Effects of moderate exercise and oat β-glucan on innate immune function and susceptibility to respiratory infection

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Davis, J. M., E. A. Murphy, A. S. Brown, M. D. Carmichael, A. Ghaffar, and E. P. Mayer. Effects of moderate exercise and oat β-glucan on innate immune function and susceptibility to respiratory infection. Am J Physiol Regul Integr Comp Physiol 286: R366–R372, 2004. First published October 9, 2003; 10.1152/ajpregu.00304.2003.—Both moderate exercise and the soluble oat fiber β-glucan can increase immune function and decrease risk of infection, but no information exists on their possible combined effects. This study tested the effects of moderate exercise and oat β-glucan on respiratory infection, macrophage antiviral resistance, and natural killer (NK) cell cytotoxicity. Mice were assigned to four groups: exercise and water, exercise and oat β-glucan, control water, or control oat β-glucan. Oat β-glucan was fed in the drinking water for 10 days before intranasal inoculation of herpes simplex virus type 1 (HSV-1) or euthanasia. Exercise consisted of treadmill running (1 h/day) for 6 days. Macrophage resistance to HSV-1 was increased with both exercise and oat β-glucan, whereas NK cell cytotoxicity was only increased with exercise. Exercise was also associated with a 45 and 38% decrease in morbidity and mortality, respectively. Mortality was also decreased with oat β-glucan, but this effect did not reach statistical significance. No additive effects of exercise and oat β-glucan were found. These data confirm a positive effect of both moderate exercise and oat β-glucan on immune function, but only moderate exercise was associated with a significant reduction in the risk of upper respiratory tract infection in this model.

IT HAS BEEN HYPOTHESIZED that moderate exercise may increase the activity of various immune cell parameters and thus decrease the risk for upper respiratory tract infection (URTI), while intense exercise may decrease the activity of these same parameters and increase the risk of infection (28), although the strength of evidence especially involving human studies has been questioned (3). Good evidence from controlled experimental studies in animals supports a negative effect of prolonged intense exercise on the risk of URTI that may result from a decrease in macrophage antiviral resistance (9, 19) and antigen presentation (6), natural killer (NK) cell cytotoxicity (17) and/or antigen specific cytokine response to induced URTI (17). However, only limited evidence exists on the effects of moderate exercise on URTI, and there are no data from controlled animal experiments involving specific responses to an induced infection.

Moderate exercise may enhance resistance to infection by activating the release of immunostimulatory factors such as growth hormone, prolactin, and cytokines (20), which in turn activate various immune cell populations. Evidence has shown that cell populations of the innate immune system appear to be most responsive to the effects of acute exercise (24). In general, exercise bouts of moderate duration (<60 min) and lower intensity [<60% of maximum oxygen consumption (V̇O₂,max)] have been associated with enhanced activity of immune parameters, including increased macrophage chemotaxis, adherence, oxidative metabolism (11), and phagocytic activities (11, 34), as well as increased NK cell activity (27, 39). These cells may constitute an important part of a first line of defense against URTI by nature of their phagocytic, cytotoxic, and intracellular killing capacities. In the only controlled animal study involving a specific immune response to a virus challenge, Kohut et al. (18) reported that moderate exercise training in aged animals was associated with an enhanced antigen-specific cytokine response to herpes simplex virus (HSV)-induced respiratory infection.

Various nutritional strategies have also been used to enhance immune function during exercise. Zinc (31), glutamine (5), carbohydrates (15, 25), and antioxidants (29) are among some nutritional strategies that have been examined for their immunopotentiating properties. However, these strategies have generally been examined for their potential to counteract the immunosuppression associated with intense exercise, not as an additional stimulus to further enhance immune function and overall health.

