

The role of the thymus in COVID-19 disease severity: implications for antibody treatment and immunization

Caitlyn Kellogg^{a,b} and Ozlem Equils^b

^aUniversity of California, San Diego School of Medicine, San Diego, CA, USA; ^bPublic Health Education, MiOra Foundation, Los Angeles, CA, USA

ABSTRACT

The thymus is a largely neglected organ but plays a significant role in the regulation of adaptive immune responses. The effect of aging on the thymus and immune senescence is well established, and the resulting inflammaging is found to be implicated in the development of many chronic diseases including atherosclerosis, hypertension and type 2 diabetes. Both aging and diseases of inflammaging are associated with severe COVID-19 disease, and a dysfunctional thymus may be a predisposing factor. In addition, insults on the thymus during childhood may lead to abnormal thymic function and may explain severe COVID-19 disease among younger individuals; therefore, measurement of thymic function may assist COVID-19 care. Those with poor thymic function may be treated prophylactically with convalescent serum or recombinant antibodies, and they may respond better to high-dose or adjuvanted COVID-19 vaccines. Treatments inducing thymic regeneration may improve patients' overall health and may be incorporated in COVID-19 management.

ARTICLE HISTORY

Received 5 August 2020
Accepted 27 August 2020

KEYWORDS

COVID-19; thymus; inflammation; immunity; aging; immunosenescence; longevity

History of recognition of the thymus and its role in the immune system

A deeper understanding of the thymus is critical in combating COVID-19. Immune changes that occur with thymic aging (immune senescence) lead to an inflammatory environment that underlies the pathogenesis of diseases of aging (i.e. cardiovascular disease, hypertension, chronic obstructive lung disease and arthritis),^{1–4} and people who develop these comorbidities are known to be at higher risk for severe COVID-19 infection and death.^{5,6}

Although knowledge of the thymus has existed since the time of the ancient Greeks (130–200 AD), who called the thymus *thumos*, meaning “principle of life,” or “heart, soul, passion, life”⁷ there were few efforts to understand its function until the 1960s when removal of the thymus in a mouse model was shown to lead to immune deficiency.⁸ In 1967, lymphocytes of thymic origin (T lymphocytes) were identified and recognized to enable bone marrow-derived B lymphocytes to differentiate into antibody-forming cells.⁸ In the 1980s, the AIDS epidemic and the discovery of thymic involvement in human immunodeficiency virus (HIV) infection brought further attention to the thymus.^{9,10} Treatments to boost thymic function were considered for HIV-infected individuals.⁹ However, the development of effective anti-retroviral treatments and successful suppression of HIV replication to undetectable levels cooled the interest in the thymus.

In the early 2000s, infants who underwent thymectomy for congenital heart surgery were found to develop an immune profile similar to that seen in aged individuals.¹¹ In those children, repopulation occurred with lymphocytes of extra-thymic origin with limited breadth of immune responses; the

number of naïve CD4+ helper and CD8+ suppressor T lymphocytes and T-cell receptor rearrangement excision circles (TRECs) were lower and the number of B lymphocytes was higher, though IgG1 and IgA levels were lower.^{11–13} Despite these observations, even as recent as 2010, the surgical protocols to repair congenital heart defects required partial or complete thymectomy. Today, thymectomy is routinely performed to treat patients with myasthenia gravis and thymomas. The sternal defect pectus excavatum is corrected with the Nuss procedure, in which a metal rod is inserted between the thymus and sternum, which may affect thymic function.^{14,15}

Factors influencing the development and function of the thymus

Genetic and environmental factors (prenatal and postnatal) influence thymic health. DiGeorge Syndrome (Chromosome 22q11.2 deletion syndrome) is the most common chromosomal microdeletion reported in humans, affecting approximately 1 in 4,000 individuals, and leads to cardiovascular defects and the absence or under-development of the thymus.¹⁶ Males and females are affected equally regardless of their race and ethnicity.^{17,18} However, the penetrance and disease severity varies among those affected, which is explained by novel genetic and epigenetic differences as well as differences in coding and non-coding genes including microRNAs (miRNA) and long noncoding RNAs (lncRNAs). For example, *DiGeorge Syndrome Critical Region 8*, *DGCR8*, is a gene on the long arm of chromosome 22, and it is required for miRNA biogenesis.^{19–20} A 50% reduction in *DGCR8* expression due to 22q11.2del may modulate the expression of hundreds of microRNAs (miRNAs).

MicroRNAs are a family of approximately 2000 evolutionarily conserved small noncoding RNAs (18–22 nucleotides) found in many body fluids such as serum, plasma, saliva and amniotic fluid. These molecules bind to diverse mRNA transcripts that play a role in cell function and target mRNA transcripts for degradation.^{20,21} MiRNAs play an important role in thymic organogenesis, maturation and involution,²² and conversely, aging influences miRNA levels.²³ Interestingly, miRNAs not only regulate host cellular responses but also responses to infectious agents like viruses.²⁴ There is a decrease in T cell number and increased susceptibility to infection observed in DiGeorge syndrome;¹⁶ miRNA dysfunction seen in this disease may be a contributor.

