Human cytomegalovirus infection and the immune response to exercise

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ABSTRACT

Human cytomegalovirus (HCMV) is a ubiquitous β-herpes virus that has co-evolved with its host since the very beginning of human life. The vast majority of adults worldwide carry the virus in a latent state, which has striking effects on the composition and function of both T-cells and NK-cells. While there is evidence to suggest that prior exposure to HCMV can have beneficial effects in the immune competent host, poor control of the virus may contribute to T-cell exhaustion and the early onset of immunosenescence. The interaction between HCMV and exercise has garnered a lot of recent research attention. This stemmed from observations that people with HCMV redeploy greater numbers of CD8+ T-cells in response to a single exercise bout, while NK-cell mobilization is, conversely, impaired. Moreover, athletes with latent HCMV infection may be better protected against symptoms of upper respiratory illness (URI), and it has been suggested that the host's ability to control HCMV (i.e. keeping HCMV in a latent state) may connect apparent bidirectional effects of exercise volume on host immunity and infection risk. This work has set a new paradigm that immune responses to both acute and chronic exercise might be governed by the infection history of the host. In this review, we summarize current knowledge on the effects of HCMV infection on T-cells and NK-cells and synthesize the literature on HCMV and the immune response to both single exercise bouts and prolonged periods of exercise training. We also discuss potential clinical and practical applications of this work including the use of HCMV reactivation as a biomarker of immune depression in athletes, its relevance in immunosenescence and the associated immune risk profile, and the potential for exercise to augment vaccine responses and the manufacture of immune cells for adoptive transfer immunotherapy. Although research in this area is still in its infancy, we conclude that host infection history and the ability to regulate dormant pathogens is likely to play a key role in our understanding of how the immune system responds to both acute and chronic exercise across the entire exercise volume continuum.

Keywords: Immunosenescence, T-cell, NK-cell, acute stress response; vaccination; adoptive transfer immunotherapy; athletes

Frequently Used Abbreviations
AML: acute myeloid leukemia
β_2-AR: β_2-adrenergic receptor
CCR: chemokine receptor
CD: cluster of differentiation
CM: central memory
EBV: Epstein-Barr virus
EM: effector memory
EMRA: CD45RA+ effector memory
HCMV: human cytomegalovirus
HLA: human leukocyte antigen
HNP: human neutrophil protein
HSCT: hematopoietic stem cell transplantation
HSV: herpes simplex virus
IE-1: immediate early antigen 1
Ig: Immunoglubulin
KIR: killer inhibitor receptor
KLRG1: killer-lectin like receptor G1
MHC: major histocompatibility complex
NKCA: natural Killer cell cytotoxic Activity
NKG: natural killer group
PD: programed death
pp65: phosphoprotein 65
TCR: T-cell receptor
URI: upper respiratory illness

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INTRODUCTION

Human cytomegalovirus (HCMV), also known as human her-pes-virus 5 (HHV-5), is a ubiquitous β-herpes virus that infects 40-70% of the adult population in the United States (10) and 45-100% of adults worldwide depending on age, ethnicity and geographical location (30). The virus is mostly transmitted through direct contact with bodily fluids such as saliva, urine or breast milk. It can also be contracted through sexual intercourse, organ transplantation and blood transfusions. Primary infection usually occurs asymptomatically before the virus establishes latency and persists for the lifetime of the host. Compared to other herpesviruses, HCMV has a very large and divergent genome which allows it to evade immune detection (152) and appears to persist in monocytes and CD34+ myeloid progenitor cells (129). While often dismissed as an innocuous infection in otherwise healthy people, HCMV is a major cause of morbidity and mortality in immunocompromised patients and is one of the leading causes of death after solid organ and hematopoietic stem cell transplantation (HSCT) (55, 129). The virus is also capable of periodic reactivation causing large-scale expansions of cytotoxic T-cells and NK-cells that seem to linger long after the infection has been curtailed. Consequently, and regardless of age, people with a latent HCMV infection have substantially increased numbers and proportions of CD8+ (and to some extent CD4+) T-cells with a highly differentiated phenotype; and more recently, it has been shown that latent HCMV infection markedly increases the proportion of NK-cells expressing the activating receptor, NKG2C (82). HCMV has also been implicated in *immunosenescence,* the term used to describe the progressive demise of the immune system that is associated with aging, and has been linked with weakened vaccine responses in both young and older subjects (85, 140).

The effects of HCMV on the phenotype and functional properties of T-cells and NK-cells has featured heavily in mainstream immunology literature over the last two decades. This is largely due to the striking ability of HCMV to alter the composition of T-cell and NK-cell subsets in peripheral blood, its propensity for reactivation due to physical and psychological stress, and its implied role as a 'driver' of immunosenescence (64). However, the interaction between HCMV and exercise has only recently been investigated (123). This interest stemmed from two sources: firstly, the observation that people with HCMV redeploy greater numbers of CD8+ T-cells in response to a single exercise bout, due mostly to the ability of HCMV to expand 'exercise-responsive' subsets of memory T-cells (139); and secondly, because regular exercise is associated with better immune responses in the elderly, it has been suggested that exercise interventions aimed at improving immunity may act on HCMV-dependent pathways of immunosenescence (127, 128). Thus, the impact of HCMV on the immune system has set a new paradigm that the immune response to both acute and chronic exercise may largely depend on the infection history of the host. In this review, we summarize current knowledge on the effects of HCMV infection on T-cells and NK-cells and synthesize the literature on HCMV and the immune response to both acute and chronic exercise. Finally, we discuss potential clinical and practical ramifications for this work in the context of immunotherapy, biomarker profiling of athletes, vaccination and *immunosenescence.*

HCMV and T-cells

Primary HCMV infection is characterized by an intense viral replication and a profound T-cell response that may last for several months (5), with both CD8+ cytotoxic and CD4+ helper T-cells playing central roles in the resolution of acute primary infection and the maintenance of long-term memory during viral persistence. HCMV-specific cytotoxicity is predominantly performed by CD8+ T-cells, although HCMV-specific CD4+ T-cells also have the ability to lyse infected target cells as well as maintain the upkeep of the CD8+ T-cell population (7, 135). It is clear that HCMV has a colossal impact on the memory T-cell pool, with ~10% of all memory CD4+ and CD8+ T-cells in blood being specific to HCMV proteins in individuals with even a latent infection (135). The proportion of the peripheral T-cell compartment devoted to the control of HCMV is highly variable, reported to range from <1% to over 40% in HCMV seropositive individuals (135, 145). The high variability is likely due to a number of factors including the size of the viral inoculum, the length of time the infection has been carried, and the immunocompetence of the host while infected (8).

Adequate HCMV control requires large numbers of differentiated T-cells with an effector memory (EM; $CD45RA^{neg}/CD62L^{neg}/CCR7^{neg}$) or effector memory RA^{pos} (EMRA; $CD45RA^{pos}/CD62L^{neg}/CCR7^{neg}$) phenotype, with the proportions of these cell subsets within both CD8+ and CD4+ blood T-cells remaining elevated long after primary infection. Increased proportions of EM and EMRA subsets among CD8+ T-cells have long been considered a hallmark of aging, although it is not uncommon for children or young adults infected with HCMV to have EM and EMRA proportions that are comparable or even greater than non-infected adults 25-50 years their senior (130, 131). While cells with this phenotype were long considered to be 'terminally-differentiated' or 'senescent', this does not apply, at least to a portion of HCMV-specific T-cells that are still able to secrete cytokines, proliferate in response to HCMV peptide stimulation, kill HCMV-infected target cells, and control viral dissemination *in vitro* (62, 143, 159). Moreover, even after massive clonal expansion, many EMRA cells can revert from a CD45RA to a CD45RO phenotype and re-express CCR7 (143), which challenges previous models of T-cell memory that surmise a unidirectional linear differentiation pathway (6, 115) and fail to accurately reflect the inherent plasticity of memory T-cell responses (129).

