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‘From immunosenescence to immune modulation’: a re-appraisal of the role of cytomegalovirus as major regulator of human immune function

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Abstract

In 2000 cytomegalovirus was identified as a risk factor for mortality in a seminal study of octogenarian residents in Sweden. This finding triggered a wave of additional epidemiological investigations, some of which supported this association whilst others observed no such effect. In addition, this increased risk of death in CMV seropositive people was correlated with observed changes within the T cell repertoire such that accelerated 'immuno-senescence' became a *de facto* explanation, without strong evidence to this effect. Recent years have seen a re-appraisal of these findings. Interestingly, many studies show that cytomegalovirus acts to improve immune function, most clearly in younger donors. In addition, the excess mortality in older people that is observed in CMV seropositive cohorts appears to be related primarily to an excess of vascular disease rather than impairment of immune function.

CMV is an important member of the natural 'virome' of *Homo sapiens* and has an important, and generally positive, modulatory influence on human immune function throughout most of life. However, within certain populations this influence can become negative and age, co-morbidity and environment all act as determinants of this effect. As such, it is important that new interventions are developed that can mitigate the damaging influence of CMV on human health in populations at risk.

Keywords Cytomegalovirus – immunosenescence – vascular surveillance - arteriosclerosis

Cytomegalovirus can act as a risk factor for increased morbidity and mortality in older people

Seminal observations by Wikby and colleagues showed that persistent cytomegalovirus infection was a risk factor for an increased risk of mortality in octogenarian donors in a Swedish cohort study (1). Similar findings were later observed in the nonagenarian group (2) although not in centenarians, potentially due to selection for long term survivors (3). This excess mortality in the OCTO and NONA cohorts appeared to relate to several different pathologies but the association with a herpesvirus infection was completely unexpected. Importantly, these observations correlated with a range of alterations in the peripheral lymphoid repertoire, most notably an inversion of the CD4:CD8 T cell ratio and an increased proportion of terminally differentiated CD8+ T cells (4). As such, a general assumption tended to emerge that these clinical sequelae arose secondary to relative dysfunction of the immune system and the association of CMV with immunosenescence became firmly established.

Over the last 18 years many studies have further investigated the association of CMV with the health of older people. Many of these have replicated features from the Swedish study, including an increased risk of frailty as well as elevated mortality risk in some cohorts (5-10). Our own study was undertaken on a cohort of 511 people aged 65 years or more at entry and who were followed for 18 years in the English Midlands region (11). Remarkably, despite extensive correction for confounding variables, this also observed that CMV sero-positive individuals died almost 4 years earlier than the sero-negative group. Similar findings have also been observed in the setting of murine CMV infection (12,13)

Importantly, this association between CMV and health problems in older people has not been seen in several other studies (14-18). Indeed, in some settings the carriage of CMV in older people has actually been shown to be beneficial to health (19).

In contrast, cytomegalovirus infection can also be beneficial for immune function

Paradoxically, despite the association described above between CMV infection and impaired health in older people, it is now clear that chronic infection with the virus can actually act to improve the quality of the immune response. Murine models have played an important role here, and the association between herpesvirus infection and improved control of acute pathogen challenge was described many years ago (20). More recent studies have also demonstrated that CMV can act to broaden the breadth and quality of the T cell response to antigen challenge in aged mice (21,22). These observations have now been vindicated in human studies. A systems analysis of the genetic and environmental determinants of quality of response to influenza vaccination in younger donors indicated that CMV was strongly associated with improved vaccine responses (23). In addition, in sub-Saharan Africa CMV

infection, which is almost ubiquitous within the first year of life, acts to support vaccine responsiveness and has a notable effect in overcoming the detrimental influence of EBV infection within these infants (24,25). As such, those who have EBV in the absence of CMV carriage have a markedly impaired ability to respond to protective vaccines. A role for the virus in broadening the functional profile of CD8+ T cells has also been demonstrated (26).

These associations should not be totally unexpected. CMV is a very important constituent of the human 'virome', a concept that is now well established and reflects the fact that all adults carry many chronic viruses which have an important modulatory influence on somatic function (Figure 1) (27). CMV has evolved in concert with the vertebrate lineage and is likely to have infected the vast majority of members of our own species during their lifetime. As such it is highly unlikely that evolutionary selection would not have accommodated to infection and limited any major detrimental effects to reproductive capacity. Instead, it could be suggested that the CMV genome could be regarded as an important, albeit viral, component of our working genome and could act to support physiological function in a number of ways. Indeed, CMV plays a dramatic role in maturing the peripheral immune system and primary infection in African infants leads to a profound increase in the number of CD8+ T cells, an effect that is retained for life. CMV may be regarded as a potent stimulus for induction of inflammatory Th1 CD4+ and CD8+ immune responses and, as well as the profound increase in CMV-directed cytotoxic cells within the vascular system, this is likely to modulate the quality of the immune response to heterologous antigens.

An important proviso here is the devastating effect that CMV infection can have in the setting of perinatal infection and the virus is now arguably the most important infectious cause of perinatal morbidity. This is a reminder of the remarkable destructive activity of the virus in the absence of effective immune control and the fact that this remains such an important problem is perhaps surprising given the otherwise potentially symbiotic nature of infection. It is possible that primary maternal infection during pregnancy is a relatively recent evolutionary occurrence, given the high prevalence of infection in childhood in most settings. Nevertheless, maternal viral reactivation can also lead to perinatal infection and one may have to consider that the evolutionary selection against such a potentially devastating occurrence has not been outweighed by a potential advantage of infection in relation to reproductive fitness.

Importantly, we must therefore regard CMV as a significant immunomodulatory factor within human immune function. Early on in life, particularly prior to reproductive age, CMV is likely to improve immune function by increasing the inflammatory capacity to respond to infectious challenge. However, as life progresses this attribute may become less beneficial, particularly when taken in association with the established 'inflamm-ageing' that occurs during the latter stages of life (Figure 2). Despite the fact that CMV has not been directly associated with increased systemic inflammation, it is entirely conceivable that, in some individuals, persistent carriage of the increased cytotoxic immune load associated with CMV

might have negative clinical associations. Of note, it is now clear that the cytotoxic functional capacity of CMV-specific CD4+ and CD8+ T cells actually increases across the life course (Figure 3) (28). The aetiology of this is not entirely clear but may reflect increasing differentiation of cells over decades towards increased cytotoxic phenotype. It is likely that such CMV-specific T cells contribute to CMV-induced vascular immunopathology and as such this observation provides a clear explanation for why viral carriage may be detrimental in those of older age.

Why is the epidemiology of CMV infection in older people so conflicting?

It is perplexing, and indeed not a little frustrating, that the clinical impact of CMV infection in older people has been so variable in different epidemiological studies. Greater homogeneity of association would almost certainly facilitate attempts to ameliorate this association (see below). Nevertheless, such findings also represent an opportunity to understand more about the impact and determinants of CMV carriage on human physiology and a number of factors are emerging that are likely to play an important role. These might be considered in relation to the nature of the virus, the host or indeed the environment.

CMV sequence analysis is in its relative infancy but it is now clear that the viral genome shows much more heterogeneity than had been appreciated (29, 30). It is conceivable, indeed likely, that such polymorphism may act to influence the clinical impact of infection on the host. Future studies of CMV sequence in large cohorts will therefore be of value although this remains a challenging undertaking due to the very low level of virus within healthy people. Secondly, it is now extremely well defined that the dose of the initial viral inoculum is a critical factor in determining the subsequent magnitude of the virus-specific immune response and associated memory inflation. Elegant work in which mice are infected with defined doses of CMV inoculum, starting as low as 10 virus particles, show that persistent infection is established in all cases but the magnitude of the immune response is strongly correlated to the initial inoculum (30-34). As such, an important physiological 'variable' of CMV infection in humans is likely to be the infectious dose of CMV that was received at the time of primary infection. Most natural infections arise from ingestion of breast milk and it is unclear to what extent there is heterogeneity in either the excretion of virus within this tissue or the absorption by the infant. Nevertheless, this association does deserve further study and could act as an important definitive set point for a life-long association between the host immune response and the viral load. Furthermore, the immune status of the host at the time of infection is likely to be an important determinant of the ultimate 'setpoint' of the virus-host balance. In many countries primary CMV infection now occurs in adulthood and it is not yet clear if this may act to accelerate or limit the impact of the virus on subsequent immune function (Marandu, this edition).

