Microfluidic chambers for monitoring leukocyte trafficking and humanized nano-proresolving medicines interactions

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Leukocyte trafficking plays a critical role in determining the progress and resolution of inflammation. Although significant progress has been made in understanding the role of leukocyte activation in inflammation, dissecting the interactions between different leukocyte subpopulations during trafficking is hampered by the complexity of in vivo conditions and the lack of detail of current in vitro assays. To measure the effects of the interactions between neutrophils and monocytes migrating in response to various chemotactic agents, at single-cell resolution, we developed a microfluidic platform that replicates critical features of focal inflammation sites. We integrated an elastase assay into the focal chemotactic chambers (FCCs) of our device that enabled us to distinguish between phlogistic and nonphlogistic cell recruitment. We found that lipoxin A4 and resolvin D1, in solution or incorporated into nano-proresolving medicines, reduced neutrophil and monocyte trafficking toward leukotriene B4. Lipoxin A4 also reduced the elastase release from homogenous and heterogenous mixtures of neutrophils and monocytes. Surprisingly, the effect of resolvin D1 on heterogenous mixtures was antisynergistic, resulting in a transient spike in elastase activity, which was quickly terminated, and the degraded elastin removed by the leukocytes inside the FCCs. Therefore, the microfluidic assay provides a robust platform for measuring the effect of leukocyte interactions during trafficking and for characterizing the effects of inflammation mediators.

Inflammation is a complex and finely tuned response to both infectious and noninfectious agents. The first line of defense is the innate immune system, and neutrophils are the first cells to accumulate at the site of inflammation, where they have the ability to contain invading pathogens and clear tissue debris (1). The metrics underlying these processes have been estimated using models of acute self-limited inflammation, and studies have shown that neutrophil recruitment reaches a maximum by 12 h, corresponding to the peak of inflammation. Monocytes are recruited more slowly, reaching a maximum between 24–72 h after the onset of inflammation and, in most, cases trigger the resolution phase of the inflammatory response (1, 2). Abnormal timing and dynamics of leukocyte responses are held to be the underlying causes of many inflammatory conditions, including sepsis (3), atherosclerosis (4–5), and ischemia–reperfusion injury (6). In the quest to identify and screen novel therapeutics to correct such conditions, it is critical to obtain precise measurements of both proinflammatory and proresolving processes and the underlying factors modulating leukocyte interactions.

Of increasing interest for promoting inflammation resolution is the characterization of emerging classes of lipid mediators of inflammation. These include lipoxins, resolvins, and recently developed humanized nano-proresolving medicines (NPRMs). Lipoxins are potent monocyte-specific chemottractants but are so in a non-phlogistic manner, because they are able to activate chemotaxis of monocytes without stimulating the release of proinflammatory chemokines (7) and yet can stop polymorphonuclear neutrophil (PMN) recruitment in vivo (8, 9). Resolvins are potent mediators biosynthesized from polyunsaturated fatty acids, with each potent member sharing a defining action in resolving inflammation (7, 10–12). NPRMs were first described by Norling et al. (13) as derivations of microparticles (MPs) released from neutrophils upon activation that exert proresolving and anti-inflammatory actions (14). To augment the proresolving activities of MPs, MPs were enriched with proresolving lipid mediators to produce NPRMs (8, 15).

Toward the goal of characterizing the temporal aspects of leukocyte accumulation in conditions relevant for inflammation dynamics, several techniques have been developed to study leukocyte trafficking. In vivo imaging techniques using two-photon techniques (16), micro–single-photon emission computed tomography (17), and fluorescent cell-tracking dyes have been designed for the monitoring of labeled leukocytes during distinct stages of the inflammatory response. These assays can only provide information in a limited spatiotemporal frame, and control over the conditions at the inflammation sites is very challenging. In vivo assays, such as the mouse air-pouch model (18), peritoneal lavage (DPL) (19), or bronchoalveolar lavage (BAL) (20), provide bulk measurements for leukocytes responding to a given proinflammatory stimulus. An in vivo human model for inflammatory responses, established by Segal et al., records the accumulation of cells in cantharidin-induced skin blisters (21, 22). Temporal resolution in these studies was limited to 24-h increments, and, in general, all in vivo assays lack the resolution to capture the complex leukocyte interactions taking place during the response. For the study of human leukocyte responses, the primary systems used are classic in vitro migration assays, such as the membrane filter method. Introduced by Boyden in 1962, the filter method is an end-point assay, unable to capture the detailed dynamics of the multiple cells types involved in these responses. Microfluidic devices have emerged to avoid these shortcomings and provide novel capabilities for multiple stable gradients (23), dynamic gradient changes (24), higher throughput (25), or ease of use (26). However, none of these devices has the ability to model neutrophil and monocyte interactions while trafficking toward sites of inflammation.