While many EMRA cells are specific for antigenic epitopes in the pp65 and IE-1 HCMV proteins, T-cells specific to other HCMV antigens including US3, pp71, UL28 and IE-2 are also mostly comprised of EMRA cells, indicating that HCMV-specific T-cells have a similar composition of T-cell subsets regardless of their antigen specificity (62). Moreover, it appears that HCMV-specific T-cell responses are highly varied among individuals, with some people mounting a more diverse response and others a more restricted and focused response to fewer HCMV proteins and antigenic epitopes (62). While the antigenic diversity of HCMV-specific T-cells

appears unrelated to age, older individuals tend to have a greater number of cells responding to HCMV proteins, and both the magnitude and diversity of these responses are stable over time (at least up to 2-years) (62). Nevertheless, it is still likely that HCMV, particularly if improperly controlled as a result of chronic immune depression or dysregulation, can drive T-cells to clonal and functional exhaustion (44). Telomere length is considered a robust measure of T-cell clonal capacity, with shorter telomeres indicative of excess T-cell proliferation and the impending end of the clonal lifespan (replicative senescence). While HCMV does not appear to impact T-cell telomere length in the young, average telomere lengths among isolated CD8+ T-cells were found to be shorter in HCMV-infected older individuals compared to their age-matched non-infected counterparts (142), indicating that HCMV may drive replicative senescence in some individuals. Riddell et al. measured telomere length in HCMV (NLV)-specific CD8+ T-cells contrasted by age and surface phenotype (CD45RA/CD27 combinations) and found that the NLV-specific cells from older subjects had shorter telomeres than the young across all surface phenotypes (111). Interestingly, they also reported that telomere length was shortest in the CD45RA+/CD27+ (normally considered to be a 'naïve' or 'low differentiated' phenotype) CD8+ subset, indicating that these cells had undergone excessive rounds of cell division *in vivo* and are certainly not naïve cells (111). Truly 'exhausted' T-cells that fail to undergo further proliferation or secrete cytokines in response to stimulation are likely to be phenotypically identical to those fully functional viral-specific cells displaying surface markers of high-differentiation (i.e. KLRG1, CD57). Thus, a simple identification of T-cell subset distribution through surface markers is insufficient to assess immunocompetence, but is still likely to provide a representative footprint of the host's infection history. At least in healthy individuals, aging (>65 yrs) does not appear to be associated with exhausted HCMV-specific CD8+ T-cells (121), and increased proportions of truly 'exhausted' or 'senescent' HCMV-specific T-cells might only manifest in the very old following a lifetime of poor HCMV control (96), after primary infection (5), or perhaps following a period of intense HCMV reactivation. Although apparently exhausted HCMV-specific T-cells have been found to express PD-1, blocking the receptor can restore their pro-inflammatory cytokine profile and antigen-specific proliferative responses suggesting that PD-1 associated exhaustion is reversible (5, 40). Nevertheless, in contrast to HIV-specific CD8+ T-cells, PD-1 expression is very low, or even absent, on HCMV-specific CD8+ T-cells (103, 138), indicating that HCMV does not induce clonal exhaustion in most immunocompetent people.

Recently, more attention has been paid to the impact of HCMV on the frequency and function of γδ T cells, which predominantly reside in the gut mucosa. Unlike their αβ T-cell counterparts, γδ T cells are present in the blood in relatively small numbers and, despite expressing the pan T-cell marker CD3, are mostly negative for both CD4 and CD8 (105). The long-term persistence of the Vδ2neg cell population in peripheral blood has become a hallmark feature of HCMV infection (105) and, although these are not HCMV-specific cells by strict definition, they are still capable of killing HCMV-infected fibroblasts through upregulation of endothelial protein C receptor (EPCR) and ICAM-1 on the stressed target cells (158). Aging is usually associated with a marked reduction in the frequency of γδ T cells in peripheral blood regardless of whether or not the host is carrying a latent HCMV infection (104).

HCMV and NK-cells

NK-cells express a range of inhibitory and activating surface receptors that tightly regulate their cytotoxic functions. These include killer-cell immunoglobulin-like receptors (KIR) that, despite having much less polymorphism than the TCR, are able to deliver inhibitory and/or activating signals to the NK-cell via human leukocyte antigens (HLA) expressed on healthy host cells and transformed/target cells (70). NK-cells play a crucial role in curtailing HCMV and other viral infections in immunocompetent individuals through expression of a series of activating receptors (e.g. NKG2D, DNAM-1, and NKp46) that allow them to recognize and eliminate HCMV and other virus-infected cells (33, 83). This immune selection pressure has resulted in HCMV acquiring many immune evasive strategies to avoid detection by host NK-cells (69, 81). For instance, HCMV can control the expression of several genes that code for ligands of NK-cell inhibitory and activating receptors in a manner that avoids detection and elimination (13, 83, 153). The virus has also been shown to induce expression of HLA homologues, which ligate with NK-cell inhibitory receptors to prevent destruction of HCMV-infected cells (12, 108). NK-cells have also acquired strategies of their own to override the immune evasive properties of HCMV. A striking example of this is the HLA-E-dependent expansion of NKG2Cpos/NKG2Aneg NK-cells (112). Both receptors ligate with the (non-classic) class 1b MHC molecule HLA-E (141) that is upregulated in HCMV-infected cells (137), tumor cell lines of major lymphoid and nonlymphoid lineages (80), and primary acute myeloid leukemia and multiple myeloma cells (94, 155). Signaling through the inhibitory receptor NKG2A is dominant, thus only NKG2Cpos/NKG2Aneg NK-cells are able to effectively lyse HLA-E-expressing target cells (71) . These NKG2Cpos/NKG2Aneg NK-cells are often referred to as "memory NK-cells", because their frequency remains elevated after resolution of HCMV viremia (15, 82) and NK-cells expressing Ly49H (the mouse equivalent of NKG2C) have been shown to mount apparent recall responses to HCMV (134). Whether or not these are truly 'memory' responses is subject to debate as these mechanisms are not nearly as precise as TCR/MHC/peptide interactions (136). However, it is clear that HCMV leaves a stable 'imprint' on the NK-cell KIR repertoire that allows the host to maintain long lasting HCMV control in a manner that goes well beyond typical features of innate immunity (129, 136). The clonal-like proliferation of NKG2Cpos/NKG2Aneg NK-cells in those with HCMV results in high expression of the putative terminal differentiation marker CD57 and chronic skewing of the KIR repertoire, as only licensed (i.e. cells expressing inhibitory KIR for self-HLA molecules) NK-cells proliferate (15, 32, 46). The preferential expansion of NKG2Cpos NK-cells observed with HCMV infection is unique amongst the Herpesvirus family and has not been reported in response to any other viruses (22, 27, 102).

NKG2Cpos NK-cells are not merely an artifact of HCMV infection as they also serve a protective function. For exam-

ple, a higher percentage of NKG2C^{pos} NK-cells is associated with a lower risk of acute HCMV infection in patients undergoing solid organ transplantation or HSCT (54, 63). Further, NKG2C^{pos} NK-cells taken from HCMV-infected donors show enhanced expansion and function in response to HCMV reactivation in HSCT recipients when compared to NKG2C^{pos} NK-cells derived from HCMV[neg] donors (45, 54). Thus, it seems clear that NKG2C^{pos} NK-cells play a critical role in the suppression of acute HCMV infection and achievement of long-term viral control. The functional benefits of NKG2C^{pos} NK-cells, however, go beyond their ability to contain HCMV. For example, NKG2C^{pos} NK-cells with high cytotoxicity have been observed to expand in response to active Hantavirus, Chikungunya, HIV, and Hepatitis B infections, but only in individuals previously infected with HCMV (22, 27, 102). Thus, it is suggested that HCMV infection "primes" NKG2C+ NK-cells to respond to other active viral infections, some of which are also associated with upregulation of HLA-E in infected cells (22). Beyond viral immunity, we have shown in healthy subjects that latent HCMV infection is associated with increased NK-cell cytotoxic activity (NKCA) against multiple myeloma, leukemia, and lymphoma cell lines expressing HLA-E (18), and HCMV reactivation during allogeneic HSCT has been associated with strikingly lower occurrences of relapse in acute myeloid leukemia (AML) patients (9% in patients with HCMV reactivation vs. 42% in those without) (11, 43). This is due, in part, to the HCMV-induced expansion of NKG2C^{pos}/NKG2A^{neg} NK-cells (46) which are able to effectively lyse HLA-E[pos] targets, including AML blasts (94) and several other 'liquid' cancers (80). However, it has also been reported that HCMV associated NKG2C^{pos} NK-cell expansions may be involved in the development of *de novo* head/neck and colorectal cancers in liver transplant patients (1), indicating that HCMV-induced expansion of highly cytotoxic NK-cell subsets could be a double edged sword that needs to be tightly controlled.

HCMV and Immunosenescence

For well over a decade, HCMV infection was purported to 'drive' immunosenescence (98, 99). This was largely due to the finding that HCMV serostatus predicted mortality over 2, 4 and 6 years in Swedish octogenarian and nonagenarian subjects (100). The impact of HCMV was an accumulation of the so-called senescent T-cells, an excess numbers of HCMV-specific T-cell clones, an inverted CD4:CD8 T-cell ratio, and a lower proportion of naïve cells. These parameters formed the 'immune risk profile', which predicted mortality and morbidity in several cohorts of very old subjects (157). Excess HCMV-specific T-cell clones were considered to signify restricted T-cell diversity and a polarization of the memory T-cell response to a single virus, whereas low numbers and proportions of naïve cells were thought to compromise immune responses to novel infectious agents and vaccine antigens. In this regard, HCMV was believed to take up a large portion of the 'immunological space' required by the host to mount efficient memory responses to novel infectious and vaccine antigens (28). However, it has since been established that HCMV actually has very little impact on the naïve T-cell compartment, but rather increases the pool of memory T-cells with a differentiated phenotype without affecting naïve T-cell numbers (87, 144, 146, 154). Thus, at least in younger, healthy

individuals, there is little evidence that the so-called immunological space is fixed, particularly if thymic output and homeostatic proliferation remains functional and continues to maintain the diversity of the peripheral T-cell pool.

