Published in final edited form as:

Lab Chip. 2015 June 21; 15(12): 2625–2633. doi:10.1039/c5lc00245a.

Whole Blood Human Neutrophil Trafficking in a Microfluidic Model of Infection and Inflammation

Bashar Hamza^{a,b} and Daniel Irimia^c

Daniel Irimia: dirimia@hms.harvard.edu

^aBioMEMS Resource Center, Department of Surgery, Massachusetts General Hospital, Boston, MA 02129

^bCurrent affiliation: Electrical Engineering and Computer Science Department, Massachusetts Institute of Technology, Cambridge, MA 02139

^cHarvard Medical School, Charlestown, MA 02129

Abstract

Appropriate inflammatory responses to wounds and infections require adequate numbers of neutrophils arriving at injury sites. Both insufficient and excessive neutrophil recruitment can be detrimental, favouring systemic spread of microbes or triggering severe tissue damage. Despite its importance in health and disease, the trafficking of neutrophils through tissues remains difficult to control and the mechanisms regulating it are insufficiently understood. These mechanisms are also complex and difficult to isolate using traditional *in vivo* models. Here we designed a microfluidic model of tissue infection/inflammation, in which human neutrophils emerge from a droplet-size samples of whole blood and display bi-directional traffic between this and micro-chambers containing chemoattractant and microbe-like particles. Two geometrical barriers restrict the entrance of red blood cells from the blood to the micro-chambers and simulate the mechanical function of the endothelial barrier separating the cells in blood from cells in tissues. We found that in the presence of chemoattractant, the number of neutrophils departing the chambers by retrotaxis is in dynamic equilibrium with the neutrophils recruited by chemotaxis. We also found that in the presence of microbe-like particles, the number of neutrophils trapped in the chambers is proportional to the number of particles. Together, the dynamic equilibrium between migration, reversed-migration and trapping processes determine the optimal number of neutrophils at a site. These neutrophils are continuously refreshed and responsive to the number of microbes. Further studies using this infection-inflammation-on-a-chip-model could help study the processes of inflammation resolution. The new *in vitro* experimental tools may also eventually help testing new therapeutic strategies to limit neutrophil accumulation in tissues during chronic inflammation, without increasing the risk for infections.

[†]Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

Introduction

The innate immune system performs the difficult task of neutralizing the millions of bacteria, fungi and other microbes that attempt to invade our bodies daily. Neutrophils represent the innate immune system's first line of defence against such pathogens. They continuously survey venules and lymphatic organs for chemical cues from inflamed tissues.¹⁻³ Upon activation by diffusing chemical signals from injured tissues, neutrophils are recruited and are active at injury sites for several hours,^{4,6} efficiently clearing microbes and protecting from severe infections.^{7,8} After completing their task, neutrophils must be cleared from the infection sites to restore tissue homeostasis. Neutrophil removal is commonly thought to occur exclusively through engulfment by macrophages.^{9,10} However, emerging results from live imaging of embryonic zebrafish suggest that neutrophils can also reverse migrate away from sites of inflammation¹¹⁻¹⁴, a process that can be modulated by various mediators and compounds.^{15,16}

The trafficking of neutrophils at inflammation sites can provide useful insights into the mechanisms by which inflammation develops and resolves.^{17,18} Impairments of neutrophil clearance after acute inflammation can lead to chronic diseases such as rheumatoid arthritis, chronic obstructive pulmonary disease, and atherosclerosis.¹⁹ However, despite its importance in health and disease, neutrophil trafficking at sites of inflammation is very difficult to study, hindered by the lack of adequate tools.¹ Neutrophil chemotaxis and reverse migration at sites of injury can be monitored in transparent zebrafish models.¹¹⁻¹⁴ However, the complexity of conditions during *in vivo* experiments limits our understanding of the precise stimuli that control neutrophil migration. The relevance of the observations in zebrafish to human pathology has not yet been fully evaluated. Moreover, the presence of other types of immune cells can interfere with trafficking and activity of neutrophils at the site.²⁰ In response to these limitations, microscale platforms have been developed to enable the study of human neutrophil migration directly from whole blood,²¹⁻²⁵ and the monitoring of trafficking and interactions towards chemoattractants,²⁶ and reversed migration.¹⁵ However, the study of human neutrophil bi-directional trafficking in the presence of whole blood, in inflammation and infection models has not yet been tested.

