ANTIGEN PRESENTATION

Damage limitation: new role for immunoproteasomes

New research published in *Cell* indicates that, in addition to promoting an adaptive immune response through increased antigen presentation, the main function of the immunoproteasome might be to protect cells from the damaging side effects of an innate inflammatory response.

Cellular protein homeostasis is maintained in eukaryotic cells by the degradation of short-lived or defective proteins that are targeted to the 26S proteasome by polyubiquitylation. During an inflammatory response, type I or type II interferon (IFN) stimulation alters the subunit composition of the 26S proteasome to form the immunoproteasome, which is thought to more efficiently generate MHC class I epitopes for presentation to CD8⁺ T cells and the initiation of an adaptive immune response. However, various data indicate that the immunoproteasome might have a function other than antigen processing; for example, the absence of immunoproteasomes does not affect T cell priming in some models.

This study showed that IFN stimulation induces the transient accumulation of polyubiquitylated proteins, levels of which peaked 4–12 hours after IFN stimulation and declined 24–48 hours after stimulation, concomitant with immunoproteasome induction. IFN γ preferentially stimulated the synthesis of polyubiquitin chains that were linked through lysine residue 48 (K48), and this depended on the specific induction of the ubiquitin-conjugating enzyme UBE2L6. The transient increase in cellular levels of polyubiquitylated proteins was also explained by the IFN-induced transient dissociation of the 26S proteasome and therefore decreased cellular proteasome activity until the immunoproteasome was formed after 24 hours.

In addition, the immunoproteasome was shown to be more efficient at degrading polyubiquitylated substrates than the standard 26S proteasome. Immunoproteasomes had twofold higher substrate turnover than standard proteasomes in *in vitro* degradation assays. Both *in vitro* and *in vivo*, cells that were deficient for immunoproteasome subunits were unable to efficiently clear the polyubiquitylated proteins that accumulated in response to IFN, and this resulted in the accumulation of protein aggregates after IFN γ stimulation that failed to be cleared as in wild-type cells.

These results show that the immunoproteasome functions to clear the cell of polyubiquitylated proteins that form during inflammatory conditions and thereby to prevent the formation of disruptive protein aggregates. IFN-induced polyubiquitylation was shown

to involve the production of reactive oxygen species during inflammation that cause oxidant damage to nascent cellular proteins. The level of oxidized proteins correlated with the level of polyubiquitylated proteins, and both were higher in cells that lacked immunoproteasomes. Furthermore, the addition to IFN-stimulated cells of an antioxidant, which prevents protein oxidation, decreased the accumulation of polyubiquitylated proteins. Immunoproteasome-deficient cells had greater accumulation of oxidized proteins after IFN γ stimulation than wild-type cells. So, inflammation-induced oxidative stress triggers polyubiquitylation of damaged proteins (aided by IFN-mediated upregulation of K48-linked ubiquitylation activity) that leads to their accelerated degradation by IFN-induced immunoproteasomes. So, by maintaining protein homeostasis, immunoproteasomes also increase the peptide supply for MHC class I antigen presentation.

The accumulation of oxidized proteins after IFN γ treatment in cells that lack the immunoproteasome resulted in increased caspase activity and increased susceptibility to an apoptosis inducer. The increase in apoptosis as a result of immunoproteasome deficiency was evident from the increased clinical score and earlier onset of symptoms (indicative of increased neuronal death) in mice with experimental autoimmune encephalomyelitis, thus indicating the importance of immunoproteasomes for preserving cell viability during inflammation.

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ORIGINAL RESEARCH PAPER Seifert, U. *et al.* Immunoproteasomes preserve protein homeostasis upon interferon-induced oxidative stress. *Cell* **142**, 613–624 (2010)