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REVIEW

Sex-based differences in immune function and responses to vaccination

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Females typically develop higher antibody responses and experience more adverse reactions following vaccination than males. These differences are observed in response to diverse vaccines, including the bacillus Calmette-Guerin vaccine, the measles, mumps and rubella vaccine, the yellow fever virus vaccine and influenza vaccines. Sex differences in the responses to vaccines are observed across diverse age groups, ranging from infants to aged individuals. Biological as well as behavioral differences between the sexes are likely to contribute to differences in the outcome of vaccination between the sexes. Immunological, hormonal, genetic and microbiota differences between males and females may also affect the outcome of vaccination. Identifying ways to reduce adverse reactions in females and increase immune responses in males will be necessary to adequately protect both sexes against infectious diseases.

Keywords: Estrogen, Gender, Microbiome, Sex difference, Vaccine, X chromosome

Introduction

Sex (i.e., the biological differences between males and females) and gender (i.e., cultural norms associated with being male or female) impact acceptance of, responses to and the outcome of vaccination.[1] Adult females develop higher antibody responses to vaccines than males. After either childhood or adult vaccination against influenza, yellow fever, rubella, measles, mumps, hepatitis A and B, herpes simplex 2, rabies, smallpox and dengue viruses, protective antibody responses can be twice as high in females compared to males of all ages.[1] Measures of cell-mediated immunity following vaccination are also higher in adult females than males for some vaccines.[2–4] Females develop more frequent and severe adverse reactions, including fever, pain and inflammation to vaccines.[1,5,6] Because information about adverse events is often acquired through passive reporting, it is assumed that this reflects a gender difference, in which females might be more likely to report adverse side effects than males. Alternatively, sex-based biological differences may also be involved, in which inflammatory responses to vaccines are higher in females and result in increased adverse biological reactions to vaccines in females compared with males. The goal of this review is to illustrate the breadth of sex differences in response to diverse vaccines across different age groups and suggest immunological, endocrinological and genetic mechanisms mediating these responses.

Evidence of sex-based differences in the outcome of vaccination

Sex differences are reported in response to both childhood and adult vaccination. 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 vaccines have not been systematically examined and may differ based on the age of the recipient, the vaccine used and the type of protective immune responses elicited.

Bacillus Calmette-Guerin (BCG) vaccine in children

The TB vaccine, BCG is recommended for children within the first year of life in endemic regions. The efficacy of the BCG vaccine is debated, with evidence that it protects against disseminated TB in childhood, but not adulthood.[7] In addition to protecting children against TB, BCG vaccination offers non-specific effects by significantly reducing all causes of mortality, especially in low birth weight babies.[8] There is some evidence to suggest that the non-specific long-term protective effects of the BCG vaccine on overall survival and reduced susceptibility to respiratory infections is greater for girls than boys.[9,10] How the BCG vaccine modifies immune function to provide non-targeted beneficial effects, especially in girls, has not been determined.

Measles, mumps and rubella (MMR) in children

The measles-mumps-rubella (MMR) vaccine consists of three virus strains that have been attenuated for virulence and replication.[11] While the precise vaccine schedules differ among countries, rates of MMR vaccination are reportedly similar between girls and boys in the United Kingdom,[12] but not in countries where girls have reduced access to health services compared with boys.[13] 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.[14] 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.[15] In a 9-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.[16] A study of pre-pubertal boys and girls 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 compared to females 2–4 weeks after vaccination, but this sex difference was no longer apparent 10 weeks post-vaccination.[17] At later ages (14–17 years of age), girls had higher anti-rubella IgG titers than boys, suggesting that long-term protection against rubella is greater for girls.[18] 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,[19,20] except for the risk of immune thrombocytopenic purpura, which is higher in males.[21] It has not been reported whether higher antibody responses in girls compared to boys following receipt of the MMR vaccine results in differential protection.

The Schwarz measles vaccine is a low titer viral vaccine that is only offered to infants from 9 months of age. Administration of the Edmonston-Zagreb high titer measles vaccine to infants <9 months of age was initiated by WHO in the late 1980s, in regions of West Africa.[22] 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.[22] The reduced survival related to the high titer measles vaccine occurred almost solely in females and led to the termination of the vaccine trials.

Yellow fever virus (YFV) vaccine in young adults

The YFV vaccine strain, 17D, is administered subcutaneously and replicates extensively, generating a viremia but with markedly reduced clinical symptoms of infection.[23] 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) indicated that the majority of reported adverse events occurred in adult females (61%).[24] The most commonly reported adverse side effects in females following YF vaccination were fever, pain, pruritus, headache, injection site erythema, urticaria, rash, nausea, dizziness, dyspnea and fatigue.[24] Females also reported more local inflammation than males. Because the VAERS is a passive reporting system, whether females are more likely to report adverse side effects than males must also be considered.

