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Splenic progenitors aid in maintaining high neutrophil numbers at sites of sterile chronic inflammation

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ABSTRACT

Neutrophils are constantly generated from hematopoietic stem and progenitor cells in the bone marrow to maintain high numbers in circulation. A considerable number of neutrophils and their progenitors have been shown to be present in the spleen too; however, their exact role in this organ remains unclear. Herein, we sought to study the function of splenic neutrophils and their progenitors using a mouse model for sterile, peritoneal inflammation. In this microcapsule device implantation model, we show chronic neutrophil presence at implant sites, with recruitment from circulation as the primary mechanism for their prevalence in the peritoneal exudate. Furthermore, we demonstrate that progenitor populations in the spleen play a key role in maintaining elevated neutrophil numbers. Our results provide new insight into the role for splenic neutrophils and their progenitors and establish a model to study neutrophil function during sterile inflammation. *J. Leukoc. Biol.* 100: 253–260; 2016.

Introduction

Mechanical trauma and chemical stimuli, such as those caused by in vivo implantation of medical devices, result in sterile inflammatory responses [1]. These responses begin with the activation of blood vessels around the implantation site, leading to the recruitment of immune cells from circulation [2]. Neutrophils, one of the first cells to be recruited, play an important role in responses against implanted devices but are considered to be involved solely in the acute stages, with their presence lasting a few days to, at most, 1 wk at implantation sites [3, 4], primarily as a result of their short lifespan and high turnover rate [5, 6].

Neutrophils are generated extensively and continuously in the BM [7, 8]. Aside from the BM, the lung, liver, and spleen are other tissue sites that are potential reservoirs for neutrophils [9]. Whereas the importance of BM in the production and maintenance of neutrophils is undisputed, the presence and role of neutrophils in other organs remain unclear. Neutrophils in the lung have been suggested to be involved in immunosurveillance [8, 10], whereas those in the liver are thought to be present for clearance [11]. However, the role and importance of splenic neutrophils are unclear. Preliminary evidence in a mouse model of lung adenocarcinoma suggests that splenic neutrophils and their progenitors could be involved in maintaining tumor-associated neutrophil numbers [12].

In this study, we evaluated the extent of neutrophil involvement in sterile inflammatory conditions caused by device implantation and investigated the importance of splenic neutrophils in this process. Our results provide evidence for chronic neutrophil presence at implantation sites and suggest a prominent role for splenic progenitors in maintaining high numbers of neutrophils at sites of inflammation.

MATERIALS AND METHODS

Mice

Female and male C57BL/6J, CD45.1 (B6.SJL-*Ptprc*^{a} *Pepc*^{b}/BoyJ), and IL-1R KO (B6.129S7-IL1r1^{tm1Imx}/J) mice were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). Mice, in the age range of 8–14 wk (corresponding to 20–28 g for male and 20–25 g for female), were used in

Abbreviations: BM = bone marrow, KO = knockout, PC = peritoneal cavity

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all experiments. All animal experiments were performed in accordance with protocols approved by the Massachusetts Institute of Technology and Harvard Medical School Committee on Animal Care and followed federal and state regulations.

Microcapsules

The synthesis of alginate microcapsules has been described previously [13, 14]. In brief, an electrostatic droplet generator was used to generate alginate microcapsules made of SLG20 alginate (NovaMatrix; FMC Bio-Polymer, Drammen, Norway), dissolved in 0.9% (w/v) NaCl solution, to a concentration of 1.4% (w/v). The alginate solution was passed through a 25G blunt needle at a flow rate of 0.18 ml/min and a voltage of ~5 kV into a 20 mM $BaCl_2$ cross-linking solution. The microcapsules were washed multiple times in saline to remove excess $BaCl_2$ before implantation.

In vivo model

Alginate microcapsules were surgically implanted into the PC of mice, as described [14]. In brief, alginate microcapsules (350 μl) were suspended in 500 μl saline for implantation. These microcapsules were then implanted into the PC through laparotomy. In mock controls, the animals underwent surgical procedures, as described above, with 1 modification: the injected material was ~850 μl saline (no microcapsules). In studies involving microcapsule implantation and splenectomy, the laparotomy procedure was modified as follows: a 10 mm longitudinal incision was made on the left side of the abdomen through the skin. A smaller incision was made through the abdominal musculature to expose the spleen. The spleen was pulled out of the incision site and gently teased apart from the blood vessels and omentum using forceps. The blood vessels were cauterized immediately using a cautery stick. With the use of a sterile plastic pipette, the microcapsules were implanted into the PC through the same incision site. Finally, the abdominal muscle was closed by suturing with a polydioxanone-absorbable, 5.0–6.0 monofilament thread. The external skin layer was closed using wound clips.

Retrieval of cells, tissues, and implants

Blood was collected using retro-orbital bleeding. For all other retrievals, mice were euthanized through gaseous CO_2 administration, followed by cervical dislocation. Peritoneal exudates were collected, passed through a 70 μm filter (to filter microcapsules), followed by RBC lysis, and finally, suspended in media or flow cytometry buffer (PBS containing 0.5% BSA and 2 mM EDTA) before further analysis. A single-cell suspension of the spleen was prepared through mechanical breakdown, followed by RBC lysis, and the cells suspended in media before further analysis. Tibia and femur (1 each) were collected for BM isolation. Following removal of muscle and other connective tissue, BM was flushed out into a sterile storage container in a sterile laminar flow hood using PBS containing 2 mM EDTA. Neutrophil counts from various organs indicate the following: per mouse for PC counts, per mouse for splenic counts, per tibia + femur for BM counts, and per microliter for blood counts.