Environmental factors regulate thymic development both pre- and postnatally. Gestational diabetes has the potential to cause birth defects, and newborns born to women with gestational diabetes have congenital features overlapping with 22q11.2del.¹⁷ Approximately 1 out of 5 infants with thymic aplasia with deletions on chromosome 22q11.2 and requiring a thymic tissue transplant were born to mothers with a clinical history of gestational diabetes.¹⁷ Retinoic acid treatment of pregnant women (tretinoin, isotretinoin) for acne also leads to 22q11.2-like congenital malformations in their infants.¹⁷

Postnatally, acute stress such as emotional distress,²⁵ malnutrition,²⁶ and infections²⁷ may induce transient thymic involution characterized by a reduction in thymus size. This is postulated to be caused by an acute loss of cortical thymocytes and reduced output of naïve T cells to the periphery.²⁸

Soluble factors released from lymphocytes and from non-lymphoid cells in the thymus, lymph nodes and at sites of inflammation regulate thymic function and T lymphocyte proliferation.²⁹ Interleukin (IL)-6 (previously called B cell-stimulating factor, BSF-2) is a costimulant for human thymocytes and T lymphocytes and plays a role in B lymphocyte differentiation.²⁹ At physiologic levels, IL-6 may promote thymocyte proliferation. However, during acute stress, elevated IL-6 levels may lead to thymic suppression.³⁰ One IL-6 family protein, leukemia inhibitory factor (LIF), has been shown to induce thymic suppression and is dependent both on intrathymic and systemic cortisol levels.³⁰

Physical trauma to the chest is common among children, especially males, and may lead to thymic injury. Thymic injury during youth may lead to a chronic inflammatory state and premature immune-senescence and may increase the risk for obesity, insulin resistance and metabolic syndrome.³¹ Research has shown that participation in American football may increase the risk for cardiovascular disease later in life, and repetitive blunt trauma to the chest may be a cause.³² Defensive lineman football players endure repeated chest insults, this may explain the higher rates of obesity and metabolic syndrome even among collegiate lineman.³³ Long term, regular, strenuous exercise (-approximately 2 hours of daily swimming, running and cycling plus weight, interval or skills training) has also been shown to decrease thymic function among healthy young people.³⁴ These observations may explain COVID-19 deaths observed in some healthy, athletic young adults.

The metabolic hormones leptin and ghrelin influence thymic function and involution.³⁵ For example, leptin, the satiety hormone that regulates the energy balance by inhibiting

hunger and diminishing fat storage in adipocytes,³⁶ plays a role in thymopoiesis and prevents corticosteroid-induced thymic atrophy.³⁷ There is a tight relationship between leptin levels and obesity, which is the most common comorbidity for severe COVID-19.^{31,38}

Thymic development and thymus size are also under the control of hypothalamic, pituitary and sex hormones, whose levels change over time. Both thymocytes and thymic epithelial cells express sex hormone receptors.^{39,40} The thymus is most active early in life but undergoes a steady decline in function over time,⁴¹ a phenomenon that is evolutionarily conserved among vertebrates.⁴² Thymic involution becomes most notable around the time of puberty when sex steroid production increases.⁴³ The increasing rate of childhood obesity and subsequent early start of puberty may initiate thymic senescence at a younger age. This may explain the high rate of obesity among pediatric and adult patients with severe COVID-19 disease.^{44,45}

Although there are limited data on the effect of gender on thymic involution, most animal data suggest that females have a larger thymus compared to males.^{42,43} In addition, the second X-chromosome in females encodes many immune proteins that play a role in thymic regulation of the immune system.⁴⁶ This may explain lower rates of severe COVID-19 in females. During pregnancy, under the influence of hormones, the thymus experiences dramatic changes, in which the cortex shrinks and there is an increase in cellularity in the medulla.⁴¹ Those changes may influence COVID-19 disease course in pregnant women.

Thymus and aging

Aging is characterized by thymic involution and peripheral immunosenescence that leads to inflammaging, which is characterized by chronic inflammation.⁴⁷ Increased levels of IL-6, IL-1, TNF- α and C-reactive protein contribute to the enhanced low-grade inflammation characteristic of aging.⁴⁷ With aging, the thymus turns into strands of medullary and cortical cells surrounded by adipose and connective tissue, altering the thymic cytokine milieu.^{48,49} Thymus-produced cytokines whose expression falls with aging include IL-2, IL-9, IL-10, IL-13 and IL-14.⁴⁹ Thymic cytokines that increase with aging include leukemia inhibitor factor (LIF), oncostatin M (OSM) and stem cell factor (SCF) (Table 1).⁴⁹ The thymic cytokine LIF also regulates adrenocorticotrophic hormone (ACTH), which regulates cortisol release.⁴⁹ Cortisol in turn suppresses thymopoiesis, suggesting that overproduction of thymic cytokines may lead to thymic atrophy.⁴⁹

As the productive capacity of the thymus lags, the frequency of naïve T cell production is reduced and the TCR repertoire contracts.¹ In young and even middle-aged adults, the naïve CD45RA+CD62L+CD4 positive T cell repertoire diversity has been estimated at 20 million different TCR- β chains; in the

Table 1. The effect of aging on thymus cytokine production.

