Intravascular immunity: the host–pathogen encounter in blood vessels

Michael J. Hickey and Paul Kubes‡*

Abstract | The immune system provides an essential defence against invading pathogens. However, bacteria have evolved numerous strategies to overcome this defence, many of which facilitate systemic dissemination of the pathogen. Nevertheless, the host has evolved many mechanisms to detect and protect against pathogens in the vasculature. Recent studies using new imaging approaches and new mouse models are revealing previously unappreciated functions of this intravascular aspect of the immune system. In this Review, we summarize recent work in this field, highlighting *in vivo* imaging studies that examine the behaviour of both the immune system and bacteria in the highly dynamic microvasculature.

Intravital microscopy study
An examination of biological processes, such as leukocyte–endothelial cell interactions, in living tissue. In general, translucent tissues are used, such as the mesentery or cremaster muscle, which can be exteriorized and mounted for microscopic observation.

High endothelial venule
(HEV). A specialized venule that is found in secondary lymphoid organs, except the spleen. HEVs allow continuous transmigration of lymphocytes as a consequence of the constitutive expression of adhesion molecules and chemokines at their luminal surface.

**Centre for Inflammatory Diseases, Monash University Department of Medicine, Monash Medical Centre, Clayton, Victoria 3168, Australia.*
‡Snyder Institute of Infection, Immunity and Inflammation, University of Calgary Health Sciences Centre, Calgary, Alberta T2N 4N1, Canada.
Correspondence to P.K.
e-mail: pkubes@ucalgary.ca
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The success of immune cells in eradicating bacteria that invade the body's tissues is essential for our survival. Although much progress has been made in delineating immune mechanisms, less is known about the immune response that occurs within blood vessels (termed intravascular immunity), which comprises both cellular and humoral components. This is in part because the study of this dynamic environment requires highly laborious imaging techniques. It is however clear that this is where one of the key 'battles' against bacteria is fought; the millions of blood vessels that perfuse the tissues must be patrolled by immune cells to prevent the dissemination of bacteria to different organs and consequently to protect the host. Therefore, there are many 'sentries' and 'guards' posted throughout the cardiovascular system to identify and eradicate pathogens. In this Review, we focus on the rapidly expanding body of knowledge of host–pathogen encounters in the vasculature, the mechanisms by which the intravascular immune system identifies and kills invading bacteria and how bacterial pathogens subvert or escape innate immune responses in the vasculature. This area of research has benefited tremendously from the recent advances in imaging, so much of this Review describes data that have been acquired using these technologies. Being able to image and track the invading pathogens, as well as the different immune cells in the blood vessels of the host, is providing key insights into a spectacular host–pathogen encounter.

The leukocyte recruitment paradigm

Neutrophils, the key effector cells in innate immunity against bacteria, are the first cells that are rapidly recruited to inflamed sites during an innate immune response to infection. The vasculature is perfectly positioned to attract neutrophils to sites of inflammation, and even subtle changes in the microenvironment are sufficient to induce neutrophil recruitment. The main role of neutrophils is to isolate, engulf and kill pathogens using oxidative and non-oxidative mechanisms.

Seminal intravital microscopy studies of neutrophil responses to infection or injury have identified that neutrophils must first tether to the blood vessel endothelium and then roll along the endothelium before firmly arresting at affected sites[1,2]. Once arrested, the cells leave the vasculature and migrate to distinct sites of infection (reviewed in REF. 3). Briefly, the family of selectin adhesion molecules, specifically P-selectin (also known as SELP) and E-selectin (also known as SELE) expressed by endothelial cells, facilitate the initial tethering and rolling of neutrophils in the postcapillary venules of the peripheral vasculature (FIG. 1a), and L-selectin supports lymphocyte adhesion in the high endothelial venules of lymph nodes[4]. However, despite the important role of selectins as facilitators of leukocyte rolling, there are numerous molecules that can mediate the recruitment of leukocytes independently of selectins. These include α4 integrin (also known as CD49D and ITGA4), CD44, vascular adhesion protein 1 (also known as AOC3) and, at lower shear stress, β2 integrin (also known as CD18 and ITGB2)[5].

In the liver, antibody-mediated blockade of selectins is particularly inefficient at inhibiting leukocyte recruitment[6], and in the lungs, selectin blockade similarly fails to reduce leukocyte recruitment in some but not all forms of inflammation[7,8]. A possible explanation for the selectin-independent recruitment to these sites could

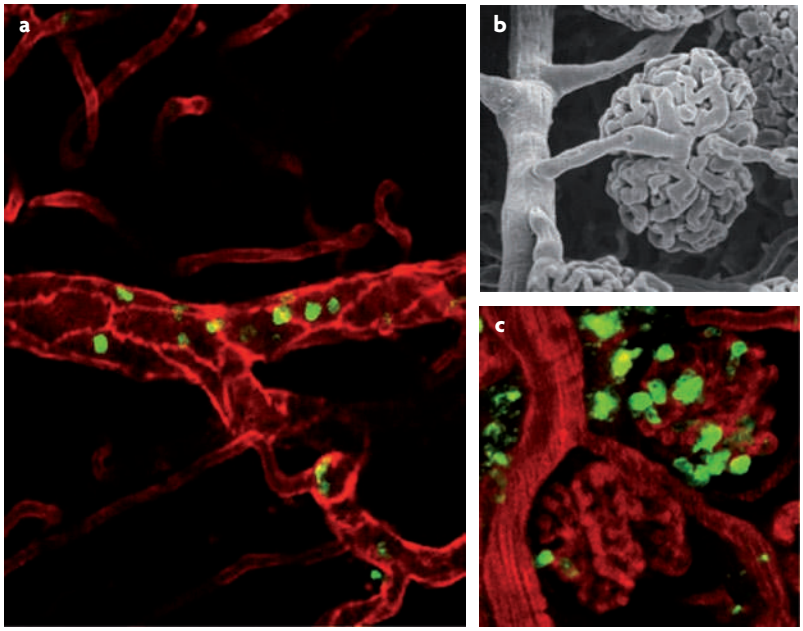

Figure 1 | **Diverse microcirculatory sites for leukocyte recruitment. a** | Multiphoton confocal microscopy image of postcapillary venules in the cremaster muscle of a lysozyme M–EGFP (enhanced green fluorescent protein) mouse, in which neutrophils express EGFP. An Alexa 594-conjugated antibody specific for platelet/endothelial cell adhesion molecule 1 (PECAM1; also known as CD31) was used to label endothelial cell junctions in the vasculature (red). $EGFP^+$ neutrophils can be seen adhering to the endothelial cell surface, both in the centre of the endothelial cells and at endothelial cell junctions. After adhesion, neutrophils typically migrate towards endothelial cell junctions, which are the best sites for neutrophil transmigration. **b** | Scanning electron microscopy image of a vascular cast of the renal glomerulus. The complex, branching nature of the glomerular capillaries, a site of leukocyte recruitment in glomerulonephritis, is apparent. **c** | Using a similar approach as in **a**, adherent neutrophils are seen within inflamed glomerular capillaries (neutrophils are stained with Alexa 488-conjugated antibody specific for GR1 (green) and the vasculature is labelled with albumin conjugated to tetramethylrhodamine isothiocyanate (TRITC; red). Neutrophil recruitment to this vascular bed occurs in the absence of rolling, a process that is normally a prerequisite for adhesion in postcapillary venules.

stem from the finding that leukocyte adhesion occurs in the capillaries of lungs and the sinusoids of the liver without the requirement for rolling. Although physical trapping of leukocytes was suggested as a possible mechanism for the recruitment of leukocytes to these sites, new evidence indicates that, at least in the liver sinusoids, the recruitment of leukocytes depends on other adhesion molecules, including CD44 and serum-derived hyaluronan-associated protein (SHAP; also known as ITIH2)[9,10]. In the glomerular microvasculature, leukocyte recruitment also occurs in capillaries (FIG. 1b,c), but through an unconventional process of immediate arrest that is mediated by platelet-derived P-selectin[11].

