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The Xs and Y of immune responses to viral vaccines

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Abstract

The biological differences associated with an individual's sex represent a major source of variation that impacts the immune response to vaccination. There are compelling clinical data illustrating that males and females differ in their innate, humoral, and cell-mediated responses to viral vaccines. Sex impacts the frequency and severity of adverse side effects of vaccination, including fever, pain, and inflammation. Pregnancy also can significantly alter immune responses to vaccines. Key aspects of the impact of sex and pregnancy on vaccination are presented using data drawn from clinical trials in humans as well as from animal models of vaccine efficacy and the groundwork is laid for future studies aimed at identifying the biological mechanisms, including genetic and hormonal factors, that underlie sex-specific responses to vaccines. An understanding and appreciation of the impact of sex and pregnancy on immune responses may alter the strategies used by public health officials to initiate efficient vaccination programs in the human population optimizing the timing and dose of the vaccine in order to maximize the number of people immunized, ensure sufficient levels of immune responses, minimize adverse side effects, and allow for more efficient protection of high priority populations.

Keywords

androgen; estrogen; gene polymorphism; hepatitis; herpes; influenza; measles; pregnancy; progesterone; sex difference; sex steroids; vaccine; virus; x-linked genes; yellow fever virus

Introduction

There are profound differences between the sexes in response to viral infections that are caused by differential disease pathogenesis (1–3). There also is increasing awareness that much of the disease attributed to virus infection results from aberrant host inflammatory

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responses (2). Consequently, heightened antiviral, inflammatory, and cellular immune responses in females, while essential for virus clearance, may underlie increased development of symptoms of disease among females as compared with males following infection (1, 3). Currently, the mechanisms mediating sex differences in response to viral infections are being examined empirically in both laboratory and clinical settings (1).

Whether responses to vaccines and other therapeutic treatments differ between the sexes is considerably less well characterized (4, 5). We propose that one of the most fundamental ways in which individuals vary with respect to vaccine-induced immune responses is based on their *sex*, which refers to the classification of males and females based on reproductive organs and functions as derived from sex chromosomal complement (6). Although studies report sex-based differences in immune responses and adverse side effects following immunization, the mechanisms mediating these differences in response to viral vaccines have not been systematically examined. Utilizing data from clinical trials, we illustrate that sex differences in response to viral vaccines are conserved and impact innate, humoral, and cell-mediated immune responses as well as adverse side effects following vaccination. There are significant gaps in our understanding of the precise mechanisms mediating sex-dependent responses to vaccines, but we provide evidence that genetic and hormonal factors impact sex-based and pregnancy-associated differences in immune responses. Finally, we highlight the areas of research critically important to advance our understanding of sex-dependent responses to vaccines. Appreciating that sex-based differences in responses to vaccination exist will have an important public health impact, as it may allow for better determination of vaccine doses which minimize adverse side effects while maximizing vaccine availability and efficacy in the general population.

Sex differences in responses to viral vaccines

Sex differences are reported in response to both childhood and adult vaccination (Table 1). Although numerous studies report that immune responses and adverse side effects following immunization differ between the sexes, the mechanisms mediating sex-based differences in response to viral vaccines have not been systematically examined and may differ based on the vaccine used and the type of protective immune responses elicited.

Yellow Fever Virus vaccine

Yellow fever virus (YFV) is a mosquito-borne virus that causes morbidity and mortality throughout the world, but is endemic to South America, the Caribbean, and Africa. The YFV vaccine strain, 17D, is administered subcutaneously and replicates extensively, generating a viremia but with markedly reduced clinical symptoms of infection (7). Of considerable concern to public health officials is the severity of adverse events following 17D vaccination. An analysis of reported adverse effects following YF vaccination to the U.S. Vaccine Adverse Event Reporting System (VAERS; 2000–2006) indicates that a majority of reported adverse events occur in adult females (61%), and typically appear within 1–2 days after vaccination (8). The most commonly reported adverse side effects in females following YF vaccination are fever, pain, pruritus, headache, injection site erythema, urticaria, rash, nausea, dizziness, dyspnea, and fatigue (8). Females also report more local inflammation

than do males, suggesting that heightened innate immune responses in females may underlie development of adverse side effects following vaccination. Because the VAERS is a passive reporting system, whether females are more likely to report adverse side effects than males must also be considered.

Understanding the human immune reponse to live, attenuated YFV 17D vaccine has been the focus of number of recent studies that have employed a 'systems biology approach' to show that innate immune gene transcriptional profiles, including the expression of toll-like receptors (TLR) and interferon (IFN)-associated genes, immediately after vaccination with YF17D predict subsquent adaptive immune responses (9, 10). These data were analyzed without consideration of the sex of the volunteers. We recently conducted a reanalysis of the publicly accessible microarray data from this study (GEO accession #: GSE13699) to test the hypothesis that women mount higher innate immune responses to vaccination than men and to illustrate that sex is a fundamental predictor of responses to vaccines that often is overlooked. In the original paper (9), 594 genes were differentially expressed between Day 0 and any time post-vaccination; our analysis (2-way ANOVA followed by contrast analyses, false discovery rate [FDR] $p < 0.05$; Partek Inc., St. Louis, MO) reveals that 660 genes are differentially expressed at either 3, 7, or 10 days after vaccination in women and only 67 genes are differentially expressed in men (Figure 1,). Using the genes that were classified into functional categories associated with innate and adaptive immunity (e.g., complement, TLR/IFN, macrophages, DCs, NK cells, B cells, and inflammation) (9), we have established that most of the reported TLR-associated genes that activate the IFN pathway are significantly upregulated in women compared with men during the first 10 days after vaccination (see Figure 1 legend for additional methodological details). If the innate immune response to vaccination predicts subsequent adaptive immune responses and protection, then our reanalysis suggest that YF17D protection may be greater in females than males. Because adverse side effects from YF17D are hypothesized to be mediated by inflammatory cytokines and chemokines, elevated innate immune responses to the vaccine may also underly the higher side effects following vaccination in females.

Data on the humoral immune responses to YFV vaccination have not demonstrated any consistent trend with respect to the vaccinee's sex (11–14), which is likely due to the robust nature of 17D vaccine replication in the host and the choice of high vaccine doses that are designed to protect both males and females from YFV. The sex differences seen in response to YFV vaccine manifest more clearly in the early innate immune response to the vaccine and in adverse side effects of vaccination, than in gross vaccine efficacy endpoints.

