Use of humanized NPY-mRFP Rat Basophilic Leukemia (RBL) IgE reporter for diagnosis of *Echinococcus granulosus* infection in the human accidental host

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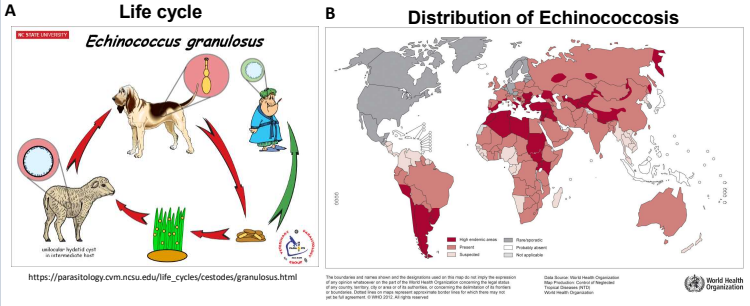
Abstract

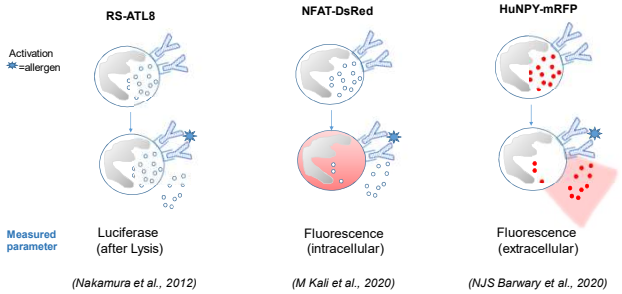
Echinococcus granulosus is the causative agent of cystic echinococcosis in humans infected as accidental hosts. Diagnosis is usually achieved using computerized imaging technologies, complemented by serological analyses. However, the latter have serious limitations in terms of specificity and, to a lesser extent, sensitivity. Even though *Echinococcus* infection induces a strong IgE response in infected hosts including human, this isotype is currently underexploited by current serological diagnostic techniques, which are mostly based on detection of parasite-specific IgG. Here, we show proof-of-principle that humanized IgE reporter systems can be used advantageously for diagnosis of cystic echinococcosis. We introduce our new RBL NPY-mRFP reporter system, which requires neither expensive substrates nor overnight incubation for detection of activation. In this reporter system, NPY-mRFP is pre-formed in granules, and activation can be conveniently assessed using fluorescence measurements as soon as 45-60 minutes after activation. Our data obtained using raw cyst fluid as antigen demonstrate the high discriminating power of IgE-based reporter systems for cystic echinococcosis diagnosis. We are currently employing several immunological and bioinformatic techniques, together with recombinant expression and protein purification, to identify known and novel *Echinococcus granulosus* allergens in cyst fluid and assess their suitability as diagnostic antigens using our reporter systems.

Echinococcus Pathogenesis and Diagnosis



Functional demonstration of allergenicity

Humanised IgE Reporter Systems: Rat basophilic leukemia cells stably transfected with FcεRI (alpha chain) and a reporter gene (luciferase or fluorescent protein)

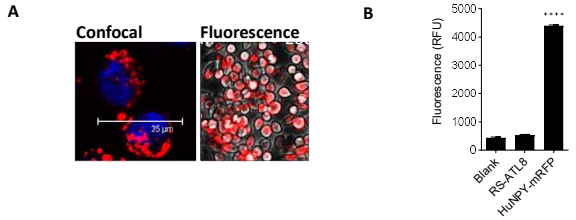


Objectives

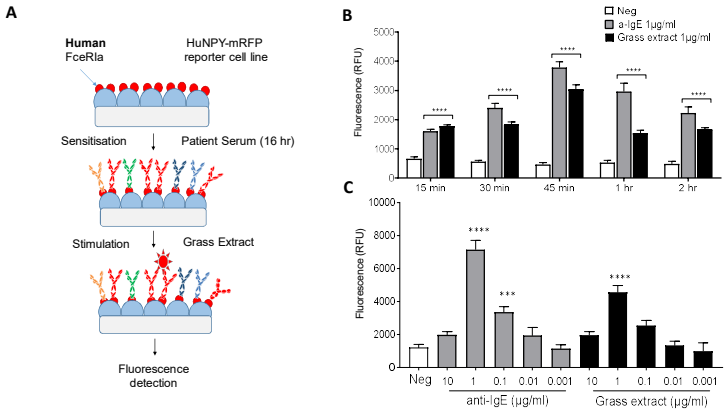
- Characterization of HuNPY-mRFP reporter system, a new diagnostic system to detect Echinococcus infection!
- Cloning and testing of Echinococcus antigens/allergens allergenicity in humanised IgE reporter system

Results

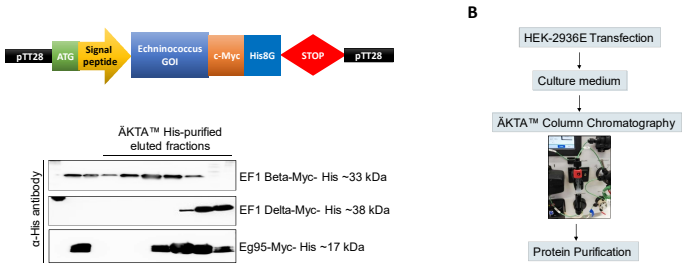
HuNPY-mRFP reporter system: Validation of mRFP expression in unstimulated cells



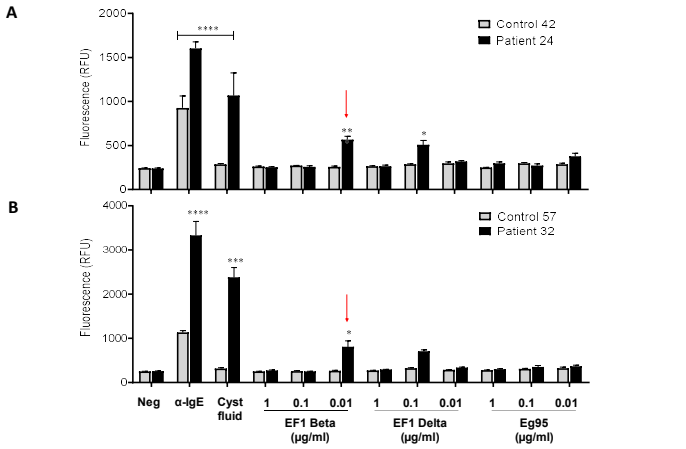
HuNPY-mRFP reporter system: Optimal timepoint and concentration of allergen stimulation



***Echinococcus granulosus* allergens expression and protein purification**



Stimulation of HuNPY-mRFP reporter cell line with *Echinococcus* EF1 Beta, EF1 Delta or Eg95



Outlook

- Further characterization of HuNPY-mRFP cells by FACS and Microscopy studies
- Cloning and expression of recombinant Echinococcus allergen candidates
- Screening of more Echinococcus allergens using several patient sera in IgE reporter assay