Title: Discovery of Monoclonal IgE Autoantibodies from Atopic Dermatitis Patients

Authors:

Derek Croote[1]
Joyce Jia Wen Wong[1]
Venu Aruva[1]
Paige Creeks[1]
Fariza Mishaal S Badloe <Fariza.Badloe@vub.be> [2]
Jessica Grossman[1]
G. Roger Thomas[1]
Henry B Lowman[1]
Jan Gutermuth <jan.gutermuth@uzbrussel.be> [2]
Inge Kortekaas Krohn <Inge.Kortekaas@vub.be> [2]

Affiliations:
[1] IgGenix, South San Francisco, CA, USA
[2] Vrije Universiteit Brussel (VUB), Universitair Ziekenhuis Brussel (UZ Brussel)
Department of Dermatology, Skin Immunology & Immune Tolerance (SKIN) Research Group
Brussels, Belgium

Conflicts of interest: DC, JJWW, VA, PC, J Grossman, GRT, and HBL are employees of and/or stakeholders in IgGenix. J Gutermuth was speaker and/or consultant for AbbVie, Almirall, BMS, Celgene, Eli Lilly, Eucerin, Janssen, LEO Pharma, L'Oreal, La Roche Posay, Nestle, Pierre Fabre, Regeneron/Sanofi, and Thermo Fisher. IKK was holder of the Fonds Wetenschappelijk Onderzoek (FWO) Post-Doctoral mandate (12W2219N), and an unrestricted Sanofi Genzyme Type 2 Innovation Grant, 2018 (0000000122).

Background

Atopic Dermatitis (AD) is one of the most prevalent inflammatory skin diseases worldwide and is characterized by eczematous rash and severe pruritus. Autoreactive IgE antibodies occur with higher likelihood and titer in AD patients compared to non-AD patients; however, the role of these autoreactive IgE antibodies in the pathogenesis of AD is unclear. Consequently, we sought to understand how AD patient characteristics, including disease severity and total serum IgE titer, correlate with the frequency and specificity of IgE autoantibodies from circulating B cells.

Methods

AD patients were selected for IgE antibody discovery based on high serum IgE titer and/or positivity in an autoreactive IgE immunoassay against keratinocyte antigens. IgE antibodies were discovered from whole blood of AD patients in an unbiased manner using the IgGenix single-cell RNA-sequencing discovery platform. Antigens to discovered antibodies were identified by multimodal screening consisting of both specific and unbiased approaches. The binding affinity of antigens to antibodies was measured by surface plasmon resonance on a Carterra LSA instrument.

Results

Keratinocyte-expressed proteins Eukaryotic Elongation Factor 2 and Galectin-7 were established as positive controls that enabled optimization of ELISA, western blot, and immunoprecipitation followed by mass spectrometry target discovery approaches. Most discovered monoclonal IgE antibodies originated from IgE plasmablasts, as opposed to IgE memory B cells, in agreement with the positive correlation between number of IgE antibodies discovered and total serum IgE titer across patients. Binding of discovered IgE autoantibodies to protein targets was shown using lysates as well as human proteome microarrays. The binding of autoantibodies to autoantigens was confirmed by measurement of binding affinity to purified, recombinant antigens.

Conclusion

We demonstrated that the IgGenix scRNA-seq platform, validated in food allergies, can be used to discover and characterize IgE antibodies in AD. Unique insight gained by target discovery using patient IgE autoantibodies opens the possibilities for novel therapeutic intervention in a highly heterogenous autoimmune disease as well as for better understanding the relationship between patient autoantibody profiles and disease subtype or endotype.