A kinetic model of bone marrow neutrophil production that characterizes late phenotypic maturation

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Acute inflammatory stimuli rapidly mobilize neutrophils from the bone marrow by shortening postmitotic maturation time and releasing younger neutrophils, but the kinetics of this change in maturation time remains unknown. We propose a kinetic model that examines the rate of change in neutrophil average age at exit from the bone marrow during active mobilization to quantify this response and use this model to examine the temporal profile of late neutrophil phenotypic maturation. Total and CD10+/CD16low circulating neutrophils were quantified in cardiac surgery patients during extracorporeal postmitotic neutrophils that are CD10+ neutrophil mean age and predicts that the proportion of mobilizable neutrophil numbers indicates that CD10 expression is directly related to the age-related acquisition of functionally relevant markers. 

bone marrow; postmitotic maturation; granulopoiesis; neutrophil recruitment; mathematical modeling

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cells at a younger average age (from a progressively earlier point in the pipeline). Changes in circulating neutrophil numbers during stimulated bone marrow release, when applied to this model of neutrophil production, can be used to predict the change in average age at which neutrophils are released in the circulation. However, this approach requires that neutrophil release from the bone marrow is the only contributor to expansion of the circulating neutrophil pool during active neutrophil recruitment. Neutrophils can also be recruited from the extramedullary marginal pool; however, these demarginalized neutrophils have no distinguishing morphological or phenotypic characteristics, preventing their quantification.

Extracorporeal circulation (ECC) during cardiac surgery provides a unique intravascular environment wherein the pulmonary vascular bed, a significant reservoir of marginal neutrophils (1), is excluded from the circulation for a substantial duration. Cardiac surgery also incorporates inhibitors and stimulants of bone marrow neutrophil release; systemic hypothermia suppresses active neutrophil recruitment from the bone marrow (3), whereas rewarming (38, 41) and reperfusion of the ischemic myocardium (25) promote recruitment. Intraoperative sampling during ECC thereby provides a useful in vivo model to evaluate acute changes in the kinetics of bone marrow neutrophil release under conditions of limited demargination. Serial sampling over a short time interval also ensures that changes in neutrophil intravascular half-life \( [t_{1/2}] \), usually 6–8 h under resting conditions (14) and time to onset of apoptosis [usually 24–48 h (27)] will not contribute to observed changes in circulating neutrophil numbers.

By investigating acute changes in the composition and size of the circulating neutrophil pool in cardiac surgery patients during ECC, we identify net expansion of the circulating neutrophil pool accompanied by an increasing proportion of neutrophils that exhibited the CD10\(^+\)/CD16\(^{low}\) phenotype. Mathematical modeling of changes in total neutrophil numbers revealed a progressive decrease in the average age at which neutrophils exit the bone marrow during ECC. Concurrent changes in numbers of CD10\(^+\)/CD16\(^{low}\) circulating neutrophils were used to predict the acquisition of CD10 expression throughout late neutrophil maturation as cellular mean age increases.

MATERIALS AND METHODS

Fluorochrome conjugated monoclonal antibodies. CD10 (H110a, mouse IgG1E\(^\)-phycoerythrin (PE) was from BD Biosciences Immunocytometry Systems (BDIS; San Jose, CA); CD16 (3G8, mouse IgG1E-PE-Cy5 and IgG1E isotypes (MOPC-21)-PE and -PE-Cy5 were from BD PharMingen (BD Biosciences Pharmingen, San Diego, CA).

Buffers, anticoagulants, and other reagents. Anticoagulants used were EDTA and citrate-theophylline-adenosine-dipyradimole (CTAD) in vacutainers (Becton-Dickinson, Franklin Lakes, NJ). The fluorescent nuclear dye Hoechst 33342 (Molecular Probes, Eugene OR), 50 mg/mL in 0.9% NaCl (Baxter Healthcare, Sydney, Australia), was used to label leukocytes in whole blood. Hanks’ balanced salt solution (HBSS) contained 10 mM HEPES, 0.5% BSA, and 1 mM sodium azide (NaN3; all from Sigma, St. Louis, MO). NaN3, 1 mM in 0.9% NaCl (Baxter Healthcare) was used during antibody incubation. All buffers, media, and reagents were Zetapore (Cuno Filter Systems, Meriden, CT) filtered for sterilization and to minimize lipopolysaccharide contamination.

