Immunoproteasomes Are Important for Proteostasis in Immune Responses

The regulated degradation of ubiquitin (ub)-tagged proteins by the ubiquitin proteasome system (UPS) represents a major pathway not only for the maintenance of proteostasis, but also for supplying peptides for MHC class I antigen presentation. This immune function of the UPS can be improved by the immunoproteasome (IP), an isoenzyme of the 26S proteasome with altered and enhanced peptide hydrolyzing activity (Figures 1A and 1E) (Deol et al., 2007; Seifert et al., 2010; Huber et al., 2012).

In their Matters Arising (Nathan et al., 2013 [this issue of *Cell*]), Nathan et al. questioned several results of our original study regarding the role of IP in preserving proteostasis upon interferon (IFN)-induced oxidative stress (Seifert et al., 2010). We showed that IFN-induced radical production augments the formation of poly-ub-conjugated, oxidant-damaged nascent proteins (defective ribosomal products [DRiPs]). The formation of DRiPs is transiently higher than their degradation rate, resulting in a transient accumulation of poly-ub-conjugates (Figure 1B). In comparison to standard proteasomes (SP), IPs eliminate DRiPs more efficiently and thereby concomitantly facilitate the MHC class I peptide supply (Figures 1A and 1E).

Nathan et al. were not able to detect an IFN-γ-induced transient increase in poly-ub-conjugates or the accumulation of ub-rich aggregates in the absence of IP. They also did not detect any effect of IP deficiency on disease manifestation in experimental autoimmune encephalomyelitis (EAE) or accelerated degradation of poly-ub-conjugates by IP. The reasons for the discrepancies are not discussed. In the majority of their experiments, Nathan et al. applied different protocols, thereby limiting a direct comparison with our results. They further did not address oxidative stress in their experiments, and in addition, a number of technical issues arise.

To visualize IFN-induced accumulation of poly-ub-conjugates, which was also observed by other groups, it is essential to counteract deubiquitylation and degradation. Moreover, the kinetics and the increase in poly-ub-conjugate formation are strictly dependent on cell viability, the latter being affected by IFN-γ-signaling-induced apoptosis (see Figure 1C and Supplemental Information). The notion that conflicting data presented by Nathan et al. could simply reflect distinct experimental setups is reinforced by the fact that one of the coauthors had replicated our findings in our laboratory when using our stimulation and buffer conditions (Spinnenhirn, 2009).

The transient accumulation of ub-conjugates is, in part, due to the increased expression of UBE2L6 (Seifert et al., 2010), which is known to conjugate both ub and the ub-like modifier ISG15 (Buchwald et al., 2010). Nathan et al. argue that IFN-γ-induced UBE2L6 is preferentially involved in ISG15 modification; however, they fail to detect IFN-γ-induced ISG15 conjugates. Our IFN response experiments (Figure 1D) clearly demonstrate that ISG15 protein conjugates do accumulate in response to IFN but that ISGylation occurs much later (24 hr) than ubiquitylation (8 hr).

Nathan et al. also claim that *β5i/LMP7*$^{-/-}$ mice are not more susceptible to EAE than wild-type mice. However, they used the abdominal area for immunization, bearing the risk of severe side effects, and, most strikingly, investigated mice from independent colonies. Genetic differences in mouse colonies and microbiotic environments do affect the disease outcome in EAE. Likewise, heterozygous breeding needs to be performed to obtain related wild-type littermates as optimal controls in EAE studies (see Extended Experimental Procedures in Supplemental Information).