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Conflict of interest statement: C.N.S. is listed as an inventor on patents related to resolvins that have been assigned to Brigham and Women’s Hospital and licensed to Resolvyx Pharmaceuticals. C.N.S. is a scientific founder of Resolvyx Pharmaceuticals and owns equity in the company. The interests of C.N.S. were reviewed and are managed by the Brigham and Women’s Hospital and Partners HealthCare in accordance with their conflict of interest policies. The other authors declare no conflict of interest.

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To address the limitations of current techniques, we designed a microscale assay that allows real-time high-resolution measurement of human leukocyte chemotaxis in response to soluble mediators (Fig. 1B). We validated these devices by showing, first, that human neutrophils accumulate more rapidly than monocytes toward specific chemoattractants, in agreement with previous observations in murine models. We next used these devices to investigate direct actions of lipoxins, resolvins, and humanized NPRMs. By incorporating an elastin degradation assay into the devices, we characterized the inflammatory phenotype of the innate immune cells recruited in response to lipid mediators in vitro, showing that leukotriene (LT)B4 and lipoxin (LX)A4 recruit functionally distinct human monocyte, and resolvin (Rv)D1 changes the interactions between neutrophils and monocytes.

**Results**

**Microfluidic Platform Design and Characterization.** Chemoattractant gradients were established between an array of 16 focal chemotactic chambers (FCCs) at the periphery and 1 central cell loading chamber (CLC) before cells were introduced into the device. The FCC, with a volume of ~2.6 nL, simulated the inflammatory focus, whereas the central CLC served as a cell source and also acted as a sink for the chemoattractant diffusing from the FCCs (Fig. 2A–C). As expected, neutrophils started to migrate toward the preestablished LT(B)4 chemotactant gradient source in the FCC minutes after being introduced into the CLC, and their numbers reached a plateau after 5 h (Fig. 2D and Movie S1). A bifurcation in the migration channel enabled us to distinguish between chemotactic and chemokinetic cell migration (Fig. 2A). Cells that follow the chemotactic gradients would turn at the bifurcation toward the FCC, whereas cells moving by chemokinesis would migrate in equal numbers toward the FCC and the exit channel leading outside of the device. The number of cells with directional migration toward the FCC was one order of magnitude larger than the number of cells that exited the device (9:1 ratio), confirming that we are measuring chemotactic cell migration. After the cells entered the FCC, their migration patterns changed, and cells appeared to be migrating randomly inside the FCC (chemokinesis).

To evaluate the dynamics and stability of the chemoattractant gradient developed between the FCC and the CLC, we primed the device with fluorescently labeled dextran (molecular mass, 1,000 Da) and measured the fluorescence levels over time. Linear gradients of chemotactant are formed along the 400-μm-long, 10 × 10 μm cross-section migration channels, and last for more than 24 h after priming the FCCs with the chemoattractant (Fig. S1A). Biophysical modeling of chemoattractant diffusion in our device using the COMSOL simulation package shows that a chemotactant gradient along the migration channel to the CLC are formed in less than 15 min for all chemoattractants, decrease by less than 10% at 6 h, and are still present at 24 h after the start of the experiments (Fig. S1B and C).

**Platform Validation with Neutrophils, Monocytes, and Standard Chemoattractants.** We validated the assay by measuring the accumulation of neutrophils and monocytes from a 2:1 heterogeneous cell mixture, toward a panel of known chemoattractants (Fig. 3). As expected, predominantly monocytes (red) were attracted toward monocyte chemotactic protein (MCP)-1 (100 nM) (Fig. 3A), whereas interleukin (IL)-8 (10 nM) and N-formyl-methionine-leucine-phenylalanine (fMLP) (100 nM) were found to elicit selectively neutrophil (blue) chemotaxis (Fig. 3B and C). A negligible number of cells entered the FCCs in the absence of a chemoattractant (Fig. 3D).