Influenza viruses

Vaccination against infection with influenza viruses occurs annually due to constant antigenic changes that accumulate in circulating virus strains (15). The most commonly used influenza vaccine is trivalent, inactivated influenza (TIV) which consists of formalin inactivated, partially purified viral components delivered via intramuscular inoculation (15). A live attenuated influenza vaccine (LAIV) also is available to individuals between the ages of 2 and 49 and is administered intranasally. Although rates of immunization against influenza viruses are similar between the sexes (16–18), humoral immune responses, most

often measured by hemagglutination inhibition titers (HAI), to influenza virus vaccines are consistently higher in women than men (4, 19–21). A randomized, prospective, single-blind study of humoral immune responses to TIV reported that healthy women (18–64 years of age) generate a more robust protective antibody response and that the response of women to a half dose of the influenza vaccine is equivalent to the antibody response of men to the full dose (20). Women consistently report more severe local and systemic reactions to influenza virus vaccines (20–24), sometimes by a factor of 2- to 3-fold or higher, which seems to be unrelated to the documented sex-associated increase adverse responses to adjuvants (25) because several of the studies utilized TIV administered without adjuvant (20, 23).

Measles, Mumps, and Rubella vaccines

The measles-mumps-rubella (MMR) vaccine consists of three virus strains that have been attenuated for virulence and replication through extensive passage in a number of different tissue culture systems (26). The current vaccine recommendations require two immunizations, the first between 12–15 months and the second at 4–6 years of age (26). Rates of MMR vaccination are similar between girls and boys (27), with the possible exception of countries where girls have reduced access to health services than boys (28). A cross-sectional study of children over 15 years of age, who received the MMR vaccine at 12–15 months after birth, indicated that the prevalence of serum IgG antibodies against measles, mumps, and rubella was significantly higher in girls than boys (29). Regression models indicate that age at the time of immunization and female sex are the two most significant predictors of antibody persistence following MMR vaccination (30). In a nine year follow-up study of children who received two doses of the MMR vaccine, the geometric mean titers of anti-mumps antibodies were equivalent in pre-pubertal girls and boys (31). A study of pre-pubertal males and females who received two doses of the MMR vaccine showed that rubella virus-specific antibody responses as well as lymphocyte proliferative responses to rubella virus peptides were transiently higher in males than females 2–4 weeks after vaccination, but this sex difference was no longer apparent 10 weeks post-vaccination (32). Whether memory responses to the MMR vaccine differ between the sexes requires further assessment. Adverse reactions, including fever, parotitis, and joint and limb pain, are higher in females than in males (6–13 years of age) up to 14 weeks after the second immunization with the MMR vaccine (33, 34), except for the risk of immune thrombocytopenic purpura which is higher in males (35).

The Schwarz measles vaccine is a low titer viral vaccine that is only offered to infants starting at 9 months of age. This measles vaccine does not protect infants against infection during the period between when maternal antibody begins to decline and immunization occurs (i.e., from 4–9 months of age). Administration of the Edmonston-Zagreb high titer measles vaccine to infants < 9 months of age was initiated by the World Health Organization in the late 1980s in regions of West Africa. A long-term follow up study of over 3,000 children inoculated with the standard, medium, and high titer measles vaccines revealed that by at least 3 years of age, mortality rates were twice as high for females than males in response to the Edmonston-Zagreb high titer measles vaccine (36). It was striking that the reduced survival related to the high titer measles vaccine compared with the standard titer vaccine occurred almost solely in females and lead to termination of the vaccine trials.

Whether females and males differed in their immunological responses to the high titer measles vaccine has not been reported.

Hepatitis A and B virus vaccines

Vaccination against hepatitis A and B viruses represents another field where immunization of large numbers of adults can provide important insights into the role of sex in vaccine-induced immune responses. The hepatitis A virus (HAV) vaccine has been in use since the early 1990s and consists of formalin inactive virus that is formulated in a number of different ways depending on the vaccine manufacturer (37). The hepatitis B virus (HBV) vaccine consists of self assembled oligomers of the HBV surface antigen (sAG) which are purified from cultures of *Saccharomyces cerevisiae* carrying the HBsAG gene (38). The in vitro correlates of protection for HAV and HBV have not been rigorously assessed but total antibody titers as determined by ELISA correlate with protection from infection.

Although rates of seroconversion are similar between the sexes (39), among adults, females consistently mount higher anti-HAV antibody responses than do males (39–43). Adverse side effects following HAV vaccination are frequent, but minimal and include fatigue, soreness at the site of injection, and edema. Whether adverse side effects in response to the HAV vaccine differ between the sexes has not been reported but because the vaccine is administered with adjuvants, it may be expected that females would report adverse side effects of greater severity and with greater frequency (25). Following vaccination against HBV, among both children and adults, anti-HBV antibody titers are higher in females than males (44–48). Immunization with the combined HAV/HBV vaccine also results in higher antibody titers among adult women than men (49, 50). In multivariate analyses, being male is a significant predictor of being ‘non-responsive’ to the HBV vaccine; thus, adult females show higher rates of seroconversion following exposure to the HBV vaccine than do males (51). Loss of antibody against the HBV vaccine during the first 10 years of life, however, is not different between males and females (52). Among elderly persons (> 60 years of age), the rate of seroconversion following vaccination with the combined HAV/HBV vaccine is similar between the sexes (53).

Herpes simplex viruses

Infection with herpes simplex virus type 2 (HSV-2) leads to the establishment of a latent infection from which the virus periodically reactivates causing genital lesions, whereas oral lesions are the primary result from infection with the related HSV-1. Vaccines based on glycoproteins B and D (gB and gD) of HSV-2 have undergone extensive clinical trials which showed no significant overall protection from infection or in the time to acquisition of infection when all study participants were included (54). When sex was considered in the time to acquisition of infection, however, protection was significantly higher in females than in males (26% versus −4%). In phase 1 and 2 studies of a recombinant gD-based HSV-2 vaccine, there was no significant protection from acquisition of HSV-2 infection in HSV-1 and HSV −2 seronegative subjects, but a sex-bias in protection was seen (overall efficacy 38%, efficacy in females 73%, efficacy in males 11%) (55). The same was true in a phase 2 trial which showed no overall efficacy, but a significant efficacy difference between HSV-1 and HSV-2 seronegative females (74%) and males (−10%). Greater side effects were

reported in females over the first 4 days following injection, but females administered the placebo also reported more side effects (56). The gD vaccine was formulated with a modified alum adjuvant which has been documented to cause greater side effects in females (25), perhaps due to a stronger innate immune responses induced in females.