Sex differences in the humoral immune responses to YFV vaccination have not been reported, which is likely due to the robust nature of 17D vaccine replication in the host and the choice of high vaccine doses. Innate immune gene transcriptional profiles, including the expression of toll-like receptors (TLR) and interferon (IFN)-associated genes, immediately after vaccination with YF17D predict subsequent adaptive immune responses,[25,26] and the expression of these TLR/IFN-associated genes is significantly higher in females compared with males after vaccination.[1] Whether the efficacy of the YFV vaccine is higher in females compared to males has not been reported.

Influenza vaccine in adults and aged individuals

The intention of receiving either pandemic or avian influenza vaccines is reportedly 2–3 times lower for females than males, even among healthcare providers.[27–30] Receipt of seasonal trivalent influenza vaccines in the United States and in several European countries is consistently lower among both young and older adult females compared with their male counterparts.[31–34] A systematic review revealed that during the 2009 H1N1 pandemic (pH1N1), receipt of the monovalent vaccine was higher in males than females worldwide.[4]

Among both younger (18–64 years) and older (>65 years) adults, females have higher hemagglutination inhibition (HAI) antibody titers than males following seasonal trivalent influenza vaccine (TIV). Receipt of either a full or half dose of seasonal TIV in adults 18–49 years of age results in HAI antibody titers that are at least twice as high in females compared to males.[35] Among older adults, receipt of TIV results in significantly higher HAI titers among older females compared to males.[6] Among older adults that received the standard seasonal TIV, higher HAI titers were associated with lower rates of hospitalization and mortality in females compared to males, suggesting that the efficacy of TIV in older adults might be higher for females.[36,37] Among older adults who receive the high-dose TIV, sex differences in HAI titers are still apparent, in which antibody responses are significantly higher in older females compared to males against each of the three influenza antigens.[38] Older females were also reported to have higher HAI antibody titers against the monovalent pH1N1 inactivated vaccine than males, resulting in a 2–3 times higher seroprotection and seroconversion rate in females compared to males.[39] Although older females produced higher antibody responses to the pH1N1 vaccine, the avidity of their antibodies after pH1N1 vaccination was significantly lower than that of older males.[40]

Passive reporting of local reactions (e.g., muscle pain, redness and inflammation) to influenza vaccines is more frequent for females than males among both younger and older adults.[41] Measurements of local erythema and induration, both of which are associated with inflammation, reveal that both younger and older adult females have larger (≥6 mm) injection site reactions to TIV than their male counterparts.[42] Systemic reactions (e.g., fever, chills, nausea, headaches and body aches) to TIV also are more commonly reported by females than males.[43] Reports of local and systemic adverse reactions, including immediate

hypersensitivity reactions, also are more frequent among adult females than males following receipt of the inactivated monovalent pH1N1 vaccine.[44–46]

Sex-based differences in immune responses

Both innate and adaptive immune responses differ between males and females and likely contribute to sex differences in the responses to vaccines. Whether sex differences in innate and adaptive immune responses are apparent among children, as has been reported for adults, has not been adequately studied.

Sex differences in innate immune responses

Pattern recognition receptors, including the Toll-like receptors, are expressed by sentinel cells (e.g., macrophages and dendritic cells [DCs]) and detect the presence of conserved microbial and viral motifs to initiate the innate immune response not only to pathogens but also to vaccine antigens.[47] The detection of lipopolysaccharide (LPS), for example, involves TLR4, which is expressed on immune cells including monocytes, macrophages and DCs. Macrophages derived from males express higher levels of TLR4 compared to female-derived cells, both constitutively and following activation.[48] Macrophages from female mice, however, express higher levels of MyD88[49] and greater p38 MAP kinase phosphorylation and, hence, activation following LPS challenge than do macrophages from males.[50] Peripheral blood mononuclear cells (PBMCs) and plasmacytoid DCs from female patients produce higher levels of type I IFNs in response to TLR7 ligands,[51,52] but lower levels of IL-10 in response to ligands for TLR8 or TLR9[53] than similarly challenged male-derived cells.

Sex biases exist at the level of inflammatory cytokine production by antigen presenting cells, including macrophages and DCs. Peripheral monocytes from male human subjects as well as peritoneal macrophages from male mice produce significantly higher levels of TNF-α, IL-1β, IL-6 and CXCL10 than cells from females.[54–57] Peritoneal macrophages isolated from male rodents produce significantly lower amounts of the anti-inflammatory prostanoids following either LPS treatment[57] or adjuvant administration[58] compared to female-derived cells. Female-derived splenic macrophages also secrete higher levels of IL-10 than do cells derived from males.[56]

In response to TIV, females have higher levels of inflammatory cytokines compared to males, which correlate with monocyte phosphorylated STAT3 levels.[4] In a humanized mouse model, TLR7 ligand-induced IFN-α production by human plasmacytoid DCs is higher in female than male hosts.[52] Treatment of postmenopausal women or mice with estradiol markedly enhances TLR7 or TLR9-mediated IFN-α production by plasmacytoid DCs in an estrogen receptor alpha (ERα)-dependent manner.[52] Furthermore, PBMCs derived from males produce less IFN-α in response to TLR7 ligands and more IL-10 after either exposure to TLR8 or TLR9 ligands or infection with herpes simplex virus 1.[53] Whether sex differences in innate immune cell activity results in differences in the outcome of vaccination requires greater consideration, especially for adjuvanted vaccines.