In contrast to the Swedish octogenarian and nonagenarian studies, a recent study completed in an elderly Dutch population found that lower frequencies of naïve and higher frequencies of late differentiated cells among the total peripheral CD8+ T-cell pool was actually associated with *increased* survival at 7-years follow up (39). Moreover, while weaker immune responses to vaccines (i.e. influenza vaccine) have been attributed to HCMV serostatus (85, 140), this has not been consistently reported (36, 149). Indeed, it was reported recently that HCMV infection *enhances* influenza vaccine responses in young adults (48). These equivocal reports, coupled with the finding that HCMV-specific T-cells with an apparent 'senescent' phenotype remain highly functional in most people, casts a great deal of dubiety on the HCMV and immunosenescence paradigm. In this regard, HCMV may actually be a 'passenger' rather than a 'driver' of immunosenescence and, at least in some cases, carrying HCMV might even be beneficial (116). Notwithstanding, the ability to control HCMV decreases with age (133), which is likely to cause large-scale T-cell clonal expansions that lead to immune 'exhaustion', inefficient vaccine responses and an immune senescent profile. It will be important for future studies to determine the impact of HCMV control, and not just serostatus, on immunosenescence. Measuring IgG antibody titers in serum is considered by some to be a crude measure that merely indicates prior exposure, and provides little information on viral load or host HCMV control over time (79). Indeed, a recent study of community dwelling elderly reported no change in HCMV antibody titers over a 12-year period (79). The presence of HCMV DNA in blood monocytes has been used as a marker of poor HCMV control, with approximately 56% of elderly individuals having HCMV DNA positive monocytes (78, 79). Moreover, a positive relationship exists between HCMV DNA+ monocytes, the numbers of HCMVpp65-specific CD8+ T-cells (78, 79), and serum neopterin (a marker of monocyte/macrophage activation) levels (77), independently of serum IgG antibody titers. Poor HCMV control might also explain the apparently weaker vaccine responses that have been reported in those with a positive IgG titer, but this has yet to be determined. A major criticism of the HCMV immunosenescence paradigm is the centric focus on the T-cell compartment, and there is a clear need to explore the relationship between HCMV and immune aging as it pertains to other lymphocytes affected by the virus, including NK-cells, B-cells and γδ T cells (8). This will be important to determine whether or not persistent HCMV infection can be considered beneficial or detrimental to immunity over the natural course of aging.

Blood Lymphocytes and Acute Exercise

Blood lymphocyte numbers increase dramatically upon engaging in a single bout of acute dynamic exercise. This exercise-induced lymphocytosis is almost instantaneous, with the mobilized lymphocytes consisting mostly of NK-cells, followed by CD8+ T-cells, CD4+ T-cells, B-cells, and lastly γδ T cells. However, when compared to lymphocyte numbers

in resting blood, the relative change in the absolute number of γδ T cells is greater than those of CD8+ T-cells but still less than NK-cells (3). Upon cessation of exercise, there is a rapid lymphocytopenia that occurs within 30-60-minutes and may persist for up to 6-24h later depending on the intensity and duration of the bout. For a more detailed overview on the lymphocyte response to acute exercise and the underpinning mechanisms, we direct the reader to the most recent ISEI position statements (150, 151). Although this response is very well characterized, the influence of infection history on the redeployment of lymphocytes and other leukocyte subtypes to single exercise bouts has only recently been investigated.

Acute Exercise Preferentially Redeploys 'Mature' subsets of T-cells and NK-cells
Within the CD8+ T-cell compartment, acute dynamic exercise has consistently been shown to evoke a preferential redeployment of antigen-specific T-cells with a differentiated effector memory phenotype (29, 124-126, 139). Cells with an EMRA phenotype are redeployed in relatively greater numbers than EM cells, followed by CM and lastly naïve cells (29). Even when other phenotypic identifiers of T-cell differentiation are used (i.e. CD27/CD28 or KLRG1/CD28 combinations), a preferential mobilization of the most differentiated, or 'late' (CD27-/CD28-; KLRG1+/CD28-) cells is evident followed by the 'intermediate' (CD27-/CD28+; KLRG1+/CD28+) cells and lastly the cells with an 'early' (CD27+/CD28+; KLRG1-/CD28+) differentiated phenotype (124, 139).

Our early interpretations of this work intimated that acute exercise preferentially redeployed 'senescent' CD8+ T-cells (124-126). This was based on prior observations that CD8+ T-cells expressing KLRG1 and/or CD57 lacked proliferative capabilities and that HCMV-specific CD8+ T-cells predominantly bore this phenotype (59, 96, 147). However, just as blocking PD-1 can restore cytokine secretion, blocking KLRG1 can restore T-cell proliferation indicating that KLRG1 is an inhibitor of T-cell clonal expansion but not a marker of replicative senescence per se (57). So although some cells bearing this phenotype may in fact be senescent, the term itself is a misnomer due to diversity of cell types, both functional and dysfunctional, that may express these surface markers. There are also other indicators that exercise mobilizes highly functional cells despite large proportions of the mobilized cells having a so-called senescent phenotype. We found that the average telomere length among isolated CD8+ T-cells was actually longer among the post-exercise cells (124), and that CD8+ T-cells in blood immediately post-exercise were still capable of secreting a wide array of cytokines following mitogen stimulation, even if bearing a CD27neg differentiated phenotype (75). Thus, the contribution of truly senescent or exhausted cells to the preferential mobilization of highly differentiated CD8+ T-cells with exercise and the physiological significance of such a response remains to be determined.

We recently reported that the redeployment of NK-cells with exercise is non-uniform, and like CD8+ T-cells, there is preferential mobilization of the most differentiated NK-cell subsets (17). NK-cell differentiation is defined by acquisition of inhibitory KIR expression followed by loss of the inhibitory receptor NKG2A (14) and gain of the "terminal differentiation" marker CD57 (23). As NK-cells differentiate, they lose the ability to proliferate and express IFN-γ in response to pro-inflammatory cytokines, while their capacity to kill a wide range of target cells increases (14, 23). In response to 30-minutes of steady state cycling exercise, NK-cell subsets were redeployed in a stepwise manner in accordance with differentiation status [highly-differentiated (KIR^{pos}/NKG2A^{neg}/CD57^{pos}) > medium-differentiated (KIR^{pos}/NKG2A^{pos}/CD57^{neg}) > low-differentiated (KIRneg-/NKG2Apos/CD57neg)] and the effect was consistent across multiple exercise intensities ranging from -5% to +15% of the individual blood lactate threshold (17). However, NKG2C^{pos} NK-cells, despite having potent NKCA against specific target cells expressing non-classical HLA-E, are redeployed with exercise in relatively few numbers regardless of differentiation status. This causes the proportion of NKG2C^{pos} NK-cells in blood to increase during exercise recovery due to a preferential egress of NK-cells lacking this receptor (17). In other words, as NK-cells are redeployed from the blood to the tissues during exercise recovery, most of the NKG2C^{pos} cells are 'left behind'. While confirming previous reports that exercise did not affect NKCA against the K562 leukemic target cell line on a per NK-cell basis (88, 92, 95) we showed that NKCA per cell was markedly elevated against the HLA-E transfected 221.AEH lymphoma cell line and multiple myeloma target cells expressing classical HLA-C (U266 and RPMI-8226) during the recovery phase of exercise (+1h after exercise cessation) (17, 18). This 1h post-exercise increase in NKCA per cell was positively associated with the proportions of NK-cells expressing the activating receptor NKG2C, and lacking inhibitory KIR for classical HLA molecules (CD158b) (17). Although it is often suggested that exercise evokes a redeployment of the most cytotoxic lymphocyte subtypes, it is somewhat of a conundrum why NKG2C^{pos} NK-cells, given their potent cytotoxic effector functions against target cells expressing non-classical HLA-E, are not preferentially redeployed with exercise as well. This appears to be due to their lower expression of the β2-adrenergic receptor (β$_2$-AR) and insensitivity to synthetic catecholamine stimulation compared to NK-cells lacking NKG2C (18). Taken together, these findings indicate that the effects of acute exercise on NK-cell function are strongly influenced by proportional shifts in NK-cell subsets and target cell expression of classical and non-classical HLA receptors.

