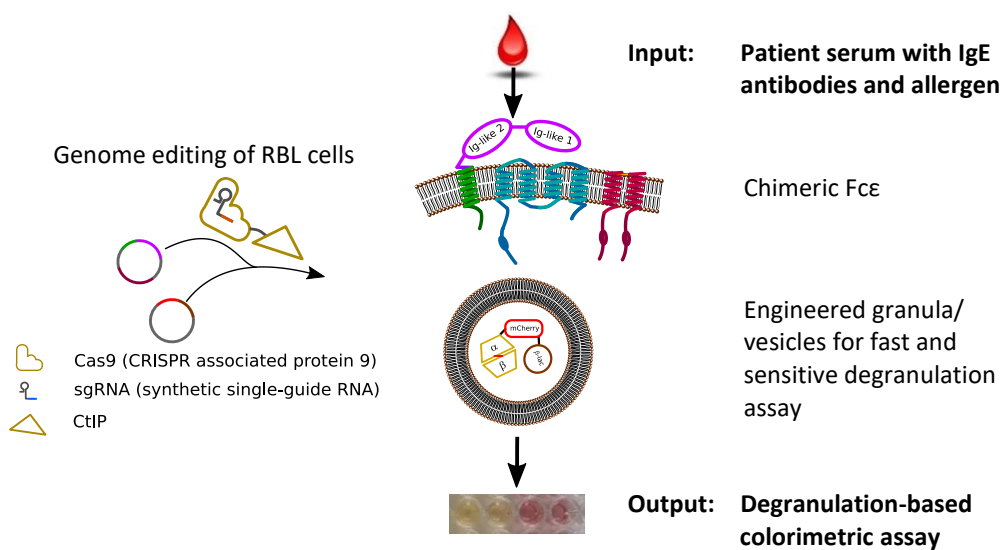


Development of a cell-based allergy test

Description

Allergies are on the rise, demanding potent diagnostic tools for screening of allergen-specific IgE antibodies in human blood samples. While skin-prick tests and serum IgE analysis are routinely performed, positive tests often poorly correlate with clinical symptoms. Consequently, functional cell-based assays are desperately needed. The rat basophil leukemia (RBL) 2H3 cell line constitutes an interesting *in vitro* system to study IgE-dependent degranulation eliminating the need to isolate mast cells from blood and tissue samples (1). Because RBL and mast cells have the same FcεRI receptor on their surface and release mediators in the same manner, RBL cells can be used to observe the acute response (2). However, due to their rodent origin this Fcεα chain is unable to bind human IgE. The aim of this project is to develop a non-invasive and fast allergen screening system. To achieve the activation of signalling cascade upon allergen-mediated clustering of human IgE antibodies, a stable RBL cell line with a chimeric FcεRI receptor will be generated using a modified CRISPR/Cas9 system (3,4). For an easy and sensitive read-out of IgE-triggered degranulation events, a fusion protein of β-hexosaminidase subunit α (Hexa), mCherry, and β-lactamase was created.



Design of Humanizing FcεRI – Adapting RBL 2H3 cells for binding human IgE while maintain signalling will be achieved by creating a chimeric FcεRI receptor retaining rat FcεRIβ chain (blue) as well as the rat anchor sequence of the FcεRIα chain (green) but replacing the IgE-binding domain (purple) as well as the FcεRIγ chain (dark red) with the human sequences. For easier and sensitive screening of degranulation events, granula will be loaded with a Hexa-mCherry-β-lactamase fusion protein.

Spectrum of Methods

- Genome editing
- Fluorescence-Activated Cell Sorting (FACS)
- Fluorescence microscopy
- Cell-based ELISA and degranulation assays

Literature

- (1) Falcone, et al., Immunol. Rev. 2018. DOI: 10.1111/imr.12628
- (2) Varricchi, et al., Immunol. Rev. 2018. DOI: 10.1111/imr.12627
- (3) Zhang, et al., Genome Biol. 2017. DOI: 10.1186/s13059-017-1164-8
- (4) Charpentier, et al., Nat. Commun. 2018. DOI: 10.1038/s41467-018-03475-7

Applications

- Medical diagnostic
- Allergen screening System

Keywords

- Degranulation
- Basophil
- RBL-2H3
- Allergy
- Immunology
- Mast cell

Interest in cooperation

- Research-based collaboration
- Contract research
- Industry-sponsored research

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