

## Gender disparity in response to anti-viral vaccines: new clues toward personalized vaccinology

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**Summary.** Sex/gender significantly affects the susceptibility and immune responses to viral diseases. Women typically develop higher innate, humoral and cellular immune responses to viral infections than men. Consistently, sex disparity in response to anti-viral vaccination has been described: after vaccination, women develop higher protective antibody titers than men. At the same time, women experience more adverse reactions to vaccination. These differences might be due to sex hormones, to genetic and epigenetic factors and/or to the features of the gut microbiota, and lead to different immune responses and sex-related outcome of vaccination. Disclosure of the mechanisms involved in sex disparity in immune responses could contribute, in the near future, to reduce adverse reactions in females and to improve the immune responses in males, adequately protecting both sexes against viral infectious diseases.

**Key words:** anti-viral vaccine, humoral immune response, cellular immune responses, innate immunity, sex hormone, X chromosome.

### *Disparità di genere in risposta ai vaccini anti-virali: nuovi indizi per una vaccinologia personalizzata*

**Riassunto.** La suscettibilità alle malattie infettive virali è influenzata dal sesso così come la risposta immunitaria alle malattie virali. Le donne infatti sviluppano, in risposta alle infezioni virali, risposte immunitarie, sia innate, che umorali e cellulo-mediate, più intense rispetto agli uomini. Analogamente la risposta ai vaccini anti-virali è diversa nei due sessi: nelle donne si raggiungono titoli di anticorpi protettivi in risposta ai vaccini significativamente più elevati che negli uomini. Tuttavia, le donne manifestano più spesso reazioni avverse ai vaccini. Gli ormoni sessuali, fattori genetici ed epigenetici e la diversa composizione del microbioma hanno un ruolo chiave e possono influenzare le risposte immunitarie e l'outcome della vaccinazione negli uomini e nelle donne. La scoperta dei meccanismi coinvolti contribuirà ad identificare i modi per ridurre le reazioni avverse ai vaccini nelle donne e ad aumentare la risposta immunitaria negli uomini.

**Parole chiave:** vaccini anti-virali, risposta immunitaria umorale, risposta immunitaria cellulare, immunità innata, ormoni sessuali, cromosoma X.

### Introduction

Vaccination is the main preventative intervention for controlling infectious viral diseases. It is well-established that many infectious diseases have successfully been reduced thanks to the widespread practice of vaccination campaigns in western countries. However, until recently, several lines of evidence clearly suggested that “biological sex” can affect the immune response to infection and, strikingly but consequently, to vaccination. At present, the relevance and mechanisms underpinning such disparities are not well understood. This is at least partially due to two major issues: i) sex disparity in vaccination, i.e. females have historically not been included in clinical trials in general, as well as in vaccine trials, and ii) inadequate dosages and schedules used, i.e. although several immune response differences between males and females have been disclosed<sup>1</sup>, vaccines are administered equally, at the same dose, in male and female recipients.

### Main features of sex disparity

As a general rule, sex disparity in response to vaccination could probably be related to the fact that the prevalence of and susceptibility to viral infections are higher in males than in females. This is true for a plethora of viral infections including Hepatitis B and C virus infections, influenza viruses, human immunodeficiency virus (HIV), and Hantavirus or West Nile virus. Even for viral infections whose incidence is not sex-biased, such as dengue and measles, a sex disparity has been detected as concerns the outcome and course of infections, worse in females than in males in terms of prevalence but more often harmful or lethal in males<sup>2-4</sup>. The mechanisms involved in the sex differences in viral infections are mostly unknown. It was however documented that males and females mount different immune responses to viral infections and it is now clear that females tend to produce more intense immune responses, either innate or adaptive<sup>5</sup>. For instance, women produce more effector cytokines, i.e. those cytokines that are produced by different types of dendritic cells and define the type of T cell effector response. In addition, women also produce higher titers of virus-specific antibodies as well as higher cell mediated immune responses than men.