β-Glucans, polysaccharides derived from the cell wall of yeast, fungi, algae, and oats, have been documented to enhance the activities of both the nonspecific and the specific immune system. However, they have received little attention in the field of exercise immunology. β-Glucan exerts its effects through direct stimulation of macrophage, neutrophil, and NK cells via β-glucan-specific receptor sites on their cell surface membranes (8, 36), such as complement receptor 3 (CR3) and dectin-1 (2). When bound, β-glucan activates these cells and sets off a cascade of immune defenses that protect the organism from various viral (37), bacterial (12), and fungal (1) challenges. The exact mechanisms are at least partially dependent on the route of administration. Protection after oral administration results primarily from ingestion of small particles of β-glucan by pinocytic M-cells located in Peyer’s patches of the small intestine. Once activated, these cells can migrate to the lymph nodes and are capable of activating other macrophages, NK cells, and T-lymphocytes via the release of cytokines (30, 32, 33). Recently in our laboratory we have found that ingestion of soluble β-glucan derived from oats reduced morbidity and mortality after an intranasal HSV-1 challenge in mice exercised to fatigue for 3 consecutive days (10).
The purpose of this study was to determine the effects of short-term moderate exercise training and oral feedings of soluble oat β-glucan on innate immune function and susceptibility to URTI in mice. This was done using a murine model of respiratory infection involving intranasal inoculation with HSV-1 (9, 10). The exercise protocol consisted of 6 consecutive days of treadmill running (1 h/day at a relative intensity of approximately 68–78% VO2 max) designed to mimic a period of moderate exercise training. A β-glucan-enriched oat bran concentrate was used because of its solubility, natural occurrence in the diet, “generally recognized as safe” designation by the US Food and Drug Administration, and documented health benefits in various pathological conditions, including diabetes and cardiovascular disease (16, 40).

METHODS

Mice. Male CD-1 mice, 4 wk of age, were purchased from Harlan Sprague-Dawley Labs and acclimated to our facility for at least 3 days before any experimentation. Mice were purchased as pathogen-free stock, and periodic screening of sentinel mice yielded negative results for common murine viral or bacterial pathogens. Mice were housed four per cage and cared for in the animal facility at the University of South Carolina Medical School. Mice were maintained on a 12:12-h light-dark cycle in a low-stress environment (22°C, 50% humidity, low noise) and given food (Purina Chow) and water (or oat β-glucan dissolved in water) ad libitum. Separate groups of mice were used for each dependent variable: in vivo susceptibility to infection (n = 24 per group), macrophage antiviral resistance (n = 18 per group), and NK cell cytotoxicity (n = 12 per group). All experiments were performed at the end of the active dark cycle.

Nutrient treatment. Mice were randomly assigned to one of the following four groups: exercise water (Ex-H2O), exercise oat β-glucan (Ex-βG), control water (Con-H2O), or control oat β-glucan (Con-βG). Ex-H2O and Con-H2O received tap water for the 10 days before inoculation/death, while Ex-βG and Con-βG mice were fed a solution of oat β-glucan dissolved in the drinking water for the 10 days before inoculation/euthanasia. The oat β-glucan solution was made from an oat bran concentrate enriched to 68% soluble β-glucan (manufactured by Nurture, Devon, PA, and supplied by Quaker Oats, Barrington, IL), which was dissolved in the drinking water at a concentration of 0.6 mg/ml and made fresh daily. The remainder of the oat bran concentrate included starch, as well as small quantities of protein, sodium, and fat. Soluble oat β-glucan is a structural polysaccharide (~2 × 106 molecular weight) found in the cell walls of the bran layer and endosperm fractions of the whole seed. Structurally, they are linear chains of β-D-glucopyranosyl units in which ~70% of the units are linked (1–4) but which also consist of β-D-cellotriosyl and β-D-cellotetraosyl residues separated by (1–3) linkages arranged in a random manner (38). Daily consumption of fluid was measured to ensure there were no differences in fluid ingestion between the water and the oat β-glucan solution. Oat β-glucan was not fed to the animals during the 21 days after inoculation. Body weight of each animal was monitored throughout supplementation and exercise period to ensure that no weight loss was experienced by any group.

Treadmill acclimation and exercise protocol. The University’s Institutional Animal Care and Use Committee approved the protocol described. On the second day of oat β-glucan/water treatment, exercise mice (Ex-H2O and Ex-βG) were acclimated to the treadmill for a period of 20 min a day. The exercise protocol consisted of a 1-h bout of treadmill running (performed in the morning, 7 AM) for 6 consecutive days. Mice ran on the treadmill (2 per lane) at a speed of 36 m/min and a grade of 8%, which elicits approximately 68–78% VO2 max (35). Electric shock was never used in these experiments as mice readily respond to a gentle tap of the tail or hindquarters encouraging them to maintain pace with the treadmill. Mice rarely require this type of continual prodding during the 1-h exercise bout. Mice in the control groups (Con-H2O and Con-βG) remained in their cages in the treadmill room throughout the exercise bouts. These mice were exposed to similar handling and noise in an attempt to control for extraneous stresses that may be associated with treadmill running. Control mice were deprived of food and water during the exercise sessions.

