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

Yishay Orr,1,2* David P. Wilson,1* Jude M. Taylor,1 Paul G. Bannon,2,3 Carolyn Geczy,4 Miles P. Davenport,1 and Leonard Kritharides1,5,6

1Department of Haematology, Prince of Wales Hospital, and Centre for Vascular Research and 4Inflammatory Diseases Research Unit, School of Medical Sciences, The University of New South Wales, Anzac Parade, Kensington;
2Department of Cardiothoracic Surgery, Royal Prince Alfred Hospital and 5The Heart Research Institute, Camperdown; 3The Baird Institute for Applied Heart and Lung Surgical Research, Newtown; and 6Department of Cardiology, Concord Repatriation General Hospital, Concord, New South Wales, Australia

Submitted 2 September 2006; accepted in final form 18 December 2006

Acute inflammatory stimuli rapidly mobilize neutrophils from the bone marrow by shortening postmitotic maturation time and releasing younger neutrophils; however, 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 circulation (ECC). Net growth in the circulating neutrophil pool occurred during the procedural (0.04 ± 0.02 × 10⁹·1⁻¹·min⁻¹), warming (0.14 ± 0.02 × 10⁹·1⁻¹·min⁻¹), and weaning (0.12 ± 0.06 × 10⁹·1⁻¹·min⁻¹) phases of ECC. When applied to our differential equation mathematical model, these results predict that neutrophil average age at exit from the bone marrow decreases continually during ECC, resulting in average neutrophil release 8.44 ± 2.20 h earlier during the weaning phase than at the beginning of ECC sampling. Modeling of concurrent changes in CD10+/CD16low neutrophil numbers indicates that CD10 expression is directly related to neutrophil mean age and predicts that the proportion of mobilizable postmitotic neutrophils that are CD10⁰ increases from 64 to 81% during these sampled 8.4 h of maturation.

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

The increased peripheral demand for neutrophils typical of acute inflammatory states is supplied predominantly by increased bone marrow neutrophil production, a response mediated acutely by increased release of preformed neutrophils. During steady-state production, mature neutrophils are released from the bone marrow at a mean age of ~12 days after precursors become neutrophil lineage-committed (9, 11). Postmitotic maturation time, which accounts for 6–8 days under basal conditions (14), is reduced by 3–4 days during active neutrophil recruitment (13, 19, 37), thereby increasing neutrophil availability for release in the circulation. Circulating neutrophil numbers can increase within 1–2 h of exposure to a leukocytosis-inducing stimulus (21), indicating a rapid onset of this abbreviation in postmitotic transit time. Although total transit time is reduced, the acceleration of neutrophil postmitotic transit, particularly the acute response during exposure to a neutrophil-mobilizing stimulus, has not been quantified. The reduction in neutrophil average age at exit from the bone marrow during active neutrophil recruitment can provide an estimate of this acute acceleration of postmitotic transit, which may afford a measure of bone marrow integrity and response to stimulation.

We (35) and others (24, 32) have demonstrated that an increased proportion of circulating neutrophils exhibit a CD10⁺ phenotype, in association with low CD16 expression (CD10⁺/CD16low) (35) during active bone marrow neutrophil recruitment. Neutrophil CD10 expression is functionally significant, since this zinc metalloproteinase (neutral endopeptidase 3.4.24.11) cleaves and inactivates multiple proinflammatory, vasoactive, and neuropeptides (6, 18, 39). Whereas CD16 expression is acquired during the metamyelocyte stage and levels gradually increase with continuing morphological maturation (40), CD10 is expressed only by segmented neutrophils (17), and an estimated 25% of maturing bone marrow neutrophils are CD10⁻ (32). The CD10⁻/CD16low subpopulation is thus morphologically heterogeneous, consisting of maturing segmented neutrophils, all band forms and some metamyelocytes. Although previous studies have established the rate at which morphological maturation occurs in the neutrophil lineage relative to time spent within the bone marrow (9–11, 28), the age-related acquisition of functionally relevant markers such as CD10 remains unreported.

Transit of neutrophil lineage-committed cells through maturation within the bone marrow, followed by their release in the circulation, is predicted to be sequential, conforming to first-in-first-out kinetics based on cellular age (4, 11, 29, 34). The increase in cellular mean age during transit can be described by a “pipeline” model of neutrophil production that is limited by a release point that defines neutrophil age at exit in the circulation (Fig. 1). During the acute phase of neutrophil recruitment, rates of cell proliferation and maturation within the bone marrow should not change, and the only means to rapidly increase circulating neutrophil numbers is to release

* Y. Orr and D. P. Wilson contributed equally to the experimental work.