HCMV Infection and Acute Exercise
HCMV Infection and the T-cell Response to Acute Exercise
The preferential mobilization of EM and EMRA cells with exercise led to the hypothesis that HCMV carriers, as a result of having greater numbers and proportions of these cell types in resting blood, would display an amplified mobilization of highly differentiated T-cells with exercise. Turner et al. were the first to show that 60-minutes of treadmill running exercise evoked a mobilization and egress of total CD8+ and EMRA CD8+ T-cells that was ~2 and ~6 times greater in those with a latent HCMV infection compared to non-infected participants (139). This amplified effect of HCMV on the redeployment of CD8+ T-cells to exercise has also been reported by our group, with the high-differentiated CD8+ T-cell subsets (KLRG1+/CD28-) accounting for the vast majority of the

HCMV effect (72, 130). Although individuals with HCMV carry other herpesvirus infections, Epstein-Barr virus (EBV) and herpes simplex virus-1 (HSV-1) serostatus does not appear to confound or alter the magnitude of the HCMV effect (16, 72). Interestingly, while aging is associated with an impaired ability to redeploy T-cells in response to a single bout of exercise (31, 84), we found that this only applies to older individuals who do not carry HCMV. On the other hand, older HCMV infected individuals mobilized CD8+ T-cells similarly to young HCMV-infected participants and almost twice as much as the non-infected young, again with the highly-differentiated subsets accounting for the effect (130). However, the redeployment (ingress and egress) of naïve/early-differentiated cells with acute exercise appears to be impaired with aging regardless of HCMV serostatus (130). HCMV infection also increases the mobilization of highly-differentiated CD4+ T-cells with exercise, although the magnitude of this response is not sufficient to amplify the redeployment of total CD4+ T-cells (72, 130, 139). It is important to note that although HCMV is associated with an amplified redeployment of differentiated T-cells, it is likely that the effects are mostly due to the unique ability of the virus to alter the composition of the peripheral T-cell pool in favour of the more exercise-responsive subtypes (72, 76). Because HSV-1 and EBV do not expand the EM and EMRA T-cell subsets in blood (37), this probably explains why these viruses are not associated with an amplified exercise redeployment of total CD8+ T-cells independently of HCMV (16, 72). It is likely, however, that other persistent viral infections that are able to expand the proportions of highly-differentiated T-cell subsets, such as Hepatitis C (6), will also contribute to an augmented redeployment of CD8+ T-cells in response to acute stress and exercise regardless of whether or not a HCMV co-infection is present. Indeed, well-treated HIV-infected men show a greater redeployment of highly differentiated CD8+ T-cells in response to acute maximal exercise compared to healthy controls (41), although it is not known if this effect is due to a HCMV co-infection as those with HIV tend to harbor HCMV also (68).

We showed that a single bout of exercise increases the number of HCMV-specific T-cells in peripheral blood 2-5-fold (130). This, in conjunction with a previous observation that the number of HCMV-specific T-cells increased after an acute psychological stress task (9), indicates that HCMV-specific T-cells are redeployed with exercise under the influence of catecholamines and β-adrenergic receptors. Phenotypic analysis of CD8+ T-cells specific to an NLV epitope of the HCMVpp65 antigen using an HLA-A2-restricted MHC class I Pentamer revealed that ~25% of resting HCMV-specific cells had a high differentiated (KLRG1+/CD28-) phenotype, increasing to ~49% immediately post exercise (130). We also found that the number of cells responding to HCMVpp65 and HCMV IE-I peptides increased dramatically post-exercise, and that the mobilized cells had broad HCMV antigen epitope specificity. In a more recent study, we stimulated a fixed number of PBMCs before and after 30-minutes of steady state cycling exercise (at +15% of the individual blood lactate threshold) with synthetic 15mer peptides specific for 4 antigens derived from HCMV (pp65 and IE-1) and EBV (LMP-2 and BMLF-1) in the presence of growth cytokines (IL-4, IL-7,

IL-15) for 8-days (Spielmann et al. Unpublished). While the expansion protocol elicited, on average, a 2.1 to 13.5-fold increase in the number of HCMV and EBV specific cells (enumerated using an IFN-γ ELISPOT assay) generated from resting PBMCs, the number of viral-specific cells present in the post exercise cultures were strikingly greater; on average, up to ~4.7 and ~70.4 times greater, for HCMV and EBV-specific T-cells, respectively (Spielmann et al. Unpublished). The number of viral-specific cells generated after 8-days of cell culture relative to the input number of T-cells and viral-specific T-cells was still greater after exercise, indicating that the exercise effect is not merely due to greater numbers of viral-specific cells among the cells stimulated with viral peptides at day 0. Moreover, the viral-specific T-cell lines expanded after exercise maintained their ability to kill autologous peptide-pulsed target cells in an MHC dependent manner, and while there was a greater proportion of EM and EMRA cells among the post-exercise T-cells at day 0, the composition of T-cell subsets did not differ between the before and after exercise cell cultures at day 8 (Spielmann et al. Unpublished). Taken together, these findings indicate that latent HCMV infection not only amplifies CD8+ T-cell redeployment in response to exercise, but that many of the mobilized cells are specific to HCMV antigens and, despite the majority of the mobilized cells having a late-differentiated phenotype, they appear to be highly functional with broad epitope diversity and the capacity for massive clonal expansion in response to peptide stimulation *in vitro* (130).

Latent HCMV infection also amplifies the redeployment of γδ T cells to a single bout of exercise in healthy young adults and, like CD8+ T-cells, the interaction effect between HCMV and exercise is independent of co-infections such as EBV, HSV-1 and parvovirus-B19 (104). Aging, however, was associated with an impaired redeployment of γδ T cells regardless of HCMV serostatus, indicating that latent HCMV infection helps maintain robust exercise-induced redeployment of CD8+ αβ T-cells but not γδ T cells with aging (104). This is likely due to the differential effects of age and HCMV infection on the γδ T cell compartment (113). Although we did not look at the relative exercise response of γδ T cell subsets (104), we expect HCMV to augment the mobilization of the Vδ2[neg] subset, given that they are overexpressed in those with HCMV and predominantly consist of the exercise-responsive EMRA phenotype (2, 4, 105, 113). It is not known, however, if γδ T cell function is affected by exercise and future studies should determine if exercise alters their proliferative responses to phosphoantigens (i.e. zoledronic acid), which are typically used to expand γδ T cells *in vitro* for immunotherapeutic purposes (61).

HCMV Infection and the NK-cell Response to Acute Exercise
Turner et al. reported that CD8+ T-cell redeployment was amplified in those with HCMV, yet total lymphocyte redeployment was unaffected (139). This provided indirect evidence that the redeployment of other lymphocyte subtypes to exercise might actually be impaired in people with HCMV. We tested this hypothesis by examining the effects of latent HCMV infection on the redeployment of NK-cells and their subtypes, and found that HCMV serostatus was associated with a strikingly impaired redeployment of NK-cells (16)

(Bigley et al., 2012). The relative blunting effect of HCMV infection on the exercise-induced redeployment of NK-cells was actually larger in magnitude than its augmenting effect on CD8+ T-cell redeployment (illustrated in Figure 1). Although we are the only group so far to report that HCMV infection inhibits NK-cell redeployment in response to acute exercise, we have found this consistently in three separate subject cohorts and shown that the effect is independent of both age and baseline blood NK-cell numbers (16, 18, 21). Moreover, HCMV only impairs the NK-cell response to exercise at intensities above the blood lactate threshold (18), the point at which blood lactate and catecholamine concentrations increase above pre-exercise levels (106). As HCMV did not affect the lactate or catecholamine response to exercise, this indicated that HCMV might be associated with decreased NK-cell catecholamine sensitivity and/or β_2-AR activity (18). To test this hypothesis, we compared NK-cell β_2-AR expres-

sion and cyclic AMP production in response to *in vitro* isoproterenol (non-preferential synthetic β-agonist) stimulation between HCMV seronegative and seropositive subjects (18). Those with HCMV had a lower expression of the β_2-AR and an impaired cyclic AMP response compared to non-infected subjects. Moreover, cyclic AMP production was inversely correlated with the proportion of NKG2C^{pos}/CD57^{pos} cells within the isolated NK-cells (18). This, in conjunction with the observation that NKG2C^{pos}/CD57^{pos} NK-cells are not preferentially mobilized with exercise, indicates that it is the accumulation of catecholamine insensitive NKG2C^{pos}/CD57^{pos} NK-cells (a cell population that is practically absent in those without HCMV) that links HCMV infection with impaired NK-cell redeployment to exercise.

