Strategies of professional phagocytes in vivo: unlike macrophages, neutrophils engulf only surface-associated microbes

Emma Colucci-Guyon1,2,*, Jean-Yves Tinevez3, Stephen A. Renshaw4 and Philippe Herbomel1,2,*

1Institut Pasteur, Unité Macrophages et Développement de l’Immunité, Département de Biologie du Développement, F-75015 Paris, France
2CNRS, URA2578, F-75015 Paris, France
3Institut Pasteur, Imagopole, Plate-forme d’Imagerie Dynamique, F-75015 Paris, France
4MRC Centre for Developmental and Biomedical Genetics and Department of Infection and Immunity, University of Sheffield, Western Bank, Sheffield S10 2TN, United Kingdom

*Authors for correspondence (emma.colucci@pasteur.fr; philippe.herbomel@pasteur.fr)

Accepted 17 May 2011
Journal of Cell Science 124, 3053–3059
© 2011, Published by The Company of Biologists Ltd
doi: 10.1242/jcs.082792

Summary
The early control of potentially invading microbes by our immune system primarily depends on its main professional phagocytes – macrophages and neutrophils. Although the different functions of these two cell types have been extensively studied, little is known about their respective contributions to the initial control of invading microorganisms before the onset of adaptive immune responses. The naturally translucent zebrafish larva has recently emerged as a powerful model vertebrate in which to visualise the dynamic interactions between leukocytes and microbes in vivo. Using high-resolution live imaging, we found that whereas macrophages efficiently engulf bacteria from blood or fluid-filled body cavities, neutrophils barely do so. By contrast, neutrophils very efficiently sweep up surface-associated, but not fluid-borne, bacteria. Thus the physical presentation of unopsonised microbes is a crucial determinant of neutrophil phagocytic ability. Neutrophils engulf microbes only as they move over them, in a ‘vacuum-cleaner’ type of behaviour. This context-dependent nature of phagocytosis by neutrophils should be of particular relevance to human infectious diseases, especially for the early phase of encounter with microbes new to the host.

Key words: Neutrophils, Macrophages, Professional phagocytes, Live imaging, Host–microbe interaction, Innate immunity, Zebrafish

Introduction
When potentially infectious microbes penetrate epithelial barriers and invade the host’s tissues, they first encounter innate antimicrobial mechanisms. These mechanisms mainly rely on the activities of the two dedicated so-called ‘professional phagocytes’, macrophages and neutrophils. The differential features of these two cell types and the molecular mechanisms underlying microbe phagocytosis and killing have been extensively studied. However, these studies have been mostly conducted in cell culture, using macrophage or neutrophil cell lines, human blood or mouse bone-marrow-derived phagocytes (Kantari et al., 2008; Nathan, 2006). Therefore, little is known about the relative contribution of macrophages and neutrophils in the initial phase of encounter with a potentially invasive microbe in vivo.

Originally introduced as a new model vertebrate organism in developmental biology (Streisinger et al., 1981), the zebrafish (Danio rerio) has emerged in the last decade as a powerful non-mammalian model to study the development and function of the immune system (Lieschke and Trede, 2009). The small size and natural translucency of swimming zebrafish larvae make it possible to follow leukocyte deployment and behaviour in vivo throughout the organism, at high resolution. As the immune system develops gradually, its adaptive arm becomes operational – in terms of ability to mount an antibody response – only when the larva develops into a juvenile fish (Lam et al., 2004). Thus, the larva has a purely innate immune system, consisting of macrophages and neutrophils (Bennett et al., 2001; Herbomel et al., 1999; Lieschke et al., 2001). It is therefore especially suitable for an in vivo investigation of innate immune responses to invading microorganisms in real time (Davis et al., 2002; Tobin et al., 2010).

