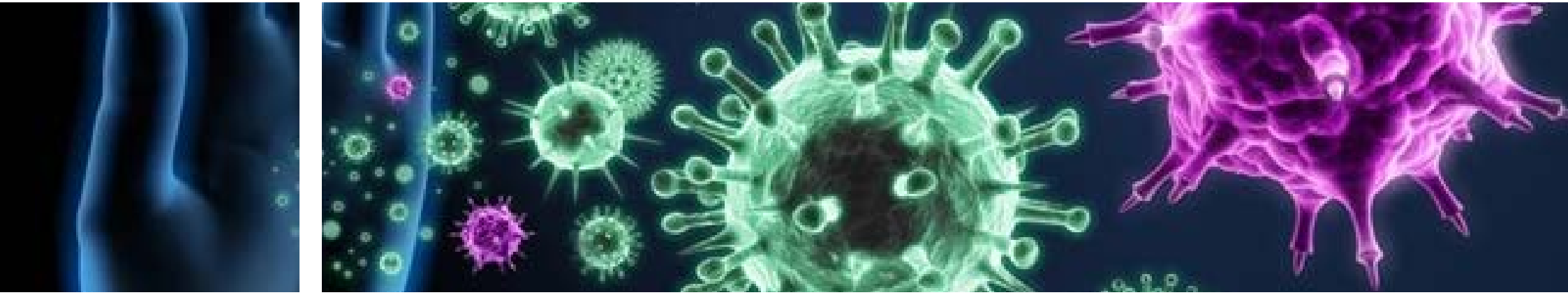
IMMUNEBALANCE



IMMUNE SYSTEM BALANCE

BIOSCIENCE GENOMICS

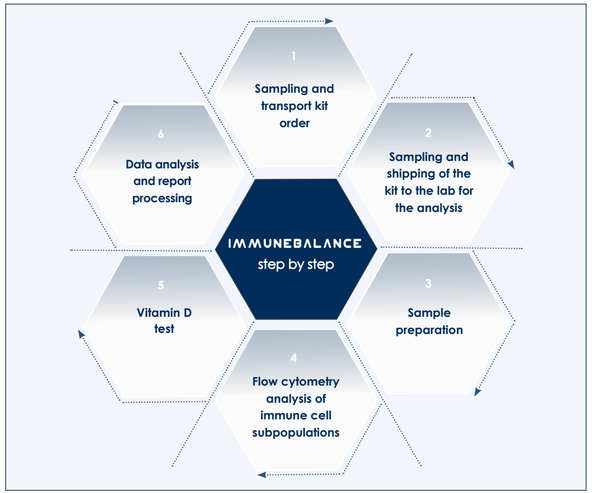
Università di Roma Tor Vergata spin off

IMMUNEBALANCE

Chemical and biological agents, together with trauma, continuously threaten health. Fortunately, the organism is equipped with a powerful weapon to counteract them: the immune system.

Aging affects immune functioning. The slow reduction of our defenses starts way before we could imagine, and the involution of some tissues responsible for immune cell production is almost complete between 40 and 50 years of age.
Promoting immune system balance, together with counterbalancing pro-inflammatory immune profiles, helps to counteract the development of age-associated degenerative, inflammatory and neoplastic diseases.

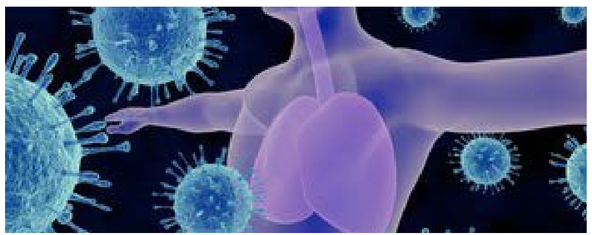
IMMUNEBALANCE by Bioscience Institute enables immune defense aging interception at every age, even when apparently healthy, for an active prevention of age-associated health issues.



