Prolonged exercise suppresses antigen-specific cytokine response to upper respiratory infection

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Kohut, M. L., G. W. Boehm, and J. A. Moynihan. Prolonged exercise suppresses antigen-specific cytokine response to upper respiratory infection. J Appl Physiol 90: 678–684, 2001.—Fatiguing exercise has been associated with an increased susceptibility to infection. This study examined the antigen-specific T-helper (Th) type 1 and Th type 2 cytokine response to herpes simplex virus (HSV) infection after an acute bout of fatiguing exercise. Male BALB/cJ mice ran on a treadmill (Ex) until voluntary fatigue (~2.5 h), and control mice were handled and remained next to the treadmill. Mice were infected with HSV 20 min after exercise. Mice were killed 2 or 7 days postinfection, and sera and spleens were taken for the determination of HSV-specific serum IgM, splenocyte cytokine production during culture with HSV, and splenocyte natural killer cell cytotoxicity. Both Th type 1 [interleukin (IL)-2, interferon-γ, IL-12] and Th type 2 (IL-10) cytokine production in spleen cell cultures, as well as natural killer cell cytotoxicity, decreased in Ex on day 2 postinfection. On day 7 postinfection, there was no difference in HSV-specific serum IgM or cytokine production by cells from control and Ex mice, with the exception of decreased IL-12 in Ex mice. These findings suggest that fatiguing exercise may alter the kinetics of antigen-specific cytokine production.

Several epidemiological studies have demonstrated that the incidence of upper respiratory infection increases within 1–2 wk after a prolonged, strenuous bout of exercise (16, 19, 20). Exhaustive exercise has also been associated with a suppression of some immune responses, and it has been suggested that the immunosuppression after fatiguing exercise may be related to the increased incidence of infection (15). There are reports of suppressed function after exercise in cells located within the respiratory tract. For example, one study in human subjects reported a decrease in the phagocytic function of cells obtained from nasal lavage fluid for several days after a 20-km race (14). A separate study using an animal model demonstrated a decrease in alveolar macrophage antiviral function for at least 8 h after an exercise bout to fatigue (3). Cells obtained from the peripheral blood, similar to cells obtained from the respiratory tract, also exhibit a decline in function after exercise. For example, natural killer (NK) cell cytotoxicity has been reported to decrease for several hours after prolonged exercise (12, 23), and lymphocyte proliferative response to mitogen also declines after exercise (17, 23). However, these exercise-induced changes in NK cell function and lymphocyte response may be related to a decrease in the number of circulating lymphocytes postexercise, rather than actual functional changes on a per-cell basis, and it is unclear whether these changes are related to altered susceptibility to infection.

Although some evidence suggests that certain non-specific immune responses, including lung macrophage antiviral function, neutrophil phagocytosis, and NK cell activity (NKCA), are reduced after an acute bout of prolonged, intense exercise, there is less information regarding the antigen-specific immune response after exhaustive exercise. One recent study examined the delayed-type hypersensitivity (DTH) response (a measure of in vivo cell-mediated immunity) to several antigens after prolonged exercise and found that the DTH response was suppressed (2). However, the humoral immune response, as measured by serum antibody production, was not altered by prolonged exercise (2, 6, 12). Based on this limited evidence, humoral and cell-mediated immune responses may be differentially altered by fatiguing exercise. It is possible that this differential modulation of cell-mediated and humoral immune responses may be related to antigen-specific cytokine production. Cell-mediated immune responses are driven by the predominant release of T-helper (Th) type 1 (Th1) cytokines [interleukin (IL)-2, interferon (IFN)-γ], whereas antibody production is driven by the release of Th type 2 (Th2) cytokines (e.g., IL-4, IL-5, IL-10). Furthermore, IL-12, released by macrophages, induces the maturation of Th1 cells and activates NK cells; therefore, IL-12 also has an important role in directing immune response. The antigen-specific Th1 and Th2 cytokine responses after exhaustive exercise have not yet been characterized, and differential cytokine responses may explain the different cell-mediated and humoral immune responses after exercise.
The purpose of this study was to examine the antigen-specific Th1 and Th2 cytokine response to herpes simplex virus type 1 (HSV-1) infection in mice after an acute bout of fatiguing exercise. Mice were infected after the exercise session to determine whether the exhaustive exercise was associated with a different or delayed Th1 and Th2 cytokine response to viral infection. This model may provide information regarding the susceptibility to infection after strenuous exercise. We also assessed both HSV-specific IgM production and nonspecific immune function (NK cell cytotoxicity).