In a previous study of zebrafish neutrophil development, we began to study neutrophil behaviour towards microbes. Upon injecting non-pathogenic Escherichia coli into the bloodstream or otic cavity of zebrafish larvae, we found that both neutrophils and macrophages were able to sense and migrate towards the injected microbes, but surprisingly, neutrophils ineffectively engulfed these bacteria, whereas macrophages engulfed them in large numbers (Le Guyader et al., 2008). Here, we analysed microbe–neutrophil interactions after microbe inoculation of zebrafish larvae by live-imaging confocal time-lapse microscopy. We found that zebrafish neutrophils very efficiently engulfed bacteria on tissue surfaces but are virtually unable to phagocytose microbes in fluid environments. In stark contrast, macrophages are able to engulf microbes regardless of how they are presented.

Results and Discussion
Unlike macrophages, neutrophils ineffectively engulf microbes in fluid-filled body cavities
To image neutrophil–microbe interactions, we performed confocal time-lapse microscopy, using transgenic mpx-GFP zebrafish larvae
in which GFP is expressed specifically in neutrophils (Renshaw et al., 2006). We first injected fluorescent DsRed+E. coli into closed cavities of the zebrafish mpx:GFP larvae into the otic vesicle, the hindbrain ventricle or the pericardial cavity (Fig. 1F). Immediately after injection, we recorded neutrophil behaviour towards the bacteria. Although neutrophils were rapidly attracted into the microbe-loaded cavity, as we previously documented (Le Guyader et al., 2008), they did not seem to engulf microbes effectively and only small phagosomes were occasionally observed in their cytoplasm. By contrast, the recruited macrophages appeared engorged with red bacteria (Fig. 1A–D). Based on their cytomorphological features, we previously showed that it is possible to distinguish macrophages from neutrophils in vivo by video-enhanced differential interference contrast (VE-DIC) microscopy (Le Guyader et al., 2008). Using this approach, we confirmed that only macrophages were full of red bacteria, although numerous neutrophils were present in the cavity, surrounded by bacteria in the cavity fluid (Fig. 1E). We then analysed neutrophil behaviour following injection of microbes in the bloodstream: the most common route of microbe delivery used so far for modelling infectious disease in zebrafish embryos and larvae. Ten minutes after the injection of DsRed+E. coli into the bloodstream of 60 hours post fertilisation (h.p.f.) mpx:GFP larvae, many bacteria were already associated with macrophages, and only few associated with the neutrophils (Fig. 1D and supplementary material Movie 1). This clear difference persisted over time (data not shown). We thus conclude that, unlike macrophages, neutrophils in zebrafish larva are virtually unable to engulf microbes from the blood or from a fluid-filled body cavity.

Neutrophils swiftly clear microbes inoculated subcutaneously

This observed neutrophil behaviour in response to infection seemed to be in marked contrast to that of mammalian neutrophils, which are considered highly phagocytic and fully competent to kill microbes (Nathan, 2006). The key to this conundrum was found by serendipity. In the course of an injection of DsRed+E. coli into the otic cavity of 72 h.p.f. mpx:GFP larvae, we also injected bacteria in the mesenchyme near the otic cavity (Fig. 2A). Neutrophils were recruited to the injected microbes as expected, but to our surprise, before entering the otic vesicle, they swiftly engulfed all the microbes present in the mesenchyme. After having engulfed the bacteria, neutrophils acquired a rounded shape and their movement slowed. We counted that 15–20 neutrophils phagocytosed 1.4×10^3 bacteria injected in the mesenchyme within 80 minutes (Fig. 2A and supplementary material Movie 2). Based on this observation, we set out to inject 1.5×10^3 DsRed+E. coli subcutaneously over a somite in 72 h.p.f. mpx:GFP larvae (Fig. 2B), and live-imaged bacteria–neutrophil interactions. At the beginning of the imaging, about 30 minutes post injection (m.p.i.), 10–20 neutrophils had already migrated to the microbe-loaded tissue (Fig. 2C, 30 minutes). Neutrophils reached the site of infection from all directions, engulfed bacteria and continued moving to phagocytose more bacteria (supplementary material Movie 3). Macrophages (GFP-negative phagocytes made visible by the engulfed DsRed+E. coli) also participated in the phagocytosis alongside neutrophils, with an apparent neutrophil to macrophage ratio of 2–3 to 1 (Fig. 2C and supplementary material Movies 3 and 5). Most, if not all, bacteria were engulfed within 1–3 h.p.i., depending on the bacterial inoculum. A subcutaneous inoculation of Gram-positive B. subtilis elicited the same neutrophil behaviour (supplementary material Movie 4).