As a consequence, females are more immune-reactive and more prone to experiencing immune-pathogenic effects of viral infections compared to male infected patients. Induction of Toll-like receptors (TLRs) associated genes and type I interferon (IFN) production are higher in females than in males<sup>6</sup>. TLRs are a class of proteins that play a key role in the innate immune system. They are single, membrane-spanning, non-catalytic receptors usually expressed by sentinel cells such as macrophages and dendritic cells, which are cells able to recognize structurally conserved molecules derived from microbes. Type I IFN proteins are mainly involved in innate immune response against viral infection, but also regulate the development of adaptive immune response. In addition, some clinical studies showed that CD4+:CD8+ T cell ratios and inflammatory Th1 responses, essential for the adaptive immune system response, are lower in males than females. On the other hand, T cells from females exhibit higher cytotoxic T-lymphocyte activity, of great importance in killing virus-infected cells, than those from men<sup>7</sup>. However, such differences are not taken into account in designing or dosing anti-viral drugs and vaccines.

Similarly to the sex bias in immune responses to viral infection, in both animals and humans, the immune responses to anti-viral vaccines might be expected to be different between male and female recipients. Scant data are available from literature and only the sex bias in humoral responses to vaccines has been reviewed<sup>8</sup>. Usually, the production of protective antibodies to several vaccinations is more elevated, about twice, in females compared to males at all ages. This is particularly true for vaccination against single-stranded RNA viruses, such as trivalent seasonal influenza vaccine, measles, mumps, yellow fever, dengue, hepatitis A and B, rubella and herpes simplex vaccinations<sup>9,10</sup>, but also against DNA viruses such as HBV and herpes simplex vaccinations. Single-strand RNA viruses are recognized by toll-like receptor 7 (TLR-7), which is encoded on the X chromosome. Thus, sex-specific antiviral responses might be affected by sex differences in pathogen recognition capabilities. This means that the ability of

men's and women's immune system to identify and counteract a pathogen invasion should be considered as a critical point in the administration of antiviral drugs and vaccines. Hence, as mentioned above, a sex bias in response to vaccination might be due to the higher levels of CD4+ lymphocytes and production of Th1 cytokines after immunization<sup>11</sup> that women have. Furthermore, the antibody responses following viral vaccines for hepatitis B and A, influenza A and herpes virus are reported to be always more intense in women than in men. In the case of herpes simplex 2 virus (HSV2), responses to vaccines showed differences between the sexes, since HSV-2 vaccine was protective against genital herpes only in women and not in men<sup>12</sup>. Some points of great interest derive from this disparity. For instance, the higher rates of seroconversion in anti-HBV vaccinated women<sup>13</sup>, both in children and adults<sup>14,15</sup>, may result in a reduced prevalence of liver cancer development in females compared to males<sup>16</sup>.

Important data on sex-associated differences in vaccinations can also be derived from studies on the influenza vaccination. It has been observed that antibody responses to seasonal trivalent inactivated vaccines are higher in women than in men<sup>8,17</sup>. An intriguing study regarding seasonal influenza vaccination also reported that U.S. women vaccinated with half dose of the trivalent influenza vaccine mounted antibody responses of similar magnitude to those obtained in men given the full dose of the flu vaccine<sup>18</sup>. These data are in line with those obtained in mice, in which females develop stronger neutralizing and total antibody responses against influenza vaccination<sup>19</sup>. This leads to a subsequent better protection of female mice against lethal challenge with the hetero-subtypic strains of influenza viruses, i.e. to cross-protection against infection with an influenza A virus serotype other than the one used for the primary infection. If this scenario is confirmed also for other vaccines, it could actually contribute to the development of a more personalized vaccination and to more appropriate vaccination campaigns. The above clearly shows that women are immune-privileged compared to men, but the other side of the coin is that

**Table 1.** Sex-related differences in vaccinations.

Virus	Antiviral vaccine	Adverse reactions	Antibody titers post-vaccination	References
Seasonal influenza virus	Trivalent (TIV)	F > M	F > M	Cook et al. <sup>17</sup> ; Klein et al. <sup>10</sup>
HBV	HBV vaccine	F > M	F > M	Klein et al. <sup>10</sup> ; Vermeiren et al. <sup>13</sup>
Hepatitis A	HAV vaccine	F > M	F > M	Klein et al. <sup>10</sup> ; Vermeiren et al. <sup>13</sup>
Herpes simplex-2	HSV-2 gD vaccine	F > M	F > M	Klein et al. <sup>10</sup>

the adverse reactions to all vaccinations, such as fever, inflammation and pain in the administration site, are reported more frequently in female recipients than in males (Table 1): this is confirmed by the last (2013) annual report of the Italian Agency of Pharmacology Surveillance (AIFA), which shows 54% of adverse reactions in women vs 46% in men, considering reactions to all viral and bacterial vaccinations, in Italy.