Flow cytometry

Single-cell suspensions from the PC, spleen, BM, and blood were prepared in PBS containing 0.5% BSA and 2 mM EDTA (staining solution). Cells were stained with a combination of the following antibodies for 20 min at 4°C: Ly6G (clone 1A8), Ly6C (HK1.4), CD11b (M1/70), CD19 (6D5), TCRβ (H57-597), CD11c (N418), F4/80 (BM8), CD115 (AFS98), CD206 (C068C2), CD117 (2B8), Sca1 (D7), CD16/32 (93), CD34 (RAM34), CD45.1 (A20), CD62L (MEL-14), CD14 (Sa14-2), and ki67 (SolA15; all purchased from BioLegend, San Diego, CA, USA, or eBioscience, San Diego, CA, USA). Additionally, cells were stained with antibodies against chemokine receptors CXCR2 (242216) and CXCR4 (247506; obtained from R&D Systems, Minneapolis, MN, USA). Flow cytometry data were collected using a BD-LSR II or BD-LSR Fortessa and analyzed using FlowJo (Tree Star, Ashland, OR, USA).

Neutrophil isolation

Neutrophils were isolated from a mixed population of PC and BM cells using a magnetic bead-based negative selection kit (Stemcell Technologies, Vancouver, BC, Canada) in accordance with the manufacturer's protocol, with 1 modification: in addition to the antibodies provided by manufacturer, we added purified anti-mouse CD115 (clone AFS98) at 1:250 dilution to increase purity of neutrophils (without the addition of the antibody, 30–40% of purified cells were not $Ly6G^+$).

Adoptive transfer experiments

Alginate microcapsules (~500 μm diameter; ~3500 microcapsules/mouse) were implanted in CD45.1 (B6.SJL-*Ptprc*^{a} *Pepc*^{b}/BoyJ) mice. One week later, C57BL/6J (CD45.2) mice were implanted with alginate microcapsules (same as in CD45.1 mice) or mock treated. Two weeks following implantation in CD45.1 mice, the PC and BM neutrophils were isolated. Cells were suspended in PBS at a concentration of 2.5 million cells/ml and administered through intraperitoneal or intravenous injections into CD45.2 mice. One or 3 d following adoptive transfer, PC cells were isolated, stained with antibodies, and analyzed using flow cytometry.

BrdU uptake

BrdU uptake and associated experiments were performed using the BrdU flow kit from BD PharMingen (San Jose, CA, USA), according to the manufacturer's instructions. One week after mice were implanted with alginate microcapsules or mock treated, 100 μl of a 10 mg/ml solution of BrdU was injected intraperitoneally. At 12 h (or 4 h or 3 d) postinjection, the PC cells, spleen, and BM were retrieved and stained with surface receptor-specific antibodies and BrdU before flow cytometry.

Parabiosis

Eight- to 9-wk-old female C57BL/6 wild-type and CD45.1 (B6.SJL-*Ptprc*^{a} *Pepc*^{b}/BoyJ) mice were joined at the flanks as described [15, 16]. In addition to postsurgical analgesics, these mice were provided with antibiotics in their drinking water.

Statistics

All data are based on at least 2 or more independent experiments, with a total of at least 5 animals/experimental group. An independent experiment is described as an experiment involving new/different batches of microcapsules, reagents, and mice and performed on a separate date. Each "n" represents an individual animal (for in vivo studies) or samples pooled together from 1 or more animals (for ex vivo studies). All data were analyzed and graphs generated using GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA). One-way or 2-way ANOVA was used for all statistical comparisons involving multiple groups.

RESULTS

Chronic neutrophil presence in PC

We recently reported an increased neutrophil presence in the PC following the implantation of microcapsules made of different materials [14]. With the use of this mouse model of peritoneal implantation, in this study, we evaluated the duration of neutrophil responses. An acute increase in neutrophils is expected following implantation of devices. However, we observed that increases in neutrophil numbers (identified as Ly6G-expressing cells [14]) were chronic, lasting at least 6 mo (~30-fold over controls), following microcapsule implantation (**Fig. 1A**). The increase in neutrophil numbers as a component of sterile inflammation was independent of the sex of mice (Supplemental Fig. 1). Furthermore, despite increased chronic presence of neutrophils in the PC, such changes were not observed in the BM, blood, or the spleen (Fig. 1B), suggesting a localized inflammation. The vast majority of Ly6G-expressing cells observed in the PC were alive (Fig. 1C, upper) and displayed canonical neutrophil morphology, consisting of lobed nuclei and