In order to quantify the neutrophil recruitment and retention in response to well-defined inflammation and infection conditions, we designed a microfluidic device in which human neutrophils migrate in response to chemoattractants and zymosan particles, directly from a droplet of whole blood. To prevent the overcrowding of the neutrophil migration paths by red blood cells from blood, we designed several mechanical obstacles that selectively prevent the entrance of red blood cells while allowing the directed and reversed migration of neutrophils. We observed, with single-cell resolution, neutrophils arriving and leaving from the chemoattractant chamber and found that the two processes occur at comparable rates. We report that the number of neutrophils that accumulate in the device is proportional to the number of free zymosan particles. Once all zymosan particles are phagocytosed, the accumulation of neutrophils reaches a plateau and subsequent neutrophils arrive and depart from the site at comparable rates. These results suggest that a novel strategy to provide large numbers of fresh neutrophils at inflammation sites while at the same time retaining just the minimal number adequate to control the phagocytosis targets.

Results

To address the need for a functional assay to study human neutrophil response to a zymosan-induced inflammation with single cell resolution, we developed a novel microfluidic device that requires less than two microliters of whole blood. The device consists of a central loading chamber (CLC), surrounded by eight neutrophil migration channel groups (19 parallel channels with dimensions: width = 10, length = 520, and height = 5 μm) leading to chemoattractant chambers (CCs) (Fig.1A, B). At the start of the experiment, the entire device is primed with chemoattractant. Afterwards, the device is washed with buffer to form gradients by diffusion between the CCs and the central loading chamber that guide the migration of neutrophils (Fig.1C). During device priming, zymosan particles are also injected with the chemoattractant and remain trapped inside the CCs after washing (Fig.1D). Through channels between chemotaxis channel groups help with efficient washing and full clearance of the chemoattractant from the central loading well. Their positioning on the periphery of the CLC was designed to minimize the possibility of stagnant chemoattractant areas after washing, which would interfere with the migration of neutrophils towards the CCs. In the absence of these channels, the chemokine remaining in the CLC after the priming and washing steps will direct some of the neutrophils away from CCs and to locations inside the device that are not being monitored. The device, made of PDMS and bonded to glass-bottom plates, is transparent to allow for a continuous monitoring of neutrophil trafficking between whole blood and the CCs. We observed the number of accumulating neutrophils inside the CCs correlates with the number of zymosan particles in the CCs (Fig.1E).

The device enables selective neutrophil migration from whole blood droplet, while confining the red blood cells (RBCs) to the blood droplet. Thus, the device accomplishes the mechanical barrier function of the vascular endothelium through a two-stages set of microscale features, without the use of endothelial cells (Fig.2A). The design takes advantage of the fact that the RBCs in the blood drop are heavier than the volume of serum they displace. Because of this density mismatch, the RBCs sediment to the bottom of the loading chamber quickly after the blood droplet is loaded in the device. Theoretically, the equilibrium position for all RBCs in a droplet is a single monolayer of cells on the flat bottom of the device. However, the surface at the bottom of the CLC is significantly smaller. We calculated that the ~ 5 million RBCs in a 1 μL droplet of blood, will cover a surface of $\sim 200 \text{ mm}^2$ when spread in a single layer, which is significantly larger than the 4 mm^2 of the CLC. Thus, the RBCs in the blood droplet are far from the equilibrium position, they will pile up in several dozen layers in the CLC, and will also push each other laterally in all available spaces. In the absence of the barriers, they would enter the migration channels and side chambers, clogging them, and reducing the ability of neutrophils to migrate.

First, two rows of posts, spaced at 30 μm distance, confine the blood droplet laterally and restrict the number of RBCs to reach the 50 μm opening outside the migration channels. A second set of features further reduces the number of advancing RBCs and confines them into single-cell columns, with all RBCs turned on their sides (Fig.2B). These columns advance from the CLC to the CCs, being pushed by the RBCs in the loading chamber, until they encounter perpendicular walls at right angle turns. The force from the settling RBCs in the

loading chamber is counteracted by the wall perpendicular to the column, and the advance of the RBCs is significantly reduced. Some RBCs that eventually “escape” around the first corner are stopped by interactions with RBCs from adjacent migration channels. A small number of RBCs escape from these channels, and advance inside the migration channels, where they could only advance by Brownian motion or vibrations from the motorized microscope stage. Significant space remains at all times between the RBCs and the walls of the channels to allow for the diffusion of chemoattractant and the passage of moving neutrophils that are guided by these gradients. Some of the jammed RBCs may be pushed further up in the migration channels by passing neutrophils.