Patients. Patients aged 35–80 yr undergoing elective, first-time cardiac surgery (n = 10; Table 1) were prospectively recruited from the Cardiothoracic Surgical Unit of Royal Prince Alfred Hospital, Sydney. Exclusion criteria were evidence of bacterial infection within the preceding 2 wk, immunosuppressive diseases, acute coronary syndrome, past treatment with immunosuppressive drugs, or chronic severe liver disease.

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male/female)</td>
<td>8/2</td>
</tr>
<tr>
<td>Age, yr</td>
<td>55.8 ± 10.2</td>
</tr>
<tr>
<td>Surgical procedure</td>
<td></td>
</tr>
<tr>
<td>CABG</td>
<td>7 (70%)</td>
</tr>
<tr>
<td>Combined CABG-valve</td>
<td>1 (10%)</td>
</tr>
<tr>
<td>Operative time, min</td>
<td>261.2 ± 41.8</td>
</tr>
<tr>
<td>ECC time, min</td>
<td>101.1 ± 28.1</td>
</tr>
<tr>
<td>Aortic cross clamp time, min</td>
<td>78.7 ± 22.1</td>
</tr>
<tr>
<td>Duration of systemic hypothermia, min</td>
<td>58.6 ± 25.1</td>
</tr>
<tr>
<td>Nasopharyngeal temperature, °C</td>
<td>31.6 ± 2.4</td>
</tr>
</tbody>
</table>

Values are given as n, %, or means ± SD; n = 10 subjects. CABG, coronary artery bypass surgery; ECC, extracorporeal circulation.
syndromes within the preceding 4 wk, severely impaired left ventricular function (ejection fraction <30%), use of any immunomodulatory medications (e.g., steroids, immunosuppressive agents), and chronic renal failure (serum creatinine >200 μmol/l). Written informed consent was obtained from all patients, and the study was approved by the institutional ethics committee.

**Surgical protocol.** Cardiac surgery with hypothermic ECC was performed through a median sternotomy under general anesthesia as previously described (35). After administration of 400 U/kg unfractionated porcine heparin (Pharmacia), ECC was established. In brief, a dual-stage cannula inserted in the right atrium provided venous drainage to the ECC circuit, whereas oxygenated blood was returned to the patient through an arterial cannula placed in the ascending aorta, bypassing the cardiac and pulmonary vascular beds. A vascular clamp was applied across the ascending aorta, cardioplegia was administered to arrest the heart, and patients were systemically cooled to 30–32°C. At the completion of the surgical procedure, patients were rewarmed to 37°C, and the aortic cross-clamp was removed, reestablishing myocardial blood flow. Patients were weaned from ECC after return of mechanical cardiac activity, at which stage normal pulmonary blood flow was gradually reestablished.

**Blood sampling.** Samples (1.5 ml) were collected from the systemic circulation via the radial arterial line of patients immediately before anesthetic induction (baseline) and sequentially at 3- to 4-min intervals during the procedural, warming, and weaning phases of ECC (Table 2 and Fig. 2); four samples were collected within each intraoperative stage. Blood was immediately anticoagulated with EDTA-CTAD, and leukocytes were labeled with 50 μl each of CD10-PE and CD16-PE-Cy5 in combination or isotype control monoclonal antibodies (MAbs; saturating concentrations) in 1 mM NaN3 on ice for 20 min, diluted to 0.75–1.0 ml with HBSS-BSA-NaNO3, and stored at 4°C in the dark for up to 4 h.

Flow cytometry was performed using an LSR bench-top flow cytometer (BDIS) within 4 h of sample collection, and Cell Quest Pro software (version 4.0.2; BDIS) was used to acquire and store data files. The validity of data over time was confirmed by daily calibration of the flow cytometer using Calibrate Rainbow beads (BDIS). A total of 1.5 × 10⁶ events were acquired per sample; leukocytes identified by positive Hoechst 33342 fluorescence and granulocyte events within the leukocyte gate, identified on the basis of their characteristic forward and side angle light scatter, were analyzed for MAb-related fluorescence. Contaminating eosinophils were excluded from the granulocyte population on the basis of absent CD10 and CD16 expression (CD10−/CD16− events); neutrophil events were identified by positive CD16 expression. The proportion of neutrophil events with positive CD10 MAb fluorescence was then determined for each sample.

