The Role of Exosomes in Semen in Suppressing Natural and Vaccine-Induced Immunity

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Abstract

Background

Exposure to semen is the primary route of transmission for many sexually transmitted infections, including HIV. Accumulating evidence suggests that components in semen directly impair leukocytes, which could compromise the protective efficacy of vaccine-induced immune responses in the mucosa. Seminal plasma contains large numbers of extracellular microvesicles or exosomes (SE). Thus, we explored the effects of SE on blood and mucosal immune responses.

Methods

SE isolated from semen donated by healthy men were pooled from multiple donors. Dendritic cells (DCs) were derived from peripheral blood mononuclear cell (PBMC) monocytes. DCs and PBMC cultures were exposed to SE and assayed for SE uptake, immune function, and cytokine production by confocal microscopy, flow cytometry, and quantitative PCR.

Results

Extracellular vesicles from semen are present at an average concentration of 2.2 x 10^{13} particles per ejaculate (n = 18 donors). These SE rapidly and efficiently entered peripheral and vaginal dendritic cells (DCs), to a lesser extent B cells, whereas uptake by T cells was poor. In PBMC cultures, SE impaired memory T cell function, reducing the production of TNF-alpha and/or IFN gamma in response to CMV, EBV, or influenza-derived peptides an average of 73% for CD4+ T cells and 55% for CD8+ T cells, in a dose-responsive manner (n = 4 donors). SE also impaired vaginal T cell responses to a superantigen. Exposing only DCs, as opposed to bulk PBMCs, to SE also blocked subsequent CD8+ memory T cell activation, reducing the proportion of cells making cytokines by 51% (n = 3 donors). This effect occurred even when only a fraction of DCs (as low as 20%) were exposed to SE.

Conclusions

SE markedly inhibited adaptive immune responses. T cell function appears to be impaired by affecting the co-stimulatory capacity of antigen-presenting cells. Understanding how programmed immune responses are altered by the presence of semen is important to developing the next generation of vaccine and preventative treatments against sexually transmitted diseases.

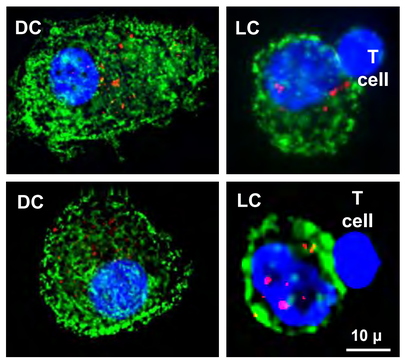
Acknowledgments

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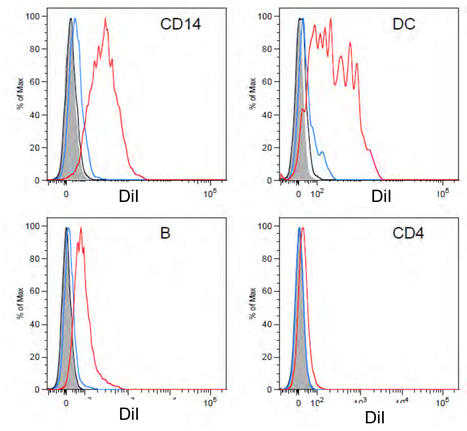
Results