In relation to potential variables within the host, genetic polymorphism within our genome would certainly be predicted to impact on an individual's ability to either prevent primary infection or control established persistent infection. Indeed, a wide range of genetic analyses are starting to reveal important associations between

genotype and both the risk of primary infection and clinical outcome (35-44). This important consideration is likely to become even more apparent during the interrogation of very rich epidemiological cohorts that are now available.

Finally, environmental factors may well have an important impact on the immune control of CMV. Indeed, the natural history of CMV infection in sub-Saharan Africa, from where our species evolved, appears to be very different from that in more developed countries. In particular, infection is acquired in almost all infants within the first year of life and is associated with a rapid differentiation of the immune repertoire (45,46). In addition, our recent studies of adults in Malawi indicate that the CD4:CD8 ratio remains stable across the lifecourse with minimal accumulation of late differentiated effector cells. As such there is no clear evidence of development of the CMV-associated 'risk memory profile' of the lymphoid repertoire across the life course. There is debate as to the clinical impact of CMV infection in this setting and it is possible that this has been grossly underestimated. CMV infection in association with HIV infection is associated with considerable health problems such as stunting of growth (47) and CMV-associated pneumonitis may be an important cause of infant mortality in some settings (48).

CMV-specific immunity: 'it's in the blood stupid!'

CMV leaves an indelible footprint on the immune repertoire. Indeed, it is very easy to tell if somebody carries CMV infection by analysing their immune profile and without recourse to looking at viral serology (49-51). Initial studies using HLA-peptide tetramers revealed the remarkable scale of the CMV-specific CD8+ T cell immune response and helped to explain some of the dramatic associations between CMV infection and immune profile that had been observed in the OCTO cohort (52). It is now appreciated that CMV drives the largest antigen-specific immune response of any pathogen within the vascular system (53-55). The consequences are dramatic and the proportion of effector/memory CD8+ T cells is typically 30% higher within individuals with chronic CMV infection, an effect that is mirrored with a similar decrease in the naive subset (56-58). This cellular and humoral response has a tendency to increase with age, in a process termed as 'memory inflation', and it is likely that this reflects a response to increased subclinical viral reactivation in older people (59). However this CMV-induced transformation in the organisation of the peripheral lymphoid profile is certainly established rapidly after primary infection and remains relatively stable throughout the majority of the lifecourse (Figure 4) (49,57). It was initially unclear why CMV would act to trigger such a huge virus-specific immune response. In retrospect the answer was probably relatively apparent many years ago. This huge metabolic investment by the immune system must almost certainly reflect a physiological requirement for immediate surveillance of the vascular tissue. This concept has been developed in an animal model which shows that vascular system is an important organ for tropism and replication of the virus and that the great majority of CMV-specific CD4+ and CD8+ T cell compartment resides within the vascular system, either within peripheral arteries or within the

blood vessels of the organs themselves (60). As such, these T cells are poised to respond extremely rapidly to viral reactivation, a function perhaps exemplified by their expression of the beta- adrenergic receptor allowing immediate mobilisation in response to physical or psychological stress, itself a trigger for viral reactivation (61). Thus we now have a clear concept of why the remarkable scale of the CMV-specific immune response actually reflects 'immune surveillance' of the vascular system (Figure 5).

When these initial observations of CMV-specific immunity within peripheral blood were observed there was a tendency to assume that this enormous immune response would be reflected throughout somatic tissues, a remarkable supposition considering that over 90% of lymphocytes reside within primary and secondary lymphoid tissue. However, the CMV-specific immune response within these tissues is more modest, although CMV-specific responses are considerable in certain organs such as lung and spleen. As such, the CMV-specific immune response is highly concentrated within the vascular system but this is not reflective of the similar magnitude of immune commitment throughout the body.

CMV infection in older people is associated with an excess of cardiovascular disease

Notwithstanding the variable association of CMV infection with increased morbidity in the elderly, there has been considerable interest in trying to understand the nature of any such pathological association. The initial focus on immunosenescence led to investigation into the relative frequency and severity of infection in the setting of CMV carriage, but this has been difficult to demonstrate. The efficacy of response to vaccine challenge has been used as a surrogate of immune competence in many studies and an impairment of influenza-specific antibody titre has been reported (62-64). However, other studies have failed to show any association between CMV carriage and either the breadth or titre of neutralising antibodies (65-67). As such, the importance of CMV infection as a determinant of immune competence remains uncertain.

In contrast, considerable data now leads towards the view that CMV acts as a risk factor for cardiovascular disease in older people (68-70). Indeed the relative risk of death from cardiovascular disease within the CFAS cohort was 1.96 in the CMV sero-positive subgroup whereas there was no increase in mortality from other broad pathologies such as infection, respiratory disease or cancer (11). The details of this excess cardiovascular mortality were not determined but this broad grouping includes disorders such as stroke, hypertensive disease and atherosclerosis. A potential association between CMV and atherosclerosis has been debated for many years this relationship remains somewhat uncertain (71-73). In contrast, considerable evidence is now emerging to suggest that CMV can act as a risk factor for the development of arteriosclerosis, a different pathology in which arterioles become stiff and which lead to an increased 'pulse wave velocity (PWV)' as each systolic heartbeat travels more rapidly through the arterial system. Increased PWV

is an important risk factor for cardiovascular disease as it acts to impair the 'buffering' of elastic arteries and increases the susceptibility of end organs to high pressures. PWV is a well-established risk factor for cardiovascular morbidity and mortality in a range of settings, most notably in patients with renal disease.

CMV seropositivity is associated with increased pulse wave velocity (74) and this is particularly notable in those with high numbers of CMV-specific T cells (75, 76). An increased 'stiffness' of arteries would be expected to lead to elevated blood pressure and indeed this has been observed in several cohorts. Our own studies detected an increase of 3.8 mmHg in systolic pressure in CMV seropositive people over the age 70 years (77). Although this increment may appear relatively modest, it is comparable or greater than that induced by other established risk factors for cardiovascular mortality such as diabetes or high salt intake and could easily explain a significant increase in cardiovascular mortality. CMV infection has always been correlated with increased blood pressure in cohorts of younger people and in a range of animal models (78, 79). However, again this association has not been observed in all epidemiological studies. Despite this, the belief that CMV may serve to drive pathology within blood vessels is relatively compelling and infection is clearly associated with endothelial dysfunction in settings such as cardiac allo-transplantation (80). Furthermore, a potential mechanism is apparent as the vascular system is a major battleground of viral infection and immunological control. The virus is tropic for endothelial cells (81) and indeed the large CMV-specific immune response within the vascular system is believed to represent 'immune surveillance' of the vascular bed (82-84).

This association of CMV with cardiovascular disease may help to explain why some of the more recent epidemiological studies of CMV infection in older people have failed to replicate earlier studies. Remarkably, morbidity and mortality from cardiovascular disease has dropped very substantially in the developed world in the last two decades. The aetiology of this is not entirely clear, but includes widespread use of statins and anti-hypertensive agents, as well as improved diet. As such, death from hypertensive disease is falling quite markedly at a global scale and therefore the relative impact of CMV on this pathology is likely to be substantially blunted.

How can the clinical impact of CMV infection in otherwise healthy people be limited in populations at risk?

Notwithstanding all of the areas of debate discussed above, it remains clear that CMV impacts negatively on the health of large numbers of the population. As such, it is entirely reasonable to start to initiate approaches that will mitigate against this problem. A key strategic decision is likely to be whether or not to limit or eradicate viral infection, or to target the virus-specific immune response as a potential mediator of immunopathology. In reality, suppression of viremia should subsequently act to reduce virus-specific immune responses and the two determinants are therefore inter-related.

Antiviral therapy is perhaps the most obvious consideration in this regard. Murine studies have shown that anti-viral drugs can profoundly suppress the degree of memory inflation following established infection and, importantly, that this also serves to improve the quality and efficacy of the immune response to a subsequent heterologous acute influenza infection. However, antiviral therapy had to be given for at least 6-12 months, representing half the lifespan of an aged mouse, and the potential mechanisms that underlie this effect are unknown (85). Our recent studies in human subjects have used valacyclovir to suppress viral replication. However, when medication was taken for six months by healthy elderly donors aged over 65 years we did not observe a contraction in the CMV-specific CD4+ or CD8+ T cell immune response in blood (In preparation). The reasons for this are not clear but may represent inadequate suppression of viral replication or the need for more prolonged treatment duration.

