Antibodies to CD52g, a Secreted Sperm coating Antigen, Agglutinate Seminal Leukocytes and Prevent their Infiltration into Vaginal Epithelium

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Abstract

Introduction:

Our program is studying the topical use of monoclonal antibodies (mAbs) for contraception and HIV prevention. mAbs directed against CD52g, an antigen secreted into the male genital tract and inserted into sperm membranes, potently agglutinate sperm and are being developed for contraceptive use. We are also seeking strategies to prevent cell-associated HIV transmission mediated by HIV-infected seminal white blood cells (sWBC). In this study, we investigated whether CD52g also attaches to sWBC, and whether anti-CD52g mAbs agglutinate these cells and/or inhibit their interaction with the vaginal epithelium.

Methods and Materials:

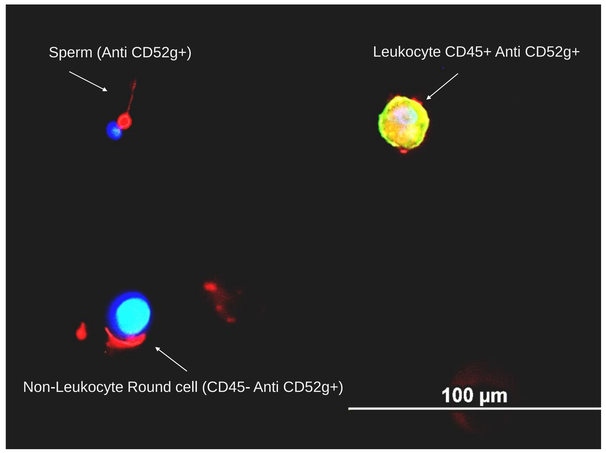
Dylight 633-conjugated MSH8, a mouse anti-CD52g mAb (gift of J. Herr), was used in Flow cytometry experiments to detect CD52g on sWBC and passive insertion of seminal plasma CD52g into the plasma membrane of monocyte-derived macrophages (MDMs). HC4, a human mAb expressed in Nicotiana (Mapp Biopharmaceuticals), was used in two functional assays: 1) agglutination of sWBC, assessed by counting the percentage of CMFDA-labelled WBC associated with sperm agglutinates in mAb-treated seminal fluid; and 2) cell attachment and infiltration assays, modeled with CD52g-coated CMFDA-labeled WBCs and EpiVaginal™ tissue (MatTek Corp), and assessed by confocal microscopy.

Infiltrated cells were counted using ImageJ software. ANOVA with post-hoc Fisher's PLSD comparison was used for statistical analysis.

Results:

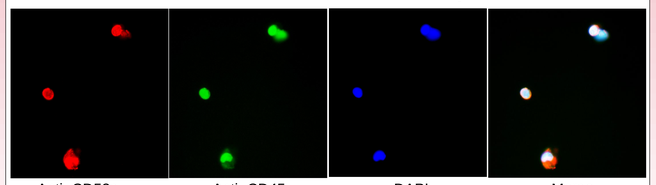
MSH8 bound to a majority of sWBCs and also to seminal plasma-treated peripheral blood mononuclear cells (PBMCs). HC4 trapped WBCs in sperm agglutinates. The antibody also significantly inhibited the attachment and infiltration of CD52g-coated WBCs into the vaginal epithelium. Control mAbs had no effect in these assays.

Fig 1. CD52g can be detected on seminal leukocytes



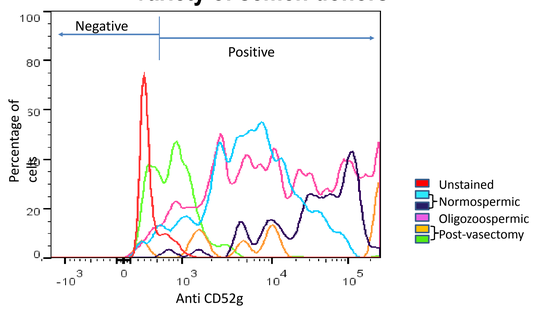
Seminal cells: sperm, leukocytes (CD45+) and other round cells (CD45-) demonstrate CD52g staining.

Fig 2. CD52g is passively inserted into cell-membranes of PBMCs incubated in seminal plasma



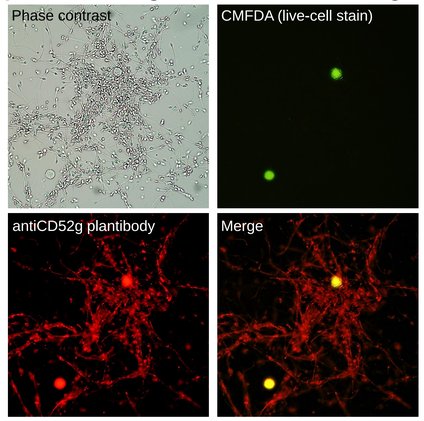
PBMCs were incubated in seminal plasma for 2 hours and then incubated with fluorescently labeled anti-CD52g and anti-CD45 antibodies. DAPI was used as a nuclear stain. CD52g was detected and co-localized with CD45 on cell surface.

