ANX005, an Inhibitory Antibody Against C1q, Blocks Complement Activation Triggered By Cold Agglutinins in Human Disease

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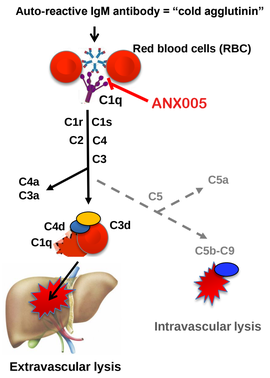
Cold Agglutinin Disease (CAD)

- Patients have auto-reactive antibodies that bind their red blood cells (RBC) at lower body temperatures
  - Particularly arms and legs in cold weather
- Antibody binding to RBC's trigger C1q binding and activation of the classical complement pathway
- RBC's coated with complement are removed by the liver or lysed within the vasculature

Important to block deposition of early complement cascade components to prevent red cell destruction



Goals

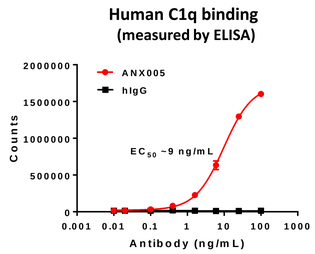
- Evaluate the ability of CAD sera to trigger complement opsonization and lysis of human RBCs
- Evaluate ANX005, a function blocking antibody against C1q, for its impact on complement deposition and hemolysis triggered in cold agglutinin-sensitized humans RBCs

Methods

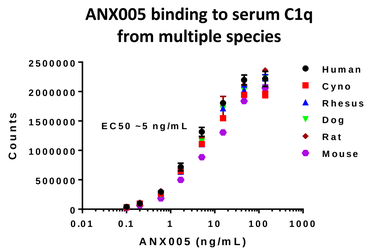
Complement deposition and hemolysis:

- hRBCs were sensitized with CAD sera for 1 hr at 4°C (15 uL sera + 15 uL hRBC)
- Complement activation was triggered by addition of 200 uL of 1/20 diluted normal human serum at 37°C for ~30 minutes
- Hemolysis was measured by release of hemoglobin into the supernatant (OD 415 nm)
- Remaining RBCs were exposed to anti-C1q, C4 and C3 antibodies and stained with fluorescent secondary antibody. Surface labeling was analyzed by flow cytometry

1 ANX005 binds C1q from multiple species with high affinity



(Binding to purified hC1q)



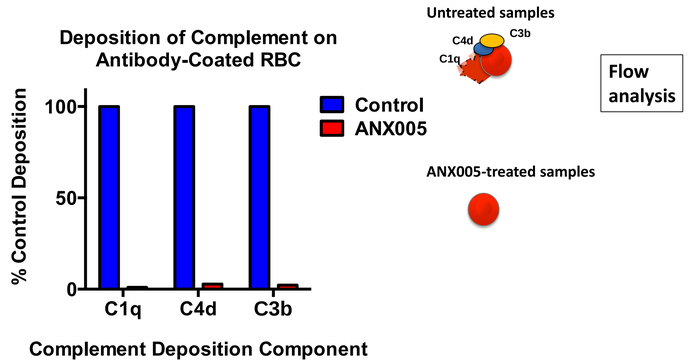
(Binding to serum-derived C1q)

