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Moss, Paul

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

Paul Moss
University of Birmingham, UK.

Professor of Haematology,
University of Birmingham
Birmingham
UK
B15 2TA

p.moss@bham.ac.uk

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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 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

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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

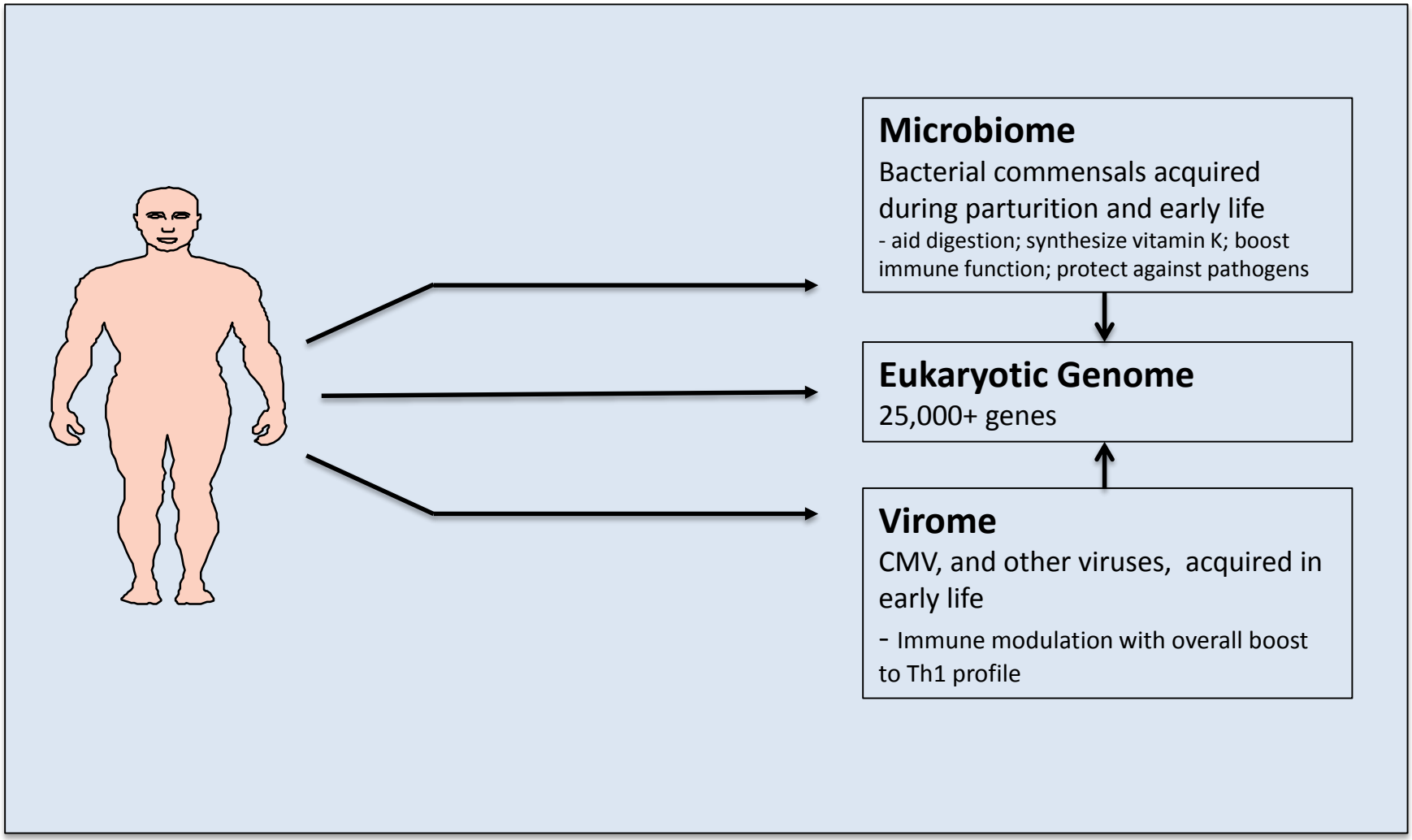

Figure 1

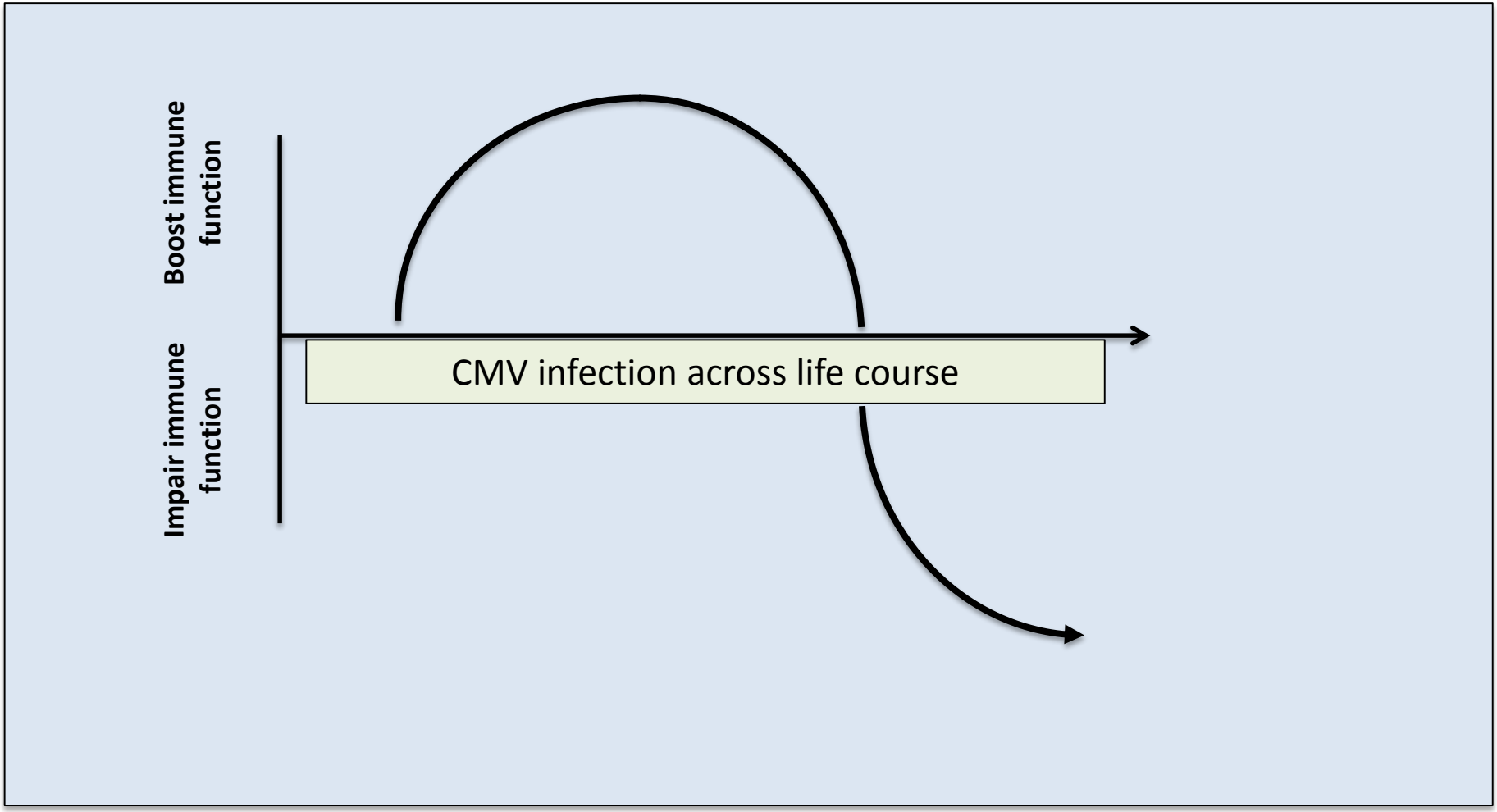
Boost immune function
Impair immune function
CMV infection across life course

