Higher antibody, but not cell-mediated, responses to vaccination in high physically fit elderly

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Keylock KT, Lowder T, Leifheit KA, Cook M, Mariani RA, Ross K, Kim K, Chapman-Novakofski K, McAuley E, Woods JA. Higher antibody, but not cell-mediated, responses to vaccination in high physically fit elderly. J Appl Physiol 102: 1090–1098, 2007. First published November 9, 2006; doi:10.1152/japplphysiol.00790.2006.—The purpose of this study was to examine whether cardiovascular fitness, independent of confounding factors, was associated with immune responsiveness to clinically relevant challenges in older adults (60–76 yr). Thirteen sedentary, low-fit (LF; maximal O2 uptake = 21.1 ± 1.1 ml·kg−1·min−1) and 13 physically active, high-fit (HF; maximal O2 uptake = 46.8 ± 3.4 ml·kg−1·min−1) older adults participated in this study. Dietary intake was assessed, and a battery of psychosocial tests was administered. In vivo antibody and ex vivo proliferative and cytokine responses to influenza (Fluzone) and tetanus toxoid (TT) vaccination and delayed-type hypersensitivity skin tests were performed. HF elderly individuals displayed a higher antibody response to two of the three strains included in the Fluzone vaccine as measured by hemagglutination inhibition, but there was no difference between groups in influenza-specific ex vivo proliferation or IFN-γ or IL-10 production. HF elderly individuals exhibited a lower IgG1 response and a tendency for a higher IgG2 response to the TT vaccine. There were, however, no differences in TT-specific ex vivo proliferation or IFN-γ or IL-10 production. In contrast, HF subjects had higher proliferative responses to phytohemagglutinin. In addition, there were no differences in delayed-type hypersensitivity responses to fungal antigens between groups. These results suggest that, after accounting for confounding factors, HF elderly individuals have higher antibody responses to Fluzone vaccine and a Th2 skewing of the antibody response to TT. There was little evidence that HF mounted better cell-mediated immune responses to the Fluzone or TT vaccine measured in peripheral blood cells or to other recall antigens in vivo.

immunity; aging; exercise; physical activity; vaccination; delayed-type hypersensitivity

DYSREGULATED IMMUNE FUNCTION occurs with advancing age in both humans and animals (35). It is well known that T lymphocyte function declines with advancing age. The best characterized of these are the well-established age-associated reductions in antigen- and mitogen-induced T-cell proliferation, IL-2 synthesis, and expression of high-affinity IL-2 receptors (16). Thymic atrophy and the associated deficiency of T-cell receptor rearrangement contribute to T-cell dysfunction in the aged by reducing the production of naive T cells seeding the periphery (3). This, coupled with the accumulation of memory or antigen-experienced T cells, leads to altered T-cell responses to pathogens and vaccines and reduced delayed-type hypersensitivity (DTH) responses (16, 20). T lymphocytes are not the only immune cells affected by aging. Our laboratory (31, 53) and others (15) have documented age-associated changes in macrophage function. For example, we have found that aging results in a reduction in the ability of macrophage’s to respond to classical activation signals like IFN-γ and LPS (31, 53). Natural killer cells from older adults and mice have been shown to be hyporesponsive to stimulation with IL-2, IL-12, and IFN-α (29, 50). Humoral immunity is also affected by aging, as demonstrated by reduced peak antibody responses to vaccines (6), poor antibody quality (30), and early loss of protection (2) compared with younger subjects. The adverse changes in immune function noted above likely contribute to the increased infectious disease susceptibility and poor vaccination responses of older subjects (35).

The realization of dysregulated immune function and the increased disease incidence in the elderly have been the impetus for interventions designed to improve immune function in this ever-expanding population (19, 36). Unfortunately, pharmacological or hormonal, genetic, and tissue grafting interventions have been impractical, costly to develop and to administer, or accompanied by adverse side effects. Alternatively, early evidence suggests that physically fit elderly (25, 37, 41, 42, 52) or sedentary older adults who engage in exercise training (8, 18, 25, 33, 51) may be able to improve immune function. However, not all longitudinal studies have documented changes in immune function (23, 38). In a recent study, Kohut et al. (26) found that a 10-mo aerobic exercise intervention improved antibody responses to influenza vaccine in previously sedentary older adults (26). Inclusion of clinically relevant immune measures (e.g., responses to vaccinations or DTH antigens) is paramount to understanding the role that exercise plays in modulating immunological vigor. Unfortunately, some previous studies have only assessed in vitro challenges to polyclonal stimuli, and others have failed to document immune responses to vaccines past a few weeks. Moreover, many previous studies have failed to control for differences in health and psychosocial status and nutrition, which likely confound the relationship between fitness and immune function.

