Linear peptides as IgG-epitope mimetic for allergic immunotherapy against birch pollen

Alessandro Streuli[1], Lara Šošić[2], Marta Paolucci[2], Agathe Duda[2], Klaus Eyer[3], Pål Johansen[2], Christian Steuer[1]

[1]Institute of Pharmaceutical Sciences, ETH Zurich; [2]Department of Dermatology, University Hospital Zurich ; [3]Department of Biomedicine, Aarhus University

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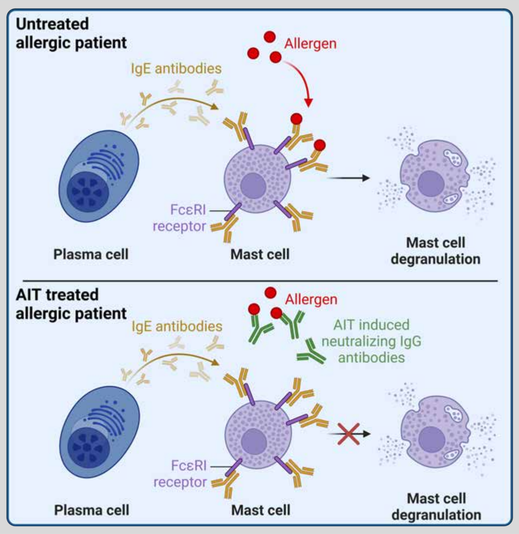
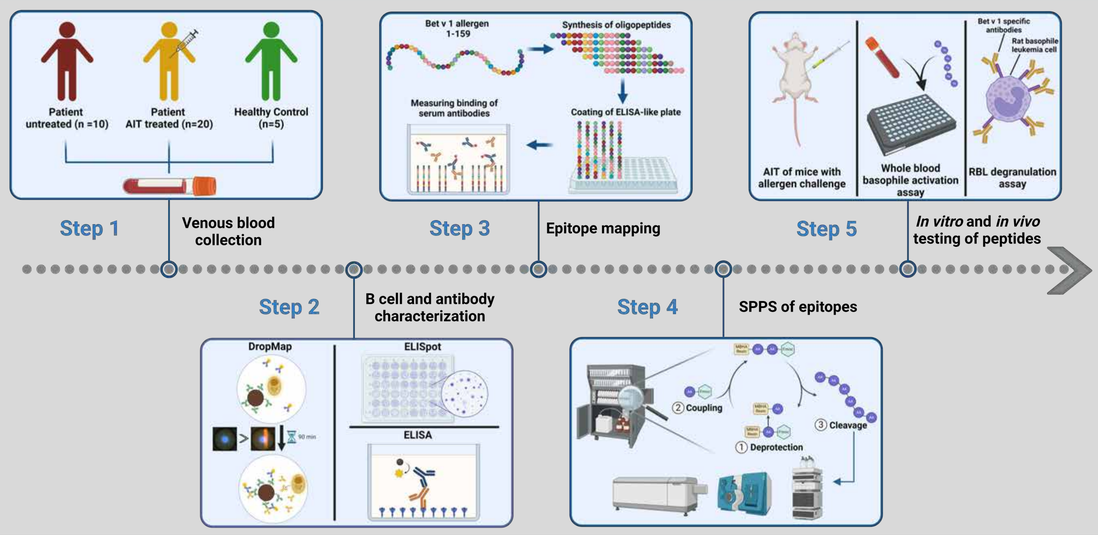
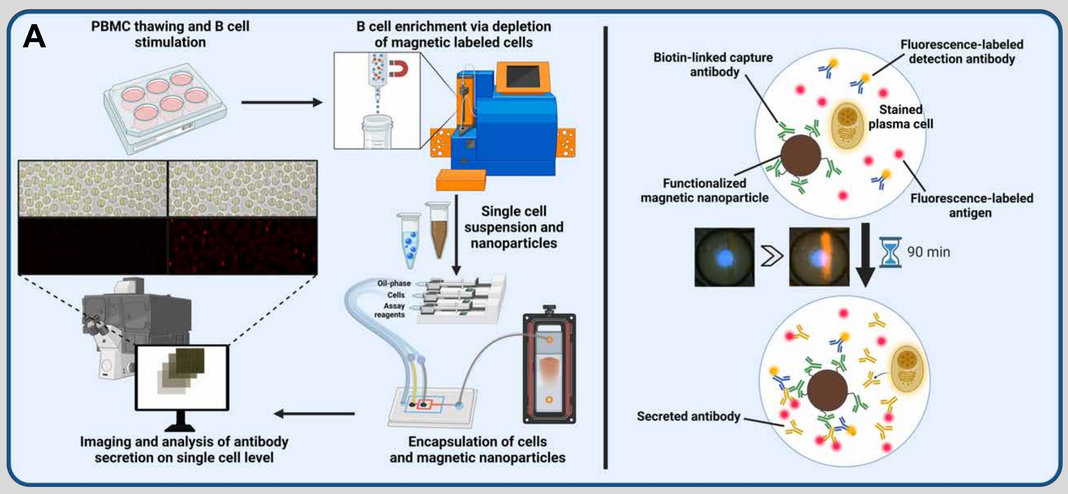
1 Introduction