Neutrophils engulf unopsonised microbes as they move over them

To explore in detail and quantify the dynamics of this phagocytosis of substrate-associated bacteria, we subcutaneously injected a smaller number of DsRed+E. coli (6×10^3) and spread them over a larger surface (Fig. 3A,B). By 3 h.p.i., all but a few (1.8%) of the injected bacteria had been engulfed by the recruited neutrophils and macrophages (Fig. 3B and supplementary material Movie 5). To try and compare the contributions and behaviour of neutrophils and macrophages despite the lack of intrinsic labelling of the latter, we traced macrophages individually through the time-lapse sequence by the

---

**Fig. 1.** Unlike macrophages, neutrophils ineffectively phagocytose E. coli injected into closed body cavities or in the bloodstream. DsRed+E. coli (red) were injected in the bloodstream of mpx:GFP zebrafish larvae, which highlight neutrophils (green). (A–D) Confocal fluorescence microscopy, maximum-intensity projection from three planes every 2 μm (A), 22 planes every 2 μm (B), 28 planes every 2 μm (C), 12 planes every 3 μm (D); dotted boxes indicate the regions magnified in the insets. (A) 5.5 hours post injection in the otic vesicle at 72 h.p.f. (B) 1 hour 20 minutes post injection in the hindbrain ventricle at 54 h.p.f. (C) 1 hour 20 minutes post injection in the pericardial cavity at 72 h.p.f. (D) 10 minutes post i.v. injection at 60 h.p.f. The yellow color mostly reflects the superimposition of red (bacteria-loaded) macrophages and GFP+ neutrophils across the Z-steps (see supplementary material Movie 1). (E) Injection in the hindbrain ventricle at 48 h.p.f. followed by wide-field fluorescence and VE-DIC microscopy. Black asterisk indicates a macrophage loaded with red bacteria; white asterisk indicates a neutrophil harboring a tiny phagosome. (F) 48 h.p.f. larva showing the injection sites (arrowheads). Cv, caudal vein; hb, hindbrain parenchyma; hv, hindbrain ventricle; ys, yolk sac; n, notochord; ov, otic vesicle; pc, pericardial cavity; ugo, urogenital opening. Scale bars: 50 μm (A,C), 75 μm (B,D), 10 μm (E).
locally coordinated movement of bacteria and associated phagosome growth outside GFP+ neutrophils that evidenced macrophage phagocytic activity. Based on this approach, quantifications of cell numbers and bacterial load in and out of phagocytes through time are presented in supplementary material Table S1 and supplementary material Figs S1–S3. Although a few neutrophils and macrophages were already present and engulfing bacteria at the injection site by the onset of confocal imaging (20 minutes p.i.), the number of neutrophils there peaked by 2 h.p.i., and that of macrophages peaked 1 hour later (supplementary material Table S1). The total bacterial load in neutrophils at any time point appeared to be 2.5–3-fold higher than in macrophages, and the mean bacterial load per cell was 1.3–2-fold higher for neutrophils than for macrophages (supplementary material Table S1). The few neutrophils and macrophages that engulfed bacteria from a well-delimited field unshared with another phagocyte allowed us to determine their rate of bacteria engulfment: thus, neutrophil 1 engulfed 200 bacteria in 46 minutes (Fig. 3C), and neutrophil 2 engulfed 350 bacteria in 78 minutes (Fig. 3D), which equates to 261 and 269 bacteria per hour, respectively. In comparison, two tracked macrophages each engulfed 50 bacteria in 30 minutes, with initial rates of 132 and 192 bacteria per hour, respectively (supplementary material Fig. S1). Thus the relative engulfment rates measured on individual neutrophils and macrophages fit well with the mean bacterial load per cell measured on the two populations of recruited phagocytes (which depends on both engulfment and bacteria destruction rates).