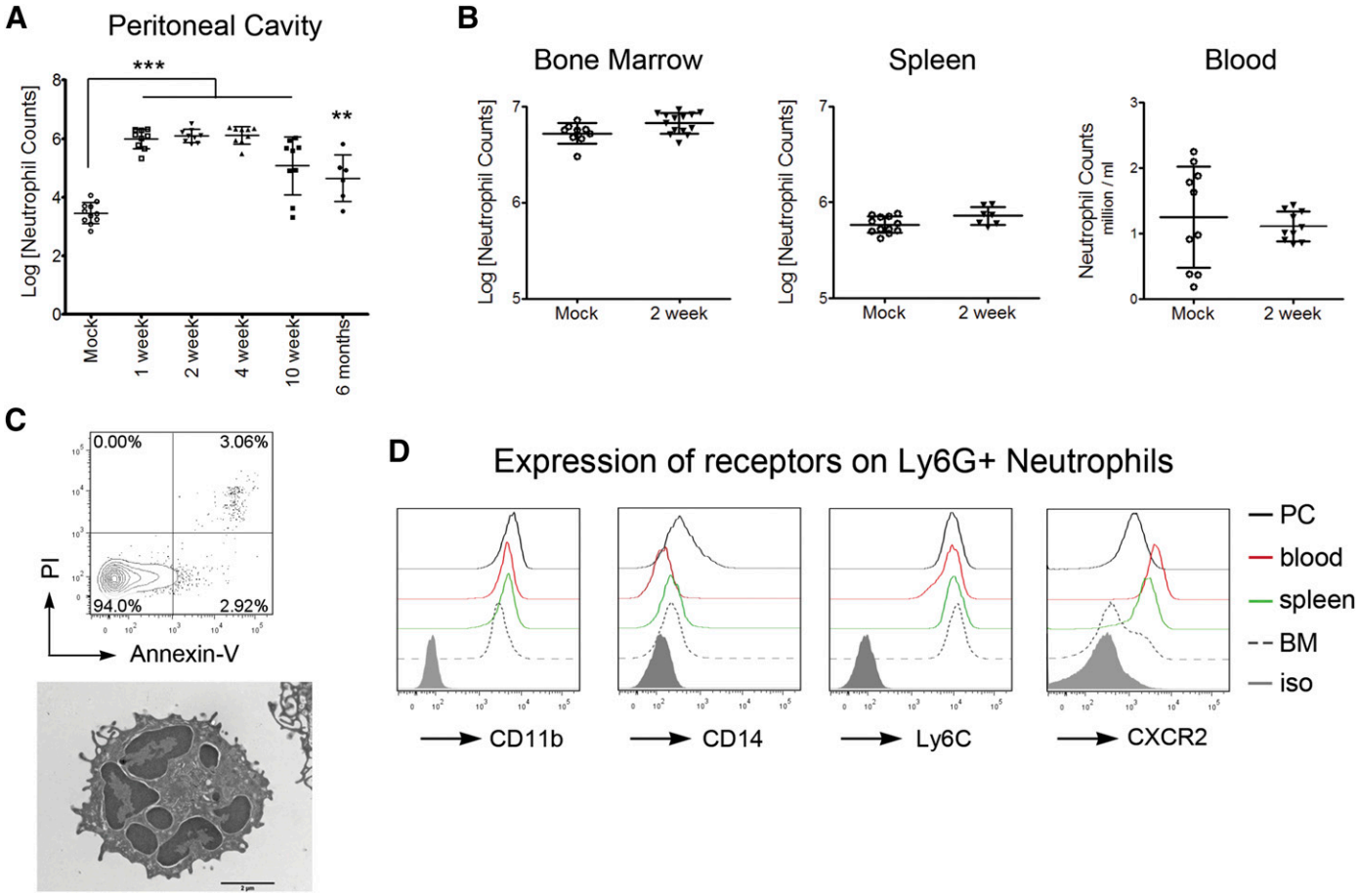

Figure 1. Chronic presence of activated neutrophils in the peritoneal exudate following microcapsule implantation. (A) Total neutrophil counts in the peritoneal exudate as a function of time. Mock and 2 wk datasets are repeat experiments, and the means are similar to historic datasets [14]. (B) Total neutrophil counts in the BM, spleen, and blood at 2 wk postmicrocapsule implantation or mock surgeries. No statistically significant difference was observed. (C, upper) Representative dot plot showing Annexin-V binding and propidium iodide (PI) staining of $Ly6G^+$ cells from the PC. (Lower) Representative electron micrograph of purified $Ly6G^+$ cells (neutrophils) isolated from the peritoneal exudate of mice implanted with alginate microcapsules. Original scale bar, 2 μm. (D) A comparison of receptor expression levels on the surface of neutrophils isolated from the PC fluid, blood, spleen, and BM. iso, Isotype. $^{**}P < 0.01$, and $^{***}P < 0.001$, presented as means ± SD. All data presented here are based on experiments involving implantation of microcapsules in male C57BL/6J mice. Data are representative of at least 3 independent experiments with $n \geq 8$ [except 2 independent experiments for 10 wk and 6 mo time points ($n = 6$)].

ruffled membranes, as confirmed through electron microscopy (Fig. 1C, lower). Additionally, neutrophils retrieved from the PC expressed characteristic surface receptors CD11b and CD14 at levels higher than and Ly6C at levels similar to neutrophils retrieved from the BM, blood, and spleen (Fig. 1D), which is suggestive of their activation status [17]. The chemokine receptor CXCR2 has been suggested to be responsible for mobilizing neutrophils from the BM into circulation, and a down-regulation of this receptor on circulating neutrophils marks them for clearance [6, 18, 19]. Notably, the expression of CXCR2 on neutrophils from the PC was higher than cells retrieved from the BM but lower than cells from the blood and spleen (Fig. 1D), potentially alluding to the terminal peripheral migratory event before clearance.