IMMUNE SYSTEM AND AGING

Our immune system is continuously challenged by internal and external factors. During aging, this phenomenon, together with the effect of years of oxidative stress exposure, promotes the so-called **inflammaging** that is low-grade *inflammation* associated with aging.
The inflammaging correlates with almost all the age-associated health issues, such as cardiometabolic and neurodegenerative diseases. Moreover, during aging immune defenses decline; this phenomenon, known as **Immunosenescence**, is associated with mortality increase and is included among the mechanisms linking aging and immune system disorders (such as autoimmune diseases and cancer).
Immunosenescence compromises the capacity to respond to new health treats, thus increasing the susceptibility to infection. Also, it is associated with increased autoantibodies production, which makes the development of autoimmune diseases easier.
Finally, during aging protective mechanisms activated by oxidative stress become less efficient, promoting the build up of senescent, dysfunctional and mutated cells. Immune responses decrease, and the risk of cancer and degenerative disease development increases.

Immune system aging reduces healthspan, that is the years of life free from chronic diseases and disability. However, aging differs from one individual to another, and the hallmarks of immunosenescence are affected by the history of individual exposure to health treats. Moreover, an apparently good health status might hide altered immune cell levels, which may be immunosenescence and inflammaging markers.
In general, both innate (e.g. **monocytes** and Natural Killer – **NK** – **cells**) and acquired immunity (mediated by **T and B cells**) slow down.



INNATE AND ACQUIRED IMMUNITY

Innate immunity provides a fast protection, representing body's first line of defense. This protection is unspecific and incomplete but is needed until the second kind of immunity (the adaptive immunity) is established. In fact, adaptive immunity takes longer to respond to health insults, but is highly specific and permanent.

Acquired immunity cells respond to the inflammatory environment generated by the innate immunity, proliferating and differentiating to eliminate the health insult. Upon terminating their work, the majority of these cells die, leaving memory cells able to mediate a rapid and protective response in case of reinfection.

Memory cells can survive for decades. Meanwhile, the immune system tries to keep blood total T cell number at a fixed level. As a consequence, chronic stimulation of immune defenses (for example, chronic viral infection) significantly reduces the space for new lymphocytes, thus the possibility to react to new health threats.

T CELLS

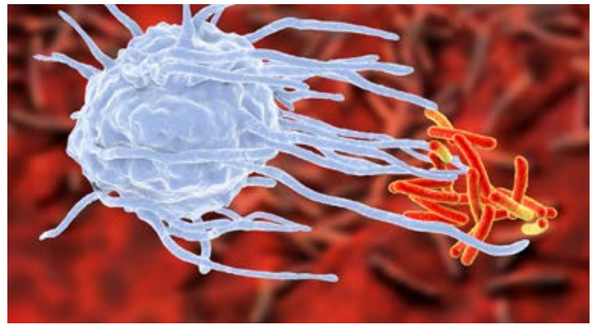
Among factors involved in age-related reduced resistance to infections are T cell population changes.

- The production of new T cells that can be activated by new antigens is reduced
- The production of molecules involved in inflammation is compromised
- T cell capability to differentiate into mature cells is reduced
- Senescent T cells display increased resistance to aptotosis and accumulate, reducing the space for new immune cells

Total T cell number can significantly decrease. However, even when total T cell reduction is not significant, the reduction of the space for new immune cells is associated with an increased risk of infection or of age-associated diseases, such as cancer.
Moreover, aging is associated with altered T cell populations percentages. Both **helper T cells (CD4+)** and **cytotoxic T cells (CD8+)** can decrease. However, the most concerning phenomenon is the inversion of CD4+/CD8+ ratio – a feature of the so-called **immune risk phenotype** (IRP).
IRP is a condition associated with aging.

High CD8+ percentages, combined with low CD4+ levels and scarce peripheral blood T cell proliferation, are associated with significant mortality increase in the elderly. CD4+/CD8+ ratio analysis enables evaluating immune system status. Its inversion (that is, values lower than 1) is a marker of increased health risk.

CD4+/CD8+ ratio can be altered by viral infection too, in particular by Cytomegalovirus (CMV) infection. In fact, **CMV positivity is among the principal causes of immune system aging.** After the first exposure, CMV persists lifelong. This leads to the chronic stimulation of CD8+ cells, which expand. CD4+ cells decrease in a compensatory way.
Even if a significant number of adults are positive to CMV, only a minor part of them is aware of this seropositivity. The analysis of CD4+/CD8+ ratio is useful also in these cases, offering information about immune system health and unveiling unsuspected impairments.



