Circulating endothelial progenitor cells are not affected by acute systemic inflammation

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Padfield GJ, Tura O, Haeck ML, Short A, Freyer E, Barclay GR, Newby DE, Mills NL. Circulating endothelial progenitor cells are not affected by acute systemic inflammation. Am J Physiol Heart Circ Physiol 298: H2054–H2061, 2010. First published April 9, 2010; doi:10.1152/ajpheart.00921.2009.—Vascular injury causes acute systemic inflammation and mobilizes endothelial progenitor cells (EPCs) and endothelial cell (EC) colony-forming units (EC-CFUs). Whether such mobilization occurs as part of a nonspecific acute phase response or is a phenomenon specific to vascular injury remains unclear. We aimed to determine the effect of acute systemic inflammation on EPCs and EC-CFU mobilization in the absence of vascular injury. Salmonella typhus vaccination was used as a model of acute systemic inflammation. In a double-blind randomized crossover study, 12 healthy volunteers received S. typhus vaccination or placebo. Phenotypic EPC populations enumerated by flow cytometry [CD34+ VEGF receptor (VEGF)R-2+ CD133+, CD14+ VEGFR-2+ Tie2+, CD45+ CD34+, as a surrogate for late outgrowth EPCs, and CD34+ CXCR-4+], EC-CFUs, and serum cytokine concentrations (high sensitivity C-reactive protein, IL-6, and stromal-derived factor-1) were quantified during the first 7 days. Vaccination increased circulating leukocyte (9.8 ± 0.6 vs. 5.1 ± 0.2 × 10⁶ cells/l, P < 0.0001), serum IL-6 [0.95 (0–1.7) vs. 0 (0–0) ng/l, P = 0.0161], and VEGF-A (60 [45–94] vs. 43 [21–64] pg/l, P = 0.0066) concentrations at 6 h and serum high sensitivity C-reactive protein at 24 h [2.7 (1.4–3.6) vs. 0.4 (0.2–0.8) mg/l, P = 0.0371]. Vaccination caused a 56.7 ± 7.6% increase in CD14+ cells at 6 h (P < 0.001) and a 22.4 ± 6.9% increase in CD34+ cells at 7 days (P = 0.04), EC-CFUs, putative vascular progenitors, and the serum stromal-derived factor-1 concentrations were unaffected throughout the study period (P > 0.05 for all). In conclusion, acute systemic inflammation causes nonspecific mobilization of hematopoietic progenitor cells, although it does not selectively mobilize putative vascular progenitors. We suggest that systemic inflammation is not the primary stimulus for EPC mobilization after acute vascular injury. Salmonella typhus; vascular injury

ENDOTHelial PROGENITOR CELLS (EPCs) are circulating mononuclear cells that are mobilized from the bone marrow in response to tissue ischemia or vascular perturbation (2, 5, 25) and have the capacity to home to sites of injury, differentiate into mature endothelial cells (ECs), and participate in vascular repair. Several studies have reported a correlation between cardiovascular risk factors and reduced circulating EPCs (10, 11, 45) with a lower EPC count being independently predictive of future cardiovascular events (48). In this rapidly evolving field of cardiovascular research, numerous cell populations have been termed “EPCs,” and a considerable degree of uncertainty surrounds their precise definition. EPCs have been largely characterized by the expression of the primitive hematopoietic progenitor markers CD34⁺ and CD133⁺ and VEGF receptor (VEGFR)-2, a surface receptor widely expressed on vascular ECs (3, 32, 45). Nonhematopoietic, CD45⁻CD34⁺ cells have also recently been identified as putative EPCs on the basis of their ability to form “late outgrowth” colonies phenotypically and functionally indistinguishable from mature EC colonies in culture (7).