To quantify the efficiency of these features, we counted the number of RBCs that penetrated into the side channels at different time points (Fig.2A). We observed, on average, 3 ± 1 RBCs enter each channel at the end of an eight-hour long experiment ($N = 3$ experiments, $N = 24$ channel groups, Fig.2C). Inside the $10 \times 5 \mu\text{m}$ migration channels (width $\times$ height), the low number of RBCs did not obstruct the diffusion of the chemoattractant necessary for neutrophil migration. Using fluorescein dye, we observed gradients lasting for at least 8 hours along the migration channels (Fig.2D). This result suggests that the infiltrating RBCs effectively reduced the channel cross-sectional area available for diffusion. By performing biophysical modelling in COMSOL Multiphysics software, we were able to verify the long-lasting gradient along the channels in the presence and absence of RBCs (Fig.2D). This low number of advancing RBCs does not pose an obstacle for neutrophil passage.

In control experiments, we observed strong neutrophil migration in response to a 0-to-100 nM LTB4 gradients in the absence of zymosan. Neutrophils chemotaxed towards the CCs, and after a short delay, retotaxed towards the central loading chamber (Fig.3A, Movie S2, ESI). We observed no neutrophil migration towards zymosan-filled CCs in the absence of LTB4. In the presence of both LTB4 and zymosan particles, a new pattern for neutrophil trafficking through the CC emerged. Neutrophils engulfing zymosan particles inside the CC did not retotax and remained inside the CC (Fig.3B, Movie S2, ESI).

We recorded the entry and exit times of each neutrophil to and from the CC, and calculated the entry and exit trafficking rates between the CC and the central loading chamber. We observed sustained rates of neutrophils entering the CCs for more than 8 hours, both in the absence and presence of zymosan particles (“Enter,” Fig.3C,D). In the absence of zymosan particles, nearly 70% of all chemotaxing neutrophils retotaxed during the duration of the experiment (“Exit” profile, Fig.3C). The majority of retotaxing neutrophils actively squeezed through the RBC-jamming region to re-enter the whole blood chamber. At approximately 3 hours after the start of the experiment, the number of trapped neutrophils reached a plateau, and the entry and exit rates became comparable for the following five hours. In the presence of zymosan particles inside the CC, almost all neutrophils entering the CCs during the first five hours were trapped inside the CC (“Enter” and “Trapped” profiles, Fig.3D). The exit rate was low for five hours, two hours longer compared to the condition without zymosan, and increased in the last three hours, when it became comparable to the entry rate (Fig.3D). It is important to note that none of the neutrophils that phagocytosed zymosan particles left the CC as all exiting neutrophils lacked a fluorescence signal that would correspond to zymosan particles (Movie S2, ESI).

We mixed different amounts of zymosan particles with LTB₄ in the CCs and monitored the trafficking of neutrophils to and from the CCs and the accumulation of neutrophils inside the CCs in response to different zymosan particle loads. To facilitate more quantitative comparisons, we defined the initial slope of the “Enter” trajectory as the “entry rate” and the slope of the “Exit” trajectory after the “inflection point” to be the “exit rate” (Fig.4A). The “inflection point” was defined by the time when all zymosan particles are phagocytosed and neutrophil trafficking switches from accumulation to equilibrium.

For low zymosan particle counts (1-15 particles), the particles were rapidly cleared in the first three hours, after which the entry and exit rates of neutrophils became comparable (Fig. 4B). For high zymosan particle counts (>30), the time required for particle clearance was significantly longer (>5 hours, Fig.4C). The recorded entry rates across all zymosan-induced burdens were comparable ($p = 0.744$). However, neutrophil entry rate in all zymosan experiments with low, medium, and high burdens (8.7 ± 0.4 , 8.1 ± 1.4 , and 9.5 ± 2.6 % , respectively) were significantly higher than the control condition (4.3 ± 0.5 , $p < 0.01$, one-way ANOVA, Fig.4D). Moreover, the inflection points were significantly delayed in experiments with low, medium, and high zymosan burden (210 ± 14 , 255 ± 63 , and 310 ± 15 min, respectively) compared to no-zymosan conditions (144 ± 17 min, $p < 0.05$, one-way ANOVA, Fig.4E). The exit rate was progressively smaller with the increase in zymosan-particle burdens (2.6 ± 0.9 , 2.0 ± 1.1 , and 1.6 ± 0.3 %, for low, medium, and high burdens, respectively) and significantly lower in the presence of zymosan compared to control conditions (4.4 ± 0.2 , $p < 0.01$, one-way ANOVA, Fig.4F)..

