The Fc function of the Human Contraception Antibody mediates interactions with cervical mucus, complement, and phagocytic cells

Emilie Mausser[1], Jai Marathe[1,2], Maria Tjilos[3], Ellena Nador[1], Joseph Politch[1], Rachel Brosnan[4], Kevin Whaley[5], Deborah Anderson[1]

[1]Department of Medicine, Boston University School of Medicine, Boston MA; [2]Department of Infectious Diseases, Boston Medical Center, Boston MA; [3]School of Public Health, Boston University School of Medicine, Boston MA; [4]Emmanuel College, Boston MA; [5]Mapp Biopharmaceuticals, San Diego, CA

Boston University School of Medicine

Boston Medical Center

Abstract

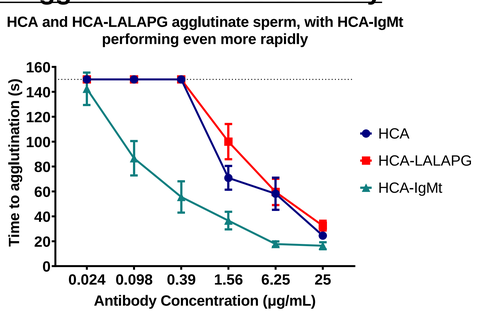
Despite many advances in contraceptive technologies, about 40% of all pregnancies are classified as unintended. To address this issue, we are developing a topical immunocontraceptive: the Human Contraception Antibody (HCA). In this study we compared the function of HCA with two variants, HCA-LALAPG and HCA-IgMt. Four different Fc function assays were used: cervical mucus penetration test, sperm immobilization test, complement-dependent cytotoxicity (CDC), and antibody-dependent cellular phagocytosis (ADCP). HCA significantly reduced the penetration of progressively motile sperm into cervical mucus compared to HCA-LALAPG. When mixed with sperm and diluted human serum, HCA and HCA-IgMt readily immobilized sperm and increased CDC was observed. Conversely, HCA-LALAPG did not immobilize sperm. HCA induced significantly more ADCP than HCA-LALAPG. These findings suggest that the contraceptive functions of HCA may be enhanced through Fc interactions with phagocytic cells, as well as with mucins and complement proteins in cervical mucus, by trapping and possibly killing sperm cells, limiting their progression through the female reproductive tract.

Introduction

We are developing a topical on demand nonhormonal immunocontraceptive product, ZB-06, a vaginal film that releases a human anti-sperm monoclonal antibody, the **Human Contraception Antibody (HCA)**. HCA binds to CD52g on mature sperm cells and has been shown to potently agglutinate sperm. It is currently in Phase I clinical trials. In addition to the HCA-IgG1, two variants have also been produced: HCA-LALAPG, a variant of HCA with a defective Fc region, and HCA-IgMt, a hexameric IgG. We are investigating possible Fc functions of HCA that may improve its ability to prevent unintended pregnancy.

Results – HCA Fab Function

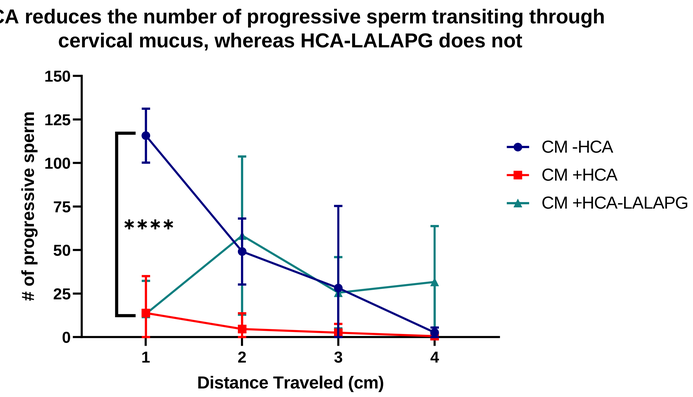
Sperm Agglutination Kinetics Assay



HCA and its variants agglutinate sperm cells in a concentration-dependent manner. HCA-IgMt agglutinates much faster at very low antibody concentrations.