In general, adhesion to the endothelium is mediated by integrins, most notably α4 integrin and β2 integrin. Adhesion occurs when rolling neutrophils detect chemokines that are presented on the surface of the endothelium following extravascular cell damage and/or infection. These 'hot spots' for adhesion, which is mediated by lymphocyte function-associated antigen 1 (LFA1; also known as αLβ2 integrin) on neutrophils, are often not the final sites for migration out of the vasculature. The adherent neutrophils often crawl (not necessarily in the direction of blood flow) to junctions and then migrate out of the blood vessel[12]. Inhibition of crawling delayed but did not eliminate emigration *in vivo*, whereas *in vitro*, crawling of monocytes was absolutely essential for subsequent migration across the endothelium and also required integrins[13].

The factors that determine where a neutrophil decides to emigrate remain unclear. Most studies suggest that neutrophils emigrate at junctions (paracellular route)[12,13], but there is also evidence indicating that neutrophils can migrate directly through the endothelium (transcellular route)[14,15]. The molecular mechanism of emigration is the least well understood and potentially the most complex aspect of leukocyte trafficking, and involves platelet/endothelial cell adhesion molecule 1 (PECAM1; also known as CD31), junctional adhesion molecules, CD99, endothelial cell adhesion molecule 1 and possibly intercellular adhesion molecules (ICAMs) and integrins (reviewed in REF. 3).

Intravascular immune 'sentinel' cells

Several populations of immune cells, including neutrophils, monocytes, invariant natural killer T (iNKT) cells, Kupffer cells and even endothelial cells, are strategically located in the vasculature to detect and respond to infection. Both basal rolling of neutrophils and intravascular crawling of monocytes, which occurs under resting conditions in some tissues as part of immune surveillance, contribute to maximizing the detection of invading microorganisms and directing subsequent cell recruitment to these sites.

Myeloid cells. A circulating cell that moves rapidly through the vasculature would have difficulty detecting subtle changes in the tissues or at the vascular wall, so cells must exit the bloodstream and roll along the vessel wall. Whether rolling occurs in non-inflamed tissues or only during inflammation is unclear, as studies in which tissues had been surgically exteriorized to enable intravital imaging showed P-selectin-mediated rolling; however, this was probably occurring in response to the surgery[16]. Some tissues, such as the skin, have constitutive expression of E-selectin and P-selectin and support basal rolling of leukocytes[17]. In the case of the skin, which is in direct contact with the external environment and billions of bacteria, any breach of the dermal barrier is likely to lead to infection. As such, constitutive rolling in the vasculature is thought to be a sentinel mechanism by which leukocytes constantly patrol the endothelium to detect evidence of infection. Whether this mechanism of constitutive rolling occurs in the intestinal tract and the lungs, which are also in direct contact with the external environment, remains unknown. In support of this idea, the extended transit time for neutrophils through the lungs suggests that they are temporarily retained in the pulmonary vasculature[18].

It is possible that basal crawling and adhesion of leukocytes in blood vessels might also function as a form of immune surveillance. Indeed, exteriorization

Shear stress
The force exerted by the flowing blood (dynes) on each unit of area of endothelial surface (cm^2); measured in dynes per cm^2.

Kupffer cell
A large, specialized ramified macrophage that lines the sinusoidal vessels of the liver. Kupffer cells regulate local immune responses and remove microbial particles, endotoxins and other noxious substances that penetrate the portal venous system.

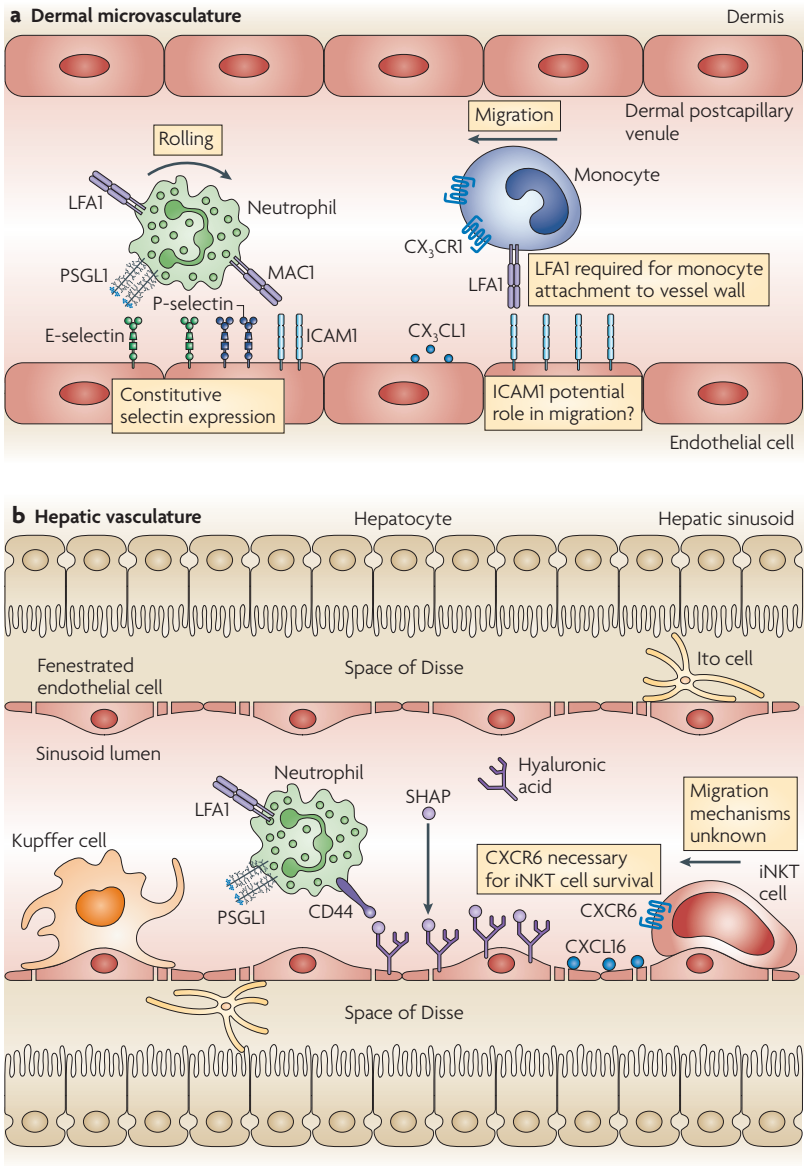

Figure 2 | **Cellular and molecular interactions for immune surveillance and recruitment in the dermal and hepatic vasculature. a** | Schematic of a dermal postcapillary venule. Neutrophils roll on constitutively expressed P-selectin and E-selectin on the dermal vasculature by interacting with ligands such as P-selectin glycoprotein ligand 1 (PSGL1). Neutrophils express lymphocyte function-associated antigen 1 (LFA1; also known as αLβ2 integrin) and macrophage receptor 1 (MAC1; also known as αMβ2 integrin and CR3), and these interact with intercellular adhesion molecule 1 (ICAM1), which is constitutively expressed by the endothelium. In addition, monocytes 'patrol' the vasculature, crawling over the endothelial cell surface through a mechanism that involves interaction between CX_3C-chemokine ligand 1 (CX_3CL1) and its receptor, CX_3C-chemokine receptor 1 (CX_3CR1). LFA1 is required to maintain monocyte attachment to the endothelial cell surface, potentially by interacting with ICAM1 expressed by endothelial cells. **b** | Schematic of a hepatic sinusoid. Kupffer cells are present in the hepatic sinusoid, where they extend processes over a wide area, but remain immobilized. Ito cells are located in the space of Disse. The sinusoidal endothelium is fenestrated, allowing plasma to readily pass into the space of Disse and come in contact with hepatocytes. Under inflammatory conditions, serum-derived hyaluronan-associated protein (SHAP; also known as ITIH2) in the hepatic sinusoidal lumen is associated with locally expressed hyaluronic acid and mediates neutrophil arrest by interacting with neutrophil-expressed CD44 (the receptor for hyaluronic acid). Invariant natural killer T (iNKT) cells are present in the liver in high numbers and spontaneously crawl through the sinusoid. CXCR6 contributes to the survival of iNKT cells in the liver, but the mechanisms underlying iNKT cell migration are unknown.

of the cremaster muscle in the absence of any other stimuli resulted in the adhesion of a high number of neutrophils within postcapillary venules[19]. Approximately 75% of these adherent neutrophils extended pseudopodia and then began to crawl randomly in all directions along the endothelium, but most cells eventually detached. *In vitro* experiments revealed that crawling of monocytes on unstimulated endothelial cell monolayers was mediated by β2 integrin (which is expressed by monocytes) and ICAM2 (which is constitutively expressed by endothelial cells)[13]. Notably, the crawling monocytes did not then migrate across the endothelium, raising the possibility that monocytes can crawl under basal conditions in blood vessels without leaving the vasculature. However, these *in vitro* experiments were carried out in the absence of shear stress, which can cause the detachment of crawling monocytes. Shear stress also increases the propensity of leukocytes to migrate out of the vasculature[20], which again raises questions about whether basal crawling occurs *in vivo*.