**METHODS**

**Animals and Exercise Protocol**

All mice (male BALB/c) were acclimated to the animal resource facility on a 12:12-h light-dark cycle for 1 wk. After this week, mice were then acclimated to treadmill running for ~10–15 min/day at a speed of 8 m/min for 1 wk before experimentation. Mice were then randomly assigned to exercise (Ex) or control (Con) groups (n = 16/group). Acute strenuous exercise consisted of one treadmill run at gradually increasing speeds until voluntary fatigue. Fatigue was defined as the point at which mice could no longer maintain pace with the treadmill belt (~2.5 h). Con mice remained in lanes next to the treadmill and were exposed to similar handling and noise stresses.

**Viral Infection**

Twenty minutes after the Ex or Con session, all BALB/c mice were infected with 50 μl of 5 × 10^5 plaque-forming units (PFU) of HSV-1 (Patton strain, originally a gift from Dr. Robert Bonneau, Pennsylvania State University Medical Center, Hershey, PA) through an intranasal route at a dose corresponding to 20% lethality. The intranasal route was chosen to mimic the typical route of viral entry in an upper respiratory infection. HSV-1 stocks were propagated in Vero cells (originally a gift from Dr. Robert Bonneau) at a multiplicity of infection of 0.01. HSV-1 stocks were stored at −70°C in medium 199 supplemented with 8% fetal bovine serum (FBS), 100 U/ml penicillin, 100 μg/ml streptomycin sulfate, 2 mM L-glutamine, and 0.075% (wt/vol) NaHCO₃ (all from GIBCO, Grand Island, NY). HSV-1 was resuspended in sterile saline immediately before infection. After infection, all mice were returned to their cages and did not exercise postinfection.

**Tissue Collection**

Two days or 1 wk after infection, mice were decapitated, and trunk blood was collected. Spleens were removed and dissociated in Hanks’ balanced salt solution (GIBCO) using a stomacher laboratory blender (Tekmar, Cincinnati, OH). Cell suspensions were passed through sterile nylon mesh to remove clumps of tissue. Cells were counted in sterile saline containing 1% Hematall (Sigma Chemical, St. Louis, MO) using a Coulter counter (Coulter Electronics, Hialeah, FL).

**In Vitro HSV-Specific Stimulation of Spleen Cells**

Spleen cells from each mouse were washed once and resuspended in RPMI 1640 supplemented with 5% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin sulfate, 2 mM L-glutamine, 0.075% (wt/vol) NaHCO₃, 20 mM HEPES, and 5 × 10^-5 M 2-mercaptoethanol (all from GIBCO). Cells were adjusted to 5 × 10^6 cells/ml, and 1 ml was added to each well of a Falcon 24-well tissue culture plate (Becton-Dickinson Labware) containing 1 ml/well of inactivated HSV-1 in supplemented RPMI media. The concentration of inactivated HSV-1 would yield 2.5 × 10^3 PFU/well (5 PFU/cell) when active. Control wells also contained 1 ml of 5 × 10^6 cells/ml but not inactivated HSV-1.

**In Vitro Lipopolysaccharide + IFN-γ Stimulation of Spleen Cells**

Spleen cells from each mouse were washed and plated in tissue culture plates as described in the previous paragraph. HSV-1 was not added to cell cultures; instead the final culture fluid contained 100 ng/ml of lipopolysaccharide (LPS) and 100 U/ml of IFN-γ. The spleen cells were then cultured for 24 h at 37°C and 5% CO₂, and supernatant was collected for the analysis of LPS + IFN-γ-stimulated IL-12 production.

**NK Cell Cytotoxicity Assay**

Spleen cells were adjusted to 2 × 10^7 cells/ml for the NK cell cytotoxicity assay. YAC-1 cells were used as the target cells in this assay. Briefly, 1.6 × 10^5 target cells were labeled with 500 μCi sodium chromate ([^3]Cr) for 1 h at 37°C. Cells were then washed three times and adjusted to a concentration of 1 × 10^6 cells/ml, and 100 μl were added to the wells of round-bottom 96-well microtiter plates. One hundred microliters of spleen cells were then added in triplicate at various effector-to-target ratios (E/T) ranging from 0.25:1 to 25:1. Total[^3]Cr release was measured in wells with 100 μl of target cells plus 100 μl of Triton X-100 (detergent). For spontaneous[^3]Cr release control, 100 μl of supplemented media were added to target cells. Microtiter plates were incubated at 37°C with 5% CO₂ for 6 h. After incubation, the plates were centrifuged at 400 g for 10 min, and 100 μl of supernatant were removed from each well and counted on a gamma counter. Percent lysis was calculated as:

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\frac{cpm \text{ sample} - cpm \text{ spontaneous release}}{cpm \text{ total release} - cpm \text{ spontaneous release}} \times 100
\]

where cpm is counts per minute.

