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SOFIA UNIVERSITY MARKING MOMENTUM FOR INNOVATION AND TECHNOLOGICAL TRANSFER

SOFIA UNIVERSITY -
MARKING MOMENTUM
FOR INNOVATION AND TECHNOLOGICAL TRANSFER

National Recovery and Resilience Plan
OF THE REPUBLIC OF BULGARIA

Selection of high affinity recombinant antibodies against human complement protein C3

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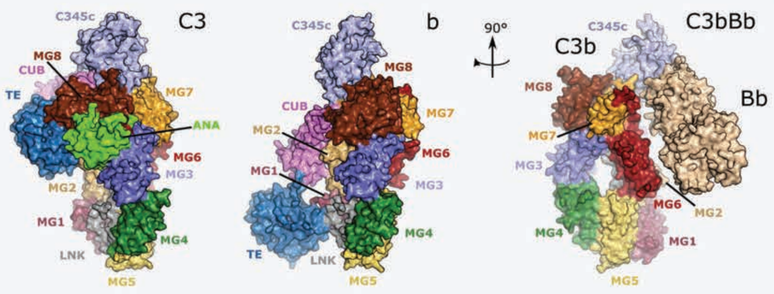
INTRODUCTION

C3 is the key protein in the activation of the Complement system and it is a contributor for the maintenance of an effectively functioning immune system. C3 is activated by a series of enzymatic reactions upon pathogen infection or cell damage. Each cleavage gives biologically active fragments, such as C3a, C3b, iC3b, C3c, and C3d. C3 and its fragments also appears to be a target for autoantibodies during the development of autoimmune diseases such as Systemic Lupus Erythematosus (SLE).

In order to study the molecular aspects of C3 autoantigenicity we aimed to select a high affinity anti-C3 antibodies from the "Griffin 1" phage display library expressing human scFv antibodies.



(Zarantonello A et al., Immunological Reviews. 2023;313:120–138.)

METHODOLOGY

- Screening the "Griffin-1" phage library expressing human scFv antibodies
- Selection of monoclonal anti-C3 scFv using a gradual decrease of the amount of the antigen C3: 12 µg in III round and 10 µg in IV round
- Transfer of selected anti-C3 scFv into a non-suppressor *E.coli* HB2151
- Induction and expression of soluble monoclonal anti-C3 scFv antibodies
- Quantitative analysis by ELISA to determine the affinity of selected scFv clones to C3
- Western Blot and Dot Blot assay with selected anti-C3 scFv antibodies against intact C3 and its smaller fragments C3b, C3c and C3d.
- Two induction approaches – IPTG induction and autoinduction, for expression of soluble anti-C3 scFv

