IT HAS BEEN HYPOTHEZIZED that regular moderate exercise lowers the risk of developing an infection, whereas excessive exercise or a sedentary lifestyle is associated with an increased susceptibility to infection (20). However, the actual results of a number of human epidemiological and observational studies are often contradictory (1). An important drawback of the epidemiological studies includes a lack of control over important variables like exposure to infectious agents, life stress, diet, and other environmental conditions. It is also very difficult to validly equate self-reported "colds, flu, or sore throat" with actual upper respiratory tract infection (URT1). Similar drawbacks exist in epidemiological studies of the acute effects of strenuous exercise and competition (1), in which the risk of URT1 is thought to increase (21, 22).

Controlled experimental studies of exercise and infection in animals generally demonstrate that forced exercise after exposure to virus or bacteria increases both mortality and severity of infection (7, 10, 24, 28). However, the effects of exercise before pathogen exposure have not been extensively studied in animals. In two studies that did examine the effects of exercise training before the administration of an infectious agent, one found enhanced survival rate and the other did not (2, 13). In addition, we are not aware of any animal studies that have examined morbidity and/or mortality after an acute bout of exercise. The animal experiments are also limited. They often utilize unrealistic exercise models (i.e., swimming with weighted tails until exhaustion and nearly drowning or running with electric shocks as motivation), introduce the infection in ways that normally do not occur in nature (i.e., through an intracerebral or intraperitoneal injection), rarely examine potential dose-response relationships, and generally fail to address plausible biological mechanisms.

Numerous studies have reported that acute exercise alters both the number of circulating leukocytes as well as various functions of lymphocytes, natural killer (NK) cells, and macrophages, and several reviews on these topics have been published (11, 29). It has been suggested that changes in immune cell number and/or function resulting from exercise may play a role in host resistance to infection (20). However, one must be cautious in assuming that alterations of a given immune parameter in vitro after a bout of exercise will necessarily result in altered host susceptibility to infection. A controlled investigation of postexercise resistance to infection after exposure to a particular pathogen, along with in vivo responses of immune cells found at sites of infection, and to the same pathogen, may provide insight into mechanisms for increased susceptibility to infection after exercise.

Within the respiratory tract, nonspecific antiviral defense is accomplished largely through the action of alveolar macrophages and the production of cytokines (16). Because of their position at initial sites of infection, these cells may be critical in determining the host susceptibility or resistance to viral infection. In our laboratory, a prior study demonstrated that activation of alveolar macrophages protected mice against pulmonary infection resulting from intranasal challenge with herpes simplex virus type one (HSV-1) (9). In addition, the difference in susceptibility to HSV-1 infection is correlated with a difference in the ability of macrophages to disseminate infection. Also, macrophages from HSV-resistant strains of mice can inhibit HSV-1 macromolecular synthesis earlier in the viral replication cycle than can macrophages from more susceptible strains of mice (25).

To investigate the effects of exercise on susceptibility to infection, the murine model of intranasal challenge with HSV-1 has been chosen. The respiratory tract infection and pathology to intranasal HSV-1 are similar to those observed in human disease (6, 19). The purpose
of the first part of this investigation was to compare morbidity and mortality among exercised vs. control mice after intranasal challenge with HSV-1. Two doses of treadmill exercise were assessed, an acute bout of short-term (30-min) exercise and an acute bout of exercise to voluntary fatigue (2.5–3.5 h). The purpose of the second series of experiments was to assess the ability of the alveolar macrophage to restrict HSV-1 viral replication (intrinsic resistance) among exercised and control mice at several time points postexercise.

METHODS

Mice. Male CD-1 mice, 4 wk of age, were purchased from Charles River Laboratories and acclimated to our facility for at least 3 days before any experimentation. Mice were purchased as pathogen-free stock, and periodic antibody screening of sentinel mice yielded negative results for common murine viral or bacterial pathogens. Mice were maintained on a 12:12-h light-dark cycle in a low-stress environment (22°C, 50% relative humidity, low noise) and were given food (Purina chow) and tap water ad libitum. All experiments were performed at the end of the active dark cycle.