LT(B)4 induced the recruitment of both monocytes and neutrophils toward the FCC, with distinct dynamics between the two cell types (Movie S2). Whereas neutrophils showed a strong and rapid response, reaching a plateau within the first 5 h, monocyte recruitment only reached a maximum after 8–9 h, and the magnitude of cell recruitment was four to five times lower (Fig. 3E). The differences in the accumulation dynamics between neutrophils and monocytes could be explained by the delayed activation of the monocytes, as well as differences in migration speed between the two cell types. Whereas neutrophils move through channels at 18 ± 5 μm/min (26) and could reach the FCC in less than 30 min, we estimated the monocyte velocity at...
5 ± 7 μm/min and calculated that monocytes would reach the FCC more than 90 min after entering the migration channels. LXA₄ induced selective monocyte recruitment that was faster and reached a plateau earlier (10 h) compared with MCP-1 (15 h; Fig. 3 A and F). Of interest, when the chemoattractant was LXA₄, we observed two plateaus in the monocyte accumulation dynamics, suggesting the possibility that LXA₄ acts as a chemoattractant to two monocyte subpopulations that migrate at different rates. Monocyte recruitment toward LXA₄ displayed the classic bell-shaped dose-dependence characteristic for chemotaxis responses to chemoattractants, with maximal recruitment observed at the 100 nM concentration (Fig. 3G). We also found that chemokinesis inside the FCC stops after ∼4 h in the presence of proresolving chemoattractants, such as LXA₄, whereas it continues well beyond 4 h in the presence of proinflammatory chemoattractants such as LTB₄ and fMLP. Delay time, accumulation time, and the final plateau values for both neutrophils and monocytes when migrating to the FCC in response to the panel of chemoattractants are summarized in Table S1.

To demonstrate that the final plateau number is characteristic of the cells behavior rather than an artifact of the device, we compared monocyte accumulation in the standard FCCs (2.6-nL volume) and a larger FCC (6.6 nL; large-to-small ratio, 2.5). The $t_{1/2}$ was approximately the same for both chambers (large-to-small ratio, 0.98), showing that the dynamics of cell accumulation are independent of the chamber size. The plateau number of monocytes increased with the increasing size of the chambers (large-to-small ratio, 1.7). However, the increase in monocyte number was not proportional to the size ratio, suggesting that final plateau numbers depend less on FCC volume than on cell characteristics (Fig. 3H).

LXA₄, RvD1, and NPRMs Significantly Reduce Monocyte and Neutrophil Chemotaxis. To quantify the modulation of monocyte trafficking in the presence of established proresolving compounds, we measured the effect of pretreatment with LXA₄ and RvD1 on the accumulation rates of monocyte toward LTB₄ as a chemoattractant (100 nM). We measured a 40% and 20% decrease in monocyte accumulation with LXA₄ and RvD1 pretreatment, respectively (Fig. 4A). The actions of LXA₄ appear to be proportional with the starting monocyte density in the CLC, with larger monocyte accumulation for the higher loading densities (Fig. 4B). We found that monocyte responses toward LTB₄ were blunted at lower seeding densities (Fig. 4B), and a density of 20,000 monocytes/chamber, which produced a monolayer of cells in the CLC, was determined to be optimal and was used for all of the following experiments.

We next used the trafficking platform to assess the actions of NPRMs (13), on both monocyte and neutrophil trafficking under coincubation conditions. When the leukocytes were exposed to NPRMs with a LXA₄ stable analog incorporated within their bilayers, the rate and numbers of monocyte recruitment toward an LTB₄ gradient were significantly ($P < 0.01$) reduced compared with LXA₄ alone (Fig. 4C). When the leukocytes were exposed to nanoparticles (NPs), which are MPs that underwent the same preparation process as NPRMs but were not enriched with proresolving lipid mediators (13), their inhibitory effect was weaker than the NPRMs. However, their inhibitory effect was still significant and likely attributable to their content of Annexin 1 (14) and is consistent with reported anti-inflammatory activity (13, 14). The average rate of cell accumulation to FCC decreased by an order of magnitude for both monocytes (down from 10 to 1 cells/h; Fig. 4C) and neutrophils (down from 50 to 5 cells/h; Fig. 4D) in the presence of NPRMs.