Sex differences in adaptive immune responses

Generally, females exhibit elevated humoral and cell-mediated immune responses to vaccination compared to males.[59] Clinical studies reveal that males have lower absolute CD3+ cell counts, absolute numbers of CD4+ T cells, CD4+:CD8+ cell ratios and helper T cell type 1 (Th1) responses.[60–63] Studies in mice further reveal that cytokine responses of CD4+ T cells often differ between males and females.[64–66] For example, female mice have been shown to produce higher Th2 cytokine responses (e.g., IL-4, IL-9 and IL-13) than males, at least following viral and parasitic infections.[67,68] Polyclonal activation of PBMCs with an exogenous antigen results in higher Th2 responses in female-derived cells compared to male-derived cells.[69] Female mice have higher proportions of regulatory T cells (Tregs) than males[70] that may underlie tolerance of fetal antigens in women during pregnancy.[71]

Females exhibit higher cytotoxic T-lymphocyte activity than males, including upregulated expression of antiviral and proinflammatory genes in T cells isolated from women as compared with T cells isolated from men.[72] Treatment-naïve women chronically infected with HIV-1 show higher levels of CD8+ T-cell activation than men when adjusted for viral load.[51] Several non-specific indicators of cell-mediated immunity are also elevated in females, with females having higher mitogen-stimulated lymphocyte proliferation, faster wound healing, and increased immunological intolerance to foreign substances than males.[73–75]

In general, females demonstrate greater antibody responses than males.[1,4,76,77] Both basal levels of immunoglobulin,[78] as well as antibody responses to vaccines, are consistently higher in females than males.[77] There are, however, reports of higher humoral responses in males than females following some vaccinations, which seem to depend on the vaccine type, age and societal status of the recipient.[77]

Mechanisms implicated in mediating sex-based differences in immune responses

Sex steroid hormones

Estradiol, progesterone and testosterone affect the effector functions of immune cells. The estrogen receptors, ERα and ERβ, for example, are expressed in various immune cells including T and B cells, natural killer cells, macrophages, DCs and neutrophils indicating responsiveness to estrogens. Activation of their cognate receptors by progesterone and testosterone also modulates the functions of T and B cells, DCs, macrophages and natural killer cells. The effects of these sex hormones are dose-dependent. This is especially relevant for estrogen and progesterone where their concentrations vary during different stages of the menstrual cycle, during pregnancy and after menopause. For example, low doses of estrogen during the luteal phase of the menstrual cycle influences Tbet expression, associated with a Th1 response, namely increased IFN-γ production, whereas higher doses of estrogen, such as during the follicular phase, down-regulate IRF1 expression, leading to Th2 polarization and IL-4, IL-5 and IL-10 cytokine production.[58] Estrogens drive expansion of Tregs and increase their suppressive effects, most notably early during pregnancy, whereas increasing progesterone levels in the second trimester lead to diminished Treg numbers.[79] Dose-dependent estrogen effects on Tregs contribute to down-regulation of the pro-inflammatory effects of Th17 cells. Recent studies have identified that human T cells exhibit a sex difference in IFN-γ and IL-17A production, i.e., Th1 vs Th17 bias, based on androgen effects on peroxisome proliferator-activated receptors (PPAR)α

and γ, with androgens increasing PPARα and decreasing PPARγ, thereby limiting IFN-γ production and increasing IL-17A production.[80]

Estrogens and progesterone/androgens likewise exert opposing effects on B cells, with estrogens implicated in promoting antibody production by B cells, mediated by Th2 associated cytokines IL-4 and IL-5 driving B cell proliferation and differentiation to plasma cells, and progesterone and androgens exerting inhibitory effects on antibody production by B cells.[4,81]

Both estradiol and testosterone are associated with enhanced functional responses by DCs,[82,83] whereas progesterone exerts immunosuppressive effects on DCs.[84] The estrogen/progesterone balance differentially affects macrophage TNF-α and NO production and estradiol exerts anti-inflammatory effects on macrophages in vitro but pro-inflammatory effects on macrophages in vivo.[85] Accumulating evidence suggests that testosterone suppresses pro-inflammatory cytokine secretion by macrophages.[86]