Increase with aging	Leukemia inhibitor factor (LIF), oncostatin M (OSM) and stem cell factor (SCF).
Decrease with aging	IL-2, IL-9, IL-10, IL-13 and IL-14.

elderly (older than 70 years),⁵⁰ the pool has severely contracted to 200,000 TCR- β specificities.⁵¹ The 95% decline in TCR repertoire diversity in CD4 + T cells in the elderly may limit functional response.⁵² Nonetheless, the overall T cell levels are maintained by a variety of peripheral thymus-independent homeostatic mechanisms⁵³ where memory T cells stimulated by cytokines undergo proliferation and differentiation.^{54,55} With the loss of critical cytokines and hormones from the thymic microenvironment, newly activated naive and memory cell populations expand in response to cognate antigenic stimulation and occupy increased fractions of the repertoire.^{54–57} Overall, these changes confer older individuals relatively preserved immunity against previously encountered antigens, but decreased immunity against new antigens, infectious agents and vaccines.⁵⁸

Interestingly, aging affects the immune system differentially between men and women. Inflammaging is accelerated among men; changes including a decline in naïve T cell numbers and increase monocyte and cytotoxic cell functions are greater in men than women, as is the decline in B-cell specific loci.⁵⁹ Also, older men have higher levels of pro-inflammatory cytokines (IL-6, IL-18) than women.⁵⁹ The genomic differences between sexes increase after age 65 years, with men having higher innate and pro-inflammatory immune activity and lower adaptive immune activity regardless of the decline of B cell frequencies.⁵⁹ These observations are persistent in different ethnic groups, which suggest that endocrine factors may be mediating these differences.^{60–62}

Thymus and COVID-19 disease

Although COVID-19 mostly affects those with comorbid conditions, even among those without comorbidities, age is a significant risk factor, and there is a direct relationship between age and COVID-19 severity and mortality.⁶ This may be explained by the inappropriate COVID-19-induced immune responses in the elderly who are already experiencing immune-senescence and inflammaging at baseline. Overall, there are many similarities between the blood cytokine profile of aging and that observed in severely ill COVID-19 patients, including an elevated IL-6, which appears to play a key role in poor COVID-19 prognosis (Table 2).^{63–66}

Assessment of thymic function to determine the risk for severe COVID-19

Although an abnormal immune response plays a large role in the pathogenesis of many comorbid conditions and the thymus is the organ for T cell lymphopoiesis, since 1800s, the white blood cell count is the only laboratory test performed during routine doctor visits to assess the immune system.⁶⁷ Thymus function is not examined during routine health visits or during the medical management of chronic diseases, such as diabetes or atherosclerosis. However, there was a great interest in thymus during the early years of HIV epidemic and there is extensive experience on the measurement of thymic function. Thymic function can be quantified by measuring the nonreplicating circle of DNA, signal joint TCR excision circle (sjTREC), in naïve T cells by performing real-time polymerase chain reactions (PCR).^{68,69} Higher

Table 2. Immune profile in aging compared to that seen in COVID-19 patients.

	COVID-19 patients	Aging
Immune cell profile		
Total T cell number	↓	↓
Total CD8 + T cell number	↓ ↓	↓ ↓
Total CD4 + T cell number	↓	↓
T reg profile	↓	↑ naturally occurring, ↓ inducible
B cell number	Normal	↓
NK cell number	Normal	↓
Cytokine profile		
IL-2	↑	↓ or normal
IL-6	↑	↑ or normal
IL-8	↑	↑ or normal
IL-10	↑	↓ or normal
TNF-A	↑	↑ or normal
IFN- γ	↑	↑ or normal

TREC number is associated with better thymic function and repertoire diversity of the memory T-cell population.⁶⁹ Thymic output may also be monitored by conducting flow cytometry in the blood and measuring naïve CD45RA and CD62 ligand positive cells.⁷⁰

Measurement of thymic function may help determine a patient's risk of developing comorbid conditions and severe COVID-19 disease and may also predict a patient's response to vaccines. The CD4 + T-cell population correlates with both the capacity to respond to vaccines and the resistance to opportunistic infections.^{71,72} Decreased thymic function may affect the response to vaccines; although most children with DiGeorge syndrome respond to live viral vaccines, the duration of immunity is much shorter.⁷³

Data on thymic health and function among patients with COVID-19 infection, including those who are younger (including children) and those without comorbidities, may help identify patients who may be at risk for more severe COVID-19 infection and who may not respond to the vaccines. The patients with low thymic function may be started on post-exposure prophylaxis or early treatment with convalescent plasma,⁷⁴ recombinant antibody⁷⁵ and/or anti-viral treatment. Patients with insufficient thymic response may better respond to high dose⁷⁶ or adjuvanted⁷⁷ vaccines.

Restoring thymus function to improve COVID-19 prognosis

The average life expectancy has increased substantially since 1900s.⁷⁸ Studies suggest that although there appears to be a maximum limit on lifespan, by targeting the biological/genetic and environmental causes of aging, humans can live longer. In the 1900s, the primary cause of death was infection. With the advent of clean water, vaccines and anti-microbials, the current leading cause of death is cancer.⁷⁹ The COVID-19 pandemic may reverse this trend. Since COVID-19-related mortality is significantly higher among people 65 years and older, treatments that restore thymic function may suppress inflammaging, COVID-19 related inflammation, and prevent severe COVID-19 disease.

