Sex, gender and infectious disease

Despite evidence of sex-specific pathogenesis, few studies of infectious diseases report or analyse sex or gender, unless it is the primary focus. Using HIV as an example, it is argued here that this leaves potentially informative data unexplored and that integrating sex and gender in analyses may accelerate research in microbial pathogenesis.

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Women are typically underrepresented in clinical trials, in part due to historical restrictions on female enrollment[1,2], which has led to a knowledge gap. Without adequate representation, our understanding of how biological sex (chromosomes and anatomy) and gender (a social construct and internal sense of self)[2] may influence the acquisition and pathogenesis of infectious diseases remains incomplete. But is consideration of sex and/or gender important in understanding microbial pathogenesis? For human immunodeficiency virus (HIV), notwithstanding the limits of the available research, decades of clinical and basic-science data provide a clear answer: sex and gender impact HIV pathogenesis[3]. Here, using HIV as the focus and including select examples from other diseases, I argue that sex and gender must be integrated into infectious disease research as a tool for discovery.

Balancing burden and representation

To date, 36 million lives have been lost in the global HIV pandemic. Of the more than 37 million people living with HIV today, over 50% are women or girls. HIV research has not reflected the burden of HIV infection among women[4] and has not consistently achieved the National Institutes of Health's goals of considering sex as a biological variable[1]. This lack of representation is at the peril of missing opportunities to identify mechanisms of disease and of deploying treatments that have been inadequately evaluated in both males and females.

Representation of women in clinical trials in HIV has also been impacted by the geographic distribution of the pandemic, with the majority of women living with HIV in under-resourced settings with less participation in treatment and cure trials. Additionally, HIV is prevalent among transgender women, also a minority of trial participants. For transgender women, there is the intersection of sex and gender with a discordance between sex chromosome complement (XY), exposure to feminizing sex hormones, and the specific cultural exposures linked to their gender[2]. A simplistic strategy of comparing cisgender women from sub-Saharan Africa with cisgender men from other sites is inadequate; the diverse socioeconomic and cultural contexts, ages, HIV virus clade and host genetics confound these comparisons on multiple levels. With these challenges, the underrepresentation of women is unsurprising and the limitations of many existing comparative studies are apparent.

Perhaps less obvious is the importance of considering sex and gender in preclinical studies. Here again, scientific rigor obligates that experimental animal models be tested in both sexes, and that the use of cell lines or human samples should account for the sex chromosome complement of the cells and the sex hormone milieu from which they are taken[5]. In vitro systems can provide a clean first inquiry into differences, as evidenced by examples from HIV research discussed below.

HIV acquisition

Globally, HIV acquisition mostly occurs at mucosal sites through sexual exposure. The sites at risk and the probability of seroconversion following a single exposure vary by sex, gender and sexual practices. Preventive strategies must be either highly effective or targeted to specific populations in which their effects are most protective. There are notable differences in some vaccine responses by sex[6], and given the challenges in HIV vaccine development, it follows that adequate inclusion for sex-stratified analyses in vaccine trials is imperative as an effect observed only in males or only in females would still potentially be of importance.

Pharmacologic prevention of HIV infection with pre-exposure prophylaxis (PrEP) is an example of unexpected impacts of sex and gender. Accelerated metabolism of the antiretroviral tenofovir in a topical microbicide by the vaginal microbiome was linked to lower preventive efficacy[7], highlighting the need to consider treatments in biological context. Gender-based behavioural differences have also featured prominently in PrEP; low rates of protection specifically in women were linked to lower adherence among women and a more stringent requirement for adherence for women to achieve protection[8]. Thus, HIV acquisition is impacted by anatomical differences, sex-based differences in vaccine responses, mucosal microbiomes and gender-based health behaviours. Some of these differences are not exclusive to HIV, or to the reproductive tract. The impact of sex-specific mucosal immunity on herpes simplex virus (HSV) acquisition is clear, and a HSV vaccine demonstrated efficacy in females, but not males[9]. Outside of the reproductive tract, sex hormones shape the gut microbiome[10]. To elucidate the interaction between the microbiome and pathogens, both preclinical and clinical trial designs need to include males and females and consider the impact of sex hormones.