Source of peritoneal neutrophils

Increased neutrophil presence in the PC in response to sterile inflammation could be a result of continuous recruitment from circulation, locally proliferating granulocyte progenitors, or long-lived neutrophils. To determine neutrophil lifespan in the PC, neutrophils purified from CD45.1 mice (Supplemental Fig. 2A) were intraperitoneally injected into CD45.2 mice that had previously received microcapsule implants (**Fig. 2A**, schematic). A small percentage of the injected CD45.1 neutrophils (~1%) was observed in the PC, 1 d following intraperitoneal adoptive transfer, and very few to no cells were observed 3 d later (Fig. 2B), suggesting that the neutrophil lifespan in the PC is short, on the order of hours to days. Furthermore, upon depletion of cells from the PC, we observed repopulation of neutrophils within 1 d (Supplemental Fig. 2B), as well as an increase in neutrophil percentages upon a secondary intraperitoneal stimulus (Supplemental Fig. 2C). Both repopulation and an increase in neutrophil proportion suggest an active increase in neutrophil numbers, which could be either a result of local cell proliferation or active recruitment from circulation.

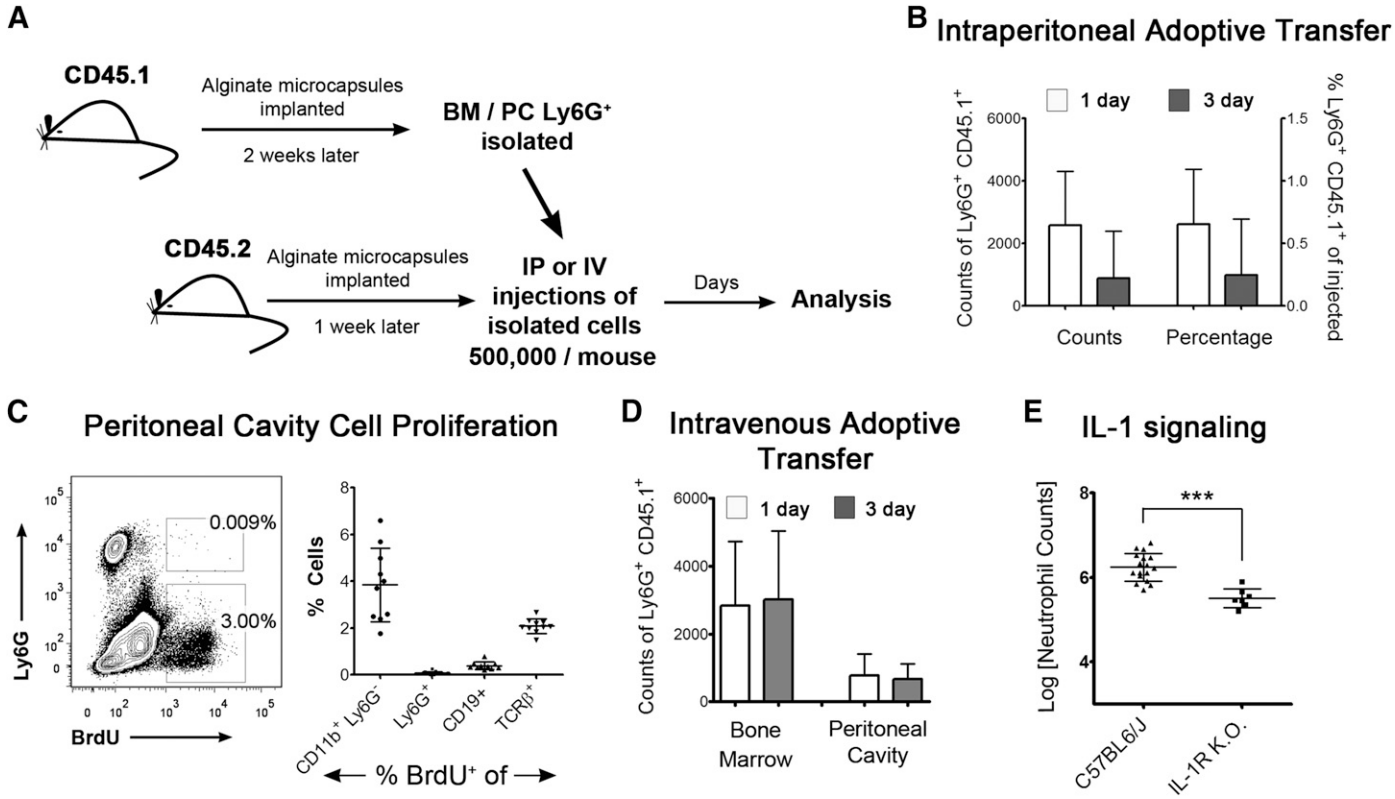

Figure 2. Chronic neutrophil presence in PC is a result of continuous recruitment from circulation. (A) Schematic describing procedures used for adoptive transfer experiments to study longevity of neutrophils in PC and their recruitment from circulation. IP, Intraperitoneal; IV, intravenous. (B) Counts (left axis) of adoptively transferred neutrophils ($Ly6G^+$ $CD45.1^+$) or percentage (right axis) of total neutrophils injected that are found in the PC, 1 or 3 d following intraperitoneal injections. Adoptively transferred neutrophils in these experiments were isolated from PC only. (C) Measure of BrdU incorporation in PC cells, 12 h postinjection of a BrdU solution and 2 wk following implantation of alginate microcapsules. (D) Counts of intravenously injected neutrophils ($Ly6G^+$ $CD45.1^+$) isolated from the BM or PC of mice that have been implanted previously with alginate microcapsules (see scheme in A). (E) Neutrophil counts in peritoneal exudates following antibody-based blocking of CXCR2 or in IL-1R KO compared with their respective controls. $^{***}P < 0.001$, presented as means ± SD. All data presented here are based on experiments involving male mice. Data are representative of at least 3 independent experiments (except for E, where data are based on 2 independent experiments), with total $n \geq 6$.