**Full blood counts.** Differential full blood counts on EDTA-CTAD anti-coagulated blood were performed with a Sysmex SF-3000 (Roche Diagnostics, Sydney, Australia) automatic full blood count analyzer. Neutrophil counts after baseline were corrected for hemodilution using the formula: (baseline hematocrit/observed hematocrit) × observed neutrophil count = corrected count.

**Statistical analysis.** Data are represented as means ± SE for n = 10 cardiac surgery patients unless otherwise stated. Results were analyzed using Prism statistical software (GraphPad Prism version 4.0 for Windows; GraphPad Software, San Diego, CA). Changes in neutrophil numbers were compared using paired t-tests (baseline vs. first intraoperative sample) and one-way repeated-measures ANOVA (comparisons within each phase of ECC). Statistical significance was defined as P < 0.05.
Mathematical modeling. The basic conceptual model of neutrophil production is that of a pipeline of neutrophils of different ages maturing in the bone marrow that enter the circulation at a certain “release point” along the pipeline. Under basal conditions, neutrophil production is constant, and neutrophils are released in a fully mature state. However, during inflammation, this baseline neutrophil production may be increased by the release of less mature neutrophils in the circulation. This equates to a left shift in the release point (Fig. 1), leading to a temporary increase in production and the emergence of less mature neutrophils in the circulation.

We denote the number of neutrophil precursor cells produced per hour from the bone marrow in the lineage toward mature neutrophils at time \( t \) by \( N(t) \) and the age of their release in circulation by \( s \). The maturation time for newly committed neutrophil precursors in the bone marrow to enter circulation is of the order of days (typically 12 days (9, 11) in the absence of inflammation); however, the duration of time covering the three phases of ECC is of the order of hours (typically 1–2 h). Thus, over the time frame of our investigation, the number of neutrophils at any maturation stage along the pipeline that could enter the circulation would not be influenced by any rise in the production of early stage neutrophils. The baseline entry rate of neutrophils from the bone marrow in the circulation is taken to be constant, \( N_0 \) (since we assume that at baseline, before surgery, the number of neutrophils in the bone marrow is in equilibrium).

We would like to model the total neutrophil level in circulation, which we define as \( N_c \). We denote this number, at time \( t \) by \( N_c(t) \). If \( 1/\delta \) is the average number of hours neutrophils will survive in circulation, established as \( \sim 6–8 \) h (2, 14) (we use an estimate of 7 h for \( 1/\delta \)), then the rate of change in the total number of these neutrophils is given by

\[
\frac{dN_c}{dt} = N_0 \left( 1 - \frac{ds}{dt} \right) - \delta N_c \tag{1}
\]

Here, the left-hand side of this equation \( (dN_c/dt) \) is the rate of change in the number of circulating neutrophils \( (N_c) \) with time. The right hand side of the equation specifies what influences the change in the number of circulating neutrophils, namely, the total production of circulating neutrophils, \( N_0 (1 - ds/dt) \), incorporating baseline bone marrow neutrophil production \( (N_0) \), adjusted for any release of less mature neutrophils because of a left shift of the release point \( (1 - ds/dt) \), and natural death or extravascular loss \( (\delta N_c) \). The \( ds/dt \) term (the time derivative of the release age \( s \) in Eq. 1 specifies the rate at which the release point in the pipeline (age at neutrophil release) is changing. Thus (1) if the release point for entry to circulation is not moving \( (ds/dt = 0) \) then neutrophils are being released at the baseline rate \( \left( N_0 \right) \); 2) if the release point is being left \( (ds/dt < 0) \) then neutrophils are being released in the circulation faster than usual (the release rate is \( >N_0 \), and at progressively younger ages; and 3) if the release point is moving right \( (ds/dt > 0) \) then the rate of neutrophil release is less than usual \( (N_0 \text{ this is not considered in our study}) \). The baseline neutrophil entry rate in the circulation, \( N_0 \), is calculated from Eq. 1 assuming that the measured pre-ECC neutrophil levels \( (N_c) \) are in equilibrium, that is, \( dN_c/dt = 0 \) and \( ds/dt = 0 \), leading to \( N_0 = \delta N_c \).

