B-1 cell (CD5+/CD11b+) numbers and nIgM levels are elevated in physically active vs. sedentary rats

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We have previously reported that voluntary free-wheel running in pathogen-free Sprague-Dawely rats increases circulating nIgM but not antigen-driven IgM (15). Furthermore, the increase in nIgM is not due to environmental enrichment, because being housed with an immobile wheel has no effect on nIgM levels (15). The mechanism(s) for how physical activity increases circulating nIgM is presently unknown. One possibility is that physical activity stimulates an increase in B-1 cell numbers.

The purpose of the following experiments, therefore, was to examine whether physical activity increases B-1 cell numbers. B-1 cell numbers were assessed in the peritoneal cavity, mesenteric lymph nodes, and spleen. These compartments were tested for the B-1 cell (CD5+/CD11b+ cells) for several reasons. First, the peritoneal cavity is the main residence of B-1 cells (5, 6, 9, 10, 16, 21). Second, the MLN is the nearest lymphoid tissue to the peritoneal cavity, and there is some evidence that, after antigenic exposure, B-1 cells migrate to the MLN, perhaps to aid the antigen response (16). Third, the spleen was tested because there is evidence that B-1 cells periodically migrate to the spleen probably to interact with the resident B-2 (conventional B cell) cell population (24).

For these studies, pathogen-free Fischer 344 rats were sedentary or had access to free wheels for 6–7 wk.

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Blood samples were taken during the course of running, and serum concentrations of circulating protein, nlgM, and IgG were measured. After 6–7 wk, rats were killed, and samples were collected from the peritoneal cavity, MLN, and spleen. nlgM concentration in the peritoneal cavity fluid was measured by using ELISA. B-1 cell numbers and percentages were assessed in each compartment by using two-color flow cytometry for coexpression of CD5 and CD11b cell surface antigens. The B-1 cell phenotype has been reported in the mouse model using the coexpression of CD5 and CD11b on peritoneal cavity, MLN, and spleen cells (2, 6, 9, 10, 16, 27, 31) using fluorescence-activated cell sorting (FACS). Although still somewhat controversial in the rat, other studies (5) have verified that rat cells coexpressing CD5 and CD11b do have a B-1 cell phenotype. We hypothesize that physical activity will both increase serum and peritoneal nlgM levels, and increase B-1 cell numbers.

METHODS

All methods were approved by the University of Colorado Animal Care and Use Committee.

Running Activity

Rats were housed individually in Nalgene Plexiglas cages (45 x 25.2 x 14.68 cm) with a stainless steel open running wheel attached (46.8 x 24.9 x 34.2 cm). Physically active rats chose to run every day during the study. Sedentary animals were housed with the same cage setup, but running wheels remained locked and immobile through the duration of the study. The caging environment met the National Institutes of Health requirements of floor space for single rodent housing. The activity of voluntarily running was chosen as the mode of exercise because, unlike forced treadmill running, it does not produce adaptations that are indicative of chronic stress in rats (25, 26). The VitalView computer program (SunRiver) recorded total daily running distances. Rats were weighed weekly.

Blood Samples

Figure 1 depicts the experimental protocols used to test the effect of physical activity on serum nlgM analysis (Fig. 1A) and B-1 cell number (Fig. 1B). Rats used in the serum nlgM analysis experiment ran for 6 wk or were sedentary controls (n = 10 rats/group). Baseline samples were collected 7 days before running onset and on days 3, 7, 14, 28, 35, and 42 after running onset. Tail vein blood samples were taken within 2 min of touching the cage. Animals were wrapped in cloth and gently restrained with a Velcro strap anchored to a table. The exposed tail was nicked with a no. 15 scalpel blade, and ~125 μl of blood were quickly milked from the tail vein. Animals were then returned to their home cages. Samples were allowed to clot at room temperature for 30–45 min and then were spun at 4,000 rpm for 30 s. Serum was removed, and samples were frozen at −20°C until assays were performed. Rats used in the B-1 cell experiment ran for 7 wk or were sedentary controls (n = 5 rats/group). Blood was taken on the day of death via decapitation.

