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The extravasation cascade revisited from a neutrophil perspective

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Neutrophil extravasation is a critical event during immune responses to infection or injury that ensures survival. However, given the destructive potential of neutrophil effector molecules such as proteases and reactive oxygen species, strict control of neutrophil recruitment is required to avoid tissue damage. Neutrophil extravasation is a multistep process of adhesive interactions between neutrophils and components of the venular wall, that is, endothelium, basement membrane and pericytes. A plethora of proteins have been identified as critical regulators of each step that provide for compensatory mechanisms thus ensuring that extravasation can happen even if one mechanism is defective. Here, we discuss recent discoveries of how extravasation is regulated with emphasis on neutrophil-specific mechanisms; and discuss potential implications for future research.

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Introduction: the classical understanding of neutrophil extravasation

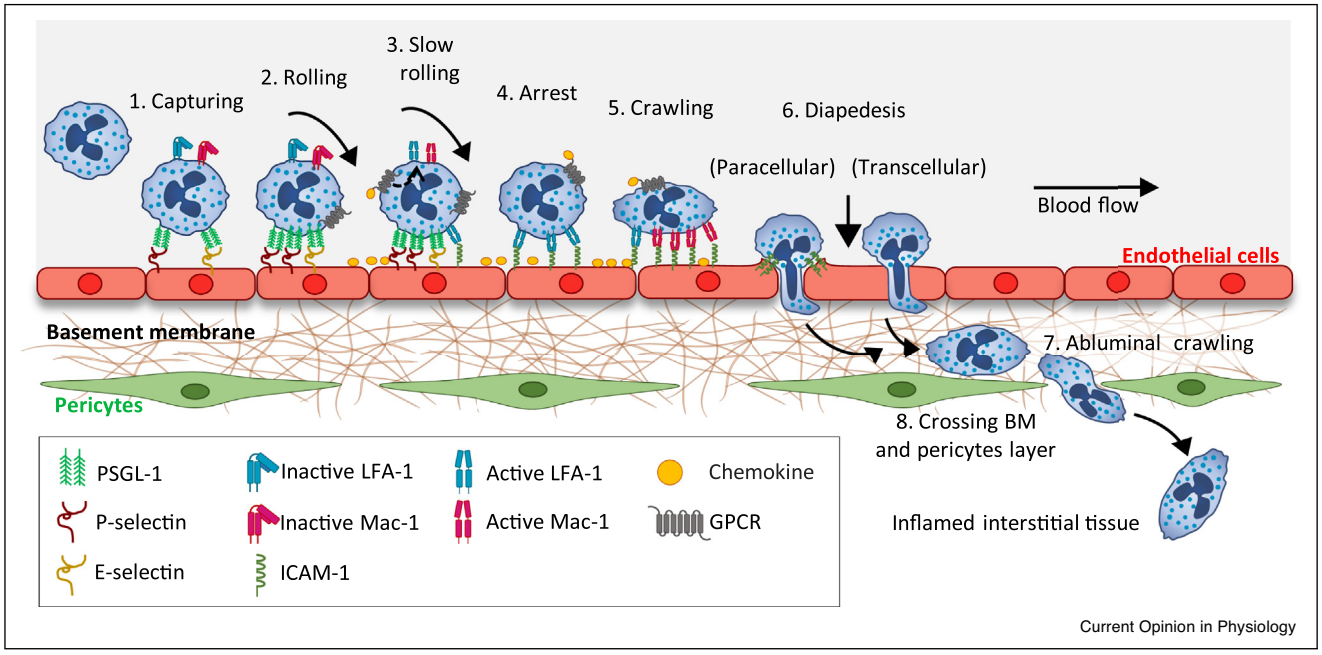
Inflammation is a physiological response to infection and injury and ensures survival. One of the first events during inflammation is the local activation of vascular endothelial cells (EC) [1,2]. Neutrophils respond quickly and bind to endothelial adhesion molecules to initiate the extravasation cascade. Neutrophils are needed in the inflamed tissue to eliminate invading microorganisms; and subsequently contribute to tissue healing [3,4]. To this end, neutrophils secrete many active molecules after reaching inflamed tissues including proteases and reactive oxygen species [5]. However, in excess, these substances also damage host tissues so that neutrophil recruitment needs to be tightly regulated.A detailed understanding of the mechanisms driving neutrophil extravasation is critical because the multiple adhesive and signaling events may serve as targets to attenuate neutrophil recruitment in chronic inflammatory diseases [6].

