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INNATE IMMUNITY

TLR9 mutations reveal a new level of self tolerance

> expression by haematopoietic stem cells (HSCs) of a TLR9 mutant that does not depend on proteolysis for its activation results in autoinflammation

The activation of Toll-like receptor 9 (TLR9) by nucleic acids requires processing of the receptor by lysosomal proteases. This was thought to ensure that TLR9 responded to foreign nucleic acids in the endolysosomal compartment rather than to extracellular self DNA at the cell surface. Mouchess *et al.* now confirm this hypothesis by showing that expression by haematopoietic stem cells (HSCs) of a TLR9 mutant that does not depend on proteolysis for its activation results in autoinflammation.

Cleavage of the TLR9 ectodomain in the endolysosome is thought to allow a membrane-proximal conformational change in response to ligand binding that facilitates the recruitment of the adaptor molecule MYD88 and initiates TLR9 signalling. So, the authors reasoned that substitution of the atypical transmembrane domain of TLR9 by the more hydrophobic transmembrane domain of TLR3 may spare the need for this conformational change. Indeed, expression of such a TLR9 mutant ($TLR9^{TM\text{-}MUT}$) in TLR9-deficient myeloid cells resulted in MYD88 recruitment to TLR9 in response to stimulation with CpG oligodeoxynucleotides, even after inhibition of proteolytic processing. This processing-independent activation of TLR9 was also triggered by plate-bound CpG oligodeoxynucleotides, indicating that $TLR9^{TM\text{-}MUT}$, unlike wild-type TLR9, can be activated both in the endolysosomal compartment and on the cell surface. Interestingly, cell-surface expression of $TLR9^{TM\text{-}MUT}$ was higher than that of wild-type TLR9.

But what are the effects of this processing-independent activation of mutant TLR9? Transfer of $TLR9^{TM\text{-}MUT}$-expressing HSCs into lethally irradiated mice resulted in an inflammatory disorder, whereas the recipients of wild-type TLR9-expressing HSCs remained healthy. The chimeric mice with $TLR9^{TM\text{-}MUT}$-expressing HSCs displayed increased cytokine levels, elevated numbers of $CD11c^+$ cells and reduced erythrocyte and B cell numbers compared with control chimaeras. Their inflammatory phenotype was not mediated by B or T cells but depended on the presence of $CD11c^+$ cells and on the expression of MYD88. Moreover, genetic deletion of members of the tumour necrosis factor (TNF) superfamily (but not of the type I interferon receptor) could partially rescue the autoinflammatory phenotype in $TLR9^{TM\text{-}MUT}$ chimaeras. Thus, the authors suggest that expression of $TLR9^{TM\text{-}MUT}$ results in the activation of dendritic cells, possibly by the extracellular TLR9 ligands that are released during irradiation-induced tissue damage. This in turn may lead to the production of pro-inflammatory cytokines, such as TNF, and the depletion of erythrocytes and B cells.

Finally, substitution of five amino acids in the transmembrane region of TLR9 was found to be sufficient to overcome the regulation of TLR9 activation by proteolytic processing, and resulted in autoinflammatory disease. The contribution of regulated TLR9 activation to self tolerance may be further evaluated by screening patients with autoinflammatory disorders for mutations in the DNA encoding the TLR9 transmembrane region.

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ORIGINAL RESEARCH PAPER Mouchess, M. L. *et al.* Transmembrane mutations in Toll-like receptor 9 bypass the requirement for ectodomain proteolysis and induce fatal inflammation. *Immunity* **35**, 721–732 (2011)