The ability of neutrophils to phagocytose microbes appeared to be coupled to their motility: neutrophils swept up microbes as they moved over them. They then rapidly concentrated the engulfed bacteria in a single large phagosome, indicating intense phagosome-to-phagosome fusion (Fig. 3 and supplementary material Movie 5). Neutrophils harboring these large...
phagosomes most often became less mobile, perhaps as a result of physical restriction of the phagosome between tissue surfaces, because these cells continued to be motile around their immobile main phagosome (supplementary material Movie 5), and could still project long membrane extensions to engulf more distantly located bacteria, which were then conveyed to the main phagosome (Fig. 3D and supplementary material Movie 5). These behavioural features of neutrophils were also observed towards Gram-positive bacteria (supplementary material Movie 4), and were also displayed by macrophages (supplementary material Movie 5, arrow).

We quantified them by measuring the speed and volume of the main phagosome of a cell over time (supplementary material Figs S2, S3). As they started to develop a sizeable phagosome, neutrophils had speeds ranging from 5 to 9 μm/minute. As their phagosome enlarged, their speed decreased to 2–6 μm/minute by 10–20 minutes later, although some then showed relapses in mobility (supplementary material Fig. S2, yellow track). By that time, their phagosome had reached a size of 150–400 μm³. Similarly, the speed of the macrophage’s main phagosome decreased from 2–6 μm/minute as they started to engulf bacteria to <1 μm/minute by 11–42 minutes later (supplementary material Fig. S3). By then, their phagosome had reached a size of 220–580 μm³.

Zebrafish neutrophils degranulate into their bacteria-laden phagosome in vivo
Mammalian neutrophils, once they have engulfed microbes, release microbicidal products from their granules into the phagosome. This process, known as degranulation, has been mostly studied in vitro, in neutrophils isolated from peripheral human blood (Faurschou and Borregaard, 2003; Nathan, 2006). We previously showed that in live zebrafish larvae, the granules of neutrophils are readily observable through VE-DIC microscopy, and that following fixation, they can be specifically stained by Sudan Black (Le Guyader et al., 2008). We now found that neutrophils that engulfed microbes showed fewer if any granules, both by in vivo VE-DIC (Fig. 4A) and by Sudan Black staining (Fig. 4B,C). Moreover, we observed that following phagocytosis, the myeloperoxidase activity initially contained in the granules

Fig. 3. Detailed behaviour of phagocytosing neutrophils in vivo. (A) 72 h.p.f. mpx:GFP larva; the imaged region is boxed. (B) DsRed+E. coli were injected subcutaneously and neutrophil–bacteria interactions imaged every 1 minute from 20 m.p.i. (left panel) to 200 m.p.i. (right panel) by confocal microscopy. Maximum-intensity projections (1.5 μm x 23 steps) are shown. Boxes 1 and 2 indicate the neutrophils magnified in C and D, respectively. (C) Time-lapse images extracted from 3D-reconstructed acquisitions. The behaviour of this neutrophil is followed here for 46 minutes, during which time it engulfed the 200 bacteria in the area limited by a dotted line, as it moved over them. Engulfed bacteria are readily concentrated into one large phagosome. (D) Time-lapse images extracted as in C. This neutrophil, which already contains numerous bacteria at the onset of imaging, then engulfed 350 bacteria in 78 minutes (top four panels; area delimited by dotted line), and swiftly concentrated them in a large phagosome; then less mobile, it still continued to internalise further bacteria by stretching its cytoplasm (129 m.p.i.; engulfed bacteria (white arrowheads) are then conveyed to the large phagosome (152–192 m.p.i.). Scale bars: 50 μm (B); 10 μm (C,D). See also supplementary material Movie 5.
often became relocalised to the phagosome (Fig. 4D). Taken together, these observations show that in vivo, zebrafish neutrophils degranulate into the phagosome following bacteria engulfment.