To determine whether local cell proliferation was occurring, incorporation of BrdU into cells in the PC was evaluated. Previously, it was shown that BrdU incorporation can be observed in cells within a few hours following injection but that the majority of $BrdU^+$ neutrophils arising from BM does not reach peripheral tissues until ~24 h following BrdU injection, with peak presence between 72 and 96 h [20, 21]. Therefore, we evaluated BrdU incorporation, 12 h after injection, by which time, locally proliferating cells would incorporate BrdU, but very few cells labeled in the BM would have reached the PC. A small but measurable percentage of $CD11b^+$ $Ly6G^-$ (non-neutrophil myeloid cells), $CD19^+$ (B cells), and $TCR\beta^+$ (T cells) cells incorporated BrdU in this time, but negligible percentages/numbers of $Ly6G^+$ (neutrophils) cells were BrdU positive, suggesting that neutrophils do not divide in the PC (Fig. 2C and Supplemental Fig. 2D). Through intravenous adoptive transfer of neutrophils isolated from the PC or BM of CD45.1 mice, we observed that a considerable number of peritoneal origin and BM origin neutrophils migrate to the PC from the blood (Fig. 2D). A potential mechanism of continuous neutrophil recruitment could involve low-grade, sterile inflammation of peritoneal epithelial cells, leading to activation of adhesion receptors on adjoining endothelial cells [22, 23]. It has been suggested that signaling through IL-1R [24] promotes migration of neutrophils through the vascular endothelium. In accordance with these reports, we observe ~5-fold reduction in peritoneal neutrophil counts for IL-1R KO mice (Fig. 2E).

To investigate further whether peritoneal granulocytic progenitor cells or neutrophils recruited from circulation were responsible for the observed chronic peritoneal presence, a parabiosis model was performed. In these experiments, a CD45.1 and a CD45.2 mouse (both female) were surgically joined together, resulting in a shared circulatory system. At 14–16 d following surgery, 1 mouse from the pair was implanted with microcapsules, and 2 wk later, the PCs of both mice were analyzed (**Fig. 3A**, schematic). Consistent with previous experiments, the mouse implanted with microcapsules had a dramatic increase in peritoneal neutrophil numbers when compared with the unimplanted mouse of the pair (Fig. 3B). Next, the level of neutrophil chimerism in the parabiotic mice was assessed. Chimerism is defined as the percentage of host cells present in donor mice (i.e., percent of neutrophils expressing the CD45.1 receptor in