Treatment with recombinant human growth hormone (rhGH) and thymus transplantation was proposed to restore thymus function among HIV-infected patients⁸⁰ and patients with DiGeorge syndrome⁸¹ respectively. Methods proposed to restore thymic function include administration of recombinant human keratinocyte growth factor (Palifermin), recombinant human hIL-7 (CYT107), recombinant human IL-22 (hrIL-22), rhGH and insulin-like growth factor-1 (IGF1), in addition to sex steroid inhibition by luteinizing hormone-releasing hormone (LHRH)-agonist (Lupron) or enzalutamide (nonsteroidal anti-androgen), adoptive transfer of precursor (pre-) T cells and thymus bioengineering.⁸² Recently in a clinical trial to improve thymic function in 10 healthy men between the ages of 51 and 65 years treatment with recombinant human growth hormone (rhGH), dehydroepiandrosterone (DHEA) and metformin showed improved immune markers.⁸³ Zinc supplementation restored thymic function in aging mouse model.⁸⁴ Currently, Interleukin7, enzalutamide and ascorbic acid plus zinc supplementation are in clinical trials to treat COVID-19.^{85–87} There is a need for concerted efforts to develop treatments targeting the thymus to improve the immune system and overall health.

Conclusions

Measurement of thymic function may help identify children and adults with immune dysfunction who are at risk of developing severe COVID-19 disease. Thymic function measurement may also be used to determine when to start convalescent COVID-19 plasma or recombinant antibody prophylaxis. Patients with low thymic function may respond better to high dose or adjuvanted vaccines, and strategies to interrupt thymic insult and restore thymic function may be incorporated in COVID-19 treatment regimens.

Disclosure of potential conflicts of interest

The authors declare no conflicts of interest.

Funding

This work was supported by MiOra public health education nonprofit.