**ELISA for Serum Anti-HSV Antibody**

Serum IgM and IgG anti-HSV antibody titers were detected by ELISA. ELISA plates (Immulon, Alexandria, VA) were coated overnight at 4°C with HSV-1 diluted in carbonate coating buffer (pH 9.6) at a concentration of 2.3 × 10^7 PFU/ml and were blocked with PBS-0.1% bovine serum albumin. Plates were washed three times with PBS-0.05% Tween between each step. Serum was diluted and added; plates were incubated overnight at 4°C. Alkaline phosphatase-conjugated goat anti-mouse IgM or goat anti-mouse IgG antibody (both from Cappel Research Products, Durham, NC) was added and then incubated overnight at 4°C. Finally, substrate (p-nitrophenyl phosphate; Sigma Chemical) was added. Absorbance was read at 405 nm by using an automated plate reader (Biotek, Winooski, VT).

**Cytokine Assays**

Cytokine assays were performed by ELISA on supernatants from HSV-stimulated and LPS + IFN-γ spleen cells. Supernatants for cytokine determination were collected from spleen cells at 24, 48, and 72 h of culture for IL-2 and IFN-γ; supernatants were collected at 48 and 72 h of culture for the analysis of IL-10; and supernatants to assess IL-12 were collected at 24 h of culture. IL-2, IFN-γ, IL-10, and IL-12
concentrations in supernatants were determined by an ELISA using monoclonal antibodies (MAb) and the protocol supplied by Pharmingen (San Diego, CA). Briefly, 96-well enhanced protein-binding ELISA plates (Corning, NY) were coated overnight with purified IL-2, IFN-γ, IL-10 (2 μg/ml), or IL-12 (8 μg/ml) capture MAb. Plates were blocked with PBS-10% FBS for 2 h at room temperature before the addition of supernatants. Between each step, plates were washed three times with PBS-0.05% Tween. Recombinant cytokine standards and samples were added in 100 μl and allowed to incubate overnight at 4°C. Biotinylated anticytokine-detecting MAb (1 μg/ml) was added, and the plates were incubated at room temperature for 45 min. Avidin-peroxidase at 2.5 μg/ml in PBS-10% FBS (Sigma Chemical) was added in 100 μl, and the plates were incubated for 30 min at room temperature. Substrate [2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid); Sigma Chemical] was added, and absorbance at 405 nm was determined using an automated plate reader (Biotek).

Statistical Analysis

Data were analyzed by using commercial software (SPSS, Chicago, IL). Statistical analyses to assess HSV-stimulated cytokine production were performed by using a three-factor (day postinfection, treatment, and hours in culture) mixed ANOVA with repeated measures on hours in culture. Statistical analysis used to assess LPS + IFN-γ-stimulated production of IL-12 was performed by using a two-factor (days postinfection and treatment) ANOVA. Serum antibody levels were compared by the use of a two-factor (treatment and serum dilution) mixed ANOVA with repeated measures for serum dilution. A two-factor (treatment and E/T) mixed ANOVA with repeated measures for E/T was used to analyze NK activity. With respect to the analysis of cytokines, if an hours × treatment, hours × day, or hours × treatment × day interaction were found, then subsequent analyses compared cytokine level at the time of optimal production in culture. Optimal production in culture was defined as the number of hours in culture that resulted in the greatest cytokine production.

RESULTS

HSV-Stimulated Cytokine Production

IFN-γ. Exercise is associated with suppressed antigen-specific IFN-γ production 2 days after exercise. A three-way ANOVA with day postinfection, hours in culture, and treatment for the cytokine IFN-γ showed a day × hours in culture interaction \(F(2,56) = 38.2, P < 0.001\) and a day × treatment interaction \(F(1,28) = 5.3, P = 0.028\). A subsequent analysis compared the amount of IFN-γ produced by cells from Ex and Con mice at the time of optimal production in culture (72 h) on days 2 and 7 postinfection using independent sample \(t\)-tests. Figure 1 shows that IFN-γ production in spleen cell cultures from Con mice was greater than that from Ex mice (\(t\)-test = 3.32, \(P = 0.005\)) 2 days after infection. An identical follow-up analysis comparing the response on day 7 postinfection demonstrated that there was no longer a difference in HSV-stimulated IFN-γ production by cells from Con and Ex mice (Fig. 1, \(t\)-test = -0.89, \(P = 0.39\)).

