Evidence for Anti-Inflammatory Effects of Exercise in CKD

João L. Viana,* George C. Kosmadakis,† Emma L. Watson,‡ Alan Bevington,‡
John Feehally,† Nicolette C. Bishop,* and Alice C. Smith†‡

*School of Sport, Exercise and Health Sciences, Loughborough University, Leicestershire, United Kingdom; †John Walls Renal Unit, Leicester General Hospital, Leicester, United Kingdom; and ‡Department of Infection, Immunity and Inflammation, University of Leicester, Leicester, United Kingdom

ABSTRACT
CKD is associated with a complex state of immune dysfunction characterized by immune depression, predisposing patients to infections, and immune activation, resulting in inflammation that associates with higher risk of cardiovascular disease. Physical exercise may enhance immune function and exert anti-inflammatory effects, but such effects are unclear in CKD. We investigated the separate effects of acute and regular moderate-intensity aerobic exercise on neutrophil degranulation (elastase release), activation of T lymphocytes (CD69 expression) and monocytes (CD86 and HLA-DR expression), and plasma inflammatory markers (IL-6, IL-10, soluble TNF-receptors, and C-reactive protein) in patients with predialysis CKD. A single 30-minute (acute) bout of walking induced a normal pattern of leukocyte mobilization and had no effect on T-lymphocyte and monocyte activation but improved neutrophil responsiveness to a bacterial challenge in the postexercise period. Furthermore, acute exercise induced a systemic anti-inflammatory environment, evidenced by a marked increase in plasma IL-10 levels (peaked at 1 hour postexercise), that was most likely mediated by increased plasma IL-6 levels (peaked immediately postexercise). Six months of regular walking exercise (30 min/d for 5 times/wk) exerted anti-inflammatory effects (reduction in the ratio of plasma IL-6 to IL-10 levels) and a downregulation of T-lymphocyte and monocyte activation, but it had no effect on circulating immune cell numbers or neutrophil degranulation responses. Renal function, proteinuria, and BP were also unaffected. These findings provide compelling evidence that walking exercise is safe with regard to immune and inflammatory responses and has the potential to be an effective anti-inflammatory therapy in predialysis CKD.


Received July 8, 2013. Accepted January 28, 2014.

N.C.B. and A.C.S. contributed equally to this work.

Published online ahead of print. Publication date available at www.jasn.org.

Correspondence: Dr. João L. Viana, School of Sport, Exercise and Health Sciences, Loughborough University, Loughborough, Leicestershire LE11 3TU, United Kingdom. Email: j.viana@lboro.ac.uk

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Although the potential anti-inflammatory effects of regular exercise in CKD patients have been recognized, research in this area is lacking, particularly in predialysis patients. Apart from the potential benefits, given that exercise is currently advocated in the routine clinical care of CKD patients, it is also paramount to determine if exercise is safe to their underlying compromised immunity and inflammatory status. Although an acute bout of moderate exercise usually exerts little influence on immune and inflammatory responses in healthy people, it is not clear what effect it may have in CKD patients. Here, we report the separate effects of acute and regular moderate-intensity aerobic exercise on measures of immunity and systemic inflammation in predialysis CKD.

**RESULTS**

**Effects of Acute Exercise**

Fifteen predialysis patients (Table 1) walked for 30 minutes on a motorized treadmill at a 1% gradient and a speed that elicited a rating of perceived exertion (RPE) in the range of 12–14 (somewhat hard). The effects of this acute bout of exercise on immune and inflammatory parameters are summarized in Table 2.

Exercise induced an increase in total leukocyte concentration, which was more pronounced postexercise (P<0.001, effect size [ES]=0.44) but still observable at 1 hour postexercise (P=0.01, ES=0.27). These effects were mainly attributable to circulating neutrophils and lymphocytes, because exercise had no effect on monocyte count. Neutrophil concentration was elevated above pre-exercise levels postexercise (P<0.001, ES=0.35) and to a greater extent, 1 hour postexercise (P=0.002, ES=0.47). Lymphocyte concentration was also elevated postexercise (P<0.01, ES=0.53) but returned to pre-exercise levels by 1 hour postexercise.

Plasma elastase concentration (unstimulated neutrophil elastase release) was elevated above pre-exercise levels postexercise (P=0.03, ES=0.73) but returned to pre-exercise levels by 1 hour postexercise. Total neutrophil elastase release after 1 hour of *in vitro* stimulation with bacterial extract showed a trend to elevation postexercise (P=0.05, ES=0.22), which was evident at 1 hour postexercise (P<0.01, ES=0.54). When these data were expressed as bacterially-stimulated elastase release per neutrophil, values were also elevated in response to exercise; however, this result was only observed at 1 hour postexercise (P=0.04, ES=0.41).

There were no effects of exercise on the percentages of CD4+ or CD8++ lymphocytes expressing CD69 after 20 hours of *in vitro* staphylococcal enterotoxin B (SEB) stimulation or the SEB-stimulated CD69 expression by either CD4+CD69+ or CD8++CD69+ lymphocytes. Likewise, CD86 and HLA-DR expressions by SEB-stimulated CD14+CD86+HLA-DR+ monocytes were also unaffected.

Exercise induced an increase in plasma IL-6 levels, which was more evident postexercise (P<0.001, ES=0.33) but still noticeable at 1 hour postexercise (P=0.004, ES=0.25). Plasma IL-10 concentration was also elevated in response to acute exercise, and although an increase was already apparent postexercise (P<0.01, ES=0.58), it was further increased 1 hour postexercise (P=0.001, ES=1.12). Exercise also induced an elevation in plasma soluble TNF-receptor II (sTNF-RII) levels, which was only evident 1 hour postexercise (P=0.03, ES=0.30). Exercise had no effect on plasma soluble TNF-receptor I (sTNF-RI) and C-reactive protein (CRP) concentrations. The exercise-induced relative changes in these plasma markers of systemic inflammation are further illustrated in Supplemental Material (Figure 1).