References

- Dai X, Zhang D, Wang C, Wu Z, Liang C. The pivotal role of thymus in atherosclerosis mediated by immune and inflammatory response. *Int J Med Sci.* 2018;15(13):1555–63. doi:10.7150/ijms.27238.
- Dai X, Hua L, Chen Y, Wang J, Li J, Wu F, Zhang Y, Su J, Wu Z, Liang C. Mechanisms in hypertension and target organ damage: is the role of the thymus key? (Review). *Int J Mol Med.* 2018;42(1):3–12. doi:10.3892/ijmm.2018.3605.
- Ying S, O'Connor B, Ratoff J, Meng Q, Fang C, Cousins D, Zhang G, Gu S, Gao Z, Shamji B, et al. Expression and cellular provenance of thymic stromal lymphopoietin and chemokines in patients with severe asthma and chronic obstructive pulmonary disease. *J Immunol.* 2008;181(4):2790–98. doi:10.4049/jimmunol.181.4.2790.
- Cosway E, Anderson G, Garside P, Prendergast C. The thymus and rheumatology: should we care? *Curr Opin Rheumatol.* 2016;28(2):189–95. doi:10.1097/BOR.0000000000000251.
- Killerby ME, Link-Gelles R, Haight SC, Schrodt CA, England L, Gomes DJ, Shamout M, Petrone K, O'Laughlin K, Kimball A, et al. Characteristics associated with hospitalization among patients with COVID-19 — Metropolitan Atlanta, Georgia, March–April 2020. *MMWR Morb Mortal Wkly Rep.* 2020;69(25):790–94. doi:10.15585/mmwr.mm6925e1.
- Petrilli CM, Jones SA, Yang J, Rajagopalan H, O'Donnell L, Chernyak Y, Tobin KA, Cerfolio RJ, Francois F, Horwitz LI. Factors associated with hospital admission and critical illness among 5279 people with coronavirus disease 2019 in New York City: prospective cohort study. *BMJ.* 2020:m1966. doi:10.1136/bmj.m1966.
- Henry George Liddell, Robert Scott, *A Greek-English Lexicon.* <http://www.perseus.tufts.edu/hopper/text?doc=Perseus:text:1999.04.0057:entry=qumo/s>
- Miller JFAP. The discovery of thymus function and of thymus-derived lymphocytes. *Immunol Rev.* 2002;185(1):7–14. doi:10.1034/j.1600-065X.2002.18502.x.
- Ye P, Kirschner D, Kourtis A. The thymus during HIV disease: role in pathogenesis and in immune recovery. *CHR.* 2004;2(2):177–83. doi:10.2174/1570162043484898.
- Grody WW, Fligel S, Naeim F. Thymus involution in the acquired immunodeficiency syndrome. *Am J Clin Pathol.* 1985;84(1):85–95. doi:10.1093/ajcp/84.1.85.
- Afifi A, Raja SG, Pennington DJ, Tsang VT. For neonates undergoing cardiac surgery does thymectomy as opposed to thymic preservation have any adverse immunological consequences? *Interact Cardiovasc Thorac Surg.* 2010;11(3):287–91. doi:10.1510/icvts.2010.237172.
- Guy-Grand D, Azogui O, Celli S, Darce S, Nussenzweig MC, Kourilsky P, Vassalli P. Extrathymic T cell lymphopoiesis. *J Exp Med.* 2003;197(3):333–41. doi:10.1084/jem.20021639.
- Eysteinsdottir JH, Freysdottir J, Haraldsson A, Stefansdottir J, Skaftadottir I, Helgason H, Ogmundsdottir HM. The influence of partial or total thymectomy during open heart surgery in infants on the immune function later in life. *Clin Exp Immunol.* 2004;136(2):349–55. doi:10.1111/j.1365-2249.2004.02437.x.
- Detterbeck FC, Zeeshan A. Thymoma: current diagnosis and treatment. *Chin Med J.* 2013;126:2186–91.
- Rea F, Marulli G, Bortolotti L, Feltracco P, Zuin A, Sartori F. Experience with the “Da Vinci” robotic system for thymectomy in patients with myasthenia gravis: report of 33 cases. *Ann Thorac Surg.* 2006;81(2):455–59. doi:10.1016/j.athoracsur.2005.08.030.
- National Organization for Rare Disorders. Complete DiGeorge syndrome. <https://rarediseases.org/rare-diseases/complete-digeorge-syndrome/>
- Du Q, de la Morena MT, van Oers NSC. The genetics and epigenetics of 22q11.2 deletion syndrome. *Front Genet.* 2020;10:1365. doi:10.3389/fgene.2019.01365.
- Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell.* 2009;136(2):215–33. doi:10.1016/j.cell.2009.01.002.
- Leung AKL, Sharp PA. MicroRNA functions in stress responses. *Mol Cell.* 2010;40(2):205–15. doi:10.1016/j.molcel.2010.09.027.
- Mendell JT, Olson EN. MicroRNAs in stress signaling and human disease. *Cell.* 2012;148(6):1172–87. doi:10.1016/j.cell.2012.02.005.
- Cortez MA, Bueso-Ramos C, Ferdin J, Lopez-Berestein G, Sood AK, Calin GA. MicroRNAs in body fluids—the mix of hormones and biomarkers. *Nat Rev Clin Oncol.* 2011;8(8):467–77. doi:10.1038/nrclinonc.2011.76.
- Xu M, Gan T, Ning H, Wang L. MicroRNA functions in thymic biology: thymic development and involution. *Front Immunol.* 2018;9:2063. doi:10.3389/fimmu.2018.02063.
- Olivieri F, Capri M, Bonafè M, Morsiani C, Jung HJ, Spazzafumo L, Viña J, Suh Y. Circulating miRNAs and miRNA shuttles as biomarkers: perspective trajectories of healthy and unhealthy aging. *Mech Ageing Dev.* 2017;165:162–70. doi:10.1016/j.mad.2016.12.004.
- Skalsky RL, Cullen BR. Viruses, microRNAs, and host interactions. *Annu Rev Microbiol.* 2010;64(1):123–41. doi:10.1146/annurev.micro.112408.134243.
- Tarcic N, Ovadia H, Weiss DW, Weidenfeld J. Restraint stress-induced thymic involution and cell apoptosis are dependent on endogenous glucocorticoids. *J Neuroimmunol.* 1998;82(1):40–46. doi:10.1016/S0165-5728(97)00186-0.