In a hallmark study, Auffray *et al.*[21] used a mouse strain in which green fluorescent protein (GFP) was highly expressed by a subset of monocytes that expresses CX_3C-chemokine receptor 1 (CX_3CR1; $CX_3CR1^{GFP/+}$ mice) and observed that the GFP^+ monocytes crawled throughout the dermal microvasculature in the absence of inflammation. This monocyte population (characterized by the $GR1^-CCR2^-CX_3CR1^+$ phenotype) has been termed 'resident' because it is present in both inflamed and non-inflamed tissues[21]. Importantly, much of this work was carried out in an ear preparation involving no surgical manipulation, which excludes the possibility that the observed monocyte behaviour was a response to surgically induced inflammation. This monocyte population could be found randomly migrating throughout the entire microvasculature, including arteries, capillaries and postcapillary venules. This migratory behaviour was notably different from that which occurs during an inflammatory response, during which inflammatory leukocyte recruitment is typically restricted to brief crawling on the venular endothelium followed by emigration.

In the same study, the number of crawling monocytes was shown to be reduced sixfold in CX_3CR1-deficient mice, which indicates that this receptor and its ligand, CX_3C-chemokine ligand 1 (CX_3CL1; also known as fractalkine), are involved in the patrolling behaviour of monocytes (FIG. 2a). Specific antibody-mediated blockade of LFA1 but not of macrophage receptor 1 (MAC1; also known as αMβ2 integrin and CR3) led to detachment of the monocytes from the endothelial cell surface, suggesting that LFA1 was essential for maintaining monocyte attachment to the endothelium under conditions of shear stress. Whether this is the only adhesion molecule that mediates crawling by $CX_3CR1^{GFP/+}$ monocytes remains uncertain. Although it is unclear whether all organs have these patrolling $CX_3CR1^{GFP/+}$ monocytes, similar crawling behaviour of monocytes has also been observed in cerebral vessels[22] and mesentery[21].

Spinning disk confocal microscopy
Form of confocal microscopy in which confocality is achieved using a spinning disk with numerous pinholes, replacing the more conventional laser scanning process that is used in many confocal microscopes. The main advantage of this form of confocal microscopy is the rapid rate of image capture (up to ~15 frames per second), which enables examination of rapidly motile cells and bacteria in the vasculature.

The study also showed that $CX_3CR1^{GFP/+}$ monocytes were important for an early and timely inflammatory response. Exposure of the mice to irritants, aseptic wounding and peritoneal infection with *Listeria monocytogenes* led to rapid (within 1 hour) migration of $CX_3CR1^{GFP/+}$ monocytes from the vasculature. In this *L. monocytogenes* infection model, emigration of $CX_3CR1^{GFP/+}$ monocytes peaked at 2 hours after infection, a time when neutrophils were only beginning to enter the peritoneum and several hours before 'inflammatory' monocytes (characterized by a $GR1^+CCR2^+$ phenotype) were observed. At 1 and 2 hours after infection, $CX_3CR1^{GFP/+}$ monocytes were the only cells that produced tumour necrosis factor (TNF), interleukin-1 (IL-1) and various chemokines, which is consistent with the view that these cells are involved in the recruitment and activation of other effector immune cells. These responses were significantly delayed in CX_3CR1-deficient mice, in which monocyte crawling is substantially reduced. Interestingly, intraperitoneal mast cells have also been suggested to be the main source of TNF and to be implicated in early neutrophil recruitment during bacterial infection[23], thereby questioning the relative importance of mast cells versus $CX_3CR1^{GFP/+}$ monocytes in the initiation of the inflammatory response.

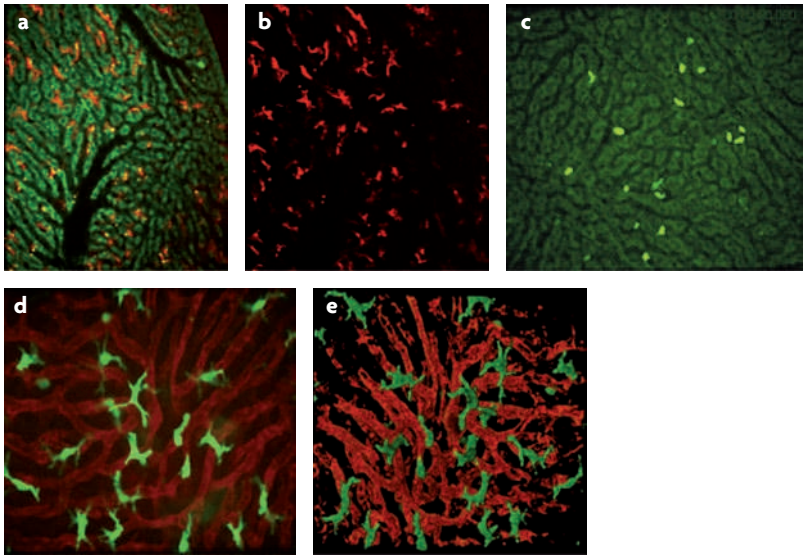

Figure 3 | ***In vivo* imaging reveals cell populations in hepatic vasculature.** *In vivo* spinning disk confocal microscopy images show the various sentinel cell populations present within and adjacent to the hepatic sinusoidal microvasculature. **a** | Image of Kupffer cells, identified by the uptake of tetramethylrhodamine dextran-loaded microspheres (red), in the hepatic sinusoids. **b** | Single colour image of the tetramethylrhodamine-stained Kupffer cells that are shown in **a**, in which their many processes can be seen. During extended periods of imaging, these cells do not seem to migrate. **c** | Invariant natural killer T (iNKT) cells (green) visualized in $CXCR6^{GFP/+}$ mice, in which green fluorescent protein (GFP) expression is restricted to CXC-chemokine receptor 6 (CXCR6)-expressing iNKT cells. These cells spontaneously migrate throughout the sinusoidal microvasculature, but cease to migrate following T cell receptor activation. **d** | Ito cells (green) visualized by GFP expression in $CX_3CR1^{GFP/+}$ mice. The sinusoidal microvasculature has been labelled with an Alexa 594-conjugated antibody specific for platelet/endothelial cell adhesion molecule 1 (PECAM1; also known as CD31) (red). Ito cells are large and have many processes that are present in the space of Disse; they may have roles in antigen presentation and immune responses. **e** | Three dimensional rendering of the hepatic microvasculature, showing the position of GFP^+ Ito cells adjacent to the hepatic sinusoids (red).

Immobilized macrophages in the vasculature. Kupffer cells are monocyte-derived cells that are found in the liver vasculature and that have an important role in removing debris, bacteria and potentially harmful substances from the blood (FIG. 2b). The liver is an anatomically and immunologically unique organ where blood containing foreign substances from the gastrointestinal tract filters through hepatic sinusoids and is scanned by Kupffer cells. Early studies showed that when bacteria or foreign particles were injected into experimental animals, most particulate matter was found in Kupffer cells[24], which at the time were described as 'fixed' tissue macrophages that resided in the liver sinusoids[25]. This idea was challenged by subsequent *in vivo* light microscopy studies, which revealed a population of crawling, non-immobilized leukocytes that behaved like Kupffer cells (that is, they phagocytosed particulate matter)[26]. However, because of the lack of specific markers to identify this subset *in vivo*, it was not possible to correlate cell phenotype with behaviour and function. Using spinning disk confocal microscopy and a selective marker for Kupffer cells (F4/80), a single, immobilized population of Kupffer cells that resided in the liver sinusoids and captured particulate matter could be detected[27]. The Kupffer cells were observed to have many processes extending into several different sinusoids, thereby providing them with a surveillance system for multiple sinusoids (FIG. 3a,b).