In agreement with our data (Figure 1E), Nathan et al. report an ~2-fold higher chymotrypsin-like peptide-hydrolyzing activity of IP, which is due to β5i/LMP7-induced structural changes. Of note, Groettrup and coworkers (Huber et al., 2012) cite our original study (Seifert et al., 2010) to support their conclusion that the β5i/LMP7 active sites are more accessible to substrates. Unexpectedly, Nathan et al. did not observe the 2-fold enhanced poly-ub-substrate turnover capacity of IP. In our in vitro degradation experiments, we used saturating substrate concentrations, thereby overruling the effects of substrate affinity, and visualized substrate turnover by immunoblotting. Nathan et al. used a substrate concentration (30 nM) far below their proposed Km values (140 and 180 nM), which does not allow the determination of differences in V_{max} values between SP and IP. Importantly, from their supplemental data, it is obvious that, in comparison to SP, ~2-fold less IP were applied for the in vitro degradation experiments.

Based on different experimental setups, including pulse-chase experiments, the turnover rate of IκB-α, and two mouse models of inflammation, we showed that IP are more efficient than SP in degrading poly-ub-conjugates (Seifert et al., 2010). In contrast, the data by Nathan et al. substantially limit a conclusion regarding the ability of IP to clear IFN-challenged cells from the accumulation of ub-rich inclusions because data on later time points in their time course are missing. The high poly-ub-substrate degradation capacity of IPs is important to prevent disease progression (Seifert et al., 2010). In support, pathological consequences due to IP functional impairment were meanwhile reported by a number of other groups (Agarwal et al., 2010; Arima et al., 2011; Hussong et al., 2011; Kitamura et al., 2011; Liu et al., 2012; Opitz et al., 2011; Zaiss et al., 2011).

SUPPLEMENTAL INFORMATION

Supplemental Information includes Extended Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.cell.2013.02.018.