The classical extravasation cascade involves selectin-mediated rolling, selectin-mediated and chemokine-mediated integrin activation leading to arrest, spreading and crawling on the endothelial surface to find permissive spots for diapedesis, abluminal crawling and breaching of the basement membrane and pericyte layer into the affected tissue (Figure 1). The extravasation cascade has been well studied and the interested reader is referred to many recent excellent reviews covering the known mechanisms occurring in EC and different types of leukocytes [1,7–11]. However, new *in vivo* imaging methods [12,13] and gene-manipulated animal models have permitted a more detailed dissection of the events during extravasation. Here, we discuss new key mechanisms through which neutrophils facilitate interactions with vascular components and transmigration (Figure 2).

Capturing, tethering, rolling

Neutrophils are captured on activated endothelium via reversible selectin-mediated catch bonds even at high shear stress created by the blood flow [14,15]. The drag force within venules pushes the neutrophil forward to initiate rolling, which is enabled by forming bonds at the front and breaking bonds at the rear of the neutrophil as the cell moves forward. Rolling is stabilized by long, thin tethers that reinforce the adhesive interaction with the endothelium to resist the blood's dragging force [3]. These tethers can turn into slings that swing around the rolling neutrophil to provide additional anchoring points at the front [16] (Figure 2a). Recently, an intravital imaging method has been developed demonstrating the common presence of tethers and slings on neutrophils [17•]. The videos confirmed *in vivo* during mild inflammation that the number of tethers is higher on neutrophils in venules with high wall shear stress; and that after most tether breaks rolling velocity is faster. Slings can also be observed *in vivo*, but at lower frequency. Explanations may include that most tethers retract before they can convert into slings; or are not observable because the transition from tether to sling is out of focus or too fast.

Figure 1



The neutrophil extravasation cascade.
Cartoon of neutrophil interactions with the venular wall during extravasation. Depicted are the well-studied adhesive interactions of capture, rolling, slow-rolling, arrest, crawling, diapedesis and post-diapedesis interactions in a simplified manner, highlighting some of the most important receptor-ligand pairs.

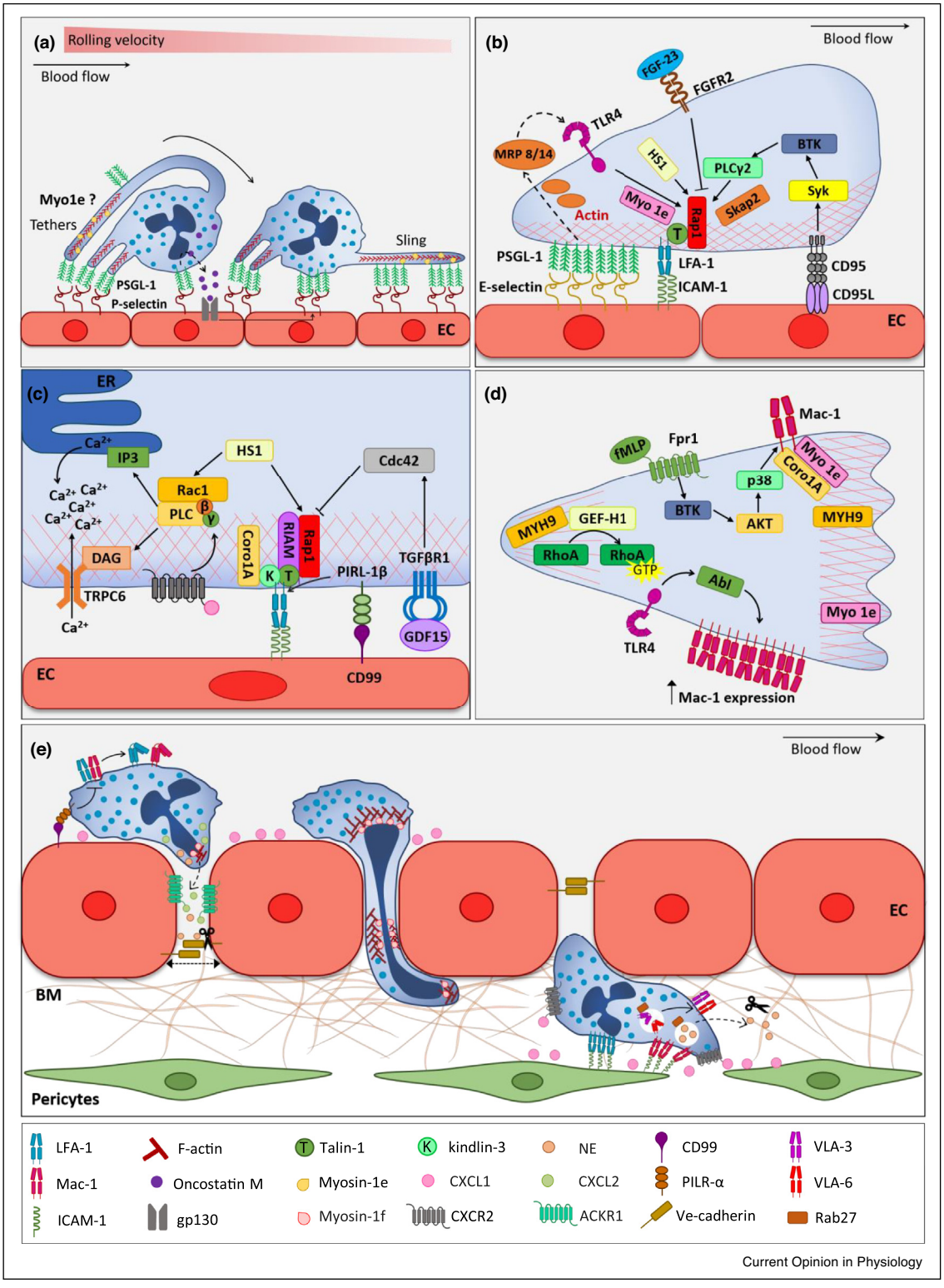
Using P-selectin-coated side-view flow chambers, this transition was observed only in 15% of all breaking tethers and occurred faster than 100 ms [18]. Of note, neutrophils roll faster when multiple tethers simultaneously break resulting in a jump forward as shown by high peaks in the rolling velocity profile over time. We have recently reported *in vivo* such a rolling behavior in myosin1e-deficient mice, termed intermittent rolling, during which rolling neutrophils frequently detach, jump, and reattach to continue rolling [19]. Considering that membrane tethers are connected to the actin cytoskeleton [20], and that myosin1e can connect membranes to the actin cytoskeleton [21], we think that myosin1e is a critical actin-binding protein (ABP) for the regulation of tether stability and tether-to-sling transition in rolling neutrophils. However, this idea needs to be tested experimentally.

