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Influence of age and physical activity on the primary in vivo antibody and T cell-mediated responses in men

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Smith, Taro P., Sarah L. Kennedy, and Monika Fleshner. Influence of age and physical activity on the primary in vivo antibody and T cell-mediated responses in men. J Appl Physiol 97: 491–498, 2004; 10.1152/japplphysiol.01404.2003.—The aging immune system is characterized by the progressive decline in the antibody and T cell-mediated responses to antigen. Little is known, however, about the benefits of exercise in aging on the generation of a primary immune response to antigen and the subsequent antibody and memory T cell-mediated response. Most in vivo immune research to date has utilized vaccines or recall antigens to elicit an immune response. Therefore, the purpose of this experiment was to examine the association of aging and physical activity on the primary antibody and T cell response to the novel protein antigen keyhole-limpet hemocyanin (KLH). Forty-six physically active and sedentary, young (20–35 yr) and older (60–79 yr) men were recruited. Subjects were intramuscularly immunized with 100 μg of KLH, and blood samples were collected at days 0, 7, 14, 21, and 28. Samples were measured for anti-KLH IgM, IgG, IgG1, and IgG2 by ELISA. On day 21 after intramuscular KLH administration, subjects received an intradermal injection with 1 μg of KLH of inflammation recorded at 24, 48, 72, 96, and 120 h to assess anti-KLH delayed-type hypersensitivity response. There was a significant reduction in all anti-KLH measures with aging except for anti-KLH IgG2. The physically active older group had significantly higher anti-KLH IgM, IgG, IgG1, and delayed-type hypersensitivity responses, but not IgG2 compared with the sedentary older group. In conclusion, regular physical activity in older men is associated with a more robust immune response to novel antigenic challenge.

immune function; vaccination; human

THE AGING IMMUNE SYSTEM is characterized by a progressive decrease in responsiveness to exogenous antigens (16, 25). This dysregulation in immune function is problematic for several reasons. A decline in antigen-specific adaptive immunity is a risk factor for increased incidence of infectious disease (12, 15). In addition, older persons are often inadequately protected from diseases because of ineffective responses to vaccination (13), a fact that is in part due to an impairment of the immunoglobulin (Ig) response (30). Both the Ig response (28) and the delayed-type hypersensitivity (DTH) response (10) to antigenic challenge are altered in older human populations, although the precise nature and cellular mechanisms of these alterations remain unknown (3).

Regular moderate exercise training (cardiovascular training, such as walking, cycling, etc.) may offset some of the immune function abnormalities found in healthy older populations (27, 33, 35). A majority of the previous work testing the effect of physical activity on immune function is limited because primarily in vitro measures of immunity were utilized. In vitro assessment of immune function can be problematic because it is difficult to link circulating blood lymphocyte response to overall immune system function (45), and changes in in vitro immune function, when cells are removed from the organism and placed in cultures, often do not predict in vivo responses, perhaps because interactions with neural and hormonal factors that are important in modulating optimal immune responses are not present. Measurement of the antibody response to vaccination and DTH response to a skin challenge provide in vivo methods to measure B cell and T cell function, respectively.

A few studies have tested the effect of physical activity on in vivo assessment of immune function. For example, Kohut et al. (22) reported an improved anti-influenza antibody response to influenza vaccination in older human populations of higher trained status. Bruunsgaard et al. (1) did not find any changes in antibody production to tetanus and pneumococcal vaccines after a race event in triathletes compared with controls. Rall et al. (32) measured DTH responses in the elderly after a 12-wk resistance training intervention; however, they did not find any changes in response to training compared with sedentary controls. One limitation of the previous work is that the antigenic challenge tested was not novel to the person and thus stimulated a secondary or tertiary immune response.