We hypothesized that highly physically fit [HF group; >50th percentile maximal oxygen uptake (VO2 max) score for appropriate age and gender] elderly men and women would exhibit a more robust immune response to clinically relevant challenges than low physically fit (LF group; <20th percentile)

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elderly individuals, while accounting for confounding variables. Groups were compared on a comprehensive panel of in vivo and ex vivo immune challenges, including humoral and cell-mediated immune (CMI) responses to influenza and tetanus toxoid (TT) vaccines and DTH testing.

**MATERIALS AND METHODS**

Subjects. Healthy, independently living elderly (60–76 yr) male (n = 13) and female (n = 13) subjects were recruited through newspaper advertisements, area community centers, local running and cycling events, and religious organizations. Exclusionary criteria included documented disease (e.g., cardiovascular disease, diabetes), medications known to alter immune responsiveness (e.g., corticosteroids), and history of conditions associated with immune dysfunction (e.g., cancer, severe arthritis, inflammatory bowel disease, etc.). As part of our screening process, we also performed complete blood cell counts and differentials and an extensive metabolic panel, including measures of fasting glucose, liver enzymes, albumin, hemocrit, hemoglobin, electrolytes, and total cholesterol. We excluded three subjects because of either abnormal blood tests (n = 2) or a positive graded exercise test (n = 1). The average number of prescribed medications taken daily by each participant was 2.7.

Previous vaccination history can have an impact on subsequent vaccine responses, so we queried our subjects about their vaccine history and asked them to retrieve vaccination records from their personal physician if possible. Ten of the 13 LF (77%) and 26 subjects in the study, 3 (2 in the HF and 1 in the LF) reported previous year’s influenza vaccination. We also asked subjects to report flu-like symptoms to us during the course of the study. Of the 26 subjects in the study, 3 (2 in the HF and 1 in the LF) reported symptoms consistent with influenza (e.g., body aches, fever, malaise). Approximately 2 wk after symptoms were reported, a blood sample was taken for hemaglutination inhibition (HI) titer analysis. In no case were antibodies against the three strains included in the vaccine elevated above that which might be expected postinfection. We could not conclude that acute illness did not confound immune function tests. No subject reported acute illness during testing.

**Blood sampling and vaccine administration.** At least 1 wk after the graded exercise test (between October 1st and 15th), subjects reported to the laboratory after an overnight fast. Each subject was asked to refrain from exercise for 48 h before any immune function testing or blood sampling. Venous blood was collected in EDTA-coated or serum separator tubes (BD Vacutainer, Franklin Lakes, NJ) for cell isolation and serum storage. After the blood draw, subjects were vaccinated intramuscularly (in the dominant arm) with Fluzone influenza vaccine (Aventis Pasteur, Swiftwater, PA) containing 15 μg of H1N1 (New Caledonia/20/99), H3N2 (Panama/2007/99), and B (Hong Kong/1434/2002) strain. In the opposite arm, they received 0.5 ml of TT (Aventis Pasteur) booster vaccination containing 5 flocculation units of TT. Subjects reported to the laboratory 6 wk and 6 mo later for fasting blood draws. Importantly, subjects were queried about their health status before and 10 days after each laboratory visit to ensure that acute illness did not confound immune function tests. No subject reported acute illness during testing.