26. Chandra RK. Nutritional deficiency and susceptibility to infection. *Bull World Health Organ.* 1979;57:167–77.
27. Savino W. The thymus is a common target organ in infectious diseases. *PLoS Pathog.* 2006;2(6):e62. doi:10.1371/journal.ppat.0020062.
28. Ansari AR, Liu H. Acute thymic involution and mechanisms for recovery. *Arch Immunol Ther Exp.* 2017;65(5):401–20. doi:10.1007/s00005-017-0462-x.
29. Lotz M, Jirik F, Kabouridis P, Tsoukas C, Hirano T, Kishimoto T, Carson DA. B cell stimulating factor 2/interleukin 6 is a costimulant for human thymocytes and T lymphocytes. *J Exp Med.* 1988;167(3):1253–58. doi:10.1084/jem.167.3.1253.
30. Gruver AL, Sempowski GD. Cytokines, leptin, and stress-induced thymic atrophy. *J Leukoc Biol.* 2008;84(4):915–23. doi:10.1189/jlb.0108025.
31. Yang H, Youm Y-H, Vandanmagsar B, Rood J, Kumar KG, Butler AA, Dixit VD. Obesity accelerates thymic aging. *Blood.* 2009;114(18):3803–12. doi:10.1182/blood-2009-03-213595.
32. Kim JH, Zafonte R, Pascuale-Leon A, Nadler LM, Weisskopf M, Speizer FE, Taylor HA, Baggish AL. American-style football and cardiovascular health. *JAHA.* 2018;7(8). doi:10.1161/JAHA.118.008620.
33. Borchers JR, Clem KL, Habash DL, Nagaraja HN, Stokley LM, Best TM. Metabolic syndrome and insulin resistance in division 1 collegiate football players. *Med Sci Sports Exerc.* 2009;41(12):2105–10. doi:10.1249/MSS.0b013e3181abdfec.
34. Prieto-Hinojosa A, Knight A, Compton C, Gleeson M, Travers PJ. Reduced thymic output in elite athletes. *Brain Behav Immun.* 2014;39:75–79. doi:10.1016/j.bbi.2014.01.004.
35. Dixit VD, Yang H, Sun Y, Weeraratna AT, Youm Y-H, Smith RG, Taub DD. Ghrelin promotes thymopoiesis during aging. *J Clin Invest.* 2007;117(10):2778–90. doi:10.1172/JCI30248.
36. Zhang F, Chen Y, Heiman M, DiMarchi R. Leptin: structure, function and biology. In: *Vitamins & hormones*. Vol. 71. Elsevier; 2005. p. 345–72. doi:10.1016/S0083-6729(05)71012-8.
37. Hick RW, Gruver AL, Ventevogel MS, Haynes BF, Sempowski GD. Leptin selectively augments thymopoiesis in leptin deficiency and lipopolysaccharide-induced thymic atrophy. *J Immunol.* 2006;177(1):169–76. doi:10.4049/jimmunol.177.1.169.
38. Rebello CJ, Kirwan JP, Greenway FL. Obesity, the most common comorbidity in SARS-CoV-2: is leptin the link? *Int J Obes.* 2020. doi:10.1038/s41366-020-0640-5.
39. Hince M, Sakkal S, Vlahos K, Dudakov J, Boyd R, Chidgey A. The role of sex steroids and gonadectomy in the control of thymic involution. *Cell Immunol.* 2008;252(1–2):122–38. doi:10.1016/j.cellimm.2007.10.007.
40. Olsen NJ, Olson G, Viselli SM, Gu X, Kovacs WJ. Androgen receptors in thymic epithelium modulate thymus size and thymocyte development*. *Endocrinology.* 2001;142(3):1278–83. doi:10.1210/endo.142.3.8032.
41. Taub DD, Longo DL. Insights into thymic aging and regeneration. *Immunol Rev.* 2005;205(1):72–93. doi:10.1111/j.0105-2896.2005.00275.x.
42. Shanley DP, Aw D, Manley NR, Palmer DB. An evolutionary perspective on the mechanisms of immunosenescence. *Trends Immunol.* 2009;30(7):374–81. doi:10.1016/j.it.2009.05.001.
43. Gui J, Mustachio LM, Su D-M, Craig RW. Thymus size and age-related thymic involution: early programming, sexual dimorphism, progenitors and stroma. *Aging Dis.* 2012;3:280–90.
44. Rottoli M, Bernante P, Belvedere A, Balsamo F, Garelli S, Giannella M, Cascavilla A, Tedeschi S, Ianniruberto S, Del Turco ER, et al. How important is obesity as a risk factor for respiratory failure, intensive care admission and death in hospitalised COVID-19 patients? Results from a single Italian centre. *Eur J Endocrinol.* 2020. doi:10.1530/EJE-20-0541.
45. Centers for Disease Control and Prevention. Coronavirus disease 2019: information for pediatric healthcare providers. <https://www.cdc.gov/coronavirus/2019-ncov/hcp/pediatric-hcp.html>