To better understand potential mechanisms of how physical activity may improve immunity, it is best to challenge subjects with a novel antigen. After antigen presentation, the B cell first produces the IgM isotype. The B cell then undergoes antibody class switching via T cell help to produce the IgG isotype. IgG consists of four subclasses in humans (IgG1, IgG2, IgG3, and IgG4). Each subclass differs in its function and cytokine requirement. IgG1, for example, is better for opsonization, whereas IgG2 is better for antigen neutralization (18). In humans, Th1 cells produce IL-2 and IFN-γ, which induces B cells to produce opsonizing antibody such as IgG1 and IgG3, whereas Th2 cells produce IL-4 and IL-10 that induce B cells to produce neutralizing antibodies such as IgG2 and IgG4 (18). If there is an improvement in IgM production with physical activity, then perhaps this is due to improved antigen presenting cell and B cell interaction. If there is a change in the response of a particular IgG subclass, then this could be reflective of changes in Th1 or Th2 cytokine production.

CD4+ T cell function and numbers decline with age (25). Moreover, the ability to form a novel T cell response is also compromised because of an increase in memory subset and a decrease in naive T cell populations over time. The generation of a DTH response after sensitization with a novel antigen provides an in vivo measure for memory T cell function of the CD4+ subset. Memory T cells will migrate to the spot of an
intradermal antigenic skin challenge after sensitization with the same antigen. The T cell then produces IFN-γ to recruit macrophages to the location (18), subsequently producing local inflammation that peaks at 48 h postchallenge and slowly resolves over the next 120 h. Improvement in this response with physical activity in the aged would suggest that there is either a restoration in the number of naïve T cells, an increase in T cell function, or an increase in macrophage recruitment.

This study proposes the use of two in vivo measures of acquired immune function in response to a novel antigen for studying the effect of aging and the potential benefits of regular moderate exercise in younger and older populations. The immune responses assessed are the in vivo generation of the primary Ig and DTH responses after antigenic challenge with keyhole limpet hemocyanin (KLH), a benign T cell-dependent protein. KLH has been previously used in humans to measure immune responses to stress (37, 38), as a vaccine conjugate (23, 31), and for bladder cancer therapy (19, 20). Reduction in the level of KLH antigen-specific antibody is suggestive of impairments in antigen presenting cell–T cell–B cell function, whereas the DTH response offers an in vivo measure of the ability of T cells to proliferate and migrate to antigen. Two experiments were performed. The first study verified that KLH immunization in humans would stimulate detectable and antigen-specific antibody and DTH responses. This was accomplished by comparing pre- and postimmunization anti-KLH IgM and IgG values, as well as comparing anti-KLH DTH responses between KLH-immunized and nonimmunized subjects. The second study tested whether there was 1) an age-associated decline in the primary antibody to a novel antigen; 2) an age-associated decline in memory T cell-mediated responses to a skin challenge (DTH) with the antigen; 3) an effect of regular physical activity on these antigen-specific responses; 4) an association of age and physical activity in antigen-specific antibody isotype (IgM and IgG) and subclass (IgG1 and IgG2); and 5) differences in specific antibody response that can be accounted for by total circulating IgM, IgG1, IgG2, IgG3, and IgG4 in aging and physical activity.

METHODS

Subjects

The experimental protocol was approved by the Human Research Committee at the University of Colorado-Boulder. Voluntary informed consent forms outlining the purpose and risks of the study were collected from all subjects. A total of 19 healthy men (n = 8) and women (n = 11) aged 20–35 participated in testing the KLH specificity (experiment 1). A total of 46 healthy men participated in the physical activity aging study (experiment 2): 10 young (aged 20–35) and 10 older (aged 65–79) sedentary and 12 young and 14 older physically active men. The sedentary men had performed no regular exercise for >2 yr. The endurance-trained men had performed regular aerobic physical activity at least three times per week for >2 yr. All subjects were healthy and free of overt disease as assessed by physical examination, medical history, resting blood pressure, and fasting blood chemistries (e.g., complete blood count with differential, lipid panel, etc.). All older subjects were evaluated for coronary artery disease via an exercise stress test with electrocardiograms and exercise blood pressures. Subjects were excluded if they were smokers, were on medications, or had high alcohol intake. Furthermore, because KLH is a derivative of sea mollusks, subjects with shellfish allergies were excluded from the study.