However, anti-viral medication has proven of more value in people at increased risk of CMV reactivation. In particular, CMV is a significant problem in patients on immune suppression for vasculitis and is associated with increased risk of heterologous infection (86). In this setting the use of valacyclovir for six months did act to completely suppress episodes of viral reactivation. Moreover this was associated with a decrease in the magnitude of both the CMV-specific CD4+CD28- T cell and improved humoral immune responses (87). A range of new drugs have now been developed which are both highly effective in CMV suppression and also very well tolerated. It is likely that these will transform the potential for ameliorating CMV-induced immunopathology and it will be interesting to see if they may one day enter clinical practice in this regard.

Concluding Remarks

CMV is a highly complex virus that has an ancient and highly intertwined relationship with our immune system. In the setting of profound immune suppression, such as that observed with transplantation or perinatal infection, viral replication is unchecked and may be associated with substantial tissue damage. However, the potential clinical importance of CMV infection in otherwise healthy immunocompetent people has been more difficult to define. Early reports demonstrating an association with increased mortality in older people have been observed in some, but not all, subsequent studies and potential determinants of this heterogeneity may be the viral inoculum, viral and host genetics and environmental factors including improved lifestyle and overall reduction in cardiovascular morbidity. CMV infection and immune surveillance is concentrated in the vascular system and this leads to a profound alteration in the lymphoid repertoire in CMV seropositive people. This has perhaps driven excessive speculation as to a negative impact of CMV on immune function whereas many studies now indicate a positive association, potentially through an inflammatory maturation of the cellular immune system. Increased infection rates in CMV-seropositive people have been difficult to demonstrate, whilst an increase in cardiovascular disease is well established. As such it may now be the time to move

away from focussing on CMV-associated immunosenescence and start to consider the complex regulation and consequence of CMV-mediated immunomodulation.

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Conflict of Interest

The author states that he has no conflict of interest

References

1. Olsson J, Wikby A, Johansson B, Löfgren S, Nilsson BO, Ferguson FG. (2000) Age-related change in peripheral blood T-lymphocyte subpopulations and cytomegalovirus infection in the very old: the Swedish longitudinal OCTO immune study. *Mech Ageing Dev.* Dec 20;121(1-3):187-201.
2. Wikby A, Johansson B, Olsson J, Löfgren S, Nilsson BO, Ferguson F (2002) Expansions of peripheral blood CD8 T-lymphocyte subpopulations and an association with cytomegalovirus seropositivity in the elderly: the Swedish NONA immune study. *Exp Gerontol* 37(2–3):445-453
3. Strindhall J, Nilsson BO, Löfgren S, Ernerudh J, Pawelec G, Johansson B, Wikby A. (2007) No Immune Risk Profile among individuals who reach 100 years of age: findings from the Swedish NONA immune longitudinal study. *Exp Gerontol* 42(8):753-761.
4. Wikby A, Mattsson IA, Johansson B, Strindhall J, Nilsson SE: (2008) The immune risk profile is associated with age and gender: findings from three Swedish population studies of individuals 20–100 years of age. *Biogerontology* 9(5):299-308
5. Strandberg TE, Pitkala KH, Tilvis RS. (2009) Cytomegalovirus antibody level and mortality among community-dwelling older adults with stable cardiovascular disease. *JAMA* 301(4):380- 382.
6. Thomasini RL, Pereira DS, Pereira FSM, Mateo EC, Mota TN, Guimarães GG, Pereira LSM, Lima CX, Teixeira MM, Teixeira AL Junior. (2017) Aged-associated cytomegalovirus and Epstein-Barr virus reactivation and cytomegalovirus relationship with the frailty syndrome in older women. *PLoS One.* Jul 10;12(7):e0180841.
7. Wang GC, Kao WH, Murakami P, Xue QL, Chiou RB, Detrick B, McDyer JF, Semba RD, Casolaro V, Walston JD, Fried LP. (2010) Cytomegalovirus infection and the risk of mortality and frailty in older women: a prospective observational cohort study. *Am J Epidemiol.* May 15;171(10):1144-52
8. Roberts ET, Haan MN, Dowd JB, Aiello AE. (2010) Cytomegalovirus antibody levels, inflammation, and mortality among elderly Latinos over 9 years of follow-up. *Am J Epidemiol.* Aug 15;172(4):363-71.
9. Feinstein L, Douglas CE, Stebbins RC, Pawelec G, Simanek AM, Aiello AE. (2016) Does cytomegalovirus infection contribute to socioeconomic disparities in all-cause mortality? *Mech Ageing Dev.* Sep;158:53-6.
10. Vescovini R, Biasini C, Telera AR, Basaglia M, Stella A, Magalini F, Bucci L, Monti D, Lazzarotto T, Dal Monte P et al.: (2010) Intense antiextracellular adaptive immune response to human cytomegalovirus in very old subjects with impaired health and cognitive and functional status. *J Immunol* 184(6):3242-3249.
11. Savva GM, Pachnio A, Kaul B, Morgan K, Huppert FA, Brayne C, Moss PA; Medical Research Council Cognitive Function and Ageing Study. (2013) Cytomegalovirus infection is associated with increased mortality in the older population. *Aging Cell.* Jun;12(3):381-7.
12. Mekker A, Tchang VS, Haeberli L, Oxenius A, Trkola A, Karrer U. (2012) Immune senescence: relative contributions of age and cytomegalovirus infection. *PLoS Pathog.* 8(8):e1002850.
13. Cicin-Sain L, Brien JD, Uhrlaub JL, Drabig A, Marandu TF, Nikolich-Zugich J. (2012) Cytomegalovirus infection impairs immune responses and accentuates T-cell pool changes observed in mice with aging. *PLoS Pathog.* 28(8):e1002849.
14. Goldeck D, Oettinger L, Janssen N, Demuth I, Steinhagen-Thiessen E, Pawelec G. (2016) Cytomegalovirus Infection Minimally Affects the Frequencies of B-Cell Phenotypes in Peripheral Blood of Younger and Older Adults. *Gerontology.* 62(3):323-9
15. Colonna-Romano G, Akbar AN, Aquino A, Bulati M, Candore G, Lio D, Ammatuna P, Fletcher JM, Caruso C, Pawelec G. (2008) Impact of CMV and EBV seropositivity on CD8 T lymphocytes in an old population from West-Sicily. *Immunity & Ageing* 5:14.