‘Vacuum-cleaner’ versus ‘flypaper’ strategy
We have thus demonstrated that in zebrafish larvae, neutrophils efficiently phagocytose only surface-associated microbes, as they move over them, in a ‘vacuum-cleaner’-like behaviour. Under these conditions, all recruited neutrophils are highly phagocytic. Recruited neutrophils appear unable to phagocytose fluid-borne microbes, engulfing only those that adhere to the walls of the infected body cavity or blood vessels. In stark contrast, macrophages are able to efficiently engulf microbes in body fluids as well as on tissue surfaces.

These findings imply that the relative importance of neutrophils and macrophages in microbe elimination will depend not only on the nature of the invading microbe, but also on the anatomical site(s) of infection. Following our initial study (Herbomel et al., 1999), the route mostly used to model microbe–host interactions and human infectious diseases in zebrafish larvae has been microbe inoculation in the bloodstream, and occasionally in the brain ventricle (Davis et al., 2002; Kanther and Rawls, 2010; Lieschke and Trede, 2009). These microbes were taken up by macrophages, with neutrophils having a minor role in the clearance of infection. Our present finding that

Fig. 4. Phagocytosing neutrophils degranulate in vivo.
(A–C) DsRed+ E. coli were injected in the mesenchyme near the caudal vein at 50 h.p.f. in mpx:GFP larvae. (A) In vivo observation. Left panels show the overlay of VE-DIC and red and green fluorescence images, shown separately in the central and right panels. GFP? neutrophils harboring a large phagosome containing DsRed+ E. coli (arrows) show no visible granules by VE-DIC. Inset: a GFP? neutrophil in the same area that did not phagocytose DsRed+ E. coli displays typical granules in constant motion. (B,C) Sudan Black staining of neutrophil granules; arrowheads indicate GFP? neutrophils that contain no E. coli and are well stained by Sudan Black; arrows indicate GFP? neutrophils that contain DsRed+ E. coli and are not (B) or only weakly (C) stained by Sudan Black, depending on their bacterial load (insets in right panels). (D) Unlabelled E. coli were injected subcutaneously at 72 h.p.f. into mpx:GFP larvae. The peroxidase activity of neutrophils was revealed with Cy3-tyramide (red), GFP by Alexa-Fluor-488-coupled anti-GFP antibody (green), and nuclei with DAPI (blue). Arrowheads indicate the typical diffuse peroxidase localisation of resting neutrophils. Arrows indicate the accumulation of peroxidase staining in the phagosomes of phagocytic neutrophils. Scale bars: 10 μm (A–C); 50 μm (D).
neutrophils efficiently phagocytose only surface-associated microbes emphasises that the design of a relevant model of infection should include a careful consideration of the site of injection. Why is the macrophage so efficient – and the neutrophil so ineffective – at clearing microbes from body fluids? First, our observation that bloodstream-injected microbes are mainly associated with macrophages within 10 minutes after the injection indicates a preferential adhesion of microbes to the macrophage, possibly as a result of macrophage-specific expression of broad-spectrum receptors such as scavenger receptors (Bowdish and Gordon, 2009). Second, we observed that, regardless of the presence of microbes, when attached to the wall of a blood vessel or body cavity, the macrophages – but not the neutrophils – continually extend large membrane vesicles into the fluid and then retract them back to the cell body (supplementary material Movie 6). These two features concur to generate a ‘flypaper’ effect: bacteria in the fluid get caught by the macrophages as they come in contact with these loose pseudopodia (Levrard et al., 2009). By contrast, neutrophils bound to vessel or body cavity walls do not show this steady generation of membrane vesicles into the fluid, which is probably associated with the general scavenging functions of macrophages. Correlatively, zebrafish larval macrophages, but not neutrophils, are constitutively highly endocytic (Le Guyader et al., 2008) and macropinocytic (data not shown).