B CELLS

B cell numbers significantly change lifelong. Immediately after birth, their total level doubles, remaining elevated until the age of 2, when it starts gradually decreasing towards adult levels. Aging is associated with a further reduction, due to the decreased production of B cells in the bone marrow.

Age-associated chronic inflammation can inhibit the production of B cells via the activity of molecules involved in inflammation control.

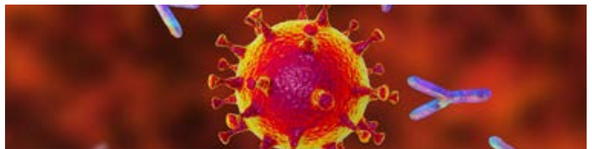
NK CELLS

Aging is associated with the increase of NK cells in peripheral blood. However, starting from the age of 50 their production decreases, suggesting a greater proportion of "old" NK cells in the elderly.

Reduced NK cell cytotoxicity is associated with morbidity and mortality increase. On the contrary, an active NK cell population is associated with longevity and good health.

It is possible to monitor some interesting changes by looking at the expression of CD56 and CD16 markers on NK cell surface. **$CD56^{bright}$ cells**, corresponding to immature NK cells, decrease, promoting a compensatory increase in the production of inflammation-related molecules.
At the same time, **CD56-CD16+ cells increase.** Also, these cells are characterized by an altered production of molecules involved in inflammation.

The redistribution of NK cell subpopulations, with decreased $CD56^{bright}$ cells and increased CD56-CD16+ cells, is typically associated with aging, and is unrelated to CMV positivity.



T CELLS, B CELLS AND NK CELLS

- T cells are white blood cells involved in acquired immunity. CD4+ cells react to bacteria, fungi, and viruses; they activate and promote the migration of other immune cells to infection sites. CD8+ cells recognize virus-infected and cancer cells, and directly kill them.
- B cells are white blood cells responsible for antibody production. Moreover, they can be involved in T cell activation and secrete molecules controlling inflammation.
- NK cells can activate other immune cells too. They produce molecules involved in inflammation, and take part in both innate and acquired immunity.

MONOCYTES

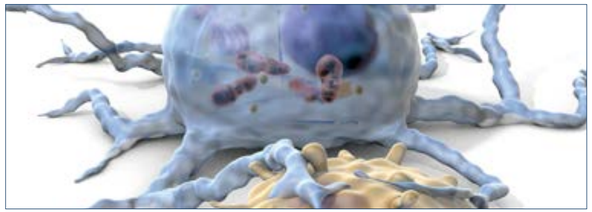
In healthy people, 95% of the monocyte pool corresponds to the "**classical" form** of these white blood cells, characterized by high CD14 and no CD16 expression **(CD14++CD16-)**. Starting from the age of 50, "**non-classical**" **CD14+CD16+ monocytes** increase; however, their function decreases, and their production of pro-inflammatory molecules increases. After the age of 60, there is a significant decrease in classical monocytes. At older ages non-classical monocytes decrease too, whereas **"intermediate" CD14++CD16+ monocytes increase**.

The increase in CD16+ (non-classical and intermediate) monocytes is associated with the development of chronic inflammation-related disorders (such as cardiovascular pathologies, obesity, diabetes, arthritis, and inflammatory bowel diseases). Fortunately, it is possible to efficiently counteract this increase.

For example, in case of obesity, both diet and bariatric surgery allow reducing CD16+ monocytes.

MONOCYTES, MACROPHAGES, AND DENDRITIC CELLS

- Produced in the bone marrow, monocytes circulate in the blood, and can differentiate into macrophages and dendritic cells.
- Once activated, they control inflammation, with both promoting and resolving function. Moreover, they phagocytate cells or toxic substances to be eliminated.



VITAMIN D AND THE IMMUNE SYSTEM

Vitamin D exerts a complex action on the immune system, promoting **a more tolerogenic state**. Immune cells can both respond to its presence and synthesize its active form.

