Takin' Out the Trash for Brain Health!

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Immunoproteasome Deficiency Results in Age-Dependent Development of Epilepsy

Leister H, Krause FF, Gil B, Prus R, Prus I, Hellhund-Zingel A, Mitra M, Da Rosa Gerbatin R, Delanty N, Beausang A, Brett FM, Farrell MA, Cryan J, O'Brien DF, Henshall DC, Helmprobst F, Pagenstecher A, Steinhoff U, Visekruna A, Engel T. *Brain Commun*. 2024;6(1):fcae017. doi:10.1093/braincomms/fcae017. eCollection 2024. PMID: 38317856

The immunoproteasome is a central protease complex required for optimal antigen presentation. Immunoproteasome activity is also associated with facilitating the degradation of misfolded and oxidized proteins, which prevents cellular stress. While extensively studied during diseases with increasing evidence suggesting a role for the immunoproteasome during pathological conditions including neurodegenerative diseases, this enzyme complex is believed to be mainly not expressed in the healthy brain. In this study, we show an age-dependent increase in polyubiquitination in the brains of wild-type mice, accompanied by an induction of immunoproteasomes, which was most prominent in neurons and microglia. In contrast, mice completely lacking immunoproteasomes (triple-knockout mice) displayed a strong increase in polyubiquitinated proteins already in the young brain and developed spontaneous epileptic seizures, beginning at the age of 6 months. Injections of kainic acid led to high epilepsy-related mortality of aged triple-knockout mice, confirming increased pathological hyperexcitability states. Notably, the expression of the immunoproteasome was reduced in the brains of patients suffering from epilepsy. In addition, the aged triple-knockout mice showed increased anxiety, tau hyperphosphorylation and degeneration of Purkinje cell population with the resulting ataxic symptoms and locomotion alterations. Collectively, our study suggests a critical role for the immunoproteasome in the maintenance of a healthy brain during ageing.

Commentary

The buildup of damaged, misfolded, and potentially toxic cellular proteins is prevented by intracellular proteolytic pathways. Among these critical pathways is the ubiquitin-proteasome system that involves covalently tagging marred or superfluous proteins with multiple ubiquitin moieties for degradation and recycling by large intracellular macromolecular complexes called proteasomes. Present in all eukaryotic cells, proteasomes are constitutively active, barrel-shaped complexes composed of a catalytic core comprised of 4 stacked rings of 7 subunits. The catalytic activity is restricted to 3 subunits—β1, β2, and β5.[1]

In contrast to the constitutively active proteasome, immunoproteasome activation is induced by the inflammatory cytokine, interferon-gamma (INF-γ). Upon INF-γ stimulation, the constitutively expressed β1, β2, and β5 catalytic subunits are replaced with their inducible homologues β1i (encoded by Lmp2), β2i (Mecl-1), and β5i (Lmp7). The functional significance of these substitutions is that the inducible immunoproteasome is more efficient in producing major histocompatibility class I antigens and thus activation of cytotoxic CD8+ T cells during adaptive immune responses. In addition to the role of the immunoproteasome in immune cells, the immunoproteasome is engaged after oxidative challenge in neuronal cultures, after brain injury, and microglial immune signaling.[2] Immunoproteasome subunits are elevated in the hippocampus of aged rodents and humans. However, it remains unclear if these elevations are an adaptative protective response to age-related stresses, injuries, or actively contribute to neurologic diseases, such as seizures and epilepsy.

With this background information, Leister and colleagues[3] asked if the immunoproteasome plays a role in preserving brain health by first assessing stock wild-type C57BL/6 mice (WT) for age-dependent increases in immunoproteasome subunits and ubiquitinated proteins. Whereas immunoproteasome subunits LMP2 and LMP7 are detected in brain tissue, brain levels were significantly lower in brain compared to the spleen and thymus, 2 organs harboring large populations of immune cells. Notably, polyubiquitinated proteins were increased in the hippocampus of 1-year old WT mice compared to young 2-month-old mice. Immunohistology revealed more LMP7 staining in hippocampal pyramidal neurons in 1-year-old mice compared