IL-2. IL-2 production decreased in spleen cells from Ex mice compared with Con mice 2 days after exercise.

The results of a three-way ANOVA revealed a day × hours in culture × treatment interaction \(F(2,56) = 6.148, P = 0.004\). An independent sample \(t\)-test was used to compare IL-2 production by spleen cells from Ex and Con mice on day 2 postinfection, at the time of optimal production in culture. The cultured spleen cells from Con mice produced significantly more IL-2 than the cells from Ex mice (Fig. 2, \(t\)-test = 2.31, \(P = 0.03\)). At later time points in culture, there was no longer a difference in IL-2 production. IL-2 production in spleen cells on day 7 postinfection did not differ between Ex and Con mice (Fig. 2, \(t\)-test = -0.91, \(P = 0.377\)).

IL-10. HSV-induced IL-10 production was reduced in Ex mice at 2 days postexercise and infection. A significant main effect of treatment was found by using a three-way ANOVA, and a significant hours in culture × day interaction was observed \(F(1,28) = 31.36, P < 0.001\). A follow-up analysis (independent sample \(t\)-test) was used to compare levels of IL-10 in superna-

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**Fig. 1.** In vitro herpes simplex virus type 1 (HSV-1)-stimulated interferon (IFN)-γ production by spleen cells from exercised (Ex) or control (Con) male BALB/cJ mice. Spleen cells were collected 2 days or 1 wk postinfection and were restimulated in vitro with ultraviolet (UV)-inactivated HSV-1. Values are means ± SE. Significant difference, Ex vs. Con, *\(P = 0.005\).

**Fig. 2.** In vitro HSV-1-stimulated interleukin (IL)-2 production by spleen cells from Ex or Con male BALB/cJ mice. Spleen cells were collected 2 days or 1 wk postinfection and were restimulated in vitro with UV-inactivated HSV-1. Values are means ± SE. Significant difference, Ex vs. Con, *\(P = 0.03\).
tants at the time of optimal production in culture (72 h) on day 2 postinfection and again on day 7 postinfection. IL-10 was greater in supernatants from Con compared with Ex spleen cells on day 2 postinfection ($t$-test = 7.36, $P < 0.001$), but was not different on day 7 postinfection ($t$-test = -0.34, $P = 0.74$, Fig. 3).

**LPS + IFN-$\gamma$-stimulated IL-12 production.** Although not induced by antigen, nonspecific IL-12 production was reduced in spleen cells from Ex mice compared with Con mice both 2 and 7 days after exercise and infection. IL-12 production from spleen cells stimulated with LPS and IFN-$\gamma$ was measured after 24 h of culture. The results of a two-way ANOVA revealed a main effect of treatment and of days postinfection, with no interaction. The spleen cells from Con mice produced more IL-12 than those from Ex mice both 2 and 7 days postinfection [$F(1,31) = 8.1$, $P = 0.008$, Fig. 4]. Spleen cells from both groups of mice produced greater amounts of IL-12 on day 7 postinfection than on day 2 [$F(1,31) = 35.11$, $P < 0.001$].

**NK Cell Cytotoxicity**

Exercise was associated with reduced NK cell function 2 days after prolonged exercise. NK cell cytotoxicity was assessed only on day 2 postinfection (Fig. 6). A two-way ANOVA demonstrated a significant main effect of treatment; the spleen cells from Con mice had greater NK cell cytotoxicity against YAC-1 lymphoma cells than did the spleen cells from Ex mice [$F(1,14) = 6.529$, $P = 0.023$].

**DISCUSSION**

The results of this investigation suggest that fatiguing exercise is associated with a transient suppression

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**Fig. 3.** In vitro HSV-1-stimulated IL-10 production by spleen cells from Ex or Con male BALB/cJ mice. Spleen cells were collected 2 days or 1 wk postinfection and were restimulated in vitro with UV-inactivated HSV-1. Values are means ± SE. Significant difference, Ex vs. Con, *$P < 0.001$.

**Fig. 4.** In vitro lipopolysaccharide + IFN-$\gamma$-stimulated IL-12 production by spleen cells from Ex or Con male BALB/cJ mice. Spleen cells were collected 2 days or 1 wk postinfection and were restimulated in vitro with lipopolysaccharide + IFN-$\gamma$. Values are means ± SE. Significant difference, Ex vs. Con, *$P = 0.008$.

