IMMUNE AGING

Time isn't kind to female T cells

While investigating sex differences in T cell aging, Mkhikian et al. identified a role for excessive IL-7 signaling and N-glycan branching in age-related T cell dysfunction in women and female mice. These findings point to the increasingly recognized importance of the effects of biological sex on immune aging, and delineate new targetable pathways in age-related immune dysfunction.

Cassandra J. McGill and Bérénice A. Benayoun

As the average lifespan continues to increase, so does the need to find effective interventions against age-related immune dysfunction. The vulnerability of older adults to infectious diseases has been especially highlighted throughout the COVID-19 pandemic, with hospitalization rates highest in individuals who are aged 65 years and older[1]. Extensive sexual dimorphism has previously been described with aging and age-related diseases[2], and in immune cell phenotypes[3]. Although aging highly associates with immune dysfunction, the effect of sex in combination with aging on immune function is still poorly understood. In a new study, Mkhikian et al.[4] find that age-associated alterations in naive T (T_N) cells are sex-dimorphic in amplitude, with older female individuals displaying reduced T_N function compared to male individuals in both mice and humans. This study provides a novel analysis of purified T cell populations with aging as a function of sex across species, and emphasizes the notion that effective therapeutic agents against immune dysfunction will probably require approaches tailored to biological sex (Fig. 1).

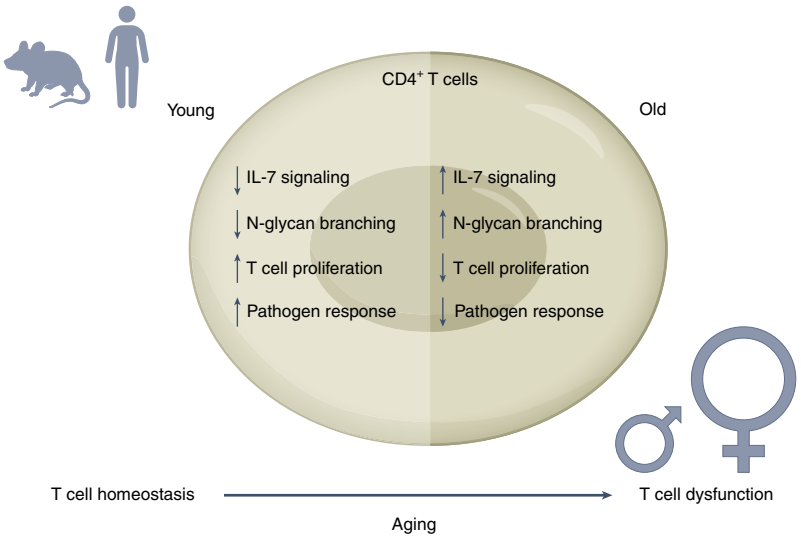

Fig. 1 | Age-related T cell dysfunction is exacerbated in women and in female mice. Mkhikian et al.[4] identified excessive IL-7 signaling in older humans and mice as a mechanism driving increased N-glycan branching of CD4⁺ T_N cells, which in turn promoted reduced T cell function. The amplitude of these defects was larger in female individuals, underlying the importance of investigating biological sex in studies of age-related dysfunction.

Previous studies have identified differences in T cell number and function with age in both mice and humans, although the mechanisms that drive these changes remained unclear[5,6]. As proteins are prepared for the cell surface or excretion, asparagine (N) residues can be modified with glycans while being processed through the ER–Golgi secretory pathway. N-glycan modifications ultimately regulate ligand production for lectins, and one in particular, galectin, has been shown to bind TCR and form a lattice, affecting clustering, signaling and endocytosis of surface receptors in T cells. As N-glycan branching levels determine the strength of the galectin lattice, T cell function can be negatively affected by N-linked glycan branching, leading to inhibition of pro-inflammatory signals and promotion of anti-inflammatory signals[7,8].

This led the authors to ask their first question: could N-glycan branching increase with age and explain age-related defects of T cells? Using L-PHA (a well-known marker for N-glycan binding), the authors compared binding in splenic mouse T cells from young versus old female and male C57BL/6 mice. Interestingly, flow cytometry analysis showed N-glycan branching increasing with age in T cells from female mice to a greater degree than in those from age-matched males. When the T cells were further separated into T_N, central memory T cell and effector memory T cell groups, the largest difference in N-glycan branching with age between female and male individuals was found in the CD4⁺ T_N cell group.

