Reversing Age-Associated Immunosenescence via Exercise

Running Title: Immunosenescene and exercise

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Abstract

Decreases in immune responsiveness with age are thought to contribute to the increased incidence and severity of infectious disease among the elderly. Several interventions, including exercise, have been proposed to restore immune function in older populations. The findings from some, but not all studies, support the possibility that exercise may attenuate immunosenescence. In recent years, the role of exercise in modulating immune response has been examined using models that may have clinical relevance, such as the response to vaccines and novel antigens. Taken together, the accumulated data suggest that exercise may be an efficacious therapy for restoring immune function in the elderly. In general, long term exercise interventions appear to show the most promise. Exercise related improvements have been reported with respect to antibody titre, T cell function, macrophage response, alterations of the T_H1/T_H2 cytokine balance, the level of pro-inflammatory cytokines, and changes in naïve/memory cell ratio. However, current data is minimal, and many questions remain including: the mechanisms that are involved, the potential clinical impact, the appropriate type or dose of exercise, and whether the benefits extend to all populations including frail, older adults. This review summarizes the major findings of these studies and proposes directions for future exploration.

Keywords: Immunosenescence; Exercise; T_H1/T_H2 cytokines; inflammation

Introduction

Age-related changes in immune function (immunosenescence) have been explored extensively in humans and a variety of animal models in the last quarter of a century. Though a large number of the observed changes involve declines in immune function, the term "immunosenescence" does not necessarily imply a deficit of immune function but more appropriately a dysregulated state. Various lymphocyte populations respond to ageing differently. Even within a specified cell type, individual activities may rise or fall with ageing. Therefore, it is important to consider immunosenescence in the organism as a whole and at the cellular level.

Many have suggested that immunosenescence may contribute to heightened disease susceptibility (both infectious and noninfectious) in the elderly. Globally,

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the human population is living longer than ever before and, consequently, the study and remediation of age-associated diseases is gaining increasing importance for both ethical and economic reasons. Numerous interventions have been suggested to counteract immunosenescence. Physical exercise is one of the more appealing interventions both in terms of efficacy, cost and logistics.

This review summarizes our current knowledge of ageing, the immune system, and exercise, and points the way towards potential avenues for future investigation. A brief overview of ageing and immune response is provided; more comprehensive reviews are published elsewhere (16, 35, 52, 76-78, 83-86, 158, 181). We begin by discussing the effects of ageing on the immune system and its specific components, using information gleaned from studies of both human and animal models. We then review data regarding the effects of exercise on immunosenescence in geriatric populations, also considering the impact of exercise on acute and chronic disease states. We conclude by considering the data in total and suggesting directions for additional research.

Ageing and the Immune System

Components of both the innate and adaptive branches of the immune system are known to exhibit functional alterations with ageing, as has been demonstrated in both human and rodent models over the last twenty-five years (16, 35, 52, 76-78, 83-86, 158, 181). Novel investigative tools and insights have allowed the specific identification of these components and the nature of their changes in recent years. The T cell population has been studied to the greatest extent and appears to be most affected by the ageing process (76, 180, 243).

Mechanisms that underlie the age-associated dysregulation are currently being investigated. The vanguard hypothesis is that decreased expression and functioning of receptors or signaling rafts (90, 101, 196, 250) and defects in signaling pathways (50, 60, 80, 122, 154, 179, 211, 225, 230, 232) may lead to altered function in immunosenescence. For example, it has been shown that NFkB levels are lower in T cells from aged mice compared to young mice (despite comparable levels of CD3 expression), and that upregulation of kinases such as protein kinase A (PKA) may lower NFκB expression and consequently the expression of downstream molecules such as interleukin (IL)-2 or IL-2Rα (CD25) (232). Various transcripts or proteins may be dysregulated (80). Declines in function and/or signal-induced relocation of other kinases and accessory molecules within the lymphocytes may instead be impaired with ageing, contributing further to cell dysfunction (154, 211, 225, 230). It is important to note that immunosenescent-associated changes in signal transduction are not usually single events – multiple intermediates or steps of the pathway are jointly effected by immunosenescence (80, 154). Changes may be further exacerbated by the shift from naïve to memory T cells in the aged (47, 63, 69, 135, 235) as well as alterations in lymphocyte genesis and development (138). Decreased expression of cell surface adhesion molecules (CAM) may inhibit cell adhesion, leading to a diminished immune response (46). Glucose utilization in lymphocytes may also be diminished with ageing (3). The immune system is not an isolated system in the living organism. Bidirectional communication with the neuroendocrine system may also impact the effects of age on immune function (144). Based on cur-

rent knowledge, multiple mechanisms likely account for immunosenescence, including factors intrinsic to the cells of the immune system as well as factors from other physiological systems.

Innate Immunity

The innate immune response is comprised of cells (such as macrophages, dendritic cells [DCs], and natural killer [NK] cells) and molecular systems (such as complement and inflammation) that respond automatically to perceived threats. Our understanding of ageing on the innate immune system is still in its infancy as most research efforts have been directed toward the adaptive immune system (85, 158, 181, 189).

Among the cells of the innate immune system, **macrophages** have been the studied to the greatest extent (139). Macrophages serve the immune system in multiple ways: (1) as antigen-presenting cells; (2) as producers of cytokines, including molecules involved in inflammation as well as B and T cell activation; (3) as producers of reactive nitrogen and oxygen species (such as nitric oxide [NO] and superoxide anion [O_2^-]) that are harmful to extracellular and intracellular pathogens; and (4) as cellular debris cleaners, such as cells that have undergone apoptosis. Implicit in the above description is that macrophages also serve as a bridge linking innate and adaptive immunity. It is interesting to note that the effects of age on macrophage function vary by tissue site (96, 126, 212). However, some common themes concerning macrophages and ageing can be discerned from the collected data.

Peritoneal macrophages isolated from rodents generally display decreased production of cytokines (IL-1, tumour necrosis factor [TNF]-α,) and reactive oxygen species when stimulated *in vitro* with mitogen, receptor-specific ligand, or virus (3, 22, 23, 43, 109, 121, 126, 196, 239). They also exhibit decreased cytostatic/cytotoxic, phagocytic, and antitumour activity (23, 58, 121, 239). Lipopolysaccharide (LPS)-stimulated IL-6 secretion by peritoneal macrophages *in vitro* does not change with age, and IL-12 appears to increase (13, 126). Macrophages from older mice, however, do produce greater amounts of PGE_2 compared with young mice, and PGE_2 is known to suppress T cell effector functions (14).

In contrast, stimulated murine alveolar and splenic macrophages generally produce higher levels of cytokines (IL-1β, TNFα, IL-6, IL-12) and nitric oxide when compared with younger counterparts (67, 96, 126, 208, 228). Exceptions to these general trends have been recorded (102, 122, 128). A more detailed comparison of the accumulated data has been presented elsewhere (126). These findings suggest that "environmental" factors specific to a tissue site may influence age-related changes in macrophage function.

To assay macrophage functions in humans, typically **peripheral blood mononuclear cells (PBMCs**; includes lymphocytes and monocytes) are collected, and macrophage function is inferred based on experimental constraints and outcomes measured. Using this methodology, macrophage function is indistinguishable from that of monocytes.

PBMCs from older humans stimulated with mitogen *in vitro* demonstrate enhanced or suppressed cytokine production on a per-cytokine basis. Production of interleukins such as IL-1β, IL-6, IL-8, IL-10, and IL-12 are increased upon ageing (36, 49, 68, 198, 200), whereas production of the cytokine IFN-α is diminished upon ageing (89, 200). However, some human studies suggest that ageing is not associated with an increase in pro-inflammatory cytokines such as IL-6 or TNF-α when health status is taken into account (13, 166).