**Effects of Regular Exercise**

The effects of regular exercise were investigated in a previously documented study. Briefly, 20 patients were assigned to a 6-month home-based walking exercise program, whereas 20 other patients continued with their habitual physical activity (control group); of these patients, 18 exercisers and 14 controls completed the study. However, given the outcomes addressed here, we have excluded patients on immunosuppressive therapy, which left data from 13 exercisers and 11 controls; their baseline characteristics are shown in Table 3.

We have previously reported that the exercise intervention improved exercise tolerance in the exercise group only. Data from the immune and inflammatory parameters investigated at baseline and 6 months for each group are summarized in Table 4. Most of the variables assessed did not differ between groups at baseline. Exceptions were CD86 expression by SEB-stimulated CD14+CD86+HLA-DR+ monocytes and plasma sTNF-RI levels (higher in exercisers) and CD69 expression by SEB-stimulated CD4+CD69+ and CD8++CD69+ lymphocytes (tendency to be higher in exercisers).

There were no effects of the exercise intervention on total and differential blood leukocyte counts or measures of neutrophil degranulation. The percentages of SEB-stimulated CD4+ and CD8++ lymphocytes expressing CD69 were also unaffected by the exercise intervention. However, CD69 expression by SEB-stimulated CD4+CD69+ and CD8++CD69+ lymphocytes was downregulated after 6 months in exercisers (P=0.02, ES=0.59 and P=0.01, ES=0.65, respectively), whereas it remained unaltered in controls (P=0.91 and P=0.27, respectively). Likewise, the expression of CD86 and HLA-DR by SEB-stimulated CD14+CD86+HLA-DR+ monocytes was also downregulated after 6

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (n men/n women)</td>
<td>12/3</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>59±10</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>80±15</td>
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<tr>
<td>Height (cm)</td>
<td>172±10</td>
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<tr>
<td>Body mass index (kg/m²)</td>
<td>27.1±5.1</td>
</tr>
<tr>
<td>eGFR (ml/min per 1.73 m²)</td>
<td>18.3±7.3</td>
</tr>
</tbody>
</table>

Data are mean±SD.
months in exercisers ($P = 0.004$, $ES = 1.27$ and $P = 0.04$, $ES = 0.72$) but upregulated in controls ($P = 0.02$, $ES = 0.76$ and $P = 0.04$, $ES = 0.51$). Comparisons of the relative changes over 6 months between groups confirmed these effects (Figure 1).

Circulating IL-6 levels tended to be reduced ($P = 0.06$, $ES = 0.40$), and IL-10 levels tended to be increased ($P = 0.07$, $ES = 0.66$) in exercisers but remained unchanged in controls ($P = 0.43$ and $P = 0.33$, respectively). Consequently, the plasma IL-6/IL-10 ratio, reflecting overall inflammatory status, was reduced in exercisers, whereas it did not change in controls (Figure 2). Plasma sTNF-RI levels were also reduced ($P = 0.04$, $ES = 0.20$), and plasma sTNF-RII showed a tendency to reduction ($P = 0.06$, $ES = 0.25$) in exercisers but not controls ($P = 0.13$ and $P = 0.50$, respectively). In contrast, plasma CRP levels were unaffected by the exercise intervention.

There were no effects of the exercise intervention on body weight, renal function, proteinuria, or BP (Table 5).

**DISCUSSION**

The impact of physical exercise on immunity and inflammation in predialysis CKD patients is unresolved. Previous research in this area is limited and has produced conflicting results, with one study reporting favorable effects of resistance exercise training\(^\text{21}\) and

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### Table 2. Effects of acute exercise on immune and inflammatory parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-Exercise</th>
<th>Postexercise</th>
<th>1 h Postexercise</th>
<th>$P$ Value(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total and differential blood leukocyte counts</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukocytes ($\times 10^9$/L)</td>
<td>6.6±1.9</td>
<td>7.5±1.9(^b)</td>
<td>7.2±2.2(^b)</td>
<td>0.001</td>
</tr>
<tr>
<td>Neutrophils ($\times 10^9$/L)</td>
<td>4.1±1.4</td>
<td>4.6±1.4(^b)</td>
<td>4.9±1.7(^b)</td>
<td>0.002</td>
</tr>
<tr>
<td>Lymphocytes ($\times 10^9$/L)</td>
<td>1.6±0.6</td>
<td>1.9±0.6(^b)</td>
<td>1.5±0.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Monocytes ($\times 10^9$/L)</td>
<td>0.6±0.2</td>
<td>0.6±0.1</td>
<td>0.5±0.2</td>
<td>0.11</td>
</tr>
<tr>
<td><strong>Neutrophil degranulation (elastase release)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma elastase ($\mu$g/L)</td>
<td>54±19</td>
<td>74±34(^b)</td>
<td>52±16</td>
<td>0.02</td>
</tr>
<tr>
<td>Total bacterially-stimulated elastase release ($\mu$g/L)</td>
<td>3398±1489</td>
<td>3765±1867</td>
<td>4359±2033(^b)</td>
<td>0.003</td>
</tr>
<tr>
<td><strong>Bacterially-stimulated elastase release per neutrophil (fg/cell)</strong></td>
<td>510±180</td>
<td>516±206</td>
<td>598±245(^b)</td>
<td>0.003</td>
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<tr>
<td><strong>SEB-stimulated CD4(^+) and CD8(^+) lymphocytes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD69(^+) cells (%)</td>
<td>23±10</td>
<td>23±11</td>
<td>23±11</td>
<td>0.81</td>
</tr>
<tr>
<td>CD8(^+)</td>
<td>24±11</td>
<td>26±14</td>
<td>24±11</td>
<td>0.71</td>
</tr>
<tr>
<td>CD69 (GMFI)</td>
<td>141±49</td>
<td>132±42</td>
<td>143±53</td>
<td>0.10</td>
</tr>
<tr>
<td>CD4(^+)CD69(^+)</td>
<td>106±51</td>
<td>101±55</td>
<td>109±60</td>
<td>0.24</td>
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<tr>
<td>CD8(^+)CD4(^+)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CD69(^+)CD8(^+)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SEB-stimulated CD14(^+)CD86(^+)HLA-DR(^+) monocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD86 (GMFI ratio to unstimulated cells)</td>
<td>1.9±0.9</td>
<td>1.8±0.8</td>
<td>1.9±0.8</td>
<td>0.98</td>
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<tr>
<td>CD86 (GMFI ratio to unstimulated cells)</td>
<td>2.2±1.0</td>
<td>2.2±0.9</td>
<td>2.2±0.9</td>
<td>0.99</td>
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<tr>
<td><strong>Plasma markers of systemic inflammation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>7.7±4.7</td>
<td>9.4±6.0(^b)</td>
<td>8.9±5.5(^b)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-10 (pg/ml)</td>
<td>3.2±0.8</td>
<td>3.8±1.1(^b)</td>
<td>4.3±1.1(^b)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>sTNF-RI (ng/ml)</td>
<td>6.9±1.8</td>
<td>7.0±1.8</td>
<td>7.0±1.7</td>
<td>0.43</td>
</tr>
<tr>
<td>sTNF-RII (ng/ml)</td>
<td>16.8±4.0</td>
<td>17.5±3.9</td>
<td>18.0±4.2(^b)</td>
<td>0.02</td>
</tr>
<tr>
<td>CRP ($\mu$g/ml)</td>
<td>2.5±2.5</td>
<td>2.4±2.3</td>
<td>2.5±2.4</td>
<td>0.38</td>
</tr>
</tbody>
</table>

