DAMPs PAMPs and LAMPs in Immunity and Sterile Inflammation

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Abstract

The importance of leukocyte trafficking in many fields of pathology is indisputable. Some treatments targeting leukocyte migration were successfully advanced through clinic while many inflammatory pathologies remain without specific therapy. This review discusses leukocyte recruitment to the site of sterile inflammation comprising the canonical trans-endothelial recruitment of bone marrow derived leukocytes, but also trans-mesothelial migration from serous body cavities. In sterile liver injury, the recruitment of various immune cells is sequential and each step in this sequence is necessary for successful tissue repair. When leukocytes become dispensable, they reversely migrate back into the blood stream, maturate in the capillaries and finally home to the bone marrow to undergo apoptosis. Equally important is a local change from a proinflammatory to a reparative program, a switch that is supervised by invariant natural killer T-cells. When the coordinated leukocyte effort fails to remove immunostimulatory molecules, inflammation persists. This leads to leukocyte-associated damage, a driver of many pathologies associated with a distinct set of lifestyle associated immunostimulatory molecular patterns that we term herein LAMPs.

Introduction

Inflammation is absolutely and categorically dependent on recruitment of leukocytes. The importance of leukocyte trafficking has been confirmed in many fields of pathology over the last 30 years (Baggiolini 2015) , including (but not limited to) auto-immune disorders (Shachar and Karin 2013), organ transplantation (Wood, Bushell, and Hester 2012), tumor immunology (Nagarsheth, Wicha, and Zou 2017; Jacquelot et al. 2018), cardiovascular diseases (Epelman, Liu, and Mann 2015), metabolic diseases (Fujita et al. 2019; Gao et al. 2019) and of course infectious diseases. Ligands and receptors coordinating leukocyte migration act as promising targets of anti-inflammatory treatment. Some small molecule inhibitors or blocking antibodies were successfully advanced through clinic for the treatment of inflammatory pathologies, although many promising molecules proved non beneficial during chronic inflammation (reviewed elsewhere (Viola and Luster 2008; Pease and Horuk 2012). Most of the mechanisms involved in leukocyte trafficking have been best characterized using inflammation triggered by infectious microbes or their evolutionarily conserved molecular patterns (Medzhitov 2008). These conserved microbial products such as LPS are also referred to as pathogen associated molecular pattern (PAMPs) and activate pattern recognition receptors (PRR). PRR signaling pathways have been well characterized as the initiators of a cascade that eventually leads to the migration of leukocytes to the site of infection. Undoubtably, defense against pathogenic microbes is vital, however an inflammatory response after sterile damage and subsequent tissue repair may be just as important for a multicellular organism's evolutionary fitness. Recent progress using in vivo microscopy and transgenic mouse reporters permitted the documentation of how the immune system rapidly reacts to sterile tissue injury. An initial inflammation with its hallmark of leukocyte recruitment is a prerequisite for effective tissue repair (McDonald et al. 2010). Inflammation in sterile injury is initiated by the same innate pattern recognition systems used to detect microbes. However, the immunostimulatory molecular patterns are distinctly associated with damage and thus called damage associated molecular patterns (DAMPs).

DAMPs are released during tissue damage (Medzhitov 2008) and initiate an inflammatory response. Sterile inflammation and subsequent tissue repair are dependent on a well-orchestrated migration sequence of leukocytes to and from the site of injury. In this review we will discuss these steps involving macrophages, monocytes, neutrophils and invariant natural killer T-cells (iNKT cells). Some steps are interdependent, for example the disruption of iNKT cell activation, will lead to impaired monocyte maturation and a consequent failure in tissue repair (Liew, Lee, and Kubes 2017). Other steps occur apparently independent of each other. Mechanistically, these steps involve the canonical trans-endothelial recruitment cascade of leukocytes from the bloodstream. However, recruitment of leukocytes from alternative routes such as from adjacent serous body cavities can be of equal importance for a successful tissue repair sequence. Furthermore, we will discuss that the in-situ switch from an inflammatory to a reparative program is another important step towards successful restitution of tissue. This local program switch is supervised by iNKT-cells who constantly sample and integrate the milieu. Then, as inflammation transitions into tissue repair, neutrophils disappear from the site of sterile injury. This may partially be due to phagocytosis by macrophages but may also be due to reverse migration of neutrophils back into the vasculature leading eventually back to the bone barrow where they are recycled. We will discuss that leukocyte traffic and tissue repair in response to simple sterile injury is highly optimized (i.e. cannot be therapeutically improved). However, in some pathologies the coordinated effort by recruited leukocytes to remove the offending agent fails, and inflammation persists. In these instances, recruited leukocytes and leukocyte-associated damage may be more foe than friend. These pathologies are often found to be associated with distinct immunostimulatory molecular patterns that are associated with industrialized western lifestyle. We will discuss and try to classify these molecular patterns named lifestyle associated molecular patterns (LAMPs) and their role in immunopathology.

