



MEETING ABSTRACT

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# PW02-029 - Single cell fluorescent immunoassay of CINCA/NOMID

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## Introduction

CINCA syndrome, also known as NOMID, is a rare auto-inflammatory disease caused by the *NLRP3* mutations. It has been known that conventional genetic analysis failed to detect disease-causing mutations in approximately 40% of patients. We have recently identified *NLRP3* somatic mosaicism on 70% of these "mutation-negative" patients in the international collaborative study (Tanaka N. and Izawa K. et al., *Arthritis Rheum*, 2011), and found no significant differences on systemic inflammation between heterozygous germline mutations and somatic mosaicism. This raises a question how a small number of *NLRP3*-mutated cells cause systemic inflammation as severely as 100% of germline mutations.

## Objectives

To solve the question, we analyzed cytokine production from the single cells, especially IL-1 $\beta$  which is the key molecule of NLRP3 inflammasome. There are the 2 forms of IL-1 $\beta$ , namely preform and mature forms, and only the latter is said to be secreted. Although the IL-1 $\beta$  in the single cell can be measured by intracellular cytokine staining, the relationship of the amount of intracellular IL-1 $\beta$  in the single cells and secreted IL-1 $\beta$  from them is still unknown. From these issues, it would be a better approach to measure the secreted IL-1 $\beta$  at a single cell level.

## Methods

In this work, we tried to establish a single cell fluorescent immunoassay system to measure IL-1 $\beta$  secretion from monocytes of CINCA/NOMID patients at a single cell level using a soft lithographic method called microengraving.

## Results

In the healthy control, very small number of cells secreted low amount of IL-1 $\beta$  by the LPS stimulation. In contrast, significant number of cells secreted high amount of IL-1 $\beta$  by the LPS stimulation in CINCA/NOMID patients with heterozygous germline mutations. We were also able to observe a large number of IL-1 $\beta$  secreting cells from patients with somatic mosaic mutations by LPS stimulation alone.

To delineate whether only the cells with a mutation on *NLRP3* are responsible for IL-1 $\beta$  secretion in the mosaics, we pick up the single cells from the microwells positive on IL-1 $\beta$  secretion by micromanipulator. By performing genetic analysis on them, we are now trying to determine whether only the mutated cells secrete IL-1 $\beta$  or the small fraction of *NLRP3*-mutated cells causes *in vivo* bystander activation of the wild type monocytes in the somatic mosaic CINCA/NOMID patients.

In addition, this method could be offered to diagnose the somatic mosaicism of CINCA/NOMID easily on the basis of single cell functional analysis, which would complement the DNA sequencing based method (Izawa K. and Hijikata A. et al., *DNA research*, 2012) that might miss some rare CINCA/NOMID cases caused by other than *NLRP3* coding region mutations.

## Disclosure of interest

None declared.

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