Fighting mycobacteria through ISGylation

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Our bodies use interferon (IFN) signalling as a central pathway to limit the spread of pathogens, such as viruses, bacteria and parasites. After pathogen exposure, IFN production leads to the activation of immune cells—such as natural killer cells, macrophages and T lymphocytes—which mediate pathogen clearance. There are two main types of IFN signalling, type I and II. In type I signalling, IFNs α and β —produced mainly by leukocytes and fibroblasts, respectively—stimulate macrophages and natural killer (NK)

cells to initiate an antiviral response. In type II signalling, IFN γ from activated T cells and NK cells potentiate type I signalling and promote inflammation. During type I signalling IFN α and IFN β bind to their cognate receptors, which results in the induction of IFN-stimulated genes (ISGs). A key function of ISGs is to interfere with viral replication, hence the name interferon. One of the most strongly induced ISGs is ISG15, a small protein consisting of two ubiquitin folds connected with a hinge spacer, thus resembling di-ubiquitin [1]. It has been proposed that

ISGylation is antiviral in mice, but its effects on human virus infection or other functional roles have been a long-standing question in the field. A study by the Casanova group provides important new insights into the role of ISG15 in humans, showing it is essential in the defence against mycobacterial disease but dispensable for other types of infection [2].

Similarly to ubiquitin, ISG15 can be conjugated to other proteins and several hundred targets have been suggested from proteomic studies [3]. However, few targets have been carefully evaluated, among them JAK1, STAT1, ERK1/2, PLC γ 1, p63, PML-RAR α , UBC13, filamin B and several viral proteins. In analogy to the ubiquitin system, ISG15 conjugation is mediated by an enzymatic cascade consisting of an E1 activating enzyme Ube1L, an E2 conjugating enzyme UbcH8 and a HECT-domain containing E3 ligase HERC5 (Fig 1A). Notably, both ISG15 and the E1/E2/E3 cascade are induced by type I IFN signalling.

Knowledge of the biological functions of ISGylation comes mainly from the analysis of knockout mice for ISG15, Ube1L and from *in vitro* studies. ISG15 $^{-/-}$ mice are more prone to infection by certain viruses, such as Sindbis, influenza A/B and herpes simplex 1 [4]. Ube1L $^{-/-}$ mice are also sensitized towards Sindbis and influenza infections [5]. Furthermore, ISG15 can inhibit the budding of certain viruses and modify viral proteins, and some viruses have developed strategies to inhibit ISGylation, underscoring the function of ISG15 and ISGylation in the antiviral response [6]. However, other viruses—such as vesicular stomatitis and lymphocytic choriomeningitis virus—have similar effects on ISG15 $^{-/-}$ and wild-type mice [7], suggesting specialized functions of ISGylation after viral infection. In addition,

Fig 1 | ISGylation and its function in the antimycobacterial response. (A) The ISG15 conjugation system. ISG15 is activated by an E1 enzyme and conjugated to substrates (green) by the action of E2 and E3 enzymes. HERC5 (HERC6 in mice) is the main ISGylating E3. (B) Mycobacterial infection induces IFN α/β production, which stimulates ISG15 synthesis and secretion in granulocytes. Secreted ISG15 can then activate NK cells to produce IFN γ , which stimulates immune system cells. See text for details. (C) HERC5 has been described to bind to polysomes and to promote cotranslational ISGylation of newly synthesized proteins. EFP, oestrogen-responsive finger protein; HERC5/6, HECT and RLD domain containing E3 ubiquitin protein ligases 5/6; HHARI, human homologue of *Drosophila* ariadne; IFN $\alpha/\beta/\gamma$, interferon $\alpha/\beta/\gamma$; IFN α R, interferon α receptor; IL-12, interleukin 12; ISG15, interferon-stimulated gene 15; Lys, lysine; mRNA, messenger RNA; NK, natural killer; TLR, Toll-like receptor; UbcH8, ubiquitin conjugating enzyme H8; Ube1L, ubiquitin activating enzyme E1-like.

a closer look at the role of ISG15 in regulating human viruses complicates the picture further: ISG15 has been found to stimulate rather than inhibit hepatitis C virus production *in vitro*, probably by preventing the degradation of viral proteins through competition between ISGylation and ubiquitylation [8]. Considering the limited number of viral infections common to both mice and man, it has been difficult to extrapolate what the *in vivo* functions of ISGylation might be in humans.

