

Aging, Persistent Viral Infections, and Immunosenescence: Can Exercise “Make Space”?

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SIMPSON, R.J. Aging, persistent viral infections, and immunosenescence: can exercise “make space”? *Exerc. Sport Sci. Rev.*, Vol. 39, No. 1, pp. 23–33, 2011. *Overcrowding the immune space with excess clones of viral-specific T cells causes the naïve T-cell repertoire to shrink, which increases infection susceptibility to novel pathogens. Physical exercise preferentially mobilizes senescent T cells from the peripheral tissues into the blood, which might facilitate their subsequent apoptosis and create “vacant space” for newly functional T cells to occupy and expand the naïve T-cell repertoire.* **Key Words:** immune risk profile, cytomegalovirus, lymphocyte apoptosis, T cell, immunology

INTRODUCTION

Immunosenescence is a canopy term that has been used to describe the biological aging and progressive dysfunction of systemic immunity. This age-related diminution of the immune system is characterized by poor vaccine efficacy, lowered immune vigilance, and greater morbidity and mortality as a result of infectious disease (18,32). During the aging process, naïve antigen virgin T cells, which are vital for mounting immune responses to novel pathogens, are gradually replaced with expanded clones of effector and effector-memory T cells that have a late-stage differentiation phenotype and limited antigenic specificity. This “overcrowding” of the so-called immune space causes the naïve T-cell repertoire to shrink, increasing host infection risk. It is now apparent that individuals carrying latent herpesvirus infections show distinct changes within the T-cell compartment that are independent of chronological age but indicative of biological age and immunosenescence (11). Persistent reactivating herpesviruses that are prevalent among humans include herpes simplex virus (HSV-1, HSV-2), Epstein-Barr virus (EBV), varicella zoster virus (VZV), and cytomegalovirus (CMV). Many of these viruses cause conspicuous infectious symptoms

to manifest upon primary infection and also on subsequent reactivations of the virus. For instance, primary EBV infection causes infectious mononucleosis (glandular fever), whereas VZV causes chickenpox upon primary infection and shingles when reactivated in later life. Other reactivating herpesviruses such as HSV-1 and HSV-2 can cause blisters (*i.e.*, cold sores) to appear on mucous membranes of the labialis and genitalia. Ironically, it is persistent infection with the clinically asymptomatic and supposedly “harmless” CMV infection that is believed to have the most deleterious effects on T-cell immunity and associated immunosenescence (11,18). Indeed, CMV seropositivity is the main latent herpesvirus to be included in the immune risk profile (IRP) — an array of immune biomarkers that have been used to predict mortality in very old humans (18). Furthermore, the numbers of CMV-specific cytotoxic T cells in blood are inversely associated with survival in the elderly (18), which bolsters the intuition that persistent CMV infection is the driving force behind immunosenescence (11).

A hallmark of aging and persistent CMV infection is the accumulation of terminally differentiated senescent T cells, which are incapable of further cell division in response to antigenic stimuli and drastically contribute to the shrinking of the naïve T-cell repertoire. To combat immunosenescence, some invasive immunotherapeutic procedures (*i.e.*, cytokine, hormone, and monoclonal antibody therapy) have been suggested to remove expanded clones of these terminally differentiated effector-memory T cells (11,17). Many of these procedures, however, are costly, high risk, and associated with a number of potentially detrimental side effects. Not to mention that the implementation of such invasive procedures in people who are not considered “ill” has some ethical issues.

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We have suggested that regular physical exercise might serve as an alternative, inexpensive, and safer strategy to combat the detrimental effects of immunosenescence that are induced by aging and reactivating viral infections (29). Despite the known beneficial effects of habitual exercise on aspects of immunity that pertain to immunosenescence (29), the underpinning mechanisms of improved immunity by exercise are not completely understood.

We (23–26), and others (1,4,33), have shown that an acute bout of aerobic exercise elicits a preferential mobilization of highly differentiated and senescent T cells (some of which are specific to CMV and EBV) from the peripheral tissues into the blood compartment before their rapid removal from the blood during the early stages of exercise recovery. The possibility that frequent shifting of senescent T cells from peripheral tissues in response to acute exercise could have accumulative long-term restorative effects on systemic immunity has not been considered. We propose that frequent bouts of acute physical exercise might serve as an adjunct approach to “make space” and expand the T-cell antigen receptor repertoire. This article summarizes the rationale behind our hypothesis that an exercise-induced mobilization of senescent T cells, and their subsequent deletion by apoptosis, could open a pathway for naïve antigen–virgin T cells to occupy the vacated space, expand the naïve T-cell repertoire, and ameliorate symptoms and biomarkers associated with immunosenescence and the IRP.

SENESCENCE OF THE IMMUNE SYSTEM

Thymic Involution and the Immune Risk Profile

The IRP evolved from the findings of the Swedish octogenarian (OCTO) and nonagenarian (NONA) longitudinal studies on biobehavioral aging, which began in the late 1990s and continue to generate interesting data to this day. All participants in these studies were selected on the grounds of good health (OCTO) or as a representation of the population (NONA), with around 10% of these considered to be in excellent health (18). An IRP was developed that could predict subsequent mortality during 3- and 5-yr periods. Characteristics of this IRP included an inverted cluster of differentiation 4 (CD4):CD8 ratio less than 1 caused by the expansion of CD8⁺ T cells with a late-stage differentiation phenotype (*i.e.*, CD27⁻, CD28⁻, CD57⁺, killer cell lectinlike receptor G1 (KLRG1⁺)), poor T-cell proliferative responses to mitogens *in vitro*, a low frequency of naïve T cells (*i.e.*, chemokine receptor (CCR)7⁺, CD45RA⁺, CD28⁺, CD62L⁺), and CMV seropositivity (18). The concept of the IRP has evolved during the last decade to incorporate additional immune biomarkers, most of which are shown in Figure 1.

T-cell development normally takes place within the thymus gland from bone marrow–derived progenitor cells, where they acquire a T-cell receptor (TCR) that fits the allelic major histocompatibility complex (MHC) molecules unique to the individual before entering the periphery as fully functional

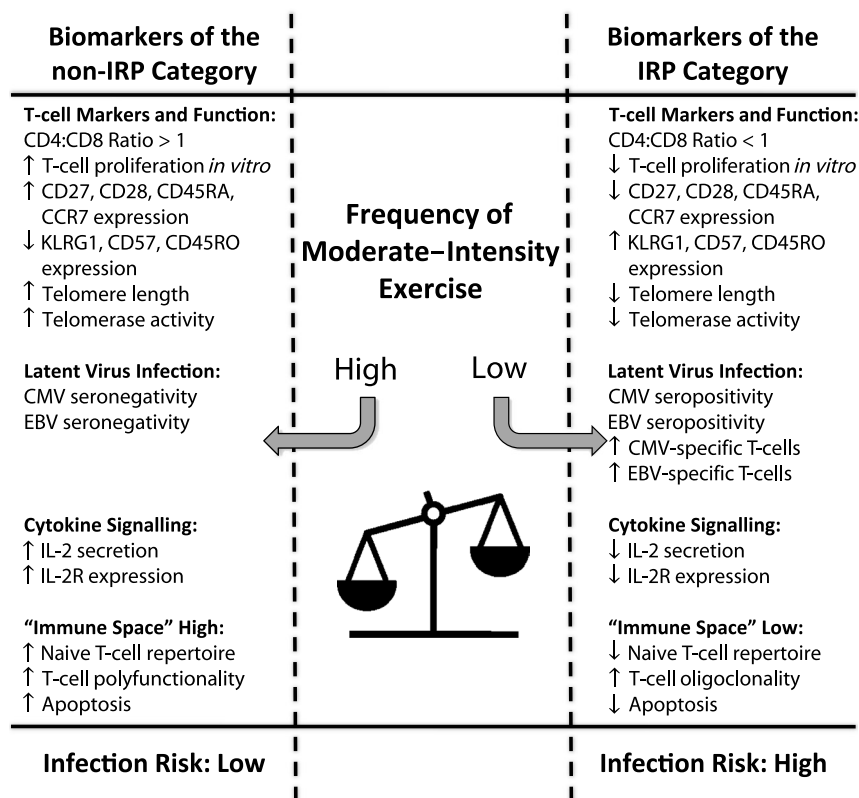


Figure 1. Hypothetical model describing how the frequency of moderate intensity exercise can influence immune system biomarkers associated with the immune risk profile (IRP). Aging and latent viral infections are associated with an increase in the number of IRP biomarkers, which in turn, is associated with increased infection risk and premature mortality. Individuals who engaged in habitual moderate intensity physical exercise throughout their lifespan are more likely to remain in the non-IRP category in later life. Conversely, sedentary individuals are more likely to enter the IRP category with aging.