ELISAs: Total nlgM, Total IgG

ELISAs were preformed by using 96-well plates (Dunex). Plates were coated with goat anti-rat IgM or IgG (Cappell) and were incubated overnight at 4°C. Plates were then washed twice with PBS and 0.05% Tween 20 (PBS-T; Sigma Chemical) and were then blocked with 5% BSA overnight at 4°C. Plates were then washed three times in PBS-T. Rat IgM and IgG (Pharmingen) were used to generate a standard curve, ranging from 10.4 to 333.33 μg/ml. Samples were assayed in duplicate and were diluted (1:2,500, 1:5,000 for serum; 1:50, 1:100 for peritoneal lavage samples) so that their values were on the linear portion of the standard curve. Plates were coated with plate sealers and incubated at room temperature for 4 h. Then, plates were washed three times with PBS-T, goat anti-rat IgM or IgG with alkaline phosphatase tag was added (Cappell), and plates were incubated another hour. Plates were then washed three times with PBS-T, and p-nitrophenyl phosphate liquid (p-NPP; Sigma Chemical) was added. Plates were incubated in the dark until the most concentrated standard read between 1.3 and 1.5 optical density on the plate reader (Molecular Devices, Emax) at 405 nm.

Protein

The Bradford assay was used to determine total serum (18) protein. Briefly, 10 μl of 1:200 dilution of sample (PBS-T) was pipetted in a 96-well plate (Dunex), and 250 μl of Bradford reagent were added. Plates were incubated for 10 min at room temperature and then were read at 590 nm on the plate reader.

B-1 Cell Assessment (CD5+/CD11b+ Cells)

Tissue Collection. Animals were gently removed from their cages and decapitated by using a guillotine within 1 min after removal. Trunk blood was taken. For the lavage, heat-sterilized tools and sterile pipettes were used. The abdominal region was sprayed down with ethanol (70%; Sigma Chemical), and the skin was removed. A small hole was cut in the abdominal wall, and 2 ml of cold sterile Hank’s balanced salt solution (HBSS; Sigma Chemical) were added. The abdominal region was then massaged for 15 s. The fluid was then retrieved via a pipette, placed in a sterile snap cap, and put on ice. A second lavage was performed with 15 ml of cold sterile HBSS. The fluid retrieved from the first small lavage

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<tr>
<td>BL</td>
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<td>B</td>
<td>Day of Running Onset</td>
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was spun at 3,000 rpm for 30 s, the supernatant was removed for assessing nIgM, and the cell pellet was resuspended with HBSS and added into the larger lavage for cell count numbers. The MLN and spleen were also removed and placed in sterile HBSS.

MLN and spleen cells were dissociated by using a modified tissue homogenizer (Bellco Glass). Cell concentrations and total cell yields were assessed by using a Coulter Counter for running distance per week throughout the duration of

FACS staining and analysis. Cells (1 × 10^6) were incubated for 30 min at 4°C in the dark with anti-CD5 FITC-conjugated (10 µl; Serotec) and/or anti-CD11b RPE-conjugated antibody (10 µl; Serotec). Double-labeled cells were washed once. Isotype controls (IgG2a, Serotec; IgG1, Linco) were included. Cells were fixed in 500 µl of HBSS and 50 µl of 37.6% formaldehyde solution at room temperature for 20 min, spun at 4,000 rpm for 1 min, and resuspended in 1.0 ml of HBSS for FACS analysis (Becton-Dickinson FACSCANTM cell sorter).

Each animal had an unstained sample to serve as its own control. Two animals from each group were used to test whether there was nonspecific binding via isotype controls. Isotype controls did not differ from unstained controls in scatter or in percentages of CD5 or CD11b staining. Thus, nonspecific binding of the anti-CD5 and anti-CD11b antibodies used in staining the retrieved cells was negligible.

Upregulation of CD5 or CD11b could be indicative of increased cellular activation (22, 28). To determine whether there were any differences in cell surface expression of CD5 or CD11b, the intensity of the signal was analyzed in two ways. First, peak mean fluorescence was determined by gating on the double-labeled population with the use of the Cell Quest analysis program. An increase in mean peak fluorescence would indicate that there is a greater density of binding sites per cell. The second method used to test for an increase in cell surface expression of CD5 and/or CD11b involved fluorescence intensity decade analysis. This method entailed measuring the percentage of cells (either CD5+ or CD11b+) found in each decade. A decade was defined as one log unit increment in fluorescence intensity. If cells upregulate cell surface expression of CD5 or CD11b, one would see an increase in the percentage of cells in the highest decade of fluorescence intensity. Both of these methods were used to determine whether physical activity changed cell surface CD5 and/or CD11b expression (22, 28).