Discussion

The microfluidic platform described here allows for the monitoring of human neutrophil trafficking in response to chemoattractant and phagocytic targets at the single cell resolution. Monitoring is performed in real time, over extended periods, and directly from a droplet of whole blood. The dynamic equilibrium between neutrophils entering and departing the chemoattractant reservoir is modulated by the presence and amount of phagocytic particles.

The ability to use whole blood is a key feature of the assay and is accomplished by two mechanisms that limit the penetration of RBCs while allowing the passage of neutrophils. An array of posts, to slow down the granular-flow of the RBCs, and an RBC-jamming region, consisting of narrow channels and 90°-turns are effective at preventing RBCs from entering the cell migration channels and chemotaxis chambers (CC). Other microfluidic devices to study the migration of neutrophils from whole blood samples only allow to study neutrophil migration on flat surfaces^{21,22,25} and are thus incompatible with the study of retrotaxis. Compared to other devices using whole blood, that were used to study neutrophil migration through channels^{23,24} the devices presented in this study extend the duration of the experiments and minimize the number of RBCs entering the migration channels. To increase the number of neutrophils migrating to the CCs and provide them with multiple choices to enter and leave these chambers, we implemented a design with several channels between the CLC and CCs. Chemoattractant gradients within the migration channels last for at least 8 hours, allowing a sufficient time to observe and study neutrophil trafficking. Moreover, the design of the RBC-jamming region accomplishes selectivity for neutrophils

since they are the only leukocytes in the blood that are able to squeeze and actively migrate through narrow openings.²⁴ The size of the channels is also comparable to the interstitial space which neutrophils are accustomed to traverse following extravasation and during tissue recruitment.²⁷

Our observations of neutrophil trafficking suggest a robust neutrophil response that accomplishes two key roles: control of infectious sites and control of inflammation. The first role is achieved by a continuous supply of fresh neutrophils and the retention of the minimum number of neutrophils necessary to neutralize the target. The continuous supply of fresh neutrophils assures that enough neutrophils are immediately available in the event of an increase in the number of targets e.g. from microbe proliferation. The retention of neutrophils after phagocytosis is also important for preventing the spread of any temporarily-surviving microbes. This is a common mechanism for neutrophils that are known to pick up several debris and microbes on their way to end-target signals.^{28,30} The change in migration patterns of neutrophils through channels after phagocytosis has also been observed and characterized before in detail.¹⁵ In previous work, we found that ~66% of neutrophils continue migrating after phagocytosis of a particle. Neutrophils continue migrating on average 500 μm after phagocytosis of a particle (~15 minutes). The results in the new system appear to be consistent with previously reported results and a comparison is included now in the discussion.

The second role is accomplished by the tuning of the dynamic equilibrium between the number of neutrophils arriving and departing the inflammation sites. This equilibrium is controlled by the local conditions in each CC. Deregulation of these mechanisms by the invading pathogens can lead to an increase in the number of trapped neutrophils, augmenting tissue damage and promoting chronic disorders.³¹ Another potential failure mode is when neutrophils take up pathogens and transport them away from the tissue.³²

The ability of neutrophils alone to achieve the dynamic balance in the absence of any other cells at the inflammation site is an important finding for our understanding of the inflammation resolution processes. Current paradigm for the resolution of inflammation is centred on the recruitment of monocytes, which stop the recruitment of neutrophils, phagocytose apoptotic neutrophils, and to promote tissue healing.^{33,34} Here we show that neutrophils alone could regulate their traffic and avoid excessive accumulation. This mechanism may provide the earliest adjustments of innate immune responses, hours before the monocytes arrive to sites of the inflammation. During prolonged inflammation, this mechanism may work in synergy with the mediators for inflammation resolution produced by monocytes. The fate of the human neutrophils leaving the inflammation sites is still unknown. Studies in zebrafish embryos have demonstrated the detection of neutrophils in many organs after local inflammation,³⁵ suggesting that neutrophils can potentially contribute to turning a local inflammatory response into a systemic multi-organ response.³⁶