A widely used but largely inferential approach to quantifying EPCs has been the EC colony forming unit (EC-CFU) assay (15). However, it is now established that EC-CFUs are predominantly composed of monocytes and lymphocytes rather than true mature ECs and have no real capacity to form perfusing vessels (35, 52). The nomenclature of this population of cells requires revision, but, nevertheless, there is compelling evidence that EC-CFUs are an important component in the cellular response to vascular injury (15, 29, 40). The role of inflammatory leukocytes in vascular repair has received much attention (9, 19, 24, 33). CD14⁺ cells expressing VEGFR-2 and the tyrosine kinase receptor Tie-2 are known to have the capacity to enhance endothelial regeneration and accelerate the restoration of vascular function (9).

The effect of inflammation on these various populations is poorly understood, with contrasting effects seen during acute and chronic inflammation. Recombinant C-reactive protein (CRP) depresses EC-CFU function in vitro (46), and, conversely, anti-TNF-α therapy enhances the mobilization and function of progenitor cells in patients with rheumatoid arthritis (1). A reduction in circulating EPCs occurs in a number of other conditions typified by chronic inflammation (13, 26, 30, 31), which would suggest that EPC dysfunction is a potential mechanism for the development of atherosclerosis in these conditions. In contrast, acute vascular injury occurring in the context of percutaneous coronary intervention (5, 29) or an acute coronary syndrome (12) induces both an acute systemic inflammatory response and the mobilization of EPCs and EC-CFUs. However, it is unclear whether the stimulus for the mobilization of these populations arises from the systemic inflammatory response or from vascular injury per se.

The aim of this study was, therefore, to investigate the behavior of a range of putative EPC populations and factors responsible for their mobilization in response to an isolated inflammatory stimulus in the absence of denuding endothelial injury. We used an established model of acute systemic inflammation, Salmonella typhus vaccination (8), to study the effects of inflammation on the behavior of EPCs in healthy volunteers.

MATERIALS AND METHODS

This study was performed with the approval of the local research ethics committee, in accordance with the Declaration of Helsinki.
and with the written informed consent of all participants. All procedures were performed in accordance with the guidelines of our institution.

**Subjects.** Twelve healthy, nonsmoking, nonobese male volunteers were enrolled into the study. All subjects were normotensive without a history of diabetes mellitus or peripheral vascular or coronary artery disease. None of the subjects had undergone typhoid vaccination in the year before the study, nor had they received vasoactive or nonsteroidal anti-inflammatory drugs in the week before the study.

**Study design.** Using a randomized balanced block double-blind crossover study design, the polysaccharide *S. typhus* vaccination (0.025 mg, Typhim Vi, Aventis Pasteur MSD, Berkshire, UK) was compared with saline placebo given by intramuscular injection at least 2 wk apart (8). Subjects attended between 8 and 10 AM for venesection and were randomly assigned to vaccine or placebo injection into the deltoid muscle of the nondominant arm. As a previous study (8) reported a peak inflammatory response occurring at 6 h after the deltoid muscle of the nondominant arm. Subjects returned at 24 and 168 h for further blood sampling. To evaluate the late effects of vaccination on EPCs, subjects were asked to return 6 h later for repeat blood sampling. To avoid confounding EPC mobilization, subjects were also asked to abstain from strenuous exertion during the study period (34).

**Blood sampling and assays.** Venous blood samples (20 ml) were collected into EDTA and serum gel for flow cytometry, mononuclear cell preparations and culture, and separation of plasma and serum. Whole blood was analyzed for total cells, differential count, and platelets using an autoanalyzer (Sysmex). ELISAs were used to quantify serum IL-6 (Invitrogen) (23), VEGF-A (Invitrogen) (6), and stromal derived factor-1 (SDF-1; R&D Systems) concentrations according to the manufacturer’s instructions (Invitrogen). Serum high sensitivity CRP (hs-CRP) was quantified using an immunoturbidimetric method (Dade-Behring, Marburg, Germany) (28).