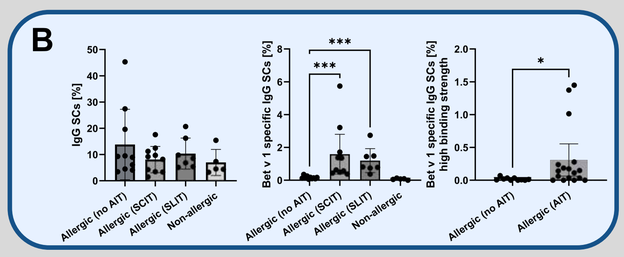
Allergies present an increasing health burden affecting nearly one billion individuals with allergen immunotherapy (AIT) as the sole causative treatment via stimulation of allergen-specific IgG antibodies[1,2]. Although these antibodies are acknowledged for their protective potential, elevated levels in AIT-treated patients do not consistently represent the success of treatment, indicating further critical factors[3]. In this study, the antibody repertoire of treated and non-treated allergic patients was characterized serologically and on a single-cell level. IgG antibodies found in sera of patients were tested for their binding capacity and strength towards Bet v 1. Four main IgG binding epitopes were identified using linear epitope mapping. The insights were used to produce six hypoallergenic peptides tested *in vitro* and *in vivo*[4].





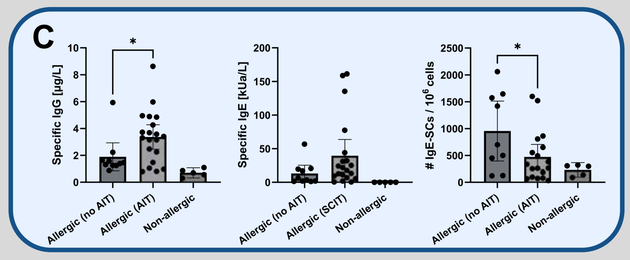
2 Antibody and B cell characterization





A: Workflow of used DropMap[5] assay to characterize memory B cells of patients.

B: DropMap results displayed more Bet v 1 specific IgG-secreting cells (SC) and particular SCs with high binding strength in both treatment groups while having constant SC over all groups.