Seminal exosomes rapidly enter antigen presenting cells



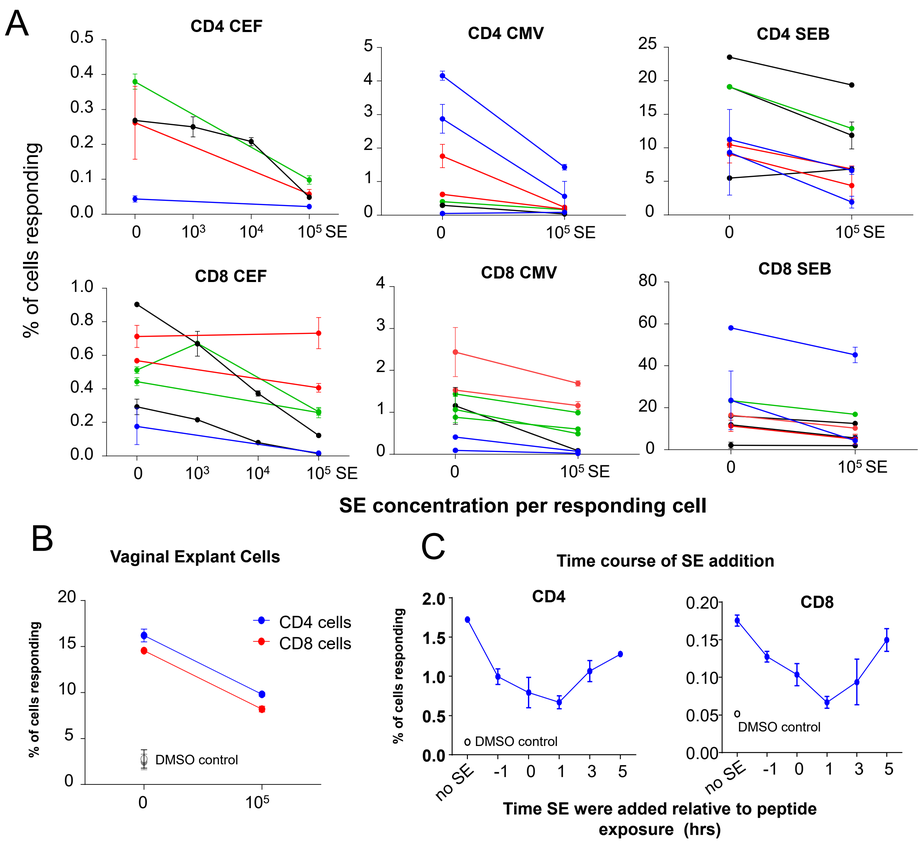
Red: DiI labeled seminal exosomes
Green: DC or LC marker

Blood-derived DC or vaginal Langerhans cells (LC) were exposed to red DiI-labeled SE for 1 hr and stained for MHC-II (green) and the nucleus (blue). Both DC and LC have exosomes internalized, apparently compartmentalized in clusters in cellular organelles. Among LC-T cell conjugates (right images), only the LCs but not the T cells (only the nucleus of which is labeled) contain SE.



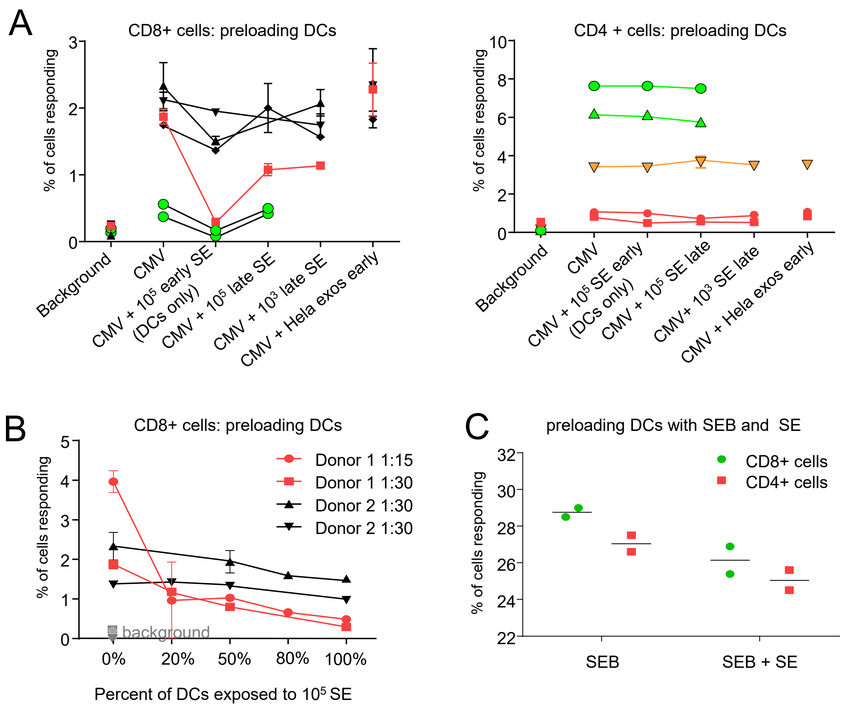
PBMCs were exposed to DiI-labeled SE for 2 hr, then stained for phenotypic markers and analyzed for SE uptake by flow cytometry. Red lines indicate exposure to 10^5 labeled SE per cell, blue lines 10^4 SE, and filled plots unexposed cells