The Bogunovic *et al* [2] study is a clear step forward in our understanding of ISG15 function in infection biology. By analysing patients with the rare paediatric syndrome, Mendelian susceptibility to mycobacterial disease (MSMD), they identified mutations in ISG15 that lead to its loss of expression and suggest that these mutations cause the disease. Importantly, these patients do not suffer from increased sensitivity to viral infections but show severe clinical symptoms when exposed to weakly pathogenic mycobacteria, such as *Mycobacterium bovis*.

As has been shown in ISG15^{-/-} and Ube1L^{-/-} mice, Bogunovic *et al* found that cells from MSMD patients lack ISG15 expression after IFN α/β stimulation, but other ISGs are induced normally, confirming that ISG15 is not essential to elicit an IFN response [2]. Furthermore, patient cell lines are not more susceptible to infection by viruses such as herpes simplex virus, Sindbis virus and vesicular stomatitis virus. Secretion of ISG15 by granulocytes from gelatinase and secretory granules is probably an important process in response to mycobacterial infection that cannot be triggered by, for example, bacterial lipopolysaccharides (Fig 1B). Monocytes and lymphocytes are known to secrete ISG15, and Casanova and colleagues show that even transfected HEK293T cells can do so—suggesting that ISG15 is both an intracellular and a secreted protein—independently of the cellular context. The main function of secreted ISG15 seems to be the triggering of IFN γ release, preferentially from NK cells, but also from T cells. Interestingly, IFN γ secretion is also stimulated by modified

ISG15 that can no longer be conjugated to target proteins. This indicates that immune cells either have an ISG15 receptor or that secreted ISG15, which is endocytosed, can induce a response without being conjugated to an intracellular target. Importantly, ISG15 secretion is lost in cells from MSMD patients and, consistently, MSMD leukocytes stimulated with mycobacteria produce greatly reduced amounts of IFN γ , which can be restored by providing recombinant ISG15. In addition, the study also demonstrates that ISG15^{-/-} mice are more susceptible to mycobacterial infection than their wild-type littermates.

Thus, the Bogunovic study identifies a common function of ISG15 in vertebrates: the ability to counteract mycobacterial infections by activating NK cells. The data also suggest that some viruses and bacteria must share pathogen-associated molecular patterns that resemble those of mycobacteria, thus initiating a similar response that involves IFN α/β , which is required for ISG15 induction. The initial activation of this mechanism by mycobacteria remains to be identified and seems to be complex, as treatment of macrophages with IFN α/β during mycobacterial infection has been shown to induce the loss of their mycobacteriostatic properties [9]; partly at odds with the conclusions from this study. These discrepancies notwithstanding, the new insights reveal an essential function for ISG15 in antimycobacterial signalling. Mycobacterial infections are hard to fight and thus the finding that ISG15 is a major effector between granulocytes and NK cells might help develop new treatment strategies. Other cellular mediators, such as the macrophage–T-cell pathway, are a crucial host defence against pathogenic (*M. tuberculosis*) and nonpathogenic mycobacteria (*M. bovis*), as well as salmonella, in other variants of MSMD. Hence, Casanova and colleagues speculate that the granulocyte–NK-cell pathway, which involves ISG15 and IFN γ , might constitute a more innate complement to the macrophage–T-cell pathway, which requires IL-12/IFN γ . However, they also report a synergistic effect of a combined

treatment of cells with ISG15 and IL-12, which rather argues that the granulocyte–NK-cell and the macrophage–T-cell systems act together. Furthermore, both granulocytes and macrophages express Toll-like receptors (TLRs) that recognize mycobacterial structures—such as TLR2 for the lipomannan of *M. tuberculosis*.

Finally, whether covalent modification of target proteins within cells by ISG15 is important during infection remains unclear. It is difficult to speculate what the main effects would be, as ISG15 modifies targets in diverse cellular pathways. Notably, the E3 ligase for ISG15 conjugation HERC5 has been shown to be associated physically with polysomes, leading to cotranslational ISGylation of newly synthesized proteins, which probably inhibits protein function in general (Fig 1C; [10]). Thus, ISG15 might have two roles in preparing cells to fight pathogens: intracellular proteome remodeling and initiating antimicrobial signalling pathways. It will be interesting to see if and how these functions intersect.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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