Future work may clarify the mechanisms responsible to the directed migration and retrotaxis inside the device. Switching the direction of the gradient between CC and CLC may be informative for the change in chemoattractant sensitivity after phagocytosis. However, such switching would require substantial changes in the design of the devices to add the

capability for removing/switching gradients. Some of the conditions have been elucidated in previous work¹⁵, when we probed the effect of different chemoattractants and concentrations on retrotax, and modulators to increase the distance travelled by neutrophils after phagocytosis. It is possible that the neutrophils secrete new chemoattractants following the interaction with zymosan particles,¹⁷ stimulating more neutrophils to migrate towards the CCs. Finally, various sources of human blood, from healthy and diseased subjects, could be tested directly and the platform could be employed for basic science as well as clinical studies. It could also represent a screening platform of candidate compounds that modulate neutrophil trafficking and help resolve inflammation.

Experimental

Microfluidic Assay Design and Fabrication

The microfluidic device for studying human neutrophil trafficking in response to zymosan particles was designed to mimic some of the biomechanical features encountered by neutrophils in tissues. The device uses a droplet of whole blood from which neutrophils emerge to migrate through small channels towards chambers of high chemokine and zymosan concentrations. The blood is introduced into a central loading inlet (diameter = 1.5 mm) surrounded by an array of posts, for slowing down RBCs' granular flow. Eight groups of straight chemotaxis channels lead to the chemoattractant chambers after passing through a succession of turns to stop further penetration of RBCs.

Devices were fabricated using standard photolithography and soft-lithography techniques to produce final molds in polydimethylsiloxane (PDMS, Sylgard, 184, Elsworth Adhesives, Wilmington, MA) on a master four-inch silicon wafer (Desert Silicon, Grandale, AZ). Briefly, the silicon wafer was spun-coated with SU-8 5 (SU-8, Microchem, Newton, MA) to produce the first layer of the design (representing the migration channel groups with the secondary RBC filtering region). A second layer of SU-8 100 photoresist was then spun and baked, following the standard protocol as recommended by the manufacturer, to define the central and chemoattractant chambers (thickness ~120 μm). PDMS base and curing agent were mixed (10:1 ratio) and poured on the master and left to de-gas for 1 hour in a vacuum chamber. After baking for 3 hours at 80° C, the PDMS layer covering the master was peeled off, punched with a 1.5mm puncher (Harris Uni-Core, Ted Pella Inc., Reading, CA) to define the inlet to the device and then with a 5 mm puncher to release the device from the PDMS slab. Donut-shaped devices were then exposed to 35 seconds of oxygen plasma along with a glass-bottom multi-well plate (MatTek Co., Ashland, MA). The PDMS device was then bonded to the multi-well plate and baked at 70° C for 15 minutes.

Device Priming

Immediately after bonding to the multi-well plate, each device was primed with Iscove's Modified Dulbecco's Medium (IMDM, ATCC, Manassas, VA) containing human-fibronectin diluted to 100nM (Sigma-Aldrich, St. Louis, MO), and 100 nM LTB₄. Devices were then left in a desiccator connected to house vacuum for 15 minutes to ensure the full removal of air bubbles from the chemoattractant chambers. To establish the gradient along the migration channels, a 1 mL syringe containing IMDM was connected to a 30G needle and with gentle

pressure, the fresh media solution replaced the chemoattractant-containing media. Through channels, to the left and right of the migration channel groups, were designed to facilitate this rinsing step. Similarly, device priming for experiments involving Alexa-Fluor-488-labelled zymosan particles (*S. cerevisiae*, Life Technologies, Grand Island, NY) was done by first reconstituting the bioparticles in a glass vial at 20 mg mL^{-1} in IMDM. The vial was then vigorously vortexed according to the manufacturer's protocol and diluted 10-50 times into the chemoattractant mixture to produce a homogenous population of zymosan particles of different densities at the chemoattractant chamber.

Whole Blood Preparation

A 40 μL sample of capillary blood was collected from healthy volunteers. The sample was immediately pipetted into a 0.5 mL Eppendorf tube containing 40 μL of IMDM, heparin anti-coagulant, and $\sim 32 \mu\text{M}$ of Hoechst stain (Hoechst, Life Technologies, Grand Island, NY). The tube was transferred to an incubator for 10 minutes at 37°C and 5% CO_2 to allow for the proper staining of the nuclei. Whole blood was then diluted again in a 1:3 ratio with IMDM solution. Finally, 1 μL of the diluted whole blood solution was gently pipetted into the central compartment of each device using a gel-loading tip. Afterwards, the whole device was fully immersed by gently adding 3-4 mL of IMDM into the well.