As macrophages carry out so many critical functions, it has been repeatedly suggested that immunosenescence-associated changes in macrophage function may contribute to older hosts' decreased capacity to clear infections (158, 181). Macrophages that are not as efficient at presenting antigen or at producing immune cell-stimulatory cytokines would delay an efficient adaptive immune response. Similarly, macrophages with diminished inflammatory cytokine or reactive oxygen species manufacturing capacity would allow pathogens greater opportunities in the host. Changes such as these may be in part responsible for higher mortality rates due to infections such as influenza and pneumonia in the elderly (253), and may explain the diminished efficacy of vaccination in this population (82).

To better understand the link between macrophages, immunosenescence, and infection, both experimental animal models of infection and observational studies in infected humans have been employed. Macrophage antiviral resistance may be increased with age (126). In models of bacterial infection, no differences have been observed between the response of macrophages from young and old mice (67, 197). Human models of infection have also been utilized to explore age-associated changes in macrophage function. Cytokine production in response to respiratory syncytial virus (RSV) *in vitro* is diminished in older volunteers compared to younger volunteers (140). When groups of old and young patients suffering bacterial pneumonia were compared, it was discovered that older patients have lower acute phase levels of several cytokines when compared to younger counterparts (89).

Dendritic cells (DCs) are another class of antigen-presenting cells important for activating T cells and also B cells. Activated DCs may be found in lymphoid tissue whereas unactivated macrophages lie throughout the peripheral tissues; once activated, they migrate to lymphoid tissue. Besides strict presentation of antigens DCs also serve as repositories of antigen, which they release over time to stimulate receptive lymphocytes in the form of iccosomes. Using rodent models, it has been shown that iccosome release is hampered in older mice and that this contributes to a diminished secondary antibody response in older mice (29), likely due in part to DCs inability to induce sufficient germinal centers in lymphoid tissue (7). Declines in DC surface expression of markers such as FcγRII may affect immune synapse formation with surface molecules on target cells, such as the B-cell receptor (BCR), and promote cell inactivation via immune tyrosine-based inhibitor motifs (ITIMs) rather than activation (7). In contrast to these results, another team has shown human monocyte-derived DCs to be similar in function, morphology, and phenotype between young and old humans (143). It was additionally demonstrated that these DCs were able to stimulate senescent T cells to activity whereas monocytes could not (143).

With ageing, numbers of **natural killer (NK) cells** in the peripheral blood decreases (221), and cell subset demographics are noticeably altered (132), including an increase in percentage of memory NK cells (220). As their name suggests, NK cells kill other cells through cytotoxic granule release. NK cell

function may increase or decrease with age depending on the parameter under scrutiny (189, 221). Further, total NK cell pool activity is maintained with ageing though per cell killing activity, and proliferative capacity declines (220). The ability to respond to IL-2 is maintained, dependent on functional outcome measure (220).

The final cell we will consider in this section is the **neutrophil,** a phagocytic granulocyte that makes up the largest portion of the white blood cell pool in humans. A study comparing the effects of age on neutrophil function in human males has shown that with increasing age, neutrophil phagocytosis increases (234) whereas neutrophil (granulocyte) superoxide anion production either decreases (160, 189) or increases (234) contingent on the stimulus employed. Granulocyte cytokine production is also heightened in centenarians as compared to middle-aged adults (160). Neutrophil chemotaxis may decline with age (217). Compared to neutrophils from young individuals, neutrophils from aged individuals are not so easily rescued from apoptosis (189).

Adaptive Immunity

Adaptive immunity is constituted by antigen-specific cells such as B-cells and T-cells and the cytokines they secrete, but also employs members of the innate immune system in its efforts as well (181). Due to a larger volume of research, more is known about the effects of age on this branch of the immune system (158). Because the adaptive branch of the immune system is dependent on the innate branch for response initiation, there are two concerns: 1) cells of the adaptive immune system are themselves directly affected by immunosenescence-associated changes and, 2) diminished innate system function may also impair the ability of B and T cells to respond to a threat.

Numbers of circulating **B cells** (the progenitors to antibody-secreting plasma cells) decline with age; germinal center B cell production also diminishes (7, 95, 204, 252). In a classic paper, investigators isolated B cells from young (~25 year old) and elderly (~85 year old) adults, stimulated them repeatedly with a *Staphylococcus* protein, and then looked for differences in B cell function. They found that B cells from older adults had similar proliferative ability but diminished plasma cell differentiation capacity as compared to young adults (62). This inability to differentiate was not due to dysfunctional T cell stimulation as co-culture experiments demonstrated (62). Some defects of ageing appear to be due to changes intrinsic to B cells. For example, signal transduction involving the B cell receptor may decline with age (242). Alternatively, cytokine production by B cells may be altered by changes in behaviour of other lymphocytes: abnormally high constitutive production of IL-12 by macrophages has been experimentally shown to drive abnormally high IL-6 and IL-10 production levels in B cells (224).

Plasma cell-produced **antibody** (Ab; also known as immunoglobulin or Ig) concentrations may or may not decrease with age, but the proportion of functional antibody does decline (219). This may be due in part, to dysfunctional stimulation (such as by DCs or macrophages) as described earlier (7, 29). In general, lower antibody titre (192) and reduced affinity have been reported (168). However, antibody subclass responses are not universally affected by ageing. Following up on a study reporting impaired IgG production in response to inactivated influenza vaccination in people, it was demonstrated that ageing decreased IgG1, but not IgG3, responses (191). However, the IgG1 subclass is best correlated with haemagglutinin-inhibiting activity (88). Similarly, IgG1 and IgG2 subclass ratios changes with age when humans are given pneumococcal polysaccharide vaccination (141). In one study, researchers probed serum samples from older adults for antibody response to common bacterial, viral, and food antigens and found no differences in serum antibody concentrations between individuals in their mid-60s to mid-80s compared to still older individuals (24). Following a second homogeneously old population for 4 years, the same scientists found no overall changes in antibody titres to the same antigens from the beginning to the end of the study (24). Further research on the level of protection afforded by antibody titre is needed to determine the clinical significance of altered antibody titre and subclass.

Most changes associated with immunosenescence involve a diverse class of cells known as **T cells**. T cells all share in common the expression of the surface marker CD3. However, within CD3 cells, two principal types of T cell may be distinguished. CD4-expressing T cells are also known as helper cells. These cells secrete many important cytokines and play crucial roles in stimulating other cells. CD4 cells themselves can be categorized on the basis of their cytokinetic profile: T_H1 (Type 1), T_H2 (Type 2), or T_H0 cells. Each group produces its own arsenal of cytokines: IFN-γ, IL-2, and IL-12 in the case of T_H1; IL-4, IL-6, and IL-10 in the case of T_H2, and both T_H1/T_H2 cytokines in the case of T_H0 (165, 200). The T_H1 response is important in assisting CD8+ T cell activation, whereas the T_H2 response drives B cell activation and antibody generation (210). CD8-expressing T cells are also known as killer T cells or cytotoxic T lymphocytes (CTLs), and these cells are involved in killing other infected cells through the release of cytotoxic molecules. CD4 cells and CD8 cells are not affected homogeneously by immunosenescence, but some age-related changes are common to both (205).

