



MEETING ABSTRACT

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PW02-025 - Programme necrosis by CAPS-associated NLRP3

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Introduction

Cryopyrin-associated periodic syndrome (CAPS), clinically characterized by neutrophil-rich urticarial rash, is associated with missense mutations in *NLRP3*. *NLRP3* is a pattern recognition receptor in the cytoplasm of cells and structurally related to plant resistance proteins, which detect pathogen- or danger-associated signals, leading to programmed cell death and hyper response at the local site in plants. In mammals, activated *NLRP3* forms inflammasome, which orchestrates the early inflammatory process via IL-1 β activation, and also cause programmed necrotic cell death termed "pyronecrosis". However, the mechanistic details are largely unknown.

Objectives

To investigate the mechanism of *NLRP3*-mediated pyronecrosis and its in vivo relevance.

Methods

We have established a system in which pyronecrosis was induced by the expression of CAPS-associated gain-of-function mutant of *NLRP3*, using a tetracycline-inducible expression (Tet-on) system. We also induced *NLRP3*-mediated cell death in mouse air-pouch and harvested the cells and fluid.

Results

Mutant *NLRP3* expression without LPS pretreatment induced only necrotic cell death but not IL-1 β secretion in this system. Silencing ASC gene by shRNA prevented pyronecrosis, while silencing caspase-1 did not. When the cell lines expressing *NLRP3* mutants by Tet-on system were treated with cathesin B inhibitor, necrotic cell death and speckle patterns of ASC oligomerization were not observed. Interestingly, when these cells were treated with

Z-VAD-fmk, the speckle patterns of ASC were seen while they were still alive.

Upon oral administration of doxycycline, injection of LPS-pretreated *NLRP3*-mutant cells into mouse air-pouch showed necrotic cell death in addition to IL-1 β release, resulting in the significant increase in numbers of neutrophils in the pouch. Interestingly, non-pretreated mutant cells, which showed necrotic cell death without mature IL-1 β release, also induced neutrophil infiltration, though smaller in number relative to neutrophil infiltration induced by LPS-pretreated mutant cells.

Conclusion

Pyronecrosis is provoked by downstream of *NLRP3*-induced ASC oligomerization, but does not require caspase-1 or IL-1 β cleavage, and that cathesin B inhibitor and Z-VAD-fmk inhibit pyronecrosis before and after ASC oligomerization, respectively. This clarifies the differences of pyronecrosis from pyroptosis which is mediated by ASC oligomerization but do not require *NLRP3*. In vivo study showed that necrotic cell death by pyronecrosis in itself can cause and exacerbate the neutrophilic inflammatory response.

Disclosure of interest

None declared.

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