Initiation of inflammation

Physical or chemical damage leads to the release of sterile damage associated molecular patterns (DAMPs). DAMPs are recognized by PRRs such as Toll-like receptors (TLRs) and cytoplasmic Nod-like receptors (NLRs) but also non-PRRs such as receptor for advanced glycation end-products (RAGE), CD44, integrins, CD91 (Table 1). PRRs are expressed on sentinels which are either immune cells including (but by far not limited to) mast cells, macrophages, dendritic cells, innate lymphoid cells and basophils. In addition, many tissues have non-immune tissue sentinel cells (Andonegui et al. 2009; Seki and Brenner 2008). Ligation of PRR on sentinel cells leads to the production of proinflammatory cytokines (TNFα and IL-1), vasoactive amines (histamine and serotonin), nitric oxide, reactive oxygen species (ROS), neuropeptides and arachidonic acid metabolites (prostaglandins and leukotrienes). Fluid-phase inflammatory pathways and platelets also contribute very early in sterile injury (Deppermann and Kubes 2018; Jenne and Kubes 2015). Damage and inflammation lead to a disruption of macro- and microbarriers (Huber-Lang, Lambris, and Ward 2018) with a consequent influx of plasma proteins and platelets. Serine proteases of the kinin, coagulation and complement cascades can be activated by DAMPs (Paidassi et al. 2008; Burk et al. 2012; Ganter et al. 2007) leading to the production of early inflammatory mediators. In addition, binding of such activated plasma products (e.g. complement C5b-9) improves the recognition of DAMPs by PRR on immune and non-immune cells. Platelet are activated by coagulation factors such as von Willebrand Factor (through the GPIb receptor), fibrinogen and fibronectin (GPIIb/IIIa receptor) as well as contact with other extracellular matrix proteins such as collagen (GPVI receptor)(Nieswandt et al. 2001; Nieswandt and Watson 2003) or by cell surface proteins normally not present in the vasculature like podoplanin (CLEC-2) (Deppermann and Kubes 2018). While the main function of platelets certainly lies in hemostasis, it is interesting to note that GPVI and CLEC2 receptors signal through an immunoreceptor tyrosine-based activation motive (ITAM), highlighting the tight link between hemostasis and inflammation from a phylogenetic perspective (Deppermann and Kubes 2018; Nieswandt et al. 2001; Nieswandt and

Watson 2003). In addition, platelets carry PRRs like TLR2 and TLR4 that can recognize PAMPs and DAMPs and contribute towards inflammation (Andonegui et al. 2005). Upon activation, platelets can influence innate immunity by secreting cytokines, chemokines and other inflammatory mediators (Wagner and Burger 2003). Moreover, platelets can recruit immune cells directly. An illustrative example is endothelial bound platelets expressing P-selectin, which binds P-selectin glycoprotein ligand-1 (PSGL1) on leukocytes and facilitates their recruitment to the site of inflammation (Zarbock, Singbartl, and Ley 2006).

Trans-endothelial migration of leukocytes to sites of sterile injury

In order to ensure blood-borne traffic to the site of sterile injury, leukocytes must breach a specific combination of barriers (Figure 1). First, leukocytes migrate through the endothelium, a process often described with the canonical steps of the leukocyte adhesion cascade: selectin-mediated rolling, chemokine-triggered activation and integrin-dependent arrest (Ley et al. 2007). Early in vitro and in vivo microscopy studies have revealed the molecular mechanisms behind this multi-step paradigm (Butcher 1991; Ley et al. 1991; Springer 1994; von Andrian et al. 1991) that applied to lymph nodes, muscle, brain and some other organs. However, more recent in vivo imaging has revealed some significant differences in places such as lung and liver where recruitment has been mostly in capillaries rolling seems to be absent. Leukocytes must subsequently transmigrate through multiple physical barriers such as the venular wall, vessel basement membrane and the pericyte sheath ultimately entering the interstitium. Increasing experimental evidence suggest that these steps are as tightly regulated (Nourshargh and Alon 2014; Nourshargh, Hordijk, and Sixt 2010). The leukocyte adhesion cascade and trans-endothelial migration has been revisited and reviewed most comprehensively by Nourshargh et al (Nourshargh, Hordijk, and Sixt 2010; Nourshargh and Alon 2014). We will summarize the key events here with importance to understand leukocyte trafficking in sterile injury (Figure 1).

