Journal Pre-proof



Sex-biased immune responses following SARS-CoV-2 infection

Rebecca L. Ursin, Janna R. Shapiro, Sabra L. Klein

PII: S0966-842X(20)30263-8

DOI: https://doi.org/10.1016/j.tim.2020.10.002

Reference: TIMI 1884

To appear in: *Trends in Microbiology*

Please cite this article as: R.L. Ursin, J.R. Shapiro and S.L. Klein, Sex-biased immune responses following SARS-CoV-2 infection, *Trends in Microbiology* (2020), https://doi.org/10.1016/j.tim.2020.10.002

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2020 Published by Elsevier.

Title: Sex-biased immune responses following SARS-CoV-2 infection

Authors: Rebecca L. Ursin, M.S.[1]‡, Janna R. Shapiro, M.S.[2,] ‡, Sabra L. Klein, PhD[1,2,3,*]

Affiliations: [1]Department of Biochemistry and Molecular Biology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA
[2]Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA
[3]W. Harry Feinstone Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, MD. USA

‡ Co-first authors
*Correspondence : Sabra L. Klein sklein2@jhu.edu .

Keywords: biological sex, SARS-CoV-2, COVID-19, immunopathogenesis

Abstract: Males are disproportionately affected by severe disease and death from SARS-CoV-2 infection. In their recent article, Takahashi et al. found sex differences in immune responses to SARS-CoV-2 and the predictors of disease progression. These findings contribute to elucidating the mechanisms that underlie the male-bias in severe disease and death from COVID-19.

Text body: The world just crossed a grim milestone, with over 1 million deaths due to COVID-19. The United States alone just surpassed 200,000 deaths, with an additional 200,000 projected by the end of 2020 [1]. While there are promising vaccine and therapeutic clinical trials, we desperately need to complement these initiatives with a deeper understanding of the immune responses that protect but that also cause pathology during SARS-CoV-2 infection. This goal is complicated by the fact that the virus does not impact everyone similarly, with age and comorbidities, such as obesity, diabetes, and heart conditions serving as risk factors for severe COVID-19 [2]. Biological sex is another factor influencing disease severity. SARS-CoV-2 causes significantly more hospitalizations, intensive care unit (ICU) visits, and deaths in males than in females, across diverse countries and age groups. Globally, for every 10 hospitalizations of adult females there are 13 in males. And most strikingly, for every 10 deaths in females, there are 14 in males [3]. It is important to note that both sex and gender contribute to disparities in COVID-19 outcomes; the former describing our biological predisposition (i.e. sex chromosome complement, sex steroid hormones, and reproductive tissues) and the latter referring to the social and behavioral characteristics (i.e. heath-seeking behaviors, mask wearing, and occupation) [2].

In their recent article, Takahashi et al. explore the immunopathogenic phenotypes exhibited by males and females in a cohort of 98 hospitalized SARS-CoV-2-positive patients, as compared to a control group of uninfected health care workers (HCW) [4]. The first study of 39 patients not admitted to the ICU was used to assess baseline immune parameters, while

repeated sampling from the larger cohort was used to assess longitudinal differences. Analysis of 71 plasma cytokines and chemokines adjusted for age and body mass index (BMI) revealed few differences between male and female patients. In unadjusted analysis, both male and female patients had higher frequencies of total and intermediate monocytes than healthy controls, while the proportion of non-classical monocytes was higher in male than female patients and HCW of either sex. High frequencies of non-classical monocytes in males were associated with lower T cell frequencies and were positively correlated with levels of CCL5, a chemokine important for recruiting T cells to inflammatory sites. Female patients had a higher proportion of activated ($CD38^{+}HLA\text{-}DR^{+}$) and terminally differentiated ($PD\text{-}1^{+}TIM\text{-}3^{+}$) T cells than female HCWs, particularly in the CD8+ T cell compartment. Clinical scores at repeated time points were used to classify patients as stabilized or deteriorated. Among males, a greater deterioration was positively correlated with age and BMI and negatively correlated with activated and terminally differentiated CD8+ T cells. Among females, disease progression was associated with the innate cytokines IL-15 and TNSF10 (TRAIL). The authors conclude that their findings demonstrate key sex differences in COVID-19 disease dynamics and provide a rationale for the pursuit of sex-specific strategies for prevention, care, and treatment of COVID-19.