Rolling neutrophils interacting with P-selectin release oncostatin M, a cytokine of the IL6 family, from granules into the circulation, where it binds to endothelial gp130 to induce clustering of P-selectin [22]. Deletion of hematopoietic oncostatin M or endothelial gp130 or application of blocking antibodies against either oncostatin M or gp130 significantly inhibits neutrophil capture and rolling [22]. This is an interesting novel paracrine mechanism by which neutrophils trigger endothelial signaling to facilitate their rolling and subsequent extravasation.

Dipeptidase-1 has been recently identified as major adhesion receptor for neutrophils in the lung and liver [23••]. Both genetic deletion and blocking antibodies inhibit neutrophil recruitment to these organs, and improve survival in endotoxemia models. On the other hand, neutrophil capture in the kidney is triggered by the neutrophil Fc-receptor FcγRIIIA [24]. In a model of anti-glomerular basement membrane (anti-GBM)-induced nephritis, neutrophils bound to deposited GBM antibodies via FcγRIIIA in an Abl/Src-dependent fashion; and inhibition of Abl/Src signaling reduced neutrophil accumulation and renal injury. These are recent examples of organ-specific neutrophil capture mechanisms highlighting the fact that reducing neutrophil recruitment is beneficial in many diseases. These data also demonstrate that therapeutic approaches targeting neutrophil recruitment must be organ-specific and disease-specific.

L-selectin is required for newly discovered neutrophil functions in lymph nodes (LN). Neutrophils recruited to draining LN in an L-selectin-dependent fashion via high endothelial venules phagocytose pathogens that breached the skin barrier and prevent their dissemination [25]. Neutrophils recirculate in LN exploiting L-selectin-mediated adhesive interactions in high endothelial venules; and a steady-state population of neutrophils within LN triggers recruitment of additional neutrophils

Figure 2



Recently discovered mechanisms driving neutrophil extravasation.
(a) Rolling. Neutrophil rolling requires the interaction of PSGL-1 and P-selectin in order to form tethers. Initial tethers become slings that swing around the rolling neutrophil and provide additional anchor points that reduce rolling velocity. Slings contain PSGL-1 patches that interact with endothelial P-selectin clusters induced by neutrophil secreted oncostatin M. Myosin-1e may link actin filaments in tethers and slings to the plasma membrane to ensure steady rolling. **(b) Slow-rolling.** Recognition of E-selectin and chemokines causes LFA-1 activation for which Rap1-mediated

in response to infection in an LTB4-dependent manner to contain the infection within LN and maintain them sterile [26]. These studies further highlight the functional versatility of neutrophils and the need for better classification of neutrophil subtypes.

