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Exercise Training and Immunosenescence

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ABSTRACT

During the aging process, a decrease in the ability of the immune system to control infection, known as immunosenescence, takes place. Paradoxically, aging also results in chronic low level inflammation and exaggerated inflammatory responses. A number of studies have investigated the effects of a variety of exercise training interventions on the immune system both in humans and using animal models of aging. Cross-sectional studies which compared masters athletes to untrained, age-matched controls found that the athletes had significantly better immune function, but these studies suffered due to the difficulty in generalizing results from highly-trained athletes to a general population of physically-active older adults. Prospective studies in humans have attempted to address this, but these studies have resulted in sometimes equivocal findings, possibly due to the differences in exercise training programs utilized. Finally, animal studies, both observational and mechanistic, have almost universally supported the exercise effect on enhancing immune status in the aged. More research is needed to determine the mechanism by which exercise influences immunity in the aged and to identify exercise training programs for use in this population. It is clear, however, that exercise is likely to be effective at boosting immunity in the elderly when undertaken regularly. KEY WORDS: Exercise, immunity, aging, immunosenescence

INTRODUCTION

A considerable body of literature exists which provides evidence for an age-related decrease in immune status resulting in a concomitant increase in morbidity and mortality. Age-related decreases in immune function are collectively known as immunosenescence and can play a role in the incidence and progression of a wide range of diseases including cancer[1], autoimmune and arthritic diseases[2], and influenza[3], among others. For example, more than three-quarters of total initial diagnoses of cancer occur in individuals age 55 and older[1]. From 1990-1998, persons aged 65 years or older accounted for more than 85% of deaths from influenza viral infection[3]. A number of strategies have been evaluated with varying efficacy in an attempt to increase survivability and decrease morbidity in populations affected with these diseases. Vaccination against influenza virus, while highly effective in younger populations, has been shown to have reduced protective effects in older populations[4]. Thymic hormone administration, while showing promise in both *in vivo* and *in vitro* testing, had only inconsistent effects on cellular and humoral immune responses in older subjects[5]. Recent advances in genetics have made way for the development

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of new therapies to combat immunosenescence, cancer, and other age-related ailments[6, 7], but such therapies are likely to be expensive, individualized, and difficult to implement in the near future.

It is important to note that not all aspects of immunity are decreased during the aging process. Franceschi et al.[8] coined the term "inflamm-aging" to describe the up-regulation of certain inflammatory factors that are associated with the development of chronic diseases during later life. Because exercise has been shown to reduce inflammation in a wide variety of these disease states including cardiovascular disease[9], obesity[10], and impaired wound healing[11], it is likely that exercise is not necessarily "immunoenhancing" but rather "immunoregulatory"; reverting a dysregulated immune system back to its youthful state.

Several lifestyle interventions have positive effects on longevity and immunity in older adults. Among the most notable is caloric restriction, which in mice when practiced without malnutrition has been shown to increase longevity, improve lymphocytic response to mitogen stimulation *in vitro*, decrease tumor incidence rate[12, 13], and reduce inflammation[14]. Additionally, nutritional supplementation with vitamin E has been shown to enhance selected markers of humoral and cell-mediate immunity, including delayed-type hypersensitivity (DTH) skin response as well as antibody responses to a number of clinically-relevant vaccines[15].

Moderate exercise has been proposed as an intervention capable of improving a number of age-related diseases and disorders and is endorsed by the American College of Sports Medicine[16, 17], the American Heart Association[18], and the American Medical Association[17] as a preventative therapy and as an adjuvant treatment for a variety of conditions affecting elderly populations. These endorsements have been informed by a wide body of literature which support the underlying hypothesis of an "inverted J-shaped" curve