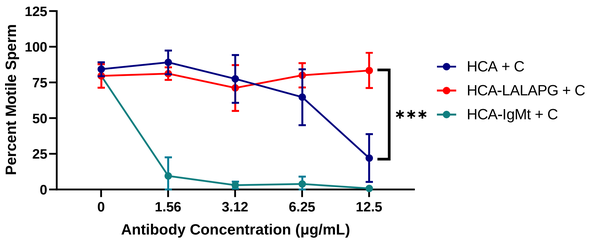
Results – HCA Fc Functions

Cervical Mucus Penetration Test

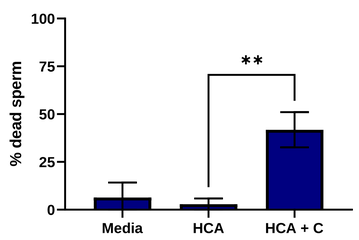


Samples of midcycle cervical mucus were loaded into flat capillary tubes by aspiration and a small interface of HCA at 12.5µg/mL added to one end. With the opposite end sealed, sperm were allowed to infiltrate through HCA and then cervical mucus. HCA significantly reduced the number of progressively motile sperm that penetrated through columns of cervical mucus. Sperm also appeared to be trapped in the cervical mucus in the presence of HCA, as evidenced by flagellar beating without forward motility. The same reduction was not seen with HCA-LALAPG, suggesting the importance of a function Fc region to interact with cervical mucus and prevent sperm progression through cervical mucus.

Sperm Immobilization Test

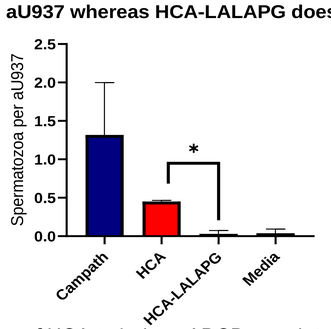


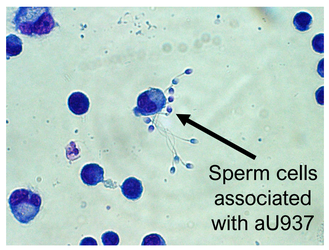
Complement-Dependent Cytotoxicity (CDC)



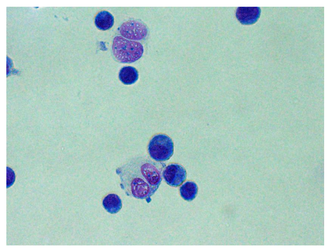
HCA is able to immobilize sperm cells in the presence of a complement source. Mixing sperm cells, HCA, and human serum caused a 40% reduction in the percent of motile sperm. As HCA-LALAPG cannot bind complement, there is no reduction in motility. Conversely, HCA-IgMt has greater valency and was able to significantly reduce the percent of motile sperm at much lower antibody concentrations. In addition to immobilizing sperm cells, HCA also appears to induce complement-dependent cytotoxicity, increasing the percent of dead sperm when complement is added.

Antibody-Dependent Cell Phagocytosis (ADCP)





HCA 25µg/mL



HCA-LALAPG 50µg/mL

The ability of HCA to induce ADCP was determined using PMA-activated U937 cells (aU937) to which antibody-treated sperm cells were added. Phagocytosis was visualized following H/E staining. Multiple sperm cells were associated with the macrophage-like cells following incubation with Campath, an anti-CD52 antibody with well characterized ADCP function. Although not as strong as Campath, HCA also had associated sperm and aU937 cells, but almost no sperm cells were present when treated with HCA-LALAPG.

Conclusion

Fab — Agglutination
- HCA agglutinates sperm via Fab
- HCA-IgMt > HCA = HCA-LALAPG

Fc — Cervical Mucus Trapping
- HCA traps sperm in CM via Fc
- HCA > HCA-LALAPG

Fc — Complement-mediated immobilization
- HCA immobilizes sperm via Fc with complement
- HCA-IgMt > HCA > HCA-LALAPG

Fc — Complement-mediated killing
- HCA can kill sperm cells via Fc with complement

Fc — Antibody-induced phagocytosis
- HCA mediates phagocytosis of sperm via Fc
- HCA > HCA-LALAPG

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