46. Fish EN. The X-files in immunity: sex-based differences predispose immune responses. *Nat Rev Immunol.* 2008;8(9):737–44. doi:10.1038/nri2394.
47. Michaud M, Balardy L, Moulis G, Gaudin C, Peyrot C, Vellas B, Cesari M, Nourhashemi F. Proinflammatory cytokines, aging, and age-related diseases. *J Am Med Dir Assoc.* 2013;14(12):877–82. doi:10.1016/j.jamda.2013.05.009.
48. Haynes BF, Markert ML, Sempowski GD, Patel DD, Hale LP. The role of the thymus in immune reconstitution in aging, bone marrow transplantation, and HIV-1 infection. *Annu Rev Immunol.* 2000;18(1):529–60. doi:10.1146/annurev.immunol.18.1.529.
49. Sempowski GD, Hale LP, Sundry JS, Massey JM, Koup RA, Douek DC, Patel DD, Haynes BF. Leukemia inhibitory factor, oncostatin M, IL-6, and stem cell factor mRNA expression in human thymus increases with age and is associated with thymic atrophy. *J Immunol.* 2000;164(4):2180–87. doi:10.4049/jimmunol.164.4.2180.
50. Hakim FT, Gress RE. Immunosenescence: deficits in adaptive immunity in the elderly. *Tissue Antigens.* 2007;70(3):179–89. doi:10.1111/j.1399-0039.2007.00891.x.
51. Naylor K, Li G, Vallejo AN, Lee -W-W, Koetz K, Bryl E, Witkowski J, Fulbright J, Weyand CM, Goronzy JJ, et al. The influence of age on T cell generation and TCR diversity. *J Immunol.* 2005;174(11):7446–52. doi:10.4049/jimmunol.174.11.7446.
52. Nanda NK, Apple R, Sercarz E. Limitations in plasticity of the T-cell receptor repertoire. *Proc Natl Acad Sci.* 1991;88(21):9503–07. doi:10.1073/pnas.88.21.9503.
53. Fry TJ, Mackall CL. Current concepts of thymic aging. *Springer Semin Immunopathol.* 2002;24(1):7–22. doi:10.1007/s00281-001-0092-5.
54. Geginat J, Lanzavecchia A, Sallusto F. Proliferation and differentiation potential of human CD8+ memory T-cell subsets in response to antigen or homeostatic cytokines. *Blood.* 2003;101(11):4260–66. doi:10.1182/blood-2002-11-3577.
55. Geginat J, Sallusto F, Lanzavecchia A. Cytokine-driven proliferation and differentiation of human naive, central memory, and effector memory CD4+ T cells. *J Exp Med.* 2001;194(12):1711–20. doi:10.1084/jem.194.12.1711.
56. Tough DF, Sprent J. Turnover of naive- and memory-phenotype T cells. *J Exp Med.* 1994;179(4):1127–35. doi:10.1084/jem.179.4.1127.
57. Mackall CL, Bare CV, Granger LA, Sharrow SO, Titus JA, Gress RE. Thymic-independent T cell regeneration occurs via antigen-driven expansion of peripheral T cells resulting in a repertoire that is limited in diversity and prone to skewing. *J Immunol.* 1996;156:4609–16.
58. Belkaid Y, Rouse BT. Natural regulatory T cells in infectious disease. *Nat Immunol.* 2005;6(4):353–60. doi:10.1038/ni1181.
59. Márquez EJ, Chung C, Marches R, Rossi RJ, Nèhar-Belaid D, Eroglu A, Mellert DJ, Kuchel GA, Bancheureau J, Ucar D, et al. Sexual-dimorphism in human immune system aging. *Nat Commun.* 2020;11(1):751. doi:10.1038/s41467-020-14396-9.
60. Piasecka B, Duffy D, Urrutia A, Quach H, Patin E, Posseme C, Bergstedt J, Charbit B, Rouilly V, MacPherson CR, et al. Distinctive roles of age, sex, and genetics in shaping transcriptional variation of human immune responses to microbial challenges. *Proc Natl Acad Sci USA.* 2018;115(3):E488–E497. doi:10.1073/pnas.1714765115.
61. Bakker OB, Aguirre-Gamboa R, Sanna S, Oosting M, Smeekens SP, Jaeger M, Zorro M, Vösa U, Withoff S, Netea-Maier RT, et al. Integration of multi-omics data and deep phenotyping enables prediction of cytokine responses. *Nat Immunol.* 2018;19(7):776–86. doi:10.1038/s41590-018-0121-3.
62. Hirokawa K, Utsuyama M, Hayashi Y, Kitagawa M, Makinodan T, Fulop T. Slower immune system aging in women versus men in the Japanese population. *Immun Ageing.* 2013;10(1):19. doi:10.1186/1742-4933-10-19.
63. Liu J, Li S, Liu J, Liang B, Wang X, Wang H, Li W, Tong Q, Yi J, Zhao L, et al. Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients. *Infectious Diseases (except HIV/AIDS);* 2020. doi:10.1101/2020.02.16.20023671