For Kupffer cells to be effective in their role of immune surveillance, they must be able to capture and phagocytose foreign material in the bloodstream while ignoring similarly sized endogenous particles that should remain in blood (for example, platelets). This is achieved through the opsonization of foreign particles by complement or antibodies. In general, the key molecules that mediate phagocytosis of opsonized particles are complement and Fc receptors, but evidence for their role in particle capture under flow conditions is limited. Recent data indicate that the Kupffer cell receptor that is crucial for complement-mediated clearance of circulating pathogens is a newly identified complement receptor known as CRIg (complement receptor of the immunoglobulin superfamily; also known as VSIG4)[28], which is expressed by Kupffer cells and a few other tissue-resident macrophages. Kupffer cells from CRIg-deficient mice could not efficiently clear *L. monocytogenes* and *Staphylococcus aureus*, allowing the bacteria to disseminate in the blood and invade other tissues. CRIg was shown to bind to complement component 3b (C3b), inactivated C3b and methylated C3, but not C3, C3a or C3d. Clearance of circulating bacteria was impaired equally, and not additively, in C3-deficient and CRIg-deficient mice, which suggests that CRIg is a C3 receptor. It is therefore clear that CRIg expression by Kupffer cells has a dominant role in C3-dependent removal of both intracellular (*L. monocytogenes*) and extracellular (*S. aureus*) pathogens from the circulation and is a crucial component of the intravascular innate immune system.

The role of Fc receptors on Kupffer cells is less clear, in part because the Fc receptor systems in mice and humans differ. Kupffer cells express high levels of Fc receptors for IgG (FcγRs) and are the main hepatic

phagocytes involved in the elimination of soluble IgG-containing immune complexes[29]. Although this is indicative of an important function for FcγRs on Kupffer cells, little is known about the roles of these receptors in bacterial elimination. Kupffer cells in patients with hepatitis have also been shown to express the Fc receptor for IgA (FcαRI). In addition, although the mouse equivalent of FcαRI has not been identified, mice that have been rendered transgenic for human FcαRI express this molecule on Kupffer cells following inflammatory stimulation[30]. Kupffer cells in FcαRI-transgenic mice could capture and phagocytose circulating bacteria coated with serum IgA[30]. FcαRI on human Kupffer cells could provide an additional line of defence for mucosal tissues by eliminating bacteria that have breached the intestinal barrier and entered the portal circulation[30].

Invariant NKT cells. Another population of leukocytes that resides in the vasculature is the NKT cell subset. NKT cells express both NK cell and T cell markers and are important innate immune cells. More than 80% of mouse NKT cells express a conserved invariant Vα14–Jα18 T cell receptor (TCR) α-chain (orthologous to Vα24 in humans)[31], and these cells are generally termed invariant NKT (iNKT) cells[32]. Most iNKT cells are found in the liver, where they make up more than 30% of the resident lymphocytes[33]. Activation of iNKT cells requires the presentation of glycolipid antigens by cells expressing the MHC class I-like molecule CD1d. Numerous glycolipids can activate iNKT cells (reviewed in REF. 33), including α-galactosylceramide (α-GalCer), which was originally discovered in extracts from the marine sponge *Agelas mauritianus*. Similar structures, including α-glucuronosylceramide from *Sphingomonas* species, α-galactosyldiacylglycerols from *Borrelia burgdorferi* (the causative agent of Lyme disease) and phosphatidylinositol tetramannoside (PIM4) found in mycobacterial membranes, have also been reported to have some iNKT cell-activating capacity. Finally, iNKT cells can also be activated by the self antigen isoglobotrihexosylceramide, which is produced by the host in response to pathogenic bacteria such as *Salmonella enterica* subspecies *enterica* serovar Typhimurium[33].

iNKT cells localize in the liver sinusoids with Kupffer cells (FIG. 3c). Geissmann and colleagues[34] engineered mice in which leukocytes expressing CXCR6 also expressed GFP (CXCR6$^{GFP/+}$ leukocytes), and in the liver most (80%) of these cells were iNKT cells. In a seminal intravital microscopy experiment, it was revealed that CXCR6$^{GFP/+}$ iNKT cells were a crawling population of leukocytes in sinusoids and continuously patrolled within these vessels, crawling randomly and independently of the direction of blood flow[34]. CXCL16, the only known CXCR6 ligand, was shown to be expressed on liver sinusoidal endothelium and to contribute to the survival and perhaps random migration of iNKT cells (FIG. 2b). Intravenous administration of α-GalCer into mice resulted in the arrest of iNKT cell migration, a response that could be prevented by CD1d blockade, suggesting that α-GalCer can be presented in the vasculature[34]. However, iNKT cells can also become activated independently of recognition of an identified glycolipid antigen presented by CD1d; for example, the cytokines IL-12 and IL-18 have been shown to function synergistically to cause arrest of patrolling iNKT cells[34,35]. Finally, iNKT cells can be activated in response to events that occur in distant organs, such as the skin, and influence the subsequent immune response. Indeed, during the initiation phase of contact sensitization, hepatic iNKT cells release IL-4, thereby activating B1 cells to produce antigen-specific IgM, which in turn activates complement to promote the recruitment of effector T cells to the skin[36].

Although CD1d is expressed by Kupffer cells, endothelial cells, hepatocytes and dendritic cells, all of which can present antigen to iNKT cells *in vitro*[37], it remains unclear which of these cells presents lipids to iNKT cells during an infection *in vivo*. Precursor dendritic cells in the circulation bind to Kupffer cells to migrate to the liver[38], where they might present antigen to iNKT cells. Furthermore, Ito cells (also known as stellate cells) have also been shown to present antigen to iNKT cells[39]. These large, star-shaped cells (FIG. 3d,e), which are found outside of the vasculature in the space of Disse, may be contacted by microvilli of intravascular iNKT cells that are extended through the fenestrated endothelium, a process described for other lymphocytes in the liver[40]. Despite these observations, spinning disk multi-channel fluorescence microscopy has revealed that iNKT cells undergo arrest on Kupffer cells, and not dendritic cells or Ito cells, following infection by pathogenic microorganisms *in vivo* (W.Y. Lee and P.K., unpublished observations). This suggests that Kupffer cells are responsible for presenting antigen to iNKT cells. Once activated through the TCR, robust production of IL-4 and/or interferon-γ (IFNγ) is induced depending on the stimulus[36,41], and this shapes the immune response by recruiting certain effector cells and inducing specific adaptive immune responses.

Endothelial cells. Over 60 trillion endothelial cells make up the largest interconnected organ in the human body, covering as much as 4,000 square metres[42]. As sentinel cells, endothelial cells would provide vast coverage for the detection of bacteria and bacterial products that enter the bloodstream. Early studies suggested that endothelial cells lacked the crucial molecules that are necessary for bacterial detection, including CD14 and several Toll-like receptors (TLRs). However, the interpretation of some of the results was complicated by the use of endothelial cells that were immortalized, serially passaged or from easily accessible large vessels. Indeed, more recent studies have revealed that primary (not passaged) endothelial cells from various organs do express CD14, TLR2, TLR4, TLR9, MD2 (also known as LY96) and the TLR signalling adaptor myeloid differentiation primary-response protein 88 (MyD88), and respond to various TLR ligands. This supports the view that they may function as sentinel cells[43–45].

Indeed, experiments using TLR4 chimeric mice in which TLR4 is expressed only by parenchymal cells (including endothelial cells) indicate that TLR4 is integral to the ability of endothelial cells to function as

C3 receptor
A receptor that mediates responses to the complement components C3 and C3a. Four C3 fragment receptors have been identified: CR1 (CD35), CR2 (CD21), CR3 (CD11b–CD18) and CR4 (CD11c–CD18). However, none of these receptors has been described to be functional on Kupffer cells.

Contact sensitization
The inflammatory immune reactions that occur in the skin after the administration of a sensitizing antigen. These reactions occur after the second and subsequent exposures to a particular sensitizing antigen and involve the recruitment and responses of effector T cells.

Ito cell
A type of pericyte that is found in the space of Disse and is the main reservoir of retinol in the liver.

Space of Disse
The space between the sinusoidal endothelial cells and hepatocytes in which Ito cells are found. Given the discontinuous nature of the sinusoidal endothelium, this space is filled with blood plasma. Hepatocytes extend microvilli into the space of Disse, thereby increasing their surface area for metabolite exchange.