Slow rolling

Under basal conditions, β2-integrins such as lymphocyte function-associated molecule-1 (LFA-1) rest in a bent, low-affinity conformation preventing them from binding its ligands. E-selectin- and chemokine-mediated signaling in rolling neutrophils induce talin-1 recruitment to LFA-1 and LFA-1 conversion to the intermediate-affinity conformation leading to initial ICAM-1 binding and slowing down rolling neutrophils [27•,28] (Figure 2b). Subsequently, sensing of optimal chemokine concentrations presented by the endothelium induces the open, high-affinity conformation of LFA-1 that involves activation of the small GTPase Ras-related protein-1 (Rap1) and presence of kindlin-3 and talin-1 leading to adhesion strengthening and firm adhesion [11]. Slowing down rolling neutrophils also requires actin dynamics [29]. For example, the ABP Src-kinase associated phosphoprotein 2 (Skap2) is required for talin-1 and kindlin-3 recruitment and β2-integrin activation [30]. Myosin-1e is required for steady rolling [19], but it remains elusive whether it does so via chemokine-induced conformational changes of β2-integrins. Both Skap2 and myosin-1e are also required for chemokine-induced arrest and macrophage-1 antigen (Mac-1)-dependent crawling, which is diminished in their absence. The ABP hematopoitic cell-specific lyn substrate (HS-1) is important for proper activation of the small GTPases Rac1 and Rap1 [31]. As both GTPases regulate integrin activation, HS1 deficiency also reduces ICAM-1 binding, leading to increased neutrophil rolling velocities, arrest and transmigration.

Absence of neutrophil plasma membrane proteins such as CD95 and the ITIM domain-containing NK receptor Ly49Q also affects slow rolling. CD95 upon recognizing its endothelial ligand CD95L induces a signaling axis involving spleen tyrosine kinase (Syk), Brutońs tyrosine kinase (Btk), phospholipase Cγ2 (PLCγ2), and Rap1 to activate LFA-1 and slow down rolling neutrophils [32]. Absence of CD95 also inhibits neutrophil effector functions leading to impaired bacterial clearance during sepsis. By contrast, absence of Ly49Q causes LFA-1 and Mac-1 hyperactivation leading to reduced rolling velocities and increased arrest, which in turn inhibits crawling and transmigration [33].

Slow rolling and firm adhesion are also controlled by the neutrophil molecules myeloid-related protein 8 and 14 (MRP8/14), which are secreted upon P-selectin glycoprotein ligand-1 (PSGL-1) binding to E-selectin. MRP8 and 14 are recognized in an autocrine manner by TLR4, which in turn activates Rap-1 and β2 integrins [34]. On the other hand, fibroblast growth factor 23 inhibits LFA-1 activation and slow rolling by activating PKA and inhibiting Rap1 downstream of FGF receptor 2 [35].

Current knowledge therefore suggests that the transition from rolling to slow-rolling is a fine-tuned process that relies not only on the action of selectins and chemokines, but also on actin dynamics and protein secretion that orchestrate the molecular events necessary to activate integrins and arrest neutrophils on the endothelial surface.

Arrest

In response to interleukin-8 (IL-8), one human neutrophil requires 4625+/−369 LFA-1 molecules in its extended, high-affinity conformation in order to arrest [36••]. LFA-1 molecules undergo this transition via intermediate states of either extended, non-high-affinity or bent, high-affinity. Superresolution microscopy combined with molecular modeling revealed that bent, high-affinity LFA-1 molecules form micro-clusters in which they face each other, and that this molecular organization requires binding to ICAM1 on neutrophils in *cis*. Of note, this molecular interaction contributes to limiting neutrophil adhesion and accumulation during inflammation. *Cis* interactions have also been described for Mac-1 with FcγRIIA that also have immune-modulatory functions by reducing neutrophil recruitment and decreasing affinity of FcγRIIA for IgG [37].