Data are mean±SD ($n=15$). GMFI, geometric mean of fluorescence intensity.

*Effect of acute exercise (one-factor ANOVA).

$^bP<0.05$ versus pre-exercise (paired $t$ tests).

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**Figure 1.** Effects of regular exercise on expression of SEB-stimulated immune cell activation markers. Relative changes over 6 months in SEB-stimulated CD69 expression by CD4\(^+\)CD69\(^+\) or CD8\(^+\)CD69\(^+\) lymphocytes (left panel) and SEB-stimulated CD86 or HLA-DR expression by CD14\(^+\)CD86\(^+\)HLA-DR\(^+\) monocytes (right panel) for the exercise (white bars) and control (black bars) groups. Data are mean±SEM ($n=13$ exercise group; $n=11$ control group). GMFI, geometric mean of fluorescence intensity.
Table 3. Effects of regular exercise: patients’ baseline characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Exercise Group (n=13)</th>
<th>Control Group (n=11)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (n men/n women)</td>
<td>8/5</td>
<td>7/4</td>
<td></td>
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<tr>
<td>Age (yr)</td>
<td>61±8</td>
<td>56±16</td>
<td>0.35</td>
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<tr>
<td>Weight (kg)</td>
<td>76.5±13.2</td>
<td>88.4±21.9</td>
<td>0.13</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>170±11</td>
<td>174±11</td>
<td>0.35</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26.6±4.7</td>
<td>29.0±5.9</td>
<td>0.28</td>
</tr>
<tr>
<td>eGFR (ml/min per 1.73 m²)</td>
<td>23.2±8.2</td>
<td>26.7±8.8</td>
<td>0.31</td>
</tr>
</tbody>
</table>

Data are mean±SD.  
*Baseline comparison between groups (independent t tests).

Table 4. Effects of regular exercise on immune and inflammatory parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Exercise Group</th>
<th>Control Group</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>6 mo</td>
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</tr>
<tr>
<td></td>
<td>Control Group</td>
<td>6 mo</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Baseline</td>
<td>Effectb</td>
<td></td>
</tr>
<tr>
<td>Total and differential blood leukocyte counts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukocytes (×10³/L)</td>
<td>6.7±2.4</td>
<td>6.6±2.1</td>
<td>0.56</td>
</tr>
<tr>
<td>Neutrophils (×10³/L)</td>
<td>4.3±1.8</td>
<td>4.2±1.6</td>
<td>0.56</td>
</tr>
<tr>
<td>Lymphocytes (×10³/L)</td>
<td>1.5±0.6</td>
<td>1.6±0.6</td>
<td>0.97</td>
</tr>
<tr>
<td>Monocytes (×10³/L)</td>
<td>0.6±0.2</td>
<td>0.5±0.2</td>
<td>0.96</td>
</tr>
<tr>
<td>Neutrophil degranulation (elastase release)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Plasma elastase (µg/L)</td>
<td>68±22</td>
<td>63±22</td>
<td>0.83</td>
</tr>
<tr>
<td>Total bacterially-stimulated elastase release (µg/L)</td>
<td>3703±1892</td>
<td>3586±2107</td>
<td>0.67</td>
</tr>
<tr>
<td>Bacterially-stimulated elastase release per neutrophil (fg/cell)</td>
<td>522±121</td>
<td>539±174</td>
<td>0.99</td>
</tr>
<tr>
<td>SEB-stimulated CD4⁺ and CD8⁺⁺ lymphocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD69⁺ cells (%)</td>
<td>24±10</td>
<td>20±8</td>
<td>0.65</td>
</tr>
<tr>
<td>CD8⁺⁺⁺</td>
<td>30±15</td>
<td>24±10</td>
<td>0.79</td>
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<tr>
<td>CD69 (GMFI)</td>
<td>175±55</td>
<td>143±53</td>
<td>0.06</td>
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<tr>
<td>CD4⁺/CD69⁺</td>
<td>110±44</td>
<td>85±32</td>
<td>0.05</td>
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<tr>
<td>SEB-stimulated CD14⁺CD8⁺⁺HLA-DR⁺ monocytes</td>
<td></td>
<td></td>
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<tr>
<td>CD86 (GMFI ratio to unstimulated cells)</td>
<td>2.1±0.8</td>
<td>1.3±0.3</td>
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<td>HLA-DR (GMFI ratio to unstimulated cells)</td>
<td>3.0±1.7</td>
<td>2.0±0.7</td>
<td>0.11</td>
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<tr>
<td>Plasma markers of systemic inflammation</td>
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<td></td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>10.3±5.2</td>
<td>8.1±4.3</td>
<td>0.63</td>
</tr>
<tr>
<td>IL-10 (pg/ml)</td>
<td>3.1±1.1</td>
<td>4.6±2.7</td>
<td>0.15</td>
</tr>
<tr>
<td>sTNF-RI (ng/ml)</td>
<td>6.2±2.5</td>
<td>5.7±2.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>sTNF-RII (ng/ml)</td>
<td>15.6±6.5</td>
<td>13.9±5.0</td>
<td>0.56</td>
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<tr>
<td>CRP (µg/ml)</td>
<td>3.5±3.3</td>
<td>2.6±2.5</td>
<td>0.90</td>
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</tbody>
</table>