Chemotaxis Imaging and Measurements

Time-lapse imaging was performed on a Nikon Eclipse Ti microscope with 10x magnification and a biochamber heated to 37°C with 5% CO_2 . Separate experiments to characterize the formation and dissipation of gradients inside the device in the presence of whole blood were performed under similar temperature and gas conditions but by replacing LTB_4 with fluorescein (Sigma-Aldrich, St. Louis, MO) of comparable molecular weight. For each experiment, at least 30 neutrophils were tracked from 3 separate devices in 3 separate wells. The entry and exit times for each neutrophil into and from the chemoattractant chamber, respectively, was recorded to produce the “Enter” and “Exit” profiles. Cells that remained at the CC were considered “Trapped”.

Statistical Analysis

“Enter”, “Exit”, and “Trapped” profiles are presented as mean values $\pm$ one standard deviation. Statistical significance of the differences between different columns of data were tested using a one-way Analysis of Variance (ANOVA). Differences were considered significant at 95% confidence level with the null hypothesis being that all mean values were the same. Once significant results were identified with the one-way ANOVA test, two-tailed t-tests were run to analyze significance between any two sets of data. Details of statistical analysis for each condition are included in the figure legends.

Conclusions

The microfluidic platform described here provides a robust and versatile experimental tool to study neutrophil trafficking in models of tissue infection and inflammation in the presence of chemical and mechanical cues as well as phagocytic targets. Novel design elements in this device, which enable the use of whole blood for the neutrophil migration assay, include the

posts and convoluted migration channels that block the advance of RBCs while enabling the migration of neutrophils. We found that the number of neutrophils departing the chambers by retrotaxis is in a dynamic equilibrium with the neutrophils recruited by chemotaxis. While the number of neutrophils trapped in the chambers is proportional to the number of microbe-like particles, our results suggest that a sophisticated system for neutrophil traffic control in tissues. This trafficking system serves to maintain a flux of fresh neutrophils at the inflammation site and represents an elegant approach to minimizing the number of neutrophils that accumulate in tissues to values that are necessary and sufficient to contain the microbes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Drs. Anna Robertson and Steven Renshaw at the University of Sheffield for the helpful discussions and Dr. Joseph Martel and Mr. Robert Kimmerling for their assistance with COMSOL-based simulations. Moreover, we thank Mr. Octavio Hurtado for assistance with device fabrication at the BioMEMS Resource Center Clean room. This work was supported by the National Institutes of Health (GM092804, EB0025003).

References

1. Amulic B, Cazalet C, Hayes GL, Metzler KD, Zychlinsky A. Annual review of immunology. 2012; 30:459.
2. Phillipson M, Kubes P. Nat Med. 2011; 17:1381. [PubMed: 22064428]
3. Summers C, Rankin SM, Condliffe AM, Singh N, Peters AM, Chilvers ER. Trends Immunol. 2010; 31:318. [PubMed: 20620114]
4. Kolaczowska E, Kubes P. Nature reviews Immunology. 2013; 13:159.
5. Kennedy AD, DeLeo FR. Immunol Res. 2009; 43:25. [PubMed: 19066741]
6. Lammermann T, Afonso PV, Angermann BR, Wang JM, Kastenmuller W, Parent CA, Germain RN. Nature. 2013; 498:371. [PubMed: 23708969]
7. Lekstrom-Himes JA, Gallin JI. The New England journal of medicine. 2000; 343:1703. [PubMed: 11106721]
8. Nemeth T, Mocsai A. Immunol Lett. 2012; 143:9. [PubMed: 22342996]
9. Gordy C, Pua H, Sempowski GD, He YW. Blood. 2011; 117:618. [PubMed: 20980680]
10. Bratton DL, Henson PM. Trends Immunol. 2011; 32:350. [PubMed: 21782511]
11. Woodfin A, Voisin MB, Beyrau M, Colom B, Caille D, Diapouli FM, Nash GB, Chavakis T, Albelda SM, Rainger GE, Meda P, Imhof BA, Nourshargh S. Nat Immunol. 2011; 12:761. [PubMed: 21706006]
12. Henry KM, Loynes CA, Whyte MK, Renshaw SA. J Leukoc Biol. 2013; 94:633. [PubMed: 23463724]
13. Shelef MA, Tazuin S, Huttenlocher A. Immunol Rev. 2013; 256:269. [PubMed: 24117827]
14. Starnes TW, Huttenlocher A. Adv Hematol. 2012; 2012:398640. [PubMed: 22844288]
15. Hamza B, Wong E, Patel S, Cho H, Martel J, Irimia D. Integr Biol (Camb). 2014; 6:175. [PubMed: 24419464]
16. Robertson AL, Holmes GR, Bojarczuk AN, Burgon J, Loynes CA, Chimen M, Sawtell AK, Hamza B, Willson J, Walmsley SR, Anderson SR, Coles MC, Farrow SN, Solari R, Jones S, Prince LR, Irimia D, Rainger GE, Kadirkamanathan V, Whyte MK, Renshaw SA. Sci Transl Med. 2014; 6:225ra29.