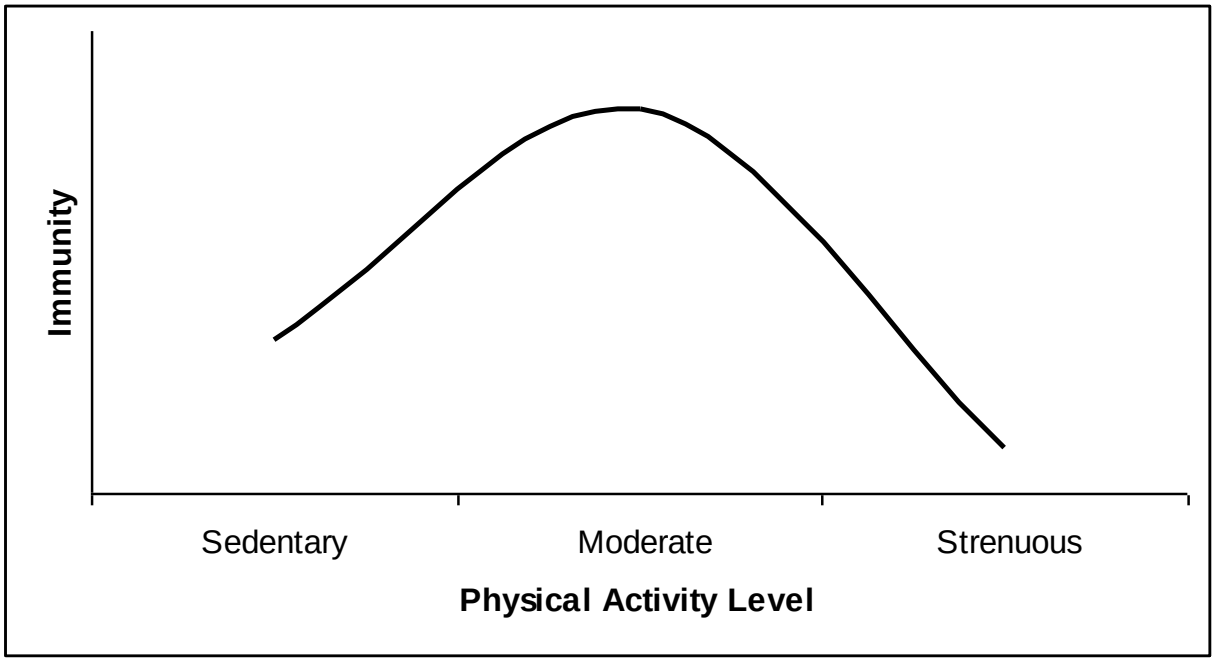

Figure 1: The "inverted J-shaped curve" which demonstrates the hypothesized immune responses to exercise level in elderly individuals. Moderate physical activity has been shown to increase the ability of the immune system to respond to infection, although strenuous physical activity may suppress immune status.

(Figure 1) in which regular moderate exercise improves immune function and overall health in elderly populations. Although the mechanisms by which moderate exercise training can have a beneficial effect on immune function in aged populations are still under intense study and debate, evidence from human cross-sectional, human prospective, and animal studies shows that physical activity can be immunopotentiating and anti-inflammatory and can prevent or reduce morbidity and decrease mortality the elderly. A small number of studies which have examined the effects of only a single bout of exercise on immune status in the elderly will not be reviewed.

HUMAN CROSS-SECTIONAL STUDIES:

Cross-sectional studies provide observations on the association between a physically active lifestyle and aging. In regard to exercise and immunosenescence, cross sectional studies have examined *in vitro* Natural Killer Cell Activity (NKCA) and lymphocyte function, in vivo antibody responses to vaccination and inflammatory markers.

Aging increases the total number of circulating NK cells, but impairs their cytotoxic activity due to a suppressed response to endogenous activators such as IL-2 and IL-12[19]. The cross-sectional data on the impact of exercise on NK cell function in older persons is equivocal. Nieman et al. first reported a 54% higher basal NKCA in highly trained older female athletes compared to sedentary controls, but observed no statistical difference in the number of circulating NK cells[20]. In contrast, Shinkai et al. found no difference in NKCA between older recreational athletes and sedentary people[21]. A follow up study, however, by Shinkai et al. supported Nieman's results, in that 60-70 year old runners exhibited a slightly higher per-cell NKCA compared with the age-matched controls[22]. While Nieman reported no difference in circulating NK cell number, Yan et al. observed higher concentration of CD16+CD56+ NK cells in elderly exercisers versus sedentary controls[23]. The most probable cause of discrepancy in these findings is the various exercise intensities, as there is undoubtedly a difference between recreational athletes and highly competitive athletes. To the best of our knowledge, no studies have examined the effects of exercise training on NK cell sensitivity to stimulation. As NK cell number has been shown to be independently associated with development of and survival after infection in the elderly[24], the exercise effects on NK cell number and activity are potentially important in this population.