Next, the authors sought to identify what could be causing the age-related increase in N-glycan branching to occur more dramatically in female mice. To narrow down the potential mechanism, they first asked whether the differences came from cell-intrinsic or cell-extrinsic factors. They observed that isolated CD4⁺ T cells from young versus old female mice cultured for three days to equalize external factors showed attenuated age-related differences in N-glycan branching, suggesting that cell-extrinsic factors were major drivers of these differences in female mice. With this in mind, the authors profiled the transcriptomes of purified CD4⁺ T_N and effector memory T cells from young and old female and male mice. Consistent with observed sex differences in the aging phenotypes of T cells, there was minimal overlap between differentially expressed genes with age across sexes. Notably, in CD4⁺ T_N cells, female-specific age-related differentially expressed genes included genes involved in the interleukin-7

(IL-7) signaling pathway. The IL-7 signaling pathway has previously been implicated in N-glycan branching regulation[9], and the transcriptional data were consistent with excessive IL-7 signaling in T_N cells from old female mice.

With IL-7 signaling being a potential mediator of age-related sex dimorphism of T cells, the next question was clear: is elevated IL-7 signaling sufficient to increase N-glycan branching? Conversely, does the dampening of IL-7 signaling rescue the excessive N-glycan branching observed in old female mice? As predicted, after administering a stabilized form of IL-7 in young mice for two weeks, N-glycan branching levels in $CD4^+$ T_N cells increased. Conversely, treatment of old female mice with an anti-IL-7 monoclonal antibody (which blocks IL-7 signaling[10]) brought branching levels down to that of young cells both in the blood and spleen.

What could be causing increased IL-7 signaling with age in female individuals? In both mice and humans, T_N cell production is based in the thymus and IL-7-dependent proliferation occurs in the periphery. However, although thymic production of T_N cells persists throughout mouse adulthood, thymic production decreases dramatically in humans early on, allowing the periphery to produce the majority of new T_N cells in adult individuals[11]. The authors posited that reduced thymic output and/or decreased estrogen levels may contribute to increased IL-7 signaling and increased N-glycan branching in older female individuals. While thymectomy did not affect IL-7 signaling nor N-glycan branching, ovariectomy led to a slight increase of IL-7 signaling levels in young mice — although N-glycan branching was unaffected. Although the aged environment may interact with these factors in unpredictable ways, these results do not support a major role of thymic output and/or estrogen levels in promoting the age-related increase in N-glycan branching of T_N cells. However, the wide interval of mouse ages in the young group (7–32 weeks) in this study may not be ideal to determine the effect of sex hormones on sex differences with aging, as young female mice do not reach stable hormonal status before about 14 weeks of age or later.

Although it was clear that N-glycan branching increased with age, especially in female mice, the authors asked an important corollary question: what are the downstream effects of increased N-glycan branching? Consistent with decreased functionality, $CD4^+$ T cells from aged female mice exhibited a reduced reaction to activation signals. Interestingly, this effect was reversed upon treatment with kifunensine (KIF) (a mannosidase I inhibitor that blocks N-glycan branching[12]), further supporting the notion that increased N-glycan branching underlies T cell dysfunction. To confirm this finding in an independent manner, the authors used a mouse model with a T-cell-specific deletion of *Mgat2*, which encodes a branching enzyme whose deletion leads to reduced N-glycan branching. Consistent with KIF-treatment observations, aged *Mgat2*-deletion mice also showed improved T cell function, as measured by CD69 levels.

Up to this point, the authors had demonstrated that (1) N-glycan branching increases with age, and this increase is larger in female mice; (2) increased IL-7 signaling with aging promotes N-glycan branching, independent of thymic involvement or estrogen levels; and (3) age-related increased N-glycan branching leads to T cell dysfunction in vitro. But what about in vivo and during times of infection? Using *Mgat2*-deficient and matched control mice, the authors infected mice with *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium). They found that aged female control mice fared worse than the matched *Mgat2*-deficient mice, with increased lethality and evidence of *S.* Typhimurium dissemination to multiple organs. Thus, limiting branching levels can improve the ability of T cells from aged female mice to stop the dissemination of *S.* Typhimurium in vivo.