Data are mean±SD (n=13 exercise group; n=11 control group).  
*Baseline comparison between groups (independent t tests).  
*Effect of regular exercise (two-factor ANOVA: time×group interaction).  
*P<0.05 versus baseline (paired t tests).

two other studies showing no effects of aerobic exercise training22,23 on plasma IL-6 and/or CRP levels. Here, we describe a more detailed examination of the impact of both acute and regular walking exercise on immunity and inflammation in CKD.

Effects of Acute Exercise
The main findings were that exercise enhanced neutrophil responsiveness to a bacterial challenge without affecting T-lymphocyte and monocyte activation and induced a systemic anti-inflammatory environment.

The observed effects of exercise on blood leukocyte counts were comparable with the effects usually reported in healthy individuals.19,24–26 As consistently reported,19,27 plasma elastase concentration was elevated immediately after exercise, indicating spontaneous neutrophil activation (degranulation in vivo) with return to resting levels within 1 hour. Considering that neutrophils are primed in CKD,28 this observation is particularly important, because it suggests that the exercise-induced neutrophil activation was only transient. Bacterially-stimulated elastase release per neutrophil was unaltered immediately after exercise but increased 1 hour later, indicating enhanced neutrophil responsiveness to a bacterial challenge in the postexercise period. This finding is in contrast to the reported reductions in stimulated neutrophil degranulation after numerous exercise protocols.19,27 Given that neutrophil dysfunction is believed to increase susceptibility to infection in CKD,29,30 a potential protective effect in the postexercise period is encouraging.

T lymphocytes and monocytes exhibit signs of both functional depression and activation in CKD.2,3 Importantly, our study suggests that acute exercise does not hinder T-lymphocyte and monocyte immune competence, and it does not aggravate their preactivated state.
In summary, a single bout of walking exercise in predialysis CKD patients seems to be safe from an immune and inflammatory perspective, improves immunosurveillance, and exerts anti-inflammatory effects.

Effects of Regular Exercise

The main findings of this study were that regular exercise downregulated T-lymphocyte and monocyte activation and that these effects were accompanied by improvements in systemic inflammation.

The exercise intervention had no effect on in vivo or in vitro neutrophil degranulation responses. In CKD, plasma elastase levels are elevated,39–41 and primed neutrophils are associated with inflammation and oxidative stress.26 Our findings indicate that regular exercise does not exacerbate the activation state of resting neutrophils (although transient spontaneous neutrophil activation occurs during each exercise bout). In addition, although the improved neutrophil responses observed after acute exercise do not translate into a chronic adaptation, given that neutrophil deficiencies may contribute to the high incidence of infections in CKD,30 it is reassuring that regular exercise does not hinder neutrophil immune competence. Furthermore, it is still possible that there is a window of protection after each exercise bout.

The observation that regular exercise downregulated T-lymphocyte activation is a very promising effect considering that it has been consistently shown that T lymphocytes are chronically elevated,33 raising a question about the safety of another exercise-induced increase. However, it is now clear that contracting skeletal muscle is the main source of the IL-6 in circulation in response to exercise,34,35 and muscle-derived IL-6, the first so-called myokine described,36 seems to mediate metabolic and anti-inflammatory effects both locally and systemically in an hormone-like fashion.8,32 In contrast to the cytokine cascade in sepsis, where IL-6 appearance is preceded by proinflammatory TNF-α and IL-1β, in response to exercise, IL-6 is typically the first cytokine present in the circulation, and then, it is followed by the cytokine inhibitors IL-1ra and sTNF-R as well as the anti-inflammatory IL-10.8,32 The kinetics of the cytokine response to exercise reported here are consistent with this finding. The greatest increase in IL-6 observed immediately after exercise was followed by a marked increase in IL-10 1 hour later, with a small increase in sTNF-RII also detected at this time. However, CRP was unaffected by exercise, which is expected, because alterations in CRP are often not seen until a few hours or even the day after exercise.37,38

Table 5. Effects of regular exercise on clinical parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Exercise Group</th>
<th>Control Group</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>6 mo</td>
<td>Baseline</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76.5±13.2</td>
<td>74.9±13.5</td>
<td>88.4±19.5</td>
</tr>
<tr>
<td>eGFR (ml/min per 1.73 m²)</td>
<td>23.2±8.2</td>
<td>23.7±10.0</td>
<td>26.7±8.8</td>
</tr>
<tr>
<td>uPCR (mg/mmol)</td>
<td>59±97</td>
<td>59±72</td>
<td>85±116</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>127±17</td>
<td>126±13</td>
<td>136±20</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>73±10</td>
<td>72±6</td>
<td>77±9</td>
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</table>

Data are mean±SD (n=13 exercise group; n=11 control group). uPCR, urine protein-to-creatinine ratio.

*Baseline comparison between groups (independent t tests).