Statistical Analysis

StatView 5.0 was used for all analyses. Repeated-measures ANOVA was used on weight, protein, IgM, and IgG. ANOVA was used on all single time point protein, IgM, and IgG, and for all cell flow cytometry analyses. Post hoc by Fisher’s protected least significant difference test was done on blood time course nIgM. Pearson’s correlation analyses were used to determine whether there was a relationship between average distance run and peritoneal nIgM, circulating nIgM, and/or CD5+/CD11b+ cell numbers. Significance for all tests was set at P = 0.05.

RESULTS

Running Distance and Body Weight

As shown in Table 1, animals used for serum nIgM analysis ran from 6 to 22 km/wk and increased their running distance per week throughout the duration of the study [F(9,5) = 2.19, P < 0.0001]. Similarly, animals used for B-1 cell assessment ran from 5–12 km per wk and also increased weekly running distances over time [F(4,6) = 5.2, P = 0.003].

Consistent with previous reports, all animals gained body weight across time [serum nIgM study: F(1,5) = 458.1, P < 0.0001; B-cell study: F(1,6) = 52.5, P < 0.0001], and the active animals gained less weight [serum nIgM study: F(1,5) = 39.4, P = 0.0001; B-1 cell study: F(1,6) = 3.6, P = 0.005]. Body weight data are not presented.

Table 1. Average weekly running distances for physically active rats

<table>
<thead>
<tr>
<th>Week</th>
<th>Average Distance Run, km/wk</th>
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<tbody>
<tr>
<td>1</td>
<td>5.9 ± 0.5</td>
</tr>
<tr>
<td>2</td>
<td>15.2 ± 1.0</td>
</tr>
<tr>
<td>3</td>
<td>12.9 ± 1.4</td>
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<tr>
<td>4</td>
<td>11.6 ± 1.6</td>
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<tr>
<td>5</td>
<td>21.6 ± 3.5</td>
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<tr>
<td>6</td>
<td>17.6 ± 3.1</td>
</tr>
<tr>
<td>7</td>
<td>14 ± 1.1</td>
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</tbody>
</table>

Values are means ± SE. Adult male Fischer 344 rats were housed with either a locked (sedentary) or mobile (physically active) running wheel for 42 (n = 10 rats/group) or 56 (n = 5 rats/group) days.

Serum Protein, nIgM, IgG, and Peritoneal nIgM

Serum nIgM analysis. Consistent with our previous results, free-wheel running had no effect on total serum protein (Fig. 2A). There were no changes between groups [F(1,18) = 0.601, P = 0.45], over time [F(1,5) = 1.63, P = 0.015], or a time-by-activity interaction [F(1,5) = 0.11, P = 0.99]. However, as shown in Fig. 2B, serum nIgM was elevated in the physically active rats compared with sedentary rats. There was a difference between activity condition [F(1,18) = 5.5, P = 0.031], a change over time [F(1,5) = 3.85, P = 0.003], and a trend toward a time-by-activity interaction [F(1,5) = 1.97, P = 0.09]. Post hoc analyses (Fisher’s protected least significant difference test) revealed that physically active animals had significantly higher nIgM on days 28 (P = 0.03), 35 (P = 0.037), and 42 (P = 0.013) after free-wheel running onset than sedentary controls. There was no correlation between average distance run and the average amount of circulating nIgM (r = 0.13, P = 0.74). Serum IgG (Fig. 2C) was not altered between groups [F(1,18) = 0.61, P = 0.45], over time [F(1,5) = 1.68, P = 0.134], nor was there a time-by-activity interaction [F(1,5) = 0.17, P = 0.99]. As shown in Fig. 2D, 6 wk of activity vs. remaining sedentary significantly increased peritoneal cavity concentrations of nIgM [F(1,18) = 6.5, P = 0.02]. Once again, there was no correlation between average distance run and amount of peritoneal nIgM (r = 0.10, P = 0.80).