Treadmill Exercise Test

All experiment 2 subjects participated in a graded treadmill exercise test with a modified Balke protocol. Maximal oxygen consumption was measured via computer-assisted open-circuit spirometry. Heart rate, blood pressure, and rating of perceived exertion were measured at each workload.

Administration of KLH

KLH is a widely used protein isolate that has been used extensively in humans (4–6, 31, 37, 38). KLH (Pierce Biotechnology, Rockford, IL) was conjugated to alum adjuvant (Pierce Biotechnology) according to manufacturer instructions. Briefly, 100 μg of KLH were adsorbed to 900 μg of alum in a ratio as previously published (36) and injected intramuscularly in the deltoid via a 1/2-in. 23-gauge needle. In experiment 1, a subset of subjects (n = 7; 3 men and 4 women) were immunized, with the remaining serving as negative controls (n = 12; 5 men and 7 women). All subjects in experiment 2 (n = 46) were immunized.

Blood Sampling

Ten milliliters of blood were collected via intravenous forearm draws at days 0 (baseline) and 21 for experiment 1 and at days 0, 7, 14, 21, and 28 for experiment 2 after KLH injection. Blood was allowed to clot and then was spun at 3,000 rpm for 10 min, and serum was separated into 1-ml aliquots and frozen at −20°C for later analysis of antibody.

Anti-KLH Antibody ELISA

Experiment 1 serum concentrations of anti-KLH IgM and IgG were measured at days 0 and 21 via ELISA. Experiment 2 anti-KLH IgM was measured at days 0, 7, 14, and 21, whereas anti-KLH IgG IgG1, and IgG2 were measured at days 0, 7, 14, 21, and 28 via ELISA. Ninety-six-well microtiter plates (Nunc, Rochester, NY) were coated with 1 μg of KLH in 100 μl of carbonate coating buffer per well and incubated for 2 h at 37°C then overnight at 4°C. Plates were washed four times with PBS-Tween 20 buffer and blocked with 1% dry skim milk in PBS. Plates were washed an additional four times, and 100 μl of sera were added at the appropriate dilutions (1:100 for IgM, 1:200 for IgG, 1:2 for IgG1, and 1:1 for IgG2). Plates were incubated for 45 min and washed six more times. One hundred microliters of horse-radish peroxidase-conjugated anti-human IgM (lot no. 080, DAKO, Carpinteria, CA), IgG (DAKO lot no. 128), IgG1 (lot no. 20167766, Zymed, South San Francisco, CA), and IgG2 (Zymed lot no. 20264084) secondary antibody was added at a concentration of 1:500 in 1% dry skim milk in PBS-Tween to the appropriate plates and incubated for 45 min. Plates were washed six more times, and 100 μl of 2,2'-azino-di(3-ethylbenzthiazoline)sulfonic acid (ABTS)+H2O2 in citrate buffer (Zymed ABTS substrate kit for horse-radish peroxidase) was added to all wells. Plates were incubated for 15–30 min and read at an optical density (OD) of 405 nm. Anti-KLH antibody is presented as absolute OD for experiment 1 to allow for the inspection of the levels of nonspecific binding in sera of preimmunized subjects. Anti-KLH antibody was calculated for each subject as a subtraction of anti-KLH primary Ig and DTH responses (4) an association of age and physical activity in antigen-specific antibody isotype (IgM and IgG) and subclass (IgG1 and IgG2); and 5) differences in specific antibody response that can be accounted for by total circulating IgM, IgG1, IgG2, IgG3, and IgG4 in aging and physical activity.

