

Immune Cells Have Sex and So Should Journal Articles

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Males and females have the same immunological cells, proteins, and pathways in place to protect against the development of disease. The kinetics, magnitude, and skewing of the responses mounted against pathogens, allergens, toxins, or self-antigens, however, can differ dramatically between the sexes. Generally, females mount higher innate and adaptive immune responses than males, which can result in faster clearance of pathogens but also contributes to increased susceptibility to inflammatory and autoimmune diseases in females compared with males. Hormonal and genetic factors contribute significantly to sex differences in immune function and disease pathogenesis. In particular, the expression of X-linked genes and microRNA as well as sex steroid hormones signaling through hormone receptors in immune cells can affect responses to immunological stimuli differently in males and females. Despite data illustrating profound differences between the sexes in immune function, sex differences in the pathogenesis of disease are often overlooked in biomedical research. Establishing journal policies that require authors to report the sex of their cells, animals, and subjects will improve our understanding of the pathogenesis of diseases, with the long-term goal of personalizing treatments for immune-mediated diseases differently for males and females in an effort to protect us equally. (*Endocrinology* 153: 0000–0000, 2012)

Let us begin with a few facts: 80% of patients with autoimmune diseases are women (1); greater than 60% of adult patients with asthma are women (2); worldwide, women are 2–6 times more likely to die from H5N1 avian influenza (3); and the risk of death from all malignant cancers is 1.6 times higher for men (4). In most cases, we do not know the precise mechanism mediating these dimorphisms in disease pathogenesis. We do know that when cells of the immune system are exposed to a virus, bacterium, parasite, innocuous allergen, toxin, or self-antigen, innate recognition and responses as well as downstream adaptive immune responses differ between males and females. Females typically develop higher innate (5, 6), cell-mediated (6–8), and humoral (9, 10) immune responses than males, which can reduce pathogen load and accelerate pathogen clearance (11–13), but can be detrimental by causing immune-mediated pathology (6, 14) as

well as autoimmune or inflammatory diseases (1, 2, 16). Immunological differences between the sexes are hypothesized to reflect endocrine-immune interactions as well as genetic differences between the sexes. Despite significant immunological differences between the sexes, a majority of studies in immunology either do not disaggregate and analyze data by sex or do not report the sex of their subjects (17). The status quo is to assume that the sexes do not differ (17), which has hindered our understanding of the pathogenesis of immune-related diseases and the underlying mechanisms.

Immune responses differ between the sexes

Innate immunity

Males and females differ in their innate immune responses, suggesting that some sex differences may be germ-line encoded. For example, innate detection of nu-

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Abbreviations: APC, Antigen-presenting cell; DC, dendritic cell; E2, estradiol; IFN, interferon; miRNA, microRNA; NF- κ B, nuclear factor κ B; NK, natural killer; P4, progesterone; PR, P4 receptor; SLE, systemic lupus erythematosus; T, testosterone; Th1, helper T cell type 1; Th2, helper T cell type 2; TLR, toll-like receptor.

cleic acids by pattern recognition receptors differs between the sexes (6, 18). There are differences between the sexes in the induction of genes associated with toll-like receptor (TLR) pathways and antiviral type I interferon (IFN) responses (9, 19), with cells from females showing a 10-fold greater level of expression than cells from males (9). Studies of both humans and rodents illustrate that the number and activity of innate immune cells, including monocytes, macrophages, and dendritic cells (DC) as well as inflammatory immune responses in general are higher in females than males (20–22). Antigen-presenting cells (APC) from females are more efficient at presenting peptides than are APC from males (23). In contrast, females often have lower natural killer (NK) cell activity than males (24).