B-1 cell assessment. Rats that were killed after 7 wk of free-wheel running activity had no changes in serum protein [F(1,9) = 0.2, P = 0.67; Fig. 3A] or serum IgG [F(1,9) = 3.35, P = 0.11; Fig. 3C]. Again, serum nIgM (Fig. 3B) was significantly higher in the physically
Fig. 2. Adult male Fischer 344 rats were housed with either a locked (sedentary) or mobile (physically active) running wheel for 42 days (n = 10 rats/group). Blood was collected via the tail vein 3 days before running onset and on days 3, 7, 12, 28, 35, and 42 of activity. Serum levels of protein (A), nlgM (B), IgG (C), and peritoneal nlgM (PeriC; D) were measured by using Bradford and ELISA assays. Physical activity reliably elevated nlgM on days 28, 35, and 42 of activity. Peritoneal nlgM was elevated by activity as well. Values are means ± SE. *P < 0.05. In D, solid bar = sedentary subjects; open bar = physically active subjects.

active animals compared with sedentary controls [F(1,9) = 11.7, P = 0.01], and there was no correlation between average distance run and serum nlgM (r = −0.55, P = 0.39). Figure 3D shows there was a trend for more nlgM in the peritoneal cavity of active rats [F(1,9) = 4.1, P = 0.07], and no correlation between average distance run and the amount of peritoneal nlgM (r = −0.24, P = 0.74).

**Cell Flow Cytometry Analysis**

Total number of cells retrieved. As shown in Fig. 4A, there were more cells retrieved from the peritoneal cavity of active animals [F(1,8) = 9.09, P = 0.017] compared with sedentary controls. There were no differences in the number of cells retrieved from the MLN [F(1,8) = 2.39, P = 0.2] nor in the total number of cells retrieved from the spleen [F(1,8) = 0.015, P = 0.9].

Number and percentage of CD5+ /CD11b+ cells. Figure 4, B and C, shows that in the peritoneal cavity there were a greater number CD5+/CD11b+ cells in active animals [F(1,8) = 6.68, P = 0.03] and a greater percentage of CD5+/CD11b+ cells [F(1,8) = 9.98, P = 0.01]. There was no correlation between average distance run and number (r = −0.39, P = 0.56) or percentage (r = −0.33, P = 0.63) of CD5+/CD11b+ cells. In the MLN, there was a significantly greater number of CD5+/CD11b+ cells in the physically active rats [F(1,8) = 5.59, P = 0.05] but not in percentage of cells [F(1,8) = 1.01, P = 0.35]. In the spleen, there was not a consistently detectable CD5+/CD11b+ cell population. No sedentary animals appeared to have this splenic population, and only three of the five physically active rats had a detectable population. The figures depicting this population for the spleen only use values for the three rats with a detectable population.

Number and percentage of CD5+ /CD11b− cells. Figure 5 illustrates the number and percentage of CD5+/CD11b− cells. In the peritoneal cavity, physical activity did not alter CD5+ cell number [F(1,8) = 1.03, P = 0.34] or percentage [F(1,8) = 1.03, P = 0.34] compared with sedentary animals. For the MLN, there was a trend toward more CD5+/CD11b− cells in the active animals [F(1,8) = 4.43, P = 0.07], with a weak trend in the percentage [F(1,8) = 3.15, P = 0.11] (Fig. 5A). In the spleen, the number of CD5+/CD11b− cells [F(1,8) = 0.03, P = 0.87] and the percentage of CD5+/CD11b− cells [F(1,8) = 0.01, P = 0.91] was not changed between groups (Fig. 5B).

Number and percentage of CD5− /CD11b+ cells. Figure 6 shows the number and percentage of CD5− /CD11b+ cells. In the peritoneal cavity, there were no differences in number [F(1,8) = 1.39, P = 0.27] or percentage [F(1,8) = 0.11, P = 0.75] of CD5− /CD11b+ stained cells with activity status. For the MLN, activ-
ity did not alter CD5/CD11b+ cells in number [F(1,8) = 0.11, P = 0.75] or percentage [F(1,8) = 0.93, P = 0.36]. In the spleen, neither the number of CD5+/CD11b+ cells [F(1,8) = 1.69, P = 0.23] nor the percentage of CD5+/CD11b+ cells [F(1,8) = 0.78, P = 0.40] was altered by activity status.