**Fig. 5.** Serum IgM anti-HSV-1 antibody response in Ex or Con male BALB/cJ mice at 1:5, 1:15, and 1:45 dilution. Values are means ± SE.

**Anti-HSV IgM and IgG antibody.** Anti-HSV IgM was assessed on day 7 postinfection (Fig. 5). There was no difference in serum IgM anti-HSV antibody between Ex and Con mice [$F(1,14) = 0.013$, $P = 0.911$]. IgG anti-HSV antibody was assessed on day 7 postinfection. However, serum levels were undetectable at this time point. Therefore, the IgG results are not shown.

**Fig. 6.** Effect of exercise treatment on natural killer cell cytotoxicity at various effector-to-target ratios on day 2 postinfection. NKCA, natural killer cell activity; %lysis, percentage of YAC-1 target cells killed in a standard $^{51}$Cr release assay. Values are means ± SE. Significant difference, Con vs. Ex, *$P = 0.023$.
of immune function and/or a delayed immune response. To our knowledge, these findings are the first to demonstrate that antigen-specific cytokine production is suppressed after one session of prolonged exercise. Cytokine production appears to be suppressed in Ex mice 2 days postinfection and postexercise; however, at 7 days postinfection, there is no longer a difference between Ex and Con mice, with the exception of production of IL-12. Although we did not assess morbidity and mortality in these experiments, our laboratory has previously shown that mice exercised to fatigue and subsequently infected with the same virus (HSV) experience a greater morbidity and mortality than do control mice or mice exercised for a shorter period of time (3). Therefore, it is possible that the exercise-associated decline in cytokine production on day 2 postinfection is related to the exercise-induced increase in mortality that our laboratory observed in a previous investigation. Although epidemiological data suggest that the incidence of infection increases after a marathon is run (16, 19), a clinical trial involving controlled exposure to virus after prolonged exercise and simultaneous assessment of immune function is necessary to determine whether humans also experience suppressed immune response and increased susceptibility to infection.

Although the findings from some epidemiological studies suggest that the susceptibility to upper respiratory infection increases after very intense, prolonged exercise (16, 19, 29), there are limited experimental data demonstrating significant exercise-induced suppression of antigen-specific immune response. In two published studies, however, human subjects received an immunization to tetanus toxoid shortly after completing a bout of strenuous exercise. Approximately 2 wk after the immunization, there was no difference in serum antibody titers in exercised compared with control subjects (2, 6). However, in one of these studies, Bruunsgaard et al. (2) did observe a decrease in the DTH response 48 h after the prolonged exercise session. Cell-mediated immune responses, including DTH responses, are favored by the production of Th1 cytokines (IL-2, IFN-γ, IL-12), whereas antibody production is driven by Th2 cytokines (such as IL-10). Based on the investigation by Bruunsgaard et al., coupled with other research showing that stressors such as foot shock can be associated with a suppression of Th1 but not Th2 cytokines (1), we hypothesized that exhaustive exercise might be associated with a decrease in the production of only the Th1 cytokines in the Th2 cytokine-dominant BALB/cJ strain of mice used in these experiments. However, we observed a decrease in both Th1 and Th2 cytokines 2 days after exercise. In a similar study using the C57Bl/6J strain of mice (Th1 dominant), we also observed a suppression of the antigen-specific production of both Th1 and Th2 cytokines 2 days after exercise and infection (unpublished observations). One recent study in humans demonstrated decreased production of IL-2 and IFN-γ 3 h postexercise but no difference in cytokine production 20 h postexercise (26). However, mitogens were used to induce cytokine production, and, therefore, we cannot directly compare our results to these findings. It is possible that the production of antigen-specific cytokines is decreased for a longer period of time (48 h) postexercise, whereas the lymphocyte response to non-specific stimuli such as mitogens may be more short term (several hours).

At 7 days postexercise, the antigen-specific cytokine production was no longer different between Ex and Con mice. Taken together, these findings suggest that, in our model of HSV infection, strenuous exercise is associated with a delay or a suppression of the early antigen-specific response, but 1 wk after the exercise session cytokine production and serum antibody titer are no longer affected by exercise. In this investigation, we cannot determine whether the immunosuppressive response at day 2 has clinical significance; however, our laboratory’s previous experiments using the same model of exercise and infection did show an increase in mortality among mice exercised to fatigue (3). It appears that fatiguing exercise is associated with lagging kinetics of antigen-specific cytokine production, which are evident 2 days postexercise but no longer apparent 7 days postexercise. In pilot experiments, we also observed that antigen-specific cytokine production 10 days after infection was not different between Ex and Con groups. It would be of interest to examine further the time course of the exercise-associated delay in cytokine production, which may be examined with a broad parametric design. In addition, although we examined antigen-specific immunomodulation after one session of exercise in this study, in future studies it would be worthwhile to examine the effects of chronic, prolonged, exhaustive exercise on virus-induced cytokine production.