17. Chtanova T, Schaeffer M, Han SJ, van Dooren GG, Nollmann M, Herzmark P, Chan SW, Satija H, Camfield K, Aaron H, Striepen B, Robey EA. Immunity. 2008; 29:487. [PubMed: 18718768]
18. Weninger W, Biro M, Jain R. Nature reviews Immunology. 2014; 14:232.
19. Serhan CN. Nature. 2014; 510:92. [PubMed: 24899309]
20. Tauzin S, Starnes TW, Becker FB, Lam PY, Huttenlocher A. J Cell Biol. 2014; 207:589. [PubMed: 25488917]
21. Kasuga K, Yang R, Porter TF, Agrawal N, Petasis NA, Irimia D, Toner M, Serhan CN. Journal of immunology. 2008; 181:8677.
22. Agrawal N, Toner M, Irimia D. Lab on a chip. 2008; 8:2054. [PubMed: 19023468]
23. Jones CN, Hoang AN, Dimisko L, Hamza B, Martel J, Irimia D. J Vis Exp. 2014
24. Hoang AN, Jones CN, Dimisko L, Hamza B, Martel J, Kojic N, Irimia D. Technology (Singapore World Sci). 2013; 1:49. [PubMed: 24809064]
25. Sackmann EK, Berthier E, Schwantes EA, Fichtinger PS, Evans MD, Dziadzio LL, Huttenlocher A, Mathur SK, Beebe DJ. Proceedings of the National Academy of Sciences of the United States of America. 2014; 111:5813. [PubMed: 24711384]
26. Jones CN, Dalli J, Dimisko L, Wong E, Serhan CN, Irimia D. Proceedings of the National Academy of Sciences of the United States of America. 2012; 109:20560. [PubMed: 23185003]
27. Friedl P, Weigelin B. Nat Immunol. 2008; 9:960. [PubMed: 18711433]
28. McDonald B, Pittman K, Menezes GB, Hirota SA, Slaba I, Waterhouse CC, Beck PL, Muruve DA, Kubes P. Science. 2010; 330:362. [PubMed: 20947763]
29. Heit B, Robbins SM, Downey CM, Guan Z, Colarusso P, Miller BJ, Jirik FR, Kubes P. Nat Immunol. 2008; 9:743. [PubMed: 18536720]
30. Lee WL, Harrison RE, Grinstein S. Microbes and infection / Institut Pasteur. 2003; 5:1299. [PubMed: 14613773]
31. Serhan CN, Chiang N, Van Dyke TE. Nature reviews Immunology. 2008; 8:349.
32. Shannon JG, Hasenkrug AM, Dorward DW, Nair V, Carmody AB, Hinnebusch BJ. mBio. 2013; 4:e00170. [PubMed: 23982068]
33. Cauwe B, Martens E, Proost P, Opdenakker G. Integr Biol (Camb). 2009; 1:404. [PubMed: 20023747]
34. Soehnlein O, Lindbom L. Nature reviews Immunology. 2010; 10:427.
35. Yoo SK, Huttenlocher A. J Leukoc Biol. 2011; 89:661. [PubMed: 21248150]
36. Mulero V, Sepulcre MP, Rainger GE, Buckley CD. J Leukoc Biol. 2011; 89:645. [PubMed: 21532050]