naïve T cells. The thymus is responsible for replenishing the naïve T-cell compartment throughout life, thus ensuring that a diverse repertoire is maintained to help combat invading novel pathogens. Unfortunately, however, the thymus undergoes age-associated atrophy immediately from birth, the rate of which accelerates after puberty and eventually leaves very little thymic mass by the age of 50–60 yr. At this time, there is a marked decline in peripheral naïve T-cell numbers (25), indicating that the thymus is no longer maintaining effective naïve T-cell homeostasis. A consequence of this is an increased homeostatic proliferation and differentiation of resident memory cells that occupy the immune space and leave the older host at an increased risk of infection because of a severely restricted T-cell repertoire. The drastically lowered numbers of naïve T cells in the elderly severely impair their immune system to recognize and respond to novel pathogens (*i.e.*, influenza), increasing morbidity and mortality as a result of infectious disease.

T-Cell Differentiation and Senescence

The appearance of senescent T cells occurs because of excess clonal expansions that occur as part of a normal immune response to reactivating or invading pathogens throughout the lifespan. This, coupled with a reduction in newly functional naïve T cells being released from the atrophying thymus, contributes to the shrinking of the naïve T-cell repertoire (*i.e.*, lowered immune space). Antigenic stimulation causes T cells to proliferate (undergo clonal expansion via cell division) and differentiate into effector T cells that perform specialized functions, such as cytokine secretion, recognition and killing of target cells, and the activation of macrophages and antibody-producing B cells. The progressive erosion of chromosome telomeres caused by repeated rounds of cell division is one way in which proliferative arrest (senescence) is induced in T lymphocytes. Telomeres are DNA nucleoprotein complexes that form the physical ends of linear eukaryotic chromosomes. They function to protect the chromosome ends from degradation and end-to-end fusion that could potentially lead to chromosomal translocations, perturbations of cell growth, and malignancy. As a result, critically shortened telomeres trigger mechanisms for senescence, causing the cell to undergo proliferative arrest, leading to premature biological aging and impaired immunity. As such, repeated exposure to antigenic growth stimuli throughout the lifespan (*i.e.*, reactivating latent viral infections) leads to greater rounds of cell division and premature senescence. Paradoxically, telomere erosion is coupled with an increased resistance to apoptosis, allowing these senescent T cells to accumulate in the blood and tissues at the expense of naïve antigen-virgin T cells (Fig. 2).

Senescent T cells are identified in blood by their expression of the cell surface receptors CD57 and the killer cell lectinlike receptor G1 (KLRG1) and absence of the costimulatory molecule CD28 (25,26). KLRG1 inhibits clonal expansion of CD8⁺ T cells and is expressed on a population of effector-memory T cells that have previously undergone excessive rounds of cell division but are incapable of further cell division. Repeated antigenic exposure (*i.e.*, throughout the lifespan) increases the frequency of senescent T cells in blood and tissues. After the resolution of a viral infection, the nor-

mal process is for excess clones of effector T cells to die by apoptosis, although some of them become long-lived central memory cells that recirculate the tissues in the event of a subsequent encounter with the same infectious agent. However, in response to certain reactivating herpesviruses, a conundrum exists in that many excess T-cell clones do not go down the normal route of postinfection apoptosis but become part of the memory T-cell pool and exacerbate the shrinking of the naïve T-cell repertoire (Fig. 2). As these senescent T cells still retain immediate effector cell functions (*i.e.*, killing of viral-infected cells) and are highly pro-inflammatory, their accumulation in blood and tissues also might contribute to a number of pathologies associated with inflammation.

Persistent Viral Infections and the Impact of CMV

After primary infection, many viruses are capable of evading the immune system to persist in the host. In some instances, these infections become chronic (*i.e.*, human immunodeficiency virus, hepatitis C), during which there is a lack of immune containment, allowing the virus to continuously replicate and generate ubiquitous viral antigens. Other persistent infections become latent when very little viral replication occurs and almost no systemic viral antigens are present or discernible to the immune system. Many latent infections, however, have the potential to be reactivated (*i.e.*, caused by stress), which allows for increased viral replication to occur and more viral particles to be present systemically. Persistent herpesviruses, which are a large family of double-stranded DNA viruses, are believed to be major contributors to the diminution of the naïve T-cell repertoire. There are currently eight known herpesviruses that infect humans and establish lifelong latency. Although all latent herpesviruses are capable of being reactivated, it is persistent CMV infection that causes the most deleterious changes within the T-cell compartment (11). CMV is an intermittently reactivating β -herpesvirus infection that persists in cells of the myeloid lineage and epithelial cells of the salivary glands and the kidney. It infects between 45% and 75% of the Western adult population (as determined by CMV immunoglobulin G seropositivity) (5), although the incidence of infection varies with age and geographic location (5,34). Primary infection can occur in the developing fetus or from person to person through bodily fluid exchange. The virus can shed in urine, blood, tears, semen, breast milk, and saliva, allowing it to be transmitted via breast feeding, sexual contact, or blood transfusion. CMV reactivation is clinically asymptomatic in healthy hosts, although continued control of the virus requires vigilant immune surveillance and strong T-cell responses that when repeatedly stimulated, because of viral reactivation, could exhaust their proliferative capacity, leading to premature senescence (Fig. 2).

The IRP is associated with a 100% infection rate with CMV compared with 85% in age-matched subjects assigned to the non-IRP category (18). Many of the late-stage differentiated T cells in those with CMV are specific for CMV antigens, with as many as 15% of all CD8⁺ T cells in peripheral blood reacting against a single CMV epitope (5,17). Absolute T-cell numbers are 20% greater in CMV-infected compared with noninfected elderly donors to their