16. Matheï C, Adriaensen W, Vaes B, Van Pottelbergh G, Wallemacq P, Degryse J. (2015) No relation between CMV infection and mortality in the oldest old: results from the Belfrailstudy.. *Age Ageing*. Jan;44(1):130-5.
17. Collerton J, Martin-Ruiz C, Davies K, Hilkens CM, Isaacs J, Kolenda C, Parker C, Dunn M, Catt M, Jagger C, von Zglinicki T, Kirkwood TB. (2012) Frailty and the role of inflammation, immunosenescence and cellular ageing in the very old: cross-sectional findings from the Newcastle 85+ Study. *Mech Ageing Dev*. Jun;133(6):456-66
18. Cicin-Sain L, Sylwester AW, Hagen SI, Siess DC, Currier N, Legasse AW, Fischer MB, Koudelka CW, Axthelm MK, Nikolich-Zugich J, Picker LJ. (2011) Cytomegalovirus-specific T cell immunity is maintained in immunosenescent rhesus macaques. *J Immunol*. Aug 15;187(4):1722-32
19. Matheï C, Vaes B, Wallemacq P, Degryse J. (2011) Associations between cytomegalovirus infection and functional impairment and frailty in the BELFRAIL Cohort. *J Am Geriatr Soc*. Dec;59(12):2201-8
20. Barton ES, White DW, Cathelyn JS, Brett-McClellan KA, Engle M, Diamond MS, Miller VL, Virgin H. W. (2007) Herpesvirus latency confers symbiotic protection from bacterial infection. *Nature*. 447:326–329.
21. Marandu TF, Oduro JD, Borkner L, Dekhtiarenko I, Uhrlaub JL, Drabig A, Kröger A, Nikolich-Zugich J, Cicin-Sain L. (2015) Immune Protection against Virus Challenge in Aging Mice Is Not Affected by Latent Herpesviral Infections. *J Virol*. Nov;89(22):11715-7
22. Smithey MJ, Venturi V, Davenport MP, Buntzman AS, Vincent BG, Frelinger JA, Nikolich-Zugich J. (2018) Lifelong CMV infection improves immune defense in old mice by broadening the mobilized TCR repertoire against third-party infection. *Proc Natl Acad Sci U S A*. Jul 17;115(29):E6817-E6825
23. Furman D, Jovic V, Sharma S, Shen-Orr SS, Angel CJ, Onengut-Gumuscu S, Kidd BA, Maecker HT, Concannon P, Dekker CL, Thomas PG, Davis MM. (2015) Cytomegalovirus infection enhances the immune response to influenza. *Sci Transl Med*. Apr 1;7(281):281ra43.
24. Holder B, Miles DJ, Kaye S, Crozier S, Mohammed NI, Duah NO, Roberts E, Ojuola O, Palmero MS, Touray ES, Waight P, Cotten M, Rowland-Jones S, van der Sande M, Whittle H. (2010) Epstein-Barr virus but not cytomegalovirus is associated with reduced vaccine antibody responses in Gambian infants. *PLoS One*. Nov 17;5(11):e14013.
25. Miles DJ, Sanneh M, Holder B, Crozier S, Nyamweya S, Touray ES, Palmero MS, Zaman SM, Rowland-Jones S, van der Sande M, Whittle H. (2008) Cytomegalovirus infection induces T-cell differentiation without impairing antigen-specific responses in Gambian infants. *Immunology*. Jul;124(3):388-400.
26. Pera A, Campos C, Corona A, Sanchez-Correa B, Tarazona R, Larbi A, Solana R. (2014) CMV latent infection improves CD8+ T response to SEB due to expansion of polyfunctional CD57+ cells in young individuals. *PLoS one*. 9:e88538
27. Cadwell, K. The virome in host health and disease. (2015) *Immunity*.19;42(5):805-13.
28. Hosie L, Pachnio A, Zuo J, Pearce H, Riddell S, Moss P. (2017) Cytomegalovirus-Specific T Cells Restricted by HLA-Cw*0702 Increase Markedly with Age and Dominate the CD8+ T-Cell Repertoire in Older People. *Front Immunol*. Dec 11;8:1776.
29. Cudini J, Roy S, Houldcroft CJ, Bryant JM, Depledge DP, Tutill H, Veys P, Williams R, Worth AJJ, Tamuri AU, Goldstein RA, Breuer J. (2019) Human cytomegalovirus haplotype reconstruction reveals high diversity due to superinfection and evidence of within-host recombination. *Proc Natl Acad Sci U S A*. Feb 28.
30. Renzette N, Pokalyuk C, Gibson L, Bhattacharjee B, Schleiss MR, Hamprecht K, Yamamoto AY, Mussi-Pinhata MM, Britt WJ, Jensen JD, Kowalik TF. (2015) Limits and patterns of cytomegalovirus genomic diversity in humans. *Proc Natl Acad Sci U S A*. 2015 112(30):E4120-8.

31. Redeker A, Remmerswaal EBM, van der Gracht ETI, Welten SPM, Höllt T, Koning F, Cicin-Sain L, Nikolich-Žugich J, Ten Berge IJM, van Lier RAW, van Unen V, Arens R. (2018) The Contribution of Cytomegalovirus Infection to Immune Senescence Is Set by the Infectious Dose. *Front Immunol.* Jan 10;8:1953.
32. Böhm V, Simon CO, Podlech J, Seckert CK, Gendig D, Deegen P, Gillert-Marien D, Lemmermann NA, Holtappels R, Reddehase MJ. (2008). The immune evasion paradox: immunoevasins of murine cytomegalovirus enhance priming of CD8 T cells by preventing negative feedback regulation. *J Virol.* 82(23):11637-50
33. Verena Böhm, Christof K. Seckert, Christian O. Simon, Doris Thomas, Angélique Renzaho, Dorothea Gendig, Rafaela Holtappels, Matthias J. Reddehase. (2009). Immune Evasion Proteins Enhance Cytomegalovirus Latency in the Lungs *J Virol.* 83(19): 10293–10298.
34. Christopher M. Snyder, Kathy S. Cho, Elizabeth L. Bonnett, Jane E. Allan, Ann B. Hill. (2011). Sustained CD8+ T Cell Memory Inflation after Infection with a Single-Cycle Cytomegalovirus. *PLoS Pathog.* 7(10): e1002295
35. Joanne Trgovcich, Michelle Kincaid, Alicia Thomas, Marion Griesl, Peter Zimmerman, Varun Dwivedi, Valerie Bergdall, Paul Klenerman, Charles H. Cook. (2016). Cytomegalovirus Reinfections Stimulate CD8 T-Memory Inflation. *PLoS One.* 2016; 11(11): e0167097
36. Goldeck D, Larsen LA, Christiansen L, Christensen K, Hamprecht K, Pawelec G, Derhovanessian E. (2016) Genetic Influence on the Peripheral Blood CD4+ T-cell Differentiation Status in CMV Infection. *J Gerontol A Biol Sci Med Sci.* Dec;71(12):1537-1543
37. Scepanovic P, Alanio C, Hammer C, Hodel F, Bergstedt J, Patin E, Thorball CW, Chaturvedi N, Charbit B, Abel L, Quintana-Murci L, Duffy D, Albert ML, Fellay J; Milieu Intérieur Consortium. (2018) Human genetic variants and age are the strongest predictors of humoral immune responses to common pathogens and vaccines. (2018) *Genome Med.* Jul 27;10(1):59.
38. Misra MK, Mishra A, Pandey SK, Kapoor R, Sharma RK, Agrawal S. (2015) Genetic variation in Micro-RNA genes of host genome affects clinical manifestation of symptomatic Human Cytomegalovirus infection. *Hum Immunol.* Oct;76(10):765-9.
39. Studzińska M, Jabłońska A, Wiśniewska-Ligier M, Nowakowska D, Gaj Z, Leśnikowski ZJ, Woźniakowska-Gęsicka T, Wilczyński J, Paradowska E (2017) Association of TLR3 L412F Polymorphism with Cytomegalovirus Infection in Children. *PLoS ONE* 12 (1):e0169420
40. Jabłońska A, Paradowska E, Studzińska M, Suski P, Nowakowska D, Wiśniewska-Ligier M, Woźniakowska-Gęsicka T, Wilczyński J, Leśnikowski ZJ (2014) Relationship between toll-like receptor 2 Arg677Trp and Arg753Gln and toll-like receptor 4 Asp299Gly polymorphisms and cytomegalovirus infection. *International Journal of Infectious Diseases* 25:11-15
41. Mitsani D, Nguyen MH, Girnita DM, Spichy K, Kwak EJ, Silveira FP, Toyoda Y, Pilewski JM, Crespo M, Bhama JK, Abdel-Massih R, Zaldonis D, Zeevi A, Clancy CJ (2011) A polymorphism linked to elevated levels of interferon-gamma is associated with an increased risk of cytomegalovirus disease among Caucasian lung transplant recipients at a single center. *J Heart Lung Transplant* 30 (5):523-529. doi:10.1016/j.healun.2010.11.008
42. Wujcicka W, Paradowska E, Studzińska M, Wilczyński J, Nowakowska D (2017) Toll-like receptors genes polymorphisms and the occurrence of HCMV infection among pregnant women. *Virology Journal* 14 (1):64. doi:10.1186/s12985-017-0730-8
43. Hoffmann TW, Halimi J-M, Büchler M, Velge-Roussel F, Goudeau A, Al-Najjar A, Marliere J-F, Lebranchu Y, Baron C. (2010). Association between a polymorphism in the human programmed death-1 (PD-1) gene and cytomegalovirus infection after kidney transplantation. *Journal of Medical Genetics* 47 (1):54-58. doi:10.1136/jmg.2009.068841