Both CD4 and CD8 cells can be characterized as naïve (never having encountered antigen), effector (currently involved in an immune response), or memory (having been involved in an immune response previously but now waiting in case the same immune threat reappears). Changes in T cell number and function (i.e., naïve:memory ratios) are believed to play a substantial role in the age-associated decline in immune response (95, 137, 158, 202, 222). Much of this change is believed to be related to thymus involution and consequential loss of function with ageing (76, 183, 186, 204, 207, 217). Overall T cell numbers may decline with ageing, although not linearly, and some subpopulations increase whereas others decrease (235). T-cell antigen-recognizing receptors (T-cell receptors or TCRs) fall into two types, α:β and γ:δ, and relative numbers of α:β versus γ:δ T cells change with ageing (207). α:β T cell subset demographics are also altered with ageing, including a substantial drop in the relative number of naïve cells and a corollary increase in memory cells (69, 202). Beyond TCRs, T cells from older adults also express lower levels of other surface molecules such as CD25 (IL-2Rα), CD38 (a B- and T-cell marker), CD71 (a transferrin receptor present on all activated/proliferating lymphocytes), and HLA-DR (248).

Besides phenotypic changes at the level of the cell surface, immunosenescence also involves intracellular changes on the molecular level. Antigen is presented to T cells via major histocompatibility complex molecules, and it has been shown that aged T cells have a decreased ability to respond to antigen as compared to younger T cells (207). One potential explanation for this is that T cells

from older individuals are less efficient at assembling a signaling complex at the site of antigen presentation (225). Dysregulation of other intracellular signaling molecules such as PKA protein kinase C (PKC), and IκB influence gene expression and consequently T cell activity (173, 232, 233). Within 5 minutes of initial activation, T cells from old mice showed diminished intracellular calcium levels compared to young controls, suggesting deficiencies in signaling-associated ion movement (159).

Immunosenescence is associated with increased frequency of apoptosis (a form of programmed cellular suicide) in T cells overall. Heightened expression levels of "death receptors" and associated downstream molecules are associated with ageing and are likely explanations for this phenomenon (93, 94, 155). This effect has been shown to be specific to certain CD45-expressing T-cell subpopulations (CD45 is a tyrosine phosphatase) (100). Evidence suggests that T cells from young humans use different apoptotic pathways than those from older humans, implying that immunosenescence is also associated with a switch in apoptotic pathways (237). But critical recent findings also suggest that T cells from aged mice may not undergo adequate apoptosis during infection, leading to increased morbidity in older hosts (112). The memory-laden T cell pool of the aged individual may contain too many self-preserving memory T cells that take up space in the T cell pool necessary for naïve cells to respond to the infection.

Ageing is associated with the accumulation of viruses encountered throughout life that are never totally cleared from the body (such as herpes virus or *Varicella,* the cause of chickenpox and shingles). Chronic stimulation by these resident viruses may lead to the accumulation of dysfunctional and/or senescent T cells (61, 95, 107, 175, 178). This is corroborated by studies of the NK cell marker on T cells (221, 226) and studies investigating defective IL-2 secretion by aged T cells (107).

Proliferative responses of T cells have been shown to be much lower in older adults as compared to younger counterparts, that may be due in part, to altered cytokines (36, 79, 149, 200, 217). It has been shown that PBMC production of IL-10 is higher in elders, contributing to the impairment of T cell proliferation (36) IL-2 stimulates T cell proliferation. Age-related reductions in T cell proliferation have also been correlated with decreases in both IL-2 and soluble IL-2R (200). Current data suggests that although IL-2Rα (CD25) expression levels on T cell surfaces appear unchanged (200), the affinities of these receptors may be lowered in immunosenescence (79); however, others contend that CD25 expression levels do diminish with ageing (248). Regardless, reduction in IL-2 does not correlate with changes in all T-cell parameters (65). The elevated macrophage production of PGE_2 associated with ageing, is also known to quell numerous T functions (14); inhibition on PGE_2 can partially restore T-cell proliferation (97). Similarly, if cyclooxygenase is inhibited with indomethacin, T-cell proliferation is improved (97).

Cytokine production by **CD4+ T cells** changes with ageing. Individual studies utilizing cells collected from the peripheral blood of humans and stimulated *in vitro* with mitogen or virus have shown that, in general, levels of T_H1 cytokines (IL-2, IL-12, IFN-γ) decrease with age, but levels of T_H2 cytokines (IL-4, IL-10) increase with age as compared to younger counterparts (34, 106, 107, 174, 195, 200, 210, 217). However, other studies have failed to demonstrate an age-related shift in preference towards one class of cytokine over another (20, 118), or have shown simply a cross-CD4-subclass heterogeneous shift to a T_H1/T_H2 imbalance (133). A recent review of the data concluded that the current findings were inconsistent regarding a preference towards a Type 1 or a Type 2 response with ageing and more appropriately reflected simply an age-associated imbalance (81).

The age-related phenotypic and function changes in CD4+ cells include altered expression of chemokines from the CCR and CXCR classes (161). Immunosenescent CD4+ cells show a loss in CD28 expression, a costimulatory molecule required for proliferation (241). The diminished capacity of CD4+ cells to respond to antigen-presenting cells or mitogen is possibly due in part to diminished tyrosine phosphorylation capacity via decreased Tyrosene kenase t56 (Lck) or other molecule activity (211, 230).

All of these changes relate to altered functional capacity in CD4+ T cells associated with immunosenescence. For instance, naïve T cell function drops with age: upon first encounter with antigen, naïve cells from older mice produce less cytokine, proliferate less, and show lower levels of helper function compared to naïve T cells from young mice (98). Aged naïve CD4+ cells can gain partial restoration of function in the presence of supplemental IL-2, but are unable to produce normal levels of the cytokine on their own, implying that some permanent change blocking T cell IL-2 production occurs during ageing (99). Not only do CD4+ T cells themselves demonstrate increasing unresponsiveness with age, but due to their dysregulated state they may actually suppress the function of other cells instead of promoting it (213).

$CD8^+$ T cells are likewise known to change with ageing (58), but in some different ways than CD4+ T cells. Cytokine production by CD8+ cells may also be considered using the Type 1/2 dichotomy even though these cells harbor no helper T cell functions. Naïve, effector, and memory human CD8+ T cells all produce significantly higher amounts of T_H1 cytokines such as IFN-γ, IL-2, and TNF-α, whereas only memory CD8+ T cells produce significantly higher amounts of T_H2 cytokines such as IL-4, IL-6, and IL-10 (251). Similarly, if CD8 cells from young and old mice are given equal stimulation and their production of cytokines are compared, cells from older mice produce more of IL-4, IFN-γ, and TNF-α, but not IL-2 (163). Thus immunosenescence affects cytokine production by CD4+ T cells and CD8+ T cells in a different manner.

Molecular and phenotypic changes associated with immunosenescence and their functional consequences have similarly been determined in CD8+ cells. CD8+ T cell subset demographics change during the course of ageing (63, 69). Changes in subset demographics have been linked to functional changes: intracellular cytokine staining has revealed that the age-associated increase in IFN-γ production by CD8+ T cells (63) may largely be due to the $CD8^{+high}$CD28 CD57+ T cell population, which expands during ageing (9). Phenotypic changes such as loss of CD28 expression are linked to decreased proliferative ability (56, 57). Intracellularly, CD8+ cells show decreased phosphorylation capacity in response to mitogen (211). CTL activity (such as Granzyme B production) and memory declines with ageing as has been shown using influenza A virus in humans (150, 153, 190) or models of delayed-type hypersensitivity reactions in mice (15).

Several age-associated changes occurring in **γδ T cells** have been investigated with the main finding that ageing does not affect γδ T cells homogeneously.