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RESULTS

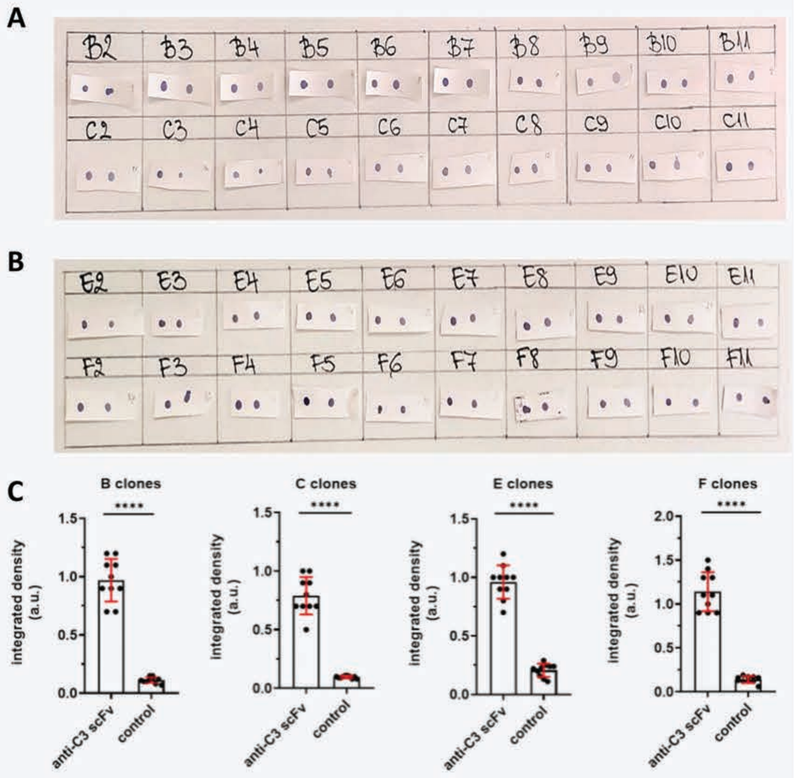

Figure 1. (A) Dot blot assay for detection of positive anti-C3 scFv antibody clones selected in round III (clones B2 - C11) and (B) anti-C3 scFv antibody clones selected in round IV (clones E2 - F11). (C) Measurement of integrated density of positive anti-C3 scFv antibodies from Dot Blot assay. 1 dot = mean value of duplicate measurement of integrated density of a sample normalized to a control. Statistical analysis was done with paired student t test, error bars: mean±SD, **** ($p \leq 0.0001$).

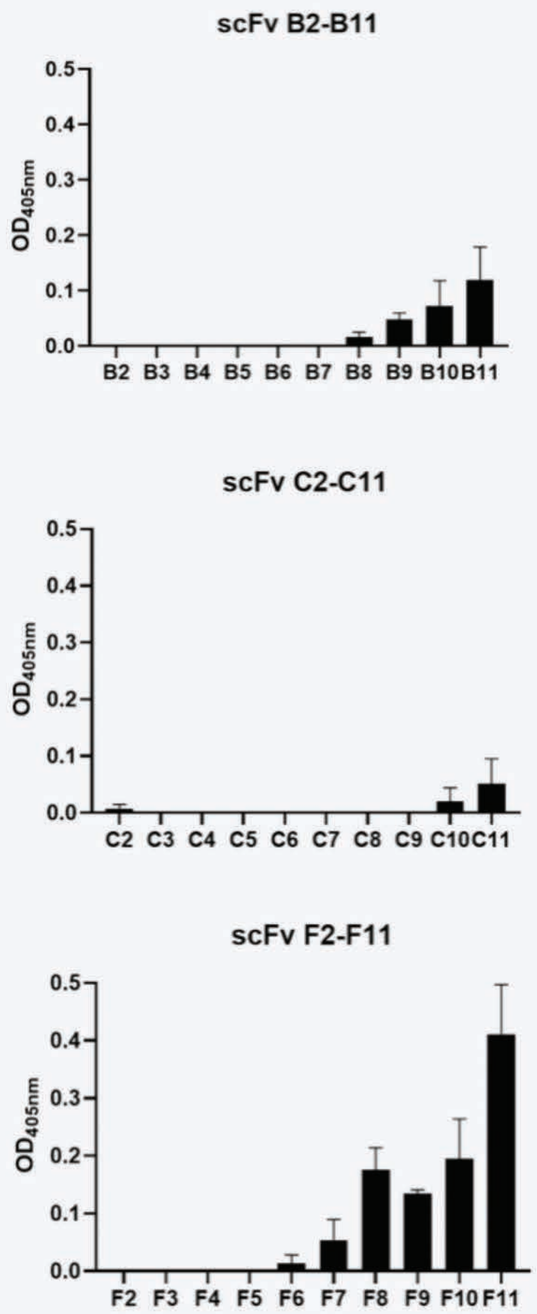

Figure 2. Comparative ELISA analysis of soluble monoclonal anti-C3 scFv antibodies from clones B2-B11, clones C2-C11 (selection round III) and clones F2-F11 (selection round IV).