Effect of regular exercise (two-factor ANOVA: time×group interaction).
Likewise, T lymphocytes from CKD patients show increased immune activation. Monocytes from CKD patients are pre-activated. In exercisers, plasma IL-6 concentration tended to be reduced after 6 months, whereas plasma IL-10 concentration tended to be increased, resulting in a significantly decreased ratio of IL-6 to IL-10 in the circulation. Plasma IL-6 levels are elevated and have consistently been shown to predict all-cause and cardiovascular mortality in CKD. Plasma levels of IL-10, one of the most important anti-inflammatory immune-regulating cytokines, are usually elevated in CKD because of persistent underlying inflammation. Hemodialysis patients with lower plasma IL-10 levels seem to be at greater risk for atherosclerosis, whereas those patients genetically predisposed to produce higher IL-10 levels show better immune balance and are at lower risk of cardiovascular events. It can be argued that the balance between IL-6 and IL-10 is a more valid parameter to draw conclusions about the overall inflammatory status. sTNF-Rs are the extracellular forms of the naturally occurring inhibitors of TNF but also, footprints of its activity and thus, not surprisingly elevated in CKD. Plasma sTNF-RI and sTNF-RII levels were or tended to be reduced after exercise, again suggesting an anti-inflammatory role of regular exercise in predialysis CKD patients.

In contrast to the favorable effects on plasma cytokines, we have failed to detect an effect of regular exercise on plasma CRP. This finding might be partly explained by the lack of an effect of the exercise intervention on body weight, because exercise without weight loss does not seem to reduce CRP. However, although it is also well established that CRP levels are elevated and predict all-cause and cardiovascular mortality in CKD, CRP is a rather nonspecific marker, and therefore, it might be difficult to detect changes in small patient cohorts. It is noteworthy that IL-6 has been consistently reported to be a stronger predictor of all-cause and cardiovascular outcomes in CKD than any other inflammatory marker, including CRP.

In summary, our findings suggest that regular walking exercise in predialysis CKD patients is safe from immune and inflammatory perspectives and that it exerts anti-inflammatory effects at both systemic and cellular levels. Obviously, this study is limited by its small sample size, and such effects need to be confirmed in larger cohorts of predialysis CKD patients. In addition, given the close links between wasting, inflammation, and atherosclerosis in CKD, future studies should also address the impact of resistance exercise. There is initial evidence that regular resistance exercise can also induce anti-inflammatory effects in predialysis CKD patients, but a more in-depth study of such effects is warranted. Ideally, investigating both exercise modes alone and combined in representative groups of patients at different CKD stages would help tailor future exercise guidelines for this population.

In conclusion, this report provides convincing evidence that exercise has the potential to be an effective anti-inflammatory therapy in predialysis CKD patients and in this way, may reduce the high risk of CVD in these very vulnerable patients. Additional research is, however, needed to fully elucidate the impact of exercise on the complex state of immune dysfunction that accompanies CKD.

**CONCISE METHODS**

**Patients**

Patients included in this report are subgroups of those patients described previously. All patients were recruited from nephrology outpatient clinics at Leicester General Hospital. Exclusion criteria were age <18 years, pregnancy, and orthopedic or cardiovascular disability that severely limited exercise capacity. The study received approval from the UK National Research Ethics Committee, and all patients gave written informed consent to participate.

The effects of acute exercise were investigated in a subgroup of patients that completed the baseline exercise test but were not receiving immunosuppressive therapy. The effects of regular exercise were investigated in a subgroup of patients that completed the 6-month study period but were not receiving immunosuppressive therapy. Patient characteristics are presented in Results (Tables 1 and 3).
Exercise Testing
All patients completed an exercise test at baseline and 6 months. At baseline, the exercise test consisted of 30 minutes of walking on a motorized treadmill at a 1% gradient and a speed that elicited an RPE in the range of 12–14 (somewhat hard).79 RPE was recorded every 2 minutes, and the treadmill speed was adjusted to maintain the RPE in the target range. The treadmill speed was also recorded every 2 minutes. At 6 months, the exercise test was repeated using exactly the same treadmill speed profile (i.e., same absolute exercise intensity), and the RPE response was recorded every 2 minutes. The effects of the exercise intervention on exercise tolerance have been previously reported.20

Exercise Program
Patients in the exercise group were prescribed a home-based exercise program, which consisted of at least 30 minutes of walking five times per week at an RPE in the range of 12–14 (somewhat hard) for a total duration of 6 months. Because most CKD patients receive β-blocker therapy, an overall heart rate (HR) range could not be used to prescribe the exercise intensity. Nevertheless, an individual HR range was established during the baseline exercise test by recording the HR response at the required RPE target range, and it was also provided to each patient in conjunction with an HR monitor. For monitoring purposes, patients were asked to keep exercise diaries, where they recorded the duration and the overall RPE of each exercise session. In addition, patients were requested to attend the hospital gym one time per month for a supervised exercise session to ensure compliance and make any necessary adjustments to the exercise program. Patients in the control group continued with their usual physical activity.

Blood Sampling, Handling, and Analysis
To investigate the effects of acute exercise, venous blood samples were obtained at rest (pre-exercise) and immediately after (postexercise) and 1 hour after (1 hour postexercise) the baseline exercise test. To investigate the effects of regular exercise, resting venous blood samples were obtained at baseline and 6 months. On both occasions, samples were collected at the same time of day, and patients were asked to refrain from exercise during the preceding 24 hours. All samples, approximately 20 ml each, were collected by venepuncture from an antecubital vein using a 21-g butterfly needle cannula and a dry syringe and immediately dispensed into two separate tubes as follows: approximately 7.5 ml into one S-Monovette tube (Sarstedt) containing K3EDTA (1.6 mg/ml) and approximately 12.5 ml into one universal tube containing heparin (16 units/ml).

Hematology
For the following analysis, K3EDTA-treated whole blood was used. Hemoglobin concentration was determined in duplicate using the cyanmethemoglobin method. Hematocrit was determined by measuring packed cell volumes in triplicate on a microhematocrit centrifuge. Plasma volume of resting blood samples was estimated from the hematocrit values. Plasma volume changes in postexercise blood samples were estimated from the hemoglobin and hematocrit values,80 and all cell counts and plasma measurements were corrected for these changes relative to the resting blood sample in the acute study only. In the exercise training study, there was no effect of time (baseline versus 6 months) on plasma volume (data not shown), and therefore, no adjustments were made. Total and differential leukocyte counts were determined using an automated hematology analyzer (Coulter AcT 5diff OV; Beckman Coulter).