Fig 3. CD52g is detected on CD45+ seminal cells from a variety of semen donors



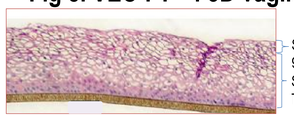
Seminal cells from multiple donors were enriched for mononuclear cells on a Ficoll gradient and then stained with a live cell stain, MSH8 and anti-CD45 Abs. Flow cytometry was performed and data analyzed on Flow-Jo software. Cells were gated on live cells, then on CD45+ and finally on MSH8+ population. Seminal cells from all donors: normozoospermic, oligozoospermic and post-vasectomy samples were positive for CD52g.

Fig 4. Leukocytes are trapped with agglutinated sperm following addition of anti-CD52g mAb



Motile sperm in seminal plasma were spiked with CMFDA stained MDMs and 100µg of HC4-N plantibody was added.
100% agglutination was observed within 30 sec and >95% MDMs were associated with sperm agglutinates.

Fig 5. VEC-FT™: 3D vaginal epithelial model



Leukocyte transmigration model: CMFDA-labeled MDMs are added to the apical surface of TNF-treated VEC-FT tissue, and infiltration is monitored by confocal microscopy. Actively infiltrating cells are observed in the suprabasal and basal layers after 4 hours.

Fig 6-I. Anti-CD52g Ab significantly decreases infiltration of MDMs into TNF-treated VEC-FT™ (ANOVA Fisher's PLSD)

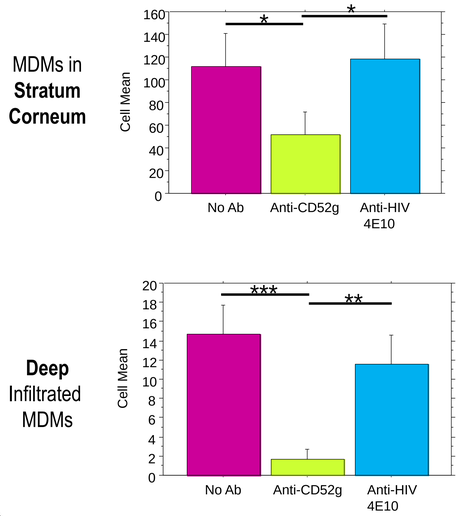
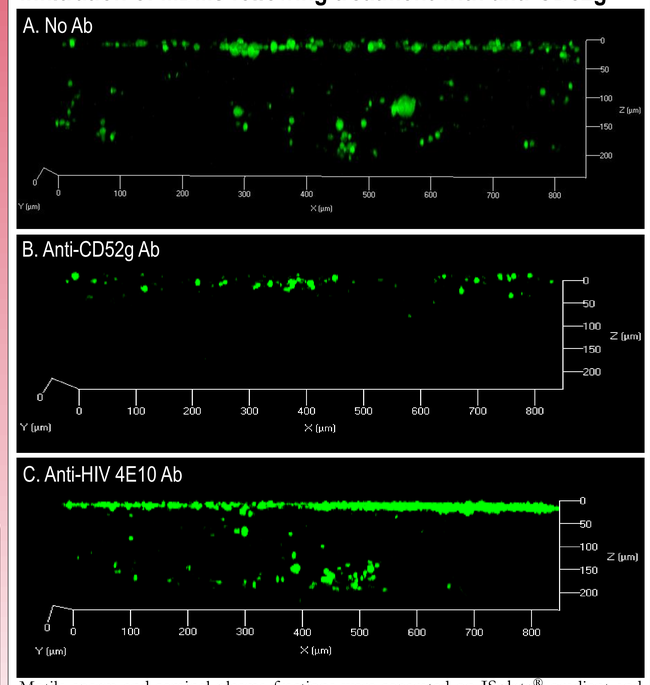


Fig 6-II. 3D reconstruction of VEC-FT™ visualized by confocal microscopy, shows decreased adhesion and infiltration of MDMs following treatment with anti-CD52g



Motile sperm and seminal plasma fractions were separated on ISolate® gradient and combined. These were spiked with CMFDA-stained MDMs and the cell suspension was applied to the apical surface of TNF-treated VEC-FT 100™ tissues that had been pretreated with HC4 (anti CD52g plantibody), 4E10 (control non-specific plantibody) or medium 1.5 hours prior to application of cells. After 4 hours of infiltration, a Z-stack was taken at 5-mm intervals through the tissue using deconvolution microscopy. Reconstructions shown in fig 6-II for (A) No antibody (B) HC4 plantibody and (C) 4E10 plantibody.

←**Fig 6-I**. shows MDM cell counts in HC4 (anti-CD52g) and 4E10 (negative control) pre-treated tissues compared to no antibody control at the level of Stratum Corneum and deep suprabasal and basal layers. Treatment with HC4 significantly decreased MDM adhesion to superficial layer and infiltration into the deeper tissues ($p<0.05$ and $p<0.0005$ respectively).

Conclusions

- Anti-CD52g was detected on cell membranes of seminal leukocytes from a variety of semen donor types including normospremic and vasectomized.
- HC4 plantibody, a potent sperm agglutinating antibody, trapped seminal WBCs with the spermatozoa.
- HC4 plantibody significantly blocked adhesion and infiltration of MDMs into EpiVaginal tissue.
- Topical vaginal application of antibodies to CD52g could serve a dual purpose use: contraception and inhibition of cell-associated HIV transmission.

This research was funded by **NIH U19 AI096398, CTSA#U54-TR001012.**