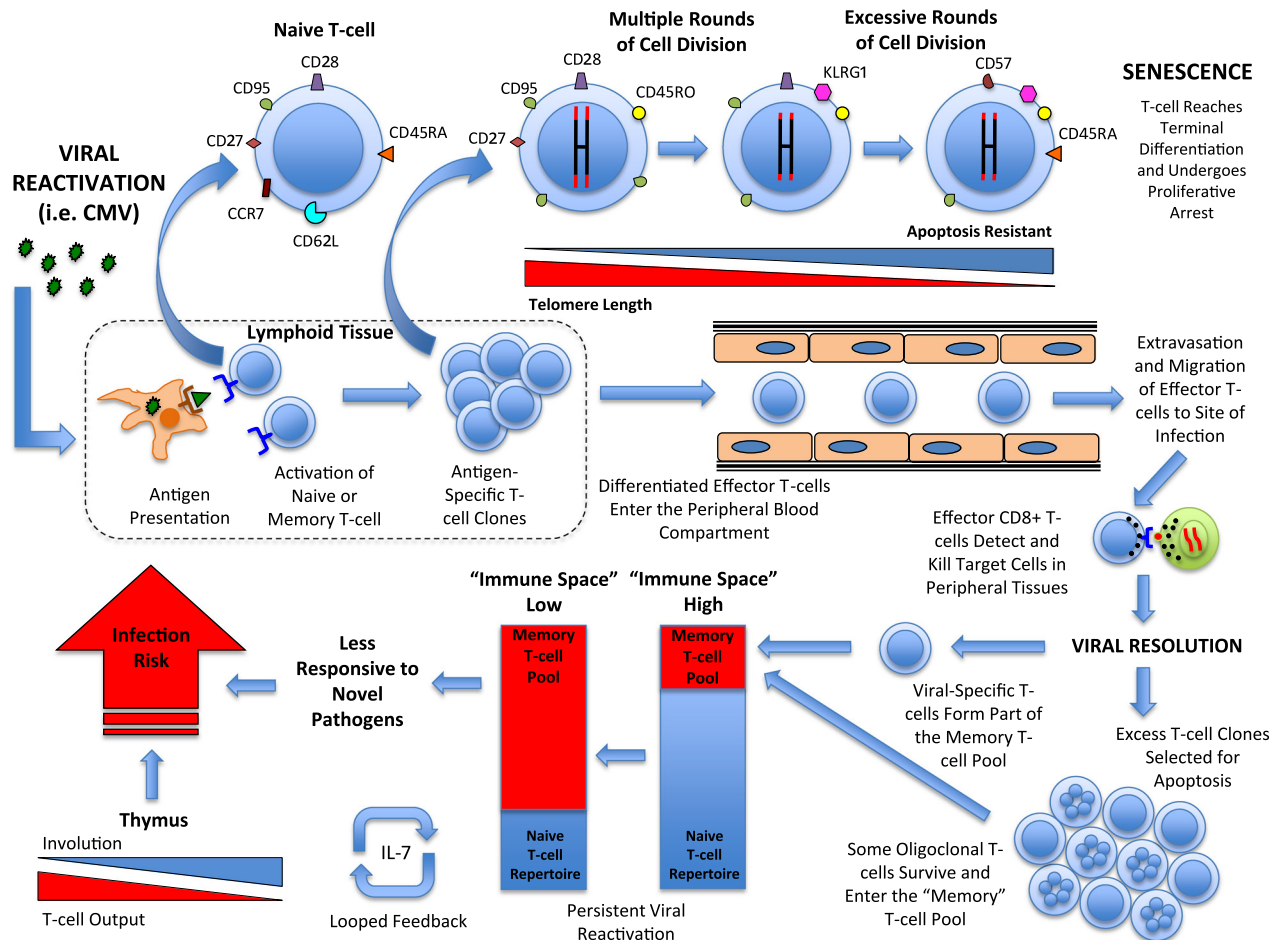


Figure 2. Immune responses to persistent viral infections lowers the immune space. naïve or memory T cells receive an antigenic stimulus in response to novel pathogen incursion or latent viral reactivation. naïve (CCR7⁺/CD27⁺/CD28⁺/CD45RA⁺/CD62L⁺) or memory (CD27⁺/CD28⁺/CD45RO⁺) CD8⁺ T cells subsequently undergo clonal expansion and differentiate into antigen-specific effector T cells. Depending on the number of cell replications that have occurred, T cells will exhibit a lowly differentiated (CD27⁺/CD28⁺/CD45RO⁺), highly differentiated (KLRG1⁺/CD27⁻/CD28⁺/CD45RO⁺), or a senescent (KLRG1⁺/CD28⁻/CD57⁺) phenotype. T cells that have undergone excess rounds of cell division have critically short telomeres, are more resistant to apoptosis (CD95^{Lo}), and undergo proliferative arrest (senescence). After clonal expansion, viral-specific CD8⁺ effector T cells migrate to sites of infection via the peripheral blood compartment and kill infected cells in the tissues. When the infection is resolved, a portion of the viral-specific effector T cells survive and become part of the memory T-cell pool, although most of the excess effector T-cell clones are selected to undergo programmed cell death (apoptosis). However, because many viral-specific terminally differentiated T cells develop an increased resistance to apoptosis, some survive and enter the memory T-cell pool via “the back door.” In response to persistent viral infections (i.e., cytomegalovirus (CMV)), the number of excess oligoclonal T-cell clones increases with each reactivation of the viruses, drastically reducing the naïve T-cell repertoire over time, manifesting as an IRP in later life. The overall effect is lowered immune responses to novel pathogens, increased infection risk, and premature mortality. Advancing the problem is the tightly controlled homeostasis of peripheral T-cell numbers by a looped feedback mechanism that involves interleukin-7 (IL-7) and the thymus. Excess T-cell numbers (caused by accumulated senescent T cells) inhibit naïve T-cell output and augments thymic involution. However, this negative feedback is reversed when peripheral T-cell numbers are low and enough thymic tissue still remains. CD, cluster of differentiation; CCR, chemokine receptor; KLRG1, killer-cell lectin-like receptor G1.

CMV-seronegative counterparts, and CMV-specific CD8⁺ T cells in older infected patients are mostly KLRG1⁺ and CD28⁻ (11), which indicates that persistent CMV infection induces senescence in CD8⁺ T lymphocytes. Conversely, most CMV-specific T cells in the young retain CD28 expression (17), indicating that these CMV-specific T cells might become senescent in later life after subsequent reactivations of the virus. As a consequence of aging, which is associated with a greater exposure to pathogens and increased frequency of viral reactivations throughout the lifespan, naïve antigen virgin T cells are gradually replaced with expanded clones of effector and effector-memory T cells that have a late-stage differentiation phenotype and limited antigenic specificity. This expansion of CMV-specific T-cell clones drastically

reduces the naïve T-cell repertoire and compromises T cell-mediated immunity to recognize and respond to novel pathogens, the resulting effect being increased morbidity and mortality mostly caused by novel pathogen incursion (i.e., influenza).

The Rationale for Making Space

The expansion of senescent CD8⁺ T-cell clones associated with persistent viral infections are believed to occupy a large proportion of the immune space that, under conditions of low pathogen exposure and fewer latent viral reactivations, would mostly be taken up by naïve T-cells. As the total number of peripheral T cells is tightly regulated, a superfluous accumulation of antigen-experienced T cells might reduce naïve T-cell

output, decreasing the number and proportion of cells capable of mounting immune responses to new pathogens. In this situation, these apoptosis-resistant and highly differentiated T cells overcrowd the immune space, resulting in a shrunken T-cell repertoire for new antigens, with an overall effect of lowered immune surveillance and increased infection risk.

Vasto *et al.* (34) reported that the immune response to CMV can account for 25% of all the CD8⁺ T-cell populations that are known to accumulate with age. Furthermore, CMV-specific CD8⁺ T cells with a senescent phenotype (*i.e.*, KLRG1⁺/CD57⁺) seem to be highly apoptosis resistant (11), suggesting that as soon as CMV-specific T cells start to occupy the immune space, removing them becomes a more difficult process. Methods to prevent, limit, or repair the damage incurred to the immune system by persistent CMV infection are currently being sought. For prevention, prophylactic vaccination and screening mothers for CMV before breast-feeding have been suggested. In those already infected, the use of antiviral drugs to reduce viral load and the targeting and deletion of KLRG1⁺CD57⁺ apoptosis-resistant viral-specific T cells by monoclonal antibody therapy have been proposed as possible methods to make space (11). This, in conjunction with other immune restoration strategies such as interleukin 7 (IL-7) therapy to increase thymic output of naïve T-cells, might help extend healthy longevity. Obvious problems with these potential interventions, however, are the costs and high-risk procedures involved, in addition to the ethical constraints of performing such invasive procedures in people who are not considered ill. We have postulated that regular physical exercise might serve as an alternative, inexpensive, and safer strategy to combat the detrimental effects of immunosenescence that are caused by frequent viral reactivations (29).

EXERCISE AND IMMUNOSENESCENCE

Despite the emergence of the IRP more than a decade ago, no study has examined the impact of regular exercise on immunity in individuals previously assigned to the IRP category. Some studies have, however, addressed some of the individual IRP biomarkers using either a cross-sectional or longitudinal experimental design involving mostly healthy but sedentary adults (29). Although it is generally accepted that habitual exercise of a moderate intensity can help prevent functional declines in systemic immunity in later life, it is not known if exercise also can help restore immune function. Cross-sectional data mostly show that IRP biomarkers (*i.e.*, T-cell responsiveness to mitogens and the naïve/memory T-cell ratio) are positively displayed in the physically active compared with the sedentary (29). Conversely, most exercise training intervention studies have found no change in similar immune parameters, thus raising concerns on the efficacy of regular exercise at restoring immune function in previously sedentary adults (29). It should be noted, however, that these studies mostly involve sedentary, albeit healthy, adults. Perhaps a more important question to ask is, can exercise restore immunity in those individuals previously identified as being at risk? Implementing an exercise training intervention in diseased cohorts or

those already assigned to the IRP category is more likely to reveal any potential immune-restorative effects of habitual exercise.

CAN EXERCISE MAKE SPACE?

