Natural killer cells

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UT(se)X differences during immune responses

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The immune system is not immune to sex differences. New research now uncovers the molecular mechanisms that underlie sex-based differences during antiviral immune responses.

Although the sex of an individual is defined based on the sex chromosome complement, and the presence of gonads and the respective gonadal hormones, sex differences are evident in multiple non-reproductive organs, including the immune system[1]. As was illustrated during the ongoing COVID-19 pandemic, male sex can be associated with more severe disease and outcomes than female sex, possibly indicating weaker antiviral responses in men[2]. In general, women mount more robust immune responses during viral infections, including those to influenza and hepatitis B viruses, and show greater drug and vaccine responsiveness; in contrast, women are more susceptible to autoimmune diseases[3]. Although hormonal differences between men and women have often been implicated in these differences[4], the underlying molecular basis was unclear. In this issue of *Nature Immunology*, Cheng et al.[5] provided strong evidence that sex chromosome dosage controls the number and activity of natural killer (NK) cells, thus regulating antiviral immune responses in a sex-dependent manner (Fig. 1).

NK cells are a type of immune cell that is important in controlling viral infections. Paradoxically, men tend to have more NK cells than women, yet are more susceptible to viral infection[6]. However, Cheng et al.[5] observed that isolated male NK cells produced lower levels of interferon-γ (IFNγ) than female cells after ex vivo stimulation with pro-inflammatory cytokines, in both humans and mice. Male NK cells also showed impaired target cell killing and production of cytotoxic molecules, such as granzyme B and perforin, indicative of overall compromised NK cell responses in male cells. Importantly, these phenotypes persisted in gonadectomized mice, thus excluding the possibility that sex-related hormone differences might account for the observed sexual dimorphism of NK cells.

A number of immune-related genes, including *TLR7* (encoding Toll-like receptor 7), *FOXP3* (encoding the transcription factor FOXP3) and *KDM6A* (encoding UTX, an epigenetic regulator with demethylase activity), as well as several microRNA molecules, are found on the X chromosome[1]. Although large portions of one of the two X chromosomes become inactivated in female mammals to correct for gene dosage effects, some genes escape from X chromosome inactivation (XCI), leading to their increased expression[7]. Therefore, Cheng et al.[5] postulated that this unequal gene expression pattern may contribute to discrete NK cell responses between sexes. Through gene expression screening, the authors discovered five genes that escape XCI in both mice and humans. Although all five were differentially expressed between the sexes, the authors focused on *Kdm6a* because it was the most downregulated XCI gene in male NK cells. Notably, these differences in *Kdm6a* transcript and UTX protein levels were independent of gonadal sex organs, indicating that UTX expression is primarily determined by X chromosome dosage.

In an elegant series of experiments, Cheng et al.[5] investigated the role of UTX in sex-related immune responses in female mice with heterozygous deletion of the *Kdm6a* gene (Utx^{Het}) specifically in NK cells (using the $Ncr1^{cre}$ mouse model[8]), which resembled male mice in their levels of UTX. Loss of one *Kdm6a* allele in female mice led to