Fenestrated endothelium
Large holes (fenestrations) of the endothelium of the hepatic sinusoids, which allow plasma in the sinusoids to freely access the space of Disse.

Toll-like receptor
(TLR). A receptor belonging to a family that recognizes conserved products that are unique to microorganisms (such as lipopolysaccharide), which are known as pathogen-associated molecular patterns. TLR-mediated events signal to the host that a microbial pathogen is present.

Box 1 | **Sepsis**

Severe sepsis is the leading cause of death in intensive care units worldwide and is responsible for over 200,000 deaths in the USA each year. Systemic bacterial infection is one of the main triggers of sepsis. This results in sequelae such as increased vascular permeability, leukocyte recruitment, tissue ischaemia, hypotension and multi-organ failure[56,94]. Although antibiotics, fluids and vasopressors (which cause vasoconstriction) are the standard of care, the prevailing view is that patients with severe sepsis often succumb to an over-exuberant systemic inflammatory response that includes the overproduction of pro-inflammatory cytokines and mediators and the inappropriate activation of immune cells, including neutrophils. Many putative anti-inflammatory therapies for this condition have been tested but have failed to show great therapeutic benefit, and severe sepsis remains an extremely difficult condition to treat.

Platelets, which are mainly involved in haemostasis, are decreased in some septic patients, and the more marked the thrombocytopenia (loss of platelets), the more severe the sepsis and the greater the mortality[95]. Although disseminated intravascular coagulation accounts for part of the thrombocytopenia, other factors also contribute[96]. Similarly to neutrophils, many platelets can be found in the lungs and the liver in both humans with sepsis and mouse models of sepsis[97,98]. This raises the possibility of an interplay between platelets and neutrophils as part of an innate immune response that is necessary for survival. However, the potential for bystander injury to self is great, and it is intriguing that activated protein C, which has both anti-thrombotic and anti-inflammatory effects, has been efficient for the treatment of a subset of severely septic patients.

sentinel cells[46]. In these chimeric mice, the presence of the TLR4 ligand lipopolysaccharide (LPS) in the blood caused endothelial cell activation, and this was sufficient to induce neutrophil recruitment[46]. Surprisingly, when TLR4 expression was restricted to bone marrow-derived cells (including macrophages and neutrophils), neutrophils were not recruited to tissues in response to LPS. However, in a separate study in which LPS was given intratracheally (such that alveolar macrophages and epithelial cells were the first to detect the TLR4 ligand), macrophage but not endothelial or epithelial cell TLR4 was required for the detection of LPS and the induction of the subsequent immune response[47]. These results support the idea that pulmonary endothelial cells function as important sentinel and effector cells in response to blood-borne bacterial products, whereas alveolar macrophages are crucial for the detection of bacterial ligands that are located in extravascular compartments.

To further examine the role of endothelial cells in perpetuating pulmonary inflammation and contributing to sepsis, transgenic mice in which nuclear factor-κB (NF-κB) activation is selectively suppressed in endothelial cells were used[48]. It was shown that lack of NF-κB activation led to reduced adhesion molecule expression by endothelial cells, reduced pulmonary neutrophil infiltration, decreased lung injury and increased survival of the mice in response to LPS or caecal ligation and puncture[48]. Despite this, the transgenic mice were not better than wild-type mice at clearing infection with *L. monocytogenes*, *Streptococcus pneumoniae* or *Salmonella enterica*. Given that excessive pulmonary inflammation is an important contributor to the sepsis-associated mortality, these findings may indicate that the endothelium is an essential contributor to life-threatening immune responses in sepsis (BOX 1), but that its role in bacterial clearance is less important than the role of other elements of the immune system[48]. In contrast to this possibility, we have recently observed that mice expressing TLR4 exclusively on the endothelium clear bacteria more effectively than wild-type mice, without any associated mortality (G. Andonegui and P.K., unpublished observations).

Caecal ligation and puncture
An experimental model of peritonitis in rodents, in which the caecum is ligated and then punctured, thereby forming a small hole. This leads to leakage of intestinal bacteria into the peritoneal cavity and subsequent peritoneal infection.

Bacterial evasion of intravascular immunity

To successfully infect the host and disseminate, invading bacteria must overcome the multifaceted intravascular innate immune system, which comprises humoral factors and various immune cells. Bacteria have evolved several ways to overcome these barriers, including avoiding detection, sending signals that 'confuse' immune cells or altering immune cell function. Although there are many mechanisms (reviewed in REFS 49,50), we discuss below those that directly affect the vasculature and leukocyte recruitment.

Diversion of intravascular immune responses. Intraperitoneal administration of LPS, which rapidly disseminates around the body through the blood, triggers neutrophil recruitment to the lungs and liver at the expense of the peritoneal cavity (potentially explaining the observed lack of recruitment to the original source of the inflammatory stimulus)[51]. Indeed, endotoxaemia results in a rapid reduction in the number of circulating neutrophils owing to their sequestration in the pulmonary and hepatic microcirculation[46], a response that is also observed during bacterium-induced septic shock[52,53]. This LPS-induced diversion of neutrophils away from the site of infection may actually aid immune evasion by bacteria. Although an underlying mechanism has yet to be determined, activated neutrophils may not be able to squeeze through the narrow lung capillaries (because their membranes are not highly deformable) and might become physically trapped. This could account for the apparent tropism of neutrophils for the lungs and liver during sepsis[54] (BOX 1). Under these conditions, neutrophils mainly stay lodged in the capillaries and do not migrate into the tissue[46,55].

The observation that inhibition of selectins and integrins does not affect neutrophil recruitment to the lungs under these conditions[46] supports the idea that passive trapping of neutrophils in narrow capillaries is responsible for this process. Alternatively, other adhesive mechanisms might be upregulated during inflammation (perhaps inappropriately) to induce neutrophil adhesion in the vessels of the liver and lungs. Indeed, neutrophils from patients with sepsis (unlike those from control individuals) express α4 integrin, which may cause them to preferentially adhere to vascular cell adhesion molecule 1 (VCAM1)[56], a molecule that is highly expressed by endothelial cells in the pulmonary vasculature. Complement activation, specifically the production of the anaphylatoxin C5a, also contributes to the recruitment of neutrophils to the lungs in models of sepsis[57]. Recent work has highlighted the difficulty of blocking this pathway: C5a activates multiple receptors[58] and is produced through the classical complement pathway by C3 activation as well as through a C3-independent, thrombin-dependent pathway[59].