64. Gong J, Dong H, Xia SQ, Huang YZ, Wang D, Zhao Y, Liu W, Tu S, Zhang M, Wang Q, et al. Correlation analysis between disease severity and inflammation-related parameters in patients with COVID-19 pneumonia. *Infectious Diseases (except HIV/AIDS)*; 2020. doi:10.1101/2020.02.25.20025643
65. Wolf J, Weinberger B, Arnold CR, Maier AB, Westendorp RGJ, Grubeck-Loebenstien B. The effect of chronological age on the inflammatory response of human fibroblasts. *Exp Gerontol*. 2012;47(9):749–53. doi:10.1016/j.exger.2012.07.001.
66. Milan-Mattos JC, Anibal FF, Perseguini NM, Minatel V, Rehder-Santos P, Castro CA, Vasilceac FA, Mattiello SM, Faccioli LH, Catai AM, et al. Effects of natural aging and gender on pro-inflammatory markers. *Braz J Med Biol Res*. 2019;52(9):e8392. doi:10.1590/1414-431x20198392.
67. French H. Leucocyte counts in eighty-three cases of appendicitis: the limitations of leucocytosis as an indication for laparotomy. *Med Chir Trans*. 1904;87:467–87.
68. Douek DC, Vescio RA, Betts MR, Brenchley JM, Hill BJ, Zhang L, Berenson JR, Collins RH, Koup RA. Assessment of thymic output in adults after haematopoietic stemcell transplantation and prediction of T-cell reconstitution. *Lancet*. 2000;355(9218):1875–81. doi:10.1016/S0140-6736(00)02293-5.
69. Douek DC, McFarland RD, Keiser PH, Gage EA, Massey JM, Haynes BF, Polis MA, Haase AT, Feinberg MB, Sullivan# JL, et al. Changes in thymic function with age and during the treatment of HIV infection. *Nature*. 1998;396(6712):690–95. doi:10.1038/25374.
70. Picker LJ, Treer JR, Ferguson-Darnell B, Collins PA, Buck D, Terstappen LW. Control of lymphocyte recirculation in man. I. Differential regulation of the peripheral lymph node homing receptor L-selectin on T cells during the virgin to memory cell transition. *J Immunol*. 1993;150:1105–21.
71. Lewin SR, Heller G, Zhang L, Rodrigues E, Skulsky E, van den Brink MRM, Small TN, Kernan NA, O'Reilly RJ, Ho DD, et al. Direct evidence for new T-cell generation by patients after either T-cell-depleted or unmodified allogeneic hematopoietic stem cell transplantations. *Blood*. 2002;100(6):2235–42. doi:10.1182/blood.V100.6.2235.
72. Roux E, Dumont-Girard F, Starobinski M, Siegrist C-A, Helg C, Chapuis B, Roosnek E. Recovery of immune reactivity after T-cell-depleted bone marrow transplantation depends on thymic activity. *Blood*. 2000;96(6):2299–303. doi:10.1182/blood.V96.6.2299.
73. Al-Sukaiti N, Reid B, Lavi S, Al-Zaharani D, Atkinson A, Roifman CM, Grunebaum E. Safety and efficacy of measles, mumps, and rubella vaccine in patients with DiGeorge syndrome. *J Allergy Clin Immunol*. 2010;126(4):868–69. doi:10.1016/j.jaci.2010.07.018.
74. Salazar E, Perez KK, Ashraf M, Chen J, Castillo B, Christensen PA, Eubank T, Bernard DW, Eagar TN, Long SW, et al. Treatment of Coronavirus Disease 2019 (COVID-19) patients with convalescent plasma. *Am J Pathol*. 2020;190(8):1680–90. doi:10.1016/j.ajpath.2020.05.014.
75. Wang C, Li W, Drabek D, Okba NMA, van Haperen R, Osterhaus ADME, van Kuppeveld FJM, Haagmans BL, Grosveld F, Bosch B-J, et al. A human monoclonal antibody blocking SARS-CoV-2 infection. *Nat Commun*. 2020;11(1):2251. doi:10.1038/s41467-020-16256-y.
76. Lee JKH, Lam GKL, Shin T, Kim J, Krishnan A, Greenberg DP, Chit A. Efficacy and effectiveness of high-dose versus standard-dose influenza vaccination for older adults: a systematic review and meta-analysis. *Expert Rev Vaccines*. 2018;17(5):435–43. doi:10.1080/14760584.2018.1471989.
77. Tregoning JS, Russell RF, Kinnear E. Adjuvanted influenza vaccines. *Hum Vaccin Immunother*. 2018;14(3):550–64. doi:10.1080/21645515.2017.1415684.
78. Roser M, Ortiz-Ospina E, Ritchie H Life expectancy. Our world in data. <https://ourworldindata.org/life-expectancy>
79. World Health Organization. Cancer. 2018 Sep 12. <https://www.who.int/news-room/fact-sheets/detail/cancer>
80. De Paoli P, Bortolin MT, Zanussi S, Monzoni A, Pratesi C, Giacca M. Changes in thymic function in HIV-positive patients treated with highly active antiretroviral therapy and interleukin-2. *Clin Exp Immunol*. 2001;125(3):440–46. doi:10.1046/j.1365-2249.2001.01615.x.
81. Markert ML, Devlin BH, McCarthy EA. Thymus transplantation. *Clin Immunol*. 2010;135(2):236–46. doi:10.1016/j.clim.2010.02.007.
82. Chaudhry MS, Velardi E, Dudakov JA, van den Brink MRM. Thymus: the next (re)generation. *Immunol Rev*. 2016;271(1):56–71. doi:10.1111/imr.12418.
83. Fahy GM, Brooke RT, Watson JP, Good Z, Vasanaawala SS, Maecker H, Leipold MD, Lin DTS, Kobor MS, Horvath S, et al. Reversal of epigenetic aging and immunosenescent trends in humans. *Aging Cell*. 2019;18(6):6. doi:10.1111/accel.13028.
84. Dardenne M, Boukaiba N, Gagnerault M-C, Homo-Delarche F, Chappuis P, Lemonnier D, Savino W. Restoration of the thymus in aging mice by in vivo zinc supplementation. *Clin Immunol Immunopathol*. 1993;66(2):127–35. doi:10.1006/clin.1993.1016.
85. InterLeukin-7 to improve clinical outcomes in lymphopenic patients with COVID-19 infection FR BL Cohort (ILIAD-7-FR). NIH: clinicalTrials.gov. <https://clinicaltrials.gov/ct2/show/NCT04407689?term=IL-7&cond=covid&draw=2&rank=2>
86. Enzalutamide treatment in COVID-19 (COVIDENZA). NIH: clinicalTrials.gov. <https://clinicaltrials.gov/ct2/show/NCT04475601?term=testosterone&cond=Covid19&draw=2&rank=4>
87. Coronavirus 2019 (COVID-19)- using ascorbic acid and zinc supplementation (COVIDAtoZ). NIH: clinicalTrials.gov. <https://clinicaltrials.gov/ct2/show/NCT04342728>