**Antibody responses.** Standard microtiter serum HI analysis, which included controls for nonspecific HI, was performed to determine anti-influenza antibody titer against each of the three strains included in the vaccine. The appropriate influenza A and B test antigens for HI were obtained as egg allantoic fluid (WHO Collaborating Center for Influenza, Centers for Disease Control, Atlanta, GA). Paired pre- and postimmunization serum samples from the same individuals were tested simultaneously for each of the test antigens. Analyses of these samples were performed at a Centers for Disease Control reference laboratory (Hackensack University Medical Center, Hackensack, NJ). Anti-TT IgG1 and IgG2 concentrations were determined in our laboratory by ELISA. Briefly, 96-well microtiter plates were coated with TT vaccine (1:10 in carbonate coating buffer) overnight followed by washing. After this, a 1:200-fold (IgG1) or a 1:100-fold (IgG2) dilution (based on previous titration experiments) of subject sera was added to the plates and incubated for 2 h at 37°C. After plates were washed, wells were incubated with a 1:200 dilution of biotin-labeled...
anti-human IgG1 or anti-human IgG2 (BD Biosciences, San Diego, CA) for 1 h at 37°C. After further washing, avidin peroxidase (Sigma, St. Louis, MO) was added to each well for 30 min at room temperature. After a wash step, substrate (TMB; BD Biosciences) was added to each well, and plates were read on a microplate spectrophotometer (Labsystems Multiskan; Fisher Scientific, Pittsburgh, PA) at 405 nm.

An identical known control sample (e.g., pooled serum) was run on each plate to correct optical density values for plate-to-plate and interassay variation. All optical density values are reported as corrected to the control.

Isolation of peripheral blood mononuclear cells. Whole blood was mixed in a ratio of three parts blood to five parts RPMI-1640 (Mediatech, Herndon, VA). This mixture was then layered onto a density-gradient Histopaque 1077 (Sigma) and centrifuged at 1,500 rpm for a duration of 30 min. The opaque interface containing the peripheral blood mononuclear cells was harvested and washed five times with RPMI-1640 containing 5% FBS (Bio Whittaker, Walkersville, MD) and 0.5 U/ml heparin (Sigma). The pellet of cells was resuspended in 20 ml of RPMI-1640-FBS-heparin. After the final wash, the cells were resuspended in AIM-V medium (Invitrogen, Carlsbad, CA). Cell viability and count were then assessed by trypan blue.

Ex vivo proliferation and cytokine production. Cells were adjusted to 2 x 10^5 cells in 150 μl of medium. Triplicate cultures were stimulated for 3 days with 0.5 (suboptimal) or 2 (optimal) μg/ml concentrations of the polyclonal mitogen phytohemagglutinin (PHA; Sigma) for 5 days with 0.5 μg hemagglutinating unit/ml of Fluzone (Aventis Pasteur) or 0.5 μg/ml TT (CalBiochem, San Diego, CA) in 50-μl volumes in 96-well round-bottom plates. Before addition, Fluzone was dialyzed against PBS at 4°C for 12 h to remove the mercury-containing preservative Thimerosal. Thimerosal, although necessary to preserve vaccine shelf-life, inhibits proliferation in culture. The concentrations of Fluzone and TT in vitro were chosen based on prior titration curves and represented a concentration that yielded suboptimal responses in older subjects (data not shown). All cultures were maintained in AIM-V medium and incubated at 37°C in 5% CO2. Immediately before the addition of 1.0 μCi of [methyl-3H]thymidine (ICN, Irvine, CA), the top 75 μl of supernatant was drawn off of each cell culture well. The supernatants were then pooled from triplicate wells and stored at -80°C for ensuing analysis of IL-10 (Th2) and IFN-γ (Th1) via ELISA. The amount of radioactivity incorporated into the cultures was determined by harvesting the contents of each well after 4-h incubation with tracer onto glass fiber filters by a PhD cell harvester (Cambridge Technologies, Watertown, MA). Radioactivity of the filters was determined with a Packard liquid scintillation counter (Packard, Meridian, CT). Net proliferation was calculated as stimulated cultures (e.g., PHA, Fluzone, TT) minus cultures containing no stimulant. The same lot numbers of PHA, Fluzone, and TT were utilized in all experiments.

DTH responses. Three intradermal injections (0.1 ml) were made in the nondominant arm of each subject by the Mantoux method, needle bevel down. The injections included saline (as a control) and two fungal antigens, candida (Candid; Allermed Labs, San Diego, CA) and trichophytin (Holllister-Stier Labs, Spokane, WA). Forty-eight hours after injection, the sites were assessed for induration in a blinded fashion by the same researcher. The area of induration was measured across the widest parts of vertical and horizontal diameters and averaged. The measurements were considered positive if they were >5.0 mm. All subjects were instructed to not use anti-inflamatory medications for 24 h before and during the 48 h after injection until the sites were read.