Activation of endothelial cells by inflammatory mediators is the primary step in leukocyte migration. The role of endothelial cells as active participants and regulators in leukocyte trafficking was reviewed by Pober et al. (Pober and Sessa 2007) and can be divided into a rapid (minutes), protein synthesis-independent type I activation, and a somewhat slower (hours) type II activation, that depends on new protein synthesis. In both types of activation, the blood vessels are locally prepared for efficient leukocyte traffic. Mechanistically this preparation comprises increased blood flow, increased vascular permeability and increased leukocyte adhesion (Pober and Sessa 2007). The former two lead to the typical clinical signs of inflammation: rubor (red color), calor (warmth), and tumor (swelling). The fourth cardinal symptom dolor (pain) is directly caused by inflammatory mediators on C-type sensory nerve fibers (Pober and Sessa 2007).

A type I activation of endothelial cells is typically mediated through G-protein coupled receptors (GPCRs) such as histamine H1 receptors. Downstream pathways include phospholipase C, inositol-trisphosphate and elevated free cytosolic Ca^{2+}, arachidonic acid (COX1) metabolism, arginine metabolism (NO production), calcium-dependent vesicle exocytosis (P-selectin) and calcium-dependent modification of cell adhesion. These events result in the production of PGI_2 and NO, both potent vasodilators, increased surface expression of P-selectin by rapid vesicle exocytosis and loosening of calcium-dependent tight and adherent junctions for leukocyte migration. The signals through GPCRs last for 20-30 minutes after which receptors become desensitized limiting the power of an inflammatory response by type I reactions alone (e.g. urticaria). A more sustained inflammatory reaction is provided by type II activation of which TNF-α and IL-1 are the typical mediators (Pober and Sessa 2007). TNF signaling in endothelial cells includes the signalosome transcription factors NFκB and AP1. IL-1 activates similar pathways but this cytokine has been implicated more in sterile in jury. Activation of these transcription factors leads to the induction of protein synthesis of E-selectin, ICAM1, VCAM1, chemokines and COX2 (Pober and Sessa 2007). Because protein synthesis is needed, a type II activation takes longer (hours). The effects of a

type II reaction are like a type I reaction and comprise vasodilation, increased leakiness, and leukocyte adhesion. In addition, a type II activation leads to the neo-synthesis of chemokines and other chemotactic cues needed for effective leukocyte recruitment and the right leukocyte subpopulation (Pober and Sessa 2007). Once established, a type II activation is not only more sustained than a type I reaction but evolves over time. For example, the expression of E-selectin is gradually decreased over time and that of VCAM1, ICAM1 and CCL2 is more prolonged, which leads to a transition from a neutrophil-rich to a mononuclear-cell-rich infiltrate. In a sterile focal hepatic injury this typically occurs between 6 and 24 hours after initial activation at the site of sterile injury (McDonald et al. 2010; Dal-Secco et al. 2015; Wang and Kubes 2016).

Capture of leukocytes by the endothelium of inflamed post-capillary venules under condition of blood flow is mediated by leukocyte expressing glycosylated selectin ligands (PSGL1, glycosylated CD44, ESL1) that bind endothelial E- and P- selectin. This process is either referred to as tethering or capture and does not completely resist flow and hence lets leukocytes roll along the vessel (Ley et al. 2007). Another important group of proteins that facilitate leukocyte recruitment are integrins. They are constitutively and subset-specifically expressed and are typically maintained in a low affinity state. Leukocyte rolling on endothelial E-selectin induces an intermediate affinity state in leukocyte integrins. This triggers low affinity bonds between leukocytes and endothelium, further slowing leukocyte rolling (Ley et al. 2007; Block et al. 2012). The decreased velocity allows for activating signals presented on the vessel wall, such as chemoattractants and chemokines to be transmitted through GPCRs on leukocytes (Campbell et al. 1998). GPCR-mediated activation of leukocytes involves a complex intracellular network and happens within milliseconds and the process has also been referred to as “inside-out-signaling”(Ley et al. 2007). Leukocyte activation is needed to develop high integrin affinity which in turn is necessary to establish firm shear-resistant adhesion between leukocytes and endothelium. Increased integrin binding (higher avidity) is the product of two events on a molecular level: a conformational change of individual integrin