Figure 2

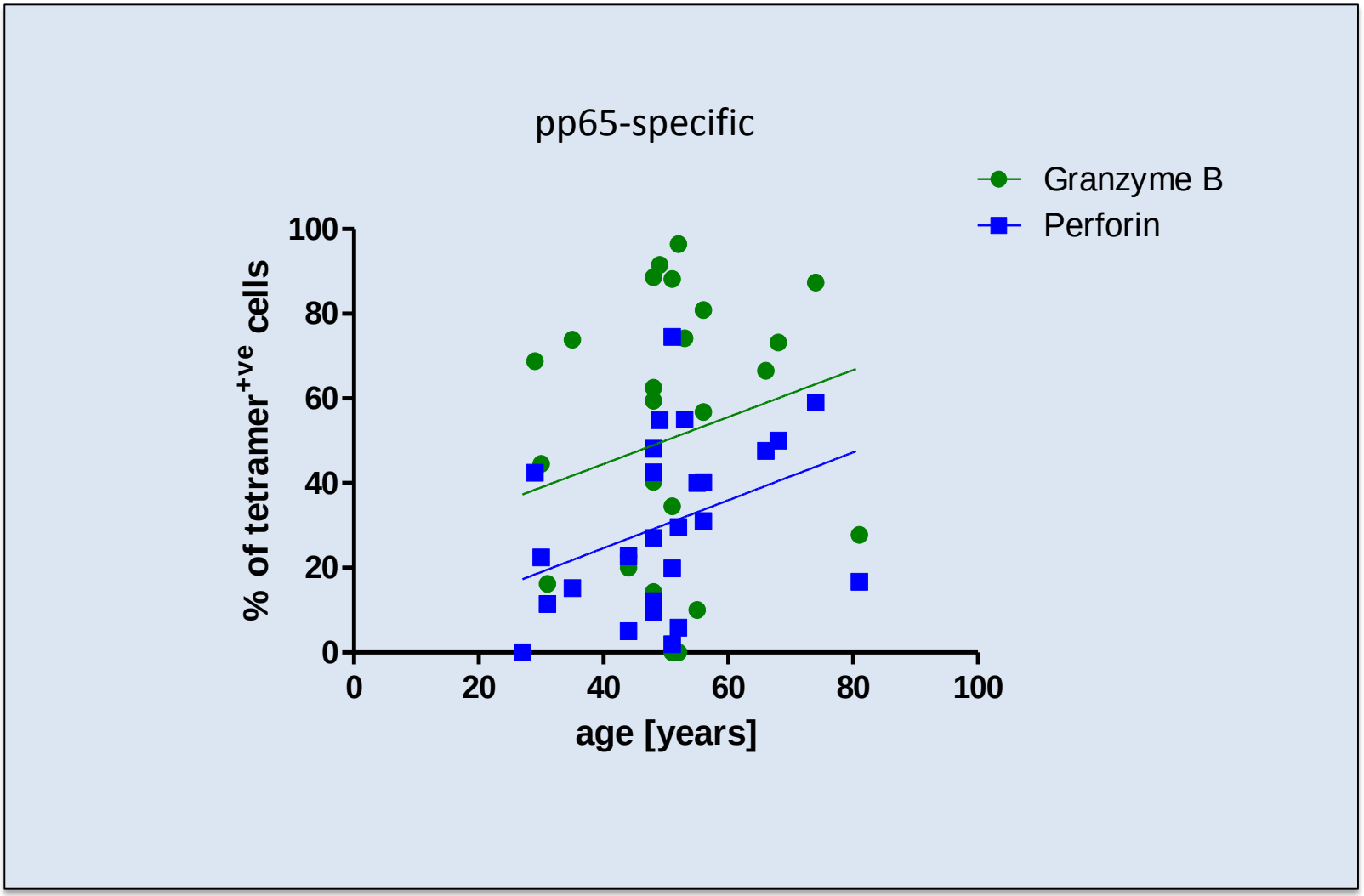
pp65-specific
Granzyme B
Perforin
% of tetramer+ve cells
100
80
60
40
20
0
0
20
40
60
80
100
age [years]