In this study, NK cell cytotoxicity was also suppressed 2 days postexercise. The effects of strenuous exercise on NK cell cytotoxicity have been assessed in numerous studies. A decrease of splenic NK cell cytotoxicity occurred 30 min postexercise in mice (24), and the results from several human studies suggest that NK cell cytotoxicity may be suppressed for up to 6 h after the exercise session (15). Our results show a decrease in NK cell function for a longer period of time than reported by others. However, unlike other studies, our animals were infected with a virus, resulting in an upper respiratory infection. Although the exposure to virus did not occur until after exercise was completed, it is possible that any potential immunosuppressive effects of strenuous exercise persist and/or are magnified in an infected animal. We are not aware of any other studies that have attempted to measure NK cell function in animals or humans suffering from upper respiratory infection after stress (exercise or psychosocial stress). One study of HIV-infected individuals noted that stress was associated with a reduction in NK cell population in infected individuals, yet this stress-induced suppression of NK cells was not observed in noninfected individuals (7). Although it has been reported that NKCA is suppressed in HIV-infected individuals (25), additional evidence suggests...
that stress further decreased the number of NK cells (7, 11). We have compared our data with the data collected from HIV-infected populations as a potential explanation for the long-term (2-day) stress-induced reduction of NKCA. However, our animals experienced pathology associated with upper respiratory infection, and we are not attempting to extrapolate our findings regarding long-term exercise-induced suppression of NK function from an animal model of upper respiratory infection to imply that long-term exercise in HIV-infected individuals results in reduced NKCA. Infection can be considered as a type of stress, and it has been shown that infection may cause an activation of the hypothalamic-pituitary-adrenal axis, resulting in elevated plasma levels of corticosteroids (21). Perhaps it has been shown that infection may cause an activation of the hypothalamic-pituitary-adrenal axis, resulting in elevated plasma levels of corticosteroids (21). Perhaps infection-induced increase in corticosteroid, combined with an exercise-induced elevation of corticosterone, led to the prolonged decrease in NK cytotoxicity.

It is possible that the decline in cytokine production and NKCA observed on day 2 is related to the elevated levels of catecholamines and corticosteroids associated with this type of fatiguing exercise. Corticosteroids are generally considered to be immunosuppressive and have been shown to reduce Th1 cytokine production or both Th1 and Th2 cytokine production (4, 13). Catecholamines may also modulate immune response. Although the specific cellular response to catecholamines is dependent on the type of adrenergic receptor expressed, in general, catecholamines elevate the intracellular concentration of cAMP, and elevated cAMP inhibits the production of IL-2 and IFN-γ (8, 15). NKCA is also inhibited by isoproterenol, a β-adrenergic agonist that elevates cAMP (9).

The timing of catecholamine exposure in relation to antigen administration is an important consideration. For example, epinephrine administration several hours before immunization altered the kinetics of antibody response, but epinephrine exposure several days before immunization inhibited the antibody response at all times (5). In our model of fatiguing exercise, it is very likely that plasma levels of epinephrine were elevated within hours of antigen administration, and it is possible that the altered kinetics of cytokine production were related to the high concentration of plasma epinephrine. A recent study also showed that exposure of Th1 cells to the β-adrenergic agonist terbutaline, which elevates intracellular cAMP levels, before activation by antigen-presenting cells inhibited IFN-γ production but did not alter IgM production (22). In our study, mice were infected with HSV 20 min postexercise. At this time, it is likely that corticosteroids and catecholamines were elevated, and it is, therefore, possible that the altered immune responses observed on day 2 after exercise and infection were related to the high concentrations of these neuroendocrine factors. We have previously demonstrated that alveolar macrophage anti-HSV function is inhibited by exhaustive exercise, but prior administration of the β-adrenergic receptor antagonist propranolol prevented this exercise-associated suppression of macrophage antiviral function (10). Future studies have been designed to clarify the role of catecholamines and corticosteroids in the exercise-associated inhibition of cytokine production.

REFERENCES