Our data notably predict that in human bacterial infections of the cerebral ventricles or pericardial cavity, macrophages are likely to be the main actors in clearing the infection. The high phagocytic efficiency of larval neutrophils on surface-associated microbes in interstitial tissue is also probably relevant to human disease. It parallels the ‘surface phagocytosis’ identified by Wood in the 1940s (Wood, 1960; Wood et al., 1946). There might also be important clinical consequences of such a phenomenon: our data suggest that the liquid environment of an abscess would frustrate the efforts of neutrophils to efficiently ingest bacteria. Might abscess formation and perhaps also biofilm formation therefore have arisen in part as a result of a specific adaptation of abscess-forming (Lowy, 1998) and biofilm-forming (Costerton et al., 1999) pathogens to avoid surface phagocytosis? Such manipulations of the environment by bacteria would ensure they are not presented on suitable surfaces for efficient phagocytosis by neutrophils. Thus the differential behaviour towards microbes of neutrophils versus macrophages that we have documented by in vivo imaging in zebrafish larvae is likely to be an underappreciated key feature of the innate immune response to bacterial infections in all vertebrates.

Materials and Methods

Zebrafish care and maintenance

The Tg(mpx:GFP)114 and Tg(lyz:DsRed)m56 transgenic zebrafish lines used in this study have been previously described (Hall et al., 2007; Renshaw et al., 2006). Zebrafish were raised and maintained according to standard procedures (Westerfield, 2000). Embryos used for imaging were raised in Volvic water with 0.28 mg/ml Methylene Blue and 0.003% 1-phenyl-2-thiourea to prevent melanin formation.

E. coli microinjection in zebrafish larvae

E. coli K12 bacteria expressing DsRed were grown in LB broth as described previously (van der Sar et al., 2003). Overnight stationary-phase culture was harvested by centrifugation (7 minutes, 5000 g). The pellet of cells was resuspended in sterile PBS. Bacterial concentration, determined by plating on solid medium, was 2–4 × 10⁹/ml, except for the experiment shown in Fig. 2C (10⁸/ml). Zebrafish larvae (48–72 h.p.f.) were anaesthetised by immersion in buffered tricaine (Sigma) and manually dechorionated if needed. They were injected with 1–2 nl of bacterial suspension, using pulled borosilicate glass micropipett (GC100F-15 Harvard Apparatus) pipettes under a stereomicroscope (Stemi 2000, Carl Zeiss, Germany) with a mechanical micromanipulator (M-152; Narishige), and a Picospritzer III pneumatic microinjector (Parker Hannifin) set at a pressure of 20 p.s.i. and an injection time of 20 ms (body cavities and subcutaneous injections) or 40 ms (bloodstream injection).

Time-lapse confocal fluorescence and wide-field VE-DIC imaging of live zebrafish larvae

Injected larvae were positioned in 35 mm glass-bottom dishes (Inagaki-Iwaki). Two methods were used to immobilise the larva in the dish: a 6% methylcellulose solution in Volvic water, added to the caudal part of the larva, or a 1% low-melting-point agarose solution covering the entire larva. The immobilised larva was then covered with 2 ml Volvic water containing tricaine. Confocal microscopy was performed at 23–26°C using a Leica SPE inverted microscope and a 16 × oil immersion objective (PL APO 16 × 0.5) (Fig. 1B–D) or a 40 × oil immersion objective (ACS APO 40 × 1.15 UV) (Fig. 2A); a Leica SP5 inverted microscope with a 40 × oil-immersion objective (HCX PL APO CS 40 × 1.25 UV) was also used to achieve higher temporal resolution (Fig. 2C, Fig. 3). Combined VE-DIC and fluorescence wide-field imaging was performed on a Nikon 90i microscope using a 60 × or a 40 × water-immersion objective, as previously described (Le Guyader et al., 2008). VE-DIC time-lapse imaging was performed on a Reichert Polysar 2 microscope using a 40 × oil-immersion objective (supplementary material Movie 6).