44. Kijpittayarit S, Eid AJ, Brown RA, Paya CV, Razonable RR. (2007). Relationship between Toll-Like Receptor 2 Polymorphism and Cytomegalovirus Disease after Liver Transplantation. *Clin Infect Dis* 44 (10):1315-1320
45. Miles DJ, van der Sande M, Jeffries D, Kaye S, Ojuola O, Sanneh M, Cox M, Palmero MS, Touray ES, Waight P et al. (2008). Maintenance of large subpopulations of differentiated CD8 T cells two years after cytomegalovirus infection in Gambian infants. *PLoS One* 3(8):2905
46. Kaye S, Miles D, Antoine P, Burny W, Ojuola B, Kaye P, Rowland-Jones S, Whittle H, van der Sande M, Marchant A. (2008) Virological and immunological correlates of mother-to-child transmission of cytomegalovirus in The Gambia. *J Infect Dis* 197(9):1307-1314
47. Gompels UA, Larke N, Sanz-Ramos M, Bates M, Musonda K, Manno D, Siame J, Monze M, Filteau S. (2012) CIGNIS Study Group. Human cytomegalovirus infant infection adversely affects growth and development in maternally HIV-exposed and unexposed infants in Zambia. *Clin Infect Dis*. Feb 1;54(3):434-42
48. Bates M, Brantsaeter AB. (2016) Human cytomegalovirus (CMV) in Africa: a neglected but important pathogen. *J Virus Erad*. Jul 1;2(3):136-42.
49. Chidrawar S, Khan N, Wei W, McLarnon A, Smith N, Nayak L, Moss P. (2009) Cytomegalovirus-seropositivity has a profound influence on the magnitude of major lymphoid subsets within healthy individuals. *Clin Exp Immunol* 155(3):423-432.
50. Hassouneh F, Lopez-Sejas N, Campos C, Sanchez-Correa B, Tarazona R, Solana R, Pera A. (2017) Differential Effect of Cytomegalovirus Infection with Age on the Expression of CD57, CD300a, and CD161 on T-Cell Subpopulations. *Front Immunol*. Jun 2;8:649.
51. Weinberger B, Lazuardi L, Weiskirchner I, Keller M, Neuner C, Fischer KH, Neuman B, Wu" rzner R, Grubeck-Loebenstein B. (2007) Healthy aging and latent infection with CMV lead to distinct changes in CD8+ and CD4+ T-cell subsets in the elderly. *Hum Immunol* 68(2):86-90.
52. Gillespie GM, Wills MR, Appay V, O'Callaghan C, Murphy M, Smith N, Sissons P, Rowland-Jones S, Bell JI, Moss PA. (2000) Functional heterogeneity and high frequencies of cytomegalovirus-specific CD8(+) T lymphocytes in healthy seropositive donors. *J Virol* 74(17):8140-8150.
53. Klenerman P, Oxenius A. (2016) T cell responses to cytomegalovirus. *Nat Rev Immunol*. Jun;16(6):367-77.
54. Sylwester AW, Mitchell BL, Edgar JB, Taormina C, Pelte C, Ruchti F, Sleath PR, Grabstein KH, Hosken NA, Kern F et al.: (2005) Broadly targeted human cytomegalovirus-specific CD4+ and CD8+ T cells dominate the memory compartments of exposed subjects. *J Exp Med* 202(5):673-685
55. Vescovini R, Biasini C, Fagnoni FF, Telera AR, Zanlari L, Pedrazzoni M, Bucci L, Monti D, Medici MC, Chezzi C et al.: (2007) Massive load of functional effector CD4+ and CD8+ T cells against cytomegalovirus in very old subjects. *J Immunol* 179(6):4283-4291.
56. Stowe RP, Kozlova EV, Yetman DL, Walling DM, Goodwin JS, Glaser R. (2007) Chronic herpesvirus reactivation occurs in aging. *Exp Gerontol* 42(6):563-570.
57. Jackson SE, Sedikides GX, Okecha G, Poole EL, Sinclair JH, Wills MR. (2017) Latent Cytomegalovirus (CMV) Infection Does Not Detrimentally Alter T Cell Responses in the Healthy Old, But Increased Latent CMV Carriage Is Related to Expanded CMV-Specific T Cells. *Front Immunol*. Jun 26;8:733.
58. Wertheimer AM, Bennett MS, Park B, Uhrlaub JL, Martinez C, Pulko V, Currier NL, Nikolich-Žugich D, Kaye J, Nikolich-Žugich J. (2014) Aging and cytomegalovirus infection differentially and jointly affect distinct circulating T cell subsets in humans. *J Immunol*. Mar 1;192(5):2143-55.
59. Parry HM, Zuo J, Frumento G, Mirajkar N, Inman C, Edwards E, Griffiths M, Pratt G, Moss P. (2016) Cytomegalovirus viral load within blood increases markedly in healthy people over the age of 70 years. *Immun Ageing*. Jan 5;13:1.

60. Smith CJ, Turula H, Snyder CM (2014). Systemic hematogenous maintenance of memory inflation by MCMV infection. *PLoS Pathog.* Jul 3;10(7):e1004233.
61. Zalli A, Bosch JA, Goodyear O, Riddell N, McGettrick HM, Moss P, Wallace GR Targeting β 2 adrenergic receptors regulate human T cell function directly and indirectly. *Brain Behav Immun.* 2015 Mar;45:211-8. doi: 10.1016/j.bbi.2014.12.001. Epub 2014 Dec 16.
62. Trzonkowski P, Mysliwska J, Pawelec G, Mysliwski A. (2009) From bench to bedside and back: the SENIEUR Protocol and the efficacy of influenza vaccination in the elderly. *Biogerontology*, 10(1):83-94.
63. Trzonkowski P, Mysliwska J, Szmit E, Wieckiewicz J, Lukaszuk K, Brydak LB, Machala M, Mysliwski A. (2003) Association between cytomegalovirus infection, enhanced proinflammatory response and low level of antihemagglutinins during the anti-influenza vaccination--an impact of immunosenescence. *Vaccine*. 21:3826–3836.
64. Merani S, Pawelec G, Kuchel GA, McElhaney JE. (2017). Impact of Aging and Cytomegalovirus on Immunological Response to Influenza Vaccination and Infection. *Front Immunol.* Jul 17;8:784.
65. den Elzen WP, Vossen AC, Cools HJ, Westendorp RG, Kroes AC, Gussekloo J. (2011) Cytomegalovirus infection and responsiveness to influenza vaccination in elderly residents of long-term care facilities. *Vaccine*. Jun 24;29(29-30):4869-74.
66. Haq K, Fulop T, Tedder G, Gentleman B, Garneau H, Meneilly GS, Kleppinger A, Pawelec G, McElhaney JE. (2017) Cytomegalovirus Seropositivity Predicts a Decline in the T Cell But Not the Antibody Response to Influenza in Vaccinated Older Adults Independent of Type 2 Diabetes Status. *J Gerontol A Biol Sci Med Sci.* Sep 1;72(9):1163-1170.
67. van den Berg SPH, Wong A, Hendriks M, Jacobi RHJ, van Baarle D, van Beek J. (2018) Negative Effect of Age, but Not of Latent Cytomegalovirus Infection on the Antibody Response to a Novel Influenza Vaccine Strain in Healthy Adults. *Front Immunol.* Jan 29;9:82.
68. Simanek AM, Dowd JB, Pawelec G, Melzer D, Dutta A, Aiello AE. (2011) Seropositivity to cytomegalovirus, inflammation, all-cause and cardiovascular disease-related mortality in the United States. *PLoS One.* Feb 17;6(2):e16103
69. Strandberg TE, Pitkala KH, Tilvis RS. (2009) Cytomegalovirus antibody level and mortality among community-dwelling older adults with stable cardiovascular disease. *JAMA* 301: 380-2.
70. Muhlestein JB, Horne BD, Carlquist JF, Madsen TE, Bair TL, Pearson RR, Anderson JL. (2000) Cytomegalovirus seropositivity and C-reactive protein have independent and combined predictive value for mortality in patients with angiographically demonstrated coronary artery disease. *Circulation.* 102:1917–1923
71. Goulenok T, Boyd A, Larsen M, Fastenackels S, Boccara F, Meynard JL, Hadour N, Samri A, Desvarieux M, Autran B, Appay V, Girard PM, Sauce D; CHIC Study group. (2015) Increased carotid intima-media thickness is not associated with T-cell activation nor with cytomegalovirus in HIV-infected never-smoker patients. *AIDS.* Jan 28;29(3):287-93
72. Ji YN¹, An L, Zhan P, Chen XH. (2012) Cytomegalovirus infection and coronary heart disease risk: a meta-analysis *Mol Biol Rep.* Jun;39(6):6537-46
73. Nieto FJ, Adam E, Sorlie P, Farzadegan H, Melnick JL, Comstock GW, Szklo M. (1996) Cohort study of cytomegalovirus infection as a risk factor for carotid intimal-medial thickening, a measure of subclinical atherosclerosis. *Circulation.* 94:922–927
74. Wall NA, Chue CD, Edwards NC, Pankhurst T, Harper L, Steeds RP, Lauder S, Townend JN, Moss P, Ferro CJ. (2013) Cytomegalovirus seropositivity is associated with increased arterial stiffness in patients with chronic kidney disease. *PLoS One.* 8(2):e55686.