Exercise protocol. The University's Institutional Animal Care and Use Committee approved the protocol described. Mice were randomly assigned to one of the following three groups: Con, control treatment; Ex-mod, treadmill exercise for 30 min at 5% grade and at a speed of 18 m/min; and Ex-ftg, treadmill exercise to the point of volitional fatigue at gradually increasing speeds, from 18 to 36 m/min at 5% grade. Similar exercise protocols have been used previously in our laboratory as models of moderate and fatiguing exercise, respectively (30). Volitional fatigue was defined as the point at which mice fail to maintain pace with the treadmill despite 2 min of gentle prodding with the hand. Electric shock was never used in these experiments, as mice respond well to a light tap on the tail or hind quarters encouraging them to maintain pace with the treadmill (30). Mice rarely required this type of continual prodding until they approached the point of fatigue. Mice in the control groups were contained in well-ventilated 4 × 12-in. Plexiglas lanes above the treadmill for an equivalent period of time. These mice were exposed to similar handling, noise, and treadmill vibrations in an attempt to control for extraneous stresses that may be associated with treadmill running. All mice were acclimated to treadmill running a minimum of two times per day for 3 days before the actual experiment as well as exposed to the Plexiglas control lanes. Mice in both the morbidity and mortality experiments (Con, n = 32; Ex-ftg, n = 32; Ex-mod, n = 23) and in the alveolar macrophage function experiment (n = 14, all groups) underwent the same exercise or control treatments as described above.

Intranasal infection with HSV-1. On the day of the experiment, mice were exposed to either the control treatment or an acute bout of exercise for either 30 min or until the point of fatigue. Immediately after the control or exercise session, mice were returned to their cages. After 15 min of rest, the mice were lightly anesthetized with ether and infected intranasally with 50 µl of a HSV-1 VR strain preparation containing 1.7 × 10^8 plaque-forming units (PFU)/ml. This dose yielded a 20% mortality rate among control mice in preliminary dose-response experiments. After infection, all mice were returned to their respective cages and placed in a P2 isolation facility for 21 days. Morbidity and mortality were monitored over this period. Several typical symptoms of illness were used to identify morbidity, namely, ruffled fur, inactivity, hunched back, and redness around eyes, nose, or mouth.

Alveolar macrophage collection, preparation, and infection with HSV-1. In the second series of experiments, mice were exposed to either the control treatment, moderate exercise (for 30 min), or exercise to fatigue. Mice were killed at one of the following time points: immediately postexercise or 3 or 8 h postexercise. These postexercise time points were chosen because nonspecific immune defense mechanisms can respond quickly (within hours) to infection. The immune parameter we measured, macrophage intrinsic antiviral resistance, is an early nonspecific resistance mechanism, serving to contain viral particles to a limited anatomical area. By 5–10 days postinfection, specific immune responses have been induced, which ultimately perform a curative function, whereas the primary function of the early nonspecific defenses is to limit spread of the infection.

Immediately after their respective treatments, mice were killed in a bell jar containing ether. Death by overetherization occurred within <2 min. Lungs were removed, and alveolar macrophages were obtained by gentle lavage of the lungs with 15–25 ml of media. The culture medium used in all experiments was RPMI-1610 (GIBCO, Grand Island, NY) containing 100 U/ml penicillin, 100 µg/ml streptomycin, and 20 mmol/l glutamine. Lung lavage cells were washed once with RPMI-1610, and any remaining red blood cells were lysed with tris(hydroxymethyl)aminomethane-aminomethane ammonium chloride, pH 7.2. Cells from mice in each group (n = 14) were adjusted to a concentration of 4 × 10^6 cells/ml in RPMI-1610 medium supplemented with 10% fetal bovine serum-RPMI (Environmental Diagnostics, Burlington, NC). Viability was determined by using trypan blue exclusion >90%, and the percentage of macrophages was calculated from a cytocentrifuge preparation followed by Diff Quick stain (Baxter Scientific, Chicago, IL). Subsequently, 200 µl of the cell preparation were added to the wells of a 96-well microtiter plate and allowed to adhere for 3 h at 37°C, at 5% CO2. After 3 h, each well was washed gently with prewarmed RPMI-1610 to remove nonadherent cells. The adherent macrophages were infected with HSV-1 KOS strain virus contained in 50–100 µl of medium at a ratio of 7–10 PFU/cell. The virus was allowed to absorb for 90 min. Prewarmed RPMI-1610 medium supplemented with 10% fetal bovine serum was added to each well (to a final volume volume of 200 µl), and the plates were incubated at 37°C, in 5% CO2 for 48 h. The HSV-1 virus used had been propagated in Vero cells. Stocks of virus were titrated on Vero cells by a plaque assay and contained 1.2 × 10^6 PFU/ml. Aliquots of the virus were stored at –80°C.