**Flow cytometric identification of EPCs.** EPCs were phenotyped using flow cytometry. Briefly, cells were directly stained and analyzed for the phenotypic expression of surface proteins using preconjugated anti-human monoclonal antibodies including anti-CD45-PercP (Becton Dickinson, Oxford, UK), anti-CD34-FITC, anti-VEGFR-2-PE, anti-Tie2-APC (R&D Systems), anti-CD-133-PE (Miltenyi Biotec, Surrey, UK), anti-CD14-FITC (Caltag Systems, Buckingham, UK), and CXCR-4-APC (R&D Systems). The fluorescence minus one technique (44) was used to provide negative controls and to establish positive stain boundaries. Undiluted samples (200 µl) were stained with antibodies for 30 min in the dark. Erythrocytes were lysed with UtiLyse solution (Dako), centrifuged at 200 g for 10 min, washed twice with PBS, and then fixed (Cell Fix solution, Becton Dickinson). Data were analysed using FlowJo (Treestar). Leukocytes were identified using their characteristic forward and side scatter profile. For CD45+ CD34+ CD133+ and VEGFR-2+ quantification, the proportion of leukocytes cells bearing each epitope was determined individually using the side scatter profile plotted against the appropriate fluorescence channel (Fig. 1). Boolean principles were used to determine automatically double- and triple-stained populations. EPCs were defined on the basis of double staining, CD34+ VEGFR-2+, and by the alternative, triple-positive definition, CD34+ CD133+ VEGFR-2+. As a surrogate for late outgrowth EPCs, we determined the frequency of CD45+ cells expressing CD34, VEGFR-2 and Tie2 expression on CD14+ cells were assessed using quadrant analysis (Fig. 1). To explore the mechanism...
RESULTS

Subjects (mean age: 26 ± 1 yr) had similar baseline profiles for each phase of the study, although the total leukocyte concentrations was slightly higher before placebo (Table 1).

S. typhus vaccination induces an inflammatory response. Vaccination was well tolerated, and there were no major adverse events during the study period. After vaccination, subjects reported mild flu-like symptoms, although these were not associated with any change in temperature, heart rate, or blood pressure. S. typhus vaccination induced a leukocytosis in all subjects that was maximal at 6 h (change: 4.6 ± 1.1 × 10⁶ leukocytes/l, P < 0.0001) and remained at 24 h (P = 0.037). This was driven predominantly by a neutrophilia (change: 4.3 ± 1.1 × 10⁶ neutrophils/l, P < 0.0001) and, to a lesser extent, by a monocytes (change: 0.19 ± 0.03 × 10⁶ monocytes/l, P = 0.004). Saline placebo injection had no effect on the differential cell counts (Table 2). After vaccination, there was an increase in serum IL-6 [0.95 (0–1.7) vs. 0 (0–0) ng/l, P = 0.016] and VEGF-A [60 (45–94) vs. 43 (21–64) pg/l, P = 0.006] concentrations at 6 h that returned to baseline levels by 24 h. Serum hs-CRP concentrations increased later, with a peak at 24 h [2.7 (1.4–3.6) vs. 0.4 (0.2–1.8) mg/l, P = 0.0001], and returned to baseline by 1 wk. Saline placebo injection had no effect on the cytokine profile (P > 0.2; Fig. 2).

CD34⁺ EPC phenotype. Six hours after saline placebo, CD34⁺, CD34⁺VEGFR-2⁺, and CD34⁺CD133⁺VEGFR-2⁺ cell concentrations had fallen by 19.5 ± 8.5% (P = 0.033), 42 ± 9.5% (P = 0.001), and 53.6 ± 12.5% (P = 0.037), respectively (Fig. 3). Concentrations were comparable with baseline at 24 h and 1 wk. Six hours after vaccination, CD34⁺ populations did

Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Vaccine</th>
<th>P Value</th>
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<tbody>
<tr>
<td>Male gender, %</td>
<td>100</td>
<td></td>
<td></td>
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<tr>
<td>Age, yr</td>
<td></td>
<td>26 ± 1.2</td>
<td>0.9</td>
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<tr>
<td>Temperature, °C</td>
<td>36 ± 0.2</td>
<td></td>
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<tr>
<td>Blood pressure, mmHg</td>
<td>136/79 ± 2/3</td>
<td>130/73 ± 4/3</td>
<td>0.130.13</td>
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<tr>
<td>Pulse rate, beats/min</td>
<td>76 ± 4</td>
<td>71 ± 9</td>
<td>0.16</td>
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<tr>
<td>Total leukocytes, ×10⁶ cells/l</td>
<td>5.6 ± 0.3</td>
<td>5.1 ± 0.2</td>
<td>0.02</td>
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<tr>
<td>Neutrophils</td>
<td>3.1 ± 0.2</td>
<td>2.7 ± 0.2</td>
<td>0.06</td>
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<tr>
<td>Lymphocytes</td>
<td>1.9 ± 0.1</td>
<td>1.7 ± 0.2</td>
<td>0.9</td>
</tr>
<tr>
<td>Monocytes, ×10⁶ cells/l</td>
<td>0.45 ± 0.02</td>
<td>0.45 ± 0.04</td>
<td>0.9</td>
</tr>
<tr>
<td>IL-6, ng/l</td>
<td>0.3 ± 0.2</td>
<td>0.5 ± 0.4</td>
<td>0.5</td>
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<tr>
<td>VEGF-A, pg/l</td>
<td>55.8 ± 10</td>
<td>47.6 ± 10</td>
<td>0.07</td>
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<tr>
<td>Stromal-derived factor-1, ng/l</td>
<td>2040 ± 56</td>
<td>2036 ± 45</td>
<td>0.95</td>
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<tr>
<td>High sensitivity C-reactive protein, mg/l</td>
<td>0.7 ± 0.2</td>
<td>0.5 ± 0.2</td>
<td>0.4</td>
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<tr>
<td>CD34⁺, ×10⁶ cells/l</td>
<td>3.38 ± 0.28</td>
<td>2.92 ± 0.24</td>
<td>0.16</td>
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<tr>
<td>CD34⁺VEGFR-2⁺, ×10⁶ cells/l</td>
<td>0.51 ± 0.09</td>
<td>0.38 ± 0.04</td>
<td>0.17</td>
</tr>
<tr>
<td>CD34⁺CD133⁺VEGFR-2⁺, ×10⁶ cells/l</td>
<td>0.14 ± 0.04</td>
<td>0.15 ± 0.06</td>
<td>0.85</td>
</tr>
<tr>
<td>CD45⁻CD34⁺, ×10⁶ cells/l</td>
<td>0.48 ± 0.06</td>
<td>0.39 ± 0.04</td>
<td>0.23</td>
</tr>
<tr>
<td>CD34⁺CXCR-4⁺, ×10⁶ cells/l</td>
<td>0.67 ± 0.1</td>
<td>0.97 ± 0.16</td>
<td>0.13</td>
</tr>
<tr>
<td>CD14⁺, ×10⁶ cells/l</td>
<td>390 ± 30</td>
<td>350 ± 30</td>
<td>0.43</td>
</tr>
<tr>
<td>CD14⁺VEGFR-2⁺, ×10⁶ cells/l</td>
<td>50 ± 10</td>
<td>60 ± 10</td>
<td>0.51</td>
</tr>
<tr>
<td>CD14⁺CXCR-4⁺, ×10⁶ cells/l</td>
<td>2 ± 0.5</td>
<td>1 ± 0.2</td>
<td>0.25</td>
</tr>
<tr>
<td>CD14⁺CXCR-4⁺VEGFR-2⁺, ×10⁶ cells/l</td>
<td>0.06 ± 0.2</td>
<td>0.7 ± 0.2</td>
<td>0.42</td>
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<tr>
<td>Colony-forming units per well</td>
<td>25 ± 6</td>
<td>33 ± 11</td>
<td>0.6</td>
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Values are means ± SE. VEGF, VEGF receptor.

underlying any changes in circulating EPCs in response to vaccination, we also determined the surface expression of the SDF-1 receptor, CXCR-4, on CD34⁺ cells. The absolute concentration of cells per milliliter of blood was calculated by reference to the total leukocyte count.

