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The influence of sex and gender on immunity, infection and vaccination

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Abstract
Sex/gender significantly contribute to shape the immune responses, contributing to differences in the pathogenesis of infectious diseases in males and females, the response to viral vaccines and the prevalence of autoimmune diseases. Females typically develop higher innate, humoral and cellular immune responses to viral infections and in response to vaccine. At the same time, women are more prone to autoimmune diseases and experience more adverse reactions to vaccination. Hormonal, genetic and environmental factors between males and females may affect the immune responses and the sex-related outcome of vaccination. Knowledge of the mechanisms involved in sex disparity in immune responses will contribute to identify the ways to reduce adverse reactions in females and to improve the immune responses in males. This is necessary to adequately protect both sexes against the immune-mediated and infectious diseases with the long-term goal of personalizing the therapies for males and females.

Key words
- sex differences
- gender differences
- immunity
- viral diseases
- vaccine

INTRODUCTION

The immune system and its coordinated response are shaped by a variety of endogenous and exogenous factors, modulators and challenges. Sex differences play a central role in determining how the male and female immune systems are regulated and respond to stimuli. One of the most frequent differences is that females exhibit more vigorous innate [1, 2], cell-mediated [3], and humoral immune responses [4, 5] to antigenic challenges than males, which can reduce pathogen load and accelerate pathogen clearance but can lead to a consequent increase in immune-related pathology [2, 6] such as autoimmune or inflammatory diseases [7-9]. In the medical field sex refers to the genetic and biological status of XX or XY organisms while gender refers to the social and cultural differences between females and males. As both factors play together, they contribute to the immunological dimorphism. [10, 11]. Despite accumulating evidence support sex-based differences in immune responses, in the susceptibility to infectious diseases and in the prevalence of autoimmune diseases, a majority of immunological studies either do not disaggregate and analyze data by sex or do not report the sex of their subjects [12]. The fundamental differences in the immune systems of males and females are attributed not only to differences in sex hormones, but are related to X chromosome gene contributions and the effects of environmental factors [13, 14].

Here we review the main differences in immune system biology between males and females, considering biological and genetic makeup as well as the role of gender as an immunomodulator. Moreover, we review sex-based differential immune responses to viral infection and vaccination.

SEXUAL DIMORPHISM IN IMMUNITY

It is well known that sexual dimorphism occurs both in human and animal models [15]. Males and females exhibit the same immune cells but the responses against bacteria, virus, parasites, allergens and self-antigen are markedly different among the sexes. It is well known, that females develop a lower burden of microbial infections by mounting higher and prolonged humoral and cell mediated immune responses [3] and generally mount higher innate immune responses [1, 2] than males. The heightened immune response that makes females more resistant to infections, however, also rendered them more susceptible to immune-mediated diseases such as autoimmune disorders and inflammatory diseases [7-9].

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Innate immunity

Innate immunity, the first line of defense against infections, is rapid and short lived and is not characterized by immunological memory. Granulocytes, monocytes, macrophages, dendritic cells (DCs) and natural killer (NK) cells are acknowledged to be involved in innate immunity and their function includes phagocytosis, induction and regulation of immune response, destruction of infected cells and cytokine production to amplify the non-specific and specific immune responses. Several differences exist between females and males in their innate immune responses including differences in cell number and activity, as well as in cytokine production. Differences between the sexes are shown in the induction of genes associated with toll-like receptor (TLR) pathways and antiviral type I interferon (IFN) responses with cells from females showing an higher level of expression of these molecules than cells from males [4, 16]. More recently, Griesbeck M *et al*. demonstrated that plasmacytoid DCs derived from females, produced significantly more IFN-α in response to TLR7 ligands than plasmacytoid DCs derived from males, resulting in stronger immune activation [17]. It has been demonstrated that females show a higher phagocytic activity of neutrophils and macrophages and antigen-presenting cells (APC) are more efficient respect to APC from males [18]. In contrast, males have an increased NK cells activity and produce higher levels of pro-inflammatory cytokines, such as IL-6 and TNFα by monocytes and macrophages, although contrasting data are reported in this regard [18, 19]. It has been reported that male monocytes stimulated with LPS showed an increased IL-12 secretion compared to female monocytes [19].