Data analysis. All statistical tests were performed with SPSS version 13.0. All nonnormal-dependent variables in raw data form (e.g., anti-influenza HI antibody titers) were log 2 transformed and analyzed with parametric tests. Because we had both a between-subject (HF and LF groups) and within-subject (time: baseline, 6 wk, and 6 mo) design, we conducted a series of 2 x 3 mixed-model repeated-measures (RM) ANOVAs using a general linear model. Post hoc t-tests with Bonferroni correction were utilized in the event of significant group x time interactions. Additionally, we conducted RM-analysis of covariance analyses and included body fat, physical activity, psychological and nutritional indexes, gender, and age as covariates to control for possible contributions to differences in immune responses between the different physical fitness groups. Independent group t-tests were performed to document differences between groups on descriptive variables. Statistical significance was set at P < 0.05 for all tests. All data are presented as means ± SE.

RESULTS

Descriptive data. Our sample contained eight men and five women in the HF group and five men and eight women in the LF group (χ² = 1.3, P = 0.22). Preliminary analyses revealed no differences in immune function responses between genders; therefore, data for men and women were pooled for subsequent analyses. As can be seen in Table 1, there were no significant differences between groups in age, resting diastolic blood pressure, maximal systolic blood pressure, treadmill time, or rating of perceived exertion at maximum. The HF group exhibited significantly higher VO2 max scores, work capacity and maximal heart rates, ventilation, and respiratory exchange ratios than the LF group. In the LF group, there were 10 subjects in the 10th percentile and 3 subjects in the 20th percentile of fitness based on VO2 max scores and gender-normative data for those >60 yr of age (1). In the HF group, there was one subject each in the 50th, 60th, and 70th percentiles, two subjects in the 80th percentile, and eight subjects in the 90th percentile. Thus it appears that our two groups can be accurately classified as low and high fit. In addition, the HF group also had significantly lower percent body fat, resting heart rate, and systolic blood pressures. There were no significant differences between groups for complete blood cell counts, fasting glucose, liver enzymes, albumin, hematocrit, hemoglobin,

<table>
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<th>Variable</th>
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<th>High Fitness</th>
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<tr>
<td>n</td>
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<td>13</td>
</tr>
<tr>
<td>Age</td>
<td>67.9 ± 1.2</td>
<td>64.8 ± 1.2</td>
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<tr>
<td>Body weight, kg</td>
<td>82.9 ± 4.5</td>
<td>68.6 ± 4.5*</td>
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<td>34.4 ± 2.1</td>
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<td>1.14 ± 0.01†</td>
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<td>43 ± 2†</td>
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<td>Tlim, s</td>
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<td>16.2 ± 0.4</td>
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<td>PASE</td>
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<td>193 ± 13†</td>
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Values are means ± SE; n, no. of subjects. LF, low fit; HF, high fit; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; VO2max, maximal oxygen uptake; METS, metabolic equivalent; V̇E, minute ventilation; RER, respiratory exchange ratio; RR, respiratory rate; RPE, rating of perceived exertion; PASE, Physical Activity Scale for the Elderly; max, maximal. *HF significantly different from LF at P ≤ 0.05; †P ≤ 0.01 via independent t-test.
electrolytes, or total cholesterol (data not shown). It should be noted that all subjects were healthy and free from reported diseases such as cardiovascular disease, diabetes, and cancer. Moreover, there were no differences between groups in the number of prescribed medications that they were taking.

Psychosocial data. We found no statistically significant differences between fitness groups for any of the psychosocial measures, including the SF-12 measuring subject perceptions of physical and mental health, the NEO Five Factor Personality Inventory, happiness (MUNSH), depression (GDS), PSS, and SPS assessments (data not shown).

Dietary data. From the food records, we assessed dietary intakes for macro- and micronutrients. There were no statistically significant differences for total kilocalories and intake of fat (total, mono-, or polyunsaturated), carbohydrates or proteins (in kcal, g, g/kg body wt, or percent), sodium, potassium, calcium, iron, zinc, or vitamins A, B, C, D, or E. The only variables that were different among the HF and LF groups were percent intake of saturated fat (9 ± 0.8% vs. 11 ± 0.7% in the HF and LF groups) and intake of vitamin K (42 ± 8 vs. 207 ± 65 μg). Although statistically significant, the physiological meaning of a 2% difference in saturated fat intake is questionable, and both are above the recommended 7% of kilocalories for those at risk for cardiovascular disease (14). Similarly, whereas vitamin K’s role in blood clotting is well known, little information is available regarding its influence on immune function. Therefore, based on these data and despite large differences in cardiovascular fitness, these two groups exhibited similar dietary behaviors.