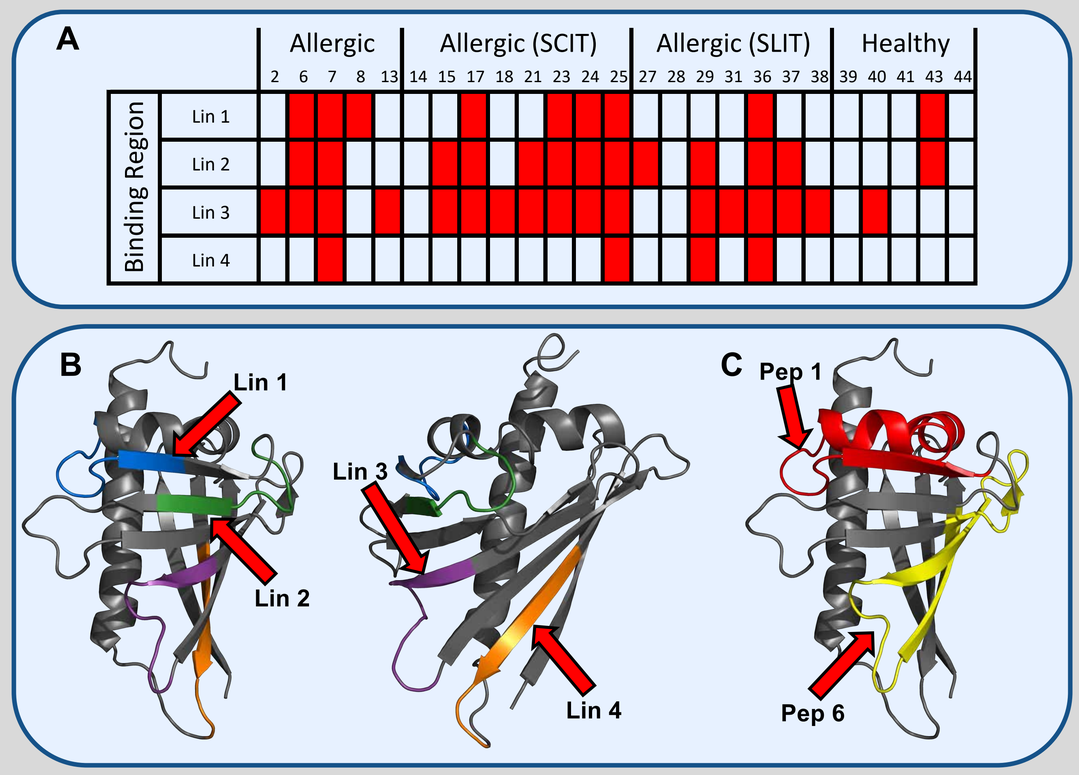
C: ImmunoCAP measurements showed higher Bet v 1 specific IgG and IgE levels in allergic patients with and without treatment. ELISpot results showed a similar trend in the B cell memory.

3 Epitope mapping

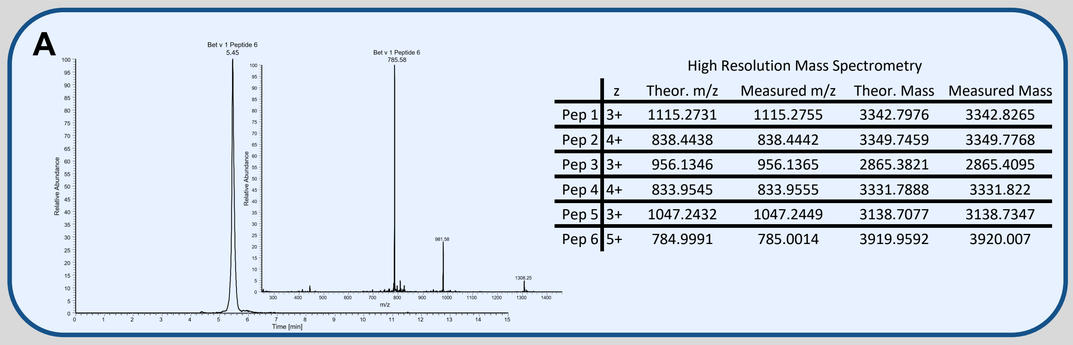
A: Binding of IgG antibodies in patients either treated via subcutaneous AIT (SCIT) or sublingual AIT (SLIT) towards linear Bet v 1 epitope indicated four novel IgG binding sites (Lin 1-4).

B: Visualisation of identified binding regions (PDB = 1BV1). Lin 1 is indicated in blue, Lin 2 in green, Lin 3 in purple, and Lin 4 in orange.

C: Visualisation of 2 out of 6 defined linear epitope mimetics (PDB = 1BV1).