As concerns the mechanisms involved in sex-based differences in the immune responses to vaccination described above, sex steroid hormones, genetic and epigenetic regulation and microbioma have been proposed as possible key players and they will briefly be discussed here below.

### Sex steroid hormones

Sex steroid hormones are thought to contribute to differences in both humoral and cellular immune responses to infection and vaccination in men and women<sup>8</sup>. In fact, animal and clinical studies have documented the relevant interplay between sex hormones and immune responses<sup>20</sup>. Consistently, sex-hormones also influence responses to anti-viral vaccines. In particular, it has been observed that estrogens, mainly 17-beta-estradiol (E2), can regulate both proliferation and apoptotic cell death of immune cells. It has actually been demonstrated since 1983<sup>21</sup> that E2 inhibits B cell apoptosis, thus increasing antibody production by B cells, and has dose-related effects on T-cell functions. This modulatory activity of E2 can contribute to the integrity and function of peripheral blood lymphocytes interfering with or bolstering both innate and adaptive immunity<sup>22</sup>. The majority of the immune cells, including T and B lymphocytes, neutrophils, macrophages, dendritic cells (DC) and NK, express estrogen receptors<sup>1</sup>. Thus, the activation by steroid hormones of their respective alpha or beta receptors modulates effector functions of the immune cells, reviewed in<sup>7</sup>. It has been reported that low doses of estrogens induce Th1 immune responses characterized by increased IFN-gamma production; whereas high doses of estrogen lead to Th2 polarization of the immune responses, with increased IL-4, IL-5 and IL-10 cytokines production, which activate the antibodies production by B cells and drive B cell proliferation and differentiation to plasma cells. Furthermore, estrogen levels influence the expansion of regulatory T cells (Tregs cells), which are T lymphocytes in charge of preventing autoimmune diseases and maintaining immunologic self-tolerance, including the maternal-fetal tolerance. The activity of Tregs is also influenced by increasing progesterone levels during pregnancy with consequent remission of autoimmune diseases, but leading to an increased susceptibility to

infectious diseases, such as influenza or malaria<sup>10,23</sup>. Androgen receptors are also expressed by T and B lymphocytes: therefore, it has been suggested that male hormones, such as testosterone, may also exert an immunomodulatory activity<sup>24</sup>. The reported lines of evidence indicate that androgen has effects opposite to estrogen on the immune system, since they limit IFN-gamma production: testosterone has shown to have an overall suppressive effect on the immune system, in particular on viral and host antigens, and to stimulate Th1 responses as well as activation of CD8+ T cells<sup>11</sup>. Th1 cells play a pivotal role in protecting an individual from infections by bacteria, fungi, and viruses, therefore the suppressive effect of androgen on Th1 cells may explain the higher susceptibility to viral infection in men than in women.

As concerns B cells, it has been suggested that sex steroid hormones can affect these cells in an opposite way, as estrogen promotes antibodies production by B cells through promotion of B cell proliferation mediated by IL-4 and IL-5. In fact, estradiol increases immunoglobulin-producing cells, by decreasing apoptosis of immature B cells<sup>11</sup>. Conversely, androgens and progesterone inhibit antibodies production by B cells. Therefore, to generalize, sex steroid hormones potently influence the immune system: estrogens have immune-enhancing effects whereas progesterone and androgens have immunosuppressive effects.

All in all, this scenario might explain the higher immune reactivity of women to infectious diseases and their increased susceptibility to autoimmune diseases, as well as the more intense immune responses to vaccination in females than in males. Accordingly, based on epidemiological evidence, women lose their immune privilege after menopause, when the hormonal milieu substantially changes<sup>13</sup>. However, the effect of hormonal levels in response to vaccines in aged recipients cannot be generalized to all vaccines and it requires more in-depth investigation.

### Genetic and epigenetic regulation

It is well-known that a large number of immune-related genes are located on the X chromosome. These include: the IL-2 receptor, which is expressed at the surface of activated T cells and natural killer (NK) cells; the toll-like receptors TLR-7 and TLR-8 which ligate single stranded RNA (ssRNA) molecules, such as ssRNA genome of viruses; the CD-154 (or CD-40 ligand), which is expressed on T- and B- lymphocytes and on DC and whose interaction with CD-40 receptor modulates the adaptive immune response in terms of activation of T cells and proliferation of B cells and antibodies production; FOXP-3, a transcription factor controlling Treg

development and function, and IRAK (Interleukin-1 receptor-associated kinase), which is an activator of TLR-mediated signal transduction. As a consequence, the X-linked genes play a key role as determinants of immune competence. Several transcriptional and translational control effectors, such as Histone deacetylase (HDAC) and NFkB (nuclear factor kappa-light-chain-enhancer of activated B cells), which are involved respectively in post-translational chromatin DNA modifications and transcriptional control of genes involved in inflammatory responses, are also encoded on the X chromosome. Consequently, since males are XY and females are XX, any mutation or polymorphism of X-linked genes may have more immune consequence for males than for females<sup>25</sup>.