75. Yu HT, Youn JC, Kim JH, Seong YJ, Park SH, Kim HC, Lee WW, Park S, Shin EC. (2017) Arterial Stiffness Is Associated With Cytomegalovirus-Specific Senescent CD8+ T Cells. *J Am Heart Assoc.* Aug 28;6(9).
76. Yu HT, Park S, Shin EC, Lee WW. (2016) T cell senescence and cardiovascular diseases. *Clin Exp Med.* Aug;16(3):257-63.
77. Firth C, Harrison R, Ritchie S, Wardlaw J, Ferro CJ, Starr JM, Deary IJ, Moss P. (2016) Cytomegalovirus infection is associated with an increase in systolic blood pressure in older individuals. *QJM.* Sep;109(9):595-600.
78. Cheng J, Ke Q, Jin Z, Wang H, Kocher O, Morgan JP, Zhang J, Crumpacker CS. (2009) Cytomegalovirus infection causes an increase of arterial blood pressure. *PLoS Pathog* 5: e1000427.
79. Li S, Zhu J, Zhang W, Chen Y, Zhang K, Popescu LM, Ma X, Lau WB, Rong R, Yu X, Wang B, Li Y, Xiao C, Zhang M, Wang S, Yu L, Chen AF, Yang X, Cai J. (2011) Signature microRNA expression profile of essential hypertension and its novel link to human cytomegalovirus infection. *Circulation.* Jul 12;124(2):175-84.
80. Petrakopoulou P, Kübrich M, Pehlivanli S, Meiser B, Reichart B, von Scheidt W, Weis M. (2004) Cytomegalovirus infection in heart transplant recipients is associated with impaired endothelial function. *Circulation.* 110(11 Suppl 1):II207-12.
81. Pampou S, Gnedoy SN, Bystrevskaya VB, Smirnov VN, Chazov EI, Melnick JL, DeBakey ME. (2000) Cytomegalovirus genome and the immediate-early antigen in cells of different layers of human aorta. *Virchows Archiv : an international journal of pathology* 436: 539-52.
82. Pachnio A, Ciaurriz M, Begum J, Lal N, Zuo J, Beggs A, Moss P. (2016) Cytomegalovirus Infection Leads to Development of High Frequencies of Cytotoxic Virus-Specific CD4+ T Cells Targeted to Vascular Endothelium. *PLoS Pathog.* Sep 8;12(9):e1005832.
83. Bolovan-Fritts CA, Spector SA. (2008) Endothelial damage from cytomegalovirus-specific host immune response can be prevented by targeted disruption of fractalkine-CX3CR1 interaction. *Blood* 111: 175-82.
84. Reed RG, Greenberg RN, Segerstrom SC. (2017) Cytomegalovirus serostatus, inflammation, and antibody response to influenza vaccination in older adults: The moderating effect of beta blockade. *Brain Behav Immun.* Mar;61:14-20.
85. Beswick M, Pachnio A, Lauder SN, Sweet C, Moss PA. (2013) Antiviral therapy can reverse the development of immune senescence in elderly mice with latent cytomegalovirus infection. *J Virol.* Jan;87(2):779-89.
86. Dimitrios Chanouzas, Michael Sagmeister, Lovesh Dyal, Phoebe Sharp, Lucy Powley, Serena Johal, Jessica Bowen, Peter Nightingale, Charles J. Ferro, Matthew D. Morgan, Paul Moss, Lorraine Harper (2018) The host cellular immune response to cytomegalovirus targets the endothelium and is associated with increased arterial stiffness in ANCA-associated vasculitis *Arthritis Res Ther.* 20: 194.
87. Dimitrios Chanouzas, Michael Sagmeister, Sian Faustini, Peter Nightingale, Alex Richter, Charles J Ferro, Matthew David Morgan, Paul Moss, Lorraine Harper. (2019) Subclinical Reactivation of Cytomegalovirus Drives CD4⁺CD28^{null} T-Cell Expansion and Impaired Immune Response to Pneumococcal Vaccination in Antineutrophil Cytoplasmic Antibody–Associated Vasculitis *J Infect Dis.* Jan 15; 219(2): 234–244.

Figure Legends

Figure 1 *Homo sapiens* comprises a rich ecosystem and symbiotic relationships with the microbiome and virome have an important influence on homeostasis

Figure 2 CMV infection may potentially have a beneficial or detrimental influence on immune function at different stages of life. CMV infection is usually acquired early in life and in childhood and young adults appears to boost immune function. In certain settings, infection can lead to tissue damage and impaired immune function in older people

Figure 3 The cytotoxic activity of CMV-specific T cells within blood increases during ageing. The figure shows the percentage expression of granzyme B and perforin expression within CMV pp65-specific CD4+ T cells in people at different ages

Figure 4 Cytomegalovirus alters the proportion of naïve and memory/effector T cells in blood. The older model of gradual inflation (left) has been largely replaced by a more immediate and sustained change which may then increase further in older age (right)

Figure 5 The concept of the large number of CMV-specific cytotoxic cells within blood as mediating 'immune surveillance' of the vascular system. CMV can infect endothelial cells and replication is triggered by 'stress'. This is countered by large numbers of immune cells maintained within the vascular system which act to rapidly suppress replication.