Neither study was able to demonstrate sex-specific differences in total antibody, neutralizing antibody, or T cell responses to vaccination (54–56); it is important, however, to note that an in vitro correlate of protection for HSV-2 vaccines has yet to be defined and may differ between males and females due to anatomical differences and differences in local antibody production in the site of virus infection. In contrast, HSV-2 seropositive females have been shown to have significantly more T cells and stronger T cell responses (i.e., IFN-γ production) to three out of four HLA-DR-restricted gD peptides (57). It may be that a detailed comparison of the female response to gD vaccine could provide important information regarding the nature of the protective immune responses that need to be induced by a HSV-2 gD based vaccine.

Animal models have been instrumental for illustrating that the hormonal milieu at the time of vaccination impacts protection against HSV-2 infection. In ovariectomized female mice, immunization with an attenuated strain of HSV-2 only protects against challenge with wild-type HSV-2 when females are treated with progesterone (P4), but not 17β-estradiol (E2). P4, administered either alone or in combination with E2, reduces HSV-2 replication in the reproductive tract by increasing the number of dendritic cells (DCs) and T cells in the vaginal lamina propria and increasing titers of gB-specific vaginal IgA (58). In HLA-DR*0101 and HLA-DR*0401 transgenic mice, following ocular infection with HSV-1, CD4+ T cell responses are higher in females than in males and immunization of transgenic mice with a cocktail of three immunodominant gD peptide epitopes that are more highly expressed in women than in men increases CD4+ T cell immune responses against HSV-1 (57). Further, immunization of female mice with regular estrous cycles with a recombinant adenovirus vector expressing HSV gB results in higher titers of gB-specific vaginal IgA during estrus than during either diestrus or proestrus and exogenous administration of P4 to female mice at the time of immunization protects females from lethal intravaginal HSV-2 challenge (59), indicating that sex steroids affect induction of protective immunity following vaccination against HSVs.

Vaccination and pregnancy

The induction of immune responses to vaccines may be altered by pregnancy. E2 and P4 concentrations are considerably higher during pregnancy than during other times in the female reproductive cycle and increase over the course of pregnancy, with highest levels achieved during the third trimester (Figure 2). These hormonal changes that occur during pregnancy are hypothesized to underlie some of the distinct immunological changes associated with pregnancy. Elevated levels of P4 stimulate synthesis of progesterone-induced binding factor (PIBF) by lymphocytes. PIBF increases over the course of pregnancy and drops significantly after birth, but in pathological pregnancies that result in preterm labor, abortion, or hypertension, concentrations of PIBF are low (60). High concentrations of PIBF promote differentiation of $CD4^+$ T cells into helper T cell type 2 (Th2) cells that produce

high concentrations of cytokines, including IL-4, IL-5, and IL-6 (61). The Th2 bias that occurs during pregnancy is paralleled by a reduction in Th1 responses (e.g., production of IFN-γ) (62, 63) (Figure 2). If the Th2 bias during pregnancy is altered, by infection for example, this can result in preterm labor or abortion (64). Elevated Th2 responses also correlate with increased antibody responses during pregnancy (65). Concurrent with the increase in Th2 responses during normal pregnancy is an increase in the activity of regulatory T cells at the maternal-fetal interface (66). Migration of regulatory T cells to the pregnant uterus is mediated by human chorionic gonadotropin (hCG), which is a chemoattractant protein secreted by the blastocyst after fertilization (67). Regulatory T cells are hypothesized to orchestrate immune tolerance of the fetus during pregnancy. Whether these hormonal and immunological changes that occur during pregnancy alter responses to vaccination must be considered.

Pregnant women and their fetuses may have increased susceptibility to a number of viral infections that require Th1 responses for effective control and clearance. Specific recommendations for vaccinating pregnant women are provided by the Centers of Disease Control (68). In general, there are no contraindications for vaccination with inactivated or subunit vaccines, but vaccination with live, attenuated vaccines is not recommended, unless the risk of infection with the pathogen is deemed excessively high. Concerns over the increased susceptibility of a fetus to infection with a live, attenuated viral vaccine strain drives this moratorium although clinical data supporting or refuting this claim are scarce. In general, vaccination of pregnant women serves to both protect women from increased susceptibility to virus infections and protect fetuses from acquiring an infection. Also of concern are the possible negative side effects of vaccination on the outcome of pregnancy, including an increased probability of preterm labor, low birth weight, and spontaneous abortion (69).

The impact of pregnancy on immune responses to vaccines as well as the effect of vaccination on the outcome of pregnancy must be weighed and considered in all studies of vaccine efficacy. Pregnant women are not immunosuppressed, but rather their immune responses are shifted toward an anti-inflammatory phenotype, which will likely influence cell-mediated and humoral responses to vaccines (Figure 2). Because the hormonal and immunological milieu of pregnant women is strikingly different from that of non-pregnant women, these populations should be considered separately when analyzing vaccines efficacy. Further, when interpreting clinical data from pregnant and non-pregnant women, consideration must be given to the fact that the etiology of adverse side effects following vaccination may differ as well.

Mechanisms of sex-based differences in immunity

Sex differences in innate immunity

Males and females differ in their innate immune responses (Figure 3). For example, innate detection of nucleic acids by pattern recognition receptors (PRRs) differs between the sexes (70). Utilization of global expression analyses of sex differences in innate immunity in animal models reveals profound differences between the sexes in the induction of genes associated with TLR pathways and induction of type I IFN responses (71). Steroid hormones

bound to their receptors can bind to specific hormone response elements in the promoter regions of hormone-responsive genes. Putative androgen and estrogen response elements (EREs) are present in the promoters of several innate immunity genes, suggesting the propensity for sex steroids to directly affect sex differences in innate immune responses (71).