Figure 3

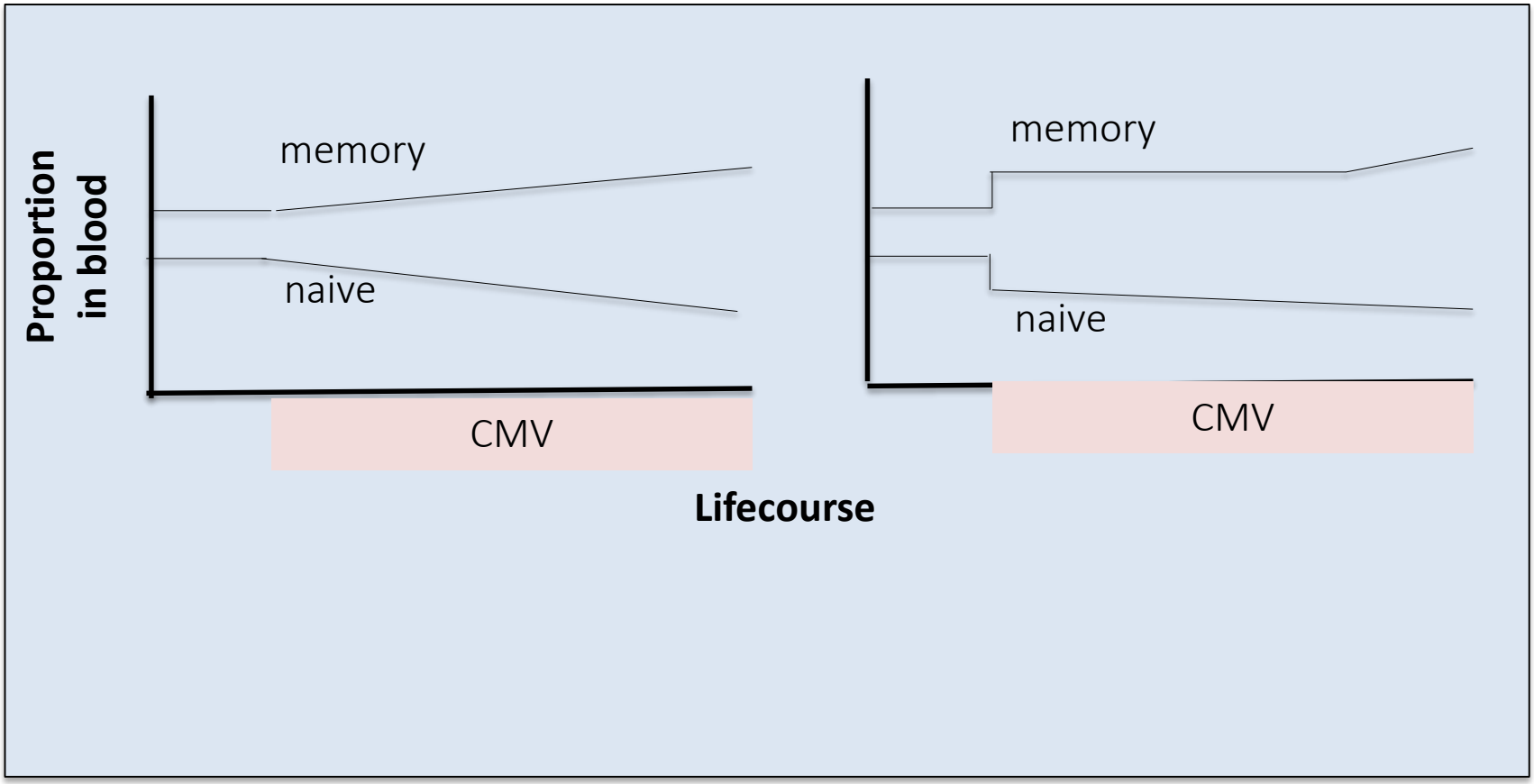
Proportion in blood
memory
naive
CMV
memory
naive
CMV
Lifecourse