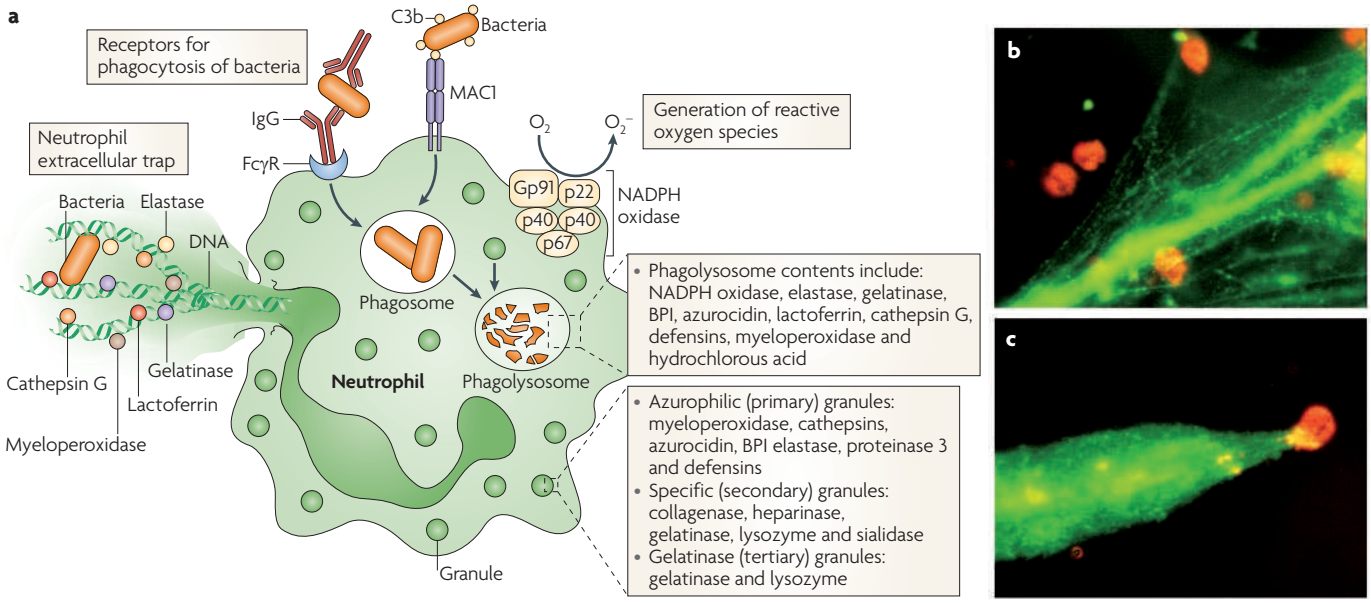

Figure 4 | **Neutrophil extracellular traps: an additional antibacterial weapon. a** | Schematic overview of neutrophil antibacterial systems. Neutrophils express numerous receptors that aid bacterial phagocytosis, including macrophage receptor 1 (MAC1; also known as αMβ2 integrin and CR3), which binds complement component 3b (C3b) on the surface of opsonized bacteria. Neutrophils also express Fc receptors for IgG (FcγR), which enable binding of IgG-coated particles and immune complexes. These molecules facilitate the uptake of opsonized bacteria into specialized membrane-bound compartments, known as phagosomes. Phagosomes subsequently fuse with lysosomes and neutrophil granules, which contain a wide range of proteolytic and antibacterial enzymes and peptides, thereby forming phagolysosomes. NADPH oxidase, which is present in the membrane of the phagolysosome, generates reactive oxygen species, which, together with various enzymes such as myeloperoxidase, result in the production of further potent oxidants that destroy the bacteria. In addition, neutrophils can extrude neutrophil extracellular traps (NETs), which are fibrous extracellular meshworks that consist of chromatin coated with proteases, including myeloperoxidase, elastase and cathepsin G. These trap and kill bacteria and degrade bacterial virulence factors. **b,c** | Images of NET formation by neutrophils under shear stress conditions, following interaction with lipopolysaccharide-activated platelets. NETs are visible by staining of extracellular DNA with Sytox Green. Images are reproduced, with permission, from *Nature Medicine* REF. 55 © (2007) Macmillan Publishers Ltd. All rights reserved. BPI, bactericidal/permeability-increasing protein.

Inhibition of effector cell function. Neutrophils have an impressive range of toxic molecules that contribute to killing bacteria. Neutrophils capture and phagocytose bacteria and subsequently internalize them into phagolysosomes, in which microbial killing takes place. The protein constituents of phagolysosomes include NADPH oxidase (which generates reactive oxygen species), myeloperoxidase, proteases (such as elastase and cathepsin G) and antimicrobial proteins (including defensins, azurocidin and bactericidal/permeability-increasing protein)[60]. However, the importance of these well-described mechanisms to killing bacteria in the blood is unclear. Furthermore, capture of bacteria by neutrophils in the dynamic setting of blood flow may be inefficient. However, a new trapping mechanism that might increase the efficiency of bacterial capture in the blood has been proposed recently[55]. This involves the release of web-like structures termed neutrophil extracellular traps (NETs) that consist of chromatin coated with proteases, which might help to trap and kill pathogens[61] (FIG. 4).

Original descriptions suggested that NETs took hours to form, were dependent on the generation of reactive oxygen species that destroyed the nuclear envelope and resulted in the lysis and death of the neutrophil[62]. A more recent study showed that NETs could be formed under conditions of shear stress *in vitro* and in capillaries of the lungs and liver *in vivo*[55]. The formation of NETs was shown to be extremely rapid (within minutes) in response to LPS or septic plasma and to require platelet binding to, and transactivation of, the neutrophils (BOX 1). This was not associated with lysis or death of the neutrophil. Once sufficiently activated, the platelets bound to neutrophils and triggered NET formation, thereby markedly increasing the bacterium-trapping capacity of the neutrophils. Platelet TLR4 is activated by 100–1,000 fold more LPS than neutrophil TLR4, which raises the possibility that platelets function as a 'barometer' in the blood, becoming activated only during serious systemic infections. This subsequently would induce neutrophils to release their proteolytic and chromatin material, and thereby increase the capacity of the innate immune system to trap and kill circulating bacteria[55]. Interestingly, platelet binding to neutrophils occurred mainly in the capillaries and sinusoids of the lungs and liver, which suggests that the tropism of neutrophils for these organs during sepsis might be a coordinated strategic move by the immune system to help catch and kill bacteria in the blood, rather than an evasion strategy by bacteria to divert immune cells.

NETs are extremely adhesive and as such avidly bind Gram-positive bacteria (such as *S. aureus*, group A *Streptococcus* species and *S. pneumoniae*) and Gram-negative bacteria (such as *Shigella flexneri* and *S.* Typhimurium), as well as fungi (such as *Candida albicans*) (reviewed in REF. 63). Because chromatin forms the structural basis of NETs (FIG. 4), treatment with DNases degrades these structures and greatly reduces microbial killing[61]. It is intriguing that bacteria that do not produce DNases tend to be less pathogenic[64]. For example, DNase-expressing strains of group A *Streptococcus* species and *S. pneumoniae* can escape killing by NETs, whereas their DNase-deficient counterparts cannot, and the former are more virulent. The reverse is also true: induced expression of DNase in DNase-deficient bacterial strains results in a marked increase in bacterial proliferation *in vivo*[65]. Furthermore, *S. pneumoniae* strains that produce DNase can disseminate far more effectively in the blood than *S. pneumoniae* that do not. In addition, the *S. pneumoniae* polysaccharide capsule may offer further protection because it reduces binding to NETs[66].

Avoiding intravascular innate immune cells by binding to the endothelium. In an attempt to escape the intravascular immune system and colonize the host to establish a replicative niche, many pathogens have evolved the ability to adhere to endothelial cells and then enter tissues. A wide variety of Gram-negative bacteria, including many strains of *Escherichia coli*, have evolved an interesting way of adhering to the endothelium that is not dissimilar to the way in which immune cells adhere through selectins[67]. Bacterial pili or fimbriae, which are structural extensions that have lectin adhesins at the tips, express FimH protein for binding to monomannose residues of host cells under flow conditions. Counter-intuitively, the duration of these biological adhesive interactions, known as catch bonds, are increased by tensile mechanical force[68]. In other words, there is a threshold level of shear stress that is necessary to enable firm adhesion of *E. coli* to the host endothelium. Although not yet shown, it is possible that these adhesive interactions might underlie bacterial tropism, for example, the tropism of meningococci for the endothelium in the brain, but not the liver, vasculature owing to the different levels of shear stress in these organs.

The interactions of Gram-positive bacteria with endothelial cells do not involve fimbriae but may still involve as-yet-unidentified catch bonds. *S. aureus* is a Gram-positive extracellular pathogen that can cause endovascular infection, resulting in diseases such as endocarditis[69]. Intriguingly, bacterial growth has been noted at sites of abnormally high shear stress, suggesting shear stress-dependent adhesion. *In vivo* imaging studies have shown that *S. aureus* that is introduced into the blood rapidly adheres to endothelial cells under flow conditions[69]. This could be mediated by fibronectin-binding proteins, which promote adhesion to several biological substrates, including endothelial cells[70]; adhesion to the endothelium is decreased in the absence of these proteins[71].

The adhesion of other Gram-positive bacteria is most efficient in inflamed blood vessels. For example, *S. pneumoniae* can adhere to resting endothelial cells by interacting with endothelial cell-expressed carbohydrates, but can only invade when endothelial cells are activated during inflammation. This is because *S. pneumoniae* express phosphorylcholine, a component of platelet-activating factor, on their cell walls and can therefore bind to PAF receptor expressed by endothelial cells[72].

Finally, numerous non-bacterial microorganisms adhere to the endothelium as a means of avoiding clearance in the spleen. Erythrocytes infected with the malarial parasite *Plasmodium falciparum* avoid passing through the spleen by undergoing rolling and adhesive interactions (similar to those between leukocytes and endothelial cells in the postcapillary venules) because they express *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) on their membranes. The parasites subvert endothelial cell adhesion molecules, including ICAM1 and CD36, for this process, as was shown *in vivo* under physiological flow conditions in the microvessels of human skin grafted onto immunodeficient mice[73].