Figure 1. Whole-blood neutrophil retrotaxis assay

(a) PDMS-based microfluidic assay consists of an array of eight chemoattractant chambers (green) positioned around a central blood sample compartment (bright red, diameter = 1.5 mm). Migration channels (width = 10, length = 520, and height = 5 μm) between the chambers and central compartment help establish the chemoattractant gradients that attract neutrophils from the whole blood sample to the chambers. Through channels on both sides of the chemotaxis channel groups allow for removal of excess blood to the surrounding medium. (B) 3D sketch of one side of the PDMS-based microfluidic device. An array of PDMS posts with spacing of 30 μm represent the primary filtering mechanism slowing down the RBC granular flow. RBC jamming section characterized by narrow channels with 90°-bends represent the secondary filtering mechanism for RBCs. Neutrophil migration channels (blue) hold chemoattractant gradients diffusing from a 400 × 40 × 120 μm (length × width × height) chemoattractant chamber (green). (C) Initial fluorescent images of the chemotaxis channels at t = 0 minutes. Dashed line is referenced in Fig.2C (D) Example of zymosan particle trapping in the CC at t = 0 minutes. (E) Number of neutrophils accumulating in the CCs is proportional to the zymosan particle count.

Figure 2.

RBC filtering mechanism and establishing long-lasting gradients. (A) Montage image representing a time-lapse of the RBC-jamming region with a single Hoechst-stained neutrophil (blue) actively migrating past the RBCs towards the 100 nM LTB₄-filled chemoattractant chamber (CC). (B) To prevent the advance of RBCs to the migration channels and allow the unrestricted passage of neutrophils to and from the CC, we designed a two step system of posts and convoluted, flat channels to block the advance of RBCs. The posts break the “pile” of RBCs from the CLC and reduce the forces pushing the RBCs into the channels. The column of RBCs in the channels (e.g. RBCs marked 1-6) is jammed at the right angle of the channels, which walls counteract most of the forces that push the RBCs inside the channels. The width of the migration channels is larger than the width of the RBCs, such that space is available for the diffusion of the chemoattractant and passage of the neutrophils. (C) RBC counts per one migration channel over time (N = 3 experiments, N = 24 channel groups). (D) Intensity profile measurement along the migration channel at times 0 and 480 minutes (black and red dots, respectively). Concentration profile modelling result along the migration channel in the presence of RBCs at times 0 and 480 minutes (green and blue lines, respectively).

Figure 3.

Neutrophil migration patterns change in the presence of zymosan particles. (A) Migration tracks for two neutrophils towards 100 nM LTB4 (no zymosan). Chemotaxis (upward-pointing black arrow) is followed by retrotaxis (downward-pointing blue arrow) towards the central whole-blood loading chamber. (B) Migration tracks for two neutrophils towards 100 nM LTB4-zymosan filled (bright green particles) CC. Red circles represent the end of the migration trajectory due to phagocytosis. Trafficking profiles of recruited neutrophils to zymosan-free CCs (C) and zymosan-filled CCs (D). “Enter” profiles represent the entry times to the CC of chemotaxing neutrophils, “Exit” profiles represent the exit time from the CC of retreating neutrophils, and “Trapped” represent the difference between the “Enter” and “Exit” profiles. “Exit” trajectory decreases significantly when compared to the control zymosan-free control condition.

Figure 4. Delayed retrotaxis during high zymosan-induced burdens

(A) Quantification of neutrophil trafficking dynamics in the proposed microfluidic assay including the entry and exit rates, and the inflection point at which neutrophils start exiting the CCs and return to blood. (B, C) Neutrophil “Enter” (black), “Exit” (blue) and “Trapped” (red) profiles over time for two different zymosan-induced burdens differentiated based on the number of particles at the CC. At high zymosan particle counts, a high overlap between the “Exit” and “Enter” profiles is observed for at least 6 hours (D) Entry rate is uniform across different zymosan burdens, but significantly higher than the control condition. (E) Inflection time point increases with the increase of the number of zymosan particles at the CC. (F) Exit rate for zymosan experiments decreases with the increase in zymosan count. Difference in exit rate between the three zymosan burden conditions was not significant. Difference between all zymosan burden and control (no zymosan) was significant. * $p < 0.05$, ** $p < 0.01$. One-way Analysis of Variance test followed by two-tailed t-test. $N = 3$ experiments, $N > 30$ cells/experiment.