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M.86. Optimizing Nanoparticles for Intravaginal Mucosal Immunization

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Intravaginal (ivag) vaccine delivery, which might be an effective way to confer local protection against STD's contracted via the reproductive tract, is limited by the mucus gel barrier, the hormone cycle, and the harsh environment that leads to low residence-time for administered agents. To meet these challenges, we formulated polymeric devices such as fast-diffusing nanoparticles that quickly penetrate the mucus gel, and also long-lasting implants that can slowly release agents into the lumen of the reproductive tract. In this study, we highlight the optimization of nanoparticles made from PLGA (d~150 nm) for ivag delivery by surface modification with PEG to enhance transport through fresh human cervical mucus. Lumen retention time for PEG-modified particles in mice following ivag delivery suggests that these particles are comparable to mucoadhesive (avidin-modified), and 3x better than unmodified PLGA particles ($p<.05$). The amounts of particles associated with mucus and within the epithelium also differ significantly across formulations. Using OVA as a model antigen, antibody production (IgG, IgA) in the vaginal wash and serum following immunization by nanoparticles was compared to those delivered by local implants. Our results demonstrate the potential of ivag immunization to induce local and systemic protection, and the use of PEG-modified nanoparticles as ivag vaccine delivery vehicles.

M.87. Analysis of Mucosal Cellular Immunity to Human Papillomavirus (HPV) Oncoprotein E7 in Cervical T Cell Isolated from Patients with Cervical Intraepithelial Neoplasia (CIN)

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Mucosal cellular immunity (MCI) is required for clearance of cervical intraepithelial neoplasia (CIN), a precursor lesion of cervical cancer. No study has addressed MCI to HPV in cervical mucosa. We here established an assay for mucosal CTL to HPV E7 in cervical T cell. Cervical mucosal cells were collected by Cytobrush from CIN patients and suspended into RPMI media. Monocyte fraction was isolated by Percoll centrifugation. The fraction was analyzed for surface antigen by flow cytometry. Using eighteen synthetic peptides covering entire HPV16 E7 sequence overlapping by 4-8 aa, HPV16 E7-specific IFNγ-producing Th1 cells or GranzymeB-producing CTL were measured by ELISPOT. 2×10^5–1×10^7 (median: 3×10^6) of cervical mucosal monocyte were isolated. Proportion of CD3+ and CD19+ cells in the monocyte were 10–20% and 0.3–5.0%, respectively. Interestingly, 10–15% of the monocyte was CD45+ Integrin α4β7+, indicating gut mucosal-derived lymphocyte. By stimulation with some sequential E7 peptides, IFNγ- and GranzymeB-producing cells were observed in the cervical T cells of all CIN patients. The cervical T cells from most CIN patients recognized E7-CTL epitope for HLA-A02 or -A24. We demonstrated HPV16 E7-specific CTL in the cervical T cells from CIN patients. Our assay will provide correlation of cervical MCI to clinical remission of CIN.

M.88. Typing of TNF Single Nucleotide Polymorphism in Cervical Squamous Intraepithelial Lesions

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Cervical cancer is a health problem in Mexico, with an incidence of 50 cases per 100,000 women. The disease begins with human papillomavirus (HPV) infection, followed by a low-grade squamous intraepithelial lesion (LG-SIL), high-grade SIL (HG-SIL), and finally the development of a carcinoma in situ. In addition to the HPV type, there are genetic factors, such as single nucleotide polymorphisms (SNPs) of the Tumor Necrosis Factor (TNF), that may be involved in the development of cervical cancer. In order to determine if the SNPs in position -308 (G/A) of TNF-α and +252 (G/A) of TNF-β were associated with SIL in HPV positive Mexican women, we studied 211 healthy women without HPV infection and 189 HPV positive patients (split into 129 with LG-SIL and 60 with HG-SIL). TNF and HPV typing was done using PCR-RFLP. Results were statistically analyzed with a X2 test, Fisher's exact p test, and Hardy-Weinberg equilibrium. HPV16 was the most frequent type in patients (50.38%). The allele TNF-α -308G was increased in HG-SIL ($pc<0.05$, OR 3.04 CI 95% (1.01–10.21)) when compared with healthy women. The allele TNF-α -308G could be a genetic marker for the development of HG-SIL in Mexican women.

M.89. Acidity and Lactic Acid in Healthy Cervicovaginal Mucus Inactivate Genital Tract Pathogens

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We have previously measured the acidity, lactic acid and acetic acid concentrations of healthy cervicovaginal mucus under hypoxic conditions, consistent with the low oxygen concentration in the vagina. Here we report the results of tests we have made of the microbicidal activity of physiologic acidity, lactic acid and acetic acid concentrations against 11 species of bacteria associated with the common cervicovaginal infection, bacterial vaginosis, as well as 4 species of vaginal lactobacilli, and 4 STI pathogens (*N. gonorrhoeae, T. vaginalis, H. ducreyi*, and HSV-2). Physiological acidity alone caused significant reduction in viability of 10 BV-associated microbes, but the addition of a physiologically typical concentration of lactic acid abolished all viability of these microbes, and almost completely abolished viability of *N. gonorrhoeae, H. ducreyi* and HSV-2. In contrast, all 4 lactobacilli species retained complete viability. Addition of acetic acid, however, produced little or no increase in microbicidal activity compared to acidity alone.