We suggest that habitual physical exercise can exert its beneficial effects on aging immunity from both a preventive and a restorative mechanism. First, from a preventive perspective, regular exercise might help restrict the opportunity for latent viral reactivations and thus lower their occurrence throughout the lifespan. This may be caused by an indirect effect (*e.g.*, exercise is known to positively impact other factors that are known to cause viral reactivation such as stress) and/or a direct effect via some yet to be identified pathway. Restricting the potential for CMV reactivation with exercise would, in turn, inhibit superfluous viral-specific T cells from overcrowding the immune space. Second, from a treatment perspective, exercise may help target the dysregulated apoptosis-resistant oligoclonal T cells by mobilizing them from the peripheral tissues to a site of execution, where they subsequently undergo apoptosis, making “vacant space” for newly generated naïve T cells to take occupancy. For this process to take place, it is proposed that there are three distinct phases that must occur: 1) a selective mobilization of senescent T cells from the peripheral tissues to the blood compartment during exercise; 2) extravasation of senescent T cells from the circulation and their subsequent apoptosis in peripheral tissues during exercise recovery; 3) subsequent generation of naïve T cells to replace the deleted senescent cells. A schematic diagram of this proposed process is depicted in Figure 3, and the evidence available to support this perspective is outlined in the remaining sections of this review.

Lymphocytes and Acute Exercise

Acute exercise elicits a now stereotypical biphasic change in the total blood lymphocyte count. At the onset of exercise, there is a rapid mobilization of lymphocytes into the blood, resulting in an elevated blood lymphocyte count (lymphocytosis). The mechanisms that underpin exercise-induced lymphocytosis are fairly well characterized and appear to be governed by increased sympathetic nervous system activity and the resulting secretion of catecholamines (*i.e.*, the hormone adrenaline and the neurotransmitter noradrenaline) (1,2). In addition to exercise, lymphocytes also rapidly ingress the peripheral blood compartment in response to acute psychological stress or β -agonist infusion, and the use of nonselective β -blockers, such as propranolol, are known to blunt the lymphocytosis that occurs in response to these acute stressors — adding to the evidence that this response is regulated by adrenergic mechanisms (1,2). In particular, lymphocyte subtypes with high cytotoxic capabilities (*i.e.*, natural killer cells (NK cells), CD8⁺ T cells, $\gamma\delta$ T cells) seem to be highly stress responsive when compared with other lymphocyte subtypes (*i.e.*, CD4⁺ T cells, B cells) that have a limited cytotoxic capacity and are mobilized in relatively fewer numbers. These cytotoxic cells also express higher levels of the β_2 -adrenergic receptor and certain adhesion/activation molecules compared with the noncytotoxic cells. This allows

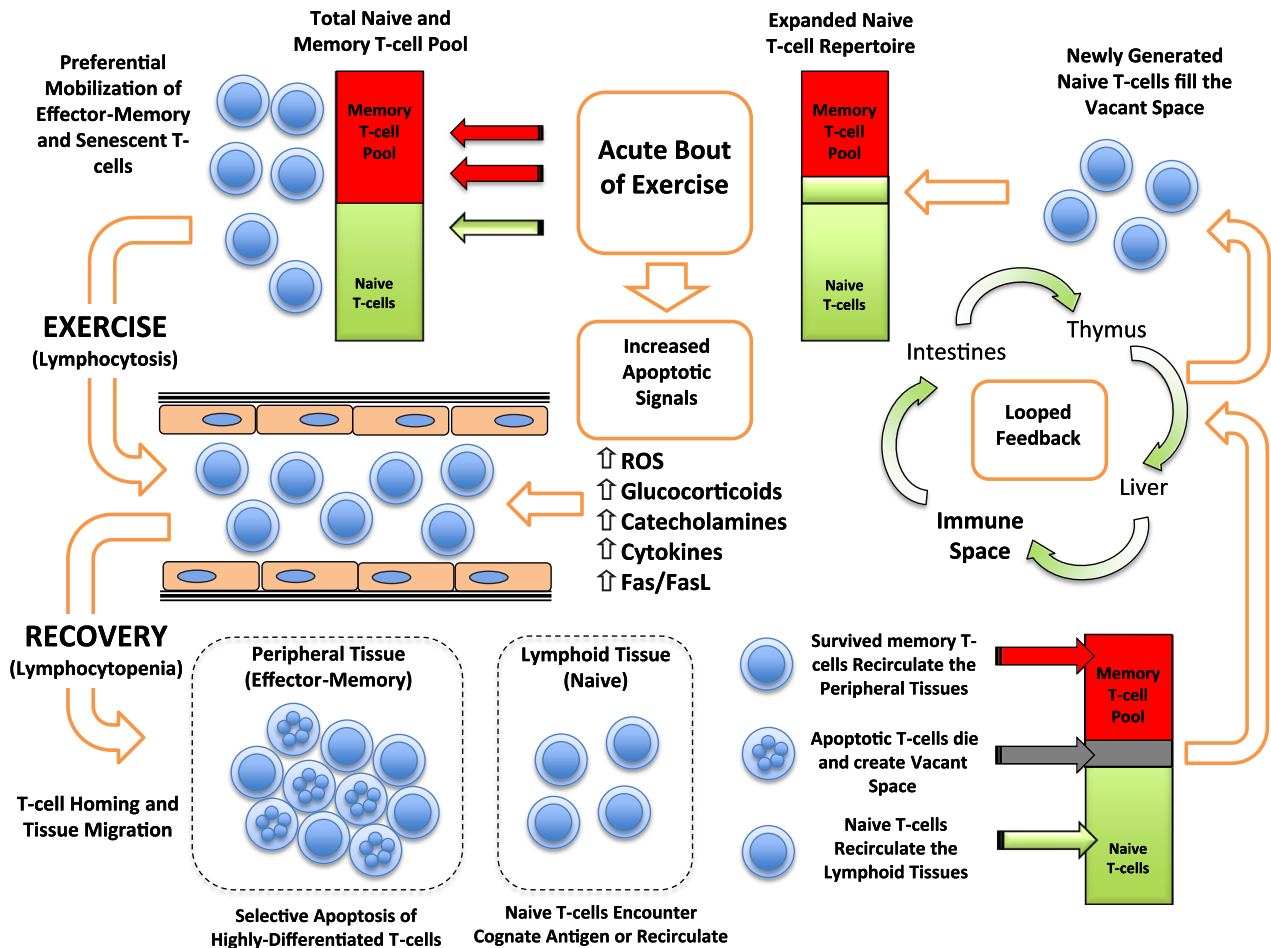


Figure 3. Hypothetical model depicting the impact of repeated bouts of acute exercise on the immune space. Acute exercise elicits the preferential mobilization of highly differentiated and viral-specific senescent T cells from the peripheral tissues into the blood compartment (lymphocytosis) under the influence of catecholamines. Exercise, in turn, increases the production of reactive oxygen species (ROS), glucocorticoids, and proinflammatory cytokines, thus exposing the senescent T cells to a milieu of proapoptotic stimuli. Cell surface death receptors (fas/fas ligand (fasL)) are upregulated on senescent T cells, which also incur mild oxidative DNA damage in the blood. These apoptosis-susceptible cells, along with undamaged naïve and memory T cells, egress the blood compartment during the recovery phase of exercise (lymphocytopenia) and migrate to specific tissues. A portion of these senescent T cells subsequently undergo apoptosis in the peripheral tissues, thus creating vacant space. Consequently, lowered T-cell numbers drive the positive feedback loop, increasing naïve T-cell output from the thymus or sites of extrathymic T-cell development (*i.e.*, liver, intestines). These newly generated T cells fill the vacant space and contribute to an expanded naïve T-cell repertoire. Repetitions of this process in response to habitual exercise reduce the frequency of senescent T cells over time, lowering infection risk and increasing healthy longevity.

them to bind to adrenaline or noradrenaline and preferentially ingress the blood compartment in response to acute stress. The reasons for this preferential mobilization of specific lymphocyte subtypes are not fully understood, but it has been postulated that the ingress of these potent cytotoxic cells occurs because of increased immunosurveillance requirements in certain tissues after periods of acute stress (1,2,33).

During the early stages of exercise recovery (usually within 30–60 min after exercise cessation), the blood lymphocyte count is known to fall below resting values (lymphocytopenia) (24–28,33) and, in response to a very demanding exercise, also below clinically lower limits (28). The mechanisms for this are not as well understood (discussed later in this article) as those that underpin lymphocytosis but increased hypothalamic-pituitary-adrenal axis activity and the subsequent secretion of cortisol are believed to play a role (6). Furthermore, although the blood lymphocyte count normally returns to resting values within 6–24 h after cessation of

exercise, the cells and tissues responsible for this restoration of the blood lymphocyte count also are not known.