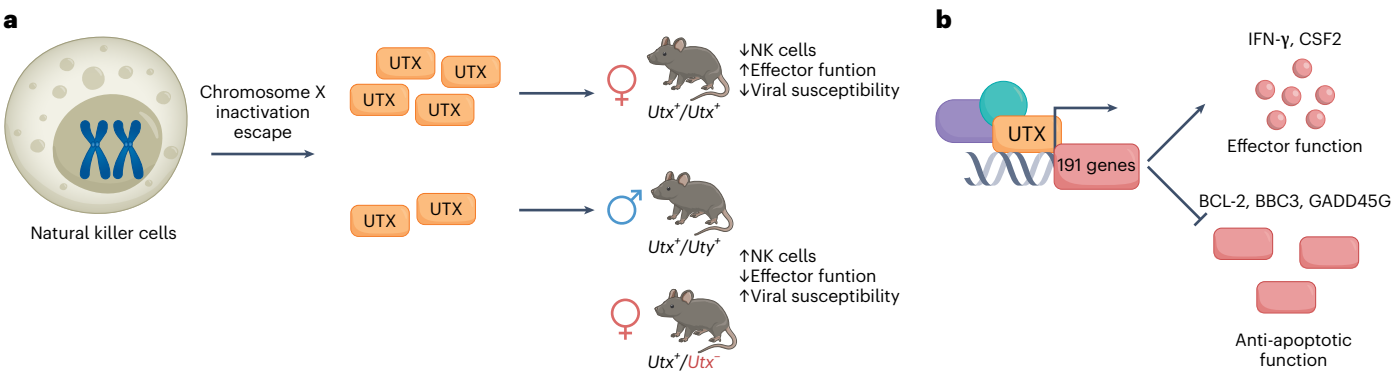

Fig. 1 | The X-linked protein UTX controls NK cell number and functions in a sex-dependent manner. a, *Kdm6a* is located on the X chromosome but escapes X chromosome inactivation in female mice and humans. Female mice have fewer NK cells but are less susceptible to viral infection, whereas male mice have more copious NK cells with less cytotoxic capacity. Female mice with heterozygous deletion of the *Kdm6a* gene in NK cells showed similar NK cell number and function to male mice, suggesting that UTX is a critical mediator of sex-related immune responses. In addition, using mice with complete deletion of UTX in NK cells, the authors discovered that UTX reduced NK cell frequency, but promoted NK effector function, in both sexes. Importantly, UTY cannot compensate for the reduced UTX dosage in male mice. **b**, UTX acts as an epigenetic regulator that affects NK cell homeostasis and function. UTX binds to 191 genes as part of a multiprotein complex, which includes lineage-specific transcription factors, altering their chromatin accessibility and expression. Among its target genes, UTX promotes expression of genes responsible for NK effector functions, while suppressing important anti-apoptotic genes.

increased NK cell numbers, as well as impaired cytokine production and cytotoxicity upon stimulation, as compared to what is seen in wild-type female mice; however, these phenotypes were similar between female Utx^{Het} and wild-type male mice, showing that reducing the genomic *Kdm6a* copy number was sufficient to normalize NK cell responses between sexes. Both phenotypes were further enhanced in both male and female mice with homozygous deletion of *Kdm6a* in NK cells, in a cell-intrinsic manner, while mice engineered to have NK cells with conditional deletion of UTX (Utx^{NKD} mice) were also more susceptible to murine cytomegalovirus (MCMV) infection. In contrast to its function in other lymphocytes[9], UTX regulated NK cell responses in a demethylase-independent manner, because a catalytically inactive UTX mutant did not alter NK cell number or functionality. Together, these results demonstrated that differential UTX expression between male and female NK cells contributes to the different antiviral immune responses observed between sexes. Of note, these results also indicate that UTY, which is the Y-linked homolog of UTX, cannot compensate for the reduced UTX dosage in male mice.

Splenic NK1.1$^+$ NK cells can be further distinguished into functional subsets based on their expression of CD27 and CD11b, which have different properties in regard to proliferation, cytotoxicity and cytokine production[10]. These results raised the question of whether the observed difference in NK cell functionality between the sexes and in the presence or absence of UTX were a consequence of altered NK cell development. However, Cheng et al.[5] found no difference in the representation of NK mature subsets either between male and female mice or in the absence of one or two copies of UTX, indicating that UTX does not change the developmental pathway of NK cells. These results were corroborated in mice with acute tamoxifen-induced deletion of UTX prior to MCMV infection, which resulted in reduced IFNγ production by NK cells. Therefore, these findings suggest that UTX regulates the response of mature NK cells during viral infection, without contributing to NK cell development.

To investigate how UTX may control NK cell number at steady state, Cheng et al.[5] interrogated the ability of NK cells from Utx^{NKD} mice to survive and proliferate. Although UTX did not affect NK cell proliferation, it promoted apoptosis, as indicated by decreased levels of active caspase 3 in Utx^{NKD} NK cells. Subsequent experiments showed that in wild-type male and Utx^{NKD} NK cells, the level of the anti-apoptotic molecule BCL-2 increased, compared to that in wild-type female NK cells, even in gonadectomized mice. Importantly, UTX bound to *Bcl2* regulatory regions and altered its chromatin accessibility and transcription. In addition to *Bcl2*, whole transcriptomic analysis showed that several genes related to cell death pathways were also differentially expressed in Utx^{NKD} NK cells, indicating that UTX controls the homeostatic NK cell number through modulation of apoptosis.