Aging significantly affects T cell functions, particularly the age-related decline in proliferative response to polyclonal mitogen and CD25 expression. Compared to NK cells, the cross-sectional data in regard to aging and exercise is relatively straightforward, suggesting an improved T cell response in physically active elderly persons. Both Shinkai and Nieman reported elderly regular exercisers exhibit a 40-50% higher proliferative response to phytohemmagglutinin (PHA) compared to sedentary controls[20, 21]. Shinkai et al. confirmed this finding with pokeweed mitogen as well[21]. In addition to polyclonal mitogens, Kohut et al. demonstrated that both vigorously- and moderately-trained aged exercisers exhibited increased lymphocyte proliferation rates in response to influenza virus vaccination[25]. Using flow cytometry, Gueldner et al. examined CD25 expression, a marker of activated T cells, in response to fixed anti-CD3 antibody in older women, and found elevated expression in the exercisers[26]. As people age, the efficacy of vaccination and response to primary antigens tends to decline, leaving older adults more susceptible to infection[27]. Determining whether regular exercise can improve vaccine responses in the elderly is of high clinical relevance. A cross-sectional study performed by our lab[28] examined a number of cell-mediated and humoral immune parameters in highly cardiovascularly-fit when compared to low fit

older adults. We found that highly fit, physically-active older adults exhibited a heightened antibody response to two of the three strains of influenza included in that year's influenza vaccination when compared to low fit[25]. Additionally, there was a greater *in vitro* proliferative response of peripheral blood mononuclear cells when stimulated with PHA. Tetanus toxoid recall vaccination caused a shift from IgG1 to IgG2 production in the high-fit subjects, which may improve protection against tetanus as IgG2 is thought to be the more potent antigen-neutralizer. Conversely, exercise failed to improve proliferative responses or cytokine production to stimulation with either influenza or tetanus toxoid vaccine *in vitro*; a finding which calls into question the clinical relevance of previous studies which have used polyclonal mitogens to stimulate proliferation. Likewise, Kohut et al. observed higher IgM and IgG responses to influenza vaccination in elderly vigorous exercisers compared to moderate exercisers and sedentary aged people[25]. Schuler et al. also found a positive correlation between antibody response and physically activity levels to the H3N2 component of the influenza vaccine[29].

While influenza vaccine response is clinically relevant, vaccine responses can be confounded by prior exposure history which is extensive in older adults. Smith et al. circumvented this dilemma by examining the antibody response to keyhole limpet hemocyanin (KLH), a T cell-dependent primary immunogen. They found that physically active older adults exhibited an increased anti-KLH IgG, IgG1, and IgM and skin delayed-type hypersensitivity (DTH) responses when compared to sedentary controls, up to 28 days post-immunization[30]. Together these data on recall and primary vaccination responses suggest that older adults who engage in regular physical activity may produce a stronger antibody response than those that are inactive.

Aging not only reduces proliferative capacity and receptor expression, but also suppresses immune cell signal transduction capacity, in particular protein kinase C (PKC). PKC is a serine/threonine kinase essential to T cell signal transduction and stimulation. Wang et al. found basal PKC activity, phorbolmyristate acetate (PMA) induced redistribution of PKC, and PHA induced stimulation of PKC were decreased in lymphocytes of aged adults, but this decrease was attenuated in older adults who were physically fit, as measured by VO_2 max[31]. Overall, these data suggest that T cells of physically active older people exhibit improved proliferative responses to mitogens, and in some studies, clinically relevant pathogens. The extent to which these effects are due to population shifts within the lymphocyte compartment is unknown.