Heterogeneous Cell–Cell Interactions Impact Migration of Monocytes and Neutrophils. To further dissect the role of heterogeneous cell–cell interactions during cell trafficking in the presence of humanized NPRM, we compared the accumulation of human neutrophils and monocyte from homogenous, as well as heterogeneous mixtures. Monocyte trafficking was enhanced under

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**Fig. 3.** Device validation. Neutrophils (blue) and monocytes (red) dynamically migrate to different chemoattractants. Graphs correspond to average cell counts in all FCCs ($n = 16$). (A–F) MCP-1 (100 nM) (A), IL-8 (10 nM) (B), fMLP (100 nM) (C), negative control (D), LTB₄ (100 nM) (Movie S2) (E), and LXA₄ (100 nM) (F). (G) Dose-response of LXA₄ as a chemoattractant to monocytes. Experiments were repeated with blood from three healthy donors for each condition. (H) Monocyte counts vs. time for two different-sized (6.6 vs. 2.6 nL) FCCs. The $t_{1/2}$ was ∼5 h for both chambers (5 vs. 5.25), and the $t_{max}$ occurred at 13 h (45 cells) and 9 h (28 cells) for the large and small FCCs, respectively.
coincubation conditions, and NPRMs incorporating either RvD1 or a LXA4 stable analog (9,12-benzo-LXA4) significantly reduced monocyte trafficking toward an LTB4 gradient (P < 0.01) (Fig. 4E). Conversely, neutrophil trafficking toward an LTB4 gradient was reduced in coincubation conditions (P < 0.01) (Fig. 4F). Here, exposure of the neutrophils with either RvD1- or 9,12-benzo-LXA4-containing NPRMs significantly reduced neutrophil accumulation in response to LTB4 under both mono- and coincubation conditions. At the same time, the 9,12-benzo-LXA4 incorporated in NPRMs was observed to be more potent at inhibiting neutrophil chemotaxis (Fig. 4E). Using fluorescently labeled NPRMs, we conducted real-time monitoring of these nanostructures following incubation with human monocytes. We observed that monocytes carried these nanostructure with them from the CLC into the FCC, and we observed as many as one intact NPRM to every three monocytes recorded in the FCC (Movie S3). This finding illustrates a potential mechanism of action of these nanostructures whereby migrating monocytes could redistribute the NPRMs into an inflammatory site.

Elastase Functional Assessments In Situ. To distinguish between phlogistic and nonphlogistic recruitment of monocytes, we developed a functional assay for measuring the levels of elastase produced in real time by monocytes accumulating in the nanoliter chambers. Elastase, a serine protease that hydrolyses amides and esters, is an enzyme up-regulated following phlogistic activation of monocytes (27). We validated this assay by quantifying elastase release in neutrophils, a cell type known to secrete high levels of this protein upon activation. We observed a significant increase in fluorescence levels during neutrophil recruitment toward LTB4 (Fig. 5A, Movie S4, and Fig. S2A). During monocyte trafficking toward LTB4, we also measured elevated elastase activity, suggestive of an activated monocyte phenotype. Elastase activity in monocytes was two orders of magnitude lower than activity detected in neutrophils and appeared to be more localized around only a subset of the monocytes recruited into the FCC (Fig. 5B, Movie S5, and Fig. S2B). A calibration between cofluorescence units and concentration of elastase (units per milliliter) was established with R = 0.95 (Fig. S2C). In longer-duration experiments, we determined that the fluorescence loss during imaging over 10 h is less than 10% from the starting levels. A heterogeneous population of neutrophils (blue) and monocytes (bright field) migrate to LTB4 (100 nM) and produce a relative fluorescent signal an order of magnitude less than neutrophil monoculture (Fig. 5 C and D). Monocytes attenuate neutrophil elastase production, as is known to occur in vivo (28).

When neutrophils were pretreated with RvD1, a proresolving lipid, migration to LTB4 decreased by two-thirds (63 ± 12%), and the production per cell of elastase decreased by two orders of magnitude. Approximately half (45 ± 5%) of neutrophils stopped moving and had degraded nuclear structure after 15 h (Fig. 5E). Monocytes (bright field) pretreated with RvD1 migrated (44 ± 22%) less to LTB4 and produced a third less elastase. Monocyte chemokinesis was also reduced, and monocytes stopped migrating after entering FCCs and remained in the first third of the total FCC surface (Fig. 5F). When a heterogeneous population of neutrophils and monocytes migrated to RvD1, elastase production surged almost as high as in neutrophil monoculture and then attenuated quickly after 10 h (Fig. 5 G and H).