Genetic and epigenetic regulation

In addition to hormonal influences, genetic and epigenetic factors contribute to sex-based differences in an immune response to vaccination. Specifically, the sex-based immune response differences observed between pre-pubertal boys and girls, and post-menopausal women and elderly men, suggest other contributions beyond sex hormones. A large number of immune-related genes encoding proteins, including IL-2 receptor-γ chain, IL-3 receptor-α chain, IL-13 receptor-α chains, TLR7, TLR8, GATA1, IRAK1, CD40 ligand and FOXP3 are located on the X chromosome.[59] A number of critical transcriptional and translational control effectors, which function downstream of activated cytokine receptors, are encoded on the X chromosome. The implications are that X-linked genes are determinants of immunocompetence. Given that males are XY and females XX, any damaging mutations or polymorphisms to X-linked genes are more likely to have an immune consequence in males compared to females.[87] There is evidence that X chromosome inactivation, the process whereby gene dosage is addressed in XX females, is not entirely random, but that skewed X chromosome inactivation may favor elimination of mutant genes and the persistence of intact genes by clonal maintenance.[88] The androgen receptor is encoded on the X chromosome.[89] Both estrogen and androgen receptors bind to hormone response elements upstream of target genes and recruit methyltransferase and histone acetyltransferase enzymes that regulate gene transcription. The combined effects of hormones influencing the epigenetic regulation of gene expression, and gene composition on the X chromosome potentially differing between XX females and XY males, will determine an immune response to vaccination.

Considerable attention has focused in recent years on the contributions of non-coding microRNAs (miRNAs) to the control of gene expression, by either repressing mRNA translation or triggering mRNA degradation. The X chromosome contains 10% of the approximate 800 miRNAs in the genome; whereas the Y chromosome contains only 2 miRNAs.[90] Accordingly, the prevalence of miRNAs on the X chromosome that includes a large number of immune-related genes inevitably influences sex-based differences in immune responses. Certainly, miRNAs are critical regulators in immune cell development and function.[91] Most notable are the X-chromosome encoded miRNA-18 and -19, implicated as miRNAs that have a role in sex-based differences in immunity, specifically potentiating inflammatory responses through the control of repressors of NF-κB signaling.[92,93]

Microbiome

Recent studies have provided insights into the relationship between human microbiota and the host's immunophenotype,[94] with supporting evidence for sex-specific relationships.[90] The human microbiota is composed of microbial communities in different habitats including the skin, gut, oral cavity, genitals that vary according to sex and time. Both diet and antibiotic use produces shifts in microbiota.[95] Age-related gastric atrophy affects the gastric microbiota, as do age-related vaginal changes pre- and post-menopause. Notably, bacteria can metabolize sex hormones, mediated by hydroxysteroid dehydrogenase enzymes that regulate the balance between active and inactive steroids.[96] Consequently, microbiome composition will directly influence an immune response, in a sex-specific manner. Antibiotics use will affect this bacteria-regulated hormonal metabolism.

Accumulating evidence indicates that hormonal status can shape microbiome composition; the onset of puberty and concomitant hormone-specific changes result in sex-specific microbiome profiles.[97] These sex-specific and hormonally directed microbiomes appear to influence the immunophenotype because in a spontaneous mouse model of type I diabetes the adoptive transfer of male-enriched gut commensals into females resulted in systemic hormonal changes and protected against disease.[98,99]

Viewed together, site-specific microbiome composition will affect an immune response in a sex-specific manner. Data are accumulating that site-specific microbiota have a role in modulating immune responses both locally and systemically. The impact of the host microbiota on a vaccine response, therefore, necessitates investigation.[100] Differences in the immune response outcomes, i.e., efficacy of oral vaccines against poliovirus, rotavirus and cholera in different geographical locations were ascribed to differences in gut microbiota.[100,101] Other studies have shown that probiotics enhanced antibody responses to oral vaccines against rotavirus, poliovirus, *Salmonella* and *Vibrio cholera*.[101] Likewise, probiotics improved immune responses in infants given oral vaccines against diphtheria, tetanus, *Haemophilus influenza* B and hepatitis virus B.[101]

Future directions

Males and females are biologically different and this likely contributes to sex-specific vaccine outcomes. Among children, adults of reproductive ages and older adults, females develop higher antibody responses and experience more adverse reactions to vaccines than males. Although there are distinct effects of sex steroid hormones on immune responses, including responses to vaccines,[4] the lack of age-related changes in the sex differential effects of vaccines suggest that genetic or other factors are likely to be involved. Utilization of primary cell culture systems, including immune cell subsets from males and females would be one way to address hormonal versus genetic factors influencing vaccine activation and effector functions. There is also growing interest in how sex-based differences in the microbiome composition affect immune responses to vaccines over time and during menstrual cycle, pregnancy and menopause. While specific details

about the mechanisms mediating how males and females differ in response to vaccination are lacking, it is apparent that the design of vaccines and vaccine strategies should be sex-specific, to reduce adverse reactions in females and increase immunogenicity in males. A greater understanding of the biological factors mediating sex differences in responses to vaccines may influence gender-biased acceptance and uptake of some vaccines, both of which are often lower in females compared to males.[102]

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References

1 Klein SL, Jedlicka A, Pekosz A. The Xs and Y of immune responses to viral vaccines. Lancet Infect Dis 2010;10:338–49.

2 Umlauf BJ, Haralambieva IH, Ovsyannikova IG et al. Associations between demographic variables and multiple measles-specific innate and cell-mediated immune responses after measles vaccination. Viral Immunol 2012;25:29–36.

