Binding, Internalisation and Intracellular Routing of Monovalent and Bivalent SNAP-Tag Fusion Antibodies in CD64+ M1 and M2 Macrophages.

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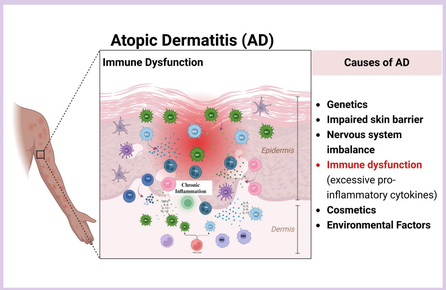
Introduction

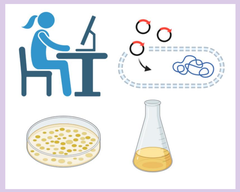
- Macrophages are the principal inflammatory cells, the most abundant and major contributors to chronicity.
- M1 and M2 overexpress high-affinity FCγR1 (CD64) receptors and an increased expression of human CD64 has been reported in several chronic diseases such as atopic dermatitis (Kiekens *et al.*,2000).



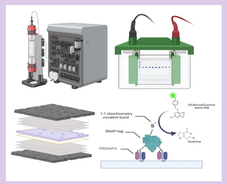
- We **hypothesized** that targeting only activated M1 and M2 macrophages through CD64 antibody recognition could generate curative treatment for CIDs.
- **Aim**: To assess how two different macrophage subpopulations bind, internalize, and engage in intracellular activities of mono- and Bivalent antibodies with and without KDEL peptide

Methods

1. Generation of SNAP-Tag fusion proteins

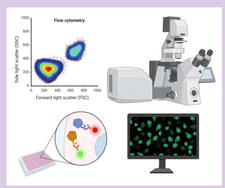


2. Transfection and protein expression



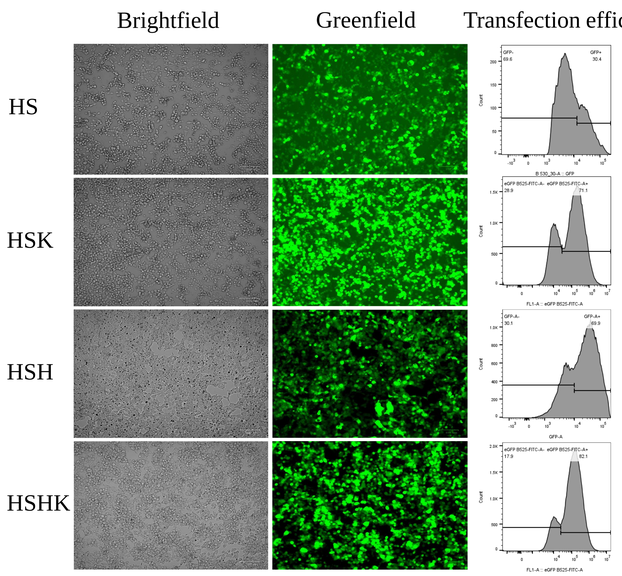
3. Protein purification and conjugation

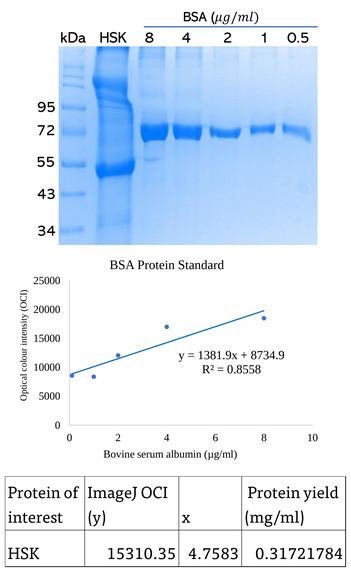
4. *In vitro* testing in HL60/U937 cell lines
   Ex vivo testing in PMBCs