Acquired immunity

Acquired immune response develops later compared with innate response, persists longer, generates memory and is characterized by specificity. T and B lymphocytes, the cellular components of the acquired immunity, originate from a common bone marrow stem cell. T cells represent the main mediators of the cellular immune response while B cells are the mediators of the humoral immune response. Usually, females exhibit more vigorous humoral responses and cell mediated immune responses to antigenic stimulation, vaccination, and infection than do males [4, 20]. Both basal levels of Ig [21] as well as antibody responses to viruses and vaccines are higher in women than men [4, 5, 20]. Moreover, females have on average higher frequency of circulating $CD4^+$ T cells than men and clinical studies reveal that males have lower $CD3^+$ and $CD4^+$ T cell counts, $CD4^+$ to $CD8^+$ T cell ratios, and helper T cell type 1 (Th1) responses than females [22, 23]. Cytokine productions in response to infections is enhanced in females compared with males [15].

BIOLOGIC MECHANISMS OF SEX DIFFERENCES

Immunological differences between the sexes have been focused on two main influences: endocrine-immune interactions as well as genetic differences between the sexes.

Immunomodulatory effects of sex hormones

Sexual hormones, *i.e.* estrogen, progesterone, and testosterone affect immune cells both quantitatively and qualitatively modulating their coordinated responses. Sex steroids alter immune cells performance through the binding to specific receptors, both nuclear and non-nuclear.

The innate immune system is suppressed by estrogen. In particular, estrogen signaling inhibits the transcription of the Fcγ RIIIA gene thus reducing the ability of monocytes to produce IL-1β, IL-6 and TNF [24]. Furthermore, it has been shown that the production of these cytokines is decreased during the follicular phase of the ovarian cycle, and it is increased in the luteal phase [18].

Estrogen also suppresses neutrophils in a biphasic fashion: during menstruation there are decreased numbers of circulating neutrophils [25], in the follicular phase neutrophil endometrial infiltration increases [26], and during post-ovulation as the early corpus luteum develops, there is further neutrophil infiltration [27]. Estrogen boosts the expression of nitric-oxide synthase [28] and therefore of nitric oxide [29], and it impairs the chemotactic ability of neutrophils in culture. Moreover, chemotaxis of neutrophils appears enhanced by progesterone, while testosterone had no modulating effect [29]. Molloy and colleagues have demonstrated decreased neutrophil apoptosis in women compared to men [30].

Elevated levels of progesterone and estrogen affect NK cell activity. High dosage of estrogen induces a suppressive activity down-regulating the expression of NK cells activating receptors CD69, NKp46, NKG2DL and 2B4 [31], while low dosage shows no effect.

Estrogen also has a significant impact on the differentiation and activation of DCs. Bengtsson *et al*. evaluated the effect of estrogen on human monocyte-derived DCs. Exposure of the immature DCs to estrogen enhanced their IL-6, IL-8 and MCP-1 secretion and increased their T lymphocyte stimulatory capacity [32]. The ability to increase T cell stimulation was confirmed and explained by an estrogen-driven up-regulation of MHC II in DCs [33].

Hormonal influences on the function of B lymphocytes have been quantified analyzing immunoglobulin levels. It is well known that females produce more elevated circulating levels of antibodies than males [21]. Kanda and colleagues [34] reported that estrogen enhances IgG and IgM production in both males and females, directly and stimulating monocytes to produce IL-10. Testosterone, on the other hand, appears to inhibit IgM and IgG production both directly and indirectly by reducing the production of IL-6 by monocytes [35]. These hormonal influences can account for the discrepancies between females and males not only in autoimmune diseases [4], but also in response to infectious diseases and to vaccination. It has been reported that estrogen increases the expression of CD22, SHP-1 and Bcl-2, the mediators of B-cell survival, while impairs mediators of B-cell apoptosis such as PD-1. This could trigger the survival of auto-reactive B-cell clones leading to autoimmune diseases [36, 37]. Testosterone

plays an opposite role impairing B-cell differentiation in response to pokeweed mitogen in human PBMC *in vitro* [38].