HCMV also affects NKCA both at rest and in response to exercise. The increased proportions of NKG2C^{pos} NK-cells in
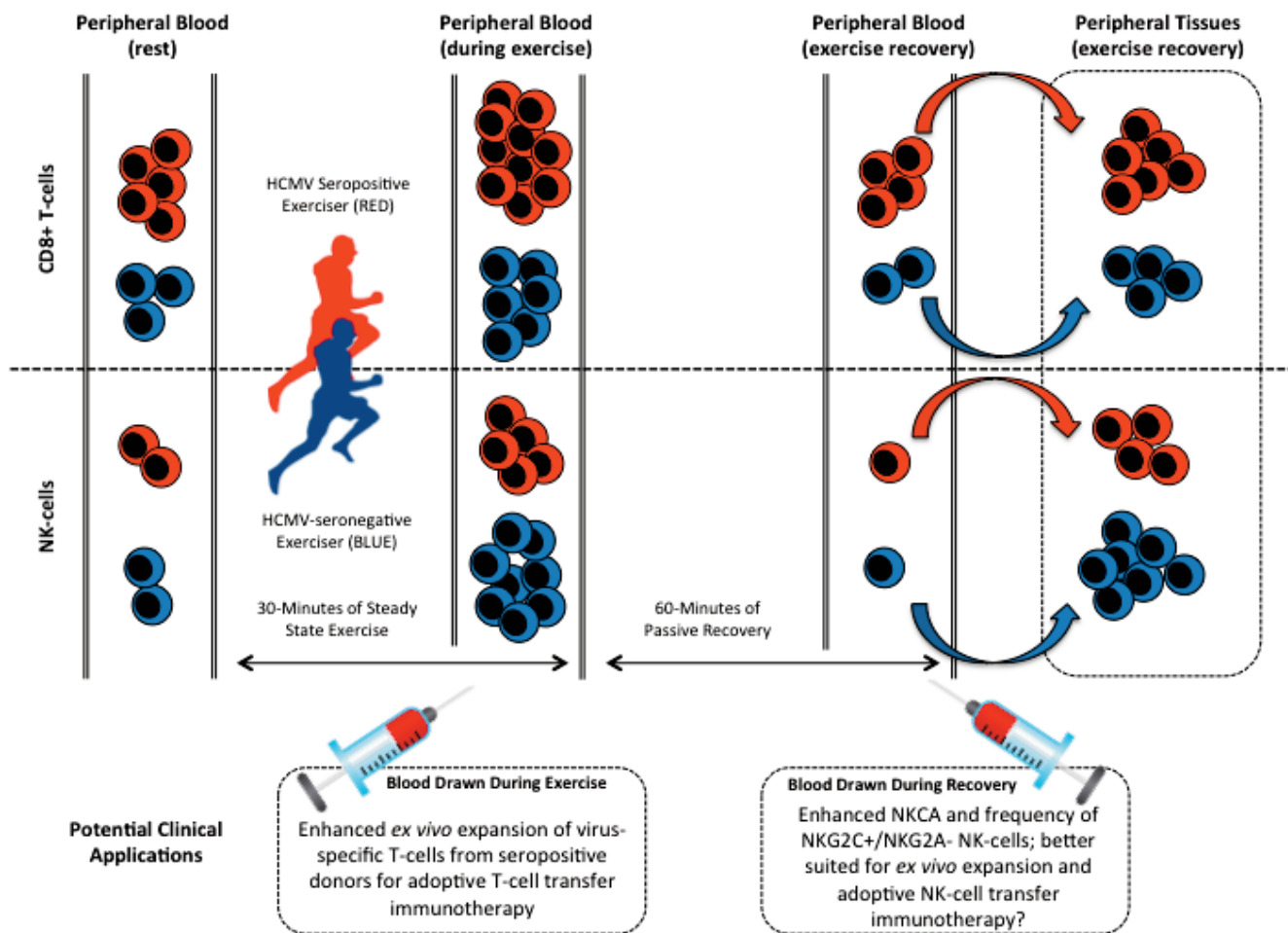

Figure 1. Divergent effects of HCMV infection on the exercise-induced redeployment of CD8+ T-cells and NK-cells and their potential clinical applications. HCMV seropositive participants mobilize 1.5-2.5 x more CD8+ T-cells than HCMV seronegative participants in response to fixed intensity steady state exercise, although the relative change in CD8+ T-cell number is similar between HCMV seropositive and seronegative participants (~2-fold increase in total CD8+ T-cells). HCMV increases the number and proportion of the catecholamine-sensitive EM and EMRA CD8+ subtypes, making the CD8+ T-cell population as a whole more responsive to exercise. The mobilized cells contain 'primed' virus-specific T-cells that may be obtained from the blood during exercise before they would normally egress the blood and infiltrate the peripheral tissues. These cells can then be used to augment the ex vivo expansion of multi-virus-specific T-cells for use in the allogeneic adoptive transfer immunotherapy setting. In contrast, HCMV seronegative participants mobilize 2-4 x more NK-cells than HCMV seropositive participants despite having similar resting NK-cell numbers. This appears to be due to a greater proportion of catecholamine-insensitive NKG2C+ NK-cells in people with HCMV. Although NK-cell cytotoxic activity (NKCA) does not change at the individual cell level during exercise, individual NKCA in blood is enhanced during exercise recovery. This is due to an increased proportion of NKG2C+/NKG2A- NK-cells in blood that are able to effectively recognize and kill HLA-E expressing tumor target cells. NK-cells with these phenotypic and functional properties might be better suited for ex vivo expansion and allogeneic adoptive NK-cell transfer.

those with HCMV enhances resting NKCA against a wide-range of tumor target cells *in vitro*, with the magnitude of the HCMV effect being positively associated with HLA-E expression on the target cells (18). However, when compared to baseline, only those without HCMV demonstrated increased NKCA per cell against the U266, RPMI-8226, and 221.AEH target cell lines 1h post-exercise. Thus, due to marked changes in the composition of NK-cell subsets, both acute exercise and HCMV are able to enhance NK-cell function, although the effects are not synergistic (18).

Divergent effects of HCMV on the Exercise-Induced Redeployment of T-cells and NK-cells
We have shown that latent HCMV infection has dichotomous effects on the redeployment of NK-cells and T-cells in response to a single exercise bout (Figure 1). While HCMV-specific CD8+ T-cells appear to be highly stress-responsive, the so-called HCMV-specific (NKG2C^{pos}) NK-cells respond poorly to catecholamines and are redeployed in comparatively fewer numbers with exercise. From a historical perspective, physical exertion was performed only when hunting, working or evading predators and is therefore considered to be an evolutionary conserved mechanism to 'prime' the immune system during situations when physical injury and infection are more likely to occur (38). As such, the current dogma is that exercise mobilizes lymphocytes and other leukocytes that have high tissue migration and effector functions as part of the 'flight or fight' response to facilitate immunosurveillance, promote wound healing and regenerate damaged tissue (24). It is perplexing, therefore, why NKG2C^{pos} NK-cells, given their importance in controlling viral infections such as HCMV and their ability to recognize and destroy malignant cells expressing HLA-E (e.g. multiple myeloma, AML) (94, 155), are 'left behind' in the bloodstream while other NK-cell subsets are redeployed to the tissues during exercise recovery. Interestingly, it is not just the exercise responses of NK-cells and T-cells that are divergent in those infected with HCMV, it is the immune response to HCMV itself.

Despite the well-established benefits of NKG2C^{pos} NK-cells to HCMV containment and overall immunity, the proportion of NKG2C^{pos} NK-cells is highly variable amongst HCMV-seropositive individuals (53). One likely explanation for this variation is that HCMV-specific NK-cell and T-cell responses are reciprocally related in subjects with good viral control (20), most likely due to the rheostat-like capacity of NK-cells to limit viral-specific T-cell responses (148). Thus, people with HCMV can contain the virus through an NK-cell or T-cell-mediated response, but not both. Considering the link between HCMV-driven T-cell responses and immunosenescence (116, 156), and the broad functionality of NKG2C^{pos} NK-cells (46), it is likely that an NK-cell-mediated response to HCMV would be preferable in most cases. However, due to the poor exercise responsiveness of NKG2C^{pos} NK-cells, it is precisely these subjects who drive the impaired NK-cell response to exercise in those with HCMV.

Does HCMV Infection Affect Other Immune Responses to Acute Exercise?
Although HCMV has profound effect on the composition and function of the blood lymphocyte compartment, neutrophils and monocytes are also highly exercise responsive and their redeployment with exercise might be affected by HCMV as well, particularly monocytes which are believed to harbor the virus during latency. However, we did not find HCMV serostatus to affect neutrophil or monocyte numbers following a 75-km cycling time trial (76). Moreover, neutrophil and monocyte phagocytosis and oxidative burst activity, as well as plasma levels of the cytokines IL-6, IL-8, IL-10 and TNF-α, and the lipid peroxidation marker F_2-isoprostanes, increased after the exercise bout but were not affected by HCMV serostatus (76). The concentration and/or secretion of salivary antimicrobial proteins (AMPs) such as salivary IgA, LL-37, HNP 1-3, lactoferrin, α-amylase and lysozyme are known to increase after a single bout of exercise (67). However, in a retrospective analysis of this cohort (67), in which ~53% of the participants were found to be HCMV seropositive, previous exposure to the virus had no impact on either the resting or the exercise-induced change in the concentration or secretion of these salivary AMPs (Kunz et al. unpublished). Thus, although the number of studies investigating the effects of infection history on other immune responses to exercise is small, it has so far only been shown that HCMV infection impacts the redeployment and function of T-cells and NK-cells in response to a single bout of exercise.

HCMV Infection and Chronic Exercise
Few studies have examined the impact of HCMV on immune responses to long-term exercise training. Participation in moderate-intensity regular exercise has been associated with a less-differentiated T-cell profile (132), while, conversely, habitual high volume exercise training has been associated with a more-differentiated T-cell profile and reduced thymic output (26, 107), which are hallmark features of immunosenescence. Thus, at least from the available cross-sectional data, it appears that moderate intensity exercise has anti-immunosenescence effects whereas high-volume exercise, such as the type practiced by highly competitive athletes, has mostly pro-immunosenescence effects. Although current empirical data is lacking, we postulate that viral control will be strongly linked to this bidirectional relationship between exercise volume and immunosenescence (Figure 2). Here we discuss the potential effects of high and low/moderate volume exercise training on the host's ability to control HCMV and other herpesviruses and how these might be linked to the apparent bidirectional effects of exercise volume on host infection risk.