(Figure 2 Legend Continued) talin-1 recruitment is required. Several newly discovered signaling proteins regulating this process are depicted. Moreover, actin-binding proteins (ABP) such as Skap2, HS1 and Myosin-1e have recently been identified as critical regulators of slowing down rolling neutrophils. **(c) Arrest.** Chemokine-induced dissociation of G-proteins from GCPR and induction of calcium flux from intracellular and extracellular sources induce the high-affinity, extended LFA-1 conformation in a Rac1-dependent and Rap1-dependent fashion. GDF15-induced activation of TGFβR can inhibit LFA-1 activation via cdc42. ABP such as talin-1, Skap2, coronin-1A and HS1 are important for full LFA-1 activation. **(d) Crawling.** BTK, AKT and p38 drive Mac-1 activation upon chemokine recognition. ABP such as coronin-1A, Skap2 and Myosin-1e regulate crawling. Neutrophil polarization is important for crawling: actin remodeling at the leading edge requires myosins such as MYH9 and Myosin-1e, and uropod formation is controlled by GEF-H1-mediated RhoA activation. TLR4 activation causes Abl1-dependent Mac1 overexpression to trigger crawling. **(e) Diapedesis.** Paracellular diapedesis is controlled by neutrophil-released CXCL2 that binds to endothelial junctional ACKR1 to guide neutrophils into the abluminal compartment. Neutrophil elastase (NE) degrades VE-cadherin and basement membrane (BM) proteins. Abluminal crawling is facilitated by interactions of VLA3 and VLA6 with BM laminins and LFA-1/Mac1 interactions with pericyte ICAM-1. Neutrophils finally leave the vascular wall by crossing through regions of low BM protein expression and pericyte gaps. For all panels: Arrows = activation, dotted arrows = autocrine loop, capped lines = inhibition, question mark = likely, but experimentally unproven mechanism, scissors = proteolytic degradation. Symbols are explained in the legend below the panels.

Integrin transmembrane domains are essential for full activation. Thus, mutating the transmembrane domain has been suggested to prevent the conformational change of LFA-1 induced by talin1 binding to the cytosolic domain that triggers extension and high-affinity conformation of the extracellular domain. Indeed, a proline mutation in the transmembrane domain of the β2 integrin that generates a flexible kink to uncouple communication between the cytosolic and extracellular domains inhibits chemokine-induced LFA-1 transition into the extended conformation, but not the transition into the high-affinity conformation. Consequently, full activation of LFA-1 containing this mutation is severely impaired and rolling HL60 cells can not slow down and arrest in response to IL-8 [38]. Of note, talin-1 depletion impairs both extension and high-affinity conformation suggesting that the proline mutation preventing extension only reflects one of talin-1 functions, and another talin1-induced mechanism must exist that triggers the conformational change for high affinity. Talin-1 needs to interact with membrane-anchored active Rap1 and its effector protein RIAM to efficiently activate integrins. Mice expressing a mutated talin-1 unable to bind Rap1 show defective integrin activation leading to reduced neutrophil adhesion and transmigration *in vivo* [39•]. This observation is in line with our previous finding that defective Rap1 activation in the absence of the ABP HS1 also causes defective adhesion and transmigration [31], although a causal relationship remains to be proven. Another ABP that binds to the cytoplasmic tail of β2 integrins is coronin-1A. In mice, it is enriched in neutrophil-EC contact zones during LFA-1-dependent arrest [40]. It also participates in Mac-1 affinity regulation, adhesion strengthening, and intraluminal crawling, without regulating calcium flux. The ABP vinculin is also important for neutrophil arrest, spreading, and polarization *in vitro*, but seems to be dispensable *in vivo* [41]. The fact that some ABP appear to have specific (actin-related) functions in a context-dependent manner, with differences *in vitro* and *in vivo*, adds another degree of complexity to their roles during neutrophil extravasation.

In mice, neutrophil arrest occurs predominantly in response to chemokines such as CXCL1 and CXCL2 [42••] that are recognized by G-protein-coupled receptors (GPCR) that contain all G proteins except Gαi3 [43]. These G proteins are critical for the acquisition of the high-affinity conformation of LFA-1 and its clustering [44]. One of the central events in chemokine-induced integrin activation is the generation of calcium flux from intracellular reservoirs and the extracellular space (Figure 2c). GPCR activation induces G protein dissociation and subsequent formation of a signaling complex containing Rac1, PLCβ2 and PLCβ3 to generate inositol-1,4,5- triphosphate (IP3) and diacylglycerol (DAG) [43]. While IP3 regulates calcium release from intracellular reservoirs, DAG controls activation of the transient receptor potential channel 6 (TRPC6) to import calcium from the extracellular space. TRPC6 is essential for CXCL1-induced Rap-1 activation and ICAM-1 binding *in vitro*, and for CXCL1-induced arrest *in vivo* [45]. On the other hand, the calcium release-activated calcium channel protein 1 (Orai-1) induces calcium flux in response to LFA-1 engagement leading to morphological changes in adherent neutrophils. This calcium flux increases with shear stress and adhesive strength suggesting that neutrophils sense mechanical forces generated by LFA-1 engagement to regulate calcium signaling and subsequent polarization and spreading to direct neutrophils to the region of the inflamed vessel where transmigration is required [46]. This is another example of how mechano-sensing contributes to neutrophil responses.