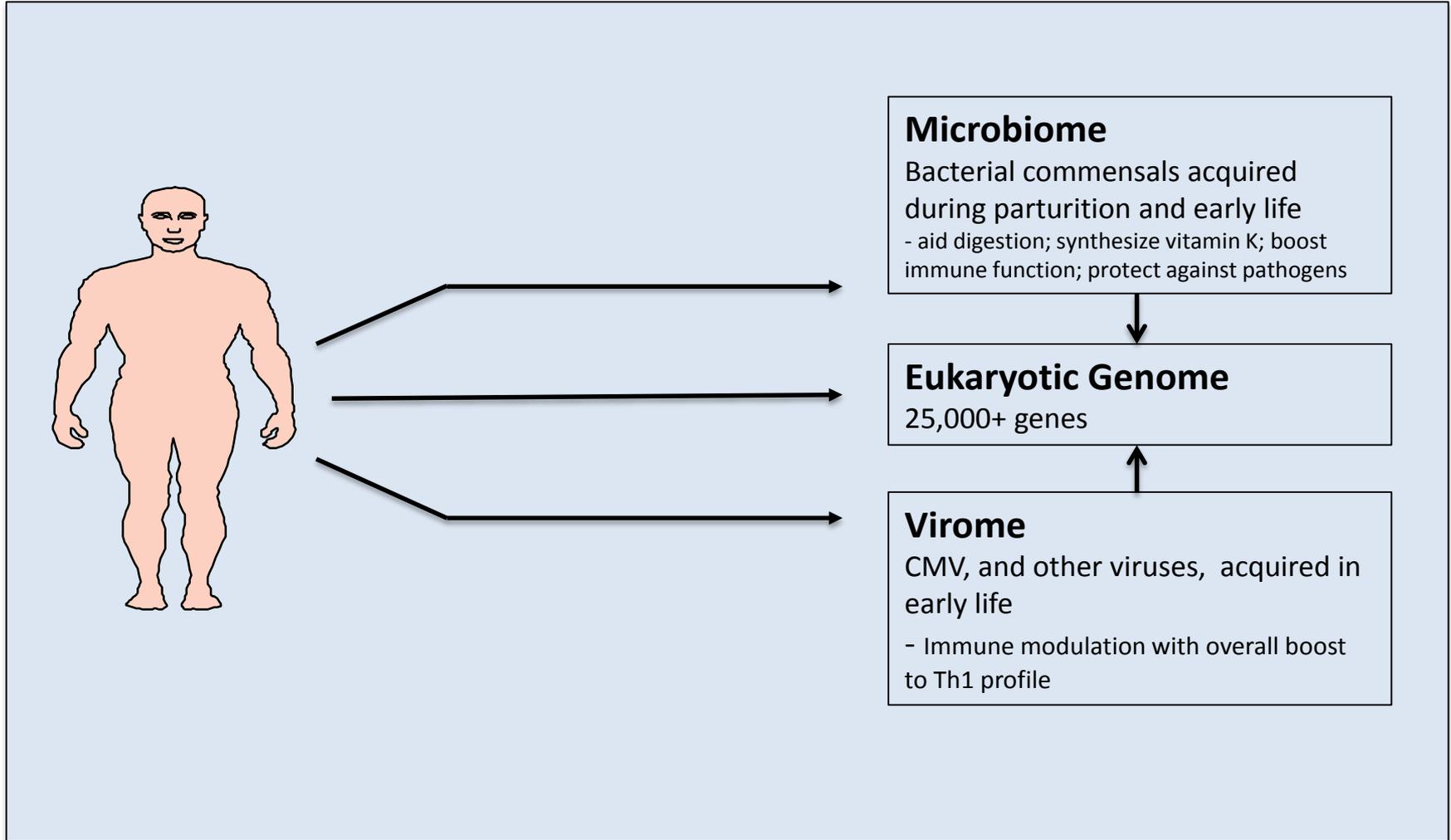


Figure 1

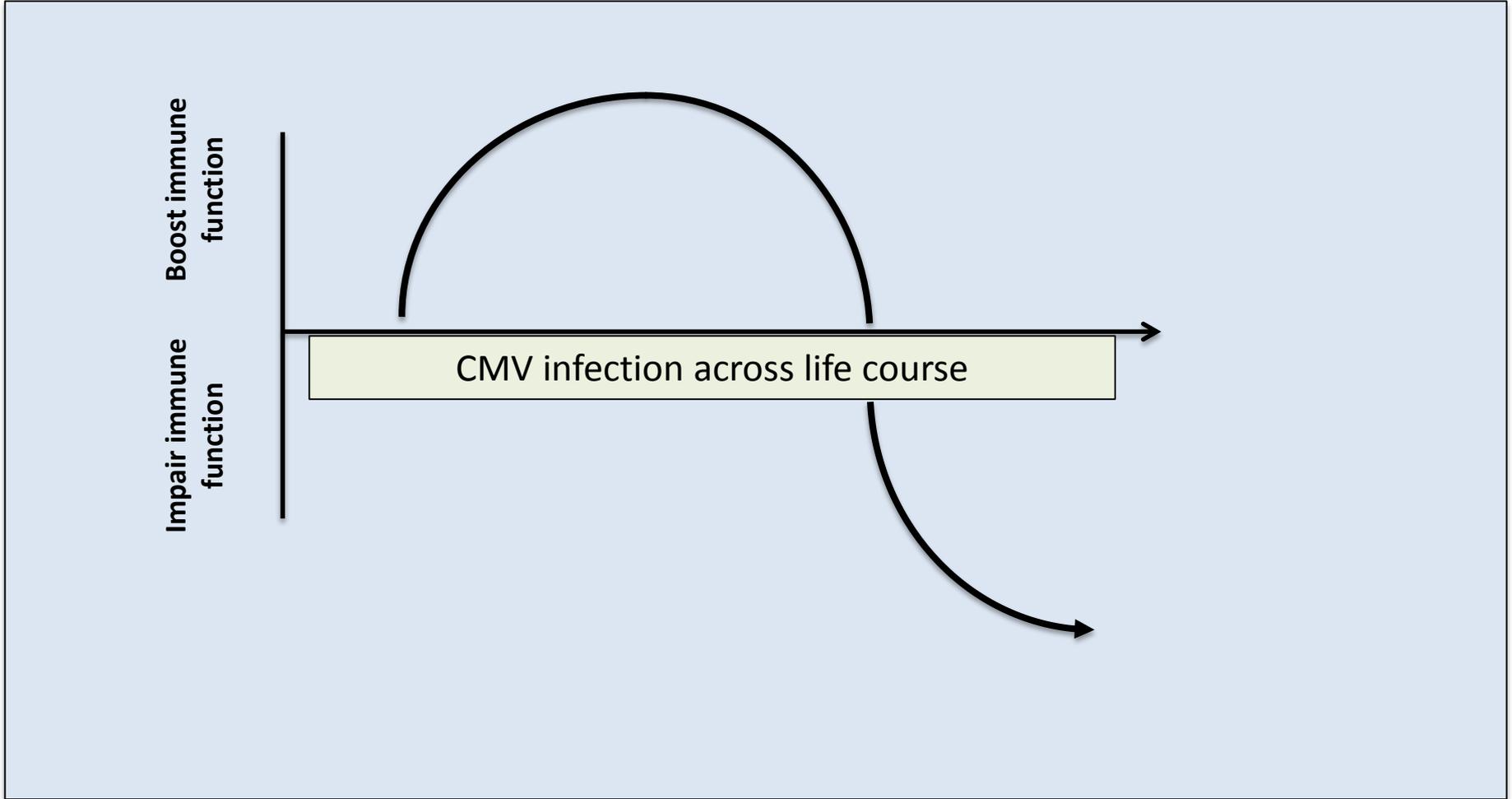


Figure 2

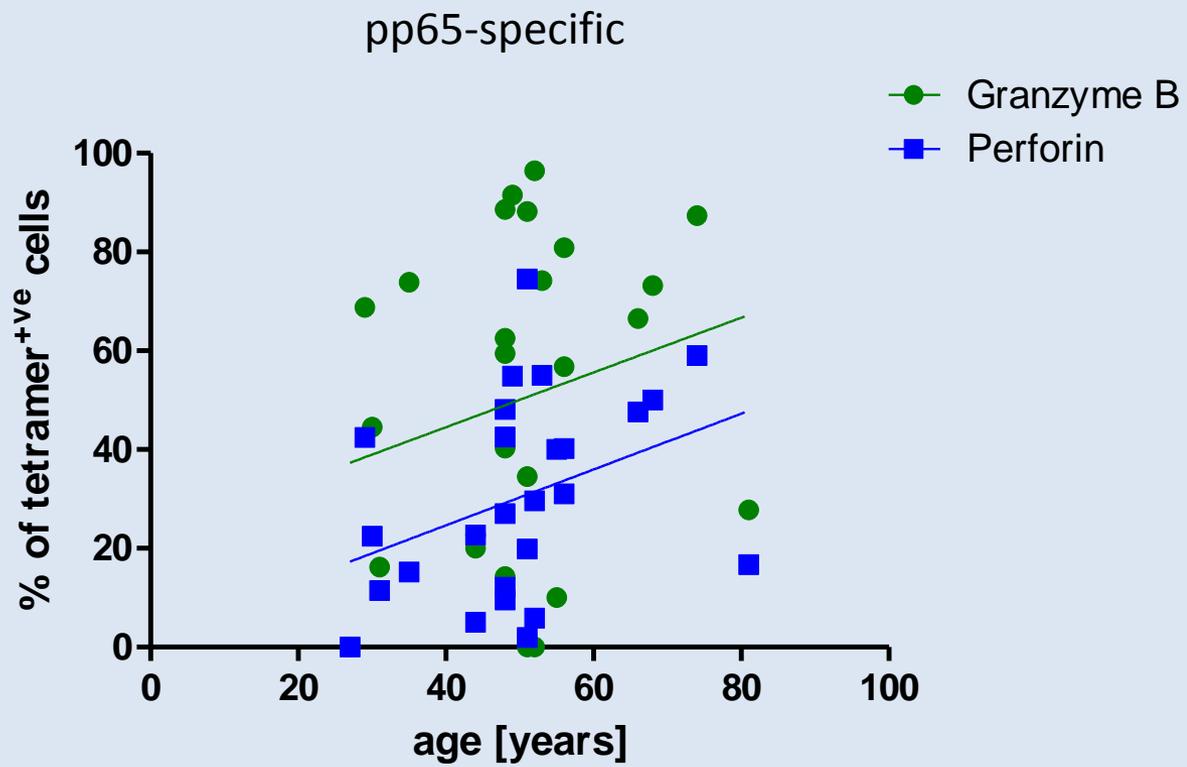