heterodimers (higher affinity) (Shamri et al. 2005) and second, an increased integrin density achieved by lateral mobility and increased expression (higher valency) (Constantin et al. 2000). High affinity neutrophil integrins such as α4,β7, VLA4 (α4,β1) and LFA1 (αL,β2) bind their respective endothelial ligands MADCAM1, VCAM1 and ICAM1 (Ley et al. 2007). This firm binding leads to the leukocyte arrest under flow conditions, a process also referred to as adhesion (Ley et al. 2007). It is well established that integrins act as signal transducers that regulate cell adhesion and motility (Shattil 2005; Giagulli et al. 2006). This process, referred to as "outside-in-signaling" involves the formation of a signalosome and a plethora of intracellular signaling pathways such as SRC kinases and PI3 kinase and results in leukocyte adhesion strengthening and adhesion spreading (Ley et al. 2007).

Migration through the vessel wall consists of three distinct barriers: endothelium, endothelial basement membrane and pericyte sheath (Nourshargh, Hordijk, and Sixt 2010). This process is regulated by endothelial cells and their associated pericytes as well as perivascular tissue inflammatory sentinels. Effector leukocytes, once firmly attached, initiate a polarized motility that enables them to move either directly through the endothelial wall or within the venular lumen. The process of attachment and lateral movement within inflamed vessels is referred to as crawling. Crawling leukocytes follow cues within the inflamed vessel to exit as close as possible to the nidus of the sterile injury. It has been proposed that this increases efficiency of leukocyte effector function and reduces collateral damage in healthy zones (McDonald and Kubes 2011). Crawling is directed by chemokine and lipid chemoattractant gradients and the generation of multiple millipede-like contacts is integrin-dependent (Schenkel, Mamdouh, and Muller 2004; Phillipson et al. 2006; Shulman et al. 2009) and potentiated by shear-stress (Fine et al. 2016). On the molecular level crawling is a tightly regulated process involving canonical actin-myosin machinery (Shulman et al. 2009). Following and integrating a trail of breadcrumbs the crawling leukocytes repeatedly extend ventral protrusions through junctions between adjacent endothelial cells or into the endothelial cell body, and if the chemotactic gradient is right, the leukocyte will breach the

endothelium (Nourshargh and Alon 2014). In the peripheral circulation leukocytes mainly breach the endothelium between adjacent endothelial cells (paracellular route, 80-90%) with trans-cellular migration being relatively rare (Muller 2011). In addition, to the aforementioned inflammatory type I and type II activation of the endothelium, endothelial cells further reduce barrier properties by leukocyte-driven molecular changes (Nourshargh and Alon 2014). For the completion of the paracellular trans-endothelial migration a series of endothelial cell-cell junctions must be breached by the leukocyte. This tightly regulated process involves spatiotemporal and functional changes of adherent (e.g. VE-cadherin) and tight junctions (JAM family, ESAM, claudin). When intravascular crawling is disrupted, leukocytes will still transmigrate, but because they are incapable of reaching junctions they migrate directly through the endothelial cell, a process referred to as transcellular pathway. It involves the formation of leukocyte podocytes also referred to as podosomes and an endothelial cell organelle called vesiculo-vascular organelle (VVO) that forms transcellular pores through which leukocytes can migrate (Carman et al. 2007; Millan et al. 2006; Feng et al. 2002)