Adaptive immunity

Generally, females exhibit greater humoral and cell-mediated immune responses to antigenic stimulation, vaccination, and infection than do males (9, 25). Both basal levels of Ig (26) as well as antibody responses to viruses and vaccines are consistently higher in females than males (9, 10, 25). Clinical studies reveal that men have lower CD3⁺ and CD4⁺ cell counts, CD4⁺ to CD8⁺ cell ratios, and helper T cell type 1 (Th1) responses than women (7, 27–29). 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), helper T cell type 2 (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 (13, 30–32). Female mice also have higher proportions of regulatory T cells than males, at least in response to certain viruses (33). Females exhibit higher cytotoxic T cell activity along with up-regulated expression of antiviral and proinflammatory genes, many of which have estrogen response elements in their promoters (8).

Mechanisms of sex differences in immune function *Sex steroids*

The prevailing hypothesis for immunological differences between the sexes is that sex steroids, particularly testosterone (T), estradiol (E2), and progesterone (P4), influence the functioning of immune cells. 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 DC (35). The binding of sex steroids to their respective steroid receptors directly influences cell signaling pathways, including nuclear factor- κ B (NF- κ B), cJun, and interferon regulatory factor 1, resulting in differential production of cytokines and chemokines (35).

Androgens, including dihydrotestosterone and T, suppress the activity of immune cells. Exposure to T reduces NK cell activity (36), surface expression of TLR4 on macrophages (37), and the synthesis of proinflammatory products, including TNF- α and nitric oxide synthase (38). In contrast, T and dihydrotestosterone increase the synthesis of antiinflammatory cytokines, such as IL-10 (38, 39). Men with androgen deficiencies have higher inflammatory cytokine (*e.g.* IL-1 β , IL-2, TNF- α) concentrations, antibody titers, and CD4⁺ to CD8⁺ T cell ratios than healthy men (40–42). Signaling through the androgen receptor antagonizes transcriptional factors (*e.g.* NF- κ B and cJun) that mediate the production of proinflammatory and antiviral cytokines (43).

Estrogens, including E2, have most of their biological effects by binding to one of two intracellular receptors, estrogen receptor- α or estrogen receptor- β . E2 also regulates innate immune responses, including production of proinflammatory cytokines, by selectively up- and down-regulating microRNA (miRNA) (44). Estradiol affects the differentiation and functioning of APC, including DC (45, 46). E2 can have bipotential effects on inflammatory responses, with low doses enhancing proinflammatory cytokine production (*e.g.* IL-1, IL-6, and TNF- α) and Th1 responses and high or sustained concentrations reducing production of proinflammatory cytokines and augmenting Th2 responses and humoral immunity (47). E2 inhibits transcription of proinflammatory genes by inhibiting NF- κ B activity and recruiting steroid receptor coactivators that act as transcriptional repressors (48, 49). High E2 also attenuates production of chemokines and recruitment of leukocytes and monocytes into several tissues (50–52). High E2 enhances the expansion of regulatory T cells in mice (53) and humans (54) and can stimulate antibody production by B cells (55).

P4 is typically regarded as antiinflammatory. P4 receptors (PR) have been identified in many immune cells (34), with differential expression between the sexes. The expression of PR is higher in DC from female than male rats, which may explain why P4 is better able to suppress the activity (*e.g.* secretion of TNF- α) of DC from female rats (56). P4 can bind to glucocorticoid receptors, which are more abundant in the immune system than are PR, and may represent an alternative mechanism for P4-induced changes in immune function (57). P4 inhibits TLR-induced cytokine production as well as surface receptor expression via PR and glucocorticoid receptors in DC (57). Progesterone suppresses innate immune responses, including macrophage and NK cell activity as well as NF- κ B signal transduction (58, 59). Elevated concentrations of P4 during pregnancy inhibit the development of Th1 immune responses that can lead to fetal rejection and pro-

mote production of Th2 immune responses, including IL-4 and IL-5 production (60, 61). In mice, the activity of regulatory T cells is increased in pregnant females and in non-pregnant females exposed to pregnancy levels of P4 (62). Progesterone also suppresses antibody production (55).

