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Antibody-dependent cellular phagocytosis of sperm mediated by a contraceptive anti-sperm monoclonal antibody and its engineered variants

Ellena Nador, Emilie Mausser, Kevin Whaley, Deborah J. Anderson

INTRODUCTION

Of the 210 million pregnancies that occur globally each year, about half are unintended. There is a need for a new generation of effective contraceptives[1]. A novel human anti-sperm monoclonal antibody, the Human Contraception Antibody (HCA), is a promising candidate for a reversible, non-hormonal female contraceptive due to its potent sperm-agglutinating activity[2] (Figure 1). Recent preclinical research and a phase 1 clinical trial testing the safety and efficacy of an HCA vaginal film demonstrated that the antibody is safe, remains active in the female genital tract, and prevents sperm migration through cervical mucus[3]. Parent HCA is an IgG1 subclass antibody, but multimeric variants are currently being explored to increase potency (Figure 2). Despite thorough characterization of several contraceptive mechanisms[4], understanding HCA activation of antibody-dependent cellular phagocytosis (ADCP) is crucial for further development, since this may stimulate undesirable downstream immune responses such as inflammation and sperm-antigen presentation which could in turn promote endogenous sperm immunity and contraceptive irreversibility. Here, we characterize the ability of IgG1 HCA, as well as multimeric and Fc-silenced variants, to activate sperm phagocytosis to inform the selection of an optimal candidate for an HCA-based contraceptive.

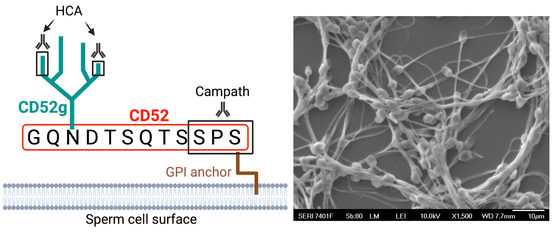
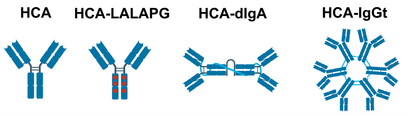


Figure 1. Human Contraception Antibody (HCA).
Left: CD52g (green), the male reproductive tract-specific epitope of HCA on the surface of mature sperm cells.
Right: SEM image of HCA-agglutinated sperm.



Variant	Agglutination	Complement-dependent sperm immobilization	Mucus trapping
HCA-IgG1	+	+	++
HCA-LALAPG	+	-	-
HCA-dIgA	++	N/A	+++
HCA-IgGt	+++	+++	+++

Figure 2. Summary of HCA variant activity.
HCA-IgG1, parent antibody; HCA-LALAPG, an IgG with three amino acid substitutions in the Fc region that ablate Fc receptor binding; dimeric IgA HCA; and HCA-IgGt, a hexameric variant of HCA engineered using the IgM tailpiece.

METHODS

The sperm phagocytosis assay was adapted from Oren-Benatoya et al.[5] with modifications (Figure 3). In brief, sperm cells in either media or antibody solution were applied to PMA-stimulated U937 effector cells. Following a 30 min incubation, cultures were washed and incubated in PBS for another 30 min. Effector cells were then incubated in trypsin to remove non-specifically bound sperm. Slides were treated with a Romanowsky stain and observed under a light microscope at 200X magnification. The number of spermatozoa associated with 100-300 macrophages were counted for each treatment group. Associated spermatozoa were defined as those that were either attached to the surface of a cell (i.e. in the beginning of the phagocytosis process), or engulfed/internalized. Experiments were repeated thrice using semen samples from different donors.

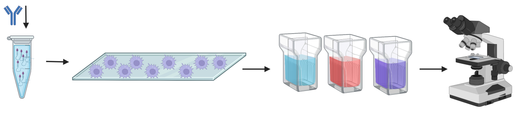
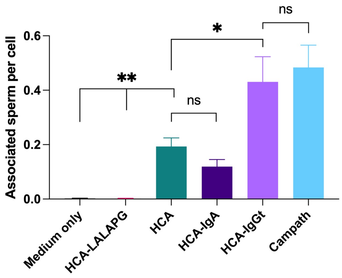


Figure 3. Antibody-dependent sperm phagocytosis assay.
Sperm cell suspensions are treated with antibody and incubated with effector cells for 30 min, followed by wash step and trypsin treatment for 5 min to remove nonspecific sperm binding. A Romanowsky stain series is applied to slides to image and quantify phagocytosed sperm.

RESULTS

Parent HCA was able to mediate sperm phagocytosis at 25 µg/ml, but not to the same degree as the positive control (about 30% of that seen with Campath treatment) which elicited the highest level of ADCP. No sperm phagocytosis was observed in the HCA-LALAPG or medium-only conditions as expected, since the LALAPG Fc mutations abrogate Fc receptor binding. Among the variants tested, HCA-IgGt exhibited the most ADCP activation, similar to Campath, and was significantly higher than IgG1 HCA. HCA-dIgA elicited a similar level of ADCP as IgG1 HCA. Attached and engulfed sperm were clearly visible by microscopy in the IgG1 HCA, HCA-LALAPG, and HCA-dIgA treatments.



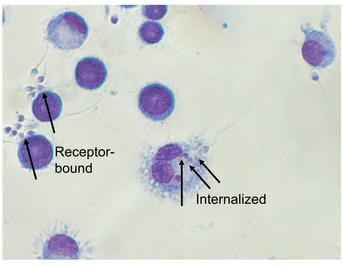


Figure 4. Antibody-dependent cellular phagocytosis of HCA variants.
Left: Sperm phagocytosis assay. Sperm suspensions were treated with antibody conditions at 25 µg/ml.
Right: Representative image of ADCP assay quantification (HCA-IgGt), 200X magnification.
All data are expressed as mean ± SEM of three independently performed experiments, *p<0.05, ** p<0.01, ns=non-significant.

CONCLUSIONS

We characterized ADCP among relevant HCA variants to further understand their biologic profiles and evaluate alternative candidates for an HCA contraceptive. IgG1 HCA, HCA-dIgA, and HCA-IgGt all activated sperm phagocytosis. Strong ADCP activity induced by HCA-IgGt treatment was likely due to multimerization of Fc regions. HCA-LALAPG did not induce phagocytosis due to its Fc-silencing mutations. Macrophages and other antigen presenting cells are widely prevalent in the female reproductive tract. Therefore, though certain HCA variants like IgGt increase avidity, they may also pose a risk for host formation of anti-sperm antibodies as a consequence of HCA-dependent sperm phagocytosis. Fc-mutated variants like HCA-LALAPG may be safer, more conservative alternatives to HCA to be used in a contraceptive product, though recent clinical trial results suggest that certain HCA Fc functions are important for contraception. Development of new Fc variants that further customize the desired HCA clinical profile are underway.

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BOSTON UNIVERSITY

AUTHOR INFO

EN, EM, DJA: Boston University Chobanian & Avedisian School of Medicine, Boston, MA
linkedin.com/in/ellenanador/

KW: ZabBio, Inc., San Diego, CA

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