Studies of both humans and rodents illustrate that the number and activity of immune cells as well as inflammatory immune responses in general are higher in females than males and may explain why women are more likely to develop inflammatory autoimmune diseases (72–74). For example, macrophages derived from female, but not male, mice preferentially secrete IL-12 following nonspecific T cell activation (75). Antigen presenting cells from females are more efficient at presenting peptides than are APC from males (76). In contrast, women with regular menstrual cycles as well as women tested during the luteal phase of their menstrual cycle have lower NK cell activity than men (77, 78). These studies merely provide a glimpse into the ways in which the innate immune response differs between the sexes.

Sex differences in adaptive immunity

Generally, females exhibit greater humoral and cell-mediated immune responses to antigenic stimulation, vaccination, and infection than do males (Figure 3) (5). Both basal levels of immunoglobulin (Ig) (79) as well as antibody responses to viruses and vaccines are consistently higher in females than males (1, 4). Clinical studies reveal that men have lower $CD3^+$ and $CD4^+$ cell counts, $CD4^+$:$CD8^+$ cell ratios, and Th1 responses (80–83). Studies in mice further reveal that cytokine responses of $CD4^+$ T cells often differ between males and females with females reportedly exhibiting higher Th1 (i.e., IFN-γ), Th2 (i.e., IL-4), and regulatory T cell (i.e., IL-10) responses than males depending on stage of infection or type of antigen encountered (84–86). Female mice also have higher proportions of regulatory T cells than males, at least in response to certain viruses (e.g., coxsackievirus) (87). Females exhibit higher cytotoxic T cell activity along with upregulated expression of antiviral and proinflammatory genes, many of which have EREs in their promoters (88).

Sex steroids modulate sex differences in immunity

The prevailing hypothesis for immunological differences between the sexes is that sex steroids, particularly testosterone (T), E2, and P4, influence the functioning of immune cells (Figure 3). Sex steroids alter the functioning of immune cells by binding to specific receptors, which are expressed in various lymphoid tissue cells as well as in circulating lymphocytes, macrophages, and DCs (89). The binding of sex steroids to their respective steroid receptors directly influences cell signaling pathways, including NF-κB, cJun, and IRF1, resulting in differential production of cytokines and chemokines (90, 91).

Androgens, including dihydrotestosterone (DHT) and T, suppress the activity of immune cells (84, 92–95). In contrast, T and DHT increase synthesis of anti-inflammatory cytokines, such as IL-10 (92, 96). Men with androgen deficiencies have higher inflammatory cytokine (e.g., IL-1β, IL-2, TNF-α) concentrations, antibody titers, and $CD4^+$:$CD8^+$ T cell ratios than healthy men (97–99). Signaling through the androgen receptor antagonizes transcriptional

factors (e.g., NF-κB and cJun) that mediate the production of proinflammatory and antiviral cytokines (90).

Estrogen receptors (ERs) are expressed in various lymphoid tissues as well as in circulating lymphocytes, macrophages, and DCs (89). There are two subtypes of the receptor for estrogens, ERα and ERβ, that are differentially expressed in subsets of immune cells (100); thus, the differential effects of estrogens on immune function likely reflect both the concentration of the hormone and the distribution and type of ER in the immune cell. Exposure of NK cells or macrophages to physiological concentrations of E2 increases the synthesis of proinflammatory cytokines (101–103). E2 can have bipotential effects, with low doses enhancing proinflammatory cytokine production (e.g., IL-1, IL-6, and TNF-α) and high concentrations reducing production of these cytokines (104). Estrogens affect the differentiation and functioning of DCs, which are critical cells that influence the nature and magnitude of the immune response to a vaccine. In vitro exposure to E2 affects the differentiation of bone marrow precursor cells into functional DCs (105, 106) and increases the synthesis of chemokines by immature DCs (107), but can downregulate antiviral responses (108). Treatment of ovariectomized mice with E2 increases the number and activity of subsets of DCs (109, 110).

Generally, low E2 concentrations promote Th1 responses and cell-mediated immunity and high concentrations of E2 augment Th2 responses and humoral immunity (111). E2 at physiological concentrations induces the expression of the transcriptional factor T-bet and production of IFN-γ by Th1 cells though IL-27, but not IL-12, in splenocytes from mice (112). Conversely, at high doses, E2 reduces the expression of IRF1 which downregulates IFN-γ production (91) and may explain the dose-dependent effects of E2 on Th1 and Th2 immune responses. Estrogens enhance the expansion of $CD4^+CD25^+$ T cells in mice (113) and humans (114). Treatment of mice with high doses of E2 decreases production of IL-17 by Th17 cells (115). Estrogens at physiological concentrations can stimulate antibody production by B cells, partially by inhibiting T cell suppression of B cell functions (116). Levels of Ig are highest prior to ovulation in women (117). E2 also activates the expression of activation-induced deaminase (AID) which enhances somatic hypermutation and class switch recombination in B cells (118). Although the impact of E2 on B cell activity and development of antibody-mediated autoimmune diseases have been characterized, considerably less is known about the role of E2 in modulating antibody responses to vaccines.

P4 can have both stimulatory and suppressive effects on the immune system, but is typically regarded as immunosuppressive. P4 receptors (PR) have been identified in epithelial cells, mast cells, eosinophils, macrophages, DCs, and lymphocytes (89). PR distribution may explain sex-specific effects of P4. For example, the expression of PRs is higher in DCs from females, which may explain why P4 is better able to suppress the activity (e.g., secretion of TNF-α) of DCs from female than male rats (119). P4 suppresses innate immune responses as well as NF-κB signal transduction (90, 109, 120–125). Elevated concentrations of P4 during pregnancy (Figure 2) modulate the development of Th1 immune responses and promote production of Th2 immune responses, including IL-4 and IL-5 production (126, 127). P4 also suppresses antibody production (116).

Host genes affect sex differences in immunity

The extent to which the differences in the gene complement between females and males underlie sexually dimorphic immune responses to vaccines is underappreciated (Figure 3). Whether sex chromosomal genes modulate sex differences in responses to vaccines has not been reported but appear to impact the development of autoimmune disease in animal models. Utilization of mice with the sex determining region Y (*Sry*) gene either deleted (XY-*Sry*) or translocated to an autosomal region (XX*Sry*) enables investigators to separate gonadal sex (i.e., the presence of ovaries or testes) from sex chromosome complement (i.e., XX or XY) (128, 129). The effect of sex chromosome complement on susceptibility to experimental autoimmune encephalitis (EAE) is associated with reduced production of Th2 cytokines, including IL-4, IL-5, and IL-13, and increased expression of *IL13ra2* (i.e., a gene encoded on the X chromosome) on macrophages and DCs of XX mice (129). The *Tlr7* gene is another gene encoded on the X chromosome that may escape X inactivation, resulting in higher expression levels of *Tlr7* in females than males (130). Consequently, DCs isolated from women produce more IFN-α in response to TLR7 ligands, including HIV-1 encoded TLR7 ligands, than do DCs from men (131). Polymorphisms in Y chromosome genes also affect sex-dependent susceptibility to autoimmune disease (132).