Peripheral blood γδ T cell numbers decline with age; concurrently, a larger proportion of cells express the $CD69^+$ marker of activation (201). Relative numbers and proportions of γδ T cell subsets change with time and some exhibit reduced proliferative abilities, but cytotoxic abilities are maintained with age (4). TNF-α, but not IFN-γ, production increases with age in this group (4).

Another unusual class of T cells are the **NK T cells**, which have an unvariant αβ receptor but also express the NK marker (189). With age numbers of NK T cells increase, as does their ability to produce IL-4 (189). It is thought that chronic stimulation by viruses that are never totally cleared by the host (mentioned earlier) may cause increases in NK marker expression on T cells and thus formation of immunosenescent NK T cells (221, 226).

Functional Relevance of Immunosenescence

It has been proposed that immunosenescence may explain the increased susceptibility of older adults to bacterial (such as pneumonia) and viral (such as influenza) infections (35, 57, 83, 87, 156, 200), as well as higher rates of autoimmune disease and/or inflammatory conditions (inflamm-ageing) (25, 77, 83, 243). Infection is often more severe in elderlies, sometimes manifesting symptoms unique to this population (83). Immunosenescence may put older adults at increased risk for certain types of cancer (16), but some evidence suggests that humans in their 90s or above (centenarians) may actually become more resistant to cancer due to additional changes with ageing (40, 95). Low levels of constitutive inflammation may be due to immunosenescence-associated alterations in plasma protein synthesis, including acute phase proteins as has been shown in rats (177), or constitutive expression of inflammatory cytokines such as IL-1 and TNF-α (25, 198, 200).

Numerous interventions have been suggested to counteract age-associated immunosenescence (18, 104), including exercise (see references cited in next section), vaccination (88, 119), caloric restriction (59, 161, 223, 236), dietary or herbal supplementation, including antioxidants such as vitamin E (14, 103, 104, 111, 162, 209), hormone manipulation (104), and other immunomanipulative techniques (6, 52, 104, 105, 186). Excluding exercise for the moment, the most studied of these interventions has been vaccination, typically against either influenza or pneumonia.

It has been demonstrated both clinically and experimentally that vaccine efficacy rates are lower in older adults (35, 119, 151, 164), though they significantly reduce hospitalizations and mortality (145, 156, 172). Several of the immunosenescence-associated changes detailed earlier are believed to be responsible for this. Impaired DC function affects not only its ability to present antigen but also the effector capabilities of its targets, B cells and T cells (7). Plasma cell antibody response to vaccination is thus diminished, resulting in decreased antibody titres in blood or secretions (192, 217). Less naïve T cells and more memory cells, a large proportion of which are partially dysfunctional, impair vaccine response (48). CTL function, essential for viral clearance, also declines with age an may abrogate a robust vaccine response, but this too is contingent on certain factors such as strain of virus (57, 153, 192). Lower levels of cytokine production in response to vaccination, such as IFN-γ (175) and IL-2 in older vaccinees, may correlate with some (152) but not all immune parameters (65). Other researchers have found no differences in the serum levels of influenza vaccine-stimulated IL-6 and IL-10 across ages (131).

Using an aged mouse model, researchers found several immune parameters impaired following intranasal challenge with influenza, including NK cell activity, CTL activity, and IFN-γ and IL-12 production (51). Virus-specific antibody and IL-4 production, by contrast, were higher, although antibody titres were not high enough to confer adequate protection (51). It has also been shown in aged mice that so many memory T cells exist than upon virus infection, not enough T cells undergo apoptosis to provide space for virus-specific effector and/or memory cells to proliferate (112).

Exercise and Immunosenescence

In comparison to the other methods enumerated just previously, exercise is a very attractive intervention for a number of reasons (92). Exercise is non-invasive, and exercise may be conducted in many different types of environments. In addition, exercise has important health benefits for many other chronic diseases including arthritis, heart disease, stroke, peripheral vascular disease, diabetes, osteoporosis, and pulmonary disease (12).

Studies exploring the potential role of exercise in amelioration of immunosenescence have only recently been initiated. Several reviews have been previously published reviewing the potential effects of exercise on immunosenescence (27, 147, 170, 171, 214-216, 238, 247). However, a recent expansion of research on this topic has necessitated a re-assessment of the current literature.

Immune Response to Single Bouts of Exercise

Some researchers have concluded that immune response to acute exercise in elders is maintained with age (183) whereas others contend that it is similar but reduced in elders (37). As single bouts of exercise are not expected to reverse immunosenescence, we will consider here their effects on immune function only fleetingly, combining data from both human and mouse studies. In general, older adults still demonstrate immunoresponsiveness to a single bout of exercise, although the magnitude of this response is smaller compared with young controls (149). Acute exercise in older subjects is known to increase NK cell activity (41, 227) and circulating numbers of neutrophils (30, 32); potentially increase secondary antibody response to booster injections (115); change circulating T cell populations (37, 149) and induce leukocytosis (26, 37); and modulate T cell proliferation (37, 45, 149).

Long-term exercise and immune function (Human Studies)

Cross Sectional studies

Several cross sectional studies have evaluated immune responsiveness in older adults who regularly participate in exercise compared to those who remain sedentary. The impact of physical activity on NK cell function in older adults is not clear. NK cell cytotoxicity of K562 tumour targets is thought to be maintained with ageing, although there may be some decline of NK activity on a per cell basis (220). In one study, competitive female athletes over 65 years of age demonstrated higher NKCA (natural killer cell activity) than sedentary controls

(171). Shinkai et al. (214) evaluated NKCA in elderly runners and did not find a significant difference between runners and controls. However, in a follow-up study, a slightly greater NKCA on a per cell basis was found in 50-59 year old runners compared to controls (216). Recently, it has also been shown that the concentration of CD16+CD56+ NK cells was greater in elderly subjects (over 60 years of age) that exercised on a regular basis in comparison to age-matched controls (249). Together, these studies suggest that exercise may slightly enhance NKCA in older adults, although additional studies are needed to confirm this possibility. There is minimal data regarding the effects of physical activity on other innate defences in humans. One group has shown that the age-associated decline in neutrophil phagocytic function is attenuated by exercise in adults aged over 60 (249), yet further research in this area is needed.

The influence of exercise participation on T cell proliferation in response to mitogens has also been evaluated. Greater lymphocyte proliferation in response to the mitogens phytohemagglutinin (PHA) and pokeweed mitogen (PWM) has been reported in older recreational male runners compared to controls (214, 216). Similar findings were reported with respect to PHA-induced lymphocyte proliferation in older competitive females athletes (171). Another T cell functional measure, the expression of CD25 (IL-2a receptor) may decline with advancing age (79, 237). One study observed that T cells from active older women showed a greater CD25 expression on T cells compared to less active. T cell signal transduction involving protein kinase C (PKC) has also been reported to decline with age (242). To investigate whether age-related changes in lymphocyte **protein kinase C (PKC)** activity may play a role in immunosenescence, one team studied PKC activity from both cytosolic and membranous fractions of lymphocytes isolated from older men and compared basal or stimulated (with PHA or phorbol myristate acetate [PMA]) activity. They found that older men had consistently lower PKC activity levels across all cellular locations and stimulation schemes, but that individuals who exercised regularly had a lesser magnitude of reduction as compared to the younger controls (240).