EC-CFUs. EC-CFUs were generated from peripheral blood mononuclear cells as previously described (15). Colonies were counted on day 5 in a minimum of three wells by observers blinded to the subjects’ clinical profiles. Colonies were extensively characterized using a battery of surface markers widely used to identify a mature endothelial phenotype, such as CD105, endothelial nitric oxide synthase, Tie-2, and CD146 and the hematopoietic markers CD45 and CD68. Direct staining was performed using acetylated LDL and the lectin Ulex europaeus I agglutinin. Briefly, EC-CFU were fixed with methanol and permeabilized using blocking buffer with added Igepal (Sigma-Aldrich). Colonies were incubated with primary antibodies for 2 h at room temperature and washed with PBS (Sigma-Aldrich). Relevant anti-mouse or anti-rabbit antibodies conjugated to alexafluor dyes (Molecular Probes, Invitrogen) were used as secondary antibodies. Nuclei were counterstained with 4',6-diamidino-2-phenylindole, and colonies were mounted with Permafluor (Thermo Scientific).

Data analysis and statistics. Continuous variables are reported as means ± SE or median and interquartile range as appropriate. Because of the crossover design, data were analyzed to confirm the absence of period effects before comparisons. Statistical analyses were performed with GraphPad Prism (Graph Pad Software) using two-way ANOVA and a two-tailed Student’s t-test or Mann-Whitney paired U-tests where appropriate. Vascular injury after percutaneous coronary intervention resulted in an ~300% increase in EC-CFUs, with an increase in CRP of a similar magnitude (29). The sample size (n = 12), based on power calculations derived from our previous study (29), gives an 80% power to detect a smaller but meaningful 75% difference in the number of EC-CFUs at a significance level of 5%.

Table 2. Change from baseline in differential leukocyte counts at 6 h

<table>
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<tr>
<th></th>
<th>Placebo</th>
<th>Vaccine</th>
<th>P Value</th>
</tr>
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<tbody>
<tr>
<td>Total leukocytes</td>
<td>0.42 ± 0.26</td>
<td>4.7 ± 0.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>0.45 ± 0.22</td>
<td>4.3 ± 0.45</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>0.06 ± 0.09</td>
<td>0.13 ± 0.08</td>
<td>0.06</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0.04 ± 0.03</td>
<td>0.19 ± 0.03</td>
<td>0.004</td>
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Values are means ± SE (in ×10⁶ cells/l).
not fall ($P > 0.2$), and, by 1 wk, circulating CD34$^+$ cells had increased by 22.4 ± 6.9% ($P = 0.04$). CD34$^+$VEGFR-2$^+$ and CD34$^+$CD133$^+$VEGFR-2$^+$ subpopulations did not change during the study period ($P > 0.2$; Fig. 4).

The late outgrowth precursor CD45$^-$CD34$^+$ similarly fell at 6 h after placebo by 7.4% ± 11; however, this did not reach statistically significance. There were no differences in CD45$^-$CD34$^+$ cells after vaccination compared with placebo ($P > 0.2$; Fig. 4).

Circulating CD34$^+$CXCR-4$^+$ cell concentrations were similar before vaccination or placebo. While the CD34$^+$CXCR-4$^+$ cell concentration followed a trend similar to that of CD34$^+$ cells, the proportion of CD34$^+$ cells expressing CXCR4 was not significantly changed throughout the study period ($P = 0.78$; Fig. 4). Serum SDF-1 concentrations were similarly unaffected by vaccination or placebo (Fig. 2).

**CD14$^+$ EPC phenotype.** After vaccination, we observed an increase in circulating CD14$^+$ cells that peaked at 6 h ($P = 0.005$). A median of 0.18% (interquartile range: 0.08 – 0.24%) of CD14$^+$ cells expressed Tie-2 and VEGFR-2, although the levels of this population did not change in response to vaccination ($P = 0.2$). Similarly, CD14$^+$Tie2$^+$ and CD14$^+$VEGFR-2$^+$ populations were unaffected by vaccination ($P > 0.2$; Fig. 5).