In vivo titration of HSV-1. Intranasal inoculation of HSV-1 VR strain in the mouse is an established experimental model of respiratory infection. Although HSV is not a common respiratory virus in humans, it can cause various pathological conditions in human such as meningoencephalitis, hepatitis, esophagitis, tracheobronchitis, and pneumonia as well as being associated with cases of adult respiratory distress syndrome (23). The intranasal route of administration was chosen to mimic the typical route of entry for viral infection. HSV-1 was propagated in Vero cells and stored at ~7°C in medium supplemented with 10% fetal bovine serum and 2% penicillin, streptomycin, and l-glutamine. The virus was titrated by administering 50 μl of various stock viral dilutions to additional mice in an initial experiment to determine the lethal dose. Morbidity and mortality were monitored for 21 days.

Intranasal infection with HSV-1. On the day of the experiment, mice (n = 24 per group) were exposed to either control treatment or exercise treatment for 1 h. Immediately after the exercise test, mice were returned to their cages. Fifteen minutes later, mice were lightly anesthetized with halothane and inoculated intranasally with 50 μl of HSV-1 VR strain. The dose yielded a 40–50% mortality rate among control mice in preliminary dose-response experiments. The actual dose (PFU/ml) of this virus was not specifically determined in this experiment. However, a similar 20% lethal dose (LD50) preparation of this virus strain contained 1.7 × 10^5 plaque-forming units (PFUs)/ml (9). Preliminary evidence in our laboratory also indicates that a dose of 1.28 × 10^6 PFUs of a similar preparation of this virus yielded an average of 1.55 × 10^6 PFUs/lung in control animals (n = 5) versus 1.06 × 10^6 PFUs/lung in exercise animals (n = 5) 3 days postinoculation, suggesting a role of exercise in reducing viral yield after a viral challenge. The pathogenesis and symptomatology of infection after intranasal inoculation of HSV have been well characterized (9, 23). After infection the mice were returned to their respective cages and housed in an isolated P2 facility. All animals were monitored twice daily for a period of 21 days for signs of morbidity and mortality. Several typical symptoms of illness were used to identify morbidity, including ruffled fur; redness around the eyes, nose, or mouth; hunched back; and decreased activity. Mice that did not display any of these symptoms were considered healthy. These easily identifiable symptoms generally develop simultaneously with a quick onset (within 24 h), and death follows within 1–2 days. It is rare for animals at this age to recover once they exhibit these symptoms. Under these circumstances, it is very difficult and usually not very informative to be more specific about the development and severity of symptoms. However, it was important to at least document the possibility that the experimental treatments, especially oat β-glucan, may have either delayed time to death once animals were sick and/or allowed for full recovery. This would obviously have important immunological implications.

Peritoneal macrophage antiviral resistance. On the day of the experiment, mice (n = 12 per group) were exposed to either control treatment or exercise treatment. Immediately after exercise or rest, mice were euthanized in a bell jar by halothane overdose. Death occurred within <1 min. Peritoneal macrophages were collected, prepared, and infected with HSV-1 as previously described (9). Briefly, peritoneal macrophages were obtained by lavage of the peritoneal cavity with 5 ml of culture media. Peritoneal lavage cells were washed and red blood cells were lysed with Tris-oxalate-chloride, pH 7.2. Cells from two animals of the same group were pooled to obtain enough cells. Cells in each pool were adjusted to a concentration of 2 × 10^6 cells/ml in cell culture media. Viability was...
determined using trypan blue exclusion >90%. Subsequently, 200 μl of the cell preparation was added to the wells of a 96-well microtiter plate and allowed to adhere at 37°C and 5% CO2. After 12 h, each well was washed gently to remove nonadherent cells. The adherent macrophages were infected with HSV-1 KOS strain contained in 50 μl of medium. The virus was allowed to absorb for 90 min. Pre-warmed RPMI-1640 supplemented with 10% fetal bovine serum was added to each well (to a final volume of 250 μl), and the plates were incubated at 37°C and 5% CO2 for 72 h. The HSV-1 used had been propagated in Vero cells and titrated on macrophages. A dose that resulted in a macrophage antiviral resistance of 50% was chosen for this experiment. Aliquots of the virus were stored at ~80°C Seventy-two hours after infection with HSV-1, antiviral resistance was quantified by a neutral red dye uptake assay as previously described (9).