Alveolar macrophage antiviral resistance neutral red assay. Forty-eight hours after infection with HSV-1, a cytopathic effect was observed in the macrophages. The degree of cytopathic effect was quantified by a neutral red dye uptake assay (15). Briefly, cell monolayers were washed twice with RPMI-1610 and stained for 2 h with 0.006% neutral red diluted in RPMI-1610. The stained monolayers were washed, and the dye was extracted by lysing cells with 200 µl of a 50:50 mixture of Sorensen’s citrate buffer (0.1 M citric acid, 0.2 M NaOH, pH 4.1) and ethanol. The optical density was read on a Dynatech MR5000 microplate reader at 530 nm. The cytopathic effect was evaluated by calculating a viability index, which is expressed as the ratio of dye uptake by infected cells to dye uptake by uninfected cells as follows:

\[
\text{Viability index} = \frac{\text{optical density of infected cells}}{\text{optical density of uninfected cells}} \times 100
\]
Statistical analysis. The $\chi^2$ analysis was used to determine the significance of differences between groups in percent morbidity and mortality at the end of the 21-day postinfection period. The Kruskal-Wallis analysis was used to detect significant differences in survival between the experimental groups across the 21-day postinfection period. Differences in macrophage antiviral resistance were analyzed by using one-way analysis of variance with Newman Keuls post hoc tests to examine individual group differences.

RESULTS

Exercise and morbidity/mortality. Exercise until fatigue and subsequent intranasal administration of HSV-1 clearly resulted in greater morbidity and mortality. Figure 1B illustrates that Ex-ftg mice experienced a significantly greater overall mortality (41%) compared with Con mice (16%) at 21 days postinfection ($\chi^2$ analysis, $P < 0.05$). Mortality among mice that exercised for only 30 min (Ex-mod group) (9%) was not different from Con mice (16%). A comparison of morbidity among the three groups showed similar results (Fig. 1A). Again, Ex-ftg mice exhibited higher morbidity (50%) by day 21 postinfection than did control mice (25%) ($P < 0.05$), whereas mice exercising for the shorter period of time (Ex-mod) did not demonstrate a morbidity rate different than in the Con group (13% vs. 25%). Morbidity among the mice was only slightly higher than mortality, as most mice that became sick died within several days.

Figure 2 shows the time course of mortality in the three groups of mice. There were no differences in the onset of mortality among the three groups. However, survival times were significantly different ($P < 0.01$) among the three groups, with more Ex-ftg mice dying sooner than either Con or Ex-mod mice.

Overall, these results suggest that mice exercised until the point of voluntary fatigue, when exposed to HSV-1, experienced a decrease in survival over the time course of the experiment as well as increased morbidity and mortality at the end point of the experiment (day 21 postinfection).

Alveolar macrophage antiviral function. In this series of experiments, mice were subjected to the same exercise or control protocols, but were killed at three time points postexercise; then alveolar macrophages were isolated, and their intrinsic antiviral function was examined. Figure 3 compares the antiviral function (expressed as a viability index) of alveolar macrophages from mice killed immediately after exercise and at 3 and 8 h thereafter. Clearly, the viability index in both groups of exercised mice (Ex-ftg and Ex-mod) killed immediately postexercise is significantly less than in Con mice ($P < 0.05$). Antiviral function in mice killed 3 h after the exercise session is also suppressed in Ex-ftg mice or in Ex-mod animals, in contrast to the Con group ($P < 0.05$). However, when mice were killed at 8 h postexercise, the suppression in antiviral function persisted only in the mice exercised to fatigue (Ex-ftg < Con, $P < 0.05$). Alveolar macrophage antiviral function in mice exercised for the shorter period of time (Ex-mod) was no longer different than in Con mice.

DISCUSSION

The results from the first series of experiments suggest that a single acute bout of prolonged strenuous exercise until the point of voluntary fatigue followed by exposure to respiratory viral infection results in greater morbidity and mortality than either no exercise or short-term exercise. Both the duration and intensity of exercise differed between the two exercise groups (Ex-mod and Ex-ftg). The time to voluntary fatigue in Ex-ftg mice ranged from 2.5 h to slightly over 3 h, whereas the Ex-mod mice ran for only 30 min. The intensity of exercise was held constant at a speed of 18 m/min at 5% grade for Ex-mod group. Mice in the Ex-ftg group began running at the same intensity and grade,
but every 30 min thereafter the speed was increased by 3 m/min until voluntary fatigue. The exercise protocol used in Ex-mod group should elicit 55–65% maximal oxygen uptake in mice, whereas the intensity of exercise at fatigue in Ex-ftg animals will be 68–78% maximal oxygen uptake (27). It is not possible to determine from our experiments whether intensity, duration, or some particular combination of intensity and duration is required to elicit changes in susceptibility to upper respiratory infection. However, the evidence implies that this response may be a threshold effect. If a dose-response relationship between exercise and infection existed, one might expect to observe a moderate increase in mortality in the Ex-mod group along with a more dramatic increase in mortality in mice exposed to fatigue (Ex-ftg group). This clearly was not the case.