3 Zhang X, Castelli FA, Zhu X et al. Gender-dependent HLA-DR-restricted epitopes identified from herpes simplex virus type 1 glycoprotein D. Clin Vaccine Immunol 2008;15:1436–49.

4 Furman D, Hejblum BP, Simon N et al. Systems analysis of sex differences reveals an immunosuppressive role for testosterone in the response to influenza vaccination. Proc Natl Acad Sci USA 2014;111:869–74.

5 Poland GA, Ovsyannikova IG, Jacobson RM. Adversomics: the emerging field of vaccine adverse event immunogenetics. Pediatr Infect Dis J 2009;28:431–2.

6 Cook IF, Barr I, Hartel G et al. Reactogenicity and immunogenicity of an inactivated influenza vaccine administered by intramuscular or subcutaneous injection in elderly adults. Vaccine 2006;24:2395–402.

7 Roth A, Garly ML, Jensen H et al. Bacillus Calmette-Guerin vaccination and infant mortality. Expert Rev Vaccines 2006;5:277–93.

8 Aaby P, Roth A, Ravn H et al. Randomized trial of BCG vaccination at birth to low-birth-weight children: beneficial nonspecific effects in the neonatal period? J Infect Dis 2011;204:245–52.

9 Biering-Sorensen S, Aaby P, Napirna BM et al. Small randomized trial among low-birth-weight children receiving bacillus Calmette-Guerin vaccination at first health center contact. Pediatr Infect Dis J 2012;31:306–8.

10 Stensballe LG, Nante E, Jensen IP et al. Acute lower respiratory tract infections and respiratory syncytial virus in infants in Guinea-Bissau: a beneficial effect of BCG vaccination for girls community based case-control study. Vaccine 2005;23:1251–7.

11 Watson JC, Hadler SC, Dykewicz CA et al. Measles, mumps, and rubella–vaccine use and strategies for elimination of measles, rubella, and congenital rubella syndrome and control of mumps: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 1998;47:1–57.

12 Martineau A, White M, Bhopal R. No sex differences in immunisation rates of British south Asian children: the effect of migration? BMJ 1997;314:642–3.

13 Naik VA, Kowli SS, Solanki MJ et al. Exposition of the status of girl child in urban area. J Postgrad Med 1991;37:216–8.

14 Dominguez A, Plans P, Costa J et al. Seroprevalence of measles, rubella, and mumps antibodies in Catalonia, Spain: results of a cross-sectional study. Eur J Clin Microbiol Infect Dis 2006;25:310–7.

15 Mossong J, O'Callaghan CJ, Ratnam S. Modelling antibody response to measles vaccine and subsequent waning of immunity in a low exposure population. Vaccine 2000;19:523–9.

16 Davidkin I, Valle M, Julkunen I. Persistence of anti-mumps virus antibodies after a two-dose MMR vaccination. A nine-year follow-up. Vaccine 1995;13:1617–22.

17 Mitchell LA. Sex differences in antibody- and cell-mediated immune response to rubella re-immunisation. J Med Microbiol 1999;48:1075–80.

18 Poethko-Muller C, Mankertz A. Seroprevalence of measles-, mumps- and rubella-specific IgG antibodies in German children and adolescents and predictors for seronegativity. PLoS One 2012;7:e42867.

19 Khalil MK, Al-Mazrou YY, Al-Ghamdi YS et al. Effect of gender on reporting of MMR adverse events in Saudi Arabia. East Mediterr Health J 2003;9:152–8.

20 Benjamin CM, Chew GC, Silman AJ. Joint and limb symptoms in children after immunisation with measles, mumps, and rubella vaccine. BMJ 1992;304:1075–8.

21 France EK, Glanz J, Xu S et al. Risk of immune thrombocytopenic purpura after measles-mumps-rubella immunization in children. Pediatrics 2008;121:e687–92.

22 Knudsen KM, Aaby P, Whittle H et al. Child mortality following standard, medium or high titre measles immunization in West Africa. Int J Epidemiol 1996;25:665–73.

23 Domingo C, Niedrig M. Safety of 17D derived yellow fever vaccines. Expert Opin Drug Saf 2009;8:211–21.

24 Lindsey NP, Schroeder BA, Miller ER et al. Adverse event reports following yellow fever vaccination. Vaccine 2008;26:6077–82.

25 Gaucher D, Therrien R, Kettaf N et al. Yellow fever vaccine induces integrated multilineage and polyfunctional immune responses. J Exp Med 2008;205:3119–31.

26 Querec TD, Akondy RS, Lee EK et al. Systems biology approach predicts immunogenicity of the yellow fever vaccine in humans. Nat Immunol 2009;10:116–25.

27 Opstelten W, van Essen GA, Ballieux MJ, Goudswaard N. Influenza immunization of Dutch general practitioners: vaccination rate and attitudes towards vaccination. Vaccine 2008;26:5918–21.

28 Ailes EC, Newsome K, Williams JL et al. CDC Pregnancy Flu Line: Monitoring Severe Illness Among Pregnant Women with Influenza. Matern Child Health J 2014;18:1578–82.