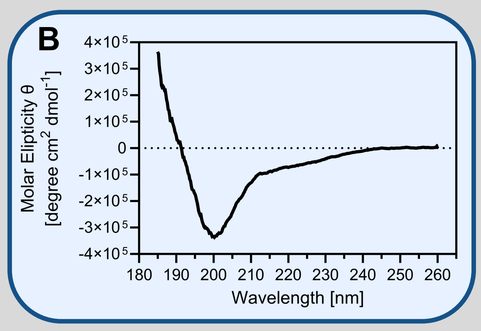
4 Synthesis of defined epitopes



High Resolution Mass Spectrometry

	z	Theor. m/z	Measured m/z	Theor. Mass	Measured Mass
Pep 1	3+	1115.2731	1115.2755	3342.7976	3342.8265
Pep 2	4+	838.4438	838.4442	3349.7459	3349.7768
Pep 3	3+	956.1346	956.1365	2865.3821	2865.4095
Pep 4	4+	833.9545	833.9555	3331.7888	3331.822
Pep 5	3+	1047.2432	1047.2449	3138.7077	3138.7347
Pep 6	5+	784.9991	785.0014	3919.9592	3920.007

A: Mass accuracy of successfully synthesised linear peptides was performed via LC-MS and high-resolution mass spectrometry. Representative LC-MS results for peptide 6 are shown. The table depicts the high resolution MS results for all peptides. Purity was determined to be >95% via LC-UV, and peptide sequence identity was validated via LC-MS/MS.



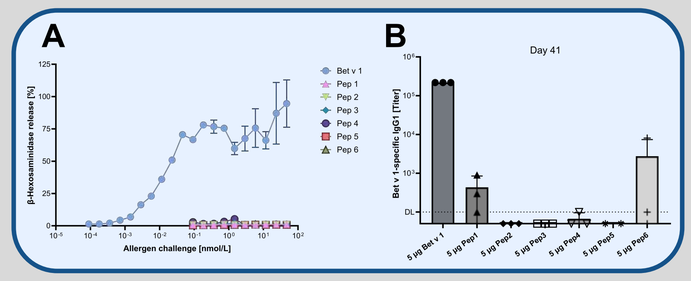
B: CD spectra of peptide 6, 50 µM in PBS (pH 7.4) with NaF, at 25 °C. Results were evaluated using the BeStSel web server and the structure was determined as predominantly uncoiled with smaller amounts of antiparallel and turn parts[6].

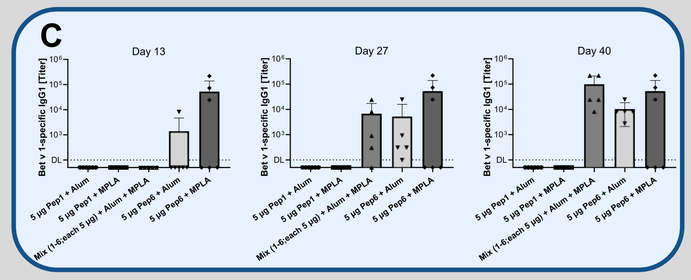
5 *In vitro* and *in vivo* testing

A: RBL degranulation assay showed that Bet v 1 protein, but none of the six peptides triggered β-hexosaminidase release.

B: Mice were immunised three times with each peptide and alum as adjuvant. Native Bet v 1 protein was used as positive control. Immune responses were detected for peptides 1 and 6.

C: Titer of mice immunised with peptide 1, peptide 6, or a mix of all peptides with either alum or MPLA as adjuvant. Peptide 1 showed no Bet v 1 specific IgG 1 titer. Immunisation using peptide 6 induced Bet v 1 specific IgG 1 in all mice.





6 Conclusion and Outlook

We characterized the antibody and the memory B cell repertoire of 30 hay fever patients via ImmunoCAP, ELISpot, and on a single-cell level via DropMap. Further, the serum of patients was used to identify four novel IgG bindings regions on Bet v 1 via epitope mapping. Six corresponding linear peptides were synthesised as epitope mimetics via SPPS. Degranulation assays showed no allergenicity induced by the six peptides. Peptides 1 and 6 showed induction of Bet v 1 specific IgG 1 antibodies in the first round of *in vivo* testing which could be confirmed in the second round for peptide 6. The potency of desensitisation via AIT of peptide 6 still needs to be tested.

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Contacts

ETH Zurich, Pharmaceutical Analytics, Institute of Pharmaceutical Sciences
Vladimir-Prelog-Weg 1-5/10, CH-8093, Zürich
AS: alessandro.streuli@pharma.ethz.ch, Tel.: +41 44 633 73 28
CS: christian.steuer@pharma.ethz.ch, Tel: +41 44 633 74 19