Effector-Memory and Senescent T-Cell Mobilization

Up until recently, data on the differentiated T-cell subsets mobilized by acute exercise were limited to the surface expression of CD45RA and CD45RO as crude markers of naïve and memory T cells, respectively. We were the first to show that acute exercise elicits a preferential mobilization of effector-memory CD8⁺ T-cell subsets expressing the markers of senescence KLRG1 and CD57 on their surface, indicating that highly differentiated and senescent T cells are more responsive to acute physical stress than naïve or lowly differentiated T cells (*i.e.*, CD28⁺) (26). We also found that these cell populations preferentially egress the blood during the recovery phase of exercise as the proportions of KLRG1- and CD57-positive cells among CD8⁺ T cells fell below baseline 1 h after exercise cessation (26). After this work, we found that

older adults (aged 50–60 yr) had a greater proportion of KLRG1⁺, CD57⁺, and CD28⁻ cells among the total CD8⁺ T-cell population compared with the young (aged 19–24 yr), at rest and immediately after exercise, and that both age groups preferentially mobilized these cells into the blood compartment in response to acute exercise (25). As senescent T cells are known to preferentially recirculate peripheral tissues (15), it was concluded from this observation that the greater frequency of senescent T cells in the old is probably not localized to the peripheral blood compartment but also is representative of other bodily tissues (25).

More recently, the ability of T cells at different stages of differentiation to respond to an acute stressor has gained interest. After an antigenic stimulus, naïve T cells differentiate into memory and effector cells, and their stage of differentiation can be determined via their cell surface phenotypes (Fig. 1). T cells are typically stratified by assessing combinations of CD45RA and CCR7 surface expression: naïve (CD45RA⁺CCR7⁺), central memory ((CM) CD45RA⁻CCR7⁺), effector-memory ((EM) CD45RA⁺CCR7⁻), and terminally differentiated effector memory ((TEMRA) CD45RA⁺CCR7⁻) T cells (21). In response to acute exercise, Campbell *et al.* (4) recently showed that there is a preferential mobilization of TEMRA and EM CD8⁺ T cells into the blood compartment compared with naïve and CM cells. Exercise performed at a higher intensity (85%) was found to mobilize greater numbers of TEMRA and EM CD8⁺ T cells compared with exercise at the lower (35%) intensity (4). However, although CD45RA and CCR7 are useful markers of T-cell differentiation, identifying CD45RA⁺/CCR7⁻ T cells as a terminally differentiated (*i.e.*, senescent) subset is contentious because of the fact that cells with this phenotype still demonstrate efficient levels of proliferation after TCR activation (7). At present, the combined expression of KLRG1 and CD57 or the expression of KLRG1 in the absence of CD28 appears to be more accepted markers of T-cell senescence, because cells with this phenotype are incapable of further division, lack expression of CD27 and CCR7,

and have a low expression of CD127 (10). Furthermore, KLRG1 seems to be a reliable marker of a T cell with antigen specificity to a chronic, latent, or active virus because more than 92% of CMV, EBV, and HIV-specific T cells express KLRG1, whereas T cells specific to influenza (a resolved infection that does not establish latency) express KLRG1 at much lower frequencies (40%–73%) (10).

We propose that the combined expression of KLRG1, with either CD28 or CD57, may be used to identify naïve, memory (highly differentiated), and senescent (terminally differentiated) T cells among CD8⁺ T cells in human blood (Fig. 4). Using this phenotype, we sought to confirm if the KLRG1⁺ T cells preferentially mobilized with exercise were a terminally differentiated or a highly differentiated memory subset (24). Although KLRG1⁺/CD57⁻ CD8⁺ T cells (effector cells destined to be long-lived memory cells) were mobilized into the blood with exercise, the relative change was significantly less (1.3 ± 0.4-fold increase) than that observed for KLRG1⁺/CD57⁺ senescent T cells (1.9 ± 0.6-fold increase) (24). These data, in conjunction with the findings of Campbell *et al.* (4), indicate that the exercise responsiveness of CD8⁺ T-cell subsets is governed by their stage of differentiation and, most likely, also by adrenergic pathways. Indeed, studies incorporating acute psychological stress (*i.e.*, public-speaking tasks) and β-adrenergic agonist infusion models have reported similar effects with regard to the mobilization of highly differentiated T cells (1,2). The preferential mobilization of T cells with a highly differentiated phenotype might be explained by altered expression of the β₂-adrenergic receptor. Microarray analysis has shown a sevenfold and fivefold up-regulation in the β₂-adrenergic receptor gene (*ADRB2*) in TEMRA and CM cells, respectively, when compared with naïve T cells (20). Moreover, senescent T-cells (*i.e.*, KLRG1⁺/CD28⁻) are known to have formidable cytotoxic capabilities and express far greater levels of cytolytic granules such as perforin and granzyme B compared with memory (*i.e.*, KLRG1⁺/CD28⁺) or naïve (*i.e.*, KLRG1⁻/CD28⁺) T cells (10), supporting the idea that CD8⁺ T cells with high

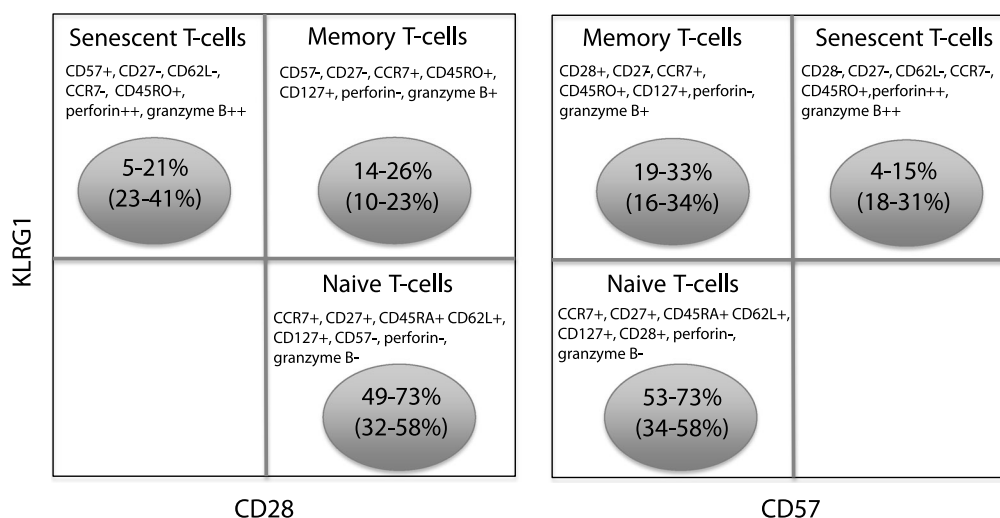


Figure 4. Identifying “naïve” (KLRG1⁻/CD28⁺/CD57⁻), “memory” (KLRG1⁺/CD28⁺/CD57⁻), and senescent (KLRG1⁺/CD28⁻/CD57⁺) CD3⁺/CD8⁺ T cells in human blood by combinations of KLRG1/CD28 or KLRG1/CD57 surface expression. Percentage values are those typically found in CMV-seronegative healthy adult males aged 20–35 yr. Values in parentheses are those from CMV-seropositive males of the same age range (23). + indicates positive; -, negative; ++, highly expressed. CD, cluster of differentiation; KLRG1, killer-cell lectin-like receptor G1; CMV, cytomegalovirus.

effector capabilities are preferentially mobilized in response to acute exercise.