X-linked genes and genetic polymorphisms

Although direct effects of gonadal steroids cause many sex differences in immune function, some sex differences might be caused by the inherent imbalance in the expression of genes encoded on the X and Y chromosomes (63). After a challenge, such as ischemic stroke, there is greater activation of X-linked genes in immune cells from females than males (64). Many genes on the X chromosome regulate immune function and play an important role in modulating sex differences in the development of immune-related diseases (65). These immune-related genes code for proteins ranging from pattern recognition receptors (*e.g.* *Tlr7* and *Tlr8*) to cytokine receptors (*e.g.* *Il2rg* and *Il13ra2*) and transcriptional factors (*e.g.* *Foxp3*) (66). There is higher expression levels of *Tlr7* in females than males (67), and DC isolated from women produce twice as much IFN- α in response to TLR7 ligands, including HIV-1 encoded TLR7 ligands, than do DC from men (6). Polymorphisms in Y chromosome genes also affect sex-dependent susceptibility to autoimmune disease (68). The expression of X-linked genes might also be affected by X-linked miRNA. miRNA are small noncoding RNA that regulate genes expression at a posttranscriptional level and play a critical role in maintaining immunological homeostasis. Dysregulation of miRNA expression may underlie development of immune-mediated diseases, ranging from cancers to autoimmune diseases (69). There are a disproportionately higher number of miRNA located on the X chromosome than on any autosomal chromosome, which is hypothesized to contribute to sex-specific development of immune-mediated diseases (69). Interpretation of sex differences in the expression of X-linked genes, however, is challenging because sex hormones or sex chromosome complement can still contribute to the observed differential gene expression (70).

Sex chromosomal complement (*i.e.* XX or XY) contributes to differential susceptibility to diseases between the sexes. In human males, the number of X chromosomes is a determinant of risk of systemic lupus erythematosus (SLE), in which the prevalence of SLE is higher in men with more than one X chromosome (71). Use of mice with the sex-determining region Y (*Sry*) gene either deleted (XY-*Sry*) or translocated to an autosomal region (XX*Sry*) also enables investigators to separate gonadal sex (*i.e.* the presence of ovaries or testes) from sex chromosome complement (72, 73). In these animal models, the sex chromo-

some complement affects susceptibility to some autoimmune and infectious diseases (73, 74).

Polymorphisms or variability in sex chromosomal genes as well as in autosomal genes that encode for immunological proteins contribute to sex differences in immune responses (75). For example, sex-based differences in human leukocyte antigen alleles contribute to the higher antibody responses of females than males to vaccination (76). Gene polymorphisms are associated with sex differences in susceptibility to bacterial (77) and viral (78) infections. Whether differential selection pressures acting on each sex cause sex-based differences in the expression of gene variants, hormone-dependent effects, or epigenetic mechanisms remain to be determined.

Disease pathogenesis differs between the sexes

Inflammatory diseases

Inflammatory-mediated diseases are caused by excessively high proinflammatory responses, including production of cytokines and chemokines as well as infiltration of immune cells, after exposure to a pathogen, allergen, toxin, or other environmental contaminants. If these immune responses become too high or remain elevated for too long, then this can cause pathology (6, 14). For example, more than 60% of patients with asthma are females (2). Females also have an approximately 30% greater risk of developing inflammatory bowel diseases, such as Crohn's disease, than males (16). As a result of elevated immunity among females, many inflammatory-mediated diseases, including eczema, allergy, asthma, and inflammatory bowel diseases are more frequent and more severe in females than males, with disease severity often changing at puberty, during the menstrual cycle, and after menopause (2, 16, 80).

Autoimmune diseases

Almost 80% of all patients with autoimmune diseases are women (65), with the female bias being highly pronounced for Sjögren's syndrome (9:1), Hashimoto's thyroiditis (8:1), SLE (8:1), rheumatoid arthritis (7:1), and multiple sclerosis (6:1) (65). The increased susceptibility of females to autoimmune diseases, like thyroiditis, SLE, and multiple sclerosis, can be recreated in animal models, which reveal profound and complex effects of sex steroid hormones and sex chromosomal complement on immune responses and development of disease (1).