In monocytes, it works as an antioxidant, and inhibits the production of inflammation-related molecules. Moreover, it seems to be involved in the epigenetic regulation of the response to antigens. In macrophages, it participates in the regulation of the production of molecules involved in inflammation. Finally, it regulates NK cell activity, and reduces the risk of autoimmune diseases and of pathogen-induced damage by acting on neutrophils.
Vitamin D regulates T and B cell functioning too. Its effects include increased production of anti-inflammatory molecules and the downregulation of the production of pro-inflammatory molecules, immune response, and autoimmune reactions.

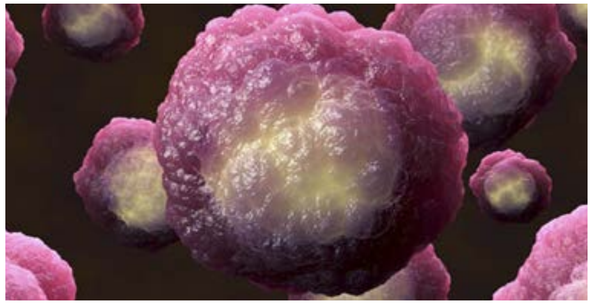
Vitamin D deficiency, which can be discovered by 25-(OH)D measurement, is associated with increased infection susceptibility and with the prevalence of autoimmune diseases. On the contrary, avoiding it promotes good immune functioning.

VITAMIN D SOURCES

Fulfilling vitamin D daily requirement is not a simple task. There are few foods containing this nutrient: salmon, rainbow trout, swordfish, mackerel, herrings, eggs, tuna, milk, and bovine liver are the richest sources. However, vitamin D is mostly synthesized in the skin by the action of sunbeam UV-rays.

Unfortunately, people spend less time in the open air than in the past, and they need to protect their skin from cancer risk by blocking UV-rays with adequate sunscreens. Moreover, latitude, season and skin pigmentation influence skin vitamin D synthesis. All these factors can limit the exposure to UV-rays. That is why vitamin D deficiency is such a widespread condition.

Unfortunately, **up to 40% of adults suffer from low serum vitamin D level**. This widespread vitamin D deficiency is caused by a combination of vitamin D-rich foods scarcity and poor UV-synthesis.
Aging comes with an even greater risk of severe vitamin D deficiency and of vitamin D deficiency-related problems, such as bone fractures, infections, cardiovascular diseases, diabetic retinopathy, migraine, cancer, and frailty.
Supplementation represents an effective strategy to correct this deficiency and promote a good immune system functioning. For example, daily supplementation has been associated with protection against acute respiratory tract infections.



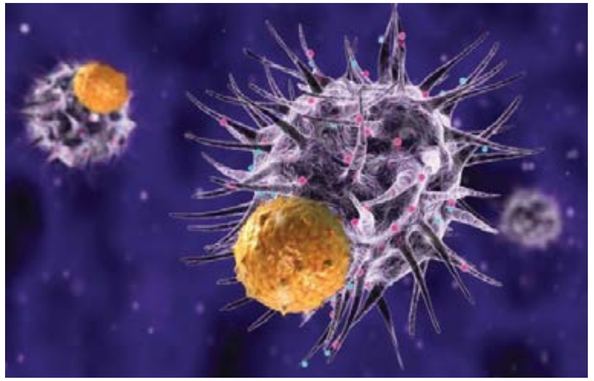
IMMUNEBALANCE

IMMUNEBALANCE by Bioscience Institute enables immune balance monitoring in healthy people, regardless of age, by the simultaneous analysis of total leukocytes, T and B cells, CD4+/CD8+ T cell ratio, monocytes CD14 and CD16 expression, NK cells, and vitamin D levels.