Polymorphisms or variability in sex chromosomal genes as well as in autosomal genes that encode for immunological proteins can influence immune responses to vaccines as well as development of adverse side effects (133). For example, sex-based differences in HLA alleles contribute to the higher antibody responses of girls than boys to measles vaccination (134). Polymorphisms in the *Il10* gene are associated with differential antibody responses in girls; conversely, polymorphisms in the *Il4r* gene are better predictors of antibody responses in boys (135). In response to the HAV vaccine, polymorphisms in the *Il10* promoter as well as the age and sex of an individual are significant predictors of the strength of the antibody response (136). In response to the mumps vaccine, girls generate higher antibody responses than do boys and polymorphisms in the cytokine receptor genes *Il12rb1* and *Il12rb2* are associated with increased antibody production (137). Whether sex-based differences in the expression of gene variants are caused by differential selection pressures acting on each sex, hormone-dependent effects, or epigenetic mechanisms remain to be determined (135). Polymorphisms in immune-related genes provide a novel area for future studies examining the genetic mechanisms that mediate sex differences in immune responses and adverse side effects following vaccination.

Conclusions

Despite data supporting a role for sex in the response to vaccines, most studies do not document sex-specific effects in vaccine efficacy or induced immune responses. There may be several likely reasons for the lack of sex-specific analyses: 1) sex may not be considered a significant variable when statistical analyses of vaccine-induced immune responses are performed; 2) representation of female and male subjects in a vaccine trial may not provide the appropriate statistical power to delineate sex-specific alterations in immune responses or adverse events; 3) because the inherent goal of vaccine efficacy studies is to determine the vaccine dosage that generates the strongest immune responses in the largest number of

subjects, the robust immune responses to such large doses of vaccine may mask the sex-specific alterations in immune responses; and 4) many vaccine studies focus only on general immune responses, such as antiviral antibody titers in serum or total T cell responses, at late times post vaccination, which will not identify sex-specific alterations in the kinetics of the induced immune responses.

There are three areas of research which could drive future studies of sex-specific immune responses to vaccines. Table 2 summarizes the major areas of research and the key questions regarding the role of sex, hormones, genes, and pregnancy as factors influencing immune responses to vaccines. Clinical studies of vaccine efficacy in humans represent the most direct and relevant way of assessing the role of sex in immune responses to vaccines. Reanalyzing existing clinical data using sex as an independent variable for all parameters could elucidate a number of sex-dependent responses as demonstrated by our analysis of YFV vaccine-induced innate immune responses (Figure 1). By incorporating more frequent biological sample collection and a more extensive analysis of the immune response to vaccines, the temporal changes associated with sex-specific immune responses could be uncovered. The availability of a number of small animal model systems that mimic human disease, along with the expanding availability of transgenic and knockout mice, will allow for detailed, mechanistic studies on sex-dependent immune responses to vaccines. The effects of pregnancy on vaccine efficacy is an area of research that is not actively undertaken in humans due to the perceived and real risks to the mother and fetus, but that could be readily assessed using small animal models. Because many different cell types contribute to the immune response to a vaccine, it is important to develop primary cell culture systems that will assess the sex-dependent responses of individual cell or tissue types to immune stimuli. Care must be taken to maintain these cultures in conditions that will allow for the determination of sex-dependent immune responses, which will drastically increase our understanding of cell-type specific, sex-dependent immune responses.

Rates of vaccination are generally similar between the sexes, suggesting that the societal and behavioral differences can be separated from the biological sex differences in response to vaccines. Our awareness of the profound variability of immune responses in human populations is growing. Advances in our ability to monitor human immune responses now allow us to identify and investigate the factors that could explain heterogeneous responses to vaccines. The impact of sex and pregnancy should be considered in all guidelines and recommendations for vaccines and represent an opportunity for researchers and public health officials to better understand and explain individual variability in vaccine efficacy.