HIV pathogenesis and cure

Beyond acquisition, several features of HIV infection differ between females and males — notably a lower viral load among females[3]. Despite this lower level of viraemia, disease progression and $CD4^+$ T-cell decline occurs at a similar rate for both sexes, making early viral-load-based treatment guidelines inappropriate for women[11]. Why lower HIV viral loads drive $CD4^+$ T-cell depletion in females is not fully understood, but it may reflect differences in immune activation. For example, one key innate sensor of HIV RNA, Toll-like receptor 7 (TLR7), has sex-specific features: after stimulation through TLR7 in vitro, female cells produce more of the antiviral cytokine interferon-α (IFNα) than male cells[12]. TLR7 is encoded on the X chromosome and can be biallelically expressed, giving females a higher gene dosage and an advantage in the case of a hypofunctional allele. The latter has been highlighted during the SARS-CoV-2 pandemic through the identification of rare TLR7 variants that are associated with severe disease in males[13]. These data illustrate that interactions between microorganisms and immune sensors are not a monolith. Despite

the shared, fundamental goal of defence against pathogens, host characteristics that include sex confer immune pressures that introduce variation in the immune response, even at the most basic level of the innate recognition.

What do these subtle differences in immune response between males and females mean for HIV pathogenesis? It is notable that rare phenotypes of spontaneous viral control are more commonly observed in females[3], including the recently described case reports of 'exceptional' elite controllers, who have little or no detectable replication competent HIV in the absence of anti-retroviral therapy[14]. The higher frequency of these outcomes in females suggests that there are consequences of the sex-based variations in immune responses. The oestrogen receptor (ESR1) was identified as a regulator of HIV latency through in vitro studies[15], highlighting the role of preclinical studies. Despite these data, in studies of elite control and the HIV reservoir, sex-stratified analyses are scant and enrolment of cisgender and transgender women is low. This is a missed opportunity. Many experimental therapies directed at HIV cure target the host immune system, not the virus. The efficacy and safety of these host-directed therapies (HDTs) are determined by host characteristics, and ignoring diversity is not possible. HIV cure is not alone in this shift. Multiple immunomodulatory therapies have been deployed against COVID-19, with documented heterogeneity in response between males and females, and HDT is an area of growing focus in tuberculosis. If the frontiers of infectious disease therapeutics are at the interface between the microorganism and the host responses, variation on both sides of this equation must be included in study design.

Moving the field forward

Classical studies of microbial pathogenesis are centred on a reductive approach based on models of disease, isolating one or a few variables of high interest while holding all other features constant. This is the clearest pathway to define the mechanism, but the results are inherently limited. These models answer focused questions with certainty, but the results may not be relevant in the context of human disease. From the opposite end of the spectrum, clinical cohorts identify features associated with outcomes, but are limited by the diversity of the participants and often cannot reach causal conclusions. Despite years of study, we still cannot predict who will become a spontaneous controller of HIV infection. In this case and in many others, truly advancing the understanding of microbial pathogenesis will mean untangling the host–pathogen relationship while accounting for multiple sources of variability, including biological sex and gender. This is critical to ensure that new therapies are safe and efficacious for all people, regardless of sex or gender.

Several steps can be taken to better integrate these variables across the spectrum of infectious diseases research, including ensuring that sex-stratified data are clearly reported, using both male and female animals in model systems, reporting the sex of cells used for in vitro studies and ethically recruiting more cisgender and transgender women into clinical trials. Required reporting by funding agencies and journals will have the greatest impact on several of these.

For trial enrolment, community engagement tailored to women must be coupled with specific and rational enrolment metrics by sex and gender to realize the goal of more diverse trial participants. The scientific and ethical mandates are clear, and the promise of novel discovery is substantial. ❐

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Competing interests

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