Image processing and analysis

The 4D files generated by the time-lapse acquisitions were processed, cropped, analysed and annotated using the LAS-AF Leica software. Acquired Z-stacks were projected using maximum intensity projection and exported as AVI files. Frames were captured from the AVI files and handled with Photoshop and Illustrator software to mount figures. AVI files were also cropped with ImageJ software, then compressed and converted into QuickTime movies with the QuickTime Pro software. Three-dimensional volume reconstruction (Fig. 3 and supplementary material Movies 4, 5), cell and phagosome tracking, and fluorescence quantifications were performed on the 4D files using Imaris software (Bitplane AG, Zurich, Switzerland) and custom MATLAB (Natick, MA) scripts.

Sudan Black staining, detection of endogenous peroxidase activity and immunohistochemistry

Zebrafish larvae were fixed with 4% formaldehyde in phosphate-buffered saline (PBS) for 1 hour 45 minutes at room temperature, rinsed in PBS and processed for Sudan Black staining, tyramide-based detection of endogenous peroxidase, whole-mount immunohistochemistry for GFP and DAPI staining of nuclei, as described previously (Le Guyader et al., 2008).

We thank Francesco Colucci, Geneviève Milon, Marc Lecuit and Véronique Witko-Sarsat for critical reading of the manuscript and helpful discussions, Karima Kiss, Valérie Briolat and Jean-Pierre Levrard for their advice and support, Dorothee Le Guyader for help with the Sudan Black and immunohistochemistry staining, Chris Hall and Phil Crosier for the Tg:DsRed transgenic zebrafish line, Kit Pogliano for the AD316 B.subtilis:F 5 and Wilbert Bitter for the E.coli:DsRed− bacteria strains. J.-Y.T. was funded by the European Commission, under auspices of WPI (S. Shorte, Institut Pasteur) in the FP7 Project MEMI.


Time after phagocytosis onset (min)

Bacteria engulfed
Table S1. (see Fig. 3 and supplementary material Movie 5) Recruitment and relative contributions of neutrophils and macrophages to the clearance of 6x10^3 DsRed^+ E. coli bacteria injected subcutaneously.

<table>
<thead>
<tr>
<th>Time (min post-injection)</th>
<th>Total bacteria fluorescence</th>
<th>Fluorescence in neutrophils</th>
<th>Number of neutrophils</th>
<th>Fluorescence in macrophages</th>
<th>Number of macrophages</th>
<th>Free bacteria fluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>100 %</td>
<td>8%</td>
<td>5</td>
<td>3%</td>
<td>3</td>
<td>89 %</td>
</tr>
<tr>
<td>60</td>
<td>77 %</td>
<td>29%</td>
<td>20</td>
<td>9%</td>
<td>11</td>
<td>39 %</td>
</tr>
<tr>
<td>120</td>
<td>55 %</td>
<td>30%</td>
<td>34</td>
<td>12%</td>
<td>17</td>
<td>13 %</td>
</tr>
<tr>
<td>200</td>
<td>36 %</td>
<td>24%</td>
<td>31</td>
<td>10%</td>
<td>25</td>
<td>2%</td>
</tr>
</tbody>
</table>

Bacterial fluorescence in each compartment through time is expressed relative to the total bacterial fluorescence at t=20 min pi (onset of the imaging), taken as 100.