These findings suggest that even a single bout of prolonged, strenuous exercise results in increased susceptibility to infection. Although a 3-h run to our defined point of fatigue in the mouse cannot be directly compared with a 3-h run in a human, there are similarities between the results from our model and human epidemiological studies of this nature. The studies in humans that do report increased incidence of URTI after a single exercise session are those that observe this change only after very prolonged, fatiguing exercise, such as running a marathon or ultramarathon (21, 22). Increased incidence of infection has not been observed after short-term moderate exercise in humans, and we did not observe an increase in morbidity/mortality in our mouse model of moderate exercise. If fact, Ex-mod mice demonstrated what appears to be a trend toward decreased morbidity/mortality compared with Con mice. Considering the epidemiological evidence regarding exercise and infection published thus far (1, 20), along with the findings reported in this study, athletes completing a prolonged endurance event may experience an increased susceptibility to infection for some period of time after the event. The potential benefits of moderate exercise, while promising, are less clear. Further study is needed to clarify the role of moderate exercise on the risk of developing infection.

To our knowledge, this is the first study employing an animal model that demonstrated an enhanced susceptibility to infection after only a single bout of exercise. In humans, two studies have demonstrated increased incidence of URTI after a single competitive running event (21, 22). Other animal studies, in which rodents were exposed to an infectious agent after exercise, did so after several weeks of exercise training. In one of these studies, mice voluntarily trained on an exercise wheel for 2.5 wk and were then infected with Salmonella typhimurium. The exercised mice exhibited an increase in survival rate compared with sedentary control mice (2). In the other study (13), rats forced to swim for 4 wk before infection with Streptococcus pneumoniae exhibited no difference in lethality rate from untrained rats. It is difficult to compare the results from these two studies with our findings, considering the fact that our protocol consisted of only a single exercise session, whereas the other two studies employed numerous sessions of exercise over several weeks. It is likely that both regular exercise training as well as single bouts of strenuous prolonged exercise can alter susceptibility to infection.

Other factors to consider in studies of strenuous exercise are the psychological aspects associated with prolonged exercise and competition. Psychological stress is also correlated with a higher incidence of upper respiratory infection (4). Only one of the studies in humans, which observed an increased incidence of URTI in runners after they completed a marathon, attempted to assess the role of additional psychological stress. In this study, runners were categorized as part of a “perceived low-stress” group if they reported feeling “definitely better” in the following three categories: sleep, overall feelings, and energy and stress level since they began regular exercise training. However, the odds of acquiring an infectious episode in the week after the marathon were not different between the perceived low-stress group and the other runners (21). Although the assessment of psychological stress used
in this study may be somewhat limited, this finding
can suggest that exercise as a factor by itself can alter
susceptibility to infection above and beyond any addi-
tional effects of psychological stress. In rodents, the
previously mentioned study that observed an increase in
survival rate after several weeks of exercise training
and subsequent exposure to S. typhimurium used volun-
tary running on an exercise wheel as the mode of
exercise (2). In contrast, the other investigation, which
found no change in survival rate among exercised vs.
sedentary animals, utilized forced swimming in rats as
the mode of exercise (13). Whereas a direct comparison
between these studies is difficult to make because of
differences in animal species and bacterial strains, it is
possible that the additional “psychological stress” asso-
ciated with forced swimming and the fear of drowning
in the second study, in contrast to voluntary running
used in the first study, may have masked any single
effect of exercise training. In our study, although “forced
exercise” was used in the sense that mice were required
to maintain pace with the treadmill belt, no additional
adverse stimuli were used. Appropriate care was taken
to thoroughly acclimate mice to the treadmill appar-
atus (at least six brief exposures to treadmill running
before the experiment). In addition, the control mice
were exposed to the same environment throughout the
running session; they were contained in similar size
Plexiglas lanes mounted directly above the treadmill,
experiencing similar handling, treadmill noise, and
vibration. Although these precautions were taken, it is
still not possible to completely rule out the possibility
that some psychological stress associated with long-
term running that was not present during short-term
exercise contributed to the changes in morbidity and
mortality. Because this issue is difficult to address in an
animal model, future studies of exercise and infection
should address other indexes of stress, including hor-
monal markers and, perhaps, assessment of psychologi-
cal state (in humans) to sort out the potential impact of
this factor.