NK cell flow cytometric assay. The methods for analysis of NK cell activity by flow cytometry were adapted for use in mice from those described by Chang et al. (7). On the day of the experiment, mice (n = 12 per group) were either exposed to control treatment or exercise treatment. Mice were euthanized 30 min after treatment by halothane overdose. Spleens were removed, weighed, and immediately homogenized in RPMI-1640, and the homogenate was centrifuged at 1,100 rpm for 10 min. Blood cells were lysed with Tris-ammonium chloride, pH 7.2, and washed once in RPMI-1640. Remaining lymphocytes were placed in a T75 flask in an incubator at 37°C and 5% CO2 until use. Stained cells were used within 24 h of staining.

RESULTS

Nutrient consumption and weight gain. There were no differences in the average amount of fluid consumed by each group. Over the course of the 10-day fluid treatment, Ex-H2O mice consumed an average of 5.65 ± 1.02 ml/day, Ex-OBG consumed 5.6 ± 1.00 ml/day, Con-H2O consumed 5.88 ± 0.58 ml/day, and Con-OBG consumed 6.15 ± 0.66 ml/day. These results indicate that 24-h fluid consumption was not affected by the dissolved oat β-glucan or the moderate exercise. This is also reflected by a lack of difference in body weight across the groups. Weight gain over the course of the acclimation phase and 6-day exercise period was 2.88 ± 0.67 g in Ex-H2O, 2.71 ± 1.2 g in Ex-OBG, 3.92 ± 1.26 g in Con-H2O, and 4.00 ± 0.97 g in Con-OBG. Although food intake was not measured in this study, it seems unlikely that differences in fluid and food intake are likely to have important influences on the important outcome measures in this study.

Morbidity. The results of the experiment showed that there were differences in morbidity across the groups over the 21-day postinfection period. Figure 1 illustrates the time course in morbidity for the four groups. Intranasal administration of HSV-1 after short-term moderate exercise training resulted in a decrease in morbidity compared with resting controls (P = 0.0002).
Exercise mice (Ex-H2O) experienced only a 13% incidence in morbidity while 58% of control mice (Con-H2O) exhibited symptoms of morbidity. Short-term moderate exercise clearly resulted in a decrease in morbidity over the 21-day postinfection period. Consumption of oat β-glucan for 10 days before inoculation did not further decrease the symptoms of morbidity; there was no difference between the Ex-H2O group (13%) and the Ex-OβG group (21%). There was no difference between Con-H2O and Con-OβG.

Mortality. Similar treatment effects were found for mortality (i.e., time to death) over the 21-day postinfection period. Figure 2 illustrates the time course in mortality across the four groups. Intranasal administration of HSV-1 after 6 days of moderate exercise resulted in a decrease in mortality ($P = 0.0018$) compared with the control mice. Ex-H2O mice showed a mortality rate of 8% over the 21 days compared with 46% in the Con-H2O mice. Consumption of oat β-glucan for 10 days before inoculation did not further decrease mortality in the exercise animals; there were no differences between Ex-H2O (8%) and Ex-OβG (21%). However, oat β-glucan administration did show a trend ($P = 0.21$) toward decreasing mortality in the control mice, prolonging the survival time of resting animals. Con-H2O mice showed a mortality rate of 46% while Con-OβG mice experienced a mortality rate of only 33%.

Peritoneal macrophage antiviral resistance. In this experiment peritoneal macrophages were isolated from the four groups of mice, and their intrinsic antiviral resistance was examined. Figure 3 compares the antiviral resistance (expressed as a viability index) of peritoneal macrophages from mice killed immediately after exercise. Clearly, the antiviral resistance in mice exercised moderately for 6 days (Ex-H2O) is significantly greater than in control mice (Con-H2O) ($P = 0.002$). Oat β-glucan consumption for 10 consecutive days did not further enhance the benefits of exercise; there was no difference between the exercise groups (Ex-H2O and Ex-OβG). However, resting mice consuming oat β-glucan dissolved in the drinking water for 10 days before death (Con-OβG) had a significantly greater macrophage antiviral resistance than resting mice drinking water (Con-H2O) ($P < 0.001$).