29 Chor JS, Ngai KL, Goggins WB et al. Willingness of Hong Kong healthcare workers to accept pre-pandemic influenza vaccination at different WHO alert levels: two questionnaire surveys. BMJ 2009;339:b3391.

30 Beau AB, Hurault-Delarue C, Vidal S et al. Pandemic A/H1N1 influenza vaccination during pregnancy: a comparative study using the EFEMERIS database. Vaccine 2014;32:1254–8.

31 Endrich MM, Blank PR, Szucs TD. Influenza vaccination uptake and socioeconomic determinants in 11 European countries. Vaccine 2009;27:4018–24.

32 Merrill RM, Beard JD. Influenza vaccination in the United States, 2005–2007. Med Sci Monit 2009;15:PH92–100.

33 Bean-Mayberry B, Yano EM, Mor MK et al. Does sex influence immunization status for influenza and pneumonia in older veterans? J Am Geriatr Soc 2009;57:1427–32.

34 Jimenez-Garcia R, Hernandez-Barrera V, de Andres AL et al. Gender influence in influenza vaccine uptake in Spain: time trends analysis (1995–2006). Vaccine 2010;28:6169–75.

35 Engler RJ, Nelson MR, Klote MM et al. Half- vs full-dose trivalent inactivated influenza vaccine (2004–2005): age, dose, and sex effects on immune responses. Arch Intern Med 2008;168:2405–14.

36 Wang CS, Wang ST, Chou P. Efficacy and cost-effectiveness of influenza vaccination of the elderly in a densely populated and unvaccinated community. Vaccine 2002;20:2494–9.

37 Chan KH, Zhang AJ, To KK et al. Wild type and mutant 2009 pandemic influenza A (H1N1) viruses cause more severe disease and higher mortality in pregnant BALB/c mice. PLoS One 2010;5:e13757.

38 Falsey AR, Treanor JJ, Tornieporth N et al. Randomized, double-blind controlled phase 3 trial comparing the immunogenicity of high-dose and standard-dose influenza vaccine in adults 65 years of age and older. J Infect Dis 2009;200:172–80.

39 Chudasama RK, Patel UV, Verma PB. Characteristics of hospitalized patients with severe and non-severe pandemic influenza A (H1N1) in Saurashtra Region, India (Two Waves Analysis). J Family Med Prim Care 2013;2:182–7.

40 Khurana S, Verma N, Talaat KR et al. Immune response following H1N1pdm09 vaccination: differences in antibody repertoire and avidity in young adults and elderly populations stratified by age and gender. J Infect Dis 2012;205:610–20.

41 Cook IF. Sex differences in injection site reactions with human vaccines. Hum Vaccin 2009;5:441–9.

42 Cate TR, Couch RB, Parker D, Baxter B. Reactogenicity, immunogenicity, and antibody persistence in adults given inactivated influenza virus vaccines - 1978. Rev Infect Dis 1983;5:737–47.

43 Nichol KL, Margolis KL, Lind A et al. Side effects associated with influenza vaccination in healthy working adults. A randomized, placebo-controlled trial. Arch Intern Med 1996;156:1546–50.

44 Elenkov IJ, Wilder RL, Bakalov VK et al. IL-12, TNF-alpha, and hormonal changes during late pregnancy and early postpartum: implications for autoimmune disease activity during these times. J Clin Endocrinol Metab 2001;86:4933–8.

45 Fofie AE, Fewell JE, Moore SL. Pregnancy influences the plasma cytokine response to intraperitoneal administration of bacterial endotoxin in rats. Exp Physiol 2005;90:95–101.

46 Griffiths PD, Ronalds CJ, Heath RB. A prospective study of influenza infections during pregnancy. J Epidemiol Community Health 1980;34:124–8.

47 Suresh R, Mosser DM. Pattern recognition receptors in innate immunity, host defense, and immunopathology. Adv Physiol Educ 2013;37:284–91.

48 Aomatsu M, Kato T, Kasahara E, Kitagawa S. Gender difference in tumor necrosis factor-alpha production in human neutrophils stimulated by lipopolysaccharide and interferon-gamma. Biochem Biophys Res Commun 2013;441:220–5.

49 Zheng R, Pan G, Thobe BM et al. MyD88 and Src are differentially regulated in Kupffer cells of males and proestrus females following hypoxia. Mol Med 2006;12:65–73.

50 Angele MK, Nitsch S, Knoferl MW et al. Sex-specific p38 MAP kinase activation following trauma-hemorrhage: involvement of testosterone and estradiol. Am J Physiol Endocrinol Metab 2003;285:E189–96.

51 Meier A, Chang JJ, Chan ES et al. Sex differences in the Toll-like receptor-mediated response of plasmacytoid dendritic cells to HIV-1. Nat Med 2009;15:955–9.