T helper (Th) cells undergo an age-related decline in absolute number, as well as a skew toward a Th2 cytokine profile characterized by increased production of IL-4, which may explain the increased susceptibility to viral infection in older adults as skewing of the T cell response towards a Th2 profile has been associated with increased disease severity in certain viral infections[32]. Ogawa et al. reported exercise-trained aged subjects had higher IFN-γ^+CD-4^+ cells and higher $CD8^+$IL-2^+ cells compared to their sedentary matched controls [33], while Shinkai et al. found elderly runners also exhibited higher PHA stimulated IL-2, IFN-γ, and IL-4 production compared to elderly sedentary[21]. These data indicate that older regular exercisers may have an improved Th1 response which may enhance cell mediated immunity, improve defense against intracellular pathogens, and reduce the risk of viral infections.

Inflammation: In more recent years, it has become evident that elevated levels of proinflammatory cytokines, namely IL-6, TNF-α and the hepatic acute phase C-reactive protein (CRP) are associated with numerous chronic diseases, and are elevated in older individuals[34]. Data from several cross-sectional studies in aged adults demonstrates that higher levels of

physical activity and/or cardiovascular fitness are inversely correlated with serum inflammatory markers such as IL-6, TNF-α, and CRP. Regular physical activity has been also been shown to result in an increase in the circulating levels of several anti-inflammatory cytokines such as IL-10 and IL-1ra, possibly as a result of increased circulating IL-6 in the absence of increases in circulating TNF-α[35]. Moreover, exercise training interventions have been shown to decrease CRP levels in older adults[36], suggesting that exercise may partially ameliorate the chronic low-level inflammatory state seen in this population.

SUMMARY OF HUMAN CROSS-SECTIONAL STUDIES

A primary limitation within these cross-sectional studies is that comparing master athletes to control subjects may not maintain external validity when trying to determine the effect of moderate physical activity on immune function in the aged population. That is, masters athletes are an atypical population and cannot be compared to a "normal" physically fit older population. With the recent public health focus on regular moderate exercise (30 min/day, 5 days/wk), futures studies must examine populations which represent moderately trained older adults. This alone will increase the number of available subjects, and shed light on more realistic associations between physical activity and immune function. As is always the case, additional limitations arise with the cross-sectional approach including the existence of confounding factors such as nutritional status, genetics, psychosocial factors, and other environmental factors which possibly skew the interpretation of physical activity on improved immune function.

HUMAN PROSPECTIVE STUDIES:

Prospective studies are able to definitively determine whether exercise training interventions influence immune function in aged adults. Numerous exercise studies, ranging from eight weeks to two years duration and including both aerobic training and strength training, have explored the impact of regular exercise training on functional aspects of the aging immune system.

NCKA and T Cell Proliferation: Our lab examined the effects of 6 months aerobic exercise training (60-65% VO_2 max, 40 min/day, 3 times/week) or flexibility training on previously sedentary older adults (65± 1 yrs)[37]. Measures of both innate immunity (NKCA) and adaptive immunity (T cell proliferation) were analyzed at pre/post intervention time-points. Both the aerobic and flexibility group demonstrated a small increase in *in vitro* T cell proliferation in response to PHA and Con A mitogens, but the aerobic group exhibited a larger change in proliferation across several doses of Con A stimulation. In regard to innate immunity, the aerobic group exhibited a trend for increased NKCA, but the results did not reach statistical significance. In contrast, Fahlman et al. observed no effect on NKCA or Con A induced T cell proliferation in a group of elderly nuns who underwent 10 weeks of walking (70% Heart Rate Reserve)[38]. The nuns represented an ideal study population as both the exercise and control group maintained very similar diets and lifestyles; the authors hypothesized the intensity and duration of the exercise intervention was insufficient to induce immune adaptations. Supporting this data is a comparable study by Nieman et al., which assigned aged sedentary females to 12 weeks of walking or calisthenics[20]. Despite a 12.6% increase in VO_2max, the walking group exhibited no training effect on NKCA or T cell proliferation to Con A. The conflicting results seen in these studies indicate the importance of training duration and intensity when designing a program to elicit functional immunological adaptations, as longer-term interventions (>6 months) of moderate intensity seem to be the

most likely candidates to beneficially-alter immune function.