The Impact of Latent Viral Infections

It is now well established that latent viral infections, particularly CMV, are associated with a greater frequency of highly differentiated and senescent T cells in the periphery. Given that these cell types are preferentially mobilized into the blood compartment in response to acute exercise, we hypothesized that CMV serostatus would impact on the mobilization of highly differentiated and senescent T cells with exercise (23,29). This also has been of interest to Bosch and colleagues, who recently showed that young CMV-seropositive subjects demonstrated a near twofold higher ingress and subsequent egress (during the early stages of exercise recovery) of CD8⁺ T cells compared with their non-infected counterparts in response to 60 min of treadmill running (33). This amplified response caused by CMV was largely explained by the greater frequency of highly differentiated and effector-memory T cells in the infected participants, who demonstrated a greater ingress and subsequent egress of CD45RA⁻/CD27⁻ (EM) and CD45RA⁺/CD27⁻ CD8⁺ (highly differentiated EM) T cells because of exercise (33). Although this study clearly demonstrated the influence of CMV status on the exercise-induced mobilization of T cells with specific phenotype characteristics, a limitation was the failure to confirm that the greater number of EM T cells mobilized in the CMV-seropositive subjects was caused by an influx of cells specific to the virus. Using MHC class I pentamers (synthetic human leukocyte antigen molecules) that are labeled with an antigenic peptide, we found that acute exercise-mobilized viral-specific CD8⁺ T cells in both CMV- and EBV-infected participants (23). The numbers of pp65⁺ (CMV epitope) and LMP-2 (EBV epitope) cells within the CD8⁺ T-cell compartment was 158% and 200% greater than the preexercise values, respectively (23). We also found a greater relative egress of senescent (KLRG1⁺/CD57⁺/CD28⁻) CD8⁺ T-cells within 1 h of exercise recovery in those infected with CMV (23). This might indicate that CMV infection, in addition to increasing the frequency of senescent T cells, also could impact on their tissue migrating potential in response to an acute stressor.

Taken together, it is well established that acute exercise elicits a preferential mobilization of effector-memory and senescent T cells compared with naïve T cells, which are mobilized in fewer numbers. Some of these T cells also are specific to CMV and EBV antigens, indicating that exercise evokes the mobilization of viral-specific CD8⁺ T cells. These potent cytotoxic cells that have limited antigen specificity are the same cell types that are believed to overcrowd the immune space because of aging and persistent viral infections (particularly CMV) (25,26). Although this is advocated to be an evolutionary response to fight-or-flight situations that might facilitate a swifter immunologic riposte to pathogen incursion in the event of tissue injury (2), we contend that frequent T-cell shifts with acute exercise also could have accumulative long-term restorative effects on systemic immunity. Although the present article proposes that the mobilization of senescent viral-specific T cells in response to frequent bouts of acute exercise could be the first step toward

an expanded naïve T-cell repertoire, fully ascertaining the fate of these mobilized cells after their egress from the blood is required before this hypothesis can be accepted.

CAN EXERCISE DELETE SENESCENT T CELLS?

Exercise-Induced Lymphocytopenia

During the last decade, identifying a mechanism to explain the consistently observed effect of exercise-induced lymphocytopenia has been of interest to those working in the exercise and stress immunology field (14,27,33). After the initial report by Mars *et al.* (14), lymphocyte cell death by apoptosis was considered a primary candidate, but this is now uncertain because of the number of recent studies that have documented a lymphocytopenia in response to acute exercise without any concomitant evidence of blood lymphocyte apoptosis (27). A more accepted mechanism for this lymphocytopenia is a selective extravasation of specific lymphocyte subsets, leaving the blood compartment to recirculate the peripheral tissues (13,28). Indeed, it is known that lymphocytes that preferentially egress the peripheral blood compartment (*i.e.*, CD8⁺ T cells, NK cells) have a heightened expression of certain cell surface activation and adhesion molecules that facilitate their transmigration (28), allowing them to pass through adjacent endothelial cells and into the tissues. Animal studies indicate that the extravasated lymphocytes migrate to peripheral tissues such as the lungs and intestinal Peyer patches after exercise (12,13), presumably as part of an increased immunosurveillance response to acute stress. Indeed, adrenergic mechanisms are purported to play a role as adrenaline infusion partially mimicked the T-cell migratory responses to exercise (13). However, the role of cortisol in exercise-induced lymphocytopenia is more perplexing than first thought. Although infusing cortisol in humans elicits a lymphocytopenia, this tends to induce a selective decline in the numbers of naïve T cells that display a lymphoid-homing phenotype (6), which is in contrast to the cell types that preferentially egress the blood during the recovery phase of exercise (*i.e.*, effector-memory and senescent T cells). In our studies, the proportions of KLRG1⁺, CD57⁺, and CD28^{null} CD8⁺ T cells was lower than baseline at 1 h postexercise, indicating that the exercise-induced lymphocytopenia is caused by a preferential egress of T cells with an effector-memory or terminally differentiated phenotype (25,26). This was investigated in more detail by Turner *et al.* (33), who reported a 60% decrease in late differentiated effector/memory cells compared with a 29% decrease in naïve T cells within 1–2 h after a 60-min treadmill running protocol. Although acute exercise elicits the preferential extravasation of highly differentiated and senescent T cells, the homing destination of these T cells is not known, nor is the fate of these cells when they reach the peripheral tissues after their egress from the blood.

T-Cell Apoptosis and Exercise

Although lymphocytes do not appear to undergo apoptosis in the bloodstream (27), the potential for specific lymphocyte subsets to undergo activation-induced cell death in the peripheral tissues after their egress from the blood is a distinct

possibility. This is supported by mouse exercise models that have shown T cells mobilized by acute exercise to preferentially migrate toward intestinal Peyer patches (12,13), and that acute exercise, in turn, causes intestinal lymphocyte apoptosis (9,12). This contention is bolstered by a large number of human studies that have documented blood lymphocyte DNA damage, increased oxidative stress-induced apoptosis, and changes in cell surface death receptors after a single bout of acute exercise. Blood lymphocytes are exposed to a milieu of proapoptotic signals during exercise that include increased levels of glucocorticoids, catecholamines, inflammatory cytokines, and reactive oxygen species (Fig. 3), which could elicit changes at the molecular level, leaving certain T-cell populations more susceptible to both intrinsic and extrinsic pathways of apoptosis after their egress from the blood. Cell signaling via the cell surface death receptor CD95 (Fas/Apo-1) and its ligand (CD95L) is capable of initiating caspase-8 that leads to DNA fragmentation and subsequent apoptosis. Changes in the surface expression of CD95 in blood lymphocytes after exercise have been reported, indicating that exercise might alter T-cell apoptosis susceptibility. The involvement of the CD95 pathway has been confirmed in a recent animal study. Kruger *et al.* (12) reported increased T-cell apoptosis in spleen, bone marrow, lung, and Peyer patches after exercise in mice. The percentage of lymphocytes positive for CD95 and CD95L was elevated in Peyer patches, whereas CD95⁺ and CD95L⁺ cells also were increased in the lymph nodes and lung, respectively. Using CD95-deficient MRL/pr-mice, the exercise-induced apoptosis of T cells was prevented in the spleen, lung, bone marrow, and lymph nodes but not in Peyer patches (12), indicating that CD95 signaling is involved in the regulation of T-cell apoptosis in certain tissues after acute exercise. In humans, acute exercise also has been shown to activate caspase-8 and caspase-9 in blood lymphocytes without evidence of apoptosis, further indicating that lymphocytes that egress the blood during the recovery phase of exercise could be destined for subsequent apoptosis in the peripheral tissues (35).

Acute bouts of high-intensity exercise also are known to cause mild DNA damage in blood lymphocytes. Using single-cell gel electrophoresis assays, many studies have documented single-strand DNA damage in as many as 30% of blood lymphocytes immediately after an acute bout of exercise (14). Fragmentation of nuclear DNA with exercise, probably via mechanisms linked to oxidative stress, could provoke apoptosis of the extravasated lymphocytes. Only Wang and Lin (35) have investigated the susceptibility of human blood lymphocytes mobilized by exercise to undergo apoptosis. Believing this response to be driven by reactive oxygen species (ROS), it was found that lymphocytes obtained after an acute bout of intensive exercise are more susceptible to *in vitro* apoptosis when stimulated with physiological doses of the prooxidant hydrogen peroxide (H₂O₂) (35).