52 Seillet C, Laffont S, Tremollieres F et al. The TLR-mediated response of plasmacytoid dendritic cells is positively regulated by estradiol in vivo through cell-intrinsic estrogen receptor alpha signaling. Blood 2012;119:454–64.

53 Torcia MG, Nencioni L, Clemente AM et al. Sex differences in the response to viral infections: TLR8 and TLR9 ligand stimulation induce higher IL10 production in males. PLoS One 2012;7:e39853.

54 Asai K, Hiki N, Mimura Y et al. Gender differences in cytokine secretion by human peripheral blood mononuclear cells: role of estrogen in modulating LPS-induced cytokine secretion in an ex vivo septic model. Shock 2001;16:340–3.

55 Moxley G, Posthuma D, Carlson P et al. Sexual dimorphism in innate immunity. Arthritis Rheum 2002;46:250–8.

56 Kahlke V, Dohm C, Brotzmann K et al. Gender-related therapy: early IL-10 administration after hemorrhage restores immune function in males but not in females. Shock 2000;14:354–9.

57 Marriott I, Bost KL, Huet-Hudson YM. Sexual dimorphism in expression of receptors for bacterial lipopolysaccharides in murine macrophages: a possible mechanism for gender-based differences in endotoxic shock susceptibility. J Reprod Immunol 2006;71:12–27.

58 Du JT, Vennos E, Ramey E, Ramwell PW. Sex differences in arachidonate cyclo-oxygenase products in elicited rat peritoneal macrophages. Biochim Biophys Acta 1984;794:256–60.

59 Fish EN. The X-files in immunity: sex-based differences predispose immune responses. Nat Rev Immunol 2008;8:737–44.

60 Wikby A, Mansson IA, Johansson B et al. The immune risk profile is associated with age and gender: findings from three Swedish population studies of individuals 20–100 years of age. Biogerontology 2008;9:299–308.

61 Villacres MC, Longmate J, Auge C, Diamond DJ. Predominant type 1 CMV-specific memory T-helper response in humans: evidence for gender differences in cytokine secretion. Hum Immunol 2004;65:476–85.

62 Amadori A, Zamarchi R, De Silvestro G et al. Genetic control of the CD4/CD8 T-cell ratio in humans. Nat Med 1995;1:1279–83.

63 Das BR, Bhanushali AA, Khadapkar R et al. Reference ranges for lymphocyte subsets in adults from western India: influence of sex, age and method of enumeration. Indian J Med Sci 2008;62:397–406.

64 Roberts CW, Walker W, Alexander J. Sex-associated hormones and immunity to protozoan parasites. Clin Microbiol Rev 2001;14:476–88.

65 Araneo BA, Dowell T, Diegel M, Danes RA. Dihydrotestosterone exerts a depressive influence on the production of interleukin-4 (IL-4), IL-5, and gamma-interferon, but not IL-2 by activated murine T cells. Blood 1991;78:688–99.

66 Barrat F, Lesourd B, Boulouis HJ et al. Sex and parity modulate cytokine production during murine ageing. Clin Exp Immunol 1997;109:562–8.

67 Hepworth MR, Hardman MJ, Grencis RK. The role of sex hormones in the development of Th2 immunity in a gender-biased model of *Trichuris muris* infection. Eur J Immunol 2010;40:406–16.

68 Huber S, Sartini D. T cells expressing the Vgamma1 T-cell receptor enhance virus-neutralizing antibody response during coxsackievirus B3 infection of BALB/c mice: differences in male and female mice. Viral Immunol 2005;18:730–9.

69 Giron-Gonzalez JA, Moral FJ, Elvira J et al. Consistent production of a higher TH1:TH2 cytokine ratio by stimulated T cells in men compared with women. Eur J Endocrinol 2000;143:31–6.

70 Frisancho-Kiss S, Davis SE, Nyland JF et al. Cutting edge: cross-regulation by TLR4 and T cell Ig mucin-3 determines sex differences in inflammatory heart disease. J Immunol 2007;178:6710–4.

71 Kallikourdis M, Betz AG. Periodic accumulation of regulatory T cells in the uterus: preparation for the implantation of a semi-allogeneic fetus? PLoS One 2007;2:e382.

72 Hewagama A, Patel D, Yarlagadda S et al. Stronger inflammatory/cytotoxic T-cell response in women identified by microarray analysis. Genes Immun 2009;10:509–16.

73 Graff RJ, Lappe MA, Snell GD. The influence of the gonads and adrenal glands on the immune response to skin grafts. Transplantation 1969;7:105–11.

74 Krzych U, Strausser HR, Bressler JP et al. Effects of sex hormones on some T and B cell functions as evidenced by differential immune expression between male and female mice and cyclic pattern of immune responsiveness during the estrous cycle. Prog Clin Biol Res 1981;70:145–50.

75 Blankenhorn EP, Troutman S, Clark LD et al. Sexually dimorphic genes regulate healing and regeneration in MRL mice. Mamm Genome 2003;14:250–60.