It is reasonable to fit the measured data to (and consider that the population of neutrophils is changing according to) a linear expression in each phase of sampling during ECC. By estimating the growth of neutrophil numbers during each sampling phase, we can then estimate the neutrophil production required to generate the observed growth rates. If the growth in circulating neutrophil numbers is linear during each phase, then \( N_c = \alpha + \beta t \) (\( \alpha \) and \( \beta \) are calculated from linear regression) and the “velocity” of the release point for the pipeline describing neutrophil age at release (i.e., the rate at which a left shift of the release point is occurring) \( ds/dt = 1 - 1/N_0 [\beta + \delta (\alpha + \beta t)] \). The best-fitting straight line through the data points for each phase of ECC sampling is calculated (see Fig. 4A) determining the line’s slope (\( B \) and \( y \)-intercept \( (\alpha) \); given that the number of neutrophils can then be interpolated at any time, using Eq. 1 and rearranging for \( ds/dt \), we have a mathematical expression for the velocity of the neutrophil release point (entry age in the circulation) at any time. Finally, the age of release, “\( s \)” at any time is calculated by mathematical integration of its velocity \( ds/dt \).

To estimate the age profile of acquiring CD10 expression in the bone marrow, we extended our mathematical model. The proportion of neutrophils of age \( s \) in the bone marrow that are CD10\(^+\) is denoted as \( p(s) \). Hence, initially, at baseline, the proportion of neutrophils released that are CD10\(^+\) is \( p(0) \approx 0.02 \), and it can be assumed that this proportion, \( p \), increases toward a value of one with decreasing neutrophil age (that is, all newly produced postmitotic neutrophils are CD10\(^+\) at the commencement of maturation in the bone marrow). Next, the change in the number of CD10\(^+\) neutrophils measured in the circulating pool \( [N_c^{CD10+}(t)] \) can be modeled similarly to the total number of neutrophils, namely,

\[
\frac{dN_c^{CD10+}}{dt} = N_0 p(s) \left( 1 - \frac{ds}{dt} \right) - \delta N_c^{CD10+}.
\]

Accordingly, we estimate the proportion of CD10\(^+\) neutrophils, \( p(s) \), at any given point \( s \) in the pipeline by

\[
p(s) = \frac{\frac{dN_c^{CD10+}}{dt} + \delta N_c^{CD10+}}{N_c \left( 1 - \frac{ds}{dt} \right)} = \frac{\frac{dN_c}{dt} + \delta N_c}{N_c \left( 1 - \frac{ds}{dt} \right)},
\]

which is calculated from the best-fitting straight lines through the data points for total and CD10\(^+\) neutrophil counts during each phase of ECC. Here, \( dN_c/dt + \delta N_c \) refers to the overall production rate of neutrophils as determined by the rate of production required to result in the observed increase in the slope of the data plus the production required to account for the death rate of neutrophils, so that \( p(s) \) is the ratio of the overall production rate of CD10\(^+\) neutrophils to the overall production rate of total neutrophils.

RESULTS

Although bone marrow neutrophil release is stimulated during ECC (5, 20), an early, transient neutropenia is frequently observed upon its commencement (20, 22). Consequently, intraoperative sampling was commenced 32.9 ± 6.2 min after ECC was established to ensure that any acute neutrophil sequestration was complete and flux between circulating and marginal neutrophil pools had reached equilibrium. There were \( 3.90 \pm 0.28 \times 10^9/l \) neutrophils within the circulating pool at the pre-ECC baseline, of which 2.2 ± 0.4% were CD10\(^+\). Total circulating neutrophil numbers were slightly reduced to \( 3.08 \pm 0.26 \times 10^9/l \) in the first intraoperative sample \( (P = 0.03; \text{Fig. 4A}) \); however, the CD10\(^-\)/CD16\(^low\) subpopulation increased from \( 0.08 \pm 0.02 \times 10^9/l \) at baseline to \( 0.28 \pm 0.02 \times 10^9/l \) (at \( P = 0.003 \)) to represent 16.4 ± 1.8% of the circulating pool at this stage (Figs. 3B and 4A).

