



2011 Research Grant Program Winning Abstract

Isolation of HIV Envelope-Specific Mucosal B Cells from Colostrum of HIV-Infected, Lactating Women

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Objective:

To isolate HIV envelope-specific B cells from colostrum of HIV-infected women and characterize the ontogeny of HIV envelope-specific monoclonal antibodies in breast milk

Background:

The moderately successful HIV vaccine trial completed in Thailand last year (RV144 vaccine trial) strongly implicated the ability of vaccine-elicited virus-specific antibody responses to protect against mucosal HIV transmission. However, the characteristics and function of transmission-blocking mucosal antibodies have not been defined due to difficulties in isolating mucosal B cells and adequate amounts of mucosal antibodies. Breast milk is a rich, easily accessible source of mucosal IgG and secretory IgA, antibodies that contribute to passive protection of infants from neonatal pathogens. In fact, functional HIV-specific antibody responses in breast milk may contribute to the protection of the majority of breastfeeding infants from HIV acquisition, despite months of chronic, daily exposure to virus in breast milk (Fouda). Moreover, B cells present in the mammary gland originate in the gut-associated lymphoid tissue and therefore represent the gastrointestinal B-cell population.

Thus, isolation and characterization of HIV envelope (Env)-specific monoclonal antibodies in milk of HIV-infected women will elucidate the specificity and virus transmission-blocking functions of Env-specific antibodies at the mucosal surface. Investigation of functional mucosal HIV Env-specific monoclonal antibodies will elucidate the virus epitope targets and antibody functions necessary for blocking mucosal HIV transmission.

In preliminary studies, we isolated B cells from colostrum of an HIV-infected, lactating woman by flow cytometric single cell sorting. However, after screening the specificity of the immunoglobulins produced by the B cells, there was a low frequency of HIV envelope-specific B cells (1 to 3%). Therefore, isolation of HIV envelope-specific B cells through fluorescently-tagged antigen labeling and colostrum B-cell staining would be a more efficient way to identify novel HIV-specific mucosal antibodies.

Hypothesis:

We hypothesize that HIV envelope-specific colostrum B cells can be isolated by antigen labeling and single-cell flow cytometric sorting and used to generate novel mucosal HIV envelope-specific antibodies

Specific aims:

1. Fluorescent labeling of HIV envelope protein and design of a memory B-cell phenotyping panel as reagents for HIV-specific colostrum B-cell isolation



A transmitted/founder clade C HIV envelope protein will be biotin labeled and stained with a streptavidin/fluorescent molecule complex using the appropriate kits. This reagent will be titrated and optimized for flow cytometry using an HIV Env-specific hybridoma cell line. In addition, we will design a panel of fluorescent antibodies to identify memory B cells in colostrum, including anti-CD20 (B-cell molecule), anti-CD27 (memory B-cell molecule), and IgD (naïve B-cell molecule). This panel will be tested in breast milk cell pellets of uninfected lactating women.

2. Isolation and characterization of HIV envelope-specific memory B cells from colostrum of HIV-infected, lactating women

Cryopreserved breast milk cell pellets from 20 to 30 HIV-infected, lactating women will be thawed and stained with the optimized B-cell phenotyping fluorescent antibody panel and the fluorescently-labeled HIV envelope protein. The HIV envelope-specific colostrum B memory cells will be enumerated and single-cell sorted into a 96-well plate containing RNA preservation solution using a BD FACSAria™ cell sorter. Human immunoglobulin variable region primers will be used to amplify the antigen-specific portion of the antibody produced by each sorted B cell. These PCR products will then be used to transiently transfect 293T cells with an IgG1 backbone in order to produce a small amount of the colostrum B-cell antibodies for screening. The transfection supernatants will be screened for HIV envelope reactivity by ELISA. Reactive immunoglobulin variable region genes will be cloned into an IgG1 backbone for large scale monoclonal antibody production. The reactive monoclonal antibodies will then be screened for anti-HIV functions including HIV envelope binding, neutralization, and antibody-dependent cell cytotoxicity.

We expect that this process of HIV envelope-specific B-cell staining and single-cell sorting of colostrum cells will be more efficient than nonspecific B-cell isolation at identifying mucosal anti-HIV envelope monoclonal antibodies and determining their breadth and function. Therefore, through this process, we expect to identify a number of novel mucosal HIV-specific monoclonal antibodies with unique anti-HIV functions.

Feasibility:

Since the colostrum cell pellets from HIV-infected women are already collected and IRB approvals are in place, we can begin this work immediately after receiving reagents. A BD FACSAria cell sorter is available for this work in the flow core. Moreover, our lab is equipped with the biolevel 2 laboratory space for sample processing and computers with FlowJo™ analysis software. Moreover, PCR and immunologic assays to produce the monoclonal antibodies and determine their anti-HIV function are in place.

Future applications of data:

This work will form the basis of an NIH NIAID R01 application to determine the unique characteristics and HIV transmission-blocking potential of the isolated colostrum HIV-specific monoclonal antibodies. Specifically, the epitope specificity, autoreactive potential, and protective efficacy of the identified mucosal antibodies will be assessed in neonatal infant monkey/oral SHIV challenge studies.

BD Biosciences reagents:

Reagents that may be used for this project include multiple fluorescently labeled monoclonal antibodies and streptavidin molecules, B-cell enrichment kits, and cell



culture media and plastic ware.

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