From these findings, it is suggested that blood lymphocytes mobilized with exercise are more sensitive to both intrinsic and extrinsic pathways of apoptosis without actually undergoing programmed cell death in the blood compartment. This process might be caused by the proapoptotic stimuli induced by exercise impacting on circulating T cells and triggering early apoptotic pathways before the cell leaves the blood

compartment. As lymphocytes are known to egress the blood during the recovery phase of exercise, a proportion of these extravasated cells (having already encountered a proapoptotic stimulus) might subsequently die by apoptosis in peripheral tissues. One notable limitation of the current literature is that little attempt has been made to document exercise-induced DNA damage or apoptosis of specific lymphocyte subset populations (*i.e.*, T cells stratified by their stage of differentiation). We do not know therefore if certain lymphocyte subtypes, T cells specific to certain viruses (*i.e.*, CMV or EBV), or T-cell subsets at a particular stage of differentiation have an increased susceptibility to exercise-induced DNA damage or apoptosis after exercise. Until this is established, it will be difficult to determine whether exercise-induced apoptosis is having a positive (*i.e.*, removal of dysregulated excess oligoclonal or damaged T cells) or a negative (*i.e.*, deletion of healthy autologous cells) effect on the immune system.

One approach that could be taken for future studies is to combine the recent work of Kruger *et al.* (12,13), in which lymphocyte trafficking and death were quantified in peripheral tissues of mice after exercise, with the classic approach of Ottaway and Parrott (16), in which lymphoid cell migration kinetics in rodents were quantified *in vivo* by biomathematical modeling. The use of biomathematics to develop *in silico* models that predict senescent T-cell migration, their accumulation in the tissues, and removal from the T-cell repertoire (*i.e.*, caused by apoptosis) in response to exercise would greatly facilitate the current exercise and immunosenescence literature and serve as a way to quantify and accurately describe the theoretical framework presented in this article (Fig. 3).

Apoptosis of Senescent T Cells

Whereas it is suggested that lymphocytes mobilized by exercise are at an increased susceptibility to programmed cell death, the pertinent question is: can exercise target the dysregulated terminally differentiated T cells that are specific for CMV and other latent viruses and purge them from the T-cell repertoire to make space? In support of this proposition, it has been shown that CD8⁺ T cells defined by CD57 expression are more susceptible to anti-CD3-induced apoptosis *in vitro* (3,22). Conversely, CD28^{null} blood T cells (which are mostly CD57⁺) have been shown to robustly resist programmed cell death when stimulated with an array of apoptotic stimuli, including anti-CD95, galectin-1, IL-2 withdrawal, and moderate heat shock (30), indicating that the susceptibility of senescent T cells to apoptosis is pathway dependent. Much of the available evidence suggests that exercise is likely to induce apoptosis via mechanisms related to oxidative stress; however, no study to date has examined the apoptosis susceptibility of naïve, effector-memory, or terminally differentiated T-cell subtypes in response to acute exercise. Wang and Lin (35) showed that CD57⁺ and KLRG1⁺ lymphocytes were more susceptible to H₂O₂-induced apoptosis *in vitro* compared with lymphocytes expressing CD28, CD62L, or CD11a. Unfortunately, however, they did not explore this in an exercise context and failed to differentiate cell death of CD57⁺ and KLRG1⁺ T cells from NK cells that also express these surface receptors.

Takahashi *et al.* (31) did, however, show that EM T cells (CD45RA⁻/CCR7⁻) were more susceptible to H₂O₂-induced apoptosis than naïve T cells (CD45RA⁺/CCR7⁺), and that a pan-caspase inhibitor was able to rescue cells from death, indicating that mitochondrial apoptotic pathways are involved (31). Taken together, these data show promise that senescent T cells might be less tolerant to oxidative stress-induced apoptosis in response to exercise, although future studies are required to confirm this. In addition, the susceptibility of specific T-cell subpopulations to undergo apoptosis after exercise should be examined in relation to latent viral infections, as well as exploring the involvement of different apoptosis-inducing pathways. Given the known detrimental effects of persistent reactivating infections on T-cell immunity, individuals carrying latent infections might benefit more from the potential regulatory effects of exercise on T-cell homeostasis.

CAN EXERCISE EXPAND THE NAÏVE T-CELL REPERTOIRE?

Increased Thymic Output

One way in which accumulative bouts of acute exercise might facilitate an expanded naïve T-cell repertoire is to stimulate thymopoiesis, allowing naïve antigen-virgin cells to replace any senescent T cells that have undergone exercise-induced apoptosis. It is proposed that the homeostatic control of peripheral T-cell numbers is tightly regulated by a negative-positive feedback loop, which governs the release rate of new thymic emigrants into the periphery. When peripheral T-cell numbers are high (*i.e.*, because of accumulated senescent cells), negative feedback ensures a lowered output of new thymic emigrants (and also possibly induces thymic atrophy), further contributing to the shrinking of the naïve T-cell repertoire. Conversely, when peripheral T-cell numbers are lowered (*i.e.*, because of apoptosis of senescent T cells), positive feedback ensures an increased output of new thymic emigrants, thus contributing to an expanded naïve T-cell repertoire (Fig. 1). The exercise-induced lymphocytopenia and subsequent apoptosis of terminally differentiated T cells in peripheral tissues could be one way in which to drive this positive feedback loop, increasing thymic output as an adaptation to habitual exercise.

Any thymopoiesis induced by exercise is likely to involve IL-7, which is required for intrathymic T-cell development, increasing thymopoiesis and contributing to homeostatic rotation of naïve and memory T cells in the periphery (8). Indeed, IL-7 cytokine therapy has been shown to increase both thymic mass and the number of peripheral recent thymic emigrants in mice and humans (8). Serum levels of IL-7 are known to be elevated in states of chronic lymphocytopenia, further emphasizing the importance of this cytokine as a regulator of peripheral T-cell homeostasis. The possibility that transient periods of lymphocytopenia caused by exercise could stimulate IL-7 release and subsequent thymopoiesis have not been examined. Moreover, most studies report that peripheral blood lymphocyte counts are restored to resting values within 6–24 h after exercise cessation, but we do not know if this is caused by a resurgence of the previously vacated cells or a

replacement of these cells with T cells of different phenotype characteristics and levels of antigen specificity. The possibility that dysregulated T cells previously purged from the repertoire are subsequently replaced with recent thymic emigrants in response to exercise should be explored, as this will have important implications to our understanding of how regular exercise helps shape the aging immune system.

Extrathymic T-Cell Development

Naïve T cells are long-lived resting cells and, at least in the young, their rate of attrition is matched by the output of new thymic emigrants, thus maintaining homeostasis of the naïve T-cell repertoire. However, as previously stated, it is well known that the thymus undergoes age-associated involution, resulting in progressively fewer numbers of new thymic emigrants with increasing age. Therefore, even if exercise was able to drive this hypothetical positive feedback loop, it would be in vain if there was insufficient thymic tissue remaining to generate sufficient numbers of newly functional T cells. A growing body of evidence supports the concept of extrathymic T-cell maturation, involving tissues such as the liver and the mucosal epithelium of the small intestines. In adult humans previously thymectomized as neonates during open heart surgery, CD4⁺ T cells are lower yet CD8⁺ T-cell counts remain similar to healthy controls (19), suggesting that extrathymic T-cell development might play a role in the maintenance of the CD8⁺ T-cell compartment to partly compensate the diminished thymic output. As such, expanding the naïve T-cell repertoire in the elderly might be dependent on extrathymic T-cell recruitment. In the presence of an unrestorable thymus, this could be the pathway by which exercise expands the naïve T-cell repertoire in the elderly and those carrying a latent CMV infection, however, this awaits investigation.

SUMMARY

Aging and persistent viral infections (particularly latent CMV infection) are major contributors to immunosenescence and the associated IRP. Although it has been shown, both epidemiologically and experimentally, that individuals who engage in habitual physical exercise of a moderate intensity exhibit a lower frequency of immune biomarkers associated with the IRP, the precise mechanisms by which regular exercise exerts its positive effects on immunity are still unknown. This article proposes a theoretical framework to explain how frequent bouts of acute exercise might create immune space by removing excess clones of antigen-specific terminally differentiated T cells and potentially restore the detrimental effects of aging and persistent viral infections on T cell-mediated immunity (Fig. 3). Although we postulate that this process performed repeatedly because of habitual physical exercise might blunt, or even restore, the diminution of the naïve T-cell repertoire that is associated with age and persistently reactivating viral infections, it is acknowledged that many future experimental studies are required before this hypothesis can be accepted. To corroborate this theory, it is essential for future research to determine the susceptibility of dysregulated viral-specific and senescent T cells to undergo

apoptosis in response to exercise and whether exercise can restore thymic output or stimulate extrathymic T-cell maturation, particularly in the elderly and those with persistent CMV infections. Moreover, the development of biomathematical models to estimate the kinetics of senescent T-cell migration and their retention and turnover in peripheral tissues in response to exercise would be illuminating. Such studies are required to determine if exercise can indeed make space and contribute to immune restoration and healthy longevity in the presence of persistent viral infections.