During ECC, active bone marrow neutrophil release is reportedly suppressed by hypothermia but stimulated by systemic rewarming (38, 41) and may be further augmented with myocardial reperfusion (25, 35). Total circulating neutrophil numbers increased during procedural \( (P = 0.03) \), warming \( (P < 0.0001) \), and weaning \( (P = 0.06) \) phases of ECC (Fig. 4A) relative to numbers present at the start of each phase. The rate of increase in circulating neutrophils appeared to be linear and was similar between operative stages (Table 3 and
Fig. 4A), indicating steady growth in the circulating neutrophil pool throughout ECC. At the completion of sampling, circulating neutrophil numbers had increased to $9.39 \pm 1.54 \times 10^9/l$ (Fig. 4A), an increment of 2.46-fold relative to baseline. The number of circulating CD10$^+/CD16^{low}$ neutrophils increased progressively and similarly during procedural ($P < 0.0001$), warming ($P < 0.0001$), and weaning ($P = 0.0008$) phases of ECC (Table 3 and Figs. 3, B-D, and 4A). The increase in the CD10$^+/CD16^{low}$ subpopulation also appeared to be linear throughout ECC (Fig. 4A). The rates of change in total and CD10$^+/CD16^{low}$ neutrophil numbers (Fig. 4A) were not significantly altered after reestablishment of normal pulmonary blood flow upon weaning from ECC (Fig. 2), indicating a negligible contribution to circulating neutrophil numbers from pulmonary demargination.

Basal bone marrow neutrophil production, based on an intravascular $t_{1/2}$ of 7 h (2, 14), was calculated to be $0.009 \pm 0.001 \times 10^9 \cdot l^{-1} \cdot min^{-1}$ [$(3.90 \pm 0.28 \times 10^9/l \times 6(=1/7/h)) \div 60$ min/h]; this is equivalent to a production rate of $0.95 \pm 0.07 \times 10^9 \cdot kg^{-1} \cdot day^{-1}$, based on a blood volume of 71 ml/kg, and is consistent with prior reports (14, 42) (Table 3). There was net growth in the circulating neutrophil pool during procedural ECC, which increased further with warming and remained similar throughout the weaning phase (Table 3). Assuming that flux between circulating and marginal neutrophil pools, intravascular $t_{1/2}$, and time to onset of apoptosis all remained constant during the brief intraoperative sampling interval, net growth in the circulating neutrophil pool during ECC could be attributed to increased bone marrow neutrophil production. We calculated that neutrophil production by the bone marrow increased during procedural ECC and was further augmented with warming but remained relatively constant during the weaning phase (Table 3). The CD10$^+/CD16^{low}$ subpopulation contributed a progressively increasing propor-
tion of bone marrow neutrophil production during each stage of ECC (Table 3).

The short intraoperative sampling interval necessitates that the calculated increase in bone marrow neutrophil production was produced by augmented release of preformed neutrophils (and band forms) and not by increased proliferation of neutrophil precursors. Neutrophils must exit the bone marrow at a progressively earlier maturational age to generate such an increase in production. The total number of neutrophils within the circulating pool at any given time can be used to predict the average age of neutrophils upon their release from the bone marrow based on our mathematical model. We predict that the release point defining neutrophil mean age at release (Fig. 1) moved with a negative velocity (underwent a “left shift” in the pipeline), defined as the rate of change of the average age (in hours) of released neutrophils as a function of time (in hours), to a progressively earlier point in the pipeline during ECC (Fig. 4B). Based on our model fit to the data, the release point had a negative acceleration that was similar and approximately constant for each stage. Consequently, the average age at which neutrophils were released from the bone marrow during the weaning phase of ECC was reduced by \( 8.44 \pm 2.20 \) h relative to the release age at the beginning of ECC sampling (Fig. 5).

