms for the other variants are SYNTENIN-1 independent, how do those mutations yield TLR7- and 8-mediated autoinflammation? To address the first question, Rael et al. (2024) showed that R336C is subject to a high rate of K63 ubiquitination and proposed that the tighter binding between UNC93B1 and SYNTENIN-1 somehow disrupts regulatory steps that lead to an accumulation of TLR7-UNC93B1 complexes. The other variant from this report was unchanged in its interaction with SYNTENIN-1 compared to WT, but because Rael et al. (2024) could not catch a break with a straightforward mechanism, T93I had to be a counterintuitive scenario as well. Instead of binding to UNC93B1-T93I tighter, the interaction between TLR7 and its mutated chaperone was appreciably weaker. To explain this, the authors pointed to a hydrogen bond that forms between UNC93B1 and TLR7 at T93, which might restrict the ability of TLR7 to bind its ligand. They suggest that TLR7s bound to UNC93B1-T93I are potentially better primed to signal upon activation.

For the remaining variants unaffected by SYNTENIN-1, the mechanisms, while incompletely described, were more intuitive. David et al. (2024) showed that the interaction between most of the UNC93B1 mutants they reported and TLR7/8 was higher than WT at baseline in THP1 monocytes and in a 293FT overexpression system. Although there were exceptions that underscore how complex and specific TLR regulation by UNC93B1 can be, there is enough here in these first reports to suggest a method to the madness.

Fortunately for all future efforts to understand the genetic drivers of TLR-mediated autoinflammation, Rael et al. (2024) built a functional map of *UNC93B1* with an alanine scan in knock-in murine macrophages that revealed which regions are important for each TLR. Moreover, the regulatory patterns observed in the screen accurately reflected the behavior of the patients described in both studies overall. As more genetic variation within the TLR regulatory network is discovered, predictive tools like this will prove their worth and impact everything from basic research to critical clinical decisions that immediately affect patients.

These reports confirmed that dominant missense mutations in *UNC93B1* can significantly turn up the dial on TLR activity to the point of disease, but they only scratch the surface when it comes to understanding what appears to be a highly nuanced trafficking network. Future work is poised to explore a more complete roster of UNC93B1 interacting partners and dig into the role UNC93B1 plays in regulating TLR expression and turnover after the ER. The inclusion of SYNTENIN-1 and suspicion around the hydrogen bond with TLR7 that is potentially disrupted by UNC93B1-T93I were clever mechanistic hypotheses that underscore the nuanced regulation of the system. To make things even more complicated, there is also the possibility that TLR trafficking is not uniform across cell types. For example, TLR3 activity was only mildly elevated by T93I and unaltered by the rest of the mutants tested. Would TLR3 gain of function be more prominent in neurons or other cell types in which there is an established connection between TLR3 and autoinflammation?

These important discoveries point us to a more complete understanding of UNC93B1 biology, but further detailed genetic, biochemical, and cell type–specific investigations will offer insights into why certain mutations preferentially impact one TLR over others and may help identify new drug targets and treatment options for patients struggling with these conditions.

References

Brinkmann, M.M., et al. 2007. *J. Cell Biol.* https://doi.org/10.1083/jcb.200612056

David, C., et al. 2024. *J. Exp. Med.* https://doi.org/10.1084/jem.20232066

Fukui, R., et al. 2011. *Immunity*. https://doi.org/10.1016/j.immuni.2011.05.010

Fukui, R., et al. 2009. *J. Exp. Med.* https://doi.org/10.1084/jem.20082316

Lee, B.L., et al. 2013. *Elife*. https://doi.org/10.7554/eLife.00291

Majer, O., et al. 2019. *Nature*. https://doi.org/10.1038/s41586-019-1612-6

Pelka, K., et al. 2018. *Immunity*. https://doi.org/10.1016/j.immuni.2018.04.011

Rael, V.E., et al. 2024. *J. Exp. Med.* https://doi.org/10.1084/jem.20232005