While most exercise training studies focus on aerobic exercise, a small number of studies have utilized strength training with the goal of enhancing immune function in aged adults. Rall et al. conducted a 12 week resistance training study in older individuals (69 yrs), and observed no effect in NKCA or PHA stimulated T cell proliferation[39]. A more recent study, however, by McFarlin et al. demonstrated an increase in NKCA in elderly females following 10 weeks of resistance training [40]. It should be noted that the McFarlin study consisted of higher intensity resistance training compared to Rall et al., perhaps suggesting an intensity threshold necessary to elicit functional immune adaptations.

T Helper Cells: Aging is typically associated with decreased circulating Th cells and a skew towards a Th2 profile, which inhibits optimal response to invading pathogens[32]. Of particular importance is the decreased expression of CD28 which plays an essential role in the differentiation of Th cells toward a Th1 phenotype. Reductions in Th1 cell numbers have been associated with reduced responses to vaccination in the elderly[41]. Exercise interventions have begun to examine the effect of training on CD28 expression in older adults. Shimizu et al. observed elevated levels of $CD4^+$, $CD28^+CD4^+$ cells, and $IFN\gamma^+CD4^+$ cells following 6 months of combined aerobic and resistance exercise compared to a sedentary control group[42]. These results indicate moderate exercise training may increase the expression of CD28 on Th cells, thus causing a Th1 skew, and potentially reducing the risk of viral infections in elderly people. In peripheral blood mononuclear cells stimulated *in vitro*, Drela et al.[43] found that 2 years of regular (2x/wk) physical activity increased intracellular IL-2 response in elderly women, suggesting that regular exercise might improve T cell responses to infection in this population. Kapasi et al, however, found no effect of a mixed modal exercise program in frail elderly (88 yrs) nursing home patients on CD28 expression or circulating Th cells[44]. One plausible explanation, suggested by Senchina and Kohut, is that the beneficial effects of exercise are only observed prior to frailty, supporting interventions earlier in the aging process[45].

In vivo Cell Mediated Immunity: Few studies have examined the impact of exercise on in vivo measures of cell mediated immunity. Chin et al. combined mild exercise with an enriched diet in an elderly population, and found a small exercise effect on the DTH response - the exercisers maintained DTH response over the 17 week intervention period, while the control group exhibited a decline[46]. Data from the above mentioned Rall et al. study supports this, as the researchers observed no change in DTH response to multiple antigens[45]. An important aspect of these studies is the clinical relevance of DTH response, which has long been used as an overall indicator of the strength of cell-mediated immunity and is supported by the negative association between DTH response and subsequent mortality[19]. However, a major limitation to using DTH as a functional measure of cell mediated immunity is the large variability typically observed, making it difficult to interpret studies with small sample numbers. Clearly further study is warranted with larger samples sizes to determine the effects of exercise training on cell mediated immunity in aged individuals.

Antibody Titer and Response: Cross-sectional evidence suggests that being physically active or cardiovascularly-fit leads to higher antibody responses to vaccination has provided rationale for longitudinal studies. Kohut et al. demonstrated that a 10 month aerobic exercise training program (65-75% Max HR) increased anti-influenza antibody titer at 1 and 3 months post-immunization compared to control subjects[25, 47]. These researchers also demonstrated an increased granzyme B level in the exercise group, which is indicative of improved cytolytic T cell function. In a

relatively large (n=144) clinical trial from our laboratory, we found that while cardiovascular training did not increase the peak antibody response to influenza vaccination in older adults, it did extend influenza seroprotection throughout the entire influenza season[48] when compared to a flexibility control group. This is clinically important because in addition to reduced peak antibody responses, the elderly also exhibit a faster reduction in protective antibody levels over time[49]. Participants in the cardiovascular exercise group also experienced reduced upper respiratory tract symptom severity throughout the flu season. Using a smaller cohort of the trial, we demonstrated that 10 months of aerobic exercise training increased the IgG, IgG1, and IgM primary antibody responses to KLH when compared to a flexibility control group[50]. In addition to improved vaccine response, exercise training may also increase salivary IgA levels, which represent the first line of defense against invading respiratory pathogens. Shimizu et al. conducted a 6 month moderate intensity cycling program in older males and females, and found that exercise induced a significant increase (~40%) in post-intervention IgA levels, where as there was no intervention effect for the control group[51]. Taken together, these results support the conclusion that regular moderate intensity aerobic exercise training may be able to offset some of the immune derangements seen with aging and improve responses to vaccination. What is unclear is how exercise is exerting its effect.