The age-related profile of CD10 expression during late neutrophil maturation in the bone marrow is uncertain. Concurrent changes in total and CD10\(^{+}\)/CD16\(^{low}\) circulating neutrophil numbers obtained during ECC sampling were incorpo-
DISCUSSION

The bone marrow postmitotic compartment contains a large reserve of functional, although not necessarily mature, neutrophils available for release in the circulation when demand is acutely increased (7, 8). Mobilization of neutrophils from the bone marrow is known to abbreviate total postmitotic transit time by 3–4 days (13, 19, 37); however, expansion of the circulating pool occurs within 1–2 h of exposure to a leukocytosis-inducing stimulus (21), indicating that more acute reductions in transit time may occur. We anticipated that such an acute reduction in transit time may be quantifiable by determining the previously unknown rate of change in postmitotic maturation time during active neutrophil recruitment. Neutrophil postmitotic transit time was evaluated in terms of mean cellular age by modeling circulating neutrophil numbers during ECC, a stimulus that induces bone marrow neutrophil recruitment (38, 41) in association with limited demargination (15, 16) and which permits repeated and frequent sampling. Our model predicts the rate of change in the average age at which neutrophils exit the bone marrow during active neutrophil recruitment. It thereby quantifies the acute acceleration and consequent abbreviation of postmitotic transit time by examining exit from the end of a temporal pipeline of bone marrow

Table 3. Changes in circulating neutrophil pool and bone marrow neutrophil production during ECC

<table>
<thead>
<tr>
<th>Phase of ECC</th>
<th>Total Circulating Neutrophils at Beginning of Phase (×10^9)</th>
<th>Net Circulating Neutrophil Pool Growth Rate (×10^7·1·min)</th>
<th>Bone Marrow Neutrophil Production Rate (×10^7·1·min)</th>
<th>CD10+/CD16^low Neutrophils in Circulating Pool at Beginning of Phase (×10^9)</th>
<th>CD10+/CD16^low Neutrophils at Beginning of Phase, % total circulating neutrophils</th>
<th>Contribution of CD10+/CD16^low Neutrophils to Neutrophil Production, % released neutrophils</th>
</tr>
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<tbody>
<tr>
<td>Pre-ECC baseline</td>
<td>3.90±0.28</td>
<td>0</td>
<td>0.009±0.001</td>
<td>0.08±0.02</td>
<td>2.2±0.4</td>
<td>2.2±0.4</td>
</tr>
<tr>
<td>Procedural</td>
<td>3.08±0.26</td>
<td>0.04±0.02</td>
<td>0.047±0.022</td>
<td>0.50±0.10</td>
<td>16.4±2.7</td>
<td>19.1±2.0</td>
</tr>
<tr>
<td>Warming</td>
<td>4.47±0.70</td>
<td>0.14±0.02</td>
<td>0.151±0.025</td>
<td>0.96±0.16</td>
<td>22.1±3.8</td>
<td>26.8±2.4</td>
</tr>
<tr>
<td>Weaning</td>
<td>7.96±1.32</td>
<td>0.12±0.06</td>
<td>0.138±0.058</td>
<td>2.30±0.45</td>
<td>30.6±4.9</td>
<td>35.5±1.8</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 10 patients averaged over 4 time points/patient during each phase. *Bone marrow neutrophil production during each phase was derived from the observed total circulating neutrophil numbers, intravascular \( t_c \) and rate of increase in neutrophil numbers (as described in Mathematic modeling). Specifically, the production rate is \( N_c(t) = \frac{1}{t_c} \) \( \frac{\partial t}{\partial t} = \beta + \delta(\alpha + \beta) \), where \( \alpha + \beta \) is the best-fitting straight line through the data points. The mean production rate ± SE for n = 10 patients is reported. †Proportion of neutrophil production contributed by CD10+/CD16^low neutrophils was calculated for each patient at each sample time point according to \( p(s) = \frac{\Delta N_{CD10^+} + \Delta N_{CD16^low}}{\Delta N_c} = \frac{\beta + \delta N_{CD16^low}}{\beta + N_c} \), where \( \beta \) is the slope of the best-fitting line through the CD10^+ data for each patient. The neutrophil production rates of 0.009 ± 0.001, 0.047 ± 0.022, 0.151 ± 0.025, and 0.138 ± 0.058 x 10^9·1·min^-1 are equivalent to 0.95 ± 0.07, 4.82 ± 2.21, 15.41 ± 2.57, and 14.06 ± 5.89 x 10^8·kg^-1·day^-1.
neutral production rather than quantifying the entire duration of this phase.