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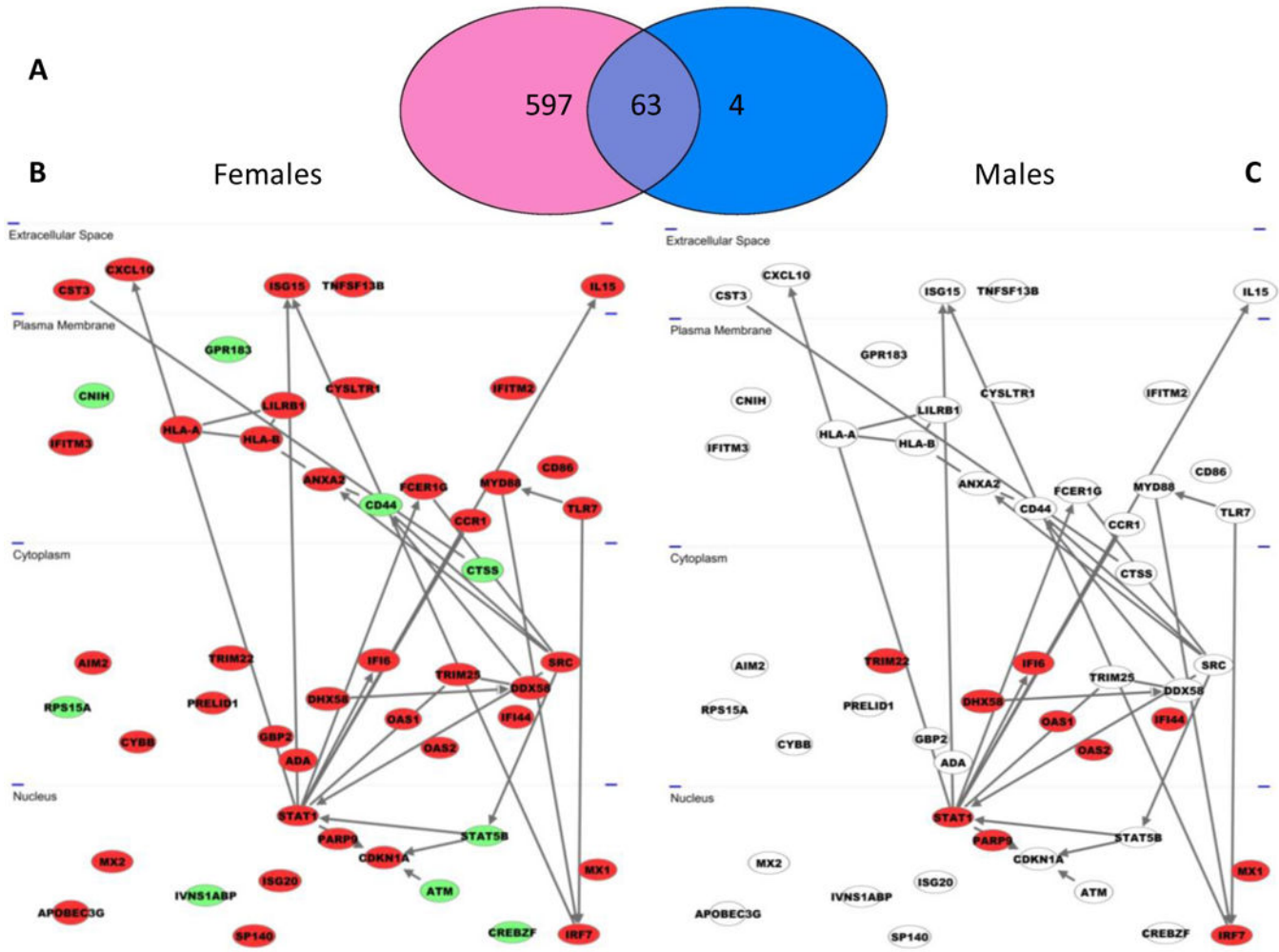

Figure 1: YFV-17D vaccination induced TLR/IFN signaling is significantly higher in women than men.

(**A**) Separate lists of genes for females and males are visualized with a Venn diagram illustrating unique and common genes expressed in women (pink) and men (blue) 3–10 days after YFV-17D vaccination. Ingenuity Pathways Analysis of innate immunity genes identified as being important predictors of adaptive responses to YFV-17D vaccination (adapted from Figure 3 and Supplemental document 2 from Gaucher et al. 2008) and the changes in expression specific to female (**B**) and male (**C**) study participants (2-way ANOVA with FDR $p < 0.05$). In the pathway analysis, node colors indicate fold change of gene expression (red = 2 to 18 fold upregulation; green = −1.2 to −2.3 downregulation; white = n.s. change from baseline) between Day 0 and 7 in women (n = 11) and men (n = 5). Illumina microarray data (GSE13699) were imported into Partek Genomics Suite and a 2-way ANOVA was conducted using time post-vaccination (i.e., Days 0, 3, 7, and 10) and sex (male and female) as the independent variables. Contrast analyses were conducted as described in the original paper (Gaucher et al. 2008) to compare gene expression values at 3, 7, and 10 days post-vaccination to Day 0 (i.e., pre-vaccination) values for females and males separately. Lists of genes were generated after establishing the False Discovery Rate $p <$ 0.05 and using a fold-change cut off of ≥2. Analyses were conducted on the Montreal

dataset; one woman was omitted because the overall gene expression Principle Component Analysis (PCA) revealed she was an outlier (YF019) and one man from Lausanne (YF10) was included in our analyses because the PCA analysis revealed that expression levels of genes from this man were similar to those from the 4 Montreal men.