Plasma Markers of Systemic Inflammation
The remaining K3EDTA-treated whole blood was centrifuged at 1500×g for 10 minutes in a refrigerated centrifuge at 4°C. The plasma obtained was aliquoted into Eppendorf tubes at 0.5 ml/tube and stored at −80°C for later determination of plasma concentrations of IL-6, IL-10, sTNF-R1, sTNF-RII, and CRP by ELISA as detailed in Supplemental Material.

Neutrophil Degranulation
One milliliter heparinized whole blood was immediately added to an Eppendorf tube containing 50 µl 10 mg/ml bacterial extract solution (stimulant, 84015; Sigma-Aldrich). The tube was sealed; blood and stimulus were mixed by gentle inversion, incubated for 1 hour at 37°C, and gently mixed again after 30 minutes. After incubation, the mixture was centrifuged for 2 minutes at 12,400×g, and the resulting supernatant was stored at −80°C before analysis. Another 1 ml heparinized whole blood was centrifuged at 1500×g for 10 minutes in a refrigerated centrifuge at 4°C, and the plasma obtained was stored at −80°C before analysis. Polymorphonuclear cell elastase concentration was determined in both bacterially stimulated and unstimulated (plasma elastase) samples using a commercially available ELISA kit (RD191021100; BioVendor GmbH) according to the manufacturer’s instructions. Bacterially stimulated and unstimulated samples were prediluted 1:1000 and 1:50 in the dilution buffer provided, respectively. All samples were assayed in duplicate, and all samples from the same patient were assayed in the same plate. The inter- and intra-assay coefficients of variation for all elastase ELISAs were 2.3% and 2.9%, respectively. Total bacterially-stimulated elastase release was calculated by subtracting plasma elastase concentration from the bacterially-stimulated elastase concentration and then, dividing by the neutrophil count to obtain the bacterially-stimulated elastase release per neutrophil.

T-Lymphocyte and Monocyte Activation
Two hundred-microliter aliquots of heparinized whole blood were cultured in 12×75-mm polystyrene round-bottomed tubes with caps (BD Biosciences) with no additive (unstimulated condition) or 1 µg/ml SEB (S4881; Sigma-Aldrich). Triplicate tubes were set up for each condition and incubated at 37°C in a humid 5% CO2 atmosphere for 20 hours. After incubation, the whole-blood aliquots were labeled in the respective culture tubes with cocktails of fluorochrome-conjugated mouse mAbs against human cell surface markers (BD Biosciences) as follows (one per condition): (1) lymphocyte surface markers: FITC-conjugated anti-CD4 (555346), phycoerythrin (PE)-conjugated anti-CD69 (555531), and PE-Cy5-conjugated anti-CD8 (555368); and (2) monocyte surface markers: FITC-conjugated anti-CD14 (555397), PE-conjugated anti-CD86 (555658), and peridinin–chlorophyll protein complex–conjugated anti–HLA-DR (347402). The remaining tube was left unstained. Labeling was carried out on ice for 20 minutes, which
was followed by erythrocyte lysis and leukocyte fixation achieved by incubating samples for 10 minutes in the dark with FACS Lysing solution (BD Biosciences). Leukocytes were subsequently washed two times in PBS containing 0.1% BSA and 2 mM EDTA and resuspended again in the same buffer for immediate flow cytometer acquisition. Samples were acquired on a flow cytometer (BD FACSCalibur) equipped with the CellQuest software package (BD Biosciences). For samples labeled with lymphocyte markers, side scatter versus forward scatter plots were used to gate on the lymphocyte population by morphology, and 30,000 lymphocytes were acquired per sample. For samples labeled with monocyte markers, 100,000 total cells were acquired per sample. Negative unstained control samples were also acquired. Software-generated data files were stored for later analyses using FlowJo version 9 for Macintosh (Treestar) as detailed in Supplemental Material.

Statistical Analyses
Data are presented as mean ± SD unless otherwise stated. All data were inspected for normality using the Shapiro–Wilk test. If a dataset was not normally distributed, statistical analysis was performed on the logarithmic transformation of the data, but data were back-transformed for presentation. Data from the acute effects of exercise were examined using a one-factor (time: pre-exercise, postexercise, or 1 hour postexercise) ANOVA with repeated measures design. For any significant unadjusted F values subsequently shown, sphericity of the data was determined using the Mauchly test, and appropriate adjustments in the degrees of freedom were made (i.e., Greenhouse–Geisser correction was applied when Greenhouse–Geisser ε was < 0.75 or Huynh–Feldt correction was used for less severe asphericity). A priori planned paired t tests with Holm–Bonferroni correction for multiple comparisons applied to the unadjusted P value were then performed between specific time points (postexercise versus pre-exercise and 1 hour postexercise versus pre-exercise).

To investigate the effects of regular exercise, baseline data were initially screened using independent t tests (exercise versus control). All data were then examined using an ANOVA with mixed design: within subjects (time: baseline versus 6 months) × between subjects (group: exercise versus control). Assumption of homogeneity was checked using Levene’s test. Where significant time × group interactions were observed, data were further examined using a priori planned t tests with Holm–Bonferroni correction as follows: paired (baseline versus 6 months) for each group and independent (exercise versus control) for 6-month data. T-lymphocyte and monocyte activation data were further examined by computing the relative (percentage of difference for 6-month data. T-lymphocyte and monocyte activation data were subsequently shown, sphericity of the data was determined using the Mauchly test, and appropriate adjustments in the degrees of freedom were made (i.e., Greenhouse–Geisser correction was applied when Greenhouse–Geisser ε was < 0.75 or Huynh–Feldt correction was used for less severe asphericity). A priori planned paired t tests with Holm–Bonferroni correction for multiple comparisons applied to the unadjusted P value were then performed between specific time points (postexercise versus pre-exercise and 1 hour postexercise versus pre-exercise).