Three recent studies have evaluated the effects of physical fitness on the immune response to antigens *in vivo*. These models may have greater physiological relevance than mitogen-induced stimulation of PBMC *in vitro*, because the *in vivo* measures of immunity evaluate the antigen-specific response, similar to actual challenge with a pathogen. In one such study, older adults that regularly performed aerobic exercise produced greater amounts of anti-influenza IgG and IgM two weeks post-immunization compared to less active or sedentary individuals (125). In this same study, lymphocyte proliferation to influenza antigen was greater in the participants reporting either regular activity or less intense activity compared to sedentary individuals. In a similar study, physical activity was assessed using the Physical Activity Scale for the Elderly (PASE), and antibody titre was measured in response to influenza immunization (206). At one-week post-immunization, the antibody titre was significantly correlated with the level of physical activity, although this correlation was not observed at 2, 4, or 6 weeks post-immunization, suggesting that exercise may alter the kinetics of immune response. The primary response to a novel antigen, keyhole-limpet hemocyanin (KLH), was recently assessed in young and old, active and inactive adults (219). With respect to anti-KLH antibody, IgM, IgG, IgG1, but not IgG2 were significantly greater in the older active adults compared with the sedentary older adults. The delayed type hypersensitivity (DTH) response to KLH was also measured in this study, reflecting the ability of T cells to proliferate and migrate in response to antigen. Again, exercise was associated with an improved DTH response. Taken together, these studies suggest that immune response to antigen challenge may be improved in older adults. In these studies, both, measures of B cell function (antibody), and T cell function (proliferation, DTH) were enhanced in the older adults that exercise regularly, perhaps providing greater protection from infection.

Prospective studies

Several prospective studies have compared infection history over a period of time, and only a few exercise intervention trials have been performed in older adults. Based on the published studies, the effects of exercise training as means of reversing immunosenescence are variable. However, it appears that long-term (> 6 months) interventions show the greatest promise. For example, in four of five intervention studies > 6 months duration, improvements of immune function were observed (2, 53, 117, 123, 244). In contrast, the findings from six of seven studies that involved shorter-term interventions were not promising in terms of enhanced immunity (17, 39, 41, 70, 73, 171, 194, 199). Therefore, unlike some physiological parameters such as the maximal oxygen uptake that improve in 8-12 weeks, a longer period of time may be necessary before adaptations in immune function occur. A comparison of the immunomodulatory effects of different types of exercise interventions or physical activity patterns over time is presented in the next section.

Infection

The influence of physical activity on risk of developing an infection in older adults has been examined in several studies. The risk of hospitalization for infectious disease was examined in 1,365 women aged 55 to 80 years. In this study, physical inactivity was associated with increased risk of infection (adj. odds ration = 4.08; 95% CI, 1.73-9.63) (136). Similarly, in a separate investigation, the total number of upper respiratory tract infections (URTI) and total number of days with URTI symptoms over a 12-month period was significantly negatively associated with daily energy expenditure in sports activity among older adults (129). Recently, the risk of developing physician-diagnosed community acquired pneumonia was evaluated in a large study population that included adults up to age 79 (8). Again, the risk of developing pneumonia decreased with increasing levels of physical activity, but only among women. In an aerobic exercise intervention trial of 12 weeks duration, elderly women assigned to a 5 days/week, 30-40 minutes at 60% of heart rate reserve, had a significantly lower incidence of upper respiratory tract infection as compared to a calisthenic (light flexibility) program (171). Recent data from our laboratory also suggested that over the course of a 10-month aerobic exercise intervention (3 days/week, 25-30 minutes at 65-75% of heart rate reserve) that began in November, participants assigned to the exercise group experienced fewer days of URTI symptoms compared to sedentary control subjects, although the n was small (n=14 exercise, n=14 control; Figure 1, Kohut et al., unpublished observations). The evidence from these prospective studies generally suggests that greater levels of physical activity are associated with reduced risk of infection. The causal link between exercise and infection remains to be

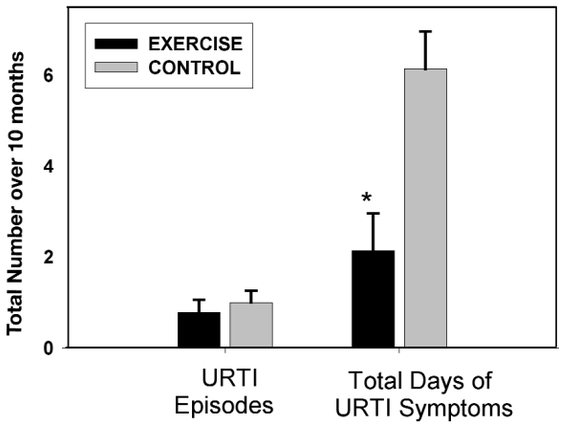

Figure 1. The total number of reported upper respiratory tract infections was compared between aerobic exercise and control subjects over the 10-month intervention period. No difference existed between groups. The total number of days with URTI symptoms was also compared between groups during this same time period. The exercise subjects reported significantly fewer days with symptoms (p=0.031). Note, illness were not physician verified and the n was small (n=14 control, n=14 exercise).

determined, however, recent studies suggest that exercise may attenuate immune decline associated with ageing.

Natural Killer cell function

The effects of exercise training on NK cell activity were examined in several studies, although the findings from some studies have suggested that total NKCA is not affected to a large extent by the ageing process. In the study by Woods et al. (244), a trend towards increased NKCA was observed in subjects completing a 6-month aerobic exercise intervention. Sixteen weeks of training with chair callisthenic exercise was also associated with increased NKCA, although no pre-intervention measures were made, thus limiting the findings from this study (41). In contrast, a twelve week aerobic exercise program, a 10-week aerobic exercise intervention, and a 10-week resistance training program did not improve NKCA (70, 73, 171). NKCA actually declined in frail older adults following 3 months of exercise in comparison to a control group (199). In summary, the impact of exercise training on NKCA in older adults is not clear, with shorter term interventions showing no change and longer term interventions suggesting a slight increase in function. In future studies, it would be worthwhile to determine if a specific time period of exercise training is necessary before changes are observed in NK function, and whether certain aspects of NK function thought to change with ageing (e.g., response to interferonα or IL-2) may be modified by exercise (21, 246).

T lymphocyte responses and related cytokines

The role of exercise training on several aspects of T cell function have been evaluated in older adults including T lymphocyte proliferation, cytotoxic T cell function, expression of activation and costimulatory markers, delayed type hypersensitivity, and cytokines (IL-2, IL-4, IFNγ). Numerous studies have demonstrated that ageing is associated with decreased T cell proliferation (55), impaired CTL function (190), reduced expression of the costimulatory molecule CD28 (56), diminished expression of the activation markers CD25 and CD69 (79, 205), and a large decline in IL-2 production (54, 229). This age-related dysfunction in the T

cell has been theorized to play a major role in altering general immune function including those associated with earlier mortality (178). The evidence to date has suggested that longer-term interventions appear to have a greater effect on T cell function than short-term exercise interventions. T cell proliferation to mitogen tended to increase following a 6-month aerobic exercise intervention (244), increased significantly following 10 months of aerobic exercise (134), but did not change after 12 weeks of similar exercise (171). In contrast, resistance training for 10-12 weeks did not alter proliferation to mitogens (73, 194). It is also of interest to note that a 32-week intervention involving endurance and strength exercises did not change proliferation among frail elderly adults (117).

A measure of T cell-mediated response *in vivo*, the DTH response, did not increase after either a 12-week resistance program (194) or a 17-week exercise program (39), although there were some concerns with the methods used to measure DTH in the second study. The effect of a longer term aerobic exercise intervention on DTH response in older adults has not yet been published, to our knowledge.