Anti-KLH DTH Response

A DTH intradermal challenge (1 μg KLH in 10 μl sterile H2O into the volar aspect of the arm) was administered 3 wk after KLH immunization via a 3/4-in. 29-gauge needle. Timing of the intradermal KLH challenge was determined from anti-KLH DTH experiments as previously published (6, 36). The diameter (mm) of the DTH response was measured 24, 48, 72, 96, and 120 h postchallenge.
Serum samples were assayed according to manufacturer instructions for total circulating IgG1, IgG2, IgG3, and IgG4 at baseline and day 21 postimmunization. Total IgG was determined by summing the total quantity of IgG subclass (IgG1–IgG4) at both preimmunization and day 21.

**Statistical Analysis**

Data were analyzed by 2 × 2 ANOVA (age × trained status) with repeated measures as indicated. When significant differences were found, Fisher’s protected least significant difference (PLSD) post hoc analyses were utilized to determine group differences. All data are expressed as means ± SE. Statistical significance was determined at \( P < 0.05 \).

**RESULTS**

**Experiment 1: Specificity of the KLH Response**

Both the anti-KLH IgM and IgG titers are elevated at day 21 postimmunization (Fig. 1A, \( P < 0.05 \)). This demonstrates that our ELISA does detect KLH-specific Ig and that KLH immunization is effective at inducing the primary antibody response. Nonspecific reactivity or cross-reactivity in preimmunized subjects resulted in an OD of 0.1–0.3; however, this was acceptable given that the overall OD range was 0–3.0. Intradermal KLH challenge resulted in a DTH reaction as measured by inflammation diameter (mm) that peaked at 48 h and persisted up to 120 h in the KLH-immunized subjects; however, nonimmunized subjects had no resultant inflammation, indicating that the DTH reaction only occurs in previously immunized subjects and is specific to memory for KLH (Fig. 1B).

**Experiment 2: Aging and Physical Activity**

**Subject characteristics.** Select subject characteristics for experiment 2 are presented in Table 1. The mean age difference between the young and older groups was 40 yr. Body mass was not related to age or body composition and was not different between the two older groups; however, physically active older subjects had lower body mass index than sedentary older subjects (\( P < 0.05 \)). Lymphocyte counts were significantly higher in the physically active older subjects compared with their sedentary counterparts (\( P < 0.05 \)) but were still within normal clinical ranges.

**Table 1. Selected subject characteristics**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sedentary</th>
<th>Physically Active</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Young (n = 10)</td>
<td>Older (n = 10)</td>
</tr>
<tr>
<td>Age, yr</td>
<td>27.6 ± 1.2</td>
<td>72.1 ± 1.2 *</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>86.0 ± 5.6</td>
<td>82.6 ± 3.0</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>20.4 ± 2.0</td>
<td>27.5 ± 1.6 *</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>27.8 ± 1.1</td>
<td>27.2 ± 0.9</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>117 ± 3</td>
<td>124 ± 4</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>78 ± 3</td>
<td>76 ± 2</td>
</tr>
<tr>
<td>V̇O₂ max, ml/kg·min⁻¹</td>
<td>42.8 ± 2.5</td>
<td>24.9 ± 0.8 *</td>
</tr>
<tr>
<td>WBC, K/μl</td>
<td>5.6 ± 0.5</td>
<td>6.2 ± 0.5</td>
</tr>
<tr>
<td>Neutrophils, K/μl</td>
<td>3.1 ± 0.4</td>
<td>3.8 ± 0.5</td>
</tr>
<tr>
<td>Lymphocytes, K/μl</td>
<td>1.8 ± 0.1</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>Monocytes, K/μl</td>
<td>0.52 ± 0.05</td>
<td>0.61 ± 0.05</td>
</tr>
<tr>
<td>Basophils, K/μl</td>
<td>0.07 ± 0.01</td>
<td>0.05 ± 0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE. BP, blood pressure; V̇O₂ max, maximal O₂ uptake; WBC, white blood cells; K, 1,000. *\( P < 0.05 \) vs. young of same trained status; †\( P < 0.05 \) vs. age-matched sedentary.
Effect of age and physical activity on the anti-KLH serum antibody response. There was a main effect of age as indicated by a reduction anti-KLH IgM \( [F(1,42) = 16.73, P < 0.001; \text{Fig. 2A}] \) and IgG \( [F(1,42) = 19.99, P < 0.0001; \text{Fig. 2B}] \) antibody response over time in the older subjects. There was a main effect of physical activity in anti-KLH IgG \( [F(1,42) = 4.74, P < 0.05] \) indicated by an increase over time that is likely due to a greater response in older physically active subjects and not of younger subjects. There was only a trend toward an increase in anti-KLH IgM \( [F(1,42) = 3.10; P = 0.0857] \) in the physically active subjects. Post hoc analysis revealed a reliably higher anti-KLH IgM (Fisher’s PLSD, \( P < 0.05)\) response by day 21 in the older physically active compared with the older sedentary group that was not significantly different than the young sedentary subjects (Fisher’s PLSD, \( P = 0.3038)\). The older physically active group had an elevated anti-KLH IgG concentration compared with the older sedentary group at both day 21 (Fisher’s PLSD, \( P < 0.05)\) and day 28 (Fisher’s PLSD, \( P < 0.05)\), which was not different on day 21 from the young sedentary (Fisher’s PLSD, \( P = 0.5572)\) and the young physically active groups (Fisher’s PLSD, \( P = 0.2951)\).