After the endothelium the leukocytes must breach two additional layers, the basement membrane and the pericyte sheath. The basement membrane is a complex network of laminins and collagen IV, deposited by endothelial cells and pericytes. Proteolytic cleavage has been postulated by some to be the mechanism by which leukocytes breach this formidable barrier, similar to cancer cell invasion (Sabeh, Shimizu-Hirota, and Weiss 2009). A growing body of imaging evidence however, supports the constitutive existence of regions within the basement membrane of the vascular beds of multiple tissues (demonstrated in cremaster muscle, mesenteric tissue, dorsal ear skin, peritoneal wall and diaphragm) that exhibit low deposition of laminin and collagen IV. These have been termed low-expression regions (LERs) and were purported to act as gateways for leukocytes allowing for emigration without having to cause massive proteolysis (Voisin and Nourshargh 2013; Voisin, Probstl, and Nourshargh 2010; Wang et al. 2006). Basement membrane crossing of leukocytes is thus becoming better understood but

additional physical (e.g. tractional force by pericytes) and enzymatic processes may be involved and provide interesting future research directions (Finsterbusch et al. 2014; Proebstl et al. 2012; Rowe and Weiss 2008). The last barrier is formed by pericytes, the second cellular component of venular walls. Pericytes surround endothelial cells in a discontinuous manner and are tightly associated with the basement membrane. Like endothelial cells, pericytes participate actively in leukocyte trafficking. They sense immunostimulatory patterns by PPRs (e.g. TLRs and NLRs) and inflammatory signals such as TNFα and IL-1 by TNFRI, TNFRII and IL-1R (Pober and Tellides 2012; Stark et al. 2013; Voisin and Nourshargh 2013). Activated pericytes express key adhesion molecules (e.g. ICAM-1, VCAM1) and chemokines (e.g. CXCL1, CXCL8, MIF) (Pober and Tellides 2012; Stark et al. 2013; Voisin and Nourshargh 2013). Pericytes play a key role in sub-endothelial neutrophil motility (Proebstl et al. 2012), an ICAM-1 – Mac-1/LFA mediated process, believed to prime leukocytes for optimized interstitial navigation and effector function (Ayres-Sander et al. 2013; Stark et al. 2013). The whole process of transmigration seems to depend on a distinct compartmentalized action of chemokines and there is evidence that a small percentage of leukocytes will abort transmigration even after reaching the pericyte space (Girbl et al. 2018)

Sequential recruitment and in-situ reprogramming of leukocytes in sterile hepatic injury

With progress in multi-photon intravital imaging and transgenic reporter mice expressing enhanced fluorescent proteins researcher have begun to move their focus further to investigate the dynamic behavior of leukocytes after they have entered the site of injury. A lot of leukocyte behavior within the injury site has been elucidated using a focal thermal injury model in the liver. We will review here the sequence of events that are observed in this model and are necessary for successful wound healing (Figure 1).

Neutrophils are the first leukocytes to be recruited to the inflammatory site (within 30 minutes) from the bloodstream (Figure 1)(McDonald et al. 2010; Lämmermann et al. 2013). After adhering at a

substantial distance from the injury, they must migrate toward the nidus of injury via the capillaries. Neutrophils show a tremendous amount of coordination within the tissue vasculature, a process believed to be mediated mainly by chemoattractant mediators. These can be roughly grouped into four families (McDonald and Kubes 2011): chemokines (primarily CXCR2 ligands for neutrophils), lipids (including leukotriene B4, LTB4), complement anaphylatoxins (C5a and C3a) and DAMPs with not all groups participating in any one model. Interestingly, while an intravascular chemokine gradient seems to be very important for successful directional migration over the initial distance within the vessel (Figure 1)(McDonald et al. 2010), a gradient of DAMPs emanating directly from the injury site provides the most potent chemotactic cue once the neutrophil is close to the wound. This DAMP gradient is paramount for an effective migration into the injury site (Figure 1) (McDonald et al. 2010; Zhang et al. 2010). This has been demonstrated for DAMPs such as N-formyl peptides. These DAMPs are recognized by the respective receptors on neutrophils including the formyl peptide receptor (FPR) (McDonald et al. 2010; Zhang et al. 2010). ATP through a purinergic receptor also helps to direct neutrophils to the injury site but this molecule has a very short half-life making the formation of a gradient quite unlikely. ATP may activate the endothelium and/or intravascular macrophage to induce the initial chemokine gradient, but this has yet to be formally demonstrated. Alternatively, the ATP could be released from the neutrophils to induce clustering of the cells. Indeed, neutrophils tend to migrate in an exponentially clustered fashion through the interstitial space, a process also referred to as neutrophil swarming (Lämmermann et al. 2013; McDonald and Kubes 2012; Lammermann et al. 2008). This autocrine signal has been reported to occur via neutrophil-derived ATP (a DAMP) and LTB4 (a lipid mediator) acting on neutrophil P2Y2 and LTB4 receptors respectively (Chen et al. 2006; Lämmermann et al. 2013). Thus, the combination of an interstitial DAMP gradient and an autocrine feed-forward signal amplification rapidly direct neutrophils through the interstitial space towards the site of injury.