Neutrophil polarization during extravasation is triggered by local generation of plasma membrane curvature in response to adhesion [47]. This local curvature elicits a cascade of signaling events culminating in myosin light chain phosphorylation and increased actomyosin contractility that triggers neutrophil arrest and polarization. Whether these events are connected to the above described LFA1-dependent mechano-sensing and calcium flux should be investigated further.

Other neutrophil membrane receptors recently identified to be implicated in extravasation are the paired immunoglobulin-like receptor β1 (PILR-β1) and PILR-α [48,49]. While PILR-β1 binding to CD99 induced by shear stress contributes to integrin activation and firm adhesion, PILR-α weakens this adhesive interaction to favor transmigration. The cytokine growth-differentiation factor 15 (GDF-15) counteracts chemokine-induced integrin activation via TGF-βRI and TGF-βRII involving a cdc42-dependent mechanism. Inhibition or gene deficiency of these two receptors prevent the inhibitory effect of GDF-15 on neutrophil integrin activation, arrest and extravasation [50]. It will be of clinical relevance to explore GDF-15 as treatment option in patients with chronic inflammatory diseases characterized by excessive neutrophil recruitment.

Crawling

CXCL1, in addition to inducing neutrophil arrest, also controls intraluminal and abluminal crawling [42••]. Neutrophils mainly depend on Mac-1 to crawl; however, recent studies provide evidence that LFA-1 also participates in this process at least *in vitro* [51,52]. Activation of the fMLP receptor Fpr-1 leads to activation of Btk, which in turn phosphorylates Akt and p38 to trigger Mac1-dependent post-adhesion strengthening and crawling [53] (Figure 2d). The Btk inhibitor PRN473 reduces intravascular crawling and neutrophil recruitment during sterile liver injury. Moreover, TLR-4 stimulation induces upregulation of Mac-1, neutrophil

accumulation in lung capillaries and *in vivo* crawling in an Abl-dependent manner. In this study, lung capillaries were identified as a niche for neutrophils to ensure rapid neutrophil responses upon bacterial insult [54••]. Inside lymphatic vessels, TNF-α induces ICAM-1 expression on the surface of the lymphatic endothelium and neutrophil crawling via Mac-1/ICAM-1 interactions [55].

During crawling, the non-muscle class-II myosin, MyH9 is enriched at lamellipodial tips and the uropod of crawling cells. Similar to Myo1e, MyH9 deficiency compromises neutrophil crawling and extravasation due to a defect in F-actin dynamics [19,56]. Myo1e depletion only reduces the number of crawling cells but not their velocity suggesting that it is rather required for the transition from arrest to crawling and not during crawling itself. By contrast, depletion of MyH9 significantly reduces neutrophil crawling distance and velocity. During chemotaxis and crawling, the formation of a leading edge and uropod requires strict regulation of F-actin dynamics in a spatiotemporal manner depending on Rac1 and RhoA signaling [57]. In this context, GEF-H1 is a guanine nucleotide exchange factor specific for Rho-A that controls neutrophil uropod contractility and crawling [58]; and Slit-Robo GTPase-activating protein 2 (SRGAP2 or ArhGAP34), a Rac1 activator, controls adhesion, neutrophil polarization and actomyosin contractility suggesting that it also regulates crawling [47]. However, more investigation is needed to decipher the exact mechanisms of actin dynamics and the precise role of ABP in this spatiotemporal control of polarization preceding crawling.

Diapedesis

Neutrophils predominantly emigrate via the paracellular route across the post-capillary endothelium. During paracellular diapedesis, neutrophils extend a pseudopod into EC contacts, bind to available endothelial junction adhesion receptor and then squeeze their body through to the basolateral side (Figure 2e). A critical requirement for breaching the endothelium is the displacement of VE-cadherin from the junctional space, for which no known neutrophil receptor exists. Removal of VE-cadherin mainly occurs by internalization [59], but in response to complement activation, locally secreted neutrophil elastase (NE) and metalloproteases degrade VE-cadherin to promote paracellular gaps that can then be exploited by neutrophils for extravasation [60].

In contrast to VE-cadherin, other endothelial junctional proteins act sequentially as ligands for neutrophil adhesion receptors. Neutrophil β2-integrins are the first to engage junctional adhesion molecules A and C (JAM-A, JAM-C), followed by homophilic interactions of PECAM-1 and then CD99 [61]. Recent studies provide evidence that neutrophil PILRs bind to endothelial CD99 to support diapedesis *in vivo*. CD99 deficiency results in the accumulation of neutrophils between EC and the basement membrane in TNF-inflamed cremaster muscles [48]. Moreover, CD99 is a heterophilic ligand for inhibitory PILR-α and thus contributes to integrin deactivation and neutrophil entry into endothelial contacts [48,49], suggesting that some adhesion receptors are critical for both initiation and termination of diapedesis.