5. Binding and internalization analysis (flow cytometry and confocal imaging)

Results

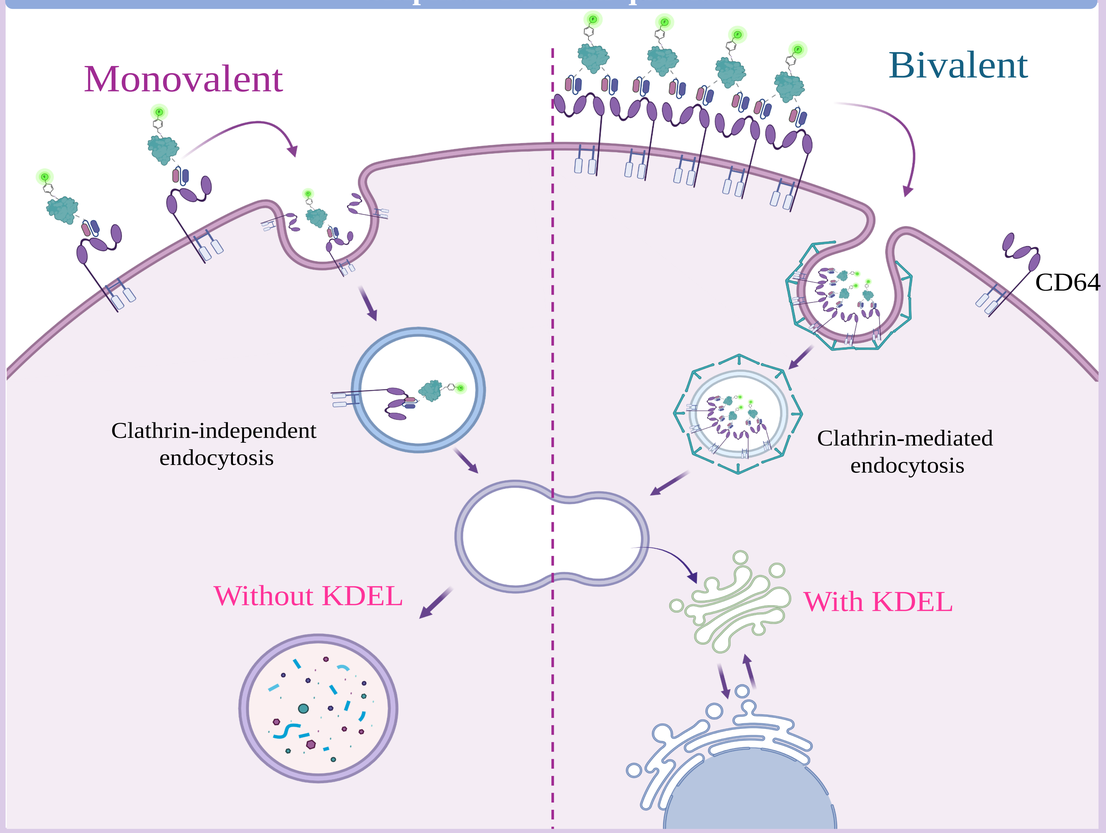




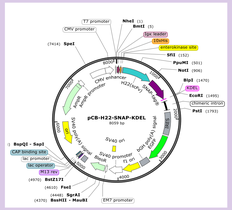
Protein of interest	ImageJ OCI (y)	x	Protein yield (mg/ml)
HSK	15310.35	4.7583	0.31721784

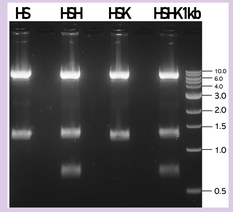
- All constructs were successfully transfected and expressed in HEK293T cells. The transfection efficiency was analyzed by flow cytometry.
- H22-SNAP-KDEL (HSK) was purified first from collected 500ml cell culture supernatant and the total yield was 0.3mg/ml)

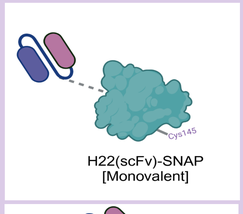
Graphical research questions

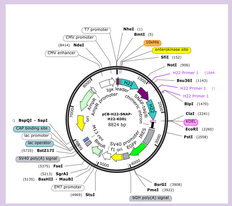


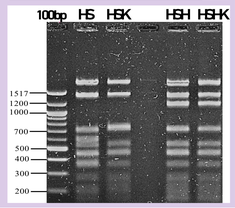
Molecular cloning

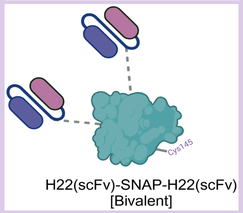












- Monovalent and bivalent single chain fragment variable of humanized IgG antibodies were successfully cloned and synthesized.
- Extracted pDNA was mapped to distinguish between mono- and bivalent with and without KDEL

Current findings

- Antibodies with KDEL showed higher protein expression both in HEK293T and HEK293 Lenti-x cell than those without KDEL.
- Purification of bivalent constructs was attempted however; the yield was very low and further purification is needed

Upcoming Experiments

- Purification and conjugation of all proteins with Benzylguanine (BG) Alexa Fluor 488
- Analyze binding of labeled proteins in HL60 and U937, a monocytic human myeloid leukemia cell line expressing CD64
- Evaluate the rate of internalization between mono- and bivalent antibody complex
- Analyze the intracellular routing in *ex vivo* differentiated M1 and M2 macrophages isolated from PBMCs.
- Evaluate the differences in localization of antibodies with and without KDEL peptide
- Lastly, evaluate the intracellular routine between M1 and M2 subpopulation

Future perspective

- The current work will provide insight into effective strategies for developing next generation Immunotherapeutics for treating chronic inflammatory diseases (CIDs).
- Depending on the outcome of intracellular routing, antibody-drug conjugates (ADCs) can be designed to take advantage of lysosomal degradation and human apoptosis-inducing enzymes can be attached for the ER routing directed by KDEL signal.

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