Importantly, the authors next asked whether these findings were restricted to mice or whether the effect of N-glycan branching on T cell function with aging could translate to humans. After isolating T cells from blood samples of women and men aged 19 to 98 years old, they observed a similar age-dependent increase of N-glycan branching in $CD4^+$ T cells, which occurred at greater levels in women. Similar to the mouse studies, removing cell-extrinsic factors dampened age-associated branching differences, suggesting extrinsic factors are also necessary for the age-related increase in branching in humans. But what about the role of differential IL-7 signaling in driving the sex differences in branching levels? When $CD4^+$ T_N and $CD8^+$ T_N cells from women were exposed to IL-7 for nine days there was no difference in T_N-cell branching. However, when the cells were exposed to both IL-7 and *N*-acetylglucosamine (GlcNAc) for nine days, increased N-glycan branching was observed in $CD4^+$ T_N cells. Serum GlcNAc levels have previously been shown to increase with age and raise N-glycan branching in activated T cells[13,14], suggesting that — at least in humans — circulating GlcNAc levels and IL-7 work together to promote N-glycan branching during aging. The authors decided to also determine whether age-related N-glycan branching also suppressed T cell activity in humans. Indeed, similar to the mouse findings, treating peripheral blood mononuclear cells from old female individuals with KIF improved T cell activation and proliferation. Altogether, the authors revealed that, in humans, age-associated increases in circulating GlcNAc and IL-7 signaling fuel increased N-glycan branching, leading to decreased function of T_N cells, a molecular mechanism new to the study of age-related immune dysfunction.

Thus, in this study, Mkhikian et al.[4] identified a new potential target for 'immunosenescence' therapeutic agents in the IL-7-driven promotion of N-glycan branching of T_N cells. Drugs promoting the reduction of N-glycan branching have already been investigated in human trials for malignancies, suggesting that intermittent exposure could be safe[15]. More generally, IL-7 levels, N-glycan branching and/or circulating GlcNAc could also represent potential therapeutic targets to reduce or reverse T cell aging.

This study illustrates why it is crucial to evaluate how biological sex interacts with conditions of interest, including aging, in both preclinical and clinical studies. As personalized medicine continues to grow in accessibility, so does evidence of sex being an important factor to take into consideration. However, another key variable that can modify drug responses and disease susceptibility is genetic background. Thus, because this study only used self-described non-Hispanic Caucasian individuals for its human arm, it will be crucial to determine whether these observations (including in terms of sex dimorphism) are conserved in other ethnic groups. Similarly, the effects of age-related comorbidities such as diabetes and neurodegenerative disorders on or in combination with this novel mechanism should also be explored. Nevertheless, this work by Mkhikian et al.[4] sets the foundation for future mechanistic studies of sex dimorphism in age-related immune dysfunction. ❐

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References

1. Garg, S. et al. *MMWR Morb. Mortal. Wkly. Rep.* **69**, 458–464 (2020).
2. Sampathkumar, N. K. et al. *Hum. Genet.* **139**, 333–356 (2020).
3. Gal-Oz, S. T. et al. *Nat. Commun.* **10**, 4295 (2019).
4. Mkhikian, H. et al. *Nat. Aging* https://doi.org/10.1038/s43587-022-XXXXX-X (2022).
5. Rodriguez, I. J. et al. *Front. Immunol.* **11**, 604591 (2021).
6. Yager, E. J. et al. *J. Exp. Med.* **205**, 711–723 (2008).
7. Lau, K. S. et al. *Cell* **129**, 123–134 (2007).
8. Morgan, R. et al. *J. Immunol.* **173**, 7200–7208 (2004).
9. Mkhikian, H. et al. *Nat. Commun.* **2**, 334 (2011).
10. Grabstein, K. H. et al. *J. Exp. Med.* **178**, 257–264 (1993).
11. den Braber, I. et al. *Immunity* **36**, 288–297 (2012).
12. Mkhikian, H. et al. *eLife* **5**, e14814 (2016).
13. Kizuka, Y. & Taniguchi, N. *Biomolecules* **6**, 25 (2016).
14. Ruhaak, L. R. et al. *PLoS ONE* **5**, e12566 (2010).
15. Goss, P. E., Reid, C. L., Bailey, D. & Dennis, J. W. *Clin. Cancer Res.* **3**, 1077–1086 (1997).

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Competing interests

The authors declare no competing interests.