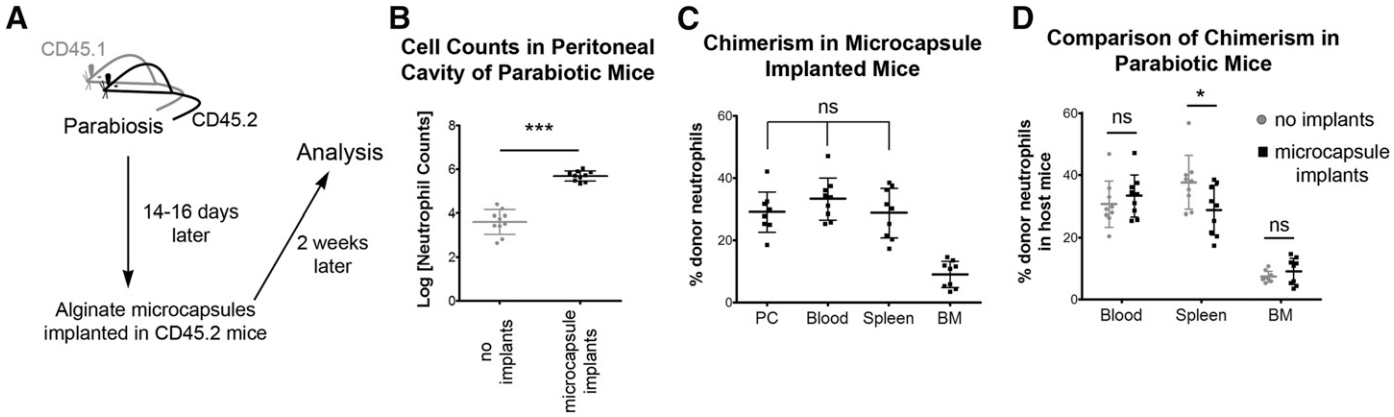

Figure 3. Parabiosis. (A) Schematic describing the parabiosis procedure to study the presence of progenitors in the PC and spleen. The parabiosis procedure was performed on CD45.1 and CD45.2 mice, and 14–16 d later, the CD45.2 mice were implanted with alginate microcapsules. Two weeks following implantation, the mice were euthanized and neutrophil levels in various organs analyzed. (B) Neutrophil counts in the PC of each mouse of the parabiotic pair. (C) Measure of chimerism (percent donor cells in host mice) in different compartments of microcapsule-implanted mice. Each dot represents a single implanted mouse of the parabiotic pair. (D) Comparison of chimerism levels in different compartments of parabiotic mice, where 1 mouse (CD45.2) of the pair received the microcapsule implants, whereas the other (CD45.1) did not. Data in parabiosis experiments are based on 2 independent experiments (total of $n = 18$ mice or 9 pairs) involving female C57BL/6J mice. ns, nonsignificant. $*P < 0.05$ and $***P < 0.001$, presented as means ± SD.

CD45.2 mice and vice versa). Whereas chimerism levels of myeloid cells reach equilibrium levels in circulation, they do not equilibrate (or chimerism levels are lower) in tissues where cells are derived from local progenitors (such as the BM) [25–27]. In the microcapsule-implanted mouse of a parabiotic pair, we observed that neutrophil chimerism levels in the PC were similar to that of the blood and spleen (Fig. 3C and Supplemental Fig. 3). Taken together, these data support continuous recruitment from systemic circulation as the primary mechanism for chronic neutrophil presence in the PC following device implantation rather than localized proliferation of resident neutrophils or their progenitors.

In the same parabiosis model, splenic neutrophil chimerism levels in microcapsule-implanted mice were observed to be significantly lower than the unimplanted mouse (Fig. 3D). Furthermore, significantly greater numbers of neutrophils in spleens of microcapsule-implanted mice (at 2 wk postimplantation), compared with spleens from saline-injected controls, expressed high levels of ki67 (**Fig. 4A** and **B**). Complementing these data, we observed that significantly increased number of neutrophils in spleens of microcapsule-implanted mice had incorporated BrdU, 12 h following intraperitoneal BrdU injection (Supplemental Fig. 4A). These data suggest the potential presence of local progenitor populations in the spleen.

Splenic progenitors

The spleen is known to contain myeloid (granulocytic and monocytic) progenitors, which could produce differentiated cells (neutrophils and monocytes) in response to inflammatory stimuli [12, 15, 28]. Consistent with these studies, myeloid progenitors were identified in the spleen of mock and microcapsule-implanted mice (**Fig. 5A**). Although no significant difference was detected in the number of splenic myeloid progenitors between mock and microcapsule-implanted mice (Fig. 5B), we observed that a greater percentage of the progenitors in microcapsule-implanted mice (at 1, 2, and 4 wk but not at 10 wk postimplantation) expresses the proliferation marker ki67 (Fig. 5C). Notably, in the BM compartment, neither an increase in total number of myeloid progenitors nor an increase in ki67-expressing progenitors was observed at any of these time points (Fig. 5B and C and Supplemental Fig. 4B).

The presence of higher numbers of mitotically active myeloid progenitors suggests a role for spleen-derived cells in actively maintaining neutrophil numbers seen in the PC in response to the inflammatory stimulus from device implantation. To test this hypothesis, the spleen was removed (splenectomy) during the peritoneal microcapsule-implantation procedure. Splenectomy with simultaneous microcapsule administration resulted in lowered peritoneal neutrophil numbers at 1 (~9-fold), 2 (~11-fold), and 4 (~3-fold) wk following implantation (**Fig. 6**). The lowered numbers were not a result of changes in systemic (as measured in the serum) G-CSF levels (Supplemental Fig. 4C)

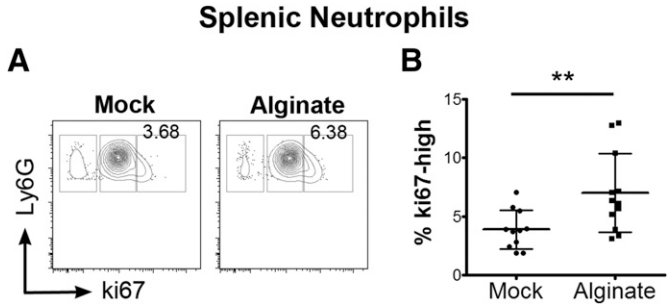

Figure 4. Splenic neutrophils. (A) Representative contour plots gated on $Ly6G^+$ cells, showing their expression of the cell division marker, ki67. (B) Quantitative comparison of the percentage of splenic neutrophils expressing ki67 between mock and microcapsule-implanted mice. Data are based on 3 independent experiments ($n \geq 6$ total mice) involving male and female mice. $**P < 0.01$, presented as means ± SD.