76 Bouman A, Heineman MJ, Faas MM. Sex hormones and the immune response in humans. Hum Reprod Update 2005;11:411–23.

77 Cook IF. Sexual dimorphism of humoral immunity with human vaccines. Vaccine 2008;26:3551–5.

78 Butterworth M, McClellan B, Allansmith M. Influence of sex in immunoglobulin levels. Nature 1967;214:1224–5.

79 Polanczyk MJ, Hopke C, Huan J et al. Enhanced FoxP3 expression and Treg cell function in pregnant and estrogen-treated mice. J Neuroimmunol 2005;170:85–92.

80 Zhang MA, Rego D, Moshkova M et al. Peroxisome proliferator-activated receptor (PPAR)alpha and -gamma regulate IFNgamma and IL-17A production by human T cells in a sex-specific way. Proc Natl Acad Sci USA 2012;109:9505–10.

81 Lu FX, Abel K, Ma Z et al. The strength of B cell immunity in female rhesus macaques is controlled by CD8+ T cells under the influence of ovarian steroid hormones. Clin Exp Immunol 2002;128:10–20.

82 Seillet C, Rouquie N, Foulon E et al. Estradiol promotes functional responses in inflammatory and steady-state dendritic cells through differential requirement for activation function-1 of estrogen receptor alpha. J Immunol 2013;190:5459–70.

83 Corrales JJ, Almeida M, Martin-Martin L et al. Testosterone replacement therapy in hypogonadal men is associated with increased expression of LAMP-2 (CD107b) by circulating monocytes and dendritic cells. Clin Endocrinol (Oxf) 2014;80:577–84.

84 Xu Y, He H, Li C et al. Immunosuppressive effect of progesterone on dendritic cells in mice. J Reprod Immunol 2011;91:17–23.

85 Dimitrijevic M, Stanojevic S, Kustrimovic N et al. The influence of aging and estradiol to progesterone ratio on rat macrophage phenotypic profile and NO and TNF-alpha production. Exp Gerontol 2013; 48:1243–54.

86 Corcoran MP, Meydani M, Lichtenstein AH et al. Sex hormone modulation of proinflammatory cytokine and C-reactive protein expression in macrophages from older men and postmenopausal women. J Endocrinol 2010;206:217–24.

87 Abramowitz LK, Olivier-Van Stichelen S, Hanover JA. Chromosome imbalance as a driver of sex disparity in disease. J Genomics 2014;2:77–88.

88 Wilson MA, Makova KD. Evolution and survival on eutherian sex chromosomes. PLoS Genet 2009;5:e1000568.

89 Lubahn DB, Joseph DR, Sullivan PM et al. Cloning of human androgen receptor complementary DNA and localization to the X chromosome. Science 1988;240:327–30.

90 Ghorai A, Ghosh U. miRNA gene counts in chromosomes vary widely in a species and biogenesis of miRNA largely depends on transcription or post-transcriptional processing of coding genes. Front Genet 2014;5:100.

91 Zhu S, Pan W, Qian Y. MicroRNA in immunity and autoimmunity. J Mol Med (Berl) 2013;91:1039–50.

92 Pinheiro I, Dejager L, Libert C. X-chromosome-located microRNAs in immunity: might they explain male/female differences? The X chromosome-genomic context may affect X-located miRNAs and downstream signaling, thereby contributing to the enhanced immune response of females. BioEssays 2011;33:791–802.

93 Gantier MP, Stunden HJ, McCoy CE et al. A miR-19 regulon that controls NF-kappaB signaling. Nucleic Acids Res 2012;40:8048–58.

94 Kabat AM, Srinivasan N, Maloy KJ. Modulation of immune development and function by intestinal microbiota. Trends Immunol 2014;pii:S1471–4906(14)00131–8.

95 Shapiro H, Thaiss CA, Levy M, Elinav E. The cross talk between microbiota and the immune system: metabolites take center stage. Curr Opin Immunol 2014;30C:54–62.

96 Garcia-Gomez E, Gonzalez-Pedrajo B, Camacho-Arroyo I. Role of sex steroid hormones in bacterial-host interactions. Biomed Res Int 2013;2013:928290.

97 Yatsunenko T, Rey FE, Manary MJ et al. Human gut microbiome viewed across age and geography. Nature 2012;486:222–7.

98 Markle JG, Frank DN, Mortin-Toth S et al. Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. Science 2013;339:1084–8.

99 Yurkovetskiy L, Burrows M, Khan AA et al. Gender bias in autoimmunity is influenced by microbiota. Immunity 2013;39:400–12.

100 Brotman RM, Ravel J, Bavoil PM et al. Microbiome, sex hormones, and immune responses in the reproductive tract: Challenges for vaccine development against sexually transmitted infections. Vaccine 2014;32:1543–52.

101 Ferreira RB, Antunes LC, Finlay BB. Should the human microbiome be considered when developing vaccines? PLoS Pathog 2010; 6:e1001190.

102 Klein SL, Pekosz A. Sex-based biology and the rational design of influenza vaccination strategies. J Infect Dis 2014;209(Suppl 3): S114–9.