In vivo influenza antibody responses. Results of HI analysis for influenza antibody responses can be found in Fig. 1. RM-ANOVA revealed significant [F_{2,48} = 5.1, P = 0.01, \eta^2 = 0.18] time × group interactions for the H1N1 (Fig. 1A, P = 0.02) and B \( F_{2,48} = 3.9, P = 0.03, \eta^2 = 0.14 \) strains (Fig. 1C, P = 0.05) included in the vaccine but not the H3N2 \( F_{2,48} = 1.4, P = 0.27, \eta^2 = 0.11 \) strain (Fig. 1B, P = 0.27). These data indicate that the HF group responded with a higher antibody response to two of the three strains included in the influenza vaccine. The change in peak titer for the three strains at 6 wk postvaccination was 2.6- vs. 1.3-fold, 1.3- vs. 1.1-fold, and 1.5- vs. 1.1-fold for the HF and LF groups, respectively. Moreover, titers against the B strain at 6 mo postvaccination tended to be higher in the HF group, indicating longer lasting protection. Prevaccine H1N1 titer was significantly \( P = 0.047 \) lower in the HF group. However, we do not believe this reflects a different exposure history between groups because there were no differences in self-reported flu vaccine exposure between groups. Moreover, there was no difference in prevaccine titer in the H3N2 strain, which was included in the Fluzone vaccine for several years before this study.

Ex vivo proliferation and cytokine responses to influenza. In addition to serum antibody titers, which are mainly protective against preventing influenza infection (48), assessment of the CMI response to influenza vaccine (e.g., proliferation and cytokine responses) gives important information about the development of antigen-specific T cells that are largely responsible for clearing infection once it is established (4). Moreover, cytokine analysis aids in the determination of whether there was a skew in the type (e.g., Th1 vs. Th2) of immune response (11). There was a significant time main effect \( F_{2,23} = 5.6, P = \)
in the repeated-measures ANOVA analysis. IL-10 production increased significantly postvaccination with respect to IFN-α/H9253 IgG1 (Th1 response) and IgG2 (Th2 response) anti-TT antibody response to a booster vaccination of TT. We found significant time main effect (F2,23 = 0.94, P = 0.41) with respect to IFN-γ production, indicating that the two fitness groups responded similarly to the vaccine. In contrast, IL-10 production increased significantly postvaccination (F2,23 = 38.6, P = <0.0001, r² = 0.78) in these older subjects, but there was no group (F1,24 = 0.36, P = 0.56) or group × time interaction (F2,23 = 0.08, P = 0.93) (Table 2).

In vivo TT antibody responses. We also examined both the IgG1 (Th1 response) and IgG2 (Th2 response) anti-TT antibody response to a booster vaccination of TT. We found significant time (F2,48 = 29.9, P = <0.0001, r² = 0.72) and time × group interaction (F2,48 = 3.6, P = 0.04, r² = 0.24) effects for anti-tetanus IgG1 antibody, such that the HF group exhibited a significantly higher IgG1 antibody response 6 wk postvaccination. There was no differences between groups 6 mo postvaccination (Fig. 3A). There was no group main effect (F1,24 = 0.1, P = 0.76), but there was a time main effect (F2,48 = 11.3, P = <0.0001, r² = 0.5) for anti-tetanus IgG2 but no group (F1,24 = 1.8, P = 0.19) or time × group (F2,48 = 0.4, P = 0.59) effect (Fig. 3B).