Pretreatment with LXA4 (100 nM) of neutrophils reduced the number of cells that migrated toward LTB4 (100 nM) and total elastase release (Fig. 5J). Monocytes migrating toward LTB4 after pretreatment with LXA4 (100 nM) accumulated in smaller numbers and aggregated close to the entrance, in a pattern comparable to that observed for RvD1 (Fig. 5K). In coincubation and pretreatment with LXA4 conditions, a smaller number of neutrophils and monocytes migrated toward the FCC, and elastase release was reduced to insignificant levels (Fig. 5K and L).

By monitoring the localized elastase activity around monocytes accumulating in the FCC in response to LTB4, we observed that mostly monocytes with high elastase activity arrived to the chamber during the first hours, whereas monocytes with low or no elastase activity accumulated over a longer period (Fig. 5B). Overall, the accumulation of two monocyte phenotypes to the FCC could be responsible for the different rates of total elastase increase over time (Fig. 5D). It is possible that the two monocyte phenotypes correspond with classic monocyte subpopulations defined based on surface markers and recently shown to express high levels of another serine protease, myeloperoxidase. We measured significantly lower elastase activity in monocytes when LXA4 was the chemoattractant stimulus (79.9% lower;
elastin degradation products out of the FCC.

... and the number of cells (tens to hundreds), as well as the min-

sensitivity of the assay to cellular activities is the result of the

clearance of elastin-degradation products by phagocytic cells can be moni-

This is accomplished by the integration of a

... and the resolution of the inflammation. The high

... the intensity of green fluorescence (D). Neutrophils alone produce two orders of magnitude

more elastase than monocytes alone and one order of magnitude more elastase than a heterogeneous population of both cell types. A negative control illustrates the lack of elastase signal from a heterogeneous population of cells in the absence of a chemoattractant gradient. Neutrophils (blue; E), monocytes (bright field; F), and neutrophils and monocytes (G) pretreated with RvD1 (10 nM) migrate to LTβ and produce various amounts of elastase quantified by the intensity of green fluorescence (H). Pretreatment with RvD1 reduced elastase secretion by two orders of magnitude for both neutrophils and monocytes. In the heterogeneous cell population, there is a transient spike of elastase secretion at the same magnitude as without RvD1 pretreatment. Neutrophils (blue; I), monocytes (red; J), and neutrophils and monocytes (K) pretreated with LXA4 (10 nM) migrate to LTβ and produce various amounts of elastase quantified by the intensity of green fluorescence (L). LXA4 pretreatment with LXA4 reduces elastase production in all cell populations by over an order of magnitude. Results are representative of two distinct experiments (n = 18 FCCs).

Discussion

The microfluidic chambers for neutrophil and monocyte traf-

... the need for external pumps (compared with other microfluidic devices) and facilitates scaling up of the assay. One potential problem in our device is that the chemoattractant gradient will change over time. A finite element model that simulates che-

... uses a cross-section of the migration channels used in the current assay (10 × 10 μm) is five times larger, it is unlikely that neutrophils could be slowed down through a steric hindrance mechanism. Overall, we consider that the slow decay of the gra-

P < 0.01) or when monocytes were pretreated with LXA4 (10 nM) (89.3% lower; P < 0.01) before migration to LTβ (Fig. S3). The reduction in elastase activity was also significant when normalized to the number of monocytes in the chamber, suggestive for a proresolving monocyte phenotype.
The complexity of neutrophil–monocyte interactions during trafficking could not be captured using traditional chemotaxis assays. Conventional assays, such as the ubiquitous transwell devices, only provide bulk measurements of migrating cells, without single-cell resolution and are end-point assays, giving no information on the temporal course of cell accumulation. Other assays, such as the fluorescence-activated cell sorter (FACS) used to study heterogeneous cell mixtures, can only measure surface markers and cannot be used to study molecules that are secreted or other functions of the cells. Emerging microfluidic devices have also focused on traditional issues, related to either chemotaxis, cell secretion, or cell–cell interactions (31); however, our device integrates all of these aspects of cellular activity in one assessor (21); Lindbom et al. (2010) Phagocyte partnership during the onset and resolution of inflammation. Nat Rev Immunol 10(6):427–439.