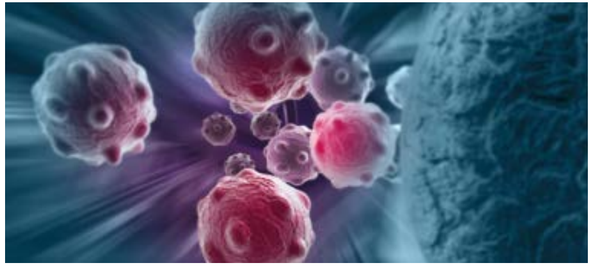
PARAMETER	DISEQUILIBRIUM
Total lymphocytes	↓
T cells	↓
CD4+ T cells	↓
CD8+ T cells	↓ or ↑
CD4+/CD8+	↓ or ↑
B cells	↓
Monocytes	
classical/intermediate	↓
intermediate/non-classical	↑
NK cells	↑
$CD56^{bright}$	↓
CD56-CD16+	↑
Vitamin D	
25-(OH)D	< 30 ng/ml (INSUFFICIENCY) < 20 ng/ml (DEFICIENCY)

The results of the test do not have diagnostic value. Rather, they provide a picture of immune defenses at the moment of blood sampling.
For each population of immune cells included in the analysis, it will be possible to compare measured levels with the values that are published in the scientific literature. By this way, IMMUNEBALANCE allows intercepting immune system imbalance.
At the same time, vitamin D level will be compared with the concentrations below which blood vitamin D is considered suboptimal (insufficiency) or low (deficiency).

Thanks to IMMUNEBALANCE it is possible to intercept deviation from healthy levels of immune cells and vitamin D before the onset of health issues. The imbalance status eventually detected can be counteracted via lifestyle-based solutions, such as the use of food supplements specifically studied to promote immune system functioning.



The final report will include a synthesis of the results of the test, a score (**IMMUNE IMBALANCE SCORE**) directly proportional to the measured immune system imbalance, and advice to promote the recovery of the balance of the immune system. Such advice should be reviewed by a qualified professional who assesses its suitability within a complete medical history.



REFERENCES

Aiello A et al. Immunosenescence and Its Hallmarks: How to Oppose Aging Strategically? A Review of Potential Options for Therapeutic Intervention. Front Immunol. 2019 Sep 25;10:2247. doi: 10.3389/fimmu.2019.02247

Aranow C. Vitamin D and the Immune System. J Investig Med. 2011 Aug; 59(6): 881–886. doi: 10.231/JIM.0b013e31821b8755

Benjamin JT et al. Neonatal Leukocyte Physiology and Disorders. In Avery's Diseases of the Newborn (Tenth Edition), 2018

Blanco E et al. Age-associated distribution of normal B-cell and plasma cell subsets in peripheral blood. J Allergy Clin Immunol. 2018 Jun;141(6):2208-2219.e16. doi: 10.1016/j.jaci.2018.02.017

Campos C et al. Effect of age and CMV on NK cell subpopulations. Exp Gerontol. 2014 Jun;54:130-7. doi: 10.1016/j.exger.2014.01.008

Ferguson FG et al. Immune parameters in a longitudinal study of a very old population of Swedish people: a comparison between survivors and nonsurvivors. J Gerontol A Biol Sci Med Sci. 1995 Nov;50(6):B378-82. doi: 10.1093/gerona/50a.6.b378

Geissmann F et al. Development of monocytes, macrophages, and dendritic cells. Science. 2010 Feb 5;327(5966):656-61. doi: 10.1126/science.1178331

Kapellos TS et al. Human Monocyte Subsets and Phenotypes in Major Chronic Inflammatory Diseases. Front Immunol. 2019 Aug 30;10:2035. doi: 10.3389/fimmu.2019.02035

Kverneland AH et al. Age and gender leucocytes variances and references values generated using the standardized ONE-Study protocol. Cytometry A. 2016 Jun;89(6):543-64. doi: 10.1002/cyto.a.22855

Li M et al. Age related human T cell subset evolution and senescence. Immun Ageing. 2019 Sep 11;16:24. doi: 10.1186/s12979-019-0165-8

Lin Y et al. Changes in blood lymphocyte numbers with age in vivo and their association with the levels of cytokines/cytokine receptors. Immun Ageing. 2016 Aug 18;13:24. doi: 10.1186/s12979-016-0079-7

Martens PJ et al. Vitamin D's Effect on Immune Function. Nutrients. 2020 May; 12(5): 1248. doi: 10.3390/nu12051248

Ma S et al. B Cell Dysfunction Associated With Aging and Autoimmune Diseases. Front Immunol. 2019; 10: 318. doi: 10.3389/fimmu.2019.00318