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Effect sizes were calculated according to Cohen with correction for sample size. Statistical significance was accepted at P < 0.05.

ACKNOWLEDGMENTS

We thank Dr. Stephen John of Royal Derby Hospital for his assistance with the BP measurements in this study.

This work was partly supported by Kidney Research UK Grant RP33/1/2007 and Portuguese Foundation for Science and Technology Grant SFRH/BD/27838/2006. At the time of writing, J.L.V. was supported by the National Institute for Health Research Diet, Lifestyle & Physical Activity Biomedical Research Unit based at University Hospitals of Leicester and Loughborough University.

The views expressed are those of the authors and not necessarily the views of the NHS, the National Institute for Health Research, or the Department of Health.

DISCLOSURES

None.

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This article contains supplemental material online at http://jasn.asnjournals.org/lookup/suppl/doi:10.1681/ASN.2013070702/-/DCSupplemental.
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Supplemental data

**Figure 1.** Effects of acute exercise: relative changes from pre-exercise in plasma markers of systemic inflammation. IL: interleukin; sTNF-R: soluble tumour necrosis factor receptor; CRP: C-reactive protein. Data are mean ± SEM (n=15). *P < 0.05 vs. pre-exercise for illustration purposes (statistical analysis performed on absolute values).
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Supplemental methods

1. ENZYME-LINKED IMMUNOSORBENT ASSAYS

Interleukin-6 and interleukin-10 assays

ELISAs for detection of IL-6 and IL-10 levels in plasma samples were developed and optimised using the capture and detection antibodies included in BD OptEIA ELISA sets (IL-6: 555220 and IL-10: 555157, BD Biosciences, Oxford, UK), National Institute for Biological Standards and Control (NIBSC) standards (IL-6: 89/548 and IL-10: 93/722, NIBSC, Potters Bar, Hertfordshire, UK) and an ELISA Amplification System (19589-109, Invitrogen, Paisley, UK). 96-well ELISA plates (Nunc-Immuno 439454 MaxiSorp) were coated with the anti-human IL-6 or IL-10 capture antibody diluted at the lot-specific recommended dilution in 0.05 M sodium carbonate buffer at 100 µl/well, sealed and incubated overnight at 4ºC. The next day, plates were washed 3 times (at 300 µl/well) with tris-buffered saline (TBS) with 0.05% Tween 20 (TBS-T) and blocked for 1 h at room temperature with 5% BSA (Probumin, Millipore, Illinois, USA) in TBS at 200 µl/well. Plates were subsequently washed 3 times with TBS-T and duplicates of samples (neat), standards (serially diluted in TBS with 10% FCS from 200 pg/ml to 0.78 pg/ml) and blank (TBS with 10% FCS) were added at 100 µl/well. Plates were again sealed and incubated overnight at 4ºC. The following day, plates were washed 6 times with TBS-T and the biotinylated anti-human IL-6 or IL-10 detection antibody diluted at the lot-specific recommended dilution in TBS-T with 1% BSA was added at 100 µl/well. Plates were incubated for 2 h at room temperature and subsequently washed 7 times with 30 seconds soaks with TBS-T. Streptavidin (SAv)-alkaline phosphatase conjugate (554065, BD Biosciences, Oxford, UK) diluted 1:2000 in TBS with 1% BSA was then added at 100 µl/well and plates were incubated for a further 1 h at room temperature. After a final wash (7 times with 30 seconds soaks with TBS-T), the substrate and amplifier solutions (prepared according to the manufacturer's instructions) were added (at 50 µl/well each) in two subsequent steps (incubated for approximately 25 min at 25ºC each). The reaction was
stopped with 0.3 M sulphuric acid (at 50 µl/well) and the plates were immediately read at 490 nm. Samples’ concentrations were determined by relation to a standard curve generated by plotting the standards’ absorbances against the log of the standards’ concentrations using a four-parameter logistic equation (Graphpad Prism version 5, Graphpad Software, La Jolla, CA, USA). All samples from the same patient were assayed on the same plate. Duplicates with a CV above 10% were repeated. The inter- and intra-assay CV were 4.4% and 3.4% for IL-6 and 5.1% and 3.8% for IL-10, respectively.

**Soluble tumour necrosis factor receptors I and II assays**

ELISAs for detection of sTNF-RI and sTNF-RII in plasma samples were developed and optimised using the capture and detection antibodies and standards included in R&D DuoSet ELISA Development kits (sTNF-RI: DY225 and sTNF-RII: DY726, R&D Systems, Abingdon, UK). 96-well ELISA plates (Nunc-Immuno 439454 MaxiSorp) were coated with the anti-human sTNF-RI or sTNF-RII capture antibody (previously reconstituted according to the manufacturer’s instructions) diluted 1:200 in 0.05 M sodium carbonate buffer at 65 µl/well, sealed and incubated overnight at 4ºC. The next day, plates were washed 4 times (at 200 µl/well) with PBS with 0.1% Tween 20 (PBS-T.1) and blocked for 1 h at room temperature with 1% BSA (Probumin, Millipore, Illinois, USA) in PBS at 100 µl/well. Plates were subsequently washed 4 times with PBS-T.1 and duplicates of samples (diluted 1:10 in PBS), standards (serially diluted in PBS with 1% BSA from 10 ng/ml to 10 pg/ml and 18 ng/ml to 18 pg/ml for sTNF-RI and sTNF-RII assays, respectively) and blank (PBS with 1% BSA) were added at 50 µl/well. Plates were again sealed and incubated overnight at 4ºC. The following day, plates were washed 4 times with PBS-T.1 and the biotinylated anti-human sTNF-RI or sTNF-RII detection antibody (previously reconstituted according to the manufacturer’s instructions) diluted 1:200 in PBS was added at 50 µl/well. Plates were incubated for 2 h at room temperature and subsequently washed 4 times with PBS-T.1. HRP Avidin D (A-2004, Vector Laboratories, Peterborough, UK) diluted 1:2000 in PBS was then added at 50 µl/well
and plates were incubated for a further 1 h at room temperature. After a final wash (4 times with PBS-T.1), an OPD substrate solution (S2045, Dako, Glostrup, Denmark), prepared according to the manufacturer's instructions, was added at 50 µl/well. Approximately 5 min after or when suitable colour has developed, the reaction was stopped with 1 M sulphuric acid (at 75 µl/well) and the plates were immediately read at 490 nm. Samples' concentrations were determined by relation to a standard curve generated by plotting the standards' absorbances against the log of the standards' concentrations using a four-parameter logistic equation (Graphpad Prism version 5, Graphpad Software, La Jolla, CA, USA). All samples from the same patient were assayed on the same plate. Duplicates with a CV above 10% were repeated, as were samples that fell beyond the dynamic range of each assay standard curve (adjusting the dilution factor accordingly). The inter- and intra-assay CV were 3.6% and 2.6% for sTNF-RI and 4.5% and 2.9% for sTNF-RII, respectively.