Cytotoxic T cell function has been shown to be essential in viral clearance, and we are aware of only one investigation that has examined CTL response to viral challenge among older adults. Following 10 months of aerobic exercise, the CTL response as assessed by Granzyme B, was greater among exercisers compared to control subjects for 2 of 3 antigens contained in the influenza vaccine (123). Given that the CTL response to influenza virus was impaired among older adults (192), an improvement in CTL function may have clinical benefits.

Cytokine production by T cells has also been reported to change with ageing, and the decline in IL-2 production has been well-documented. A recent study showed that the percentage of lymphocytes expressing intracellular IL-2 was greater in elderly women (aged 62-86) that attended an aerobic exercise program for 2 years as compared to sedentary women (53). However, the authors found no difference between exercise and control subjects with respect to the percentage of lymphocytes expressing intracellular IL-4 or IFNγ in response to PMA and calcium ionophore. One limitation to this study was the lack of baseline pre-intervention immune measures between the groups.

Other measures of T cell responsiveness have included the expression of CD25 (IL-2 receptorα), HLA-DR (component of Class II major histocompatibility molecules), and expression of the costimulatory molecule CD28. In the only published study to examine the effects of exercise on these T cell activation markers, no benefits of a 32-week endurance and strength training program were found in frail elderly subjects (116). In a separate study, 10 months of aerobic exercise or flexibility/resistance exercise did not alter expression of the early T cell activation marker, CD69 (134). However, data from the same laboratory did show that the 10-month aerobic intervention significantly increased CD25 expression, whereas 10 months of flexibility and resistance training did *not* alter CD25 expression (Figure 2; unpublished results, Lee et al). To our knowledge, this was the first data to compare immune responses associated with a long-term aerobic exercise intervention in contrast to a long-term resistance/flexibility exercise intervention.

Taken together, the results from several studies indicated that long term aerobic exercise interventions improved T cell responses among older adults. Although resistance training did not appear to improve T cell function, the dura-

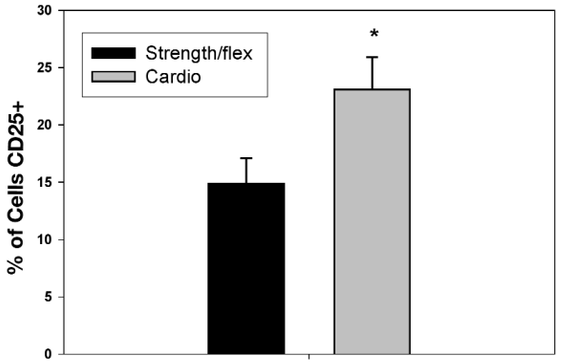

Figure 2. After a 10-month intervention, the percentage of CD25+ expressing cells was significantly greater (p=0.042) among the participants of the aerobic intervention as compared to the strength/flexibility intervention. Baseline CD25+ values were used as a covariate in the analysis.

tion of the intervention was shorter (10-12 weeks), and aerobic exercise trials of a similar duration also did not increase T cell response in older adults (171). The only data that we are aware that evaluated a longer term resistance exercise intervention is shown in Figure 2, and fewer benefits were found with resistance training compared to aerobic exercise. Additional studies regarding the role of resistance exercise would be informative. Another concern from current data was that the potential improvements of T cell function associated with aerobic exercise may not apply to frail, older adults. The two studies that included this population failed to observe significant improvements of T cell function after 17 weeks or 32 weeks of training (39, 117). Longer periods of exercise training may have been required before effects may be seen in this population or it may be that frail older adults may not be capable of exercising at a sufficient intensity to elicit changes of immune competence. Although the data has been very limited, in a comparison of lower intensity exercise with moderate/vigorous intensity exercise, immune benefits were observed only in the subjects participating in higher intensity exercise (125). Alternatively, it is possible that older adults who may be classified as suffering from frailty syndrome, have reached a stage at which it is not possible to reverse age-related declines of immune function. In summary, the data from exercise intervention trials among older adults suggests that aerobic exercise may enhance T lymphocyte responses, but longer periods of exercise are likely required before benefits are observed, and benefits may not be found in all populations.

Antibody

The effects of exercise training on antibody responses have been evaluated in two studies. In one of these studies, salivary secretory IgA levels were evaluated after 12 months of endurance and resistance training (2). The prevalence of mucosal infections appears to increase with age, and IgA on mucosal surfaces may act as one of the first lines of defence against infection. However, several studies have shown that salivary IgA concentration and secretion rates tended to *increase* with age (5, 38, 71) including IgA antibodies to specific microorganisms (185), although there was one report of diminished salivary IgA secretion rate (157). Interestingly, exercise training (60 minutes aerobic and 60 minutes resistance training per week for 12 months) was associated with an enhancement of both salivary IgA concentration and secretion rate (2). Presumably, the exercise-induced increase would be associated with greater protection from mucosal microbial pathogens, although this remains to be established.

A significant number of older adults do not develop a protective antibody titre following immunization with influenza vaccine, leaving these individuals potentially vulnerable to infection (120). Another recent study evaluated the effects of exercise training on serum antibody titre to influenza vaccine. In an exercise trial, older adults participated in a 10-month aerobic exercise intervention, and antibody titre in response to influenza vaccine was measured. At baseline, prior to the intervention, there was no difference in antibody titre between the exercise and control group. In contrast, after the exercise intervention, the exercisers responded to the annual influenza vaccine with a greater increase in antibody titre than the control group (123). Although the antibody titre among exercisers increased to a greater extent than the control subjects, the level among elderly exercisers did not reach the titre in a young comparison group. This suggests that exercise may attenuate, rather than completely reverse immunosenescence. This study may have important clinical implications given that protection from influenza infection has been correlated with higher serum antibody titre (91) and therefore, even a slight improvement in antibody response could potentially result in better protection from infection.

Inflammation and Pro-inflammatory cytokines (IL-1β, TNFα, IL-6, IL-18)

Due to the limited information on the topic of inflammation, both cross sectional and prospective studies have been included in this section. In recent years, inflammation and markers of inflammation have been associated with a number of chronic diseases; for recent reviews see (78, 130, 231). In particular, elevated inflammatory cytokines and inflammatory factors such as C-reactive protein (CRP) have been associated with, or implicated in the pathogenesis of: osteoporosis (146), diabetes (193), cardiovascular disease including atherosclerosis (19, 28, 72, 108), obesity (42), hypertension (10), frailty, disability and mortality (33, 64). An increase in monocyte production of inflammatory cytokines has been reported with advancing age (198, 203). Several epidemiological studies have suggested that exercise may minimize inflammation. For example, the NHANES III data that included 13,738 subjects showed that the odds ratios for elevated CRP were reduced to 0.85 (95% confidence interval 0.70-1.02) for those adults participating in moderate activity, and further reduced to 0.53 (CI 0.40-0.71) for those participating in vigorous physical activity (75), after adjusting for many factor including body mass index (BMI). In a smaller study, however, the inverse association between inflammatory factors and physical activity was no longer significant *after* adjusting for BMI (188). In a study focused on middle-aged and older adults, greater levels of physical activity were inversely related to CRP levels (odds ratio 0.63; 95% CI 0.43-0.93) for those participating in physical activity > 22 times per month (1). The one study that examined only those adults > 65 years of age also observed that participants in the highest quartile of physical activity had 19% lower concentrations of CRP than those in the lowest quartile of physical activity, after adjusting for disease and BMI. The epidemiological data generally supports the possibility that regular exercise may reduce levels of inflammatory mediators, and although the mechanism remains to be determined, it has been suggested that muscle derived IL-6 may play a role (182).