At the site of injury, neutrophils promote tissue healing. Although their canonical role is to battle microbes, there is a growing body of evidence to suggest that they are imperative for timely restoration of tissue architecture in sterile injury (McDonald et al. 2010; Wang 2018). Neutrophils contribute to tissue repair in three ways. First, they remove necrotic material, a process shown to be a prerequisite for effective wound healing in sterile injury (McDonald et al. 2010). Second, neutrophils are an important source of growth factors (Wang 2018; Gong and Koh 2010). For example, they significantly contribute to neo-angiogenesis in a VEGF dependent manner (Gong and Koh 2010). Third, neutrophils becoming apoptotic and being cleared by macrophages contributes to a pro-resolution program that is characterized by an anti-inflammatory cytokine signature (TGFβ, IL-10) (Wang 2018). However, neutrophils can also significantly increase tissue damage by amplifying the inflammatory response and direct release of toxic effectors (Kruger et al. 2015). Main mechanisms include the release of reactive oxygen species (ROS), proteolytic enzymes and anti-microbial proteins as well as neutrophil extracellular traps (NETs). These proteolytically coated chromatin entities are emerging as key neutrophil mediated tissue injury and may contribute to the development of many non-infectious diseases (Jorch and Kubes 2017). In a simple focal hepatic injury however, NETs were not produced and neutrophils rapidly removed necrotic tissue, paving the road for the next step towards homeostasis.

As we have discussed above neutrophils play an important role in tissue repair by clearing debris, releasing growth factors (Wang 2018) and in some injuries inducing the recruitment of other immune cells. As the necrotic tissue is cleared and DAMPs disappear, the local program switches from inflammation towards repair and neutrophils are no longer needed and must be removed. In fact, neutrophil clearance from inflamed tissue is another critical step for effective tissue repair (Wang 2018). Canonically, neutrophil clearance occurs by neutrophil apoptosis and subsequent phagocytosis by macrophages. This process is mediated by phosphatidylserine expressed on apoptotic neutrophils and enhanced by the release of alpha-defensins (Miles et al. 2009). The ingestion of apoptotic cells or

apoptotic bodies induces an anti-inflammatory tissue-repair polarization in macrophages towards an M2 repair phenotype, a state that includes the expression of TGF-β, IL-10, PGE2 and VEGF (Wang 2018; Soehnlein and Lindbom 2010). M2 macrophages, but also neutrophils and other cells, produce lipoxin A4, resolvins and protectins which inhibit further neutrophil recruitment and enhances the clearance of apoptotic neutrophils by phagocytosis (Soehnlein and Lindbom 2010). This process is also referred to as efferocytosis (Jones et al. 2016). Additional anti-inflammatory pro-resolution signaling from neutrophils to macrophages include (but are not limited to): phosphatidylserine containing microvesicles shed by neutrophils (Gasser and Schifferli 2004), chemokine sequestration through CCR5 modulation by neutrophils (Ariel et al. 2006), Annexin A1 released from neutrophil granules or in microvesicles (Rhys et al. 2018) and IL-10 (Kasten, Muenzer, and Caldwell 2010). The latter however, has been consistently observed in mice (Kasten, Muenzer, and Caldwell 2010), while the IL-10 locus in human neutrophils seems to be inactive (Tamassia et al. 2013; Davey et al. 2011). These pro-resolution signals create a resolving feed-forward loop, leading to an exponential decrease in neutrophil number by terminating immigration and increasing efferocytosis (Jones et al. 2016).