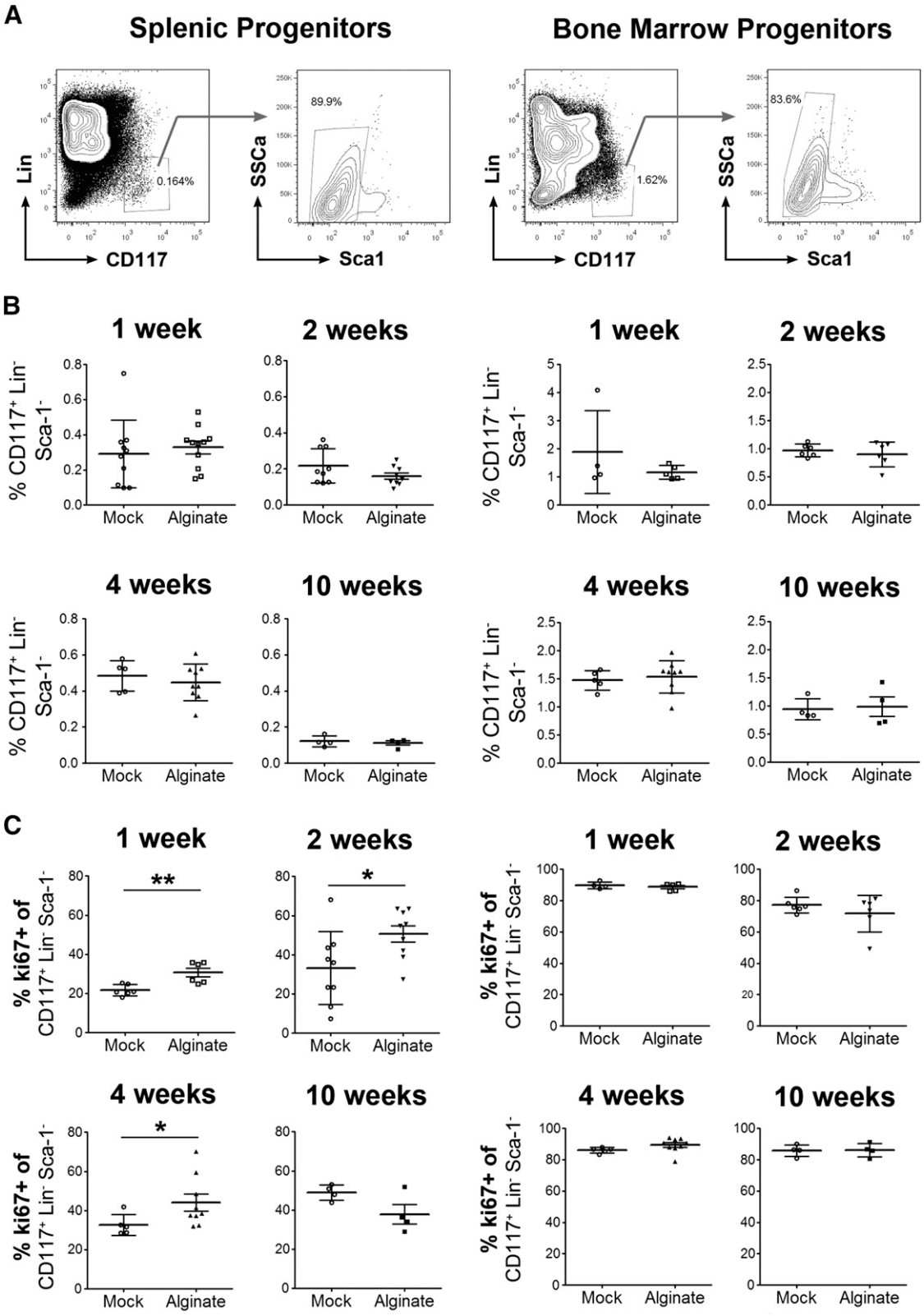

Figure 5. Myeloid progenitors in the spleen and BM. (A) Representative flow cytometry contour plots to determine myeloid progenitors ($CD117^+$ $Sca1^-$ Lin^-). Lin is lineage and indicates CD127, CD3, B220, MHC-II, CD49b, NK1.1, CD11c, Ly6C, and Ly6G. SSCa, side-scatter-area. (B) Quantification of myeloid progenitors as a percent of the total single cell population at 1, 2, 4, and 10 wk after mock or microcapsule-implantation procedure. (C) Percentage of $CD117^+$ $Sca1^-$ Lin^- cells that express ki67, measured at various time points. $^*P < 0.05$ and $^{**}P < 0.01$, presented as means ± SD. Data are representative of at least 2 independent experiments with total $n \geq 6$, except for the 10 wk time point (1 independent experiment, $n \geq 4$), involving both male and female mice.