The elastase spike from neutrophils after coincubation and RvD1 fl is more complex than predicted by the current paradigm of inflammation-resolution and enhances stability and potent anti-inflammatory effects of the cells. Emerging microfluidic devices, only provide bulk measurements of migrating cells, without single-cell resolution and are end-point assays, giving no information on the temporal course of cell accumulation. Other assays, such as the fluorescence-activated cell sorter (FACS) used to study heterogeneous cell mixtures, can only measure surface markers and cannot be used to study molecules that are secreted or other functions of the cells. Emerging microfluidic devices have also focused on traditional issues, related to either chemotaxis, cell secretion, or cell–cell interactions (31); however, our device integrates all of these aspects of cellular activity in one assessor (21); Lindbom et al. (2010) Phagocyte partnership during the onset and resolution of inflammation. Nat Rev Immunol 10(6):427–439.


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Supporting Information

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SI Text

SI Materials and Methods

Microfluidic Device Fabrication. Microfluidic devices used to measure leukocyte migration in response to chemoattractant gradients were manufactured using standard microfabrication techniques. Two layers of photoresist (SU8; Microchem), the first one 10 μm thick (corresponding to the migration channels) and the second one 70 μm thick (corresponding to the FCCs) were patterned on one silicon wafer sequentially using two photolithographic masks and processing cycles according to the instructions from the manufacturer. The wafer with patterned photoresist was used as a mold to produce polydimethylsiloxane (PDMS) (Fisher Scientific) devices, which were then bonded to the base of glass-bottom 12- or 24-well plates, using an oxygen plasma machine (Nordon-March).

Preparation of Humanized NPRMs. Human MPs were prepared (1) and added to thin lipid films in glass flasks (after organic solvent removal by rotary evaporation; 10 min; 25 °C) containing fluorescent 1,2-dioleoyl-sn-glycero-3-phospho-1-serine-N-7-nitro-2,1,3-benzoazadiazol-4-yl (100 μg; Avanti Polar Lipids) and 75,8R, 175-trihydroxy-docosa-4Z,9E,11E,13Z,15E,17Z-hexaenoic acid resolving D1 (RvD1), 1 μg (Cayman Chemical), or O-[9,12]-benzo-6-epi-lipoxin A4 (LXA4 analog) (1 μg) (2) prepared for these studies by custom synthesis (Avanti Polar Lipids). Interrcalation of RvD1 or LXA4 analog and fluorescent phospholipid was performed by aqueous energy dissemination using a sonic dismembrator (output power, 15 W; 15 min; 25 °C; Fisher Scientific). Humanized NPRMs were layered on Sephadex G50 columns (Sigma-Aldrich), and fractions were collected in 0.2-μm-filtered PBS. Incorporation of RvD1 and LXA4 analog was determined using LC-MS/MS, and fluorescent phospholipid content was confirmed by flow cytometry (FACSCanto II; BD Biosciences). NPRMs were sized using calibration beads (Corpuscular) by flow cytometry. Primary human neutrophils and monocytes were pretreated for 15 min at 37 °C with two lipid mediators, LXA4 (10 nM) and RvD1 (10 nM), known to be anti-inflammatory and proresolving. In a similar fashion, cells were pretreated with the NPRM at a ratio of 2:1 NP per cell.

Primary Human Neutrophil and Monocyte Isolation. De-identified, fresh human blood samples from healthy volunteers, aged 18 y and older, who were not receiving immunosuppressants, were purchased from Research Blood Components, LLC. Peripheral blood was drawn in tubes containing a final concentration of 5 mM EDTA (Vacutainer; Becton Dickinson) and processed within 2 h of blood collection. Using a sterile technique, neutrophils and monocytes were isolated from whole blood using HetaSep and a Ficoll-Paque gradient, respectively, followed by the EasySep Human Neutrophil and Monocyte Enrichment Kits (STEM-CELL Technologies) following the manufacturer’s protocol. The purity of neutrophils and monocytes was assessed to be >98% using the Sysmex KX-21N Hematology Analyzer (Sysmex America, Inc.). Neutrophils were stained with Hoechst (32.4 μM) (Sigma), and monocytes were stained with the PKH26 Red Fluorescent Cell Linker Kit for Phagocytic Cell Labeling (4 μM) (Sigma) following the manufacturer’s protocols. The final aliquots of neutrophils and monocytes were resuspended in RPMI plus 10% FBS (stock 50 mL FBS/450 mL RPMI) (Sigma-Aldrich) at a concentration of 20,000 cells/2 μL and kept at 37 °C during pretreatment with different proresolving molecules and NPRMs. Cells were then immediately introduced into the microfluidic device for the chemotactic assay. The ratio between neutrophils and monocytes for the heterogeneous samples was 2:1. All experiments were repeated at least three times with neutrophils and monocytes from three different donors.