Kinetics of binding to hC1q

Ka (1/Ms)	kd (1/s)	K_D (pM)
9.6 x 10^5	6.4 x 10^{-6}	6.7

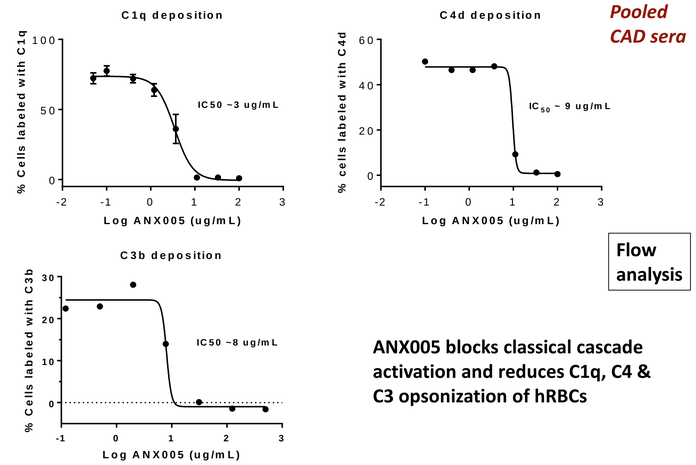
Biacore results

2 ANX005 blocks C1q, C4d & C3b deposition onto cold agglutinin-sensitized hRBCs (pooled CAD sera)



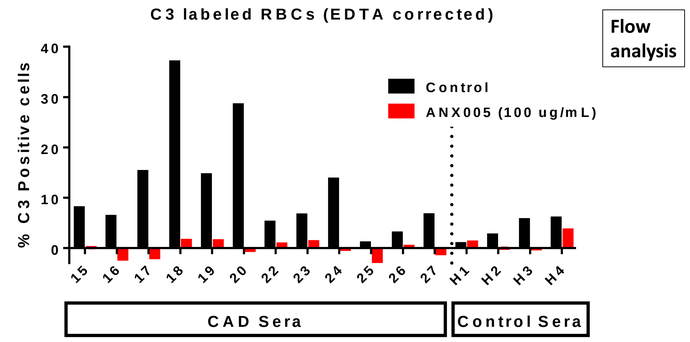
Activation of classical complement cascade by cold agglutinins from patient sera

3 ANX005 - Dose-dependent reduction in C1q, C4 and C3 deposition onto cold agglutinin-sensitized hRBCs



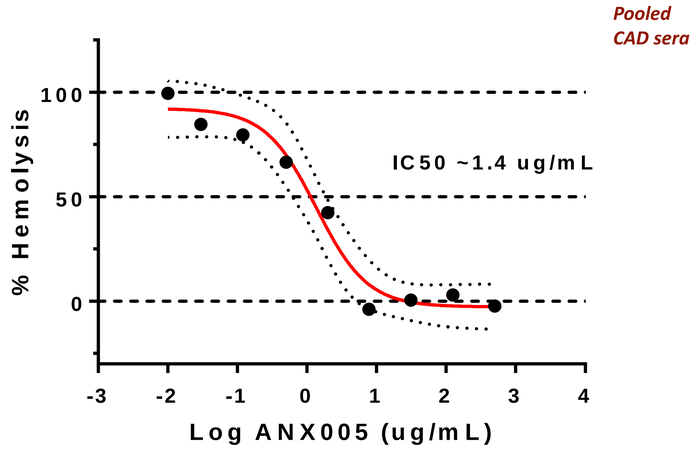
ANX005 blocks classical cascade activation and reduces C1q, C4 & C3 opsonization of hRBCs

4 ANX005 blocks C3 deposition onto human RBCs using CAD sera from individual patients



Extent of C3 deposition onto hRBCs was different using CAD sera from different subjects, but ANX005 led to robust reduction in all samples

5 ANX005 – Dose-dependent reduction in hemolysis in CAD sera sensitized hRBCs



Summary & Conclusions

- We have developed an anti-C1q antibody (ANX005) that binds with high affinity to C1q (~10 pM) and inhibits classical complement cascade activation.
- ANX005 shows a dose-dependent reduction in deposition of C1q, C4 and C3 onto cold agglutinin-sensitized human RBCs and blocks hemolysis.
- C1q inhibition with ANX005 should be explored clinically in patients with antibody mediated diseases including cold agglutinin disease.

Conflict of interest

Morie Gertz MD – Honoraria from Celgene, Millennium, Novartis, Ionis, Med Learning group, Research to Practice, Prothena
The following authors are employees of Annexon Biosciences and own equity – Haiyan Qiu, Laura Kendall, Mario Saltarelli, Ted Yednock, Sethu Sankaranarayanan

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