However, several in vivo microscopic observations are hard to reconcile with monocytic phagocytosis being the main mechanism of neutrophil clearance. First, in several sterile injury models, neutrophils entered and disappeared well before any monocytes were recruited. Second, monocyte or macrophage depletion had no impact on neutrophil disappearance. And third, careful long-term tracking of neutrophils and monocytes failed to show phagocytosis of neutrophils happening frequently enough to significantly contribute to the clearing of vast numbers of neutrophils. More recent in vivo microscopy studies have provided insight into a phenomenon referred to as reverse transmigration (rTEM)(de Oliveira, Rosowski, and Huttenlocher 2016). Neutrophils migrating back to the vasculature was based on microscopic observations in zebrafish and in vitro models using human endothelial cells and leukocytes (Mathias et al. 2006; Buckley et al. 2006). It was postulated that a constitutively present subpopulation

of ICAM1^{hi}, CXCR1^{lo} blood neutrophils was in fact a group of neutrophils that already had undergone migration and then returned back to the blood via rTEM (Buckley et al. 2006).

More recently, rTEM was visualized in mammals using in vivo microscopy and endothelial JAM-C was identified as an important regulator of rTEM (Woodfin et al. 2011). Blockade or genetic deletion of endothelial JAM-C led to increased neutrophil rTEM (Woodfin et al. 2011). Similarly, local LTB4 induced proteolytic cleavage of JAM-C by endothelial neutrophil elastase promotes rTEM into the circulation and may be decisive in dissemination of systemic inflammation (Colom et al. 2015). However, it remained unclear whether these observations were just aborted forward TEM or neutrophils that truly had completed their effector function in the interstitial space. In 2017 Wang et al formally demonstrated reverse migration from the interstitial space back to the blood stream by using photoactivatable green fluorescent protein (PA-GFP). They showed that neutrophils reversely transmigrate into the vasculature after participating in repair of thermal liver injury (Figure 2). The neutrophils then entered the free flowing blood and stopped in the lung capillaries where they upregulate CXCR4 which presumably enabled them to home back to the bone marrow where they underwent apoptosis (Wang et al. 2017)(Figure 2). Notably, reverse but not forward migration, was dependent on cathepsin C, an enzyme needed to activate various proteases (Wang et al. 2017). Impairment of proteolytic capacity results in neutrophil persistence in the injury site which was associated with disrupted revascularization (Wang et al. 2017). In addition to proteolysis, reverse migration is regulated by hypoxia-inducible factor 1α (HIF-1α) and CXCL8/CXCR2 signaling. HIF-1α activated neutrophils continue to patrol the injury site during the resolution phase, when neutrophils would normally migrate away (Elks et al. 2011). CXCL8/CXCR2 has been identified as a specific ligand/receptor pair that orchestrates chemotaxis through the interstitial space back towards blood vessels (Powell et al. 2017). Collectively, these studies suggest that reverse migration is necessary for resolution. However, some authors have suggested that reverse migration could also be a means by which sterile local inflammation (e.g. pancreatitis) may spread to

distant sites (e.g. lung) or become systemic (Wu et al. 2016). Indeed, enough peripheral injury results in many activated neutrophils being present in the lung vasculature and may cause interstitial damage to the lungs that becomes clinically apparent as acute respiratory distress syndrome. Therefore both, the molecular mechanisms of reverse migration and the specific role of reverse migration in many inflammatory pathologies needs to be further elucidated and may provide therapeutic avenues for the future.

Monocytes recruitment is the next critical step in the sequence for successful wound healing. Monocytes are thus the second immune cellular component recruited to sterile hepatic injury (Figure 1). Neutrophil recruitment seems to be necessary for subsequent monocyte recruitment in some models such as injection of intrascrotal platelet-activating factor (PAF) (Soehnlein et al. 2008). Other models however, such as the focal sterile hepatic injury, fail to demonstrate this causal link and suggest that neutrophils and monocytes have an independent program of recruitment (Dal-Secco et al. 2015). If present, this neutrophil-monocyte cross-talk is dependent on neutrophil derived azurocidin and LL-37 and FPR on monocytes (Soehnlein et al. 2008). In focal sterile hepatic injury classical proinflammatory $CCR2^{hi}$ $CX3CR1^{low}$ monocytes are recruited from the blood at 8-12 hours post-injury in a CCR2/CCL2 dependent manner but entirely independent of neutrophils (Dal-Secco et al. 2015). In addition, to the classical proinflammatory $CCR2^{hi}$ $CX3CR1^{lo}$ monocytes another $CCR2^{low}$ $CX3CR1^{hi}$ monocyte population, referred to as alternative monocytes, gradually appears over 48 hours after injury. While classical proinflammatory monocytes form a ring-like structure around the necrotic area, the second alternative monocyte population entered the injury site and promotes tissue repair (Dal-Secco et al. 2015). However, recruitment of alternative monocytes from the blood could not be demonstrated. Using a combined $CCR2^{RFP/+}$ and $CX3CR1^{GFP/+}$ reporter and rainbow hue analysis revealed a continuum of monocytes ranging from classical pro-inflammatory ($CCR2^{RFP}$) monocytes to alternative green ($CX3CR1^{GFP}$) monocytes as well as many intermediate populations including orange yellow and light green suggesting