T regulatory (Treg) cells exert immunoregulatory functions controlling the expansion of the peripheral T cell pool and its response to infections and maintaining self-tolerance controlling the expansion of auto-reactive T cell clones. Estrogen at high concentration (*e.g.* during pregnancy or the periovulatory phase of the menstrual cycle) positively influences Treg cells, increasing their frequency and number [39]. Therefore, estrogen, driving the expansion of the Treg compartment, could contribute to the increased incidence of autoimmune diseases in women [40].

Estrogen controls T cell homing by increasing the expression of certain chemokine receptors in $CD4^+$ T-cells such as the CC-chemokine receptors, CCR5 and CCR1 [41]. Estrogen also regulate Th1 and Th2 responses in a bi-phasic manner during the menstrual cycle in females. Indeed, low doses of estrogen, accompanying menstruation and during the luteal phase skew the Th response towards a Th1 polarization with associated increased cell-mediated immunity, whereas high doses of estrogen during the follicular phase, unbalance the Th cell differentiation toward a Th2 phenotype and associated humoral responses [42].

It has been reported a relationship among commensal gut microbiome, immune responses, and host hormones. Recent finding suggest that the hormonal status of the host can shape microbiome composition and reciprocally, the microbiome may exert various influence over host sex hormone levels, contributing to the differences in the immune response between females and males [43].

The role of sex chromosomes-linked genes and genetic polymorphisms

As a general rule, the gender immunological dimorphism is consequence of direct effect of sex hormones. However, it is well know that a natural difference of expression of Y and X-linked genes also contributes to sex differences in the immune response [44]. More than 1000 genes are mapped on the X chromosome *versus* only 100 on the Y chromosome and many genes on the X chromosome are immune-related coding for proteins involved in immune functions. Furthermore, naturally occurring mutations in one gene copy might produce two distinct alleles with different immunomodulatory capacity. These immune-related genes code for proteins such as TLR7 and TLR8, γ-chain subunit common to receptors for IL-2, IL-4, IL-7, IL-9, IL-15, IL-21 and transcriptional factors such as Foxp3 [13]. Females have a higher expression of TLR7 [45] respect to males, pDCs from females produce more IFN-α in response to TLR7 stimulation than pDC from males [46] and sex hormones, as written above, can modulate the IFN-α production in response to TLR7 ligands [17]. Furthermore, the expression of X-linked genes may be affected by X-linked micro-RNAs (miRNAs), which, regulating post-transcriptional mRNA gene expression, play a central role in maintaining immunological homeostasis. The X chromosome contains a higher number of miRNAs than autosomal chromosome, while Y chromosome contains only two miRNAs. It is now clear that miRNA located on the X chromosome, can contribute to sex-specific development of immuno-mediated diseases [47]. A dysregulated miRNA expression and/or function has been associated with various autoimmune diseases including systemic lupus erythematosus, rheumatoid arthritis and multiple sclerosis and estrogen may contribute to the pathogenesis of autoimmune disease such as systemic lupus erythematosus via the regulation of miRNA expression [48].

Mutations and/or polymorphisms of immune-related gene on X chromosome may be critical determinants of immune system diseases. Female carry two chromosomes, one from each parent, whereas males carry one X chromosome inherited from the mother and one from the father. The X chromosome inactivation provides dosage compensation from X-linked genes between XX females and XY males: in a female, approximately half of the cells expresses genes derived from the maternal X chromosome and the other half express genes derived from the paternal X chromosome. Thus, deleterious mutations that occur in an X chromosome-linked gene makes male more susceptible to clinically relevant diseases, whereas the phenomenon of X inactivation in females protects them from immune dysregulation.