NK cell cytotoxicity. NK cell activity was examined 30 min after the last bout of exhaustive exercise. Figure 4 demonstrates NK cell activity across the four groups, expressed as percent lysis of YAC lymphoma cells. Six days of moderate exercise was associated with a very small increase in splenic NK cell cytotoxicity at effector:target ratios of 5:1 ($P < 0.05$) and 1:1 ($P = 0.005$), but not 20:1 and 80:1, compared with nonexercised controls. Oat β-glucan consumption did not result in any change in NK cell cytotoxicity. There was no difference in the total number of splenocytes harvested between the groups, but the NK cells were not specifically counted and therefore the cytotoxicity results were not adjusted and presented on a per NK cell basis.

TNF-α. The presence of TNF-α was not detectible above 3 pg in any of the groups after moderate exercise or oat β-glucan treatment. Therefore, moderate exercise or oat β-glucan consumption was not associated with an elevation of this cytokine.
DISCUSSION

The effect of intense exercise (i.e., exercise stress) on immune function and infection risk is well studied. The data generally support the hypothesis that exercise stress can be immunosuppressive and increases the risk of infection (28). The hypothesized benefits of moderate exercise are less strong, primarily due to limited evidence of a direct benefit of moderate exercise on susceptibility to infection in response to a standardized virus exposure. The immune mechanisms that contribute to this effect are also less well understood. This study used an animal model of induced respiratory infection to determine the direct effects of a short period of moderate exercise training on morbidity and mortality after a controlled intranasal exposure to HSV-1. The respiratory tract infection and pathology in this model are similar to those observed in human disease and have been used to evaluate the efficacy of antiviral drugs (13, 23). We have used it previously to show that prolonged running to fatigue (approximately 2.5–3 h) increases susceptibility to respiratory infection that were associated with a decrease in alveolar macrophage resistance to HSV-1 (9). To our knowledge this is the first study to show a direct beneficial effect of short-term moderate exercise training on susceptibility to URTI and macrophage antiviral resistance to HSV-1. This beneficial effect of moderate exercise in conjunction with our previous study (9) that showed no effect of a single 30-min session of exercise on URTI and a suppression of macrophage antiviral resistance suggests that multiple sessions of moderate exercise may be required to enhance resistance to infection in this model. The soluble oat fiber β-glucan was also tested as a possible nutritional approach to further enhance the benefits of moderate exercise. However, while oat β-glucan was effective in enhancing macrophage antiviral resistance, the lower mortality rates did not reach statistical significance. There were no additive effects of moderate exercise and oat β-glucan in this experiment.

Other studies have examined either incidence of URTI or changes in immune cell parameters after moderate exercise, but these studies are more descriptive in nature and are less well controlled. Human studies using self-reported sickness logs have shown a decrease in incidence of URTI after moderate physical activity. In one randomized, controlled study, exercise subjects walked briskly for 45 min, 5 days/wk, and experienced one-half the days with URTI symptoms during the 15-wk period compared with that of the sedentary control group (27). Another study on the effects of energy restriction and moderate exercise on incidence of URTI found that the number of days with symptoms of URTI for subjects in the exercise groups was reduced relative to the nonexercise groups (26). Matthews et. al. (21) reported that moderate physical activity was associated with a 20–30% decrease in the annual risk of URTI in a cohort of predominantly nonathletic adults. However, in none of these studies was the virus exposure controlled. In one animal study, mice voluntarily trained on an exercise wheel for ~2.5 wk and were then infected with a standardized dose of Salmonella typhimurium. The exercised mice exhibited an increase in survival rate compared with sedentary controls (4). Our previous study found that a single 30-min bout of moderate exercise was not effective in reducing infection in a similar model (9), but this may be explained by differences in the exercise protocol (1 day vs. 6 days) and perhaps weaker virus dose (LD_{20} vs. LD_{50}) resulting in a ceiling effect.