High Volume Exercise Training and HCMV Control
Infection history is likely to have important implications for the immune system of athletes and other occupational personnel exposed to prolonged periods of high physical training loads. Indeed, Brown et al. reported that HCMV infection was more prevalent among female soccer players compared to age-matched controls (25, 26) despite training status being associated with fewer differentiated T-cells (26), while Moro-Garcia et al. (91) reported that older athletes with HCMV presented with lower numbers of CD4+ T-cells. Although measures of viral serology are becoming more common in exercise training investigations, studies that have examined the effects of high volume exercise training on latent viral reactivation in athletes are scarce, and none, to our knowledge, have focused
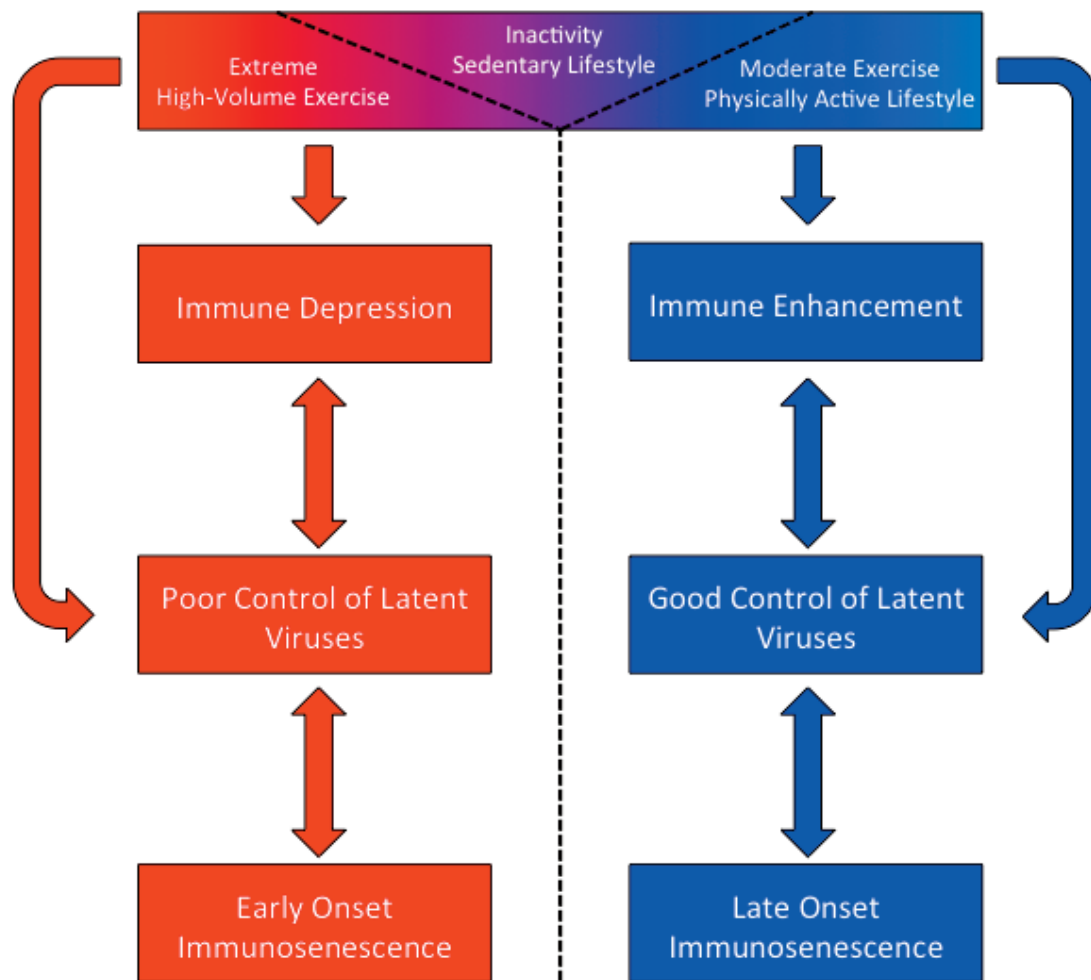
Figure 2. Theoretical framework depicting the bidirectional effects of exercise on immune function and latent viral control. Regular moderate intensity exercise and physically active lifestyles are associated with enhanced immunity that results in better control of latent viruses. Conversely, excessive high volume exercise (such as the type practiced by high performance athletes) is associated with immune impairment that may result in poor control of latent viral infections. The bidirectional effects of exercise and physical activity on immune function and viral control is likely to have important health implications across the lifespan. Individuals with strong immune function and good viral control will likely spend a greater proportion of their lives in good health (increased helathspan) with immunosenescence occurring at an older chronological age compared to those with weak immunity and poor viral control.

specifically on HCMV. In contrast, latent viral reactivation has been studied extensively in astronauts and it has been shown that crewmembers who shed viral DNA [HCMV, (EBV) and varicella-zoster virus (VZV)] have a skewed Type I plasma cytokine profile compared to their non-shedding counterparts (86). Moreover, viral DNA in astronauts has been found in samples collected even prior to spaceflight indicating that it is a stress-induced phenomenon (35). Indeed, viral reactivation has been linked with acute and chronic stress (50, 117) and these may be exacerbated by frequent training and competition (150).

Symptoms of upper respiratory illness (URI) continue to be a problem for high performance athletes. Gleeson et al. (51) reported that EBV serostatus was associated with increased self-reported symptoms of URTI in a group of elite swimmers following a 30-day period of intensive training. Further, EBV DNA was detectable in saliva (indicative of viral shedding) prior to the manifestation of URI symptoms, indicating that EBV reactivation and URI incidence during periods of intensive training could be causally related (51). However, in a fol-

low up study, prophylactic administration of the anti-viral drug Valtrex was able to prevent EBV reactivation but failed to prevent symptoms of URI (34). In contrast, a recent study found that prior exposure to both HCMV and EBV protected endurance athletes from URI during periods of increased training volume (56). In a study of 246 athletes over a 4-month winter training period, those athletes with HCMV/EBV (25% of the cohort) reported fewer URI symptom days (median: 2 vs. 4 days) compared to non-infected athletes. Seropositivity to EBV alone provided no protective effect on URI symptoms, indicating that either prior exposure to HCMV alone, or a synergistic effect between HCMV and EBV, offers protection against future illness (56). As most HCMV-infected people are co-infected with EBV (133), it is difficult to determine the effects of HCMV independently of EBV. These apparent protective effects of prior HCMV exposure against infectious symptoms in athletes may not be surprising, as greater numbers of circulating EM and EMRA CD8+ T-cells and an amplified redeployment of these cell types in response to a single exercise bout may actually enhance immunosurveillance. In addition, those with a

HCMV positive IgG antibody titer also display increased resting NKCA against a wide range of NK-cell target cells (Bigley et al., Unpublished). It is not known if any of the athletes in the He et al. (56) study displayed signs of poor viral control, as HCMV IgG antibody titers and viral shedding were not reported. Moreover, the discrepancy in findings between Gleeson et al. (51) and He et al. (56) might be due to the failure of the latter study to differentiate between latent and active forms of infection. It is possible, therefore, that prior HCMV exposure boosts immunosurveillance and reduces infection risk in the immunocompetent athlete, but if excessive training leads to immune dysregulation and HCMV or EBV is allowed to reactivate, the viral replication might overburden the immune system and increase the risk for opportunistic infections. Although Brown et al. (25) recently reported that 2-weeks of increased training volume did not alter the composition of T-cell subsets in elite level soccer players regardless of HCMV serostatus, it is not known if the HCMV positive athletes experienced a reactivation of the virus as only serostatus was determined. Thus, it is important that future studies determine the link between viral control and immune competence during periods of heavy training and competition.

Low/Moderate Volume Exercise Training and HCMV Control
As persistent HCMV infection has been implicated in the etiology of immunosenescence, it is intuitive to speculate that appropriate lifestyle interventions aimed at boosting immunity and keeping the virus in check would be advantageous to the host. While many studies have investigated the effects of long-term, moderate-intensity exercise training on various aspects of immunity, particularly in the elderly and the obese (128), it is somewhat surprising that there are currently no studies focused on viral seroprevalence or viral control. Notwithstanding, signature features of both latent and chronic HCMV infection tend to be less marked in habitual moderate-intensity exercisers compared to their sedentary counterparts (127, 128). The beneficial effects of habitual moderate intensity exercise include enhanced vaccine responses, lower numbers of differentiated T-cells, lower circulating levels of pro-inflammatory cytokines, increased mitogen-induced T-cell proliferation, and longer blood leukocyte telomere lengths (128). Hence, it is possible that HCMV regulation connects the beneficial effects of habitual moderate-intensity exercise with improvements in immune function. Future studies should test this hypothesis through randomized clinical trials utilizing longitudinal exercise training interventions.