GENDER DIFFERENCES

In addition to biological differences, we discuss the immunomodulatory role of gender. Gender interacts with biological factors, such as hormones and genetics, in the exposure, development and outcomes of the immune response. Gender refers to the differences between males and females regulated by cultural and social factors that, involving all areas of human life, consequently affect the different steps of the immune response. Exposure to various types of antigens, access to health promotion programs and health care, as well as prioritization of health needs, affect the different response of females and males to immunologic challenges [49]. Differences in the social expectations in men and woman in the household and in the workplace determine different exposure patterns to antigens. For example, the exposure to asbestos in construction works, a profession mostly performed by males, increases the incidence of mesothelioma, while in many cultures, women spend more time at home and thus are more exposed to indoor pollution from smoking and heating. Food intake and food composition also affect the immune function and in many areas of the world, the inadequate nutrition is more frequent in the woman [50]. Women in developing countries are frequently confronted with a myriad of socio-cultural factors which negatively impact on physical well-being and accessibility to appropriate health care services. From an immunological viewpoint, a lack of resources determine less access to antibiotics and chemotherapies, affecting infective, rheumatologic or cancer diseases. Poverty is one major gender bias in access to all procedures of health care in any society, thus should be considered an indirect female immunomodulatory factor.

SEX INFLUENCES THE RESPONSES TO AND OUTCOME OF VIRAL INFECTIONS

Rodent studies have shown that males are more susceptible to infections by several pathogens (bacteria, parasites and viruses) and that this difference related to sex hormones [51]. Actually, it has long been recognized [52] that the prevalence and susceptibility to some viral infections are higher in male than in female, as in the case of Hepatitis B and C viruses, HIV, Hantavirus, West Nile Virus infections, influenza viruses, that are more prevalent and intense in male than in female infected patients. However, the outcome and course of viral infections are worse in women than men, although the mortality is often higher in male patients [53]. The mechanisms involved in the sex differences in viral infections are mostly unknown.

According to the above described sex/gender differences in immune responses, women have a reduced susceptibility to viral infections because they mount stronger immune responses than men. There is a growing body of evidence from literature [3, 10] showing that both innate and adaptive immune responses to viral infections differ between males and females. In particular, the immune responses toward viruses are usually more intense in females than in males. As a consequence, women are more immune-reactive and more prone to undergo immune-pathogenic effects of viral infections, as well as to develop increased symptoms in viral infections compared to male infected patients. When a virus infects and encounters the host immune system, induction of the innate immune response, including the APCs activity and the initial inflammatory response mediated by cytokines, chemokines and IFN production, is more elevated in female than in male [53]. Following clearance of virus, during the return to homeostasis, females can maintain elevated immune responses resulting in an increased risk of immune-pathologies; in males, in contrast, lower antiviral immune responses can result in an increased risk of virus persistence compared to females.

THE RESPONSES TO VACCINATION DIFFER BETWEEN SEXES

Women have been historically under-represented in clinical trials, including vaccine clinical trials [53], that, as consequence, may have led to an inaccurate dosage of vaccines for women. The responses to vaccination have been reported to differ between sexes [54]; however, vaccines are commonly administered in a same way to male and female recipients. With regard to this, few years ago, Engler RJ and collaborators showed that young women receiving half dose of the seasonal influenza vaccination generated a stronger antibody response that was equivalent to that of men vaccinated with full dose [55]. Moreover, despite higher morbidity and mortality during influenza pandemics, probably due to elevated induction of pro-inflammatory cytokines and chemokines, women showed a better response to influenza vaccination in terms of higher levels of neutralizing antibodies [56]. Besides influenza vaccination response, sex-related differences have been observed in immunogenicity and effectiveness for several other vaccines, such as Hepatitis A and B [57], measles [58], yellow fever [59], Herpes simplex 2 [60]. Yellow fever vaccine is an interesting example, since it has been reported to elicit a stronger response in females, through the up-regulation of TLR–associated genes, that activate IFN production only in women [61]. In the case of Hepatitis B vaccination in children and adults, anti-HBV antibody titers are higher in females than males [62, 63]. Similarly, the antibody responses to seasonal trivalent inactivated influenza vaccine have been reported to be higher in women than in men and female mice also mount higher neutralizing antibody responses against either sub-lethal primary infection and to vaccination than male mice [64, 65]. However, it is not known whether the enhanced immune responses to vaccines in females are associated to a more efficacy or to long-lived protection.