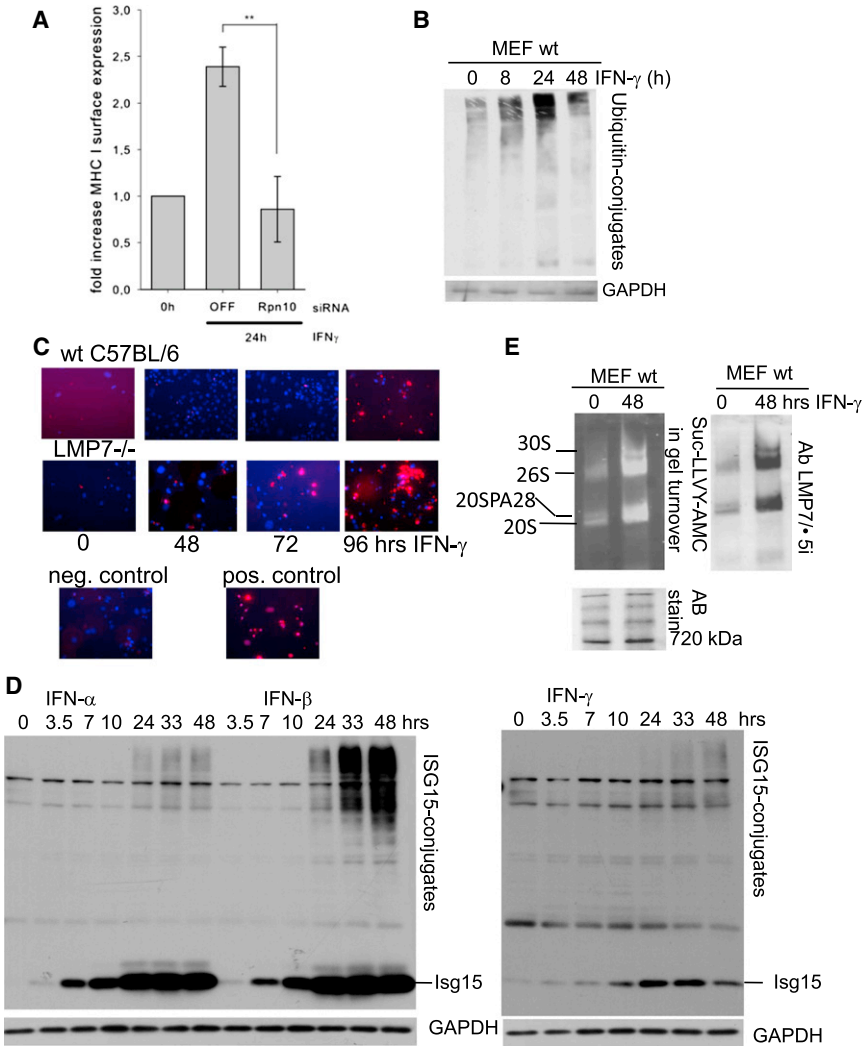

Figure 1. IFN-γ Signaling Results in Transient Accumulation of Poly-Ub-Conjugates by a Concerted Action of UPS Components followed by Increased Degradation Capacity of IP

(A) Total amounts of loaded MHC class I molecules at the cell surface correspond with UPS activity in cells. MHC class I molecules at the cell surface were measured by flow cytometry and staining with HLA-ABC antibodies (BD). Treatment of human HeLa cells with 100 U/ml human IFN-γ for 24 hr increased the MHC class I surface expression ~2-fold by increased peptide loading due to i26S proteasome peptide processing. Depletion of the ub adaptor protein Rpn10 of the i26S proteasome by siRNA resulted in a significant decrease of MHC class I surface expression in comparison to cells transfected with off-target siRNA.

(B) MEFs derived from C57BL/6 mice displayed a transient accumulation of poly-ub-conjugates in response to IFN-γ (100 U/ml) for the indicated time points, as shown by immunoblot using a ub antibody (DAKO). GAPDH served as loading control.

(C) Tunel assay of murine embryonic cardiomyocytes isolated from wild-type and *β5i/LMP7*$^{-/-}$ mice in response to IFN-γ (100 U/ml) at the indicated time points showed increased cell death in IP-deficient cells. Apoptosis in wild-type cardiomyocytes was observed at 96 hr IFN treatment and in *β5i/LMP7*$^{-/-}$ cardiomyocytes at 48 hr. All cells were grown on cover slides for 96 hr. Cells were stained using in situ cell death detection kit TMR red (Roche). Positive control: +DNAse (Roche) 400 U/ml; negative control: without terminal deoxynucleotidyl transferase (TdT).

(D) IFN-γ-mediated accumulation of ISG15 conjugates. Lysates of HeLa cells treated with IFN-α, IFN-β, and IFN-γ for the indicated time points were stained for ISG15 conjugates in immunoblots (ISG15-specific antibody laboratory stock; GAPDH loading control).

(E) Native PAGE substrate overlay (Suc-LLVY-AMC) of total lysates of MEFs untreated or in response to IFN-γ for 48 hr showed an ~2-fold increased chymotrypsin-like activity of IP (IFN-γ) compared to SP (untreated) (left). Native PAGE immunoblot stained IPs using LMP7-specific antibodies (laboratory stock) (right). The migration of different proteasome complexes is indicated; amido black (AB) stain of the blot as loading control (bottom).

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Frédéric Ebstein,[1,5] Antje Voigt,[1,2,5] Nicole Lange,[1] Annika Warnatsch,[1] Friederike Schröter,[3] Timour Prozorovski,[3] Ulrike Kuckelkorn,[1] Orhan Aktas,[3] Ulrike Seifert,[4] Peter-M. Kloetzel,[1,*] and Elke Krüger[1,*]

[1]Charité Universitätsmedizin Berlin, Charité CrossOver, Institut für Biochemie CCM, Charitéplatz 1, 10117 Berlin, Germany

[2]DZHK (German Centre for Cardiovascular Research), Partner Site Berlin, 10117 Berlin, Germany

[3]Department of Neurology, Medical Faculty, Heinrich-Heine-Universität, 40225 Düsseldorf, Germany

[4]Institute of Molecular and Clinical Immunology, Medical Faculty, Otto-von-Guericke-Universität, 39120 Magdeburg, Germany

[5]These authors contributed equally to this work

*Correspondence: p-m.kloetzel@charite.de (P-M.K.), elke.krueger@charite.de (E.K.)

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