Only anti-KLH IgG1 demonstrated an effect of training \( [F(1,42) = 14.24, P < 0.001; \text{Fig. 3A}] \) and a reliable age \( \times \) trained status \( [F(1,42) = 5.26, P < 0.05)\) interaction. Post hoc analysis revealed that older trained subjects had elevated anti-KLH IgG1 response at days 14 (Fisher’s PLSD, \( P < 0.0001)\), 21 (Fisher’s PLSD, \( P < 0.01)\), and 28 (Fisher’s PLSD, \( P < 0.001)\) compared with their sedentary counterparts. In addition, the anti-KLH IgG1 responses of the physically active older subjects were not statistically different at days 21 \( (P = 0.93)\) and 28 \( (P = 0.207)\) compared with the young physically active controls. This effect was isotype specific such that anti-KLH IgG2 responses were not affected by age \( (P = 0.513)\) or physical activity \( (P = 0.144)\) (Fig. 3B).

Effect of age and physical activity on the anti-KLH DTH response. All subject groups mounted a DTH response to the intradermal challenge over time \( [F(5,190) = 38.601, P < 0.0001; \text{Fig. 4}]\). Statistical analysis revealed a main effect of training \( [F(1,38) = 4.922, P < 0.05)\) over time and a time \( \times \) trained status interaction \( [F(5,190) = 2.699, P < 0.05)\). Anti-KLH DTH response was higher in both physical activity age groups, as indicated by a lack of main effect of aging \( [F(1,38) = 0.2306, P = 0.6339)\) or age \( \times \) trained status interaction \( [F(1,38) = 1.000, P = 0.3236)\).
revealed that the sedentary older subjects had age-related reduction in the anti-KLH DTH response at 48 h compared with the young physically active subjects (Fisher’s PLSD, $P < 0.05$). DTH response was significantly elevated in older physically active subjects compared with their sedentary counterparts (Fisher’s, $P < 0.05$) and was similar to that of the younger controls ($P = 0.6237$). However, 120 h after the intradermal injection the older physically active subjects had not fully resolved their inflammation, which was significantly elevated compared with young sedentary (Fisher’s PLSD = 2.832, $P < 0.05$) and the young physically active (Fisher’s PLSD = 3.75, $P < 0.001$) subjects.