Focusing on genetic differences, one small case series identified loss-of-function variants of the pattern recognition receptor toll-like receptor 7 (TLR7) in male patients requiring mechanical ventilation [5]. Upon stimulation *in vitro* with imiquimod, a TLR7 agonist, peripheral blood mononuclear cells isolated from the patients showed no increase in *TLR7* mRNA expression, decreased expression of transcripts in the type I interferon (IFN) pathway, and decreased production of IFN-γ, as compared to healthy controls. *TLR7* is X-linked and known to escape X-inactivation [6], suggesting a mechanism whereby men expressing a single copy of *TLR7* are at increased risk of severe disease compared to women expressing two copies. This conclusion would also support the findings in the current paper, whereby greater T cell activation in females may result from greater induction of TLR7. A larger study performed shotgun RNA sequencing from nasopharyngeal swabs collected from 430 infected individuals and 54 controls [7]. Analysis revealed 19 genes where the sex difference in expression could be attributed to infection with SARS-CoV-2, including down-regulation of B cell activity and natural killer (NK) cell-activating receptors in males, coupled with an up-regulation of transcripts that inhibit NFκB signaling.

A recent article reports that at least 10% of 987 patients with severe disease had neutralizing IgG auto-antibodies against IFN-ω and/or IFN-α, which were associated with low serum IFN levels *in vivo* and inhibited the ability of IFN-α2 to block SARS-CoV-2 from infecting Huh7.5 cells *in vitro* [8]. These auto-antibodies were not found in any of 663 patients with mild or asymptomatic disease and are estimated to be present in 0.33% (0.015-0.67%) of the general population. Strikingly, 94% of the patients with neutralizing auto-antibodies were male, providing yet another mechanism for the observed male bias in severe disease and death. Among COVID-19 patients with mild disease, a male sex bias exists during the recovery phase of COVID-19 where males produce more robust anti-SARS-CoV-2-Spike protein antibodies and greater neutralizing antibodies than females. Because Takahashi et al. saw no sex difference in

antibody titers at baseline, this points to a potential difference in antibody kinetics between the sexes [9].

Takahashi et al. provide insight into the underlying immunological mechanisms that contribute to sex differences in COVID-19. Most published reports describe heterogeneity of the immune responses to SARS-CoV-2, without analytical consideration of the host demographic factors that could be involved. The data in Takahashi et al. illustrate that host demographic factors, including sex, explain heterogeneity in the immune responses to SARS-CoV-2, which could provide novel insights into targetable factors that could mitigate disease. Pre-existing conditions, age, BMI, sex, and other intrinsic factors can all drastically alter how a viral pathogen is recognized, the magnitude and efficacy of innate and adaptive immune responses, and ultimately disease outcomes. Sex-specific treatment of all diseases, not just viruses and not just SARS-CoV-2, need to be further investigated and implemented. Furthermore, sex-disaggregated data in vaccine and therapeutic trials are crucial for developing interventions that are safe and effective for both sexes[10]. Most likely, this virus will be maintained in the environment as a seasonal pathogen, much like the 2009 H1N1 influenza pandemic virus, with long-term implications to public health. In the meantime, investigating sex differences in the immune response to SARS-CoV-2 infection has the potential to provide therapeutic insights and contribute to precision medical interventions that do not assume that we can all be treated identically to be protected equally.

References:

1. National Center for Immunization and Respiratory Diseases (NCIRD) Division of Viral Diseases (2020) COVID-19 Forecasts: Deaths. https://www.cdc.gov/coronavirus/2019-ncov/covid-data/forecasting-us.html, (accessed Oct. 2 2020).
2. Scully, E.P. et al. (2020) Considering how biological sex impacts immune responses and COVID-19 outcomes. Nature Reviews Immunology, 1-6.
3. (2020) Global Health 5050. https://globalhealth5050.org/, (accessed Oct 5 2020).
4. Takahashi, T. et al. (2020) Sex differences in immune responses that underlie COVID-19 disease outcomes. Nature, 1-9.
5. Made, C.I.v.d. et al. (2020) Presence of Genetic Variants Among Young Men With Severe COVID-19. JAMA 324 (7).
6. Souyris, M. et al. (2018) TLR7 escapes X chromosome inactivation in immune cells. Science immunology 3 (19), eaap8855.
7. Lieberman, N.A.P. et al. (2020) In vivo antiviral host transcriptional response to SARS-CoV-2 by viral load, sex, and age. PLOS Biology 18 (9), e3000849.
8. Bastard, P. et al. (2020) Auto-antibodies against type I IFNs in patients with life-threatening COVID-19. Science, eabd4585.
9. Klein, S.L. et al. (2020) Sex, age, and hospitalization drive antibody responses in a COVID-19 convalescent plasma donor population. The Journal of Clinical Investigation.

10. Bischof, E. et al. (2020) Clinical trials for COVID-19 should include sex as a variable. J Clin Invest.

Journal Pre-proof