Ex vivo proliferation and cytokine responses to TT. There were no significant time (F2,23 = 0.85, P = 0.41), group (F1,24 = 0.05, P = 0.83), or time × group effects (F2,23 = 0.47, P = 0.57) in net proliferation to TT ex vivo. It should be noted that there was high variability in this measure, including many nonresponders (Fig. 4). The cytokine response to TT ex vivo can be found in Table 3. Unlike the IFN-γ response to influenza, there was a significant time main effect (F2,48 = 29.1, P = <0.0001, r² = 0.73) when TT was used as the stimulus. Like influenza, however, there was no significant group (F1,24 = 0.49, P = 0.49) or group × time interaction (F2,48 = 0.06, P = 0.95), indicating that both groups responded similarly (Table 3). There was also a significant (F2,48 = 24.8, P = <0.0001, r² = 0.69) increase in IL-10 in response to TT in culture, with no group (F1,24 = 0.19, P = 0.66) or group × time interaction (F2,48 = 0.46, P = 0.64) (Table 3).

DTH responses. DTH skin testing has been used to assess global CMI (47), and anergy (e.g., lack of response) has been

<table>
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<th>Group</th>
<th>Pre 6 wk Post 6 mo Post</th>
<th>Pre 6 wk Post 6 mo Post</th>
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<tr>
<td>LF</td>
<td>26±3 124±19* 86±15*</td>
<td>26±3 124±19* 86±15*</td>
</tr>
<tr>
<td>HF</td>
<td>31±8 141±26* 65±20*</td>
<td>31±8 141±26* 65±20*</td>
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Values are means ± SE. HA, hemagglutinating unit; Pre and post, before and after injection. *Significantly (P < 0.05) different from Pre within group.

Table 2. Flu-induced (0.9 HA/ml) cytokine levels in LF and HF elderly individuals

Fig. 2. Net ex vivo peripheral blood mononuclear cell (PBMC) proliferation [in counts/min (cpm)] in response to 0.9 µg hemagglutinating unit/ml of trivalent influenza vaccine (Fluzone; Aventis Pasteur, Swiftwater, PA) before and after (6 wk and 6 mo) Fluzone vaccination. There was a significant time (P = 0.01), but no group (P = 0.62) or group × time interaction (P = 0.14) in the repeated-measures ANOVA analysis.

Fig. 3. IgG1 (A) and IgG2 (B) antibody responses to tetanus toxoid (TT) vaccination in HF and LF elderly men and women. OD, optical density. There were significant time (P < 0.0001) and time × group (P = 0.04) interactions for the IgG1 response to TT vaccination. There was a significant time but no group interaction effect for the IgG2 response, despite higher concentrations in the HF group.

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found to be associated with mortality due to sepsis (9, 47). As can be seen in Table 4, there were no statistical differences in the number of positive responders (e.g., >5 mm induration) between the LF and HF groups. Likewise, the induration scores of those who responded positively did not differ significantly when assessed at 48 h postinjection.

**PHA-induced proliferation.** We found a significant ($F_{1,24} = 5.4, P = 0.03$, $\eta^2 = 0.24$) increase in PHA-induced proliferation in the HF vs. the LF groups when using a high (e.g., 2.0 $\mu g/ml$) but not ($F_{1,24} = 0.53, P = 0.48$) a suboptimal (e.g., 0.5 $\mu g/ml$) concentration of PHA (Fig. 5).

**Influence of covariates on differences in immune function between groups.** On the basis of the possibility of an effect of body fat percentage, age, gender, nutritional status (e.g., total kcal, protein kcal, vitamins E and K, zinc, iron, selenium, EPA/DHA), and psychosocial status (e.g., SF-12, NEO, MUNSH, GDS, PSS, SPS) on immune responses, we performed a general linear model RM procedure on the major immune variables that demonstrated differences between the groups (e.g., antibody responses to the H1N1 and B components of the influenza vaccine, IgG1 response to tetanus vaccine, and PHA-induced proliferation) with percent body fat as a covariate. These analyses did not effectively change our significant group $\times$ time interaction.

**DISCUSSION**

There are several cross-sectional studies that have examined immune function in groups of older adults with different physical activity levels (25, 37, 40, 41, 52), but there has been only one that has compared groups with differing cardiovascular fitness (42). The consensus from these studies has been that physically active or fit elderly have more robust immune responses than their less active or fit counterparts. Unfortunately, some of these studies have not adequately documented or controlled for underlying health, psychosocial, or nutritional differences between groups, making firm conclusions about the relationship between physical activity or fitness and immune function tenuous. Underlying disease, poor nutrition, and some psychosocial factors are well known to negatively impact responses to vaccination (24, 34, 49). Some of these studies have also utilized tests in which the clinical relevance is unclear (e.g., proliferative responses to polyclonal mitogens).