Figure 3

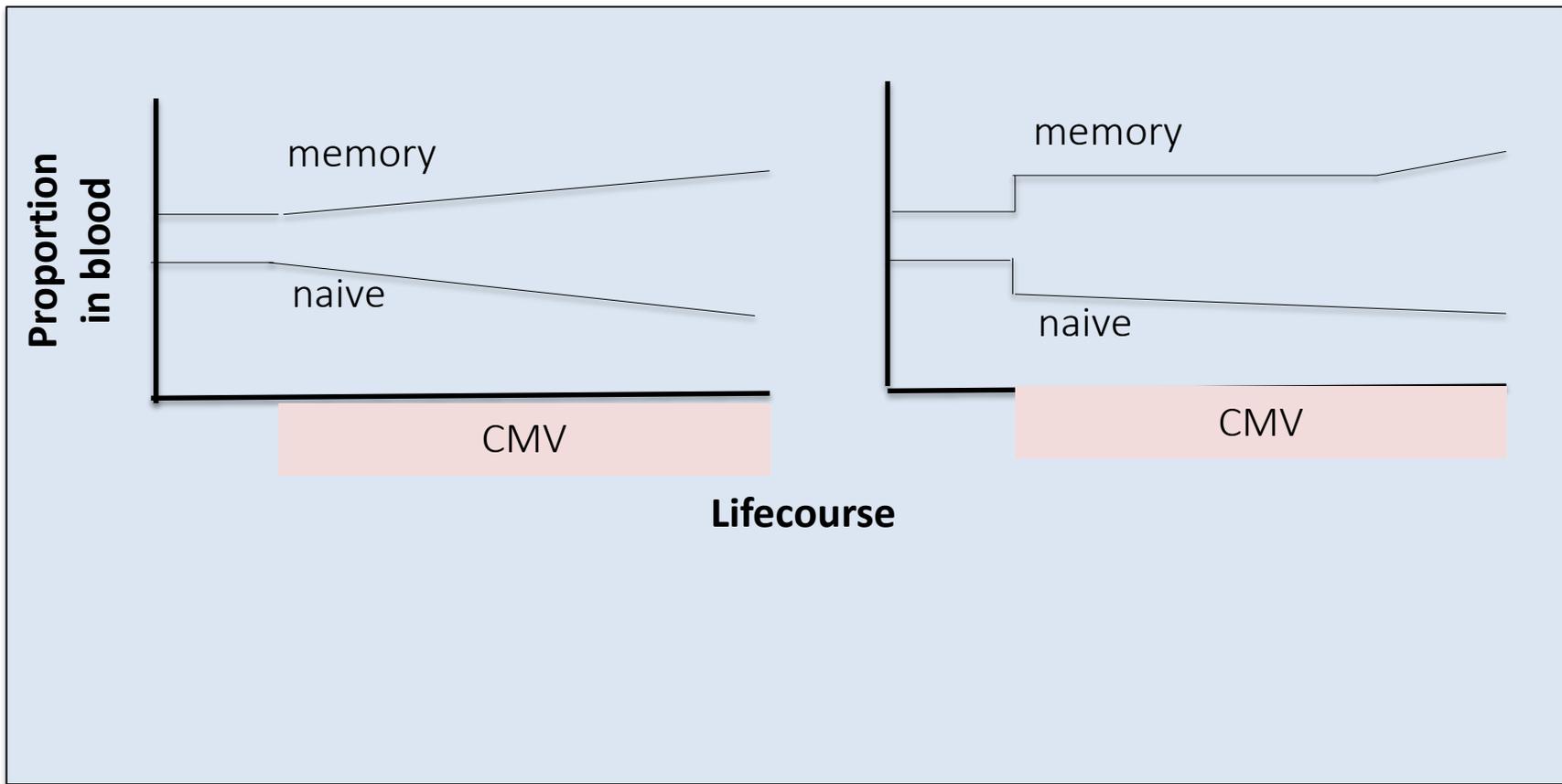


Figure 4

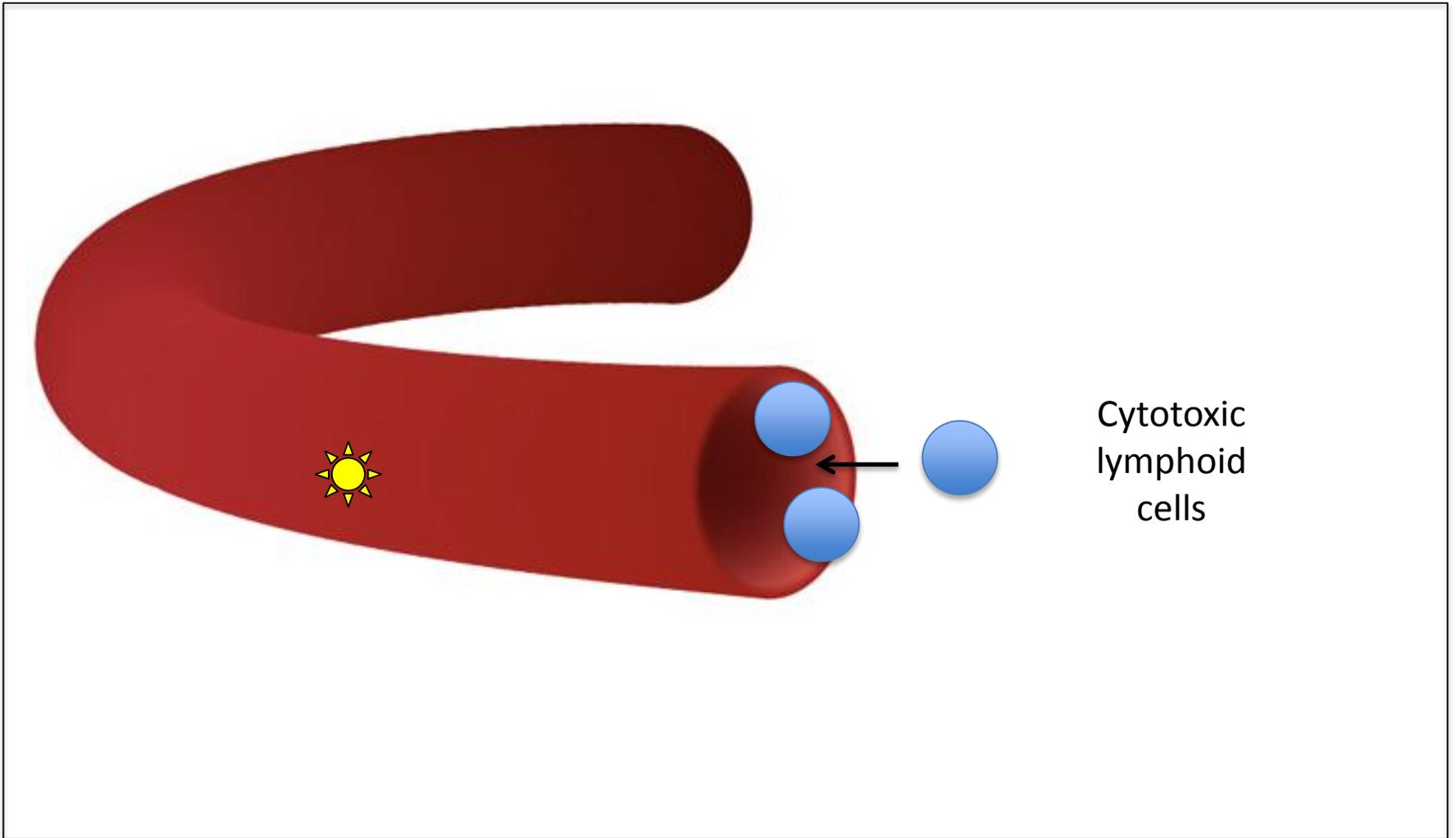


Figure 5