Inflammation: Our lab has recently published evidence[52] that 10 months of aerobic exercise was able to reduce serum CRP levels in elderly adults (age 60-83 years), concurrent with no reduction in CRP levels of a similar group which underwent only flexibility exercise. Additionally, it was found that reductions in trunk fat associated with exercise were the best predictor of the reductions in systemic inflammation, suggesting that adipose tissue accumulation may be at least partially responsible for the increases in basal inflammation seen in the elderly and other individuals.

In a similar study, Kohut et al. examined the effects of 10 months of aerobic exercise (65-80% VO_2max) in elderly men and women compared to a flexibility control group. Results demonstrated that aerobic exercise induced significant reductions in serum IL-6, CRP, and IL-18, while both interventions were sufficient to cause a decrease in serum TNF-α[53]. Combined resistance and cardiovascular exercise training programs have also been shown to reduce serum CRP in elderly adults[36]. However, strength training alone has been shown to have no effect on systemic inflammation in the elderly[39], suggesting that choice of exercise mode is extremely important in order to receive the full benefits of exercise training on low-level chronic inflammation.

It must be noted that while the presence of a chronic inflammatory disease is one of the primary causes of mortality in the aged population, a normal innate immune response is necessary to protect against infectious disease. To this extent, further studies must examine the hormetic role of exercise mode, intensity, and duration on maintaining the necessary balance between an adequate inflammatory response and chronic inflammation. Moreover, little is known regarding the mechanism of exercise's apparent anti-inflammatory effect.

SUMMARY OF HUMAN PROSPECTIVE STUDIES

While most studies, cross-sectional and prospective alike, have demonstrated enhanced immune function in physically active adults, further study is needed to definitively establish a role for exercise interventions in modulating beneficial effects on immune function in aged individuals. An underlying problem in comparing studies is the differences in exercise mode, intensity, and duration. A summary of

the data suggests that regular moderate intensity aerobic exercise for a duration of > 6 months is a probable threshold which must be accomplished to observe favorable changes in immune function. Furthermore, it must be noted that an important caveat to nearly all exercise immunology studies is the use of *in vitro* responses of peripheral immune cells to assess overall immune functioning. *In vitro* measures such as NCKA, T cell proliferation, and cytokine production lack clinical disease correlates, and thus exercise induced changes cannot be quantified in terms of disease risk or susceptibility. The recent emphasis on *in vivo* immune measures such as DTH skin response, plus the overwhelmingly positive data on the exercise effect on antibody response to vaccination shown by our lab and others, have begun to address these deficiencies, but much work is yet to be done. A notable problem with testing of immune responses in human subjects is the difficulty in establishing a mechanism for the exercise effect. This can be partially ameliorated by employing animal models to test for the specific effects seen in the human population.

ANIMAL STUDIES

In general, animal studies allow for a closer examination of the molecular mechanisms by which exercise alters immune status. These types of studies allow for much better control of all aspects of the experimental design, from exercise duration, intensity, and training modality (including but not limited to swimming, forced treadmill running, and voluntary wheel running) to the eating and social habits of the experimental animals. Additionally, recent developments in rodent genetics will allow investigators to choose specific rodent models of a variety of diseases and disorders which are relevant to gerontology research. The overwhelming majority of animal research in exercise immunology involves testing using rodent models.