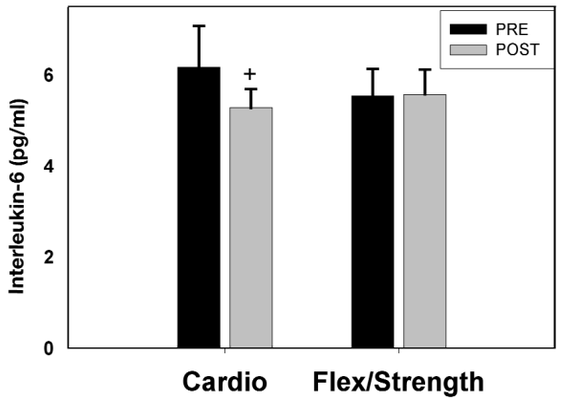

Figure 3a. The level of serum IL-6 tended to decline in the cardio group as compared to subjects in the strength/flexibility training group. A statistical trend towards a greater decrease in the cardio compared to the strength/flex group was observed (p=0.10); n=19 cardio subject, n=19 strength/flex subjects.

Several prospective studies have evaluated the impact of exercise training on inflammatory cytokines or mediators. A 10-week resistance training program in older women reduced the levels of IL-6, TNF-α, IL-1β produced by LPS-stimulated peripheral blood mononuclear cells (187). Similarly, a 3-month resistance exercise intervention among frail elderly participants decreased TNF-α mRNA, although in this study, the TNF-α was measured in muscle tissue. The authors suggested that resistance training may attenuate muscle wasting associated with advancing age, given that elevated TNF-α may be related to muscle wasting (66). A separate study of frail older adults evaluated both nutritional and exercise interventions in a 17-week randomized trial. In a subgroup of subjects who had detectable levels of serum CRP, it appeared that exercise reduced CRP by 1.3 + 1.2 mg/L, and the combined exercise/nutrition intervention decreased CRP by 8.5 + 17.0 mg/L (44). However, the number of subjects was low and the results are of questionable statistical significance. Flynn et al. (74), took an interesting approach in an experiment designed to examine the effects of resistance training on toll-like receptor 4 and CD14 mRNA expression in elderly women. Toll-like receptor 4 and CD14 are involved in signal transduction in response to bacterial LPS, resulting in the release of pro-inflammatory cytokines. After a 10-week resistance training intervention, the monocytes from the exercise participants demonstrated reduced Toll-like receptor 4 and CD14 mRNA expression. However, exercise was not associated with changes in IL-6, IL-1β, or TNF-α mRNA per leukocyte. Although this study is somewhat complicated by the inclusion of subjects taking different hormone replacement therapies or other medication in the exercise group compared to the control group, and the lack of pre-intervention measures, this is the first attempt to assess the potential effects of exercise on receptors important in LPS signaling in association with pro-inflammatory cytokines. In the only published trial that we are aware of that evaluated long-term exercise and pro-inflammatory cytokines, slightly younger participants were studied (women mean age 49.7, men mean age 48.1) (218). The cytokines were measured using peripheral blood mononuclear cells with or without the mitogen PHA. The production of the inflammatory cytokines IL-1α, TNFα, IFNγ decreased after a 6-month exercise intervention, whereas the production of the "atheroprotective" anti-inflammatory cytokines IL-10, IL-4, TGFβ increased in both PHA-stimulated and non-stimulated cell cultures. These results were promising, but need to be evaluated among older adults. Also, it is important to note that many of the studies linking pro-inflammatory cytokines and chronic disease have evaluated serum levels of these cytokines, rather than cytokines produced by peripheral blood mononuclear cells. Our laboratory has recently examined the effects of long-term (10-month) exercise interventions on serum levels of IL-6 and CRP using high sensitivity ELISA kits. A resistance/flexibility program was compared with a cardiovascular exercise intervention. Both groups exercised 3 times per week, with the cardiovascular group exercising at 65-75% of heart rate reserve for 25-30 minutes per session whereas the resistance/flexibility group performed flexibility exercises and 1-2 sets of resistance exercises (8-15 reps/set). The results are shown in Figure 3a. Serum IL-6 tended to decrease in the subjects that participated in the cardiovascular intervention, whereas no change occurred in the resistance training group (change from pre-intervention, p=0.10). Figure 3b shows a similar pattern with respect to serum CRP, a trend towards a decrease in the serum of cardiovascular subjects, but no change in the strength/flexibility trained subjects after the intervention (p=0.09).

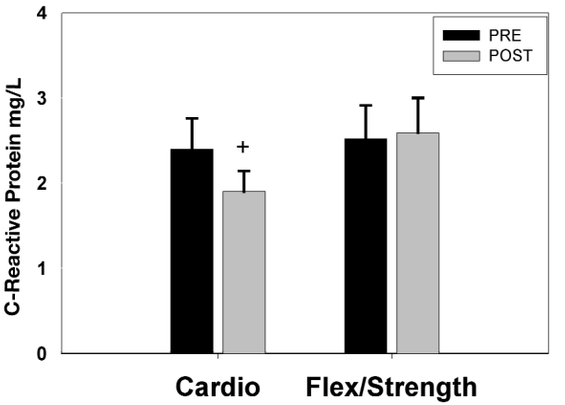

Figure 3b. The level of serum CRP tended to decline in the cardio group as compared to subjects in the strength/flexibility training group. A statistical trend towards a greater decrease in the cardio compared to the strength/flex group was observed (p=0.09); n=18 cardio subject, n=20 strength/flex subjects

The research focus on pro-inflammatory cytokines/ inflammatory mediators and their potential role in chronic disease is relatively new. The effects of acute exercise on inflammatory cytokines have been investigated in numerous studies ever since the first innovative work published by Cannon and Kluger in 1983 (31). However, it is not known whether chronic exercise can minimize the "inflammatory state" associated with many chronic diseases. Perhaps the mechanism by which exercise training minimizes the risk of developing chronic conditions such as heart disease or diabetes, involves a reduction in the inflammatory cytokines. This field of research will be improved by additional long term studies that simultaneously evaluate the influence of exercise rehabilitation on pro-inflammtory mediators and the risk of developing chronic conditions including frailty, as well as overall mortality.

Animal studies

There are numerous animal studies that have evaluated the role of exercise as an

immunomodulator, and some of these investigations have examined the incidence or severity of infection. This review will focus primarily on studies that used aged animal models and present data on the question of whether exercise can attenuate immunosenescence.

T cell proliferation and IL-2 in response to mitogens

T lymphocyte responses, particularly proliferation and IL-2 production are reduced with advancing age, both in animal models as well as humans. Both rats and mice have been used to evaluate the effects of exercise training on T lymphocyte function and the results are variable. For example 6 months of swim training in rats actually *reduced* proliferation and IL-2 production in young rats (7 months), however, there appeared to be no difference in these same immune parameters comparing exercise and sedentary older rates (18 months) (176). Two other studies that used running as the exercise mode found that 15 weeks of exercise training *increased* ConA-induced proliferation in rats 27 months of age, and that 21 months of daily exercise resulted in a trend towards increased proliferation to PHA in rats 23 months of age which became statistically significant if high and low responders were evaluated separately (167, 236). Interestingly, proliferation decreased in young (8 months) and middle aged (17 months) rats exposed to the same 15 week exercise regimen (167). A similar pattern was seen with respect to ConA-induced IL-2 such that 15 weeks of exercise improved IL-2 production in the older rats (27 months) but IL-2 declined in young or middle aged rats. The differences in age in those rats defined as old (18 months as compared to 23 or 27 months) may account for the seemingly different findings. The middle-aged rats (17 months) that also showed a decline in proliferation with exercise training in the treadmill running study were nearly the same age as the "old" rats in the previous swim training study that showed no difference in proliferation. Contrasting results were reported in a recent study that evaluated 8 weeks of treadmill running using young (4 months) and older (18 months) mice. The investigators found that exercise training was associated with decreased proliferative response to ConA or PHA in older mice, but the opposite was true in young mice (increased proliferation to PHA, but not ConA) (127). It is currently difficult to draw a conclusion regarding the role of exercise training in reversing the age-related decline in mitogen-induced proliferation, based on the current data in animal models.