We conjecture that vaginal microbicide formulations that support both acidity and lactic acid may reduce STI transmission. The broad-spectrum microbicidal action of lactic acid also suggests it might be used as a non-toxic preservative.

M.90. The Inflammatory Response to Chlamydia Infection is Enhanced by Progesterone

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The hormone-responsive human endometrial cell line ECC-1 was cultured for 24 hours with physiological concentrations of estradiol and/or progesterone then infected with *Chlamydia trachomatis* serovar D. RNA was harvested from ECC-1 cells 24 hours post-infection and subjected to microarray analysis. Changes in gene expression between progesterone treatment alone versus progesterone treatment plus *C. trachomatis* infection (set 1) and between no hormone treatment versus *C. trachomatis* infection alone (set 2) were identified. We identified 521 up-regulated genes unique to set 1, genes that were up-regulated by infection in the presence of progesterone but not increased by either infection alone or progesterone alone. Of these genes 42 were up-regulated >20-fold, 16 >50-fold and 9 >100-fold. Among the most up-regulated genes were CX3CL1, CCL2, CCL20, CXCL10, CXCL11, IL-6, IL-8, IL-17C, IL-20, EBI3 and selectin E. These genes are involved in innate immunity and inflammatory pathways and many are regulated by type 1 and type 2 interferons. We are currently investigating the expression of these genes in infected primary endocervical epithelial cells isolated at different stages of the menstrual cycle. These studies may help us to explain how the female sex hormones affect susceptibility to chlamydial infection and the inflammation that develops subsequent to infection.

M.91. Protective Role of Toll-Like Receptor 4 (TLR-4) in Experimental Gonococcal Infection of Female Mice

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Acute gonorrhea in women is characterized by a purulent cervical exudate containing PMNs with intracellular gonococci. The role of TLR4 during cervical infections is unclear since gonorrhea induces cytokines in cervicovaginal epithelial cells, which do not express TLR4. 17β-estradiol-treated BALB/c mice are susceptible to gonococcal lower genital tract infection, which leads to proinflammatory cytokine induction and vaginal PMN influx. Here we examined the role of TLR4 in modulating the innate response and course of infection in the murine gonococcal infection model. Groups of 8 BALB/c and C.C3-tlr4Lps-d/J (TLR4 mutant) mice were inoculated intravaginally with *N. gonorrhoeae* strain FA1090 or PBS. Vaginal mucus was quantitatively cultured for 10 days, and vaginal PMNs and cytokines determined. There was no difference in the duration of colonization between strains. However, a significant difference in the colonization load occurred on days 4–7 of infection with 1–2 logs more bacteria recovered from C.C3-tlr4LPS-d/J mice. Interestingly, these same mice had a significantly greater influx of PMNs. We conclude that TLR4 plays a protective role in controlling *N. gonorrhoeae* lower genital tract infection in the mouse model. While TLR4 independent signals may be capable of recruiting phagocytic cells, TLR4 is required for immune cell function and bacterial clearance.

M.92. The Effect of Female Sex Hormones on *Chlamydia Trachomatis* Gene Expression

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Female sex hormones influence both the host immune response and susceptibility to *C. trachomatis* infection. No studies, however, have investigated how the hormonal environment of the host cell affects actual chlamydial gene expression. The hormone-responsive human endometrial cell line ECC-1 was cultured for 24 hours in physiological concentrations of estradiol and/or progesterone then infected with *C. trachomatis* serovar D at an MOI of 15. Chlamydial gene expression was then investigated using an Affymetrix chlamydial gene array, developed in house. Our data shows that expression of 60 of 1175 chlamydial genes was significantly altered when host cells were cultured with hormones prior to infection, compared to infection of control ECC-1 cells. Of particular interest was the expression of 8 genes (cydA, cydB, pyk, yggV, dnaK, recA, omcB, trpB) in estradiol-supplemented cultures, which showed patterns of gene expression similar to that seen in the interferon-gamma and antibiotic-induced models of chlamydial persistence. These genes are involved in energy acquisition by the replicating Chlamydia, transition of Chlamydia from the replicating RB to the infectious EB and tryptophan metabolism, all of which are altered in chlamydial persistence. The data may help to explain why infections are more common in the estrogen-dominant phase of the menstrual cycle and suggest that estradiol favours the development of persistent infections that may allow Chlamydia to (a) resist common antibiotic therapy and (b) survive the innate immune response to infection, thereby facilitating repeated reactivation of infection that drives damaging immunopathology.