A number of studies have examined the effects of various types of exercise training on immune parameters in aged animals. An early study by Pahlavani et al.[54] examined splenic lymphocytes *in vitro* after isolation from aged rats which had been subjected to 6 months of daily swimming training. Exercise training slowed the age-related decline in lymphocyte proliferation due to mitogen stimulation compared to sedentary controls, and mitogen-stimulated interleukin (IL)-2 production was also higher in exercise trained rats compared to controls. A follow-up study, published by Nasrullah & Mazzeo[55], used the same rat model of aging but this time subjected the animals to 15 weeks of 5 days/week treadmill training at 75% maximal effort. Treadmill running improved lymphocyte proliferative response 58% compared to sedentary controls in the aged rats. IL-2 production followed a similar pattern, but natural killer cell cytolytic capacity declined in older rats and was not improved by exercise training.

Several studies have examined the efficacy of exercise training on increasing the functionality of different immune cell populations. Ferrandez & De la Fuente[56] found that swimming training increased the phagocytic capacity of macrophages elicited from the mouse peritoneum against a variety of stimuli. Exercise training increased the macrophage capacity for chemotaxis as well as for the ingestion of latex beads *in vitro*. Training also increased the ability of the macrophages to produce superoxide anion (O_2^-, a component of the immune system oxidative burst) in response to stimulation with latex beads. A previous study[57] examined the potential for exercise-induced increases in corticosterone levels to mediate the exercise effects on macrophage phagocytic capacity. Peritoneal macrophages incubated with plasma from exercise-trained mice, as well as cells incubated with corticosterone at a concentration similar to that seen post-exercise, both increased the phagocytic capacity of macrophages in

response to *Candida alibicans* stimulation *in vitro*. This suggests that exercise-induced increases in glucocorticoids, at least in the short term, may be partially responsible for the exercise effects on cellular immune function seen in other studies. In addition, our lab has shown previously that 16 weeks of treadmill exercise training can increase tumor cytolysis in mice, mainly mediated by enhancements in nitric oxide production by macrophages[58].

Additional studies of humoral and cellular responses with exercise to various pathogens have been undertaken in aged mice. Kohut et al.[59] found that 8 weeks of treadmill training in aged mice (16-18 months) did not increase anti-herpes simplex virus (HSV) IgM responses but did increase production of cytokines including IL-2 and interferon (IFN)-γ by alveolar and splenic lymphocytes in response to HSV-1 viral infection. In contrast, Barnes et al.[60] subjected rats to 10 weeks of treadmill training, and found that measures of typical training efficacy, including heart-to-body weight ratio, VO_2 max, and respiratory exchange ratio, were all improved with exercise. However, no improvements in immunological parameters including antibody response to the novel antigen keyhole limpet hemocyanin (KLH) were found when exercise-trained animals were compared to sedentary controls. These studies, plus those referenced above, indicate that both chronic treadmill and swimming exercises can have beneficial effects on immune parameters in aged mice, but that these effects are not consistent across or even within training modalities.

Given the sometimes inconsistent results when examining the effect of exercise training on immune status, combined interventions have been attempted in order to increase the effectiveness of exercise in boosting the elderly immune system. The most popular interventions have combined nutritional interventions (including dietary restrictions and nutritional supplements) with exercise training. One of the first studies in this area combined caloric restriction, already shown to have a positive influence on immunity in aged populations[12, 13], with exercise training. Utsuyama et al.[61] found that a portion of mice fed a diet consisting of 60% of the caloric intake of *ad libitum*-fed controls, when exercised daily for 21 months, had greatly increased T lymphocyte proliferation in response to mitogen stimulation compared to controls. The same mice also had somewhat increased B lymphocyte proliferation under the same conditions. Strasser et al.[62] compared a caloric restriction intervention to treadmill exercise and found that the exercise training resulted in significantly higher white blood cell counts and better lymphocyte proliferation in response to antigen stimulation when compared to the caloric restriction and *ad libitum*-fed groups. Results from these studies indicate that exercise may be somewhat more important than diet in enhancing immune status in the elderly.