Effect of age and physical activity on total IgM, IgG, IgG1, IgG2, IgG3, and IgG4. There were no differences in total circulating IgM, IgG, IgG1, IgG2, IgG3, and IgG4 antibodies between the subject groups. Furthermore, there were no differences in total antibody concentrations 21 days postimmunization between groups ($P > 0.05$). These data support the idea that significant changes in a specific immune response may occur in the absence of changes in total circulating Ig.

DISCUSSION

This is the first study to clearly demonstrate by use of a novel in vivo antigenic challenge that physical activity is associated with elevated generation of a primary antigen-specific T cell-dependent antibody and DTH responses in aging humans. The primary observations of this study are the following: 1) there is an age-related decline in the primary antibody response to the novel antigen KLH; 2) there is an age-related decline in the memory T cell response to KLH; 3) older physically active subjects have an improved antibody and DTH response compared with older sedentary subjects that is equal to that of younger subjects; 4) the changes in anti-KLH IgG production are primarily of the IgG1 subclass; and 5) the detected changes in antibody are reflective of improvements in antigen-driven responses because total antigen nonspecific Ig is not affected.

Previous studies examining vaccine efficacy in young vs. older subjects have demonstrated declines in anti-influenza (13) and no changes in anti-tetanus (2) antibody response. It is important to account for previous exposure to antigen when utilizing vaccines to compare in vivo immune responses between subject groups, because there is a high likelihood of previous encounter and resultant immunological memory. On the basis of our specificity results, the KLH response is antigen specific and is only present in immunized individuals. As such, KLH challenge is a useful tool in determining the effects of aging and trained status on the in vivo immune response in humans as well as animals. In fact, it has been recently used to assess the extent of immune dysfunction in persons with diabetes (40).

CD4$^+$ T cell function has been reported to decline with aging, and that decline contributes to the subsequent reduction in B cell Ig production (46). The decrease in T cell function is characterized by a failure to proliferate in response to antigen, possibly because of a reduction in IL-2 production (24, 29, 39). Previously, Shinkai et al. (34) suggested that trained older people have improved T cell function secondary to improved in vitro IL-2, IFN-γ, and IL-4 production. This would be consistent with our data, because our trained older subjects had improved antigen-specific IgM and IgG responses to a T cell-dependent antigen, suggesting that the physical activity may restore the anti-KLH antibody response by increasing T cell help.

Similar to the influenza vaccination study by Powers (30), our data demonstrated a reduction in specific IgG1 response in the elderly individuals. In the present study, there was a change only in the IgG1 antibody response and not in the IgG2 response. Perhaps there were no changes in the anti-KLH IgG2 responses between groups because protein-derived antigens such as KLH drive primarily IgG1 and IgG3 antibody responses in humans (47). Alternately, the differential anti-KLH IgG1 and IgG2 responses may be reflective of age-selective declines in helper T cell function. Th1 cytokines stimulate IgG1 and IgG3 production, whereas Th2 cytokines promote IgG2 and IgG4 production from B cells in humans (18). Thus the selective effect of aging and training on IgG1 may reflect a greater sensitivity of Th1 cells to the effects of age and activity. Although we were not able to directly measure the changes in anti-KLH IgG3 or IgG4 in our study, we did measure total nonspecific IgG3 and IgG4; however, these results indicated that no subject group differences in these antibody subtypes were present.