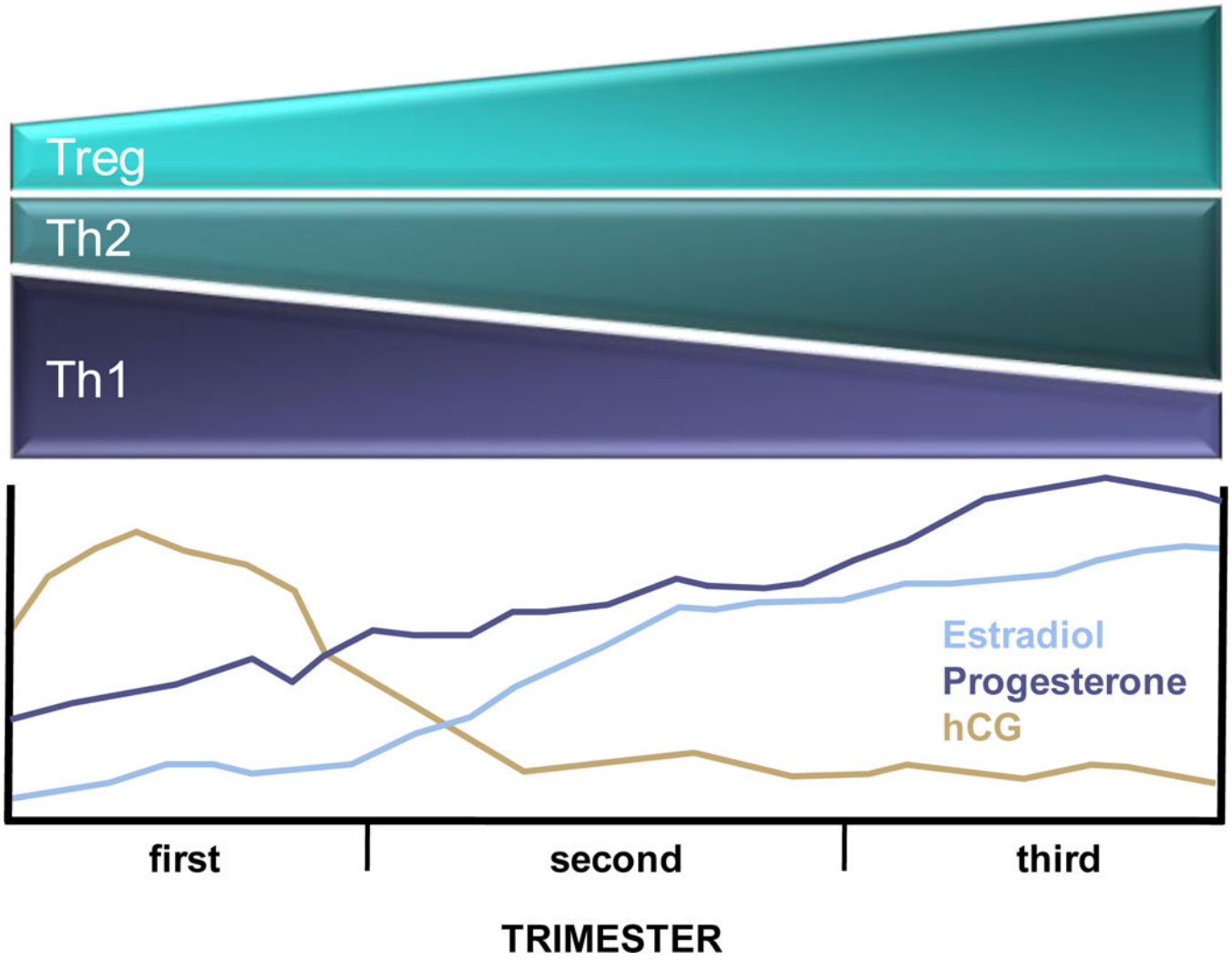

Figure 2: Hormonal changes during pregnancy affect T cell responses.

The upper panel illustrates the shift in the T helper 1 (Th1) versus T helper 2 (Th2) balance toward a Th2 -bias by the third trimester of pregnancy and the corresponding changes in regulatory T (Treg) cell activity. The bottom panel illustrates the variations in estradiol, progesterone, and human chorionic gonadotropin (hCG) during the trimesters of pregnancy. Variations in sex hormone levels can lead to significant alterations in T cell activity during pregnancy.

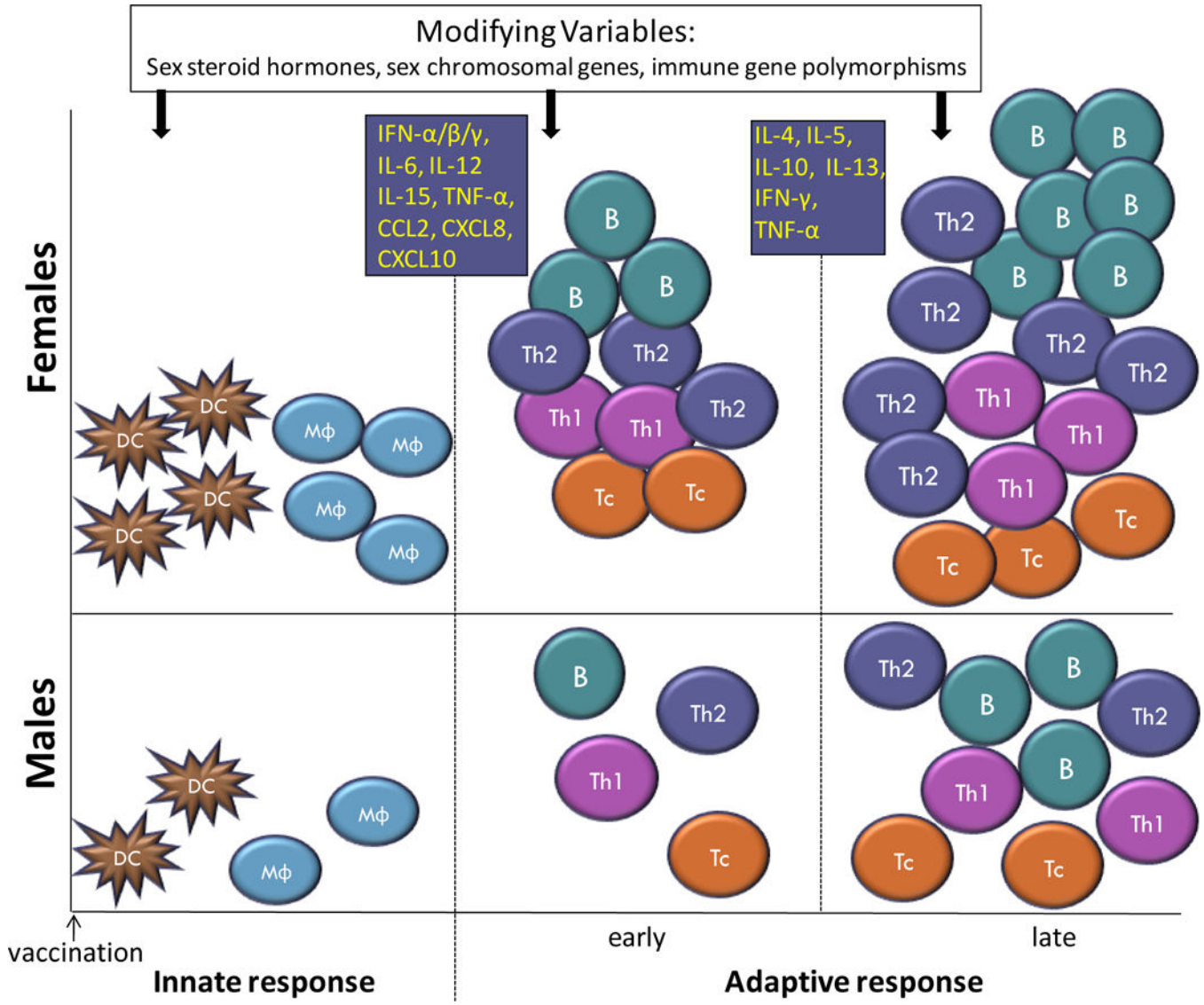

Figure 3: Sex –based differences in innate and adaptive immune responses following vaccination.