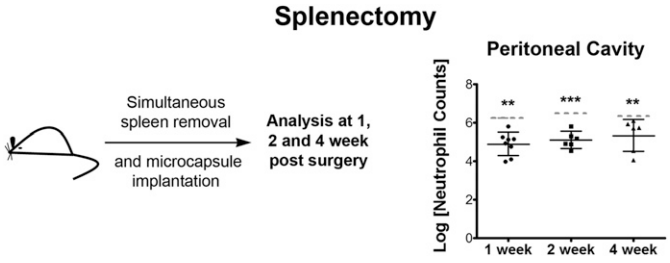

Figure 6. Splenectomy. (Left) Schematic describing experiment and (right) neutrophil counts in the PC of mice that have received a splenectomy and alginate microcapsule implants. Dotted red lines represent neutrophil counts in mice that have a spleen (based on data presented in Fig. 1). Splenectomy data are based on at least 2 independent experiments ($n \geq 6$ total mice) involving male mice only. $^{**}P < 0.05$, $^{***}P < 0.01$, presented as means ± SD.

and are likely a result of the absence of spleen-derived neutrophils following splenectomy.

DISCUSSION

A persistent, locally increased neutrophil presence in response to sterile inflammation is not common and has been reported only in response to intraperitoneal hydrocarbon oil administration [24, 29, 30]. Here, we demonstrate that a strategy involving peritoneal implantation of microcapsule devices elicits a similar chronic increase in neutrophil numbers (Fig. 1). Notably, the increase in neutrophil numbers was limited to the PC, suggesting a localized inflammation. This model of tissue-specific, chronic, and elevated neutrophil presence offers an opportunity to study the source of origin of these cells. Neutrophils at peritoneal implant sites could be site-specific, i.e., arising from long-lived neutrophils at the implant site or local neutrophil generation/proliferation, or through continuous recruitment from circulation as a result of local inflammation. Adoptive transfer studies (Fig. 2) demonstrate that neutrophil lifespan in the PC is short, on the order of hours to days, as observed previously [5, 9]. Additionally, the absence of BrdU uptake (Fig. 2) suggests that local generation of neutrophils is negligible to absent. In comparison, intravenous adoptive transfer of neutrophils and the parabiosis model strongly support continuous recruitment from systemic circulation as the mechanism for chronic neutrophil presence in the PC following microcapsule implantation.

Continuous recruitment from circulation suggests a need for increased production of neutrophils. However, neither an increase in BM neutrophil numbers (Fig. 1) nor an increase in proliferating myeloid progenitors in the BM (Fig. 5) was observed. In contrast, a larger proportion of proliferating myeloid progenitors was observed in the spleen at 1, 2, and 4 wk following peritoneal microcapsule implantation, suggesting an increased production of myeloid cells in the spleen. Corresponding with the increase in proliferating myeloid progenitors, an increased number of recently generated neutrophils were observed in the spleen. Furthermore, splenectomy (Fig. 6) resulted in lowered neutrophils numbers in the PC, demonstrating that splenic neutrophils are, at least in part, responsible for maintaining high neutrophil numbers in the PC in this model of microcapsule implantation.

Granulopoiesis in the spleen has been shown to occur in a model of subcutaneous tumor implantation [31–34]. In the same model of tumor implantation, increased granulopoiesis in the BM has also been demonstrated [32, 35, 36]. Based on these reports, it is important to note that the spleen is not solely responsible for generating neutrophils during an inflammatory event. Indeed, data presented here are in accordance with these reports. In an alternate parabiosis model, where the microcapsules are implanted before parabiosis, splenic neutrophil chimerism levels in implanted mice were observed to return to a level that is equal to the unimplanted mice by 6 wk post-implantation (Supplemental Fig. 3). Furthermore, in the splenectomy model, a reduced but still significant number of neutrophils are observed in PC following the removal of the spleen (Fig. 5). In fact, splenectomy resulted in increased BM progenitor proliferation (Supplemental Fig. 4B). Furthermore, increases in proliferating splenic progenitors are not observed at 10 wk postmicrocapsule implantation (Fig. 4). Together, these data suggest that although splenic progenitors are important in maintaining high neutrophil numbers at sites of inflammation for at least 4–6 wk, they do not have an exclusive role in neutrophil generation.

In conclusion, we show a chronic and increased presence of neutrophils at sterile inflammatory sites, resulting from microcapsule implantation that is substantially more long lasting than previously described. The increased presence of neutrophils in the PC is primarily a result of recruitment from systemic circulation and not a result of local proliferation of myeloid progenitors. Furthermore, we demonstrate that granulocytic progenitors present in the spleen play a significant role in the process of mobilizing neutrophils to peripheral sites of inflammation. This particular model of chronic neutrophil presence in peripheral tissues could be used to study neutrophil biology in vivo and help in elucidating the broader roles of splenic progenitors in sterile inflammatory reactions.

AUTHORSHIP

S.J. performed or assisted with all of the experiments and data analysis. S.A-D. and K.T. assisted with all of the experiments. B.C.T. assisted with the adoptive transfer experiments. D.L.G. and P.E.N. assisted with design of experiments and interpretation of data and helped write the paper. D.A. and U.H.v.A. designed and performed the parabiosis studies, assisted with associated data analysis, and helped write the paper. S.J., R.L., and D.G.A. designed the project, analyzed the data, and wrote the paper.

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DISCLOSURES

The authors have no conflicts of interest to declare.

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KEY WORDS:
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