As an example with regard to this, it has been reported that school age girls, who received the two doses of MMR vaccine (mumps, measles and rubella) generated higher antibody responses to mumps vaccine than boys. This was found to be a consequence of polymorphisms in the cytokine receptor genes IL-12 RB1 and IL-12 RB2<sup>26</sup>, which influence the IL-12 cytokine function in the molecular switch of T cell phenotype and in the B cell function.

The androgen receptor is encoded on the X chromosome, and since both estrogen and androgen receptors bind to the hormone response elements upstream of target genes and recruit HDAC and methyltransferases that regulate gene transcription through the gene DNA methylation, the combined effects of the hormones on epigenetic regulation of gene expression and the different gene composition between XX and XY, probably affect sex disparity in immune responses to vaccination.

### microRNA

Recent studies pointed to the contribution of short non-coding RNA, the microRNAs (miRs), in controlling gene expression through mRNA degradation or inhibition of mRNA translation. It has recently been reported that the X chromosome contains about 12% of all human encoding microRNAs, i.e. about 800-1000 miRs. Although the role of most X-linked miRs is not known, several of them have a role in cancer onset and regulate the immune responses/system at different levels<sup>27</sup>. Among them, miR-223 positively regulates granulocytic differentiation, miR-106a, miR-424, miR-542 and miR-503 control monocytopoiesis and monocytic differentiation, which can have a great impact on innate immune responses, and regulate IL-10 (an anti-inflammatory cytokine) production. Moreover, miR-18 and -19 enhance inflammatory responses through the control of NFkB signaling<sup>28,29</sup>. Dysregulation of these and other miRNAs expression on the X chromosome, by means of irregular DNA methylation during X

silencing escape, may determine the differences in immune responses between sexes. There are still several gaps in the knowledge of the X-linked miRNAs functions and of the contribution of those miRs to gender-specific predisposition for immune diseases. MiRNA mediated regulation of genes can have a high impact on cell function, therefore this is a challenging field of investigation deserving future development.

### Microbiota

The human microbiota consists of microbial communities in different habitats of the body, including the skin, gut, oral cavity, and genitals. They vary according to sex and time. Both diet and antibiotic use produce shifts in the features of microbiota<sup>30</sup>.

Recent studies provided novel insights into the relationships between the microbiota and the host immune responses. It is known that bacteria can metabolize sex hormones with consequent direct influence on the immune response. The different efficacy of oral vaccines against poliovirus, rotavirus and cholera in different geographical areas has been ascribed to differences in the microbiota of the recipients<sup>31</sup>. Some studies have shown that probiotics given together with vaccines such as diphtheria, tetanus, cholera, and Hepatitis B, improved antibody responses<sup>32</sup>. On the whole, accumulating data point to a specific role of the microbiota composition in the modulation of immune responses. Hence, this represents a further argument of great interest in the understanding of sex disparity in vaccine responses.

### Conclusions

Biological differences between males and females contribute to sex-specific vaccine outcomes. Women develop higher antibody responses to vaccines than men and experience more adverse reactions to vaccinations. Although sex steroid hormones, genetic and epigenetic mechanisms, as well as microRNAs and microbiota composition have been demonstrated to affect immune responses to vaccines over time, specific and detailed mechanisms mediating those sex-based differences are lacking so far. Additional basic biomedical research in this area should be carried out in order to adjust vaccine composition and dosage to sex-related differences in immune responses.

### Key messages

- Sex based disparity in response to anti-viral vaccine is related to the sex disparity in immune response.
- Production of protective antibodies in response to vaccines is more elevated in women than in men.
- Women are immune-privileged compared to men.
- Women experience more adverse reactions to vaccination than men.
- Sex-hormones, epigenetics, microRNAs and microbioma represent the main actors in sex disparity in response to vaccines.

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