Finally, many of the studies have partitioned groups on the basis of self-reported responses to physical activity questionnaires, and only one study (42) has directly measured cardiovascular fitness. Therefore, the aim of this study was to examine whether older adults with differing cardiovascular fitness exhibited more robust immune responses to clinically relevant challenges, while accounting for potential confounding influences that likely exist between groups of elderly men and women with vastly different fitness (or physical activity) levels.

Both antibody and CMI responses are generated after administration of the influenza vaccine. Although anti-influenza antibodies assist in preventing infection (48), CMI responses, specifically the generation of anti-influenza CD8$^+$ T lymphocytes, promote viral clearance after infection (4). Many older adults fail to respond to influenza vaccine with vigorous humoral and especially CMI responses. Moreover, an age-related shift from Th1 cytokines (including IFN-$\gamma$) to Th2...
cytokines (including IL-10) has been associated with reduced humoral and CMI responses to influenza challenge (11, 44).

Our data indicated that the HF elderly men and women in this study responded with higher antibody titers to two of the three components of the influenza vaccine but failed to respond better than the LF group with respect to influenza-induced proliferation or cytokine (IFN-γ or IL-10) production. This may indicate that HF elderly individuals are better protected from influenza postvaccination, but once infected they respond similarly in terms of viral clearance. Along these lines, another way to assess influenza vaccine efficacy is to examine whether subjects achieved a fourfold increase or whether their HI titers were elevated to a protective level (>40 HI) after vaccination (4). With the use of χ² analysis, our HF subjects exhibited significantly higher (e.g., 4-fold) responses to both the H1N1 (8/13 vs. 3/13 for HF and LF) and B (7/13 vs. 2/13 for HF and LF) strains, and, importantly, more HF subjects developed a protective titer (e.g., >40 HI) against the B strain virus compared with LF subjects (9/13 vs. 4/13 for HF and LF). There were no differences in the development of protective titers in response to the H1N1 or H3N2 strains. Our data suggest, at least with the B strain, that the HF group developed a response that would be protective against natural influenza virus infection, whereas the LF group did not.

Three other cross-sectional studies have examined the association between physical activity and immune response to influenza vaccine (25, 39, 40). In general agreement with our documented highly fit and active elderly subjects, Kohut et al. (26) found that self-reported highly active (e.g., vigorous activity >3 times/wk) older adults exhibited higher anti-influenza IgG and IgM responses 2 wk after influenza vaccination than moderately active (e.g., light activity <3 times/wk) or sedentary controls (25). They also found, like we did, that cytokine levels were unchanged across activity (or, in our case, fitness) level. In contrast to our study (although we found a trend; P = 0.14), they found that both moderately and highly active subjects had higher ex vivo vaccine-induced proliferation responses. The reason for this difference is unclear but may be due to differences in the time of assessment (e.g., 2 wk in Kohut et al. vs. 6 wk in the present study). Likewise, Schuler et al. (40) found a positive correlation between physical activity (as measured by the Physical Activity Scale for the Elderly) and H3N2 HI antibody titer 1 wk postvaccination but not at later time points. Also, this correlation was not seen in the response to the H1N1 strain. Importantly, our HF group exhibited elevated H1N1 and B strain antibodies when measured 6 mo postvaccination. This is important because many times influenza outbreaks occur at the end of the flu season (e.g., March or April) when protective antibody concentrations have diminished. In contrast to the elevated antibody response to the influenza vaccine in physically fit or active elderly men and women, Schuler et al. (39) found no differences in anti-influenza antibody responses in a group of college-aged subjects, indicating that fitness or activity may have no influence on flu vaccine responses in a younger, healthy population (39).