switching from inflammatory to alternative monocytes at the site of injury (Dal-Secco et al. 2015). Long-term in vivo imaging showed that indeed, monocytes are recruited as CCR2^{hi} cells and gradually change their phenotype towards CX3CR1^{hi} while clustered within the ring-like structure around the focal damage. This suggests a phenotypic conversion in situ from pro-inflammatory to alternative (Dal-Secco et al. 2015). The orchestration of this process was clearly happening locally, but what mechanism allowed the immune system to assess the local environment and discriminate sterile injury from infectious injury?

Invariant natural killer (iNKT) cells, a specialized subset of innate T lymphocytes, have been described as the orchestrators of immunity exhibiting both, potent immunostimulatory or immunoregulatory roles. The iNKT cells express a restricted T-cell receptor (TCR) repertoire that allows them to recognize exogenous glycolipid antigens (from pathogens), presented by CD1d, a molecule similar to major histocompatibility complex (MHC) class-I (Adams and López-Sagaseta 2011; Brigl and Brenner 2004). In addition, iNKT cells have been described to recognize self-antigen glycolipids (Facciotti et al. 2012). Depending on whether an iNKT cell is activated by a foreign or self-antigen, it starts to rapidly secrete either proinflammatory type-1 cytokine interferon-γ (IFN-γ) during infection, or type-2 cytokines such as IL-4 and IL-10 during tissue repair (Geissmann et al. 2005; Liew and Kubes 2015). Under baseline conditions CXCR6$^{GFP/+}$ iNKT cells constantly crawl within the liver sinusoids patrolling for perturbations (Geissmann et al. 2005; Lee et al. 2010; Velázquez et al. 2008). Within 8 hours after focal liver injury, they arrested their crawling and accumulated in a concentric circle around the injury zone where the inflammatory monocytes accumulated (Liew, Lee, and Kubes 2017). Several studies showed that when iNKT cells arrested indicated that they were being presented with an antigenic ligand by CD1d (Lee et al. 2010; Wong et al. 2011). This CD1d dependent antigen presentation activated the iNKT cells (increased CD69 expression) to begin to produce the appropriate cytokines (Lee et al. 2010; Liew, Lee, and Kubes 2017). Liver sinusoidal endothelial cells as well as Kupffer cells were the main (but not exclusive) cells

that presented self-antigen to iNKT cells in sterile hepatic injury. In addition, IL-12 and IL-18 also helped to activate the iNKT cells (Liew, Lee, and Kubes 2017). During infections iNKT cells were activated to produce type-1 cytokines such as IFN-γ (Lee et al. 2010). In contrast, a hepatic focal sterile injury led to the production of type-2 cytokines such as IL-4. The accumulation and activation (crawling arrest, CD69 expression) of iNKT cells and production of type-2 cytokines coincided with the switch of recruited inflammatory $CCR2^{hi}$ $CX3CR1^{low}$ monocytes to alternative $CCR2^{low}$ $CX3CR1^{hi}$ repair monocytes suggesting a cross-talk (Dal-Secco et al. 2015). Indeed, when iNKT cell activation was inhibited by genetic ablation of CD1d or through anti-CD1d, anti-IL-12 and anti-IL-18 treatment, significantly more $CCR2^{hi}$ $CX3CR1^{low}$ cells accumulated around the injury but failed to convert to a $CCR2^{low}$ $CX3CR1^{hi}$ polarization and thus fewer monocytes were able to infiltrate the injury proper resulting in impaired tissue repair (Liew, Lee, and Kubes 2017).

In summary, successful wound healing in sterile hepatic injury requires a well-timed sequence. Neutrophils remove debris and may or may not be necessary for subsequent recruitment of inflammatory monocytes. Inflammatory monocytes are recruited from the bone marrow via blood stream and accumulate around the injury. In order to enter the injury and promote tissue repair they need to be in-situ reprogrammed from inