HHS Public Access

Author manuscript

Nat Aging. Author manuscript; available in PMC 2022 September 22.

Published in final edited form as:

Nat Aging. 2022 March ; 2(3): 189–191. doi:10.1038/s43587-022-00185-0.

Time isn't kind to female T-cells

Cassandra J. McGill[1,2], **Bérénice A. Benayoun**[1,3,4,5,6,7]

[1]Leonard Davis School of Gerontology, University of Southern California, Los Angeles, CA 90089, USA

[2]Graduate Program in the Biology of Aging, University of Southern California, Los Angeles, CA 90089, USA

[3]Molecular and Computational Biology Department, USC Dornsife College of Letters, Arts and Sciences, Los Angeles, CA 90089, USA

[4]Biochemistry and Molecular Medicine Department, USC Keck School of Medicine, Los Angeles, CA 90089, USA

[5]USC Norris Comprehensive Cancer Center, Epigenetics and Gene Regulation, Los Angeles, CA 90089, USA

[6]USC Stem Cell Initiative, Los Angeles, CA 90089, USA

Abstract

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 mouse and human female T-cell dysfunction. These findings point to the increasingly-recognized importance of the impact of biological sex on immune aging and delineate new targetable pathways in age-related immune dysfunction.

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 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]. While aging highly associates with immune dysfunction, the impact of sex in combination with aging on immune function is still poorly understood. In a new study, *Mkhikian et al.*, find that age-associated alterations in naïve T-cells (T_N) are sex-dimorphic in amplitude, with older females displaying reduced T_N function compared to males in both mice and humans[4]. 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 therapeutics against immune dysfunction will likely require approaches tailored to biological sex (Figure 1).

[7]Correspondence: berenice.benayoun@usc.edu.

Competing interests

The authors declare no competing interests.

Previous studies identified differences in T-cell number and function with age in both mice and humans, although the mechanisms driving 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. Since N-glycan branching levels determine the strength of the galectin lattice, T-cell function can be negatively impacted 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 be increasing with age and explain age-related defects of T-cells? Using a well-known marker for N-glycan binding (L-PHA), the authors compared L-PHA binding in splenic mouse T-cells from young *vs.* old, female and male C57BL/6 mice. Interestingly, flow-cytometry analysis showed N-glycan branching increasing with age in female mouse T-cells to a greater degree than in age-matched males. When the T-cells were further separated into naïve, central memory, and effector memory (T_{EM}) groups, the largest difference in N-glycan branching with age between females and males was found in the $CD4^+$ T_N group.

Next, the authors sought to identify what could be causing the age-related elevated N-glycan branching to occur more dramatically in females. To narrow down the potential mechanism, they first asked whether the differences came from cell intrinsic or extrinsic factors. They observed that isolated $CD4^+$ T-cells from young vs. 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 females. With this in mind, the authors profiled the transcriptomes of purified $CD4^+$ T_N and T_{EM} 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 (DEGs) with age across sexes. Notably, in $CD4^+$ T_N cells, female-specific age-related DEGs 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 was consistent with excessive IL-7 signaling in old female T_N cells.

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, would dampening of IL-7 signaling rescue excessive N-glycan branching observed in old females? 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 females? 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 aged females. While thymectomy did not impact 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. While 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 impact of sex hormones on sex-differences with aging, since young females do not reach stable hormonal status before ~14 weeks of age or later.

Although it was clear that N-glycan branching was increasing with age especially in females, the authors asked an important corollary question: what are the downstream effects of increased N-glycan branching? Consistent with decreased functionality, female aged $CD4^+$ T-cells 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 demonstrated that (i) N-glycan branching increases with age, and this increase is larger in females, (ii) increased IL-7 signaling with aging promotes N-glycan branching, independent of thymic involvement or estrogen levels and (iii) age-related increased N-glycan branching leads to T-cell dysfunction *in vitro*. But what about *in vivo* and during times of infection? Using the *Mgat2*-deficient and matched control mice, the authors infected the mice with *Salmonella enterica* serovar Typhimurium (*S. Typhimurium*). They found that aged female control mice fared worse than the matching *Mgat2*-deficient mice, with increased lethality and evidence of *S. Typhimurium* dissemination to multiple organs. Thus, limiting branching levels can improve the ability of aged female T-cells 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 human females and males 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 females. 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 female $CD4^+$ T_N and $CD8^+$ T_N cells were exposed to IL-7 for nine days there was no difference in naïve T-cell branching; however, when the cells were exposed to both IL-7 and N-acetylglucosamine (GlcNAc) for 9 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 old female PBMCs with KIF improved T cell activation and proliferation. Altogether, the authors revealed that human age-associated increases in circulating GlcNAc and IL-7 signaling fuels 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.*, identified a new potential target for "immunosenescence" therapeutics 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.

Altogether, 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 impactful factor to take into consideration. However, another key variable that can modify drug responses and disease susceptibility is genetic background. Thus, since this study only used non-Hispanic Caucasian subjects 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.*, sets the foundation for future mechanistic studies of sex-dimorphism in age-related immune dysfunction.

Acknowledgements

Elements of the figure were generated using BioRender.com. C.J.M is supported by NIA T32 AG052374 predoctoral fellowship, and B.A.B. was supported by NIGMS R35 GM142395.

References

1. Garg S et al. Hospitalization Rates and Characteristics of Patients Hospitalized with Laboratory-Confirmed Coronavirus Disease 2019 - COVID-NET, 14 States, March 1–30, 2020. MMWR Morb Mortal Wkly Rep 69, 458–464, doi:10.15585/mmwr.mm6915e3 (2020). [PubMed: 32298251]
2. Sampathkumar NK et al. Widespread sex dimorphism in aging and age-related diseases. Hum Genet 139, 333–356, doi:10.1007/s00439-019-02082-w (2020). [PubMed: 31677133]
3. Gal-Oz ST et al. ImmGen report: sexual dimorphism in the immune system transcriptome. Nat Commun 10, 4295, doi:10.1038/s41467-019-12348-6 (2019). [PubMed: 31541153]
4. Mkhikian H et al. Sex-dimorphic impairment of T cell immunity in the elderly via synergism of interleukin-7 with N-acetylglucosamine. Nat Aging XX (2022).
5. Rodriguez IJ et al. Immunosenescence Study of T Cells: A Systematic Review. Front Immunol 11, 604591, doi:10.3389/fimmu.2020.604591 (2020). [PubMed: 33519813]

6. Yager EJ et al. Age-associated decline in T cell repertoire diversity leads to holes in the repertoire and impaired immunity to influenza virus. J Exp Med 205, 711–723, doi:10.1084/jem.20071140 (2008). [PubMed: 18332179]
7. Lau KS et al. Complex N-glycan number and degree of branching cooperate to regulate cell proliferation and differentiation. Cell 129, 123–134, doi:10.1016/j.cell.2007.01.049 (2007). [PubMed: 17418791]
8. Morgan R et al. N-acetylglucosaminyltransferase V (Mgat5)-mediated N-glycosylation negatively regulates Th1 cytokine production by T cells. J Immunol 173, 7200–7208, doi:10.4049/jimmunol.173.12.7200 (2004). [PubMed: 15585841]
9. Mkhikian H et al. Genetics and the environment converge to dysregulate N-glycosylation in multiple sclerosis. Nat Commun 2, 334, doi:10.1038/ncomms1333 (2011). [PubMed: 21629267]
10. Grabstein KH et al. Inhibition of murine B and T lymphopoiesis in vivo by an anti-interleukin 7 monoclonal antibody. J Exp Med 178, 257–264, doi:10.1084/jem.178.1.257 (1993). [PubMed: 8315381]
11. den Braber I et al. Maintenance of peripheral naive T cells is sustained by thymus output in mice but not humans. Immunity 36, 288–297, doi:10.1016/j.immuni.2012.02.006 (2012). [PubMed: 22365666]
12. Mkhikian H et al. Golgi self-correction generates bioequivalent glycans to preserve cellular homeostasis. Elife 5, doi:10.7554/eLife.14814 (2016).
13. Kizuka Y & Taniguchi N Enzymes for N-Glycan Branching and Their Genetic and Nongenetic Regulation in Cancer. Biomolecules 6, doi:10.3390/biom6020025 (2016).
14. Ruhaak LR et al. Decreased levels of bisecting GlcNAc glycoforms of IgG are associated with human longevity. PLoS One 5, e12566, doi:10.1371/journal.pone.0012566 (2010). [PubMed: 20830288]
15. Goss PE, Reid CL, Bailey D & Dennis JW Phase IB clinical trial of the oligosaccharide processing inhibitor swainsonine in patients with advanced malignancies. Clin Cancer Res 3, 1077–1086 (1997). [PubMed: 9815786]

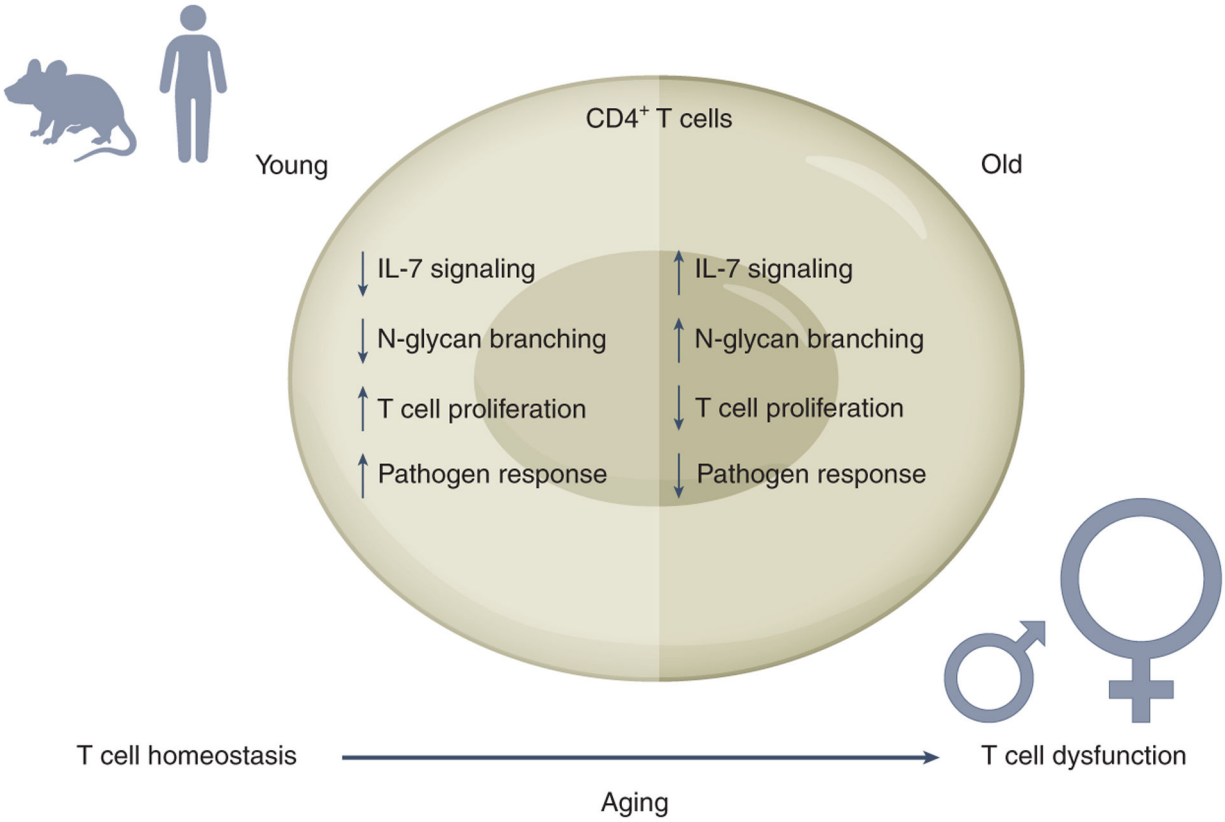

Figure 1: Age-related T-cell dysfunction is exacerbated in female mice and humans.

The study by Mkhikian and colleagues identified excessive IL-7 signaling in aged animals 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 females, underlying the importance of investigating biological sex in studies of age-related dysfunction.