Mean peak and decade fluorescence. As reported in Table 2, there were no differences in mean peak fluorescence in peritoneal cavity cells for CD5 [F(1,8) = 2.26, P = 0.18] or CD11b [F(1,8) = 0.16, P = 0.70], or when fluorescence was analyzed by decade of fluorescence intensity for CD5+/CD11b− cells [F(1,8) = 2.245, P = 0.16] or CD5−/CD11b+ cells [F(1,8) = 2.88, P = 0.13].

DISCUSSION

This is the first study to examine the impact of voluntary physical activity on the CD5+/CD11b+ cell phenotype (B-1-like cells) of the rat. The results confirm our earlier observation that physical activity increases circulating and peritoneal cavity nIgM (15). Circulating nIgM in active rats was higher in physically active animals, and nIgM was trending higher in peritoneal lavage samples. Values are means ± SE.

peritoneal cavity was elevated in animals that were physically active for 6 wk of physical activity and was trending toward being higher in animals that were physically active for 7 wk. Furthermore, these data indicate that the peritoneal CD5+/CD11b+ cell population is increased after 7 wk of wheel running. Interestingly, the average distance run did not correlate with nIgM in the peritoneal cavity or circulation, or with CD5+/CD11b+ cells, indicating that it is the activity status and not the running distance that is important in producing nIgM and B-1 cell increases. These data suggest that one potential mechanism for the increase in peritoneal and circulating nIgM after free-wheel running is an expansion of the B-1 cell population.

Additionally, physically active animals had a greater number of CD5+/CD11b+ cells removed from the peritoneal cavity. To ascertain whether this increase in cell...
numbers was due to more CD5+/CD11b+ cells, the number of double-positive cells was subtracted from the total number of cells. Interestingly, this subtraction removed the difference in total cell numbers due to activity status [F(1,8) = 1.48, P = 0.26]. Therefore, it appears that the increase in the total number of peritoneal cavity cells of active animals was due to an increase in specifically the B-1 cell (CD5+/CD11b+) population.

In the spleen, there were detectable CD5+/CD11b+ cells only in three of the physically active rats and in no sedentary rats. Perhaps the frequency in the sedentary animals was too small to detect, or, perhaps, there were no B-1 cells in the stained cell population due to chance. It is thought that there are few resident B-1 splenocytes at any one time, but rather B-1 cells periodically migrate to the spleen in small numbers (34). It is important to note that there is a small sister population of CD5low that were not investigated in these studies. It appears that this cell type is a very small percentage of peritoneal cavity cells (7, 21).

Table 2. Mean peak fluorescence and fluorescence as determined by decade of labeled peritoneal cavity cells

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<th>Sedentary</th>
<th>Physically Active</th>
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<tr>
<td><strong>CD5+</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean peak</td>
<td>632.5 ± 59</td>
<td>509.2 ± 56</td>
</tr>
<tr>
<td>Decade 1</td>
<td>80.6 ± 1.3</td>
<td>79.6 ± 1.7</td>
</tr>
<tr>
<td>Decade 2</td>
<td>13.1 ± 0.8</td>
<td>14.9 ± 1.2</td>
</tr>
<tr>
<td>Decade 3</td>
<td>4.4 ± 0.4</td>
<td>5.55 ± 0.6</td>
</tr>
<tr>
<td><strong>CD11b+</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean peak</td>
<td>3514.4 ± 541</td>
<td>3767.9 ± 178</td>
</tr>
<tr>
<td>Decade 1</td>
<td>9.37 ± 2.1</td>
<td>11.1 ± 1.2</td>
</tr>
<tr>
<td>Decade 2</td>
<td>39.7 ± 0.7</td>
<td>41.4 ± 1.5</td>
</tr>
<tr>
<td>Decade 3</td>
<td>49.8 ± 1.7</td>
<td>47.9 ± 2.1</td>
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Values are means ± SE. Adult male Fischer 344 rats were housed with either a locked (sedentary) or mobile (physically active) running wheel for 56 days (n = 5 rats/group). Peritoneal lavage samples were taken. Mean peak fluorescence for CD5 and CD11b were determined by the Cell Quest program. Decade fluorescence was determined by gating on the CD5+ or CD11b+ population and then looking at fluorescence in 3 decades (log units) of the fluorescence population. More fluorescence in decade 2 or 3 would indicate that there was a greater receptor density for the surface markers tested. There were no differences with activity condition for either CD5 or CD11b.
CD5+/CD11b− cells did not differ in the peritoneal cavity or spleen. This cell population is most likely primarily T cells (32). The MLN was trending toward a greater number of CD5+/CD11b− cells. Perhaps physical activity can slightly augment the CD5+/CD11b− population in this compartment. For CD5−/CD11b+ cells, there were no differences in any compartment tested. These cells are probably predominately macrophages/monocytes (35).