to 2-month-old mice, but the immunostaining was not quantified. There was an insignificant trend for an age-related increase in hippocampal LMP7 protein. Turning to a triple gene knockout (TKO) of the 3 inducible subunits, Lmp2$^{-/-}$, Mecl-1$^{-/-}$, Lmp7$^{-/-}$, required for immunoproteasome formation, the authors found a nearly 2-fold increase in polyubiquitinated proteins in 8-month-old TKO hippocampus compared to age-matched WT hippocampus. The ubiquitinated protein accumulation was accompanied by hyperphosphorylation of the microtubule-associated protein tau (tau) by antibody AT8 that recognizes tau phosphorylation at residues Ser 202 and Thr 205. Closer inspection of Figure 1E reveals the preponderance of AT8 immunoreactivity in the nucleus of CA3 pyramidal neurons, which might be indicative of broader tau dysregulation in the absence of the immunoproteasome. Additional information on the phosphorylation status of tau was not provided as the analysis was limited to the phosphorylation status of Ser 202 and Thr 205.

Interestingly, this study found that the lack of immunoproteasome subunits resulted in the development of epilepsy in adult mice. The authors report an age-dependent increase in behavioral seizures in female TKO mice beginning at 6 months of age. Nearly 30% of female TKO mice show seizure activity by 20 months of age whereas single Lmp7$^{-/-}$ KOs and, as anticipated, WT mice remained seizure free at this advanced age. When 1-year-old TKO mice were challenged with a single systemic injection of kainic acid, the resultant seizure severity and frequency were both enhanced compared to Lmp7$^{-/-}$ or WT mice, indicating a depressed seizure threshold. All 12-month-old TKO mice challenged died within 1 hour of kainic acid administration, but none of the 2-month-old TKO mice died after the kainic acid challenge. The seizure severities were not compared between young and old TKO, confounding interpretation of the survival studies. Examination of male TKO and littermates sufficient for all 3 inducible genes would have enhanced the key observations and strengthened the conclusions of a link between the immunoproteasome and epilepsy. Several notable aspects of the experimental design enhanced rigor, including the use of TKO tissues to validate key reagents and blinded assessment of seizure phenotypes and behavioral assays.

Despite these straightforward and informative findings reported by Leister et al, the mechanism by which the elimination of the immunoproteasome generates a seizure-prone environment warrants further study. The strong correlation between the brain pathology consisting of ubiquitinated proteins and phosphorylated tau is suggestive of a potential mechanism but is not conclusive. A possible key molecule linking immunoproteasome dysfunction and seizures is the tau protein itself. Genetic elimination of tau in the Kcna1$^{-/-}$ knockout mouse of temporal lobe epilepsy[4] results in dampened neuronal excitability, reduced seizures, and increased survival. The protective response of tau reduction has also been demonstrated using a tau-lowering antisense oligonucleotide in a mouse model of Dravet syndrome.[5] Taken together, these findings support a deleterious role of tau in seizures and survival and identify reducing tau levels as a possible therapeutic strategy.

Given that the TKO mouse has deficiencies in antigen presentation,[6] one could speculate the neurologic phenotypes reported by Leister and colleagues are caused by inadequate antigen presentation and altered immune function during the aging process. It is worth noting that a previous study provided data supporting a role for the Lmp7 subunit in promoting neuronal hyperexcitability. Selective pharmacological inhibition of Lmp5 in acute rat hippocampal and entorhinal cortical slice cultures prevented or significantly delayed seizure-like neuronal excitability induced by 4-aminopyridine.[7] Notably, this slice culture design inherently limits systemic immune cell engagement, raising the intriguing possibility that subunits of the immunoproteasome might protect against seizures independent or in additional to immune function.

Altogether, this study provides a foundation for the future consideration of the immunoproteasome in modulating seizure threshold, which may be applicable to epilepsy in general and in particular for epilepsies characterized by a profound adaptive immune response. Future studies designed to further interrogate the role of proteasomes in epilepsy will be well served by using the TKO mouse coupled with the available selective general proteasome and immunoproteasome inhibitors. Further evaluation of this idea will require careful pharmacokinetic-pharmacodynamic studies of the timing and extend of proteasome inhibition in the brain in preclinical seizure models. Previous, well-designed studies reveal a therapeutic window to achieve beneficial immune modulation in experimental seizure models. Administration of nimelsulide or celecoxib, cyclooxygenase-2 (COX-2) inhibitors, before kainic acid worsened seizure intensity, increased mortality, and did not improve postseizure memory deficits. However, COX-2 inhibition after kainic acid improved memory performance.[8,9] The realization that inflammatory activation might be a net benefit in the preseizure environment, and later deleterious, represents a key challenge and opportunity for targeting inflammatory pathways in preclinical and clinical trials.

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