We also assessed the antibody (including IgG₁ and IgG₂ as indicators of a possible Th1 to Th2 skew) and CMI response to TT vaccination. Prevention of tetanus by TT vaccination is the most effective control, and booster vaccinations are recommended every 10 yr, especially in the elderly (7). Unfortunately, when elderly receive TT booster vaccination, their peak (~3–6 wk) and longer-lasting (>3 mo) antibody responses are lower than those of younger individuals (2, 6). In the present study, we found the HF group exhibited a significantly lower peak IgG₁, and a tendency for a higher IgG₂, antibody response to the TT vaccine. These data suggest a possible fitness-associated skewing of the antibody response to TT challenge. This could potentially provide HF subjects with better protection vs. tetanus because IgG₂ is better at antigen neutralization than IgG₁, which is a better opsonin (22). To our knowledge, the only other study that has examined antibody isotype in relation to fitness or physical activity status is the study of Smith et al. (42). In contrast to our data demonstrating a trend toward a higher IgG₂ response and a lower IgG₁ response to TT vaccine in the HF group, Smith et al. found that physically active elderly patients had a higher IgG₁, but not IgG₂, and a higher DTH response to a primary vaccination with keyhole limpet hemocyanin (KLH), suggestive of a higher cell-mediated Th1-type response (42). Although unclear, the difference may be that our vaccine was a recall and theirs a primary vaccination. Despite the shift in antibody subclasses in our study, we failed to detect TT-induced differences in IFN-γ (Th1) or IL-10 (Th2) cytokines ex vivo. Interestingly, a recent study in mice demonstrated that voluntary wheel running increased antigen-specific antibody-producing B cells and prolonged IgG half-life in blood after tetanus immunization (43). They did not examine the IgG subclass response to the vaccine.

DTH skin testing has been used to assess global CMI (47), and anergy (e.g., lack of response) has been found to be associated with mortality due to sepsis (35, 47). Aged individuals lose their ability to mount a CMI response to challenge with antigens to which they have been previously exposed (17). With respect to skin testing, this deficit results in a reduction of induration (swelling and redness) or anergy (lack of skin response, i.e., induration ≤2 mm) when antigens are applied intradermally. We found no difference between fitness groups in DTH skin test responses to two fungal antigens: trichophyton and candida. Unfortunately, we did not test other bacterial or viral recall antigens, and our relatively low subject number for this type of analysis can be seen as a limitation in this study. To our knowledge, only one other study has examined the relationship between physical activity or fitness and DTH responses. Smith et al. (42) found that highly fit and active elderly individuals exhibited a greater DTH response to KLH when tested 21 days after primary KLH vaccination (42). The reason for the different response in HF subjects in that study vs. ours could be because that study measured the DTH response soon after a primary immunization, whereas our study examined recall antigens with a high number of subjects displaying anergy.

Several studies have shown that highly physically active people have higher proliferative responses to polyclonal mitogens like PHA (37, 41). Unfortunately, these studies usually only include one concentration (e.g., optimal) of mitogen. Our data agree with and extend the findings of others (37, 41) in that we found higher proliferative responses in highly fit older adults when cells were stimulated with optimal, but not suboptimal, concentrations of PHA. Interestingly, this elevated polyclonal proliferative response in HF subjects occurred despite no differences in vaccine-specific cell proliferation. This finding further detracts from the clinical relevance of poly-
clonal proliferation assay as a means of predicting antigen-specific immune responses.

Despite our accounting for major confounding variables (e.g., disease, nutritional, psychosocial status), cross-sectional studies by their very nature possess numerous limitations. Obviously, randomized longitudinal studies overcome these weaknesses. There have been a few small longitudinal studies, including one by our group (51), that have examined the influence of exercise training on immune function in previously sedentary elderly. These studies have either found augmented immune responses (8, 26, 33, 51) or have failed to document change (23, 38). Of particular note is the study of Kohut et al. (26) who found that exercise training improved the antibody to influenza vaccination. It is unclear whether this increase was related to changes in fitness, but they did report that improvements in psychosocial variables could not account for the improvement in antibody response (27), a finding consistent with the present study.

In summary, our cross-sectional data suggest that, after accounting for many potentially confounding factors, including health, nutritional, and psychosocial status, elderly men and women exhibiting high cardiovascular fitness have higher antibody responses to influenza vaccine and a Th2 skewing of antibody responses to influenza vaccine and a Th2 skewing of antibody responses to tetanus booster vaccination. In contrast to other studies, there was little evidence that HF elderly subjects mounted better ex vivo CMI responses to the influenza or tetanus vaccine. These data confirm and extend the existing literature regarding the influence of physical fitness on immune responses and lend further support for the conduct of randomized clinical trials examining immune responses in previously sedentary older adults.

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GRANTS

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