Address for reprint requests and other correspondence: L. Kritharides, Centre for Vascular Research, School of Medical Sciences, 4th Floor Wallace Wurth Bldg., The Univ. of New South Wales, Kensington, NSW 2052 (e-mail: L.kritharides@unsw.edu.au).

http://www.ajpregu.org 0363-6119/07 $8.00 Copyright © 2007 the American Physiological Society R1707

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
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 demarginated 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 (HI10a, 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 \( \mu \)M 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

### Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Sex (male/female)</th>
<th>8/2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>55.8±10.2</td>
</tr>
<tr>
<td>Surgical procedure</td>
<td>CABG 7 (70%), Valve replacement 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 during hypothermia, °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 μM Hoechst 33342 at 30°C for 10 min. Samples were then placed on ice in the dark, and cells were labeled for flow cytometry.

Flow cytometry. Antibody labeling of whole blood, performed within 2 h of sample collection, was a modification of a previously published method (26). Aliquots (5 μl) of Hoechst-labeled blood were incubated with 1 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 in the dark for 20 min, diluted to 0.75–1.0 ml with HBSS-BSA-NaH3, 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 Calibrize 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 MAAb-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 MAAb 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 4.00 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.

Table 2. Blood sampling details

<table>
<thead>
<tr>
<th>ECC Phase</th>
<th>Sampling Frequency, min</th>
<th>Duration of Sampling Phase, min</th>
<th>Physiological Status and Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procedural</td>
<td>3.8 ± 0.2</td>
<td>11.4 ± 0.6</td>
<td>Hypothermic ECC established; pulmonary vascular bed excluded; nasopharyngeal (NP) temperature ≤31.8°C</td>
</tr>
<tr>
<td>Warming</td>
<td>3.2 ± 0.2</td>
<td>9.5 ± 0.5</td>
<td>Systemic rewarming instituted; pulmonary vascular bed still excluded; temperature range: 32.2 ± 0.5 to 36.1 ± 0.3°C</td>
</tr>
<tr>
<td>Weaning</td>
<td>3.6 ± 0.2</td>
<td>10.8 ± 0.5</td>
<td>Normothermia (NP temperature ≥37°C); aortic cross-clamp released, reestablishing blood flow to the heart; blood flow gradually reestablished to pulmonary vascular bed with return of mechanical cardiac function</td>
</tr>
</tbody>
</table>

Values are given as means ± SE; (n = 10/interval); 4 samples were collected during each interval.
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 (\( N_0 \)); 2) if the release point is moving 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 (\( N_0 \); 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 in 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

\[
p(s) = \frac{\frac{dN_{C,CD10−}}{dt} + \delta N_{C,CD10−}}{N_0 \left(1 - \frac{ds}{dt}\right) + \delta N_{C,CD10−}} = \frac{\frac{dN_{C,CD10−}}{dt} + \delta N_{C,CD10−}}{N_0 \left(1 - \frac{ds}{dt}\right) + \delta N_{C}},
\]

which is calculated from the best-fitting straight lines through the data points for total and CD10− neutrophils counts during each phase of ECC. Here, \( dN_{C}/ds + \delta N_{C} \) 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 ± 0.28 × 10⁹/l neutrophils within the circulating pool at the pre-ECC baseline, of which 2.2 ± 0.4% were CD10−/CD16low (Figs. 3A and 4A). Total circulating neutrophil numbers were slightly reduced to 3.08 ± 0.26 × 10⁹/l in the first intraoperative sample (\( P = 0.03 \); Fig. 4A); however, the CD10−/CD16low subpopulation increased from 0.08 ± 0.02 × 10⁹/l at baseline to 0.50 ± 0.10 × 10⁹/l (\( P = 0.003 \)) to represent 16.4 ± 2.7% 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 $0.009 \pm 0.001 \times 10^9/l^{-1} \cdot min^{-1}$ [$(3.90 \pm 0.28 \times 10^9/l) \times \delta (=1/7/h) \div 60 \text{ min/h}$]; this is equivalent to a production rate of $0.95 \pm 0.07 \times 10^9/kg^{-1} \cdot day^{-1}$, 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 ± 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+/CD16low circulating neutrophil numbers obtained during ECC sampling were incorpo-
Changes in circulating neutrophil pool and bone marrow neutrophil production during ECC

The estimated proportion of mobilizable postmitotic neutrophils in the bone marrow postmitotic compartment declines (Fig. 4). By the weaning phase of ECC, we estimate that only 0.4% of neutrophils released from the bone marrow are CD10⁺, suggesting a rapid acquisition of CD10 expression in the final hours of neutrophil maturation.

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.