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References

- Anane LH, Edwards KM, Burns VE, et al. Mobilization of gammadelta T lymphocytes in response to psychological stress, exercise, and beta-agonist infusion. *Brain Behav. Immun.* 2009; 23:823–9.
- Atanackovic D, Schnee B, Schuch G, et al. Acute psychological stress alerts the adaptive immune response: stress-induced mobilization of effector T cells. *J. Neuroimmunol.* 2006; 176:141–52.
- Brenchley JM, Karandikar NJ, Betts MR, et al. Expression of CD57 defines replicative senescence and antigen-induced apoptotic death of CD8⁺ T cells. *Blood.* 2003; 101:2711–20.
- Campbell JP, Riddell NE, Burns VE, et al. Acute exercise mobilises CD8⁺ T lymphocytes exhibiting an effector-memory phenotype. *Brain Behav. Immun.* 2009; 23:767–75.
- Colonna-Romano G, Akbar AN, Aquino A, et al. Impact of CMV and EBV seropositivity on CD8 T lymphocytes in an old population from West-Sicily. *Exp. Gerontol.* 2007; 42:995–1002.
- Dimitrov S, Benedict C, Heutling D, Westermann J, Born J, Lange T. Cortisol and epinephrine control opposing circadian rhythms in T cell subsets. *Blood.* 2009; 113:5134–43.
- Dunne PJ, Faint JM, Gudgeon NH, et al. Epstein-Barr virus-specific CD8(+) T cells that re-express CD45RA are apoptosis-resistant memory cells that retain replicative potential. *Blood.* 2002; 100:933–40.
- Fry TJ, Mackall CL. The many faces of IL-7: from lymphopoiesis to peripheral T cell maintenance. *J. Immunol.* 2005; 174:6571–6.
- Hoffman-Goetz L, Quadrilatero J. Treadmill exercise in mice increases intestinal lymphocyte loss via apoptosis. *Acta Physiol. Scand.* 2003; 179: 289–97.
- Ibegbu CC, Xu YX, Harris W, Maggio D, Miller JD, Kourtis AP. Expression of killer cell lectin-like receptor G1 on antigen-specific human CD8⁺ T lymphocytes during active, latent, and resolved infection and its relation with CD57. *J. Immunol.* 2005; 174:6088–94.
- Koch S, Larbi A, Ozelik D, et al. Cytomegalovirus infection: a driving force in human T cell immunosenescence. *Ann. N. Y. Acad. Sci.* 2007; 1114:23–35.
- Kruger K, Frost S, Most E, Volker K, Pallauf J, Mooren FC. Exercise affects tissue lymphocyte apoptosis via redox-sensitive and Fas-dependent signaling pathways. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2009; 296:R1518–27.
- Kruger K, Lechtermann A, Fobker M, Volker K, Mooren FC. Exercise-induced redistribution of T lymphocytes is regulated by adrenergic mechanisms. *Brain Behav. Immun.* 2008; 22:324–38.
- Mars M, Govender S, Weston A, Naicker V, Chuturgoon A. High intensity exercise: a cause of lymphocyte apoptosis? *Biochem. Biophys. Res. Commun.* 1998; 249:366–70.
- Masopust D, Vezyz V, Marzo AL, Lefrancois L. Preferential localization of effector memory cells in nonlymphoid tissue. *Science.* 2001; 291: 2413–7.
- Ottaway CA, Parrott DMV. A method for the quantitative analysis of lymphoid cell migration experiments. *Immunol. Lett.* 1981; 2:283–90.
- Ouyang Q, Wagner WM, Voehringer D, et al. Age-associated accumulation of CMV-specific CD8⁺ T cells expressing the inhibitory killer cell lectin-like receptor G1 (KLRG1). *Exp. Gerontol.* 2003; 38:911–20.
- Pawelec G, Larbi A, Derhovanessian E. Senescence of the human immune system. *J. Comp. Pathol.* 2010; 142(Suppl. 1):S39–44.
- Prelog M, Keller M, Geiger R, et al. Thymectomy in early childhood: significant alterations of the CD4(+)CD45RA(+)CD62L(+) T cell compartment in later life. *Clin. Immunol.* 2009; 130:123–32.
- Riddell NE, Wallace GR, Van Stijn A, et al. Selective β -adrenergic receptor expression on human memory CD8⁺ T-lymphocyte subsets regulates mobilization and INF- γ production. In: The 9th World Congress on Inflammation, Tokyo, Japan; 2009, p. Mini Paper 7.
- Sallusto F, Lenig D, Forster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature.* 1999; 401:708–12.
- Shinomiya N, Koike Y, Koyama H, et al. Analysis of the susceptibility of CD57 T cells to CD3-mediated apoptosis. *Clin. Exp. Immunol.* 2005; 139:268–78.
- Simpson RJ, Bartlett DB, Spielmann G, McFarlin BK. Viral-specific CD8⁺ T-cells are mobilised into the peripheral blood in response to acute aerobic exercise in humans. In: 9th Symposium of the International Society of Exercise and Immunology, Tübingen, Germany; 2009, p. 49.
- Simpson RJ, Cosgrove C, Chee MM, et al. Senescent phenotypes and telomere lengths of peripheral blood T-cells mobilized by acute exercise in humans. *Exerc. Immunol. Rev.* 2010; 16:36–51.
- Simpson RJ, Cosgrove C, Ingram LA, et al. Senescent T-lymphocytes are mobilised into the peripheral blood compartment in young and older humans after exhaustive exercise. *Brain Behav. Immun.* 2008; 22:544–51.
- Simpson RJ, Florida-James GD, Cosgrove C, et al. High-intensity exercise elicits the mobilization of senescent T lymphocytes into the peripheral blood compartment in human subjects. *J. Appl. Physiol.* 2007; 103: 396–401.
- Simpson RJ, Florida-James GD, Whyte GP, Black JR, Ross JA, Guy K. Apoptosis does not contribute to the blood lymphocytopenia observed after intensive and downhill treadmill running in humans. *Res. Sports Med.* 2007; 15:157–74.
- Simpson RJ, Florida-James GD, Whyte GP, Guy K. The effects of intensive, moderate and downhill treadmill running on human blood lymphocytes expressing the adhesion/activation molecules CD54 (ICAM-1), CD18 (beta2 integrin) and CD53. *Eur. J. Appl. Physiol.* 2006; 97:109–21.
- Simpson RJ, Guy K. Coupling aging immunity with a sedentary lifestyle: has the damage already been done? — a mini-review. *Gerontology.* 2010; 56:449–58.
- Spaulding C, Guo W, Effros RB. Resistance to apoptosis in human CD8⁺ T cells that reach replicative senescence after multiple rounds of antigen-specific proliferation. *Exp. Gerontol.* 1999; 34:633–44.
- Takahashi A, Hanson MG, Norell HR, et al. Preferential cell death of CD8⁺ effector memory (CCR7⁻CD45RA⁻) T cells by hydrogen peroxide-induced oxidative stress. *J. Immunol.* 2005; 174:6080–7.
- Targonski PV, Jacobson RM, Poland GA. Immunosenescence: role and measurement in influenza vaccine response among the elderly. *Vaccine.* 2007; 25:3066–9.
- Turner JE, Aldred S, Witard O, Drayson MT, Moss PM, Bosch JA. Latent cytomegalovirus infection amplifies CD8 T-lymphocyte mobilisation and egress in response to exercise. *Brain Behav. Immun.* 2010; 24:1362–70.
- Vasto S, Colonna-Romano G, Larbi A, Wikby A, Caruso C, Pawelec G. Role of persistent CMV infection in configuring T cell immunity in the elderly. *Immun. Ageing* 2007; 4:2.
- Wang JS, Lin CT. Systemic hypoxia promotes lymphocyte apoptosis induced by oxidative stress during moderate exercise. *Eur. J. Appl. Physiol.* 2010; 108:371–82.