Cancers

Sex is an important factor in the diagnosis, pathogenesis, and prognosis of many cancers that occur outside the reproductive tract and related tissues. For a majority of

cancers at most ages, the risk of malignancy is higher for males (81). Males have a 1.6-fold greater risk of mortality from all malignant cancers than females (4). Mortality rates for a majority of nonreproductive cancers, including lip, larynx, hypopharynx, esophagus, bladder, and lung, are consistently higher among males than females (4). Male-biased mortality rates are hypothesized to reflect universal differences in the etiology of cancer (4), which may involve sex differences in viral infection, immune function, hormonal regulation, gene expression, sex chromosome complement, oxidative damage, autophagy, or a combination of factors (4, 82).

Infectious disease

Males and females differ in their susceptibility to a variety of pathogens (25). Heightened immunity in females to pathogens, ranging from malaria to HIV and influenza, contributes to how the intensity (*i.e.* pathogen load within an individual) and prevalence (*i.e.* number of infected individuals within a population) of infections are often lower for females than males (25). Females, for example, have more than 40% less HIV RNA in circulation than males (12). Despite having lower HIV loads, women who are matched to the same HIV load as men have a 1.6-fold higher risk of developing AIDS (83). There is growing awareness, however, that much of the disease attributed to infection results from aberrant host inflammatory responses (84). Excessive inflammatory responses are hypothesized to underlie severe outcome of influenza (85). During the 2009 H1N1 pandemic in Canada (which is a country noteworthy for disaggregation and analysis of data by sex), women had a 2.4-fold higher risk of death than men (86). Consequently, heightened immune responses in females, although essential for pathogen clearance, may underlie increased development of symptoms of disease among females as compared with males after infection (14). Immune responses to pathogens also vary with changes in hormone concentrations over the menstrual or estrous cycle, after contraception use, during pregnancy, and after menopause (25). Although behavioral factors can influence exposure to pathogens, hormonal and genetic effects on immune function cause differential responses to infection between the sexes.

Vaccines

The biological differences between the sexes is a major source of variation in the immune response to vaccination (9). Women consistently report more frequent and severe local and systemic reactions to viral and bacterial vaccines than men (9, 10, 15), which may reflect either a reporting bias or greater inflammatory responses among women than men (9, 34). Antibody responses to bacterial and viral

vaccines, including the seasonal trivalent inactivated influenza vaccines, are consistently at least twice as high in women as men (9, 10, 15). To highlight the value of disaggregating and analyzing data by sex, in clinical trials of a herpes simplex virus (HSV) vaccine, no overall protection from infection was observed in phase 1 or 2 trials (79). When data were analyzed by sex, the efficacy of the vaccine was 73% in women and only 11% in men, indicating that the vaccine was able to provide protection against development of symptoms associated with genital herpes in women but not in men. There currently is no approved herpes simplex virus vaccine.

Conclusions

The examples above provide merely a glimpse into how disease pathogenesis differs between males and females. There are significant gaps in our understanding of the precise mechanisms mediating sex-biased immune responses. Future research must continue to define the pathways mediating how hormones, genes, and genetic polymorphisms alter the functioning of cells and tissues to cause profound differences in the pathogenesis of disease. Many of our immunological differences are hardwired in our genome, but our hormonal environment may affect the kinetics, magnitude, and skewing of these differential responses when faced with immunological challenges. The questions that remain are the following: 1) can we therapeutically manipulate the expression of our genes or concentrations of hormones to reverse sex differences in susceptibility to disease; and 2) can information about gene expression and hormonal environment be used to tailor treatments differently for males and females? The concept of personalized medicine is not novel; what is novel is that sex may be a fundamental factor to consider when designing and administering treatments for diseases. We will achieve this end result only if journals and funding agencies continue to require that investigators report the sex of their cells, animals, and subjects.

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