McBride JA and Striker R. Imbalance in the game of T cells: What can the CD4/CD8 T-cell ratio tell us about HIV and health? PLoS Pathog. 2017 Nov; 13(11): e1006624. doi: 10.1371/journal.ppat.1006624

Montecino-Rodriguez E et al. Lymphoid-Biased Hematopoietic Stem Cells Are Maintained with Age and Efficiently Generate Lymphoid Progeny. Stem Cell Reports. 2019 Mar 5; 12(3): 584–596. doi: 10.1016/j.stemcr.2019.01.016

Morbach H et al. Reference values for B cell subpopulations from infancy to adulthood. Clin Exp Immunol. 2010 Nov;162(2):271-9. doi: 10.1111/j.1365-2249.2010.04206.x

Patel AA and Yona S. Inherited and Environmental Factors Influence Human Monocyte Heterogeneity. Front Immunol. 2019 Nov 7;10:2581. doi: 10.3389/fimmu.2019.02581

Pennock ND et al. T cell responses: naïve to memory and everything in between. Adv Physiol Educ. 2013 Dec; 37(4): 273–283. doi: 10.1152/advan.00066.2013

Poitou C et al. CD14dimCD16+ and CD14+CD16+ monocytes in obesity and during weight loss: relationships with fat mass and subclinical atherosclerosis. Arterioscler Thromb Vasc Biol. 2011 Oct;31(10):2322-30. doi: 10.1161/ATVBAHA.111.230979

Kweder H and Eidi H. Vitamin D deficiency in elderly: Risk factors and drugs impact on vitamin D status. Avicenna J Med. 2018 Oct-Dec; 8(4): 139–146. doi: 10.4103/ajm.AJM_20_18

Seaborg E. Just right: how much vitamin D is enough? In Endocrine news, Endocrine Society. 2014 Nov. https://endocrinenews.endocrine.org/nov-2014-just-right-how-much-vitamin-d-is-enough/

Seidler S et al. Age-dependent alterations of monocyte subsets and monocyte-related chemokine pathways in healthy adults. BMC Immunol. 2010 Jun 21;11:30. doi: 10.1186/1471-2172-11-30

Solana R et al. Shaping of NK cell subsets by aging. Curr Opin Immunol. 2014 Aug;29:56-61. doi: 10.1016/j.coi.2014.04.002

Stockinger B et al. The concept of space and competition in immune regulation. Immunology. 2004 Mar; 111(3): 241–247. doi: 10.1111/j.1365-2567.2004.01831.x

Strindhall J et al. The inverted CD4/CD8 ratio and associated parameters in 66-year-old individuals: the Swedish HEXA immune study. Age (Dordr). 2013 Jun; 35(3): 985–991. doi: 10.1007/s11357-012-9400-3

Thaler B et al. Differential in vivo activation of monocyte subsets during low-grade inflammation through experimental endotoxemia in humans. Sci Rep. 2016 Jul 22;6:30162. doi: 10.1038/srep30162

Valiathan R et al. Effects of Ageing on the Immune System: Infants to Elderly. Scand J Immunol. 2016 Apr;83(4):255-66. doi: 10.1111/sji.12413

Ventura MT et al. Immunosenescence in aging: between immune cells depletion and cytokines up-regulation. Clin Mol Allergy. 2017 Dec 14;15:21. doi: 10.1186/s12948-017-0077-0

Verschoor CP et al. Alterations to the frequency and function of peripheral blood monocytes and associations with chronic disease in the advanced-age, frail elderly. PLoS One. 2014 Aug 8;9(8):e104522. doi: 10.1371/journal.pone.0104522

Wikby A et al. Changes in CD8 and CD4 lymphocyte subsets, T cell proliferation responses and non-survival in the very old: the Swedish longitudinal OCTO-immune study. Mech Ageing Dev. 1998 May 15;102(2-3):187-98. doi: 10.1016/s0047-6374(97)00151-6

Wikby A et al. The immune risk profile is associated with age and gender: findings from three Swedish population studies of individuals 20-100 years of age. Biogerontology. 2008 Oct;9(5):299-308. doi: 10.1007/s10522-008-9138-6

Zhang Y et al. In vivo kinetics of human natural killer cells: the effects of ageing and acute and chronic viral infection.

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