Microfluidic Assay Preparation and Analysis. To prime the microfluidic devices, 15 min before neutrophils were introduced, each CLC was filled with and surrounded by 10 μL of the chemotactant of interest and extracellular matrix protein. The device was then placed in a vacuum for 15 min. The chemotactant filled all of the FCCs as the air was displaced. The devices were then vigorously washed three times with 1× PBS to remove any residual chemotactant that was outside of the FCCs. The device was then submerged in 0.5 mL of cell media. Neutrophils and monocytes (20,000 cells/2 μL) were then pipetted into the CLC using a gel-loading pipette tip. Neutrophil migration into the migration channel toward the FCC started immediately and was recorded using time-lapse imaging on a fully automated Nikon TIE microscope (10× magnification) with biochamber heated to 37 °C with 5% carbon dioxide gas. Monocyte migration began after a 2-h delay. Image analysis was performed using Elements software (Nikon). Cell counts in the FCC were determined automatically using Elements software.

Functional Single-Cell Elastase Assay. To determine whether monocytes recruited to the FCC were phlogistic or nonphlogistic, we implemented a functional assay in the FCC. We used EnzChek Elastase Assay Kit from Molecular Probes, in which a DQ elastase is added to the chemokine solution. We created a calibration curve relating fluorescence to elastase activity (units per milliliter) for 1-, 2-, 5-, and 8-h incubation times were created by priming the device with a known concentration of porcine pancreatic elastase. We created a calibration curve to correlate fluorescence units to elastase activity (units per milliliter) (R² = 0.9992). Neutrophils were used as a direct positive control and were measured with 2,500 fluorescence units after 5 h of culture.

Fig. S1. Diffusion modeling of chemoattractant gradient in device. (A) Finite element model (COMSOL) of diffusion of chemoattractant gradient of device and comparison with experimental measurements of small-molecular-mass (1 kDa) fluorescent dye diffusing out of the FCCs. (B) Visualization of the gradient in the migration channels as simulated using COMSOL finite element modeling software package. (C) Summary of diffusion coefficients for chemoattractants used in experiments and the time for concentration of chemoattractant in the FCC to decrease to half of its original levels.

<table>
<thead>
<tr>
<th>Molecule Type</th>
<th>Molecular Weight [kDa]</th>
<th>Diffusion Coefficient [cm^2/s]</th>
<th>[FCC] t_{1/2} [hrs]</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCP1</td>
<td>1.1</td>
<td>3.9*10^{-7}</td>
<td>34</td>
</tr>
<tr>
<td>IL-8</td>
<td>8</td>
<td>3.5*10^{-7}</td>
<td>38</td>
</tr>
<tr>
<td>fMLP</td>
<td>0.4</td>
<td>10^{-6}</td>
<td>11</td>
</tr>
<tr>
<td>LTB4</td>
<td>0.3</td>
<td>10^{-6}</td>
<td>11</td>
</tr>
<tr>
<td>LXA4</td>
<td>0.3</td>
<td>10^{-6}</td>
<td>11</td>
</tr>
<tr>
<td>Elastin</td>
<td>640</td>
<td>10^{-8}</td>
<td>&gt; 20 days</td>
</tr>
<tr>
<td>Tropoelastin</td>
<td>70</td>
<td>10^{-7}</td>
<td>8 days</td>
</tr>
<tr>
<td>Elastase</td>
<td>29.5</td>
<td>2*10^{-7}</td>
<td>4 days</td>
</tr>
</tbody>
</table>
Fig. S2. Elastase assay validation. (A) Neutrophils migrate to LTB₄ (100 nM), which stimulates elastase activity. Using the EnzChekElastase Assay Kit from Molecular Probes, a fluorescent signal is produced upon substrate cleavage by the enzyme. The sequence of time-lapse images illustrates the strong elastase activity after 5 h (Movie S4). (B) Monocytes migrate to LTB₄ and a halo around the cells is observed identifying enzyme after 10 h (Movie S5). (C) Calibration curve created by adding known concentrations of porcine pancreatic elastase to the FCC.