Figure 4

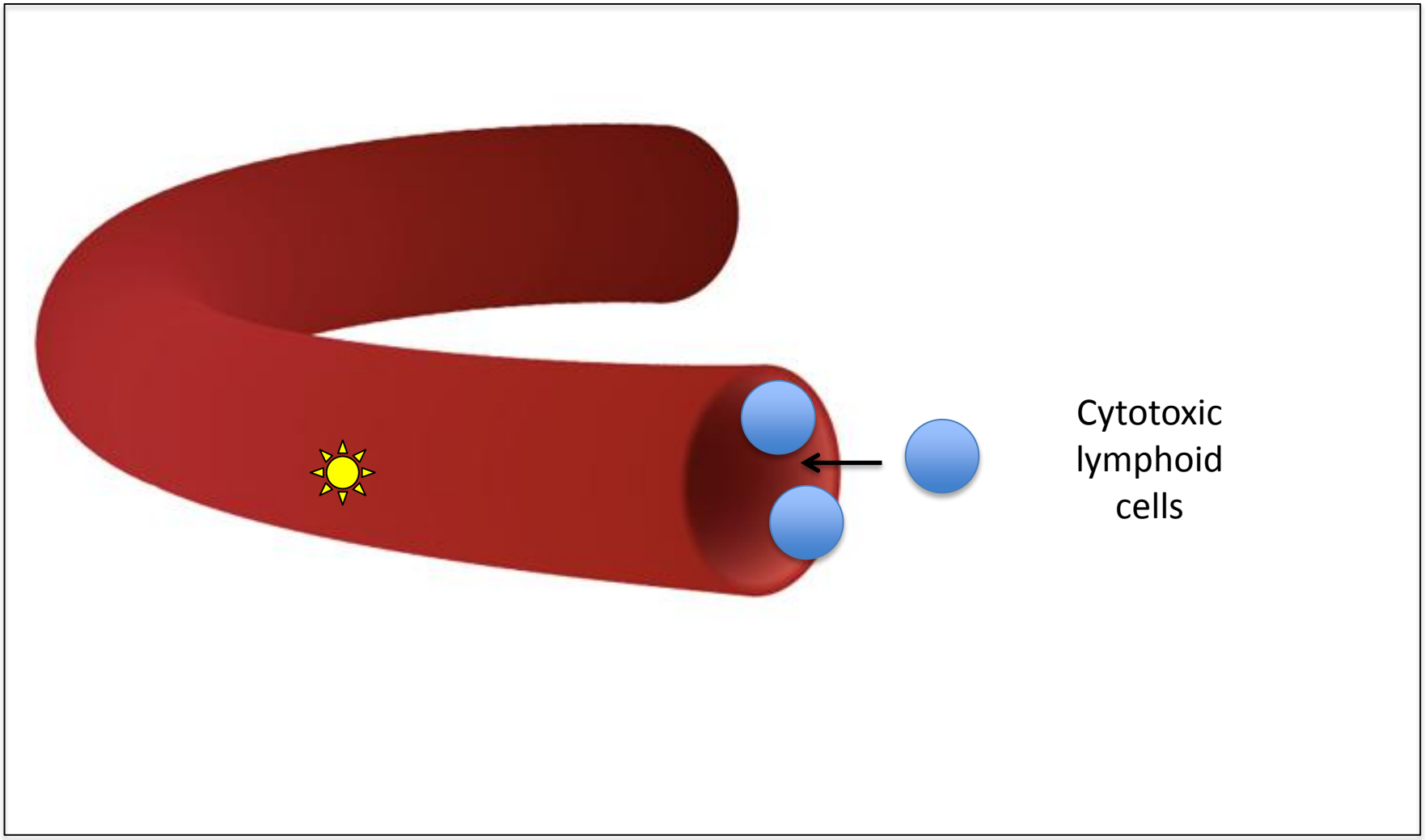

Figure 5