The narrow sampling interval of our study limits the potential for alterations in neutrophil intravascular survival to contribute significantly to growth in the circulating neutrophil pool. Because the sampling period is relatively short compared with the average lifespan of a neutrophil (i.e., ~1.7 vs. 7 h, respectively), even if neutrophil death was reduced to zero during the sampling period, this should only increase the observed number of circulating neutrophils by ~30% (vs. the ~2.5-fold observed increased observed). This permits the assumption that increased bone marrow neutrophil production is the only significant source for the expanded circulating neutrophil pool during ECC. Morphologically mature neutrophils remain in the bone marrow for ~2–3 days before their release (11), and there are sufficient postmitotic reserves to supply basal neutrophil production for at least 4–5 days (36, 43). The observed rapid increase in bone marrow neutrophil production can only be explained by an increased rate of release of these preformed neutrophils, given an anticipated delay of 3–4 days before increased proliferation within the mitotic pool would be expected to contribute (43). The overall 2.46 ± 0.42-fold increment in circulating neutrophil numbers observed was consistent with other studies investigating acute mobilization of neutrophils from the bone marrow reserve (12, 23, 33), suggesting that the response to such leukocytosis-inducing stimuli is of a relatively uniform magnitude. The progressive increase in relative size of the younger CD10−/CD16low subpopulation during net circulating neutrophil pool growth was consistent with age-dependent first in-first out kinetics for neutrophil release from the bone marrow.

Changes in phenotype during neutrophil lineage maturation have traditionally been evaluated relative to cell morphology as an indicator of maturity. However, as exemplified in previous studies (35), the CD10−/CD16low phenotype identifies greater numbers of phenotypically immature neutrophils than does cellular morphology alone. The temporal profile of phenotypic maturation may therefore provide a more detailed and accurate indicator of the rate at which mature phenotypic characteristics are acquired. By analyzing concurrent changes in total and CD10−/CD16low neutrophils in the circulating pool, our model predicts an age-related profile of CD10 expression during late neutrophil maturation that is independent of cell morphology. Assuming that CD10 expression is acquired at the same rate during steady-state neutrophil turnover and abbreviated bone marrow transit, we demonstrate that CD10 is acquired at an approximately constant rate with increasing neutrophil mean age. Moreover, phenotypic maturation appears to continue for the entire duration of postmitotic transit, since only a small proportion of circulating neutrophils are CD10− under basal conditions in this study and others (24, 30, 35). In contrast to morphological maturation changes that occur on a time scale of days, our results suggest that phenotypic changes occur at a more rapid rate, since acquisition of CD10 expression was evident on an hourly basis. Whether prematurely released CD10−/CD16low neutrophils can subsequently complete their maturation and acquire CD10 expression within the vascular space is unknown. However, mature neutrophils require at least 8 h for CD10 synthesis (31), a time frame outside the range of intraoperative sampling duration used here, and should not significantly affect our analysis.

The significant increase in CD10−/CD16low neutrophils during ECC is consistent with a substantial contribution from bone marrow neutrophil release to the observed growth in circulating neutrophil numbers. However, we cannot exclude a contribution from other sources. Although the pulmonary marginal neutrophil reserve is excluded from the circulation during ECC, demargination of neutrophils from other organ-specific vascular beds can contribute to expansion of the circulating neutrophil pool. Because ECC is associated with limited demargination (15, 16), it is likely that contributions from the marginal neutrophil pool are not likely to significantly confound our conclusions.

In conclusion, the results reported here comply with an age-related pipeline model of bone marrow neutrophil production through which cellular transit follows first in-first out kinetics and is acutely accelerated during active neutrophil recruitment. The proportion of CD10− neutrophils within the maturing bone marrow pool was directly related to the mean age of cells. This mathematical model may be applicable to in vivo analysis of acute changes in postmitotic transit time induced by other stimuli that can selectively mobilize the bone marrow neutrophil reserve.
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