Fig. S3. Monocyte migration and elastase release. (A) Monocytes migrating to LTB₄ (100 nM) produce an elastase signal of ~0.1 U/mL after 6 h. (B) Monocytes migrate to LXA₄ (100 nM), and the elastase signal is significantly reduced compared with that seen with migration to LTB₄ (100 nM). (C) Monocytes are pretreated with LXA₄, and migration dramatically decreases along with the production per cell of elastase. (D) Graph of elastase release (units per milliliter) vs. time (hours) for previously described conditions (A–C).
### Table S1. Summary of dynamic constants describing neutrophil and monocyte migration to an array of chemoattractants

<table>
<thead>
<tr>
<th>Chemoattractant</th>
<th>Function in vivo</th>
<th>Cell type</th>
<th>Delay (h)</th>
<th>Accumulation time (h)</th>
<th>Plateau no. of cells</th>
<th>Rate (cells/h)</th>
<th>t_{1/2} (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>fMLP</td>
<td>Proinflammatory; derived from bacterial protein degradation or from mitochondrial proteins upon tissue damage; strong chemoattractant induces adherence, degranulation, and production of tissue-destructive oxygen-derived free radicals in phagocytic cells</td>
<td>PMN</td>
<td>0.16</td>
<td>4</td>
<td>285</td>
<td>71</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mono</td>
<td>6</td>
<td>8</td>
<td>17</td>
<td>2.1</td>
<td>7</td>
</tr>
<tr>
<td>IL-8</td>
<td>Proinflammatory; produced by macrophages, epithelial, and endothelial cells; induces chemotaxis of granulocytes</td>
<td>PMN</td>
<td>&gt;0.16</td>
<td>3</td>
<td>173</td>
<td>57.7</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mono</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>LTB₄</td>
<td>Proinflammatory; produced from leukocytes in response to inflammatory mediators; induces the adhesion and activation of leukocytes on the endothelium, allowing them to bind to and cross it into the tissue; potent chemoattractant and induces the formation of reactive oxygen species and the release of lysosome enzymes by PMNs</td>
<td>PMN</td>
<td>&gt;0.16</td>
<td>2.5</td>
<td>282</td>
<td>112.8</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mono</td>
<td>4</td>
<td>7</td>
<td>73</td>
<td>10.4</td>
<td>6</td>
</tr>
<tr>
<td>MCP-1</td>
<td>Proinflammatory; produced by endothelial cells, mononuclear cells, mast cells, T cells, osteoblasts, and fibroblasts; attracts monocytes and basophils but not neutrophils or eosinophils; may be involved in the recruitment of monocytes into the arterial wall during the disease process of atherosclerosis</td>
<td>PMN</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mono</td>
<td>4</td>
<td>&gt;15</td>
<td>44</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>LXA₄</td>
<td>Anti-inflammatory and proresolving; biosynthesized by leukocytes; inhibits platelet activating factor (PAF)- or fMLP-induced neutrophil and eosinophil chemotaxis in vitro</td>
<td>PMN</td>
<td>9</td>
<td>3</td>
<td>5</td>
<td>2.5</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mono</td>
<td>3</td>
<td>9</td>
<td>31</td>
<td>3.4</td>
<td>4</td>
</tr>
</tbody>
</table>

For further detail on the chemoattractant mediators see refs. 1 and 2. Mono, monocyte. Ø, no migration.

**Movie S1.** Double-stained (blue, nucleus; red, membrane) neutrophils begin migrating along the chemoattractant gradient after 20 min. Cells continue to migrate into the FCC until the chamber is filled with cells by 5 h. There is no pressure gradient influencing cell migration in microfluidic device.

**Movie S1**

**Movie S2.** Neutrophils (bright field) and monocytes (red) dynamically migrate to LTB4 (100 nM). Neutrophils were imaged every 10 min, and PKH26 fluorescently tagged monocytes every hour. This in vitro model correlates to the dynamics seen in vivo where neutrophils migrate to afflicted site after injury or microbial infection. This is followed by migration of monocytes to site of inflammation.

**Movie S2**
Movie S3. Monocyte migration after interacting with a fluorescently labeled (green) proresolving nanomedicine particle (NPRM). Images were acquired every 20 min.

**Movie S3**

Movie S4. Neutrophils migrate to LTB₄ (100 nM), which stimulates elastase activity. Using the EnzChekElastase Assay Kit from Molecular Probes, a fluorescent signal is produced upon substrate cleavage by the enzyme. The movie illustrates the strong elastase activity after 5 h.

**Movie S4**
Movie S5. Monocytes migrate to LTB₄, which stimulates elastase activity. Using the EnzChekElastase Assay Kit from Molecular Probes, a fluorescent halo around the cells is observed identifying enzyme after 10 h.

Movie S5