Habitual low volume exercise may also have indirect effects on viral control through its ability to modulate human stress levels (93), as HCMV reactivation has been shown to occur during periods of acute (order of days to weeks) and chronic (order of months to years) stress (35). In a large occupational cohort that included over 300 HCMV seropositive participants, Rector et al. (109) reported that higher HCMV IgG serum antibody titers were associated with questionnaire-based measures of elevated anxiety, depression, vital exhaustion, and lower scores of mental health. These associations withstood adjustment for a large number of potential confounding factors such as age and socioeconomic status. Because exercise has been shown to ameliorate symptoms of

psychological stress and improve health and overall wellbeing (93), it is likely that frequent participation in structured exercise training or physical activity will mediate these relationships between measures of psychological stress and poor control of HCMV.

HCMV Infection and Exercise: Potential Clinical and Practical Applications
Latent Viral Reactivation: A biomarker of immune depression in athletes?
While there are several anecdotal reports of athletes contracting infectious mononucleosis, developing shingles and/or having frequent cold sores (110), no study to our knowledge has shown that these manifestations of primary or reactivated herpesvirus infections are more frequent in athletes compared to their non-athlete counterparts. However, latent viruses can often reactivate without conspicuous symptoms of infection, particularly during or shortly after periods of high physical and/or psychological stress. Other models of both acute and chronic stress have shown evidence of latent viral reactivation without accompanying infectious symptoms, such as academic stress, military training/deployment, bereavement and caregiving (35). So not only does the shedding of viral DNA have the potential to directly cause disease, but the subclinical reactivation of a latent virus may serve as a reliable indicator of immune depression in athletes and provide an early indication that they might be susceptible to opportunistic infections. HCMV reactivation can be detected through shedding of viral DNA in urine (86, 133), the presence of HCMV DNA in blood monocytes (78), and/or pp65 antigenemia in peripheral blood leukocytes (66); with changes in HCMV IgG antibody titers being used by some researchers as an indicator of viral load (20, 36, 109). Thus, the shedding of viral DNA could potentially be used to predict overtraining/underperformance and allow coaches and team physicians to make decisions on the training, nutritional and recuperation regimens of an athlete with an active herpesvirus infection. While the molecular biology techniques required to confirm active viral infections may not be practical in large groups, high performance athletes should at least be screened for HCMV serology and other persistent infections so that those previously exposed can be monitored periodically for viral load using more sensitive measures (i.e. viral antigenemia; shedding of viral DNA) as they prepare for major competitions.

Immunosenescence and the Immune Risk Profile
Whether or not the plasticity of the immune system can be modulated by exercise during the natural course of aging is of great interest to both exercise immunologists and immunogerontologists. It has been suggested that regular exercise may help prevent and/or rejuvenate 'older looking' immune systems (127, 128). Although HCMV infection features heavily in immunosenescence and the associated IRP, the contemporary view is that HCMV might be a 'passenger' rather than a 'driver' of immunosenescence, and the reactivation of HCMV and the consequential effects it has on immunity are secondary to immunosenescence itself (116). Thus, it is possible that exercise may have greater involvement in preventing immunosenescence that leads to HCMV reactivation and its effects on the peripheral T-cell pool in particular. If regular exercise is found to improve HCMV control, especial-

ly in the elderly, this could be one mechanism by which exercise helps curtail immunosenescence.

It is equally important to determine if exercise can exert rejuvenating effects downstream of immunosenescence. One possible mechanism would be for regular exercise to alter the composition of the peripheral T-cell pool in the direction of a less senescent profile. We previously hypothesized that regular moderate intensity exercise performed over a very protracted period of time (likely in the order of months to years) might 'make space' for new and fully functional T-cells by causing older, exhausted memory T-cell subsets to undergo apoptosis (122). These new cells would likely come from increased thymic output, sites of extrathymic T-cell maturation or homeostatic clonal expansion of existing T-cells (122). The 'exercise makes space' hypothesis may also have an indirect component in that regular moderate exercise may exert better viral control, thus resulting in less frequent shedding of HCMV. As the frequency of viral shedding decreases, the antigenic stimulus and the need to maintain large numbers of HCMV-specific T-cells also decreases and the HCMV-specific T-cells are therefore selectively 'deleted' over time. So, in this instance, exercise is not directly causing the deletion of excess viral T-cell clones, but the accompanying improvement in HCMV control eliminates the need for their existence in large numbers. There is evidence for this 'use them or lose them' idea with other viral infections such as adenovirus, where large numbers of adenovirus-specific cells are more likely to be found in children (due to recent infection) than adults and persistence of memory cells is relatively short-term (order of a few years) (118). However, it should be noted that HCMV-specific T-cell clonotypes may persist for up to 4-years even in healthy people with low antigenic load (58), so any alteration in the frequency of HCMV-specific T-cells with exercise as a result of better viral control may only be seen after a protracted period of time.

It is important to note that the 'exercise makes space' hypothesis is merely a theoretical framework that is a long way off from having the empirical support required to be an accepted mechanism by which exercise can rejuvenate the aged immune system. The main challenge to the credibility of this framework is that it was integrated with the dogmatic view of that time that there is a fixed 'immunological space' that restricts total T-cell numbers (28). This limitation in 'space' was thought to reflect homeostatic control of peripheral T-cell numbers as opposed to a shortage of actual physical space, but this idea has recently been challenged (87, 144, 154). It is also unknown if T-cell apoptosis is required to trigger the production of new T-cells, although a recent study by Mooren and Kruger (90) showed that adoptive transfer of apoptotic lymphocytes in rodents triggered the release of hematopoietic progenitor cells into the circulation. Whether or not apoptotic T-cells can trigger thymic output or homeostatic T-cell proliferation in a similar manner remains to be determined.

Regardless of what the underpinning mechanism might be, there are no longitudinal randomized control trials to date that show exercise training can rejuvenate older looking immune systems (128). However, as exercise training has been shown to improve vaccine outcomes in previously sedentary commu-

nity dwelling elderly (65, 160), it is likely that direct improvements in immune function are involved. Future clinical trials involving exercise training and outcome measures of immune function should focus on cohorts with an apparent 'senescent' or 'immune risk profile' at baseline, as exercise is likely to exert larger beneficial effects in these subject groups. For instance, individuals with several hallmark features of immunosenescence (i.e. inverted CD4:CD8 T-cell ratio, low naïve T-cell numbers, increased PD-1^{pos} HCMV-specific T-cells) and/or those with poor HCMV control (high HCMV IgG antibody titers, HCMV DNA[pos] monocytes) might benefit more from an exercise training intervention compared to those who, despite being chronologically older, display few signs of immunosenescence and impaired HCMV control. Moreover, a limitation of the current HCMV and immunosenescence literature is that it is polarized almost entirely to the T-cell compartment. Recent evidence has indicated that certain individuals control HCMV predominantly through NK-cells, which may shoulder the burden of HCMV control to preserve T-cell function and prevent excess T-cell clonal expansion and functional exhaustion (20). It is therefore important that future studies determine the impact of exercise and other lifestyle interventions on HCMV control and the interaction between T-cell and NK-cell responses to the virus during the natural course of aging.

Exercise as a Vaccine Adjuvant: is HCMV Infection a Mediator?

Improvements in vaccine response after exercise training have provided the strongest evidence to date that the plasticity of the immune system can be positively affected by exercise in non-diseased people. Woods et al. (160) reported that community-dwelling elderly randomized to a 10-month cardiovascular exercise-training program had increased seroprotection rates following immunization with the trivalent influenza vaccine compared to controls who performed flexibility/stretching exercise up to 24 weeks after inoculation. Kohut et al. (65) also reported that older adults immunized with a trivalent influenza vaccine before and after a 10-month aerobic exercise training intervention had a greater mean fold increase in antibody titre to H1N1 and H3N2 strains of influenza A virus compared to non-exercised controls. Several studies have shown that single bouts of exercise performed immediately prior to vaccination are also effective, but these are not always consistent (97). These studies have involved whole body dynamic exercise and localized resistance exercise designed to evoke a local inflammatory response at the site of vaccination. Exercise and vaccine studies typically focus on the influenza, tetanus toxoid, diphtheria, pneumococcal and meningococcal vaccines, and the subjects range from young, healthy adults to community dwelling elderly (97). In response to acute exercise, the majority of studies report that exercise enhances the response against those vaccine strains that elicited the poorest response in the control group, suggesting that immune responses to those vaccine antigens with low immunogenicity are most likely to be enhanced by acute exercise (97).