Seminal exosomes impede antigen-specific immune responses by CD4+ and CD8+ cells

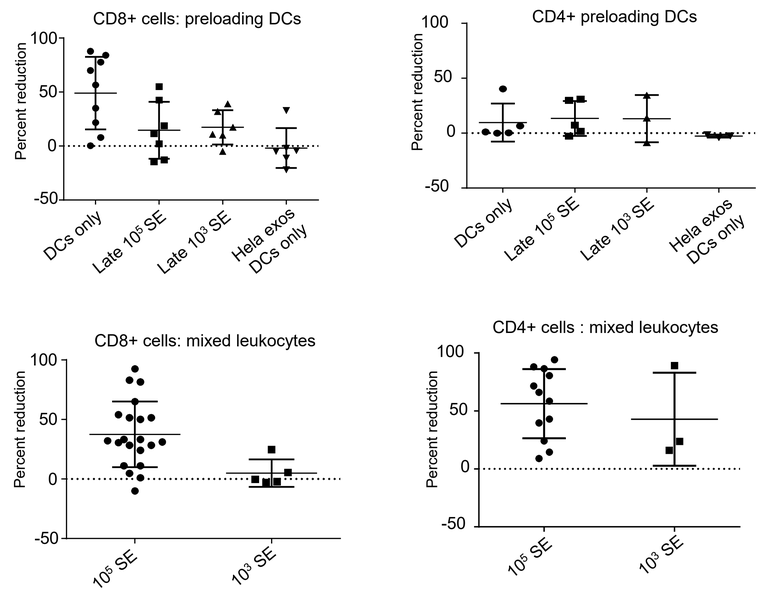


A) PBMCs were exposed to CEF or CMV peptides, or the superantigen SEB at the same time as 10^3, 10^4, or 10^5 SE per cell (or left unexposed). Production of cytokines was assessed by intracellular cytokine staining for IFNγ, IL-2, and/or TNFα, and the sum of % of cells responding is reported as total response. Each color indicates a different blood donor. **(B)** Cells isolated from vaginal explant tissue were exposed to the superantigen SEB in the presence of no SE or 10^5 SE per leukocyte for 6 hrs, then stained as in A. **(C)** PBMCs were exposed to CMV peptides at time zero and 10^5 SE per cell added at the indicated time. Cells were assessed for cytokine production at 6 hrs as in (A). .Y-axis is the total percent of T cells producing cytokines.

Exposing only DCs to seminal exosomes impairs immune responses by CD8+ T cells



A) DCs differentiated from blood monocytes were exposed to CMV peptides for 24 hrs. SE at 10^5 per cell were added at the time of antigen loading (early), or when combined with responding autologous PBMCs 24 hrs later (late). Exosomes derived from Hela cells served as a control. Each color indicates a separate blood donor. **B)** DCs were exposed to CMV peptides for 24 hours. Half of the antigen-exposed DCs were simultaneously treated with 10^5 SE/cell. SE-treated and -untreated DCs were washed, combined at the indicated fractions (x axis), and added to autologous responder PBMC at a total DC to responder cell ratio of 1:15 or 1:30. "Background" are cultures without antigen. Error bars are stand. dev. of duplicates. Figure shows only CD8+ T cell responses. **C)** DCs were exposed to the superantigen SEB for 24 hours. SE at 10^5 per cell were added at the time of exposure to SEB. Duplicate samples from one blood donor are shown



Summary of all experiments on T cell responses to viral peptides in the presence of SE. The percent reduction from cultures exposed to CMV or CEF peptides alone is plotted on the y-axis. In preloading DC plots, "DCs only" indicates that only DCs were exposed to SE. "Late" indicates that SE were added to responding autologous PBMCs at the time of mixing with antigen-loaded DCs, as in part (A). In "mixed leukocyte" plots, peptides and SE were added to PBMC cultures at the same time and cultured for 6 hours.