Anti-KLH DTH responses were higher in the physically active older group compared with the sedentary older group. This indicates that the in vivo measure of T cell-mediated immune response is associated with higher physical activity status. The DTH response is driven by memory CD4$^+$ T cells that release IL-2 and IFN-γ to signal macrophages to an area after an encounter with the recall antigen. There is also a shift in the subset of T cells from naive (CD45RA$^+$) to memory (CD45RO$^+$) with aging, resulting in a compromised ability to deal with new antigens (3, 9, 14). Because there appears to be a restoration in the in vitro T-cell cytokine production in trained elderly (35), these results provide in vivo evidence that physical activity is associated with higher T cell-mediated responses. Furthermore, the DTH measure could have an important clinical implication, because DTH anergy is a predictor of mortality in the elderly (42) and is a determinant of infectious disease risk (10).
Changes in B cell repertoire with age also contribute to the decline in antigen-specific responses (8). There is a shift in the population of B cells from B2 to B1 cells (43, 44). B1 cells contribute to the increase in autoantibodies with age. Furthermore, there is a higher occurrence of autoantibody (e.g., α-dsDNA) in older individuals compared with younger controls (17). Thus, in the case of aging, the problem is not a reduction in the total concentration of antibody but rather a decrease in functional antibody. The results from the present study support this idea. As shown in Fig. 5, aging was not associated with a decline in total Ig, rather only a decline in the generation of new antibody in response to KLH. Importantly, our study demonstrated a higher antigen-specific antibody production in the physically active older group compared with the sedentary older group and demonstrated that this increase was detected in antigen-specific antibody independent of any changes in total antibody. In addition, changes in total IgM, IgG, IgG1, IgG2, IgG3, and IgG4 over time could not account for changes in the anti-KLH IgG response. Older subjects did not have reduced total antibody, and physical activity did not increase total antibody. Assessment of total antibody is not sensitive enough to detect either aging or physical activity. Thus further questioning the validity of measuring total Ig as a means of examining the effects of an intervention on immune responses.

Fig. 5. Total serum concentrations of IgM (A), IgG (B), IgG1 (C), IgG2 (D), IgG3 (E), and IgG4 (F) were measured on day of KLH immunization and 21 days postimmunization. The changes in total antibody over time could not account for the subject group difference in the antigen-specific KLH response as there were no total increases in any Ig isotype.
function and supports the power of the primary antigen-specific in vivo immune response used in this study.

Psychological stress is an important factor in the type and magnitude of immune response in the elderly. Both Kiecolt-Glaser et al. (21) and Vedhara et al. (41) found that elderly subjects who cared for a dependent spouse and experienced a higher level of stress had a lower antibody response to influenza vaccine. Maintaining a physically active lifestyle improves health throughout the life span, but especially during times of immunocompromise (11). For example, we have previously reported that rats with 6 wk of free wheel running are protected against stress-induced suppression of anti-KLH IgG, IgG, and IgG2a (26). Furthermore, Kohut et al. (22) found a positive influence of physical activity and psychosocial status as assessed by questionnaire on the anti-influenza vaccine IgM and IgG responses in older adults. Although the present study cannot directly assess this idea, it is possible that the improved responses of our physically active older subjects were due to an improved ability to offset the effects of stress that is common in the aging population.

We initially chose to use cross-sectional methodology because we aimed to measure the primary immune response and it is difficult to utilize the measure twice in subject groups, because there would be a resultant memory response. Because of the cross-sectional design, we are not able to conclude that physical activity, per se, attenuates the aging-associated decline in the primary immune response. We did attempt to control for various health factors such as body composition, medications, blood pressure, blood chemistries, diet, and overt disease that could influence our results. Our groups were matched for all of these factors. One difference that we found between the groups was that the older sedentary subjects had lower circulating lymphocyte and basophil concentrations than the physical activity subjects. Thus the physically active older subjects were slightly more resistant to age-associated declines in lymphocytes numbers. The present study cannot determine whether such changes contributed to any of the differences to the KLH measures. Moreover, we found a significant positive correlation with maximal oxygen consumption and day 21 anti-KLH IgM (P < 0.01), day 28 anti-KLH IgG (P < 0.01), and day 28 anti-KLH IgG1 (P < 0.01) responses independent of age, lending evidence to the hypothesis that trained status may be a significant factor contributing to optimal antibody responses.

In conclusion, our model provides an in vivo demonstration of an age-related reduction in the primary antibody and memory T-cell response in humans, which may be offset by maintaining a physically active lifestyle. On the basis of our results, future work examining the influences of an exercise intervention on the primary immune response in aging is warranted.

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