While the mechanisms underpinning these exercise-induced improvements in vaccine responses are likely to be different between single exercise bouts and chronic exercise training, it

is possible that individuals with poor HCMV control (i.e. weaker immunity) will benefit most from the adjuvant effects of exercise. Although weaker immune responses to vaccines (i.e. influenza vaccine) have been attributed to HCMV serostatus (85, 140) this has not been consistently reported (36), and these equivocal findings might be due to the failure of these studies to account for HCMV control using sensitive methods such as viral shedding or the presence of HCMV DNA^{pos} monocytes (78, 86). We postulate that the inverse associations between HCMV serostatus and vaccine efficacy will be more marked in those with poor HCMV control, and that chronic exercise training will, in turn, enhance vaccine efficacy by improving HCMV control and lowering the overall burden placed on the immune system. Moreover, because vaccines already elicit robust immune responses in the vast majority of healthy people, the adjuvant effects of the acute exercise response might not be apparent in those with good HCMV control. At the very least, acute exercise might help those with HCMV mount vaccine responses that are comparable to non-HCMV infected people at rest. In a study of young healthy adults, acute eccentrically biased resistance exercise was found to enhance immune responses to the seasonal influenza vaccine in men(42), with the exercise bout affecting those with and without HCMV equally (140). This occurred despite latent HCMV infection being associated with weaker vaccine responses (140). Future research should determine the impact of both acute and chronic exercise on vaccine efficacy in the context of HCMV infection across a wide age range, considering both prior exposure and the ability of the host to keep the virus in a latent state. This will be particularly important to study in the elderly who are known to have impaired vaccine responses and are more likely to have poor control of HCMV due to immunosenescence.

Acute Exercise and Adoptive Transfer Immunotherapy
Another clinical procedure that may benefit from the immune-enhancing effects of acute exercise is adoptive transfer immunotherapy - the passive infusion of *ex vivo* expanded donor-derived or autologous immune cells to a cancer patient recipient. HSCT is used to treat many hematologic malignancies, but is associated with significant morbidity and mortality especially due to viral infections (i.e. HCMV, EBV and adenovirus), relapse, and graft-versus-host disease (GvHD) (49, 55). Viral infections and relapse can be controlled by adoptive transfer of antigen-specific T-cells that have been expanded *ex vivo* from an MHC compatible donor. Current viral-specific T-cell manufacturing processes involve stimulating PBMCs with overlapping viral antigen peptides *in vitro* to expand the population of memory T-cells with anti-viral activity (49). Clinically sufficient numbers can be obtained in 8-21 days before they are delivered to the patient. Although adoptive T-cell transfer is often successful in curtailing viral infections after HSCT, the inadequate restoration of immunity in some cases may be due to the failure to generate sufficient numbers of functional antigen-specific T-cells that are able to recognize and destroy target cells in vivo and persist in the host after transfusion. Using viral antigen peptides to expand memory T-cells from peripheral blood, we have shown that a single bout of exercise is capable of augmenting the manufacture of highly functional cytotoxic T-cell lines specific to multiple virus antigens (Spielmann et al. Unpublished). Thus, exercise

might serve as a simple and economical method to augment the rapid generation of multi-virus specific T-cells from healthy donors for subsequent adoptive transfer to immunocompromised patients after HSCT. Exercise therefore has the potential to amplify the total number of viral specific cells generated from a fixed volume of blood ensuring a faster delivery of a product that is enriched with broad virus specific activity to the patient. Moreover, exercising donors during blood collections may also reduce the need for apheresis, cost and the overall burden placed on the donor. As the mechanisms for viral-specific T-cell mobilization with exercise are likely to involve interactions between catecholamines and β-AR, it is also possible that the exercise effects might be reproducible in resting donors administered a synthetic β-AR agonist (3). Although this remains to be determined, eliminating the need for exercise would be preferable for some donors and may increase the applicability of the technique to the autologous adoptive transfer immunotherapy setting also. For instance, cancer patients required to donate their own cells for reinfusion (i.e. autologous adoptive T-cell transfer) might be too ill to perform a single exercise bout but may be able to tolerate the administration of a β-AR agonist for the purposes of mobilizing their antigen-specific T-cells to the peripheral blood prior to *ex vivo* expansion.

Although cytotoxic CD8+ and CD4+ T-cells are often preferred for adoptive transfer immunotherapy because of their antigen specificity and ability to proliferate and persist in the host after infusion, allogeneic adoptive transfer of NK-cells has also shown promise as a means of controlling or reversing the spread of multiple human malignancies including multiple myeloma, AML, and non-small cell lung cancer (60, 89, 120, 148). This immunotherapeutic procedure has shown a consistently high safety profile and has increased survival in poor prognosis cancer patients (89, 114) and is preferred by some over T-cell transfer because NK-cells do not cause GvHD (114). However, multiple issues remain that undermine the efficacy of long-term cancer treatment using adoptive transfer of NK-cells. Existing pre-transfer expansion protocols are able to generate large numbers of NK-cells (47, 119), but alloreactivity of donor NK-cells is highly variable (101, 114, 120) and expression of NKG2A is far greater than NKG2C (119), which limits the capacity of NK-cells to kill tumor cells expressing classical HLA molecules and HLA-E (101, 120). As such, the rapid expansion of alloreactive, HLA-E-targeting NK-cells needs to be improved. Our work shows that exercise has great potential as an adjuvant for NK-cell immunotherapy as it primes NK-cells to kill HLA-expressing tumor cells that are typically resistant to NK-cells (17, 18). Interestingly, however, the phenotypic and functional properties of NK-cells in the blood during the recovery phase of exercise appear better suited for the allogeneic adoptive transfer setting compared to NK-cells in blood at rest or immediately after exercise (17) (Figure 1). Indeed, 1h after completing a 30-minute cycling protocol, there are increased proportions of NKG2C^{pos}/NKG2A^{neg} NK-cells and their ability to kill target cells expressing both classic and non-classic HLA molecules is markedly elevated, particularly in HCMV seronegative donors (18). Thus, it might be better to expand NK-cells during exercise recovery as opposed to during or immediately after exercise, especially from HCMV seronegative donors,

who tend to have lower numbers of NKG2C^{pos}/NKG2A^{neg} NK-cells and lower NKCA against HLA-E-target cells at rest (18). We have also shown that a single bout of exercise can augment the manufacture of monocyte-derived dendritic cells and T-cells recognizing tumor antigens (73, 74) from healthy people, indicating that exercise has great potential as a simple and economical adjuvant to boost the manufacture of various cell types for use in the allogeneic adoptive transfer immunotherapy setting (19).

SUMMARY

It has been suggested that HCMV has likely co-evolved with its host since the very beginning of human life (136). Carrying the infection was long considered to exert mostly negative effects on immunity that may accelerate the biological aging of the human immune system and the onset of immunosenescence (64). This viewpoint has changed somewhat in that prior exposure to HCMV, provided that the host is immune competent and can adequately keep the virus in check, might actually strengthen immunity (116). Evidence for this comes from studies that have found enhanced immune responses to the influenza vaccine in young people with latent HCMV infection (48), and a putative virus-versus-tumor effect that has been documented both *in vitro* and *in vivo* (20, 43). Specifically the increase in NKG2C expression on NK-cells from people with even a latent HCMV infection enhances NKCA against certain cancer (i.e. AML) and other viruses (i.e. Hantavirus) that are characterized by the upregulation of HLA-E on malignant or transformed cells (20, 22); whereas clinical HCMV reactivation and the subsequent accumulation of NKG2C+ NK-cells have been linked to a markedly decreased risk of relapse in AML patients (43, 52). Despite the longstanding association between humans and HCMV, studies investigating the effects of exercise in the context of HCMV infection only began in earnest within the last 5-years. It has become apparent that HCMV infection has a profound influence on the redistribution of CD8+ T-cells, γδ T-cells and NK-cells in response to a single exercise bout, and that these effects are independent of co-infections with other herpesviruses such as HSV-1 and EBV (16, 72). As such, studies that are concerned with harnessing the acute stress response to improve clinical outcomes (i.e. vaccination, adoptive transfer immunotherapy, surgical outcomes) should consider the role that host infection history plays in this response.

We postulate that the bidirectional effects of exercise volume on host immunity will be directly linked to the ability of the host to control HCMV and other persistent infections (Figure 2). It is vitally important for future research to determine if regular moderate-intensity exercise training and/or physical activity can improve control of HCMV (and other latent viruses) and preserve host immunity. Moreover, whether or not improved viral control can contribute to immune rejuvenation through exercise and lifestyle interventions is a key question that still remains unanswered (127). The interactions between habitual exercise/physical activity and psychological stress on HCMV reactivation and immune function should also be thoroughly explored as this will add to our understanding of the role that lifestyle and the social environment might play in the etiology of immunosenescence. In contrast to habitual moderate-intensity exercise training and physical activity, high volume prolonged exercise training may compromise immunity and impair host HCMV control, which could lead to detrimental pro-senescence effects for the high performance athlete in both the short and long term. Although research in this area is still in its infancy, we conclude that host infection history and the ability to regulate dormant pathogens is likely to play a key role in our understanding of how the immune system responds to both acute and chronic exercise across the entire exercise volume continuum.

ACKNOWLEDGMENTS

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