Nutritional strategies have been examined for their ability to counteract the possible increase in risk of infection in response to intense exercise stress. However, no reported studies have examined their effect in combination with moderate exercise. It appears that carbohydrates may hold the most promise in maintaining immune function and host resistance to infection during periods of heavy training (15, 25). We have also recently evaluated the effectiveness of β-glucan in this regard (10). β-Glucan has been associated with enhanced protection against a wide variety of pathogenic challenges due to its mild immunostimulant properties (12, 41). However, this is the first experiment on β-glucan in response to moderate exercise. The results suggest that there are not added benefits of oat β-glucan on the risk of URTI. However, on closer inspection, this may be the result of a ceiling effect; the benefits of moderate exercise left little room for a further reduction in infection rate compared with the control animals.

In the second series of experiments, we sought to explore potential mechanisms that may explain the effects of moderate exercise and oat β-glucan on URTI. Cells of the innate immune system such as macrophages and NK cells serve as a first line of defense against most infectious agents. Macrophages have been reported to limit viral replication, including HSV-1, thus limiting further spread of infection (22). Studies from our laboratory have shown that alveolar macrophages may play an important role in this model of HSV-1 infection (9, 19). NK cells also function to recognize and kill virally infected cells as well as providing a link to effective adaptive immunity. Moderate exercise may enhance resistance to infection by activating these cells through the release of immunostimulatory factors such as growth hormone, prolactin, and cytokines (20).

Both human and animal studies support a role of moderate exercise on immune function. NK cell function has been documented to be increased in elderly subjects after moderate exercise (39). In this study we found a very small, albeit statistically significant, effect of exercise on NK cell cytotoxicity at some but not all effector-target ratios. It is therefore difficult to make firm conclusions about the relative importance of the small effect without further investigation. However, the increase in macrophage function found in this study was more substantial and extends the work of others who have found benefits of moderate exercise on macrophage function. Kohut et. al. (18) using a similar animal model of HSV-1 respiratory infection demonstrated that moderate exercise was associated with increased production of cytokines associated with an enhanced cell-mediated immune response in older mice. Moderate exercise has been associated with an increase in macrophage phagocytosis (34), as well as an increase in oxidative metabolism, microbial capacity, spontaneous mobility, chemotaxis, and attachment of this cell population (11).

The mechanism of increased host defense with β-glucan administration is also primarily attributed to the activation of macrophages, neutrophils, and NK cells (8, 32, 33). These cells contain a receptor site specific for β-glucan on their cell surface membrane such as complement receptor 3 and dectin-1 (2, 8, 36) that when ligated can exert increased macrophage, neutrophil, and NK cell activity. However, the mechanisms of stimulation are dependent on the route of administration (e.g., intravenous, intraperitoneal, or oral) and specific characteris-
tics of the β-glucan, including the source (e.g., oats, yeast, fungi, etc.), solubility, molecular mass, degree of branching, and conformation (ratio of 1 → 3 to 1 → 4 and 1 → 6 glucose-pyranosyl linkages) (38). Orally administered β-glucan enhances both peritoneal and alveolar macrophage activity through an increase in acid phosphatase activity, phagocytosis, H₂O₂ production, and IL-1 production (30, 33), as well as increasing NK cell cytotoxicity (32). This probably results from ingestion of β-glucan by pinoyctic M-cells located in Peyer’s patches of the small intestine, causing a release of cytokines that are responsible for initiating an extensive cascade of systemic immune responses (14, 30, 32, 33). It is also possible for soluble oat β-glucan to be absorbed into the lymphatic and cardiovascular systems and thereby interact directly with circulating immune cells (16, 40). Therefore, it seems reasonable that soluble oat β-glucan produces its immunopotentiating activities initially through the direct activation of various innate immune cell populations via β-glucan specific receptors on their cell surface (within the gut-associated lymphoreticular system and perhaps central circulation) and later through indirect activation via a cascade of systemic immune responses involving cytokines.

The results from this study support our hypothesis that moderate exercise training can decrease susceptibility to an induced respiratory infection in mice. These data also provide evidence for a role of macrophages and NK cells as mediators of this benefit on host protection. There were no added benefits of oat β-glucan in this experiment, but this deserves further research to evaluate the noted positive trends in both immune function and infection rates given the relatively small sample size and possible ceiling effect encountered in this experiment, especially given the known benefits of β-glucan in other infectious disease models and the fact that β-glucan was associated with enhanced macrophage anti-viral function in this experiment.

GRANTS

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REFERENCES