Stealth as a mechanism of evasion. Recently, spinning disk confocal microscopy was used to examine adhesion of the spirochete *B. burgdorferi* to microvascular endothelium under flow conditions *in vivo*[74,75]. When delivered into the circulation, *B. burgdorferi* interacted with capillaries, postcapillary venules and larger veins, but not with arterioles (FIG. 5). This was probably because of higher shear stress in the arterioles, as reducing flow induced bacterial adhesion. *B. burgdorferi* tethered and adhered to the vessels through the interaction of host fibronectin and glycosaminoglycans with the *B. burgdorferi* fibronectin- and glycosaminoglycan-interacting protein BBK32 (REFS 74,75).

A striking observation was that *B. burgdorferi* could adhere and move unopposed by neutrophils and other immune cells in the skin vasculature. In fact, neutrophils, monocytes and other circulating immune cells seemed to be completely oblivious to the bacteraemia. Although neutrophils do recognize *B. burgdorferi* and undergo activation and chemotaxis *in vitro*[76], there was a complete lack of detection *in vivo,* suggesting that *B. burgdorferi* induces potent inhibitory mechanisms. Indeed, *B. burgdorferi* are resistant to the alternative pathway of complement activation[77], and this is probably mediated by many mechanisms. Among these are the expression of several distinct outer-surface proteins (known as *B. burgdorferi* complement regulator-acquiring surface proteins) that bind the host serum complement inhibitor factor H, the degradation of C3b and C3 convertase and the prevention of membrane attack complex formation[78–80]. Several other species of bacteria, such as group A *Streptococcus* spp., have also developed similar strategies (for example, binding factor H) to inhibit complement[81]. Interestingly, our preliminary findings show that Kupffer cells do recognize, capture and ingest *B. burgdorferi* *in vivo* by unknown mechanisms (W.Y. Lee and P.K., unpublished observations).

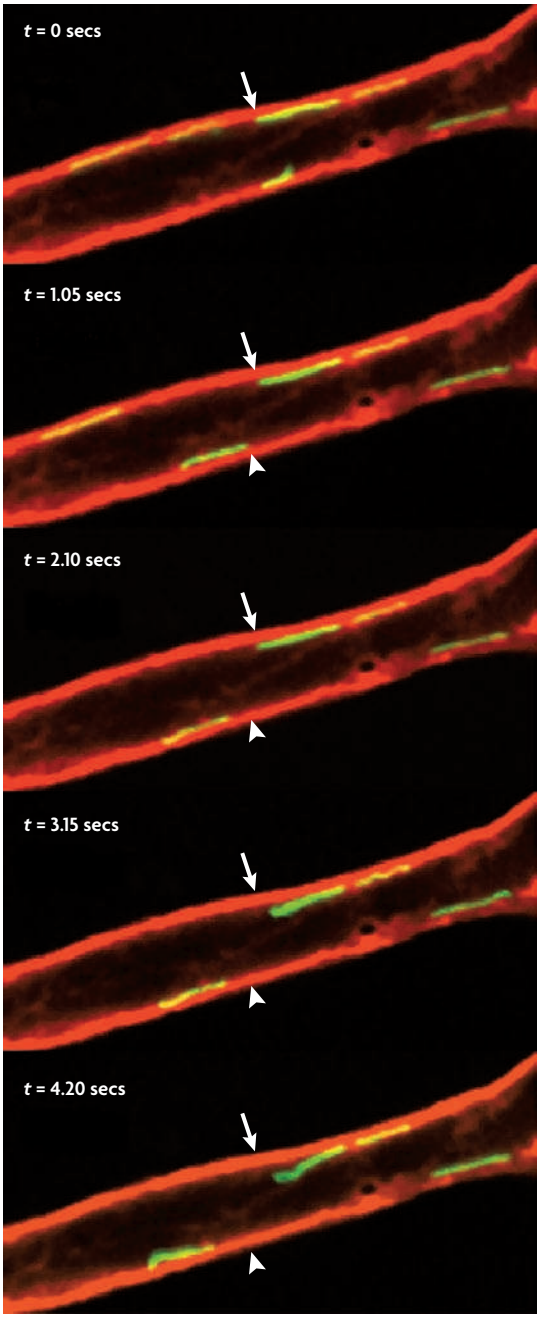

Figure 5 | **Adhesion and translocation of *Borrelia burgdorferi* in the vasculature.** Time sequence of confocal microscopy images of *B. burgdorferi* (expressing green fluorescent protein; GFP) migrating within a dermal postcapillary venule (red; labelled using Alexa 555-conjugated antibody specific for platelet/endothelial cell adhesion molecule 1). The initial positions of two bacteria are indicated by arrows or arrowheads, and the time (in seconds) shown in the upper left corner of each frame indicates the elapsed time after the initial image. Migration of the bacteria along the endothelial cell surface is apparent by the displacement from their initial positions in the later images. The opposing directions of migration show that this process is independent of the direction of blood flow. Despite the presence of these intravascular bacteria, no evidence of endothelial cell activation or leukocyte recruitment is observed.

Preventing leukocyte recruitment. Neutrophils encounter and 'prioritize' many chemoattractant gradients to pursue bacteria. For example, neutrophils adhere to the endothelium of blood vessels in response to chemokines, including CXCL8 (also known as IL-8). However, while still in the presence of this initial stimulus, they must eventually be able to respond to other chemoattractants that are generated by bacteria (such as *N*-formyl-methionyl-leucyl-phenylalanine; fMLP) to leave the vasculature and arrive at the site of infection. Neutrophils undergo chemotaxis using at least two separate signalling pathways: the phosphoinositide 3-kinase (PI3K)–phosphatase and tensin homologue (PTEN) pathway (which is stimulated by CXCL8) and the p38 pathway (which is stimulated by fMLP), with the p38 pathway dominating over the PI3K pathway[82,83]. Chemotaxis in response to CXCL8 requires that phosphatidylinositol-3,4,5-trisphosphate ($PtdIns(3,4,5)P_3$) is located at the leading edge of a migrating neutrophil and PTEN (which dephosphorylates $PtdIns(3,4,5)P_3$ to generate phosphatidylinositol-4,5-bisphosphate ($PtdIns(4,5)P_2$)) is located along the sides and uropod of the cells[84]. fMLP also induces the localization of PTEN at the sides and uropod of the cell, but does not depend on $PtdIns(3,4,5)P_3$[85]. In opposing gradients, PTEN becomes distributed throughout the cell circumference, thereby inhibiting all $PtdIns(3,4,5)P_3$ and consequently allowing preferential migration towards bacterial products in a p38-dependent manner[85]. Such prioritization is defective in *Pten*$^{-/-}$ neutrophils, resulting in compromised bacterial clearance *in vivo*. These observations indicate that neutrophils, through the actions of PTEN, prioritize and integrate responses to multiple chemotactic cues.