Facing the female immune-privilege in response to vaccination, women experience worse and more often side effect of vaccination, probably related to higher inflammatory and cellular immune responses in female recipients than in males. This was reported with a measles vaccination trial in Guinea-Bissau and Senegal in 1990s, during which only female recipients of the vaccine had twofold increase of mortality compared with the standard vaccine [66]. In Italy, the official report of post-marketing vaccines surveillance (AIFA 2013) also reported a 56% of adverse reactions in females *vs* 46 % in males towards all the recommended vaccinations.

With regard to the mechanisms involved, based on the above described effects of sex hormones on the immune system it is conceivable that they play a role in the sex disparity of response to vaccines. In fact, it has been reported that men with high testosterone levels mount lower titers of neutralizing antibodies to influenza vaccination compared to men with low levels of testosterone, suggesting an immunosuppressive role for testosterone. Conversely, estrogen at physiological concentration, can stimulate antibody production by B cells including the response to influenza vaccine administered to mice [67, 68].

In addition to the hormonal influences, sex differences observed in immune responses between pre-puberal boys and girls, and in post-menopausal women, suggest a different mechanism. As illustrated in a previous paragraph, the X chromosome contains genes encoding for immune-related molecules and transcription factors (*i.e.* for IL-2 receptor gamma chain, IL-3 receptor, TLR7, -8, CD40 ligand, FOXP3 etc.), that implicates that X chromosomes contain determinants of immune-competence. As a consequence of the XX genetic composition of female and of XY in male any damaging mutation or polymorphism to X-linked genes are more likely to have immune consequences in male compared to female [69]. With regard to this, it has been reported that girls generate higher antibody responses than boys in response to the mumps vaccine as a consequence of the polymorphisms in the cytokine receptor genes IL-12 RB1 and IL-12 RB2 [70]. Androgen receptor is also encoded on X chromosome. The sex hormones receptors bind to their hormone response element upstream of the target genes and recruit methylation enzymes, thus influencing epigenetic regulation of gene expres-

sion [71]. This mechanism, together with the different gene composition between XX and XY, probably affects the sex disparities in immune responses to vaccination.

More recent studies have provided insights into the relation between the microbiota and the host immune responses, as stated above. It is known that bacteria can metabolize sex hormones with consequent direct influence on the immune response, in sex-specific manner. The different efficacy of oral vaccines against poliovirus, rotavirus and cholera in different geographical areas have been ascribed to differences in the microbiota of the recipients [72]. Some studies have shown that probiotics given together with vaccines, such as diphtheria, tetanus, cholera, Hepatitis B, improved the antibody responses [73]. Although these results are intriguing, they deserve further studies.

CONCLUSIONS

Sex-specific determinants of immunity such as the effects of sex hormones, sex-chromosome-encoded genes and environmental exposure directly contribute to the immune cell repertoire in response to a specific insult. These factors may influence DNA methylation, expression levels of miRs, and chromatin remodeling. Moreover, these sex-specific determinants regulate microbiome composition and potentially shape immune cell functional profile, through bidirectional relationships. The consequences of changed immune cell phenotype affect sex-specific responses to vaccination, risk for autoimmune disorders and susceptibility to pathogens.

Therefore, the therapeutic intervention strategies against infections and autoimmune diseases must take these differences into account to provide optimal disease management for both sexes. Future biomedical studies need to report the sex of cells, animals and subjects to improve the understanding of the pathogenesis of diseases and thus personalize the therapies for males and females. The studies so far available on sex different responses to anti-viral therapy and prophylaxis are limited and suggest the need for additional basic biomedical research in this area.

Conflict of interest statement

The authors declare that they have no conflicts of interest.

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