Infection models and T_H1 / T_H2 cytokines

An 8-week treadmill exercise program (5 days/week, 45 minutes/session), followed by exposure to intranasal exposure to HSV-1 virus was used to determine whether the production of virus-specific cytokines was affected by exercise in young (4 month) and old (18 month) mice (127). Immune responses were measured 10 days post-infection, by culturing spleen cells with the same virus *in vitro* to elicit virus-specific responses. In young mice, IL-2 was enhanced by exercise, and in old mice the kinetics of IL-2 production appeared be changed by exercise, rather than total IL-2. In a similar study from the same group of investigators, immune responses were evaluated at 7 days post-infection. IL-2 was significantly increased among old exercised mice, and showed a trend towards an increase in young mice (124). Another T_H1 cytokine important in viral infections is interferon gamma (IFNγ). In these same experiments, the IFN-γ responses were very similar to IL-2 (old mice - significant increase 7 days post and altered kinetics at 10 days post; young mice – trend to increased 7 days post and significant increase at day 10 post). The T_H2 cytokine, IL-10 was also studied in the viral infection model. At 7 days post-infection, a slight trend towards increased IL-10 was observed in both age groups in exercised mice, whereas by 10 days post-infection, exercise significantly increased IL-10 in both young and old mice. Taken together, these findings suggested that exercise training may enhance T_H1 cytokines earlier during infection, but affect T_H2 cytokines later in the course of infection. With advancing age, it has been suggested that there is a shift in the T_H1/ T_H2 cytokine balance, particularly with a decline in the T_H1 cytokines (81). The possibility that this shift in cytokine balance may contribute to an increased rate of infections has been raised (210). Perhaps exercise reduce infection severity among aged populations by altering the T_H1/ T_H2 balance early in the course of infection, yet the data to support this possibility are currently very limited.

Macrophage function

Age-associated declines in **macrophage** function may be reversed by exercise. One study showed that moderate, regular exercise (5 days/week, 45 minutes/day, 16 weeks) in older mice increased the capacity of peritoneal macrophages to kill tumour cells *in vitro* (142). Furthermore, the results indicated that nitric oxide appeared to be responsible for the exercise-induced increase in macrophage antitumor function. In a separate study of older mice, exercise training (5 days/week, 45 minutes/day, 9 weeks) reversed the age-related decline in the LPS/IFN-γ-stimulated production of TNF-α by peritoneal macrophages (126). Interestingly, the investigators also observed that ageing was accompanied by an *increase* in alveolar macrophage LPS/IFN-γ-stimulated production of IL-12, and exercise training reduced IL-12 in older mice, similar to the response of younger mice. Peritoneal macrophage antiviral resistance to HSV-1 also appeared to increase with age. The same 9-week exercise protocol further enhanced macrophage antiviral resistance in older mice. In a separate study from the same group, ageing was associated with increased IL-12 production in spleen and alveolar cells (124). Exercise tended to decrease LPS/IFN-γ-stimulated IL-12 in both young and old mice, although this did not quite meet statistical significance (p=0.063). Although more studies of exercise and its effects on macrophages from the aged are needed, these initial studies show some promise in reversing some of the age-related alterations of macrophage function.

Antibody

Three studies have evaluated the role of exercise training on antibody production in aged animals. In an earlier study of rats, trained for 10 weeks with treadmill running, there was no change in the antibody response to KLH in the exercised compared to control rats (11). In two studies involving 8 weeks of treadmill exercise in aged (18 months) and young mice (4 months), IgM antibody to HSV-1 virus was not increased by exercise 7 days post-infection (124), but did appear to be enhanced by exercise in both young and old mice at 10 days post-infection (127). Again, the timing of antibody measure in relation to antigen challenge may influence the effects of exercise. Also, antibody titre without some measure of infection severity may be difficult to interpret.

Naïve/memory

The naïve/memory T cell ratio has been shown in numerous human and animal models to change with age, with a decline in naïve cells and an increase in memory cells. It has been suggested that the decline of naïve cells may impair the ability to response to novel antigens. Recently, Woods et al. (245) demonstrated that 4 months of exercise training significantly increased the percentage of both CD4+ and CD8+ naïve cells in old, but not young mice. Also, it appeared that the number of memory cells decreased, but the number of naïve cells did not change. Similar findings were observed after 8 weeks of moderate exercise in a different study, with the results suggesting a trend ($p=0.07$) towards a decline in the percentage of memory cells found only in older mice (127). This initial data in animal models is promising. It remains to be seen whether the same effects may be found in humans. Also, identifying the mechanisms that drive this change in the naïve/memory cell ratio is another important objective of future research in this field.

Mechanisms by which exercise may alter immunosenescence

The mechanisms responsible for enhanced immune response resulting from long-term exercise training have not been elucidated. It has been suggested that neuroendocrine factors may be one of these mechanisms (113, 148, 184). One approach used to evaluate potential mechanisms relies on pharmacological blockade of specific neuroendocrine factors to determine their role in immunomodulation. Relatively few studies have used this approach to examine exercise training-induced changes (in contrast to acute exercise). With this approach, opioid peptides and catacholamines via beta-adrenergic receptors, have been shown to contribute to the exercise training-induced modulation of immunity (110, 113, 114, 127). To our knowledge, there is only one published study that has used pharmacological manipulation to study the potential neuroendocrine mediators in the aged (127). The findings from this study suggested that beta-adrenergic receptor activation played a role in modulating antibody, mitogen-induced proliferation, and T_H1 cytokines (IL-2, IFN-γ), but not the T_H2 cytokine, IL-10. However, the role of beta-adrenergic receptors appeared to be age-dependent, because beta-adrenergic blockade in young mice did not appear to mediate the exercise-induced changes.

Summary

Overall, the combined data suggest that exercise may be an efficacious therapy for partially restoring immune function in geriatric populations, particularly when long-term exercise interventions are employed. Currently, there are insufficient data to determine: 1) whether aerobic exercise has different effects than resistance training, 2) an optimal amount of exercise that can be recommended, and 3) if the benefits of exercise are restricted to certain populations.

The results from the studies presented suggest that exercise may reverse several characteristics of immunosenecence. A reversal of the age-related decline in the naïve/memory cell ratio may contribute to an impaired response to new antigens, and data suggests that exercise influence the naïve/memory ratio. Also, the age-related decline in vaccine efficacy due to reduced antibody may be improved by exercise, as well as the T-cell and B cell response to novel antigens.

Ageing may be accompanied by an alteration in the T_H1/ T_H2 cytokine balance, particularly a decline in IL-2, and exercise appears to improve both IL-2 production and IL-2 receptor expression. Although these limited results are promising, further research is clearly needed. The mechanisms that underlie the exercise-mediated immunomodulatory effects are unexplored, with only one study suggesting a role for beta-adrenergic receptors. Many aspects of immune function remain to be further examined including dendritic cells, antigen processing and presentation, cell signaling events, costimulatory molecules, response to infectious challenge including immunopathology, etc. Clearly, this is a new and growing area of research, with substantial public health significance.

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