C-reactive protein assay

An ELISA for detection of CRP levels in plasma samples was developed and optimised using a method adapted from Pawluczyk et al.\textsuperscript{1} 96-well ELISA plates (Nunc-Immuno 439454 MaxiSorp) were coated with anti-human CRP rabbit polyclonal antibody (235752, Calbiochem, Merck Chemicals, Nottingham, UK) diluted to a final concentration of 5 µg/ml in 0.05 M sodium carbonate buffer at 65 µl/well, sealed and incubated overnight at 4ºC. The next day, plates were washed 4 times (at 200 µl/well) with PBS-T.1 and blocked for 1 h at room temperature with 1% BSA in PBS at 100 µl/well. Plates were subsequently washed 4 times with PBS-T.1 and duplicates of samples (diluted 1:100 in PBS), CRP standards (85/506, NIBSC, Potters Bar, Hertfordshire, UK, serially diluted in PBS with 1% BSA from 1 µg/ml to 1 ng/ml) and blank (PBS with 1% BSA) were added at 50 µl/well. Plates were again sealed and incubated overnight at 4ºC. The following day, plates were washed 4 times with PBS-T.1 and anti-human CRP mouse monoclonal antibody (ab8279, Abcam, Cambridge, UK) diluted 1:750 in PBS was added at 50 µl/well. Plates were incubated for 2 h at room
temperature and subsequently washed 4 times with PBS-T.1. HRP conjugated anti-mouse immunoglobulins rabbit polyclonal antibody (P0260, Dako, Glostrup, Denmark) diluted 1:1000 in PBS was then added at 50 µl/well and plates were incubated for a further 1 h at room temperature. After a final wash (4 times with PBS-T.1), an OPD substrate solution (S2045, Dako, Glostrup, Denmark), prepared according to the manufacturer’s instructions, was added at 50 µl/well. Approximately 5 min after or when suitable colour has developed, the reaction was stopped with 1 M sulphuric acid (at 75 µl/well) and the plates were immediately read at 490 nm. Samples’ concentrations were determined by relation to a standard curve generated by plotting the standards’ absorbances against the log of the standards’ concentrations using a four-parameter logistic equation (Graphpad Prism version 5, Graphpad Software, La Jolla, CA, USA). All samples from the same patient were assayed on the same plate. Duplicates with a CV above 10% were repeated, as were samples that fell beyond the dynamic range of each assay standard curve (adjusting the dilution factor accordingly). The inter- and intra-assay CV were 3.2% and 2.9%, respectively.
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2. FLOW CYTOMETRY DATA ANALYSIS

T-lymphocyte analysis
SSC vs. FSC plots of all cells acquired were used to gate on the lymphocyte population by morphology (Figure 2 a). Subsequent SSC vs. FL1 or FL3 plots of the lymphocyte population were used to gate, respectively, on the CD4 positive population (CD4+, helper T-cells; Figure 2 b) or on the CD8 brightly positive population (CD8**, cytotoxic T-cells; Figure 2 c). This gating strategy for cytotoxic T-cells was used because it is known that all lymphocytes that express CD8 at high fluorescence intensities also express CD3 and that this population forms the majority of the total cytotoxic T-cell blood pool, while on the other hand, lymphocytes that express CD8 at low fluorescence intensities also include natural killer cells.\(^2,3\) Further FL2 vs. FL1 or FL3 plots of CD4\(^+\) or CD8\(^{**}\) lymphocytes, respectively, were used to determine the percentage the CD4\(^+\) and CD8\(^{**}\) lymphocytes expressing CD69 and the geometric mean of fluorescence intensity (GMFI) of CD69 in these cells (Figure 2 d, e, f and g). The unstimulated samples were used to define the threshold of positive staining and only SEB-stimulated data are reported.

Monocyte analysis
FL2 vs. FL1 plots of all cells acquired were used to gate on the CD14\(^+\)CD86\(^+\) cells (Figure 3 a). Subsequent FL3 vs. FL1 plots of the CD14\(^+\)CD86\(^+\) cells were used to gate on the CD14\(^+\)CD86\(^+\)HLA-DR\(^+\) cells (Figure 3 b). Backgating analysis was then performed to ensure the gated cells fell on the monocyte region by morphology on SSC vs. FSC plots (Figure 3 c). Further FL2 or FL3 histogram plots of CD14\(^+\)CD86\(^+\)HLA-DR\(^+\) monocytes were used to determine, respectively, the GMFI of CD86 and HLA-DR in these cells (Figure 3 d and f). Negative unstained controls were used to define the threshold of positive staining. This analysis was carried for both unstimulated and SEB-stimulated samples and SEB-stimulated data are reported as a ratio to the unstimulated condition.
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Figure 2. T-lymphocyte flow cytometry data analysis.
Figure 3. Monocyte flow cytometry data analysis.
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