**EC-CFUs.** Mean baseline numbers of CFUs were similar before vaccination and saline placebo (33 ± 11 vs. 24 ± 6, $P = 0.6$) and remained unchanged throughout the study period ($P = 0.7$; Fig. 4). As previously described, colonies expressed a variety of endothelial and hematopoietic characteristics (3, 18, 52). These included acetylated LDL uptake and lectin binding in addition to the strong expression of Tie-2, a tyrosine kinase receptor expressed on mature ECs necessary for normal vascular development, and CD146, a transmembrane glycoprotein responsible for endothelial intracellular adhesion. CD105, or endoglin, a marker of the activated endothelium thought to be limited to proliferating cells, was widely expressed throughout the colonies. Colonies also expressed endothelial nitric oxide synthase, an enzyme responsi-
ble for the production of nitric oxide by ECs in response to shear stress. The hematopoietic nature of EC-CFUs was confirmed by the expression of the pan-leukocyte marker CD45 and the macrophage marker CD68 (Fig. 6).

DISCUSSION

EPCs, EC-CFUs, and late outgrowth colonies are mobilized in response to vascular injury, such as that occurring in the context of percutaneous coronary intervention or an acute coronary syndrome (17, 25, 29, 39, 49). These clinical events also cause a systemic inflammatory response. It remains unknown whether inflammation occurring in this context is a physiological event necessary for EPC mobilization or an opposing pathological process detrimental to vascular repair.

In the present study, we examined the behavior of a wide variety of putative vascular progenitors in response to an inflammatory stimulus using a model of acute systemic inflammation in the absence of a discrete vascular injury. Our study demonstrates a biphasic response to acute systemic inflammation, with early and late mobilization of CD14⁺ and CD34⁺ cells, respectively.

Fig. 5. Effect of vaccination on circulating CD14⁺ populations. Vaccination resulted in a significant mobilisation of CD14⁺ cells compared with placebo (P < 0.001; A). CD14⁺ subpopulations bearing Tie-2 and/or VEGFR-2 were not significantly different from placebo throughout the study period (P > 0.2; B–D). Data are presented as absolute mean changes from baseline ± SE.
However, specific vascular progenitor subpopulations, including late outgrowth colony precursors, CD45−/CD34+ progenitor cells, were unaffected by systemic inflammation.

While EC-CFUs are not mature ECs, they do have an established association with cardiovascular disease, and it is likely that EC-CFUs have a vasculoprotective role through the secretion of a variety of angiogenic factors (33). We (29) recently demonstrated that EC-CFUs are mobilized in response to discrete vascular injury that occurs during percutaneous coronary intervention and that this mobilization is associated with an acute inflammatory response. However, EC-CFUs were unaffected by the isolated inflammatory stimulus in the present study. We therefore suggest that EPC and EC-CFU mobilization in response to vascular injury is a specific phenomenon and that inflammation per se does not directly mobilize EPCs or EC-CFUs.

Acute systemic inflammation induced by S. typhus vaccination does have deleterious effects on endothelial function. The model used here has been used to demonstrate that inflammation impairs endothelium-dependent vasodilatation (16) and, furthermore, that anti-inflammatory treatment ameliorates these adverse effects (22, 47). We hypothesize that while the transient inflammatory response to S. typhus vaccination is sufficient to cause endothelial dysfunction, it is not sufficient to cause vascular denudation and, therefore, stimulate vascular progenitor cell mobilization.

The interactions between EPC mobilization and systemic inflammation remain obscure. Both EC-CFUs and late outgrowth EPCs are known to secrete proinflammatory mediators such as tissue factor and monocyte chemoattractant protein-1, a property potentiated by stimulation by other inflammatory mediators such TNF-α. This might suggest that EPCs have a possible proinflammatory role (53). Several studies have supported a direct inhibitory effect of inflammation on EPCs. An experimental in vitro study (41) has suggested that CRP can downregulate the production of angiogenic chemokines by EC-CFUs and impair EC-CFU migration toward VEGF. Mayr et al. (27) recently reported a two-thirds reduction in the proportion of circulating EPCs (CD34+CD133+VEGFR-2+) and EC-CFUs 4–6 h after an intravenous LPS infusion. In contrast, we did not detect a change in EC-CFU numbers after vaccination, nor did we find any direct effect of serum from vaccinated subjects on the migratory, proliferative, or adhesive capacity of EC-CFUs (data not shown).