For both CD5+ and CD11b+ cells, there was no alteration in the mean peak or the decade fluorescence due to activity status. This indicates that, in an unchallenged animal, B-1 cells do not have upregulated CD5 or CD11b receptors due to activity status alone.

The number and percentage of CD5+/CD11b+, CD5+/CD11b−, and CD5−/CD11b+ cells were as expected in each of the three compartments. The peritoneal cavity, where most B-1 cells reside, had the greatest percentage and number of CD5+/CD11b+ cells. As would be expected, the greatest numbers and percentages (~45%) of CD5+ cells (expressed primarily on T cells) were found in the spleen and MLN. In contrast, the peritoneal cavity had a very low number and percentage of CD5+/CD11b− cells. This too can be expected in a pathogen-free animal. The number and percentage of CD5−/CD11b+ cells (found on macrophages/neutrophils) were highest in the peritoneal cavity, which is a reservoir for these cells. CD5−/CD11b+ cells were not widely found in the spleen or MLN in these pathogen-free animals. This is to be expected, because in the absence of a challenge there would be no reason for these cells, most likely macrophages, to migrate from their resident compartment of the peritoneal cavity (8).

It is important to recognize that the effect of voluntary, habitual exercise does not generalize to B-2 (conventional B cell) cell responses. Indeed, we have previously reported (15) no impact of this type of exercise on B-1 cell responses to KLH. These results are consistent with the majority of literature, which suggests that B-1 cell responses are not altered by exercise (20, 23).

The exact mechanism(s) responsible for the changes in the B-1 cell population of voluntarily physically active animals are unknown. Increased IL-10 and decreased IL-6 are two potential mechanisms that could be responsible for the increase in B-1 cells with physical activity. IL-10 is an autocrine growth factor for B-1 cells and is elevated with strenuous exercise (29). It is possible that there is a rise in B-1 production of this cytokine with habitual moderate activity, and this leads to increased B-1 cell numbers. IL-10 levels were measured (ELISA) in the peritoneal cavity lavage, but there were no differences (sedentary: n = 10, mean = 6.4 ng/ml ± 7.2; physically active: n = 10, mean = 15.4 ± 9.6 ng/ml; P = 0.11). It is feasible that this method was not sensitive enough due to the autocrine nature of B-1 cell IL-10 release. Labeling for intracellular IL-10 would perhaps reveal differences with activity status.

IL-6, an inflammatory cytokine, appears to have an inhibitory role on the B-1 cell population (27). In genetic IL-6 knockout mice, the B-1 cell population in the peritoneal cavity is expanded, and these cells respond more vigorously to Salmonella typhimurium (2). The authors hypothesized that IL-6 is an inhibitory cytokine on the B-1 cell population. One source of IL-6 is adipose tissue (13). Habitual, moderate exercise has been shown to decrease plasma IL-6 levels, perhaps due to a reduction in adipose tissue (13, 19, 36). Additionally, rats that free-wheel run have a reduction in fat mass (14). Thus perhaps a reduction in adipose tissue in active rats leads to a decrease in IL-6 levels, and this reduction allows for a B-1 cell population increase. However, Fischer 344 rats are very lean, even in the sedentary condition (laboratory observations). To measure a change in fat mass with free-wheel running, one would have to include both weight and an estimation of fatty tissue, because there might be a decrease in fat mass without a decrease in weight due to more lean muscle mass in the active animals. This idea requires further investigation.

Increased B-1 cell numbers and thus circulating IgM stemming from habitual, moderate activity would probably benefit host immunity. B-1 cells are vital in protecting against bacteria and viral challenge. Mice without secreted IgM succumb to a survival bacterial infection (10) and have high mortality rates in a viral challenge model (3, 4). Interestingly, although IgM has been thought to be one trigger of autoimmune disease, there is now evidence that it can, in a normal animal, aid in controlling autoimmunity (11). B-1 cells are an important, often overlooked component of an effective immune response.

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