Following vaccination, the activity of innate immune cells, including dendritic cells (DCs) and macrophages (MΦ), and the production of inflammatory cytokines (e.g., interferon [IFN] α/β/γ, interleukin [IL]-6, IL-12, IL-15, and tumor necrosis factor [TNF]-α) and chemokines (e.g., CC-chemokine ligand 2 [CCL2, also called MCP-1], CX-chemokine ligand [CXCL8, also called IL-8], and CXCL10 [also called IP-10]) is elevated in females compared with males. The increased number and activity of innate immune cells in females drives heightened expansion and activity of B cells and T cells during the early adaptive immune response. Elevated T helper 2 (Th2) responses, including the production of IL-4, IL-5, IL-10, and IL-13 further expands B cell responses and drives the elevated humoral immune response in females during the late adaptive phase of the immune response. Several modifying variables including sex steroid hormones (e.g., estradiol, progesterone, and testosterone), sex chromosomal genes (e.g., *Il2rγ*, *Irak*, *Tlr7*, *Tlr8*, *Foxp3*, and *Ikkγ*), and

immune gene polymorphisms are hypothesized to mediate dimorphic innate and adaptive immune responses to viral vaccines.

Table 1:

Sex differences in the response to both childhood and adult virus vaccines

Dependent measure	Vaccine[1]	Sex difference	Age of study population[2]	References
Rate of vaccination	TIV and LAIV	F > M	Adults	(16)
		M > F	Adults	(17)
		F = M	All ages	(18)
	MMR	F = M	Infants/toddlers	(27)
Rate of seroconversion	HAV/HBV	F = M	Adults	(39)
		F = M	Elderly adults	(53)
Humoral/antibody response	TIV	F > M	Adults	(19, 20, 138)
		F > M	Elderly adults	(21, 139, 140)
	17DV	F > M	Adults	(11)
	BERNA-YF, RKI-YF, ARILVAX, YF-VAX	M > F	Adults	(12, 13)
	AP-YF, 17DD	F = M	Adults	(13, 14)
	RA27/3	M > F	After puberty	(32)
	Schwarz	F > M	Adults	(141)
	MMR	F = M	Before puberty	(31)
		F = M	After puberty	(142)
		F > M	After puberty	(29, 137)
	HPV4	M > F	Before and during puberty	(143)
	HAV	F > M	Adults	(39–43)
	HBV	F > M	Infants-puberty	(44, 45)
	HBV	F > M	Adults	(46–48, 51)
	HAV/HBV	F > M	Adults	(49, 50)
	HSV-2 gD	F > M	Adults	(55, 144)
	HDCV, PCECV	F > M	Adults	(145, 146)
	Dryvax	F > M	Adults	(147)
	attenuated Dengue virus	F > M	Adults	(148)
	attenuated VEE virus	M > F	Adults	(149)
Cell-mediated immunity	MMR	F = M	After puberty	(137, 142)
	RA27/3	M > F	After puberty	(32)
	HSV-2 gD	F > M	Adults	(57)
Adverse reaction	TIV	F > M	Adults	(20, 22, 23)
			Elderly adults	(21, 24, 150)
	17D	F > M	Adults	(8)
	MMR	F > M	Infants/toddlers	(34)
		M > F	Infants/toddlers	(35)
		F > M	Before puberty	(33)
	attenuated JE virus	F > M	Adults	(151)

Dependent measure	Vaccine[1]	Sex difference	Age of study population[2]	References
Mortality	Edmonston-Zagreb	F > M	Infants/toddlers	(36)

[1]TIV and LAIV protect against influenza virus infection; MMR protects against measles, mumps, and rubella; 17-DV, 17-DD, BERNA-YF, RKI-YF, AP-YF, ARILVAX, and YF-VAX protect against yellow fever virus; RA27/3 protects against rubella; HDCV and PCECV protect against rabies virus; Schwartz and Edmonston-Zagreb protect against measles; HPV4 protects against human papillomavirus types 6, 11, 16, and 18; HSV-2 gD protects against herpes virus type 2; Dryvax protects against smallpox; VEE= Venezuelan equine encephalitis; JE = Japanese encephalitis.

[2]Infants/toddlers = 6–59 months of age; children before puberty = 5–10 years of age; children after puberty = 10–17 years of age; adults = ≥ 18 years of age; elderly = ≥ 65 years of age

Table 2:

Future directions and recommendations

Independent Variable	Clinical Research	Basic Research	
	Human Studies	**Animal Studies**	**Cell Culture Systems**
Sex	• Analyze existing biological samples and clinical data for sex-specific variation • Design prospective studies with adequate numbers of males and females • Design prospective studies to maximize biological sample collection and frequency of sampling	• Control for confounding variables, including dose, time of vaccination, time of virus challenge, age, route of inoculation • Use of sufficient numbers of male and female animals to determine statistically significant differences in dependent measures • Determine in vitro correlates of protection	• Use primary cell cultures derived from males or females • Determine sex chromosomes of existing cell lines • Determine sex chromosome gene expression in heteroploid cell lines
Hormones	• Document the hormonal status of women at the time of immunization • Consider the effects of oral contraceptives and hormone replacement therapy on responses of women to vaccines • Examine effects of age-related declines in testosterone on responses to vaccines in men	• Determine if age-related changes in hormone concentrations affect immune responses • Effects of hormone manipulations on vaccination efficacy • Evaluate whether vaccination alters reproductive functions • Evaluate immune responses after vaccination during different stages of the reproductive cycle • Utilize hormone receptor knockout mice to establish signaling mechanisms involved in sex differences in responses to vaccination	• Culture cells under conditions where sex hormone effects can be assessed • Determine the cell type - specific effects of sex hormones on innate immune responses • Examine the effects of pharmacological agonists and antagonists of sex hormone receptors
Genes	• Examine the sex-specific effects of polymorphisms in immune-related genes • Determine the presence of hormone response elements in the promoters of immune-related genes • Examine the expression of immune-related sex chromosomal genes	•Develop transgenic mice with sex-specific alterations in gene expression • Determine the sex-specific effects of gene disruptions or alterations on immune responses • Examine sex chromosomal and autosomal gene effects on sex-based differences in responses to vaccination	• Expand the use of primary cell cultures to avoid the complications of chromosomal abnormalities associated with most transformed cell lines • Assess cell type and sex-dependent changes in immune gene activation
Pregnancy	• Determine the effect of pregnancy on a woman's response to vaccination • Evaluate immune responses to vaccines across the trimesters of pregnancy • Determine vaccine efficacy in pregnant women • Document the effects of vaccines on pregnancy outcome	• Evaluate the effects of pregnancy on responses to vaccination • Determine the relative risk/benefit ratio of vaccination to the outcome of pregnancy • Determine vaccination schedules which provide optimal protection for pregnant animals	• Develop and refine pregnancy specific cell culture models (e.g. placental cultures, umbilical vein cultures)