Mechanisms: With the increasing evidence, from both human and animal studies, that exercise improves immune function in the elderly, many investigators have begun to examine the potential mechanisms for this change. An active area of study is the effect of neuroendocrine factors such as opioids and catecholamines on immunosenescence when these factors are modulated by exercise. Itoh et al.[63] found that resting levels of the β_2-adrenergic receptor, which recognizes catecholamines and causes increased blood vessel dilation and glycogenolysis, were decreased after 3 weeks of exercise training. This down-regulation of the receptor was associated with an increased IL-12 production when peritoneal macrophages were stimulated *in vitro* with lipopolysaccharide. This effect was ameliorated when macrophages were transfected with β_2-adrenergic receptor cDNA, suggesting that a down-regulation in the receptor with exercise causes an increase in immune function when immune cells are presented with a pathogen. Kohut et al.[64] showed that blocking β-adrenergic receptors

with nadolol blunted the exercise-induced increases seen in IgM, IL-2, and IFN-γ with exercise in HSV-infected aged mice. Adrenergic blockade also decreased lymphocyte proliferation due to mitogen stimulation in aged mice. These effects were seen only in the aged group; β-blockade did not reduce immune system function in HSV-infected young mice.

Other studies have examined the effects of endogenous opioids on immune parameters. Based on previous data that showed an increase in secondary antibody response to albumin after a single exercise bout in old mice[65], Kapasi et al.[66] examined the effects of endogenous opioids on antibody response to albumin after 9 weeks of moderate exercise training. The investigators found that blocking opioid action via the opioid antagonist naltrexone caused a decrease in secondary antibody response compared to exercised mice that received a placebo. This data suggests that the actions of endogenous opioids, which are upregulated during exercise, may be partially responsible for the increased immune function seen with exercise in aged populations.

Aging is associated with a significant decline in the number of naïve ($CD44^{lo}$) T cells[67], concomitant with an increase in the number of memory T cells ($CD44^{hi}$)[68], decreasing the ability of the aged immune system to respond to antigens to which it has not been previously exposed. Evidence from our lab[69] suggests that exercise improves the ratio of naïve to memory T cells in older mice. Despite the inability of 16 weeks of exercise to reverse the age-associated loss in thymus weight, analysis of subpopulations of T cells from both the spleen and thymus of young and old mice revealed that exercise training brought about a reduction in levels of memory T cells in old but not young mice, thereby shifting the ratio of naïve to memory T cells closer to that of a younger population. A single exercise training bout did not affect the levels of memory or naïve T cells in either old or young mice, suggesting that long-term exercise training is required to achieve the results seen in this study. Currently the mechanism by which exercise affects memory T cell populations is unclear, and future studies are needed to clarify whether this effect proves to increase the functionality of the remaining naïve and memory T cell populations in aged individuals.

CONCLUSIONS

There is overwhelming evidence that some form of regular physical activity can have a beneficial effect on immune status in elderly individuals. Data from human cross-sectional studies almost universally supports the hypothesis that moderate exercise enhances immune function in older adults, but these studies suffer due to their reliance on well-trained master's athletes. These individuals, who presumably compete at a high level in their age bracket, may have better-than-average genetics and thus may not represent the normal response to exercise as might be seen in a previously-sedentary but otherwise healthy individual. As a result of this deficiency, a number of human prospective studies have been undertaken to examine the effects of starting an exercise intervention on a wide range of immunological parameters, including lymphocyte and natural killer cell function, antibody response to vaccination, and inflammation. However, while results from these studies are mostly positive, they are not as robust as those of the cross-sectional studies discussed previously, mostly due to the differences in duration and intensity of the exercise training programs used in each study. A general conclusion that can be drawn from the human prospective data is that at least 6 months of moderate-intensity exercise training is necessary to begin to see the benefits of exercise on immune function, but much more work is needed in this area before any definite conclusions can be drawn.

Animal studies overwhelmingly show that moderate exercise training can improve

immune status in the elderly, but these studies often are difficult to translate to a human population. Studies using animal models of aging and disease do have the benefit of allowing for much easier testing of the mechanisms of exercise modulation of the immune system, but studies in this area are only in the early stages, and much work is yet to be done before we fully understand the ways in which exercise is acting to enhance immunity. The reliance on *in vitro* techniques in both human and animal models is somewhat troubling, and an effort must be made in the future to greater utilize *in vivo* testing so that the results of such studies can be compared more easily to clinical measures.

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