With the second series of experiments, we sought to
explore potential mechanisms mediating the exercise-
induced changes in mortality. In this murine model of
HSV-1 infection, the virus is introduced by intranasal
administration. The immune cells located within the
respiratory tract are the first to encounter the virus,
and it is these cells and/or products of the innate
immune system that will serve as the first line of
defense against infection until specific immune re-
sponses can be induced. Unlike adaptive immunity,
which requires several days to develop effective T-cell-
mediated and humoral antiviral responses, innate de-
fense systems are effective immediately after infection.
The alveolar macrophage is an important cell of the
innate defense system located within the respiratory
tract that has the capacity to take up viruses in a
nonspecific manner. The macrophage can limit viral
replication including HSV-1, thus limiting further
spread of the infection (17, 18). In addition, prior
experiments from our laboratory have shown that
activation of the alveolar macrophage with the syn-
thetic immunostimulant muramyl tripeptide reduced
mortality after intranasal administration of HSV-1 (9).
The results from these experiments suggest that alveo-
lar macrophages may play an important role in this
model of HSV-1 infection and that further activation of
these cells can significantly reduce mortality. Con-
versely, it is not unreasonable to consider the possibil-
ity that suppression of macrophage antiviral resistance
may result in increased mortality. Therefore, we chose
to study in vitro antiviral function in alveolar macro-
phages obtained from mice after an acute bout of
exercise.

Our results demonstrated that alveolar macrophage
antiviral resistance is suppressed in exercised mice.
This finding was observed in macrophages obtained
both from mice exercised to fatigue (Ex-ftg group) and
in mice exercised for only 30 min (Ex-mod group).
Recall that the increase in mortality was only observed
in mice exercised to fatigue. Whereas at first glance
these in vitro results may appear to contradict the in
vivo mortality data, an examination of the time course
may provide one potential explanation. Macrophages
obtained from Ex-mod mice exhibited a decrease in
antiviral resistance immediately postexercise and 3 h
later. By 8 h postexercise, this suppression was no
longer present. In contrast, macrophages from Ex-ftg
mice continued to show a suppression in antiviral
resistance at the 8-h time point. Whereas no further
time points were examined, it appears that the suppres-
sion of antiviral resistance persisted over a longer
period of time in Ex-ftg compared with Ex-mod group.
Perhaps, it is this prolonged decline in function that
ultimately leads to detrimental changes in resistance
and increased mortality.

Although we did not attempt to determine how the
decline in macrophage antiviral function may occur in
response to this type of exercise, several neuroendo-
crine factors released during prolonged, strenuous exer-
cise may have altered macrophage function. Both corti-
costeroids and catecholamines have been shown to
suppress various macrophage functions, and serum
levels of these factors are elevated during this type of
exercise (12, 14). Cytokines such as tumor necrosis
factor-α, interferon-α and -β, as well as nitric oxide may
mediate resistance to HSV-1 (5, 8, 23). Stress as well as
catecholamines and/or corticosteroids can inhibit pro-
duction of these cytokines, potentially contributing to
the decrease in resistance to HSV-1 (3, 26). Studies are
currently underway to investigate the role of these
neuroendocrine factors and cytokines in this model of
HSV infection.

Other innate defense mechanisms such as NK cell
function and interferon production were not assessed in
this study. However, other findings from our laboratory
suggest that interferon production is not impaired in
alveolar macrophages infected with HSV-1 after a bout
of exercise to fatigue (unpublished observations). Spec-
ific immune responses such as T-cell-mediated immu-
nity or antibody production were not measured in this
study. It is certainly possible that exercise may alter
any one or several of these immune parameters that
could subsequently influence disease outcome. In fact, exercise has been shown to alter NK function, T-cell mitogenic responses, and antibody production (11). However, specific or nonspecific antiviral immune responses to exercise within the respiratory tract have not been examined in conjunction with respiratory infection. To our knowledge, this is the first study to demonstrate an exercise-induced alteration of any antiviral defense system within the respiratory tract in conjunction with altered susceptibility to respiratory infection after exercise. Whereas this investigation provides one potential explanation for the increase in morbidity and mortality in mice exercised to fatigue, future studies are necessary to address the specific role of alveolar macrophages and other immune components in this model of increased susceptibility to respiratory infection after exercise.

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REFERENCES