M.93. HIV-1-specific Antibodies in Exposed, but Uninfected Individuals

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Protection from HIV-1 infection in HIV-exposed seronegative (HESN) women was correlated to specific antibodies of the IgA isotype. It was proposed that the HESN sex-workers are protected

by the IgA antibodies, induced in the genital tract by repeated exposure to HIV, that may interact with and neutralize free HIV-1 in mucosal secretions or within epithelial cells. We analyzed sera and vaginal washes from 26 HIV-1-infected and 41 HIV-1-HESN women from Tanzania. These samples were evaluated, in a blinded fashion, for the presence and levels of HIV-specific IgG and IgA antibodies, using ELISA and Western blotting, and for HIV-neutralizing activity. Our results indicate that HIV-1-specific IgG and IgA antibodies were absent in both sera and cervicovaginal washes of HESN women. HIV-1-infected patients had statistically significant higher levels of total IgG, but not IgA, in both serum and cervicovaginal secretions compared to HESN subjects. HIV-neutralizing antibodies were detectable in all sera and in 3 vaginal secretions of HIV-infected women, but only marginal neutralization was observed in 4 of the 41 serum samples from HESN patients, although no HIV-1-specific antibodies were detected in these samples. These results indicate that protection of HIV-exposed but uninfected women is not antibody-mediated.

M.94. Vaginal Acidity and Lactic Acid Measured Hypoxically are More Potent Than Previously Reported

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Previous reports of vaginal pH and lactic acid have provided artifactually high pH and low lactic acid concentrations since observations were generally made under aerobic conditions, rather than the hypoxic condition of the vagina, and did not evaluate the health of the vaginal microbiota. Normal cervicovaginal secretions have been reported to have 0.2% lactic acid, 0.1% acetic acid, and a pH of 4.2. Here we report measurements made under hypoxic conditions, and controlling for changes in partial pressure of CO_2, using cervicovaginal secretions from 18 women with healthy lactobacillus-dominated microbiota as determined by a Nugent score of 0–1. We found a mean lactic acid concentration of 1.2% ± SD 0.2% (range 1.0%–1.6%), and a mean pH of 3.7 ± SD 0.3 (range 3.2–4.4). Acetic acid was generally undetectable (limit of detection = 0.005%). Aerobic conditions rapidly caused a loss of lactic acid and rise of acetic acid, presumably via bacterial oxidative conversion of lactic acid. In anaerobic secretions, pH decreased linearly with lactic acid concentration pH = 5.4–1.4 (% lactic acid), r^2 = 0.92, and the lactate D/L isomer ratio ranged between 38–62%.

M.95. Role of Integrin Receptors and Junctional Proteins in Macrophage Migration through a Vaginal Epithelial Tissue Model

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Seminal macrophages can carry HIV-1 and other STD pathogens, and may serve as Trojan Horse vectors of disease transmission. We recently developed a model for studies on macrophage attachment and infiltration into human vaginal epithelial tissue. When labeled macrophages are added to the apical (luminal) surface of human vaginal organotypic cultures (MatTek EpiVaginal™ model), their adherence and infiltration into the tissue can be documented by confocal microscopy. The purpose of this study was to identify integrin receptors and junctional proteins that may play a role in macrophage infiltration of vaginal tissue. Seminal macrophages express high levels of integrin receptors CD11/a (LFA-1) and CD11/b (MAC-1), classical counter-receptors for ICAM-1, JAM-A and JAM-C. ICAM-1, JAM-A and JAM-C are expressed by epithelial cells in the EpiVaginal model, and their distribution patterns closely mimic those in the native tissue. Treatment of EpiVaginal cultures with TNF-alpha and PMA dramatically enhanced the expression of ICAM-1 in the epithelium and lamina propria. Peptides or antibodies that block these adhesion molecules might prove to be useful components in vaginal microbicides to prevent the sexual transmission of HIV-1.

M.96. Selective Estrogen Receptor Modulators (SERMs) Regulate the Production of MIP3α and Mouse KC (IL-8) by Uterine Epithelial Cells

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SERMs are widely utilized in the treatment of estrogen dependent tumors by interacting with estrogen receptors (ER) α and/or β altering gene transcription. This study investigates the effect of a number of different SERMs on isolated uterine epithelial cell production of MIP3α/CCL20, which has antimicrobial properties, and mouse KC (IL-8) *in vitro*. Whereas estradiol treatment inhibits the production of MIP3α/CCL20 the present study demonstrates that SERMs with specificity to ERα (ICI 182,780 and Y134) stimulate the production of MIP3α/CCL20 within 24 hrs. Tamoxifen (ER agonist/antagonist) and PHTPP (ERβ antagonist) had no effect on the production of MIP3α/CCL20. This suggests that regulation of cytokines and chemokines produced by epithelial cells occurs through ER-ligand interactions, particularly ERα. As a part of these studies, we found that ICI 182,780 and Y134 inhibited KC by uterine epithelial cells. This work provides a foundation to understanding the role for ERα specific SERMs as mediators of innate immunity. Specifically, selected SERMS have the potential to enhance antimicrobial protection against pathogens without recruiting pro-inflammatory immune cells that may compromise reproductive function and/or the risk inflammation of the mucosae. Supported by NIH grant AI-013541.