Functional effects of circulating EC-CFUs and EPCs have been characterized previously. We (29) demonstrated that EC-CFUs are mobilized in response to vascular injury and that this mobilization is associated with an acute inflammatory response. However, EC-CFUs were unaffected by the isolated inflammatory stimulus in the present study. We therefore suggest that EPC and EC-CFU mobilization in response to vascular injury is a specific phenomenon and that inflammation per se does not directly mobilize EPCs or EC-CFUs.

In contrast, we did not detect a change in EC-CFU numbers after vaccination, nor did we find any direct effect of serum from vaccinated subjects on the migratory, proliferative, or adhesive capacity of EC-CFUs (data not shown). LPS infusion results in a marked monocytopenia and lymphocytopenia, whereas the inflammatory response to S. typhus vaccination in the present study involved a monocytes and had little effect on circulating lymphocytes. Given that EC-CFUs are derived from monocyte (33) and lymphocytic (19) subpopulations, it is quite possible that the reduction in EC-CFUs in Mayr et al.’s study was determined principally by a profound mononuclear cell depletion. Mayr et al. also reported a reduction in CD34+CD133+VEGFR-2+ after LPS-induced inflammation, which appears to contradict the findings of our present study. However, as CD34+CD133+VEGFR-2+ concentrations were reported relative to the total leukocyte count, which was markedly increased due to a neutrophilia (20), it is entirely possible that the absolute number of circulating progenitors was unchanged. Unfortunately, absolute numbers were not reported, preventing definitive conclusions from this study and making it difficult to compare these findings directly with our own.
We interpret the mobilization of CD34 and CD14 cells as a nonspecific response to inflammatory mediators induced by S. typhus polysaccharide exposure. CD14+ is a receptor for endotoxin predominantly expressed on circulating monocytes (50). The mobilization of monocytes is part of an acute phase response and is common to a wide variety of inflammatory, infective, and neoplastic conditions. CD34 functions as a regulator of cellular adhesion and identifies a naïve population of cells widely regarded as having "stem cell" capacity. Mobilization of CD34+ cells is similarly nonspecific and occurs in response to a variety of inflammatory stimuli. Concordant with our findings, a delayed mobilization of CD34+ cells occurs in healthy volunteers treated with granulocyte colony-stimulating factor (36) and in patients after acute myocardial infarction (39).

Numerous factors responsible for EPC mobilization, homing, and recruitment into new or injured vessels have been identified (43). Although current understanding of these processes remains incomplete, two cytokines, SDF-1 and VEGF-A, are thought to be of particular importance to EPC mediated reendothelialization through the stimulation of their cognate receptors: CXCR4 and VEGFR-2, respectively (2, 4, 14, 21, 38, 51). In the present study, S. typhus vaccination caused a significant increase in VEGF-A; however, this alone was insufficient to mobilize putative EPCs or EC-CFUs. SDF-1 is thought to act downstream of VEGF to enhance the incorporation of EPCs into sites of neendothelialization (14) and is increased in response to vascular injury (37). That SDF-1 and the concentration of circulating cells expressing its receptor, CXCR-4, were unchanged after vaccination are supportive of our hypothesis that the necessary pathways for EPC mobilization and homing are not solely activated by an inflammatory stimulus.

Finally, the concentration of circulating CD34+ populations fell over the 6-h period after placebo, confirming a previously reported circadian variation of EPCs (42). Interestingly, this circadian variation of EPCs was attenuated by acute systemic inflammation in our study, presumably through the disruption of normal trafficking of cells to and from the bone marrow.

Conclusions. Acute systemic inflammation caused by S. typhus stimulation stimulates early and late mobilization of CD14+ and CD34+ populations, respectively. However, despite significant secretion of VEGF, systemic inflammation appears to not cause mobilization of vascular progenitors, including a surrogage measure of late outgrowth colonies. This would indicate that humoral factors specific to vascular injury are necessary for EPC mobilization. We suggest that endothelial denudation or tissue ischemia is necessary for this to occur. Future studies are required to prospectively determine the role of these populations in modulating the response to acute vascular injury.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