As an epigenetic modifier, UTX controls gene expression by altering chromatin organization in potential regulatory genomic regions. To provide molecular insight into the function of UTX in NK cells, Cheng et al.[5] analyzed the transcriptomic and chromatin accessibility profiles of NK cells lacking UTX through bulk RNA sequencing (RNA-seq) and assay for transposase-accessible chromatin (ATAC) sequencing (ATAC-seq). This analysis revealed numerous genetic loci that lost or gained accessibility, while over 1,200 genes showed altered expression in the absence of UTX. Combinatorial analysis of the RNA-seq and ATAC-seq data determined that 395 genes were both differentially accessible and expressed with a positive correlation. Consistent with the impaired response of Utx^{NKD} NK cells, genes that were less accessible and showed lower expression in Utx^{NKD} NK cells were enriched for pathways associated with cytokine production, lymphocyte activation and immune effector processes. In contrast, pathways related to developmental, metabolic and biosynthetic processes were associated with genes that were upregulated in Utx^{NKD} NK cells.

To identify direct effects of UTX in NK cell function, Cheng et al.[5] further interrogated the chromatin binding profile of UTX in NK cells with anti-UTX CUT&TAG sequencing. They determined that, of the 395 genes that were accessible and expressed in a UTX-dependent manner, 191 were also bound by UTX, providing strong evidence that these genes were direct UTX targets. Among these genes, UTX bound to sets of genes associated with pathways involved in cytokine signaling and cytotoxicity, including *Ifng* and *Csf2*. Together, these data support the likelihood that UTX regulates NK cell effector function by directly regulating expression and chromatin accessibility in key effector gene loci.

To begin to understand how UTX may control transcription in NK cells, the authors performed transcription factor motif analysis of the differentially accessible peaks and the UTX-bound peaks. This analysis revealed that binding motifs for multiple transcription factors involved in NK cell function, including RUNX1, RUNX2 and T-BET, were associated with UTX-bound loci and with differential accessibility in Utx^{NKD} NK cells. Although indirect, these findings suggest that UTX interacts with NK-lineage-associated transcription factors to regulate the chromatin accessibility and transcription of NK effector genes during an immune response.

The work by Cheng et al.[5] elegantly highlights the significance of UTX in modulating NK cell homeostasis and function in a sex-dependent manner. Although other immune subsets show differences between sexes[11], there has not, as yet, been a thorough investigation of whether these differences are UTX dependent, or whether UTX is differentially expressed in other immune cell types between male and female mammals. Notably, UTX regulates the number of invariant natural killer T cells by contributing to the shaping of the lineage-specific super-enhancer landscape during their development through its demethylase activity[9]. In stark contrast, UTX functions in NK cells in a demethylase-independent manner, indicating that UTX utilizes lineage-specific mechanisms to control immune cell responses. Motif analysis provided initial insights into the protein complexes potentially underlying the described effects in NK cells. Immunoprecipitation experiments followed by mass spectrometry are required to reveal the different protein partners in NK cells as well as how these transcription factors are altered in other immune populations and different environmental contexts, such as viral infection and stress response. Nonetheless, additional studies are required to uncover the full spectrum of UTX functions in other immune cell populations.

Although personalized medicine aims to provide equal care to all individuals, numerous aspects of immune responses are currently unclear. Sexual dimorphism is a critical biological factor that must be considered when studying immune response variability and for future therapeutic strategies, especially in individuals with sex chromosome disorders and in transgender individuals. This work by Cheng et al.[5] is a significant step toward understanding the molecular basis of sexual dimorphism in the immune system, which may ultimately contribute to new treatments and therapies for infectious and autoimmune diseases.

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References

1. Wilkinson, N. M., Chen, H. C., Lechner, M. G. & Su, M. A. *Annu. Rev. Immunol.* **40**, 75–94 (2022).
2. Takahashi, T. & Iwasaki, A. *Science* **371**, 347–348 (2021).
3. Jacobsen, H. & Klein, S. L. *Front. Immunol.* **12**, 720952 (2021).
4. D'Agostino, P. et al. *Ann. NY Acad. Sci.* **876**, 426–429 (1999).
5. Cheng, M. I. et al. *Nat. Immunol.* https://doi.org/10.1038/s41590-023-01463-8 (2023).
6. Huang, Z. et al. *Proc. Natl Acad. Sci. USA* **118**, e2023216118 (2021).
7. Berletch, J. B., Yang, F. & Disteche, C. M. *Genome Biol.* **11**, 213 (2010).
8. Eckelhart, E. et al. *Blood* **117**, 1565–1573 (2011).
9. Beyaz, S. et al. *Nat. Immunol.* **18**, 184–195 (2017).
10. Hayakawa, Y. & Smyth, M. J. *J. Immunol.* **176**, 1517–1524 (2006).
11. Menees, K. B. et al. *Immun. Ageing.* **18**, 3 (2021).

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Competing interests

The authors declare no competing interests.