**DISCUSSION**

The bone marrow postmitotic compartment contains a large reserve of functional, although not necessarily mature, neutrophils that are CD10⁻ and cellular mean age. Under basal conditions, the estimated proportion of mobilizable postmitotic neutrophils that were CD10⁻ was 2.2% (Fig. 4B). We predict that this proportion increases as the average age of neutrophils within the bone marrow postmitotic compartment declines (Fig. 4B). Our model predicts that CD10 expression is directly related to cellular mean age and is acquired at an approximately constant rate during the final hours of neutrophil maturation (Fig. 6). At baseline, under steady-state conditions, we estimate that 97.8 ± 0.4% of neutrophils released from the bone marrow are CD10⁻. By the weaning phase of ECC, we estimate that only 64.5 ± 1.8% of neutrophils leaving the bone marrow are CD10⁺, suggesting a rapid acquisition of CD10 expression in the final hours of neutrophil maturation.

**KINETIC MODEL OF NEUTROPHIL PRODUCTION AND MATURATION**

<table>
<thead>
<tr>
<th>Phase of ECC</th>
<th>Total Circulating Neutrophils at Beginning of Phase (×10⁹/l)</th>
<th>Net Circulating Neutrophil Pool Growth Rate (×10⁹·1⁻¹·min⁻¹)</th>
<th>Bone Marrow Neutrophil Production Rate (×10⁹·1⁻¹·min⁻¹)*</th>
<th>CD10⁺/CD16low Neutrophils in Circulating Pool at Beginning of Phase (×10⁹/l)</th>
<th>CD10⁺/CD16low Neutrophils at % total circulating neutrophils</th>
<th>Contribution of CD10⁺/CD16low Neutrophils to Neutrophil Production, % released neutrophils†</th>
</tr>
</thead>
<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±0.18</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 t½, and rate of increase in neutrophil numbers (as described in Mathematic model). Specifically, the production rate is \( \frac{dN_c}{dt} = \beta + \delta (\alpha + \beta) \), 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⁺/CD16low neutrophils was calculated for each patient at each sample time point according to \( \rho(s) = \frac{dN_c + dN_{CD10}^{CD10-}}{dN_c + dN_c} = \frac{\beta + \delta N_{CD10}^{CD10-}}{\beta + N_c} \), 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 × 10⁹·1⁻¹·min⁻¹ are equivalent to 0.95 ± 0.07, 4.82 ± 2.21, 15.41 ± 2.57, and 14.06 ± 5.89 × 10⁹·kg⁻¹·day⁻¹.

**Fig. 5. Neutrophil exit age decreases during active recruitment from the bone marrow.** Changes in circulating neutrophil numbers were applied to our mathematical model to predict the average change in neutrophil age (hours) at release from the bone marrow as a function of time (minutes) from the beginning of the procedural phase of ECC (t = 0 min); change in age is expressed relative to neutrophil age at t = 0 min. The observed change in neutrophil age with time was consistent with a constant acceleration of the “release point,” leading to a continuously decreasing release age with time. The best-fitting straight line through the data points for each phase of ECC was determined for each patient and used to calculate the change in neutrophil release age for each patient during ECC; the mean ± SE was then calculated for all patients. The solid curve refers to the average age of release, and the dashed curves are the SEs.
CD10 expression in the bone marrow is related to mean neutrophil age. The proportion of CD10-/CD16low neutrophils within the postmitotic pool was estimated relative to neutrophil average age (hours) at release from the bone marrow during ECC. Concurrent total and CD10-/CD16low circulating neutrophil numbers from each sample during all 3 phases of ECC were applied to our mathematical model for each patient. The quotient of linear expressions (as a mathematical form predicted by our model) that best fit this data (according to GraphPad Prism version 4.00) is presented as the predicted proportion of CD10- neutrophils within the bone marrow (y-axis) vs. the number of hours neutrophils are released early relative to $t = 0$ min (x-axis).
ACKNOWLEDGMENTS

We thank flow cytometrist Leonie Gaudry for excellent technical assistance, Deborah Crewor for detailed assistance with scrutiny of our mathematical model and calculations, and Colin Chesterman and the Departments of Haematology and Flow Cytometry, Prince of Wales Hospital, Sydney, Australia, for use of equipment and resources invaluable to this project.

GRANTS

This work was supported by the National Heart Foundation of Australia, The National Health and Medical Research Council of Australia, The Royal Australasian College of Surgeons, The Australasian Society of Cardiothoracic Surgeons Research Foundation, the James S. McDonnell Foundation 21st Century Research Award/Studying Complex Systems, and a New South Wales Ministry for Science and Medical Research Infrastructure grant. M. P. Davenport is a Sylvia and Charles Viertel Senior Medical Research Fellow.

REFERENCES