Deformation and translocation of the nucleus are also required in neutrophils to pass efficiently through the narrow endothelial junctions (Figure 2e). Nuclear squeezing begins with the insertion of a nuclear lobe into the lamellipodium at the leading edge of the migrating neutrophil, followed by pushing into the endothelial junction, deformation and elongation of the nucleus, and finally retraction of the nucleus from the junction [62•,63,64]. Insertion of nuclear lobes displaces endothelial actin stress fibers and promotes formation of paracellular gaps between two adjacent endothelial cells, or transcellular pores within the body of an endothelial cell, through which neutrophils can transmigrate. Surprisingly, in this context, endothelial contractility was not required for opening of gaps [62•]. Neutrophils express low levels of lamin-A to maintain a soft nuclear lamina and allow rapid nucleus translocation during transmigration through rigid collagen barriers [64]. However, a stiffer nucleus in laminA-overexpressing HL60 cells did not affect the ability to cross an endothelial monolayer and only induced slower nuclear squeezing and enlargement of paracellular gaps [64]. Myosin1f-deficient neutrophils show impaired nuclear deformation through an endothelial monolayer and strongly reduced extravasation, without showing differences in apical adhesive interactions [63]. This contrasts data from myosin1e-deficient neutrophils that show intermittent rolling and reduced apical adhesion. Here, the transmigration defect is similar to the adhesion defect suggesting that Myo1e in addition to firm adhesion does not further affect diapedesis. Future research is required to clarify why these structurally similar class-I myosins have different functions during extravasation. A possibility includes that Myo1f enables dynamic linking of the nucleus membrane to the actin cytoskeleton that regulates deformation, whereas Myo1e rather acts at the plasma membrane to regulate initial neutrophil–endothelial interactions.

During transmigration, neutrophil sense locally presented chemokines within an inflamed environment; and this is driven by compartmentalization of chemokine depots that control the directionality of diapedesis [42••]. CXCL2 is secreted in an autocrine manner by transmigrating neutrophils within the junctional space to guide luminal-to-abluminal migration (Figure 2e). CXCL2 binds within junctions to the endothelial junctional atypical chemokine receptor ACKR1 to form a chemokine depot for self-guidance during

transmigration. The importance of CXCL2 in regulating proper uni-directionality of migration was demonstrated by the increased frequency of neutrophils aborting diapedesis in TNF-inflamed post-capillary venules when blocking CXCL2; and the same occurred in ACKR1-deficient mice [42••].

Additionally, local hyperpermeability alters the directional gradient of chemokines, that is, increased endothelial permeability leads to chemokine translocation from the interstitium into the systemic circulation, from where it directs transmigrated neutrophils back into the vascular lumen [65•]. Together, these studies show that neutrophils depend on correct local deposition and presentation of chemokines for proper luminal-to-abluminal diapedesis. These are paradigm-shifting studies of high clinical relevance because under pathological conditions with increased microvascular permeability, when correct deposition of chemokines is compromised, transmigrated and activated neutrophils can reverse-transmigrate and circulate to secondary organs, where they contribute to tissue damage [65•,66]. Thus, it is important to control local vascular permeability in inflammatory diseases to avoid aberrant neutrophil migration dynamics.

Post-diapedesis

After crossing the endothelial barrier, neutrophils need to navigate through the underlying venular basement membrane (BM) and pericyte layer to reach the inflamed interstitial tissue (Figure 2e). The BM is composed of a dynamic, heterogeneous network of collagen type IV and laminins. Neutrophils use regions of low laminin-411 and collagen IV expression as preferred sites to cross the BM [67]. Furthermore, binding of neutrophil integrins VLA-3 (α3β1) and VLA-6 (α6β1) to laminins, and NE-mediated laminin cleavage are important for neutrophil motility inside the BM. After ischemia/reperfusion injury, transmigrating neutrophils release NE within the venular BM to induce degradation of BM proteins and pore formation [8]. To this end, intracellular vesicles containing NE, VLA-3 and VLA-6 are mobilized to the cell surface; and the mammalian sterile 20–like kinase 1 (MST1) regulates this translocation in a Rab27-dependent manner [68]. Rab27 interaction with the neutrophil Src family kinases Hck, Fgr, Lyn is also important for vesicle transport [69]. Curiously, in mice-deficient for either MST1 or Src family kinases, neutrophils accumulate within the BM, thus highlighting the critical role of these integrins and proteases in aiding neutrophils to cross the BM and finish extravasation [68,69]. Whether MST1 and Src family kinases work in a coordinated fashion during vesicle trafficking remains unknown and needs further analysis.

VLA-3 also regulates adhesive forces at the head of polarized neutrophils during migration through the BM [70]. In this study, uropod elongation before retraction has been proposed as the final step of neutrophil extravasation. Moreover, VLA-3 is highly expressed on septic neutrophils; and blocking VLA-3 reduces neutrophil recruitment and improves survival during sepsis [71]. It will be interesting to study whether VLA-3 acts together with VLA-6 in a coordinated or sequential manner during neutrophil migration through the BM, and whether their functions are regulated by abluminal chemokine gradients.

Crossing the pericytes layer is the final step of neutrophil transmigration into the inflamed interstitial tissue (Figure 2e). Neutrophils crawl along pericyte protrusions rich in ICAM-1 in a Mac1-dependent and LFA1-dependent fashion, guided by the secretion and deposition of CXCL1 within the subendothelial space [42••,72]. This abluminal crawling enables neutrophils to find gaps in the pericyte layer. It is still unclear whether Mac-1 and LFA-1 act here in a similar way as during luminal interactions with the endothelium. However, it is likely that, in absence of shear stress in the subendothelial space, the activation and functionality of these β-2 integrins is governed by distinct mechanisms.

Conclusion

Neutrophil extravasation is a multistep event where each step is regulated by a plethora of different proteins. This multitude of different control mechanisms certainly serves as fail-safe to ensure that extravasation can happen even when one mechanism is defective in order to ensure our survival. This theory also explains why most protein deficiencies only cause a reduction in extravasation of 30–50%. Even absence of critical adhesion molecules (e.g. ICAM-1) does not entirely abolish extravasation. Given these partial effects, it is evident that all players need to act coordinately in 'team-work', and that one player may compensate for the absence of another. Thus, the main questions to ask are NOT if and why a certain protein affects neutrophil extravasation, but rather WHEN and HOW?

Extensive research is still ongoing to unravel how the interplay between all these mechanisms works. New imaging and genetic manipulation techniques together with improved mathematical modeling systems will support new discoveries in this exciting research field.

Conflict of interest statement

Nothing declared.

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Update

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1) **Physiological proteomics of heart failure**

James O'Reilly, Merry L. Lindsey, John A. Baugh

Volume 1, February 2018, Pages 185–197

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Given her role as Guest Editor, Merry Lindsey had no involvement in the peer-review of this article and had no access to information regarding its peer-review. Full responsibility for the editorial process for this article was delegated to David Eisner.

2) **Rapid retrograde regulation of transmitter release at the NMJ**

Richard R. Ribchester, Clarke R. Slater

Volume 4, August 2018, Pages 82–87

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Given his role as Co-Editor-in-Chief, Richard Ribchester had no involvement in the peer-review of this article and had no access to information regarding its peer-review. Full responsibility for the editorial process for this article was delegated to Richard Robitaille.

3) **The redox physiology of red blood cells and platelets: implications for their interactions and potential use as systemic biomarkers**

Miriam M. Cortese-Krott, Sruti Shiva

Volume 9, June 2019, Pages 56–66

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Given their roles as Guest Editors, Miriam Cortese-Krott and Sruti Shiva had no involvement in the peer-review of this article and had no access to information regarding its peer-review. Full responsibility for the editorial process for this article was delegated to Elizabeth Murphy.

4) **Sodium channels and transporters in the myometrium**

Chinwendu Amazu, Juan J. Ferreira, Celia M. Santi, Sarah K. England

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Given her role as Guest Editor, Sarah England had no involvement in the peer-review of this article and had no access to information regarding its peer-review. Full responsibility for the editorial process for this article was delegated to Susan Wray.

5) **Sleep and ageing: from human studies to rodent models**

Laura E. McKillop, Vladyslav V. Vyazovskiy

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Given his role as Guest Editor, Vladyslav Vyazovskiy had no involvement in the peer-review of this article and had no access to information regarding its peer-review. Full responsibility for the editorial process for this article was delegated to A. Jennifer Morton.

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6) **The effects of reward and social context on visual processing for perceptual decision-making**

Kentaroh Takagaki, Kristine Krug

Volume 16, August 2020, Pages 109–117

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Given her role as Guest Editor, Kristine Krug had no involvement in the peer-review of this article and had no access to information regarding its peer-review. Full responsibility for the editorial process for this article was delegated to Alasdair Gibb.

7) **The extravasation cascade revisited from a neutrophil perspective**

Michael Schnoor, Eduardo Vadillo, Idaira María Guerrero-Fonseca

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Given his role as Guest Editor, Michael Schnoor had no involvement in the peer-review of this article and had no access to information regarding its peer-review. Full responsibility for the editorial process for this article was delegated to Pilar Alcaide.