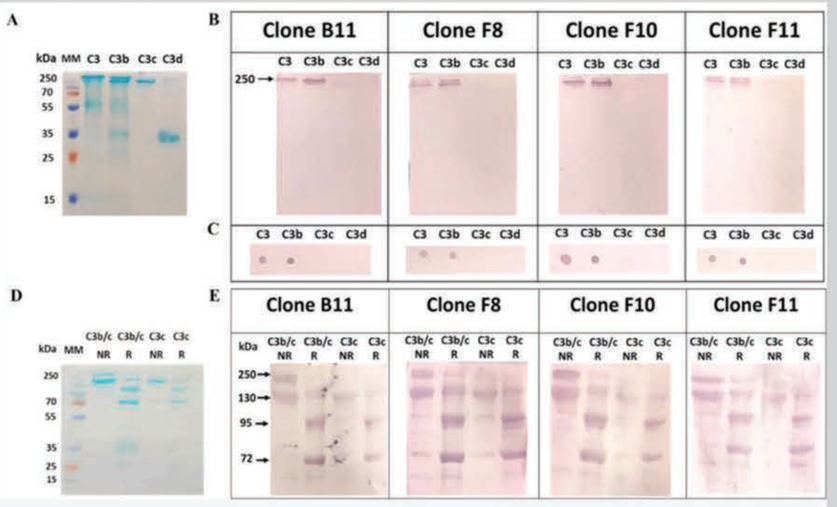

Figure 3. Immunoblotting of the selected high affinity scFv clones for the recognition of the intact C3 and its smaller fragments C3b, C3c, C3d. (A) NC with blotted proteins after non-reducing 15% SDS PAGE. Western Blot (B) and Dot Blot (C) of antigens with selected anti-C3 scFv clones. (D) NC with blotted proteins after reducing 15% SDS PAGE. (E) Western Blot of reduced antigens with selected anti-C3 scFv clones

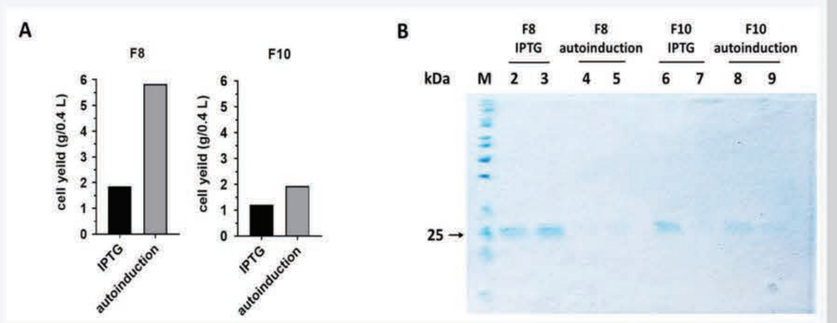

Figure 4. E. coli cell yield obtained after IPTG-induction and autoinduction of (A) clones F8 and F10 and (C) clones B11 and F11; (B) non-reducing 15% SDS-PAGE of anti-C3 scFv eluates of clones F8 and F10. Lanes: M, molecular marker; 2 and 3: clone F8 eluates from IPTG induction; 4 and 5: clone F8 eluates from autoinduction; 6 and 7: clone F10 eluates from IPTG induction; 8 and 9: clone F10 eluates from autoinduction; (D). non-reducing 15% SDS-PAGE of anti-C3 scFv eluates from clones B11 and F11. Lanes: M, molecular marker; 2, 3, 4: clone F11 eluates from IPTG induction; 5, 6, 7: clone F11 eluates from autoinduction; 8 and 9: eluates from culture growth medium of clone B11; 10 - cell lysate from IPTG induction of clone B11; 11 - cell lysate from auto-induction of clone B11. The relative protein molecular weight (kDa) was estimated with ImageJ software.

CONCLUSION

The gradual decrease of the amount of antigen the successive rounds resulted in the selection of high-affinity antibodies to C3. Comparative quantitative ELISA analysis highlighted 4 monoclonal antibodies suitable for further experimental work - B11, F8, F10 and F11. Four clones B11, F8, F10 and F11 recognized C3, C3b, but not C3c and C3d.

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