The bacterial product LPS blocks neutrophil migration towards chemokines such as CXCL8 by activating TLR4, which induces a p38-dependent inhibition of $PtdIns(3,4,5)P_3$. LPS itself does not have chemoattractant properties, so the neutrophils stop and do not migrate towards the source of LPS. In fact, LPS also blocks migration towards fMLP by an unknown mechanism[83]. In mice, although systemic administration of LPS has been shown to result in adhesion of neutrophils in the microcirculation, no transmigration into the extravascular compartment occurred[51]. Adding a chemotactic stimulus in an attempt to draw leukocytes out of the vasculature was insufficient to cause emigration[86]. The mechanism by which LPS blocks neutrophil emigration remains elusive, but it is possible that LPS-induced p38 activation sets up a PTEN 'barrier' (as described above) around the entire cell, which prevents neutrophils from responding to chemotactic stimuli; a similar stop signal is observed with TNF (an activator of p38)[87]. This suggests that the presence of LPS and TNF in tissues probably facilitates the recruitment of neutrophils from the vasculature by activating endothelial cells and macrophages and preventing neutrophils from migrating away from these sites. However, when in the blood, these mediators may serve to distract or prevent neutrophils from being recruited to the appropriate tissue. This issue is further compounded by the fact that

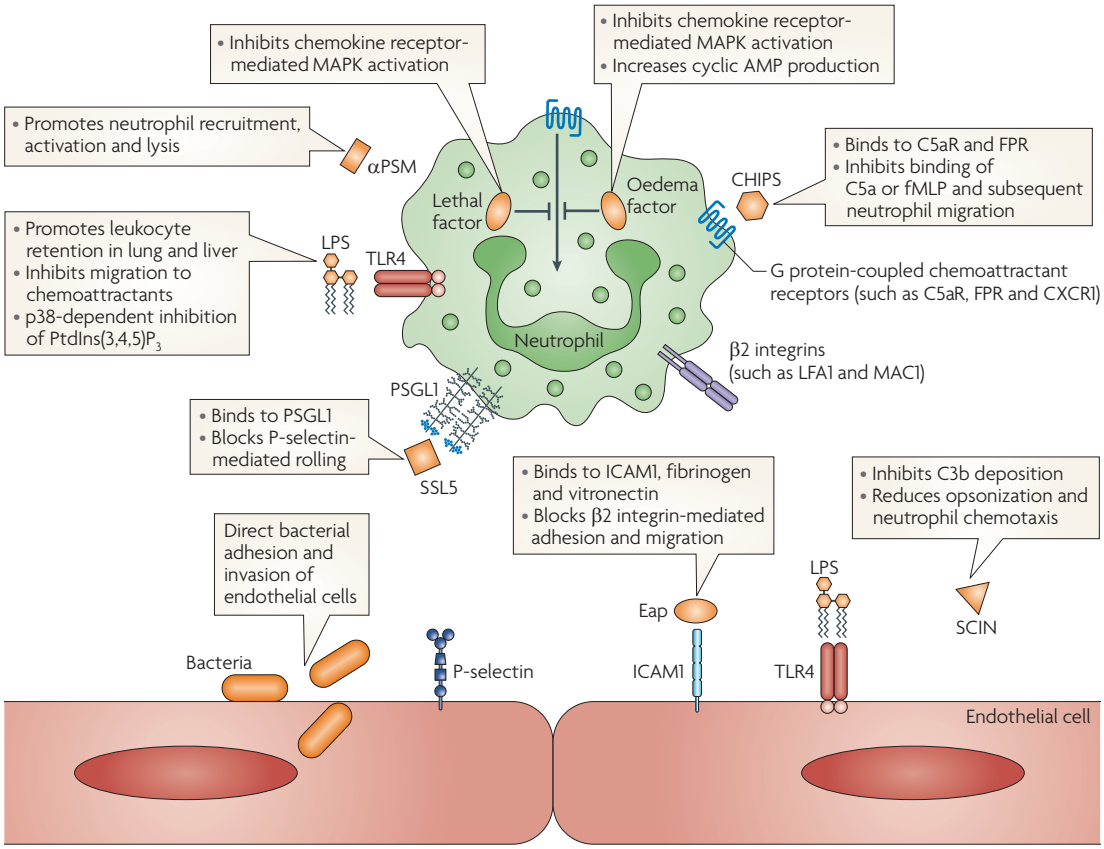

Figure 6 | **Bacterial distraction mechanisms in the vasculature.** Bacteria use a wide range of approaches to 'distract' leukocytes and reduce their ability to effectively clear bacterial pathogens. Bacteria can directly adhere to and invade endothelial cells, thereby avoiding detection by patrolling immune cells. Lipopolysaccharide (LPS)-mediated activation of Toll-like receptor 4 (TLR4) leads to neutrophil retention in the pulmonary and hepatic vasculatures, and LPS-mediated leukocyte activation triggers p38 (a mitogen-activated protein kinase (MAPK)) and inhibits leukocyte migration. *Staphylococcus aureus* produces various molecules that inhibit leukocyte recruitment: α-type phenol soluble modulin (αPSM) peptides induce recruitment, activation and subsequent lysis of neutrophils; CHIPS (chemotaxis inhibitory protein of *S. aureus*) binds to receptors for complement component 5a (C5aR) and formyl peptide (FPR), inhibiting leukocyte migration; staphylococcal superantigen-like 5 (SSL5) binds to P-selectin glycoprotein ligand 1 (PSGL1) on the surface of leukocytes and inhibits rolling; extracellular adherence protein (Eap; also known as Map) binds to intercellular adhesion molecule 1 (ICAM1) and inhibits β2 integrin-mediated adhesion to endothelial cells; staphylococcal complement inhibitor (SCIN) inhibits C3b deposition on bacteria, thereby reducing phagocytosis by neutrophils and neutrophil chemotaxis. *Bacillus anthracis* also produces toxins that reduce leukocyte migration. *B. anthracis* lethal factor cleaves members of the MAPK kinase (MAPKK) family downstream of chemokines receptors, limiting immune cell activation, whereas oedema factor causes a prolonged increase in cellular cyclic AMP, which inhibits cell migration, cytokine and oxidant production, and phagocytosis. CXCR1, CXC-chemokine receptor 1; fMLP, *N*-formyl-methionyl-leucyl-phenylalanine; LFA1, lymphocyte function-associated antigen 1; MAC1, macrophage receptor 1; PtdIns(3,4,5)P_3, phosphatidylinositol-3,4,5-trisphosphate.

LPS from some organisms can also activate complement[88], which results in the generation of chemoattractants such as C5a; this would add to the complexity of the chemotactic cues detected by neutrophils.

In addition to the distracting signals provided by LPS, leukocytes must overcome a diverse arsenal of additional bacterial products that specifically interfere with leukocyte recruitment[50] (FIG. 6). For example, clostridial species release toxins that damage the vasculature and inhibit microvascular blood flow, thereby limiting neutrophil recruitment and oxygen delivery to the affected site (which are conditions that favour the growth of these anaerobic bacteria)[89]. A more subtle set of bacterial products that combat the recruitment process is exemplified by *S. aureus*. This highly virulent pathogen expresses numerous molecules that target each step of the recruitment cascade (reviewed in REF. 50). One example is staphylococcal complement inhibitor, which stabilizes C3 convertases, thereby inhibiting C3b deposition and ultimately inhibiting neutrophil uptake of bacteria and neutrophil chemotaxis[90,91]. Some strains of methicillin-resistant *S. aureus* express α-type phenol soluble modulin peptides, which induce the recruitment and activation of neutrophils and subsequently promote neutrophil lysis[92]. Furthermore, staphylococcal superantigen-like 5 binds to P-selectin

glycoprotein ligand 1, the main ligand of P-selectin, and inhibits the rolling of neutrophils on endothelial cells[93]. The *S. aureus* protein CHIPS (chemotaxis inhibitory protein of *S. aureus*) binds to the G protein-coupled chemoattractant receptors for C5a and formyl peptides on immune cells, thereby inhibiting binding of the natural ligands for these receptors[50]. This impedes normal neutrophil chemotaxis that is associated with bacterial infection. In addition, the *S. aureus* extracellular adherence protein (Eap; also known as Map) binds to a range of proteins, including ICAM1, and inhibits β2 integrin-dependent neutrophil adhesion to endothelial cells[50]. By targeting distinct elements of the multistep process of neutrophil recruitment, the combined actions of these proteins generate a highly effective disruption of the neutrophil response that would otherwise target the infection.

Concluding remarks

The studies reviewed here serve to illustrate the complex nature of the 'war' between the immune system and invading bacteria, using the vasculature as a 'battlefield'. The recent imaging studies have markedly expanded our knowledge of this intravascular immune response, but our understanding of the mechanisms underlying the actions of bacteria within the vasculature is still at its infancy. Investigation of the tissue-specific mechanisms of bacterial interactions with endothelial cells, particularly in organs such as the brain, where infection can have devastating consequences, could be highly beneficial. Knowledge derived from studies of this nature has the potential to lead to new therapeutic modalities for sepsis, which continues to result in an unacceptably high mortality rate.

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DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
α4 integrin | β2 integrin | CRIg | CXCL8 | CXCL16 | CXCR6 | E-selectin | ICAM1 | LFA1 | PECAM1 | PTEN | P-selectin | SHAP | TNF

FURTHER INFORMATION
Paul Kubes's homepage:
http://www.ucalgary.ca/paulkubeslab
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