Dissecting the structural determinants of a class of anti-TG2 antibodies by an integrated computational and experimental approach

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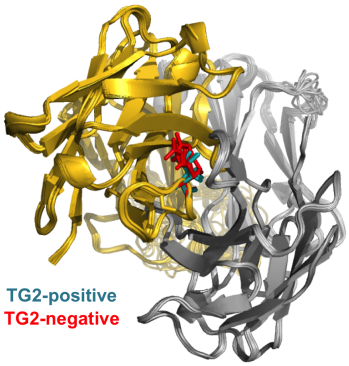
Motivation
The enzyme transglutaminase 2 (TG2) is the main autoantigen in celiac disease (CD) [1]. The presence in the serum of anti-TG2 antibodies (Abs) is a sensitive and specific biomarker for CD. TG2 epitope 1 is the most frequently targeted of four common epitopes identified from a panel of TG2-specific monoclonal antibodies derived from single plasma cells of celiac lesions. However, the structural determinants of the positivity of celiac antibodies to TG2 epitope 1 have not been elucidated yet. Herein, based on the interplay between experimental and computational analyses, we present the successful design of specific anti-TG2 Abs.
Methods
Assignment of the hypervariable loops canonical structure has been performed by DIGIT [2]. Design of H3 sequences has been made based on Hidden Markov Models (HMMs) by HMMER. Ab 3D models have been built with Modeller using the structure of the 679-14-E06 Ab as a template (PDB ID: 4ZD3, resolution 2.4 Å) [3].
Results
A set of over 100 antibody sequences was initially isolated by phage display and shown by ELISA essays to bind TG2. In this set of antibodies, different VH chains, all belonging to the IGHV5 gene family, have been selected on pairing with a limited set of VL chains from celiac Abs. To the aim of highlighting the structural determinants of these anti-TG2 antibodies, we analysed them in terms of sequence identity and especially of length and conformation of the six hypervariable loops, representing the antibody recognition site. The conformation of five of the six hypervariable loops are indeed known to exhibit a finite repertoire of conformations, known as canonical structures [4]. Since we observed a limited variability in the length, canonical structure and composition of loops L1-L3 and H1-H2, we especially focused on the length and composition of loop H3, usually playing a key role in the antigen recognition. We could thus identify a "consensus" H3 sequence, present in 40% of the analysed sequences, which is 12 residues long and features four "positivity conditions" (i.e. specific amino acids at specific loop positions). An ideal TG2-positive H3 sequence for experimental testing was obtained as a plurality-rule consensus sequence based on the HMM of the above set of sequences. Furthermore,

random Ab H3 sequences were generated by phage display in a scaffold IGHV5 sequence upon fixing the above positivity conditions and tested for positivity to the TG2 binding. Binding essays demonstrated the ability of the above criteria to produce TG2-directed Abs. We also verified that the positivity conditions were never matched in an extended repertoire of about 6500 VH antibody sequences belonging to the IGHV5 family from three healthy donators [5], assumed as a negative set. As an experimental 3D structure is available for a celiac antibody matching the conditions of our positive consensus sequence [3], we could model with high reliability the newly obtained Abs experimentally shown to be positive or negative to the TG2 recognition. Based on the analysis of these 3D models we could identify a fifth positivity criterion (see Figure) that is now under experimental investigation. The combined computational and experimental approach we adopted here led to an unprecedented successful design of TG2-directed Abs, based on a detailed insight of the structural determinants required for Ab binding to TG2 epitope 1.
Finally, preliminary binding affinity measures indicate that our strategy for the design of an ideal positive sequence produced a sequence at enhanced affinity for TG2. The contribution of each H3 position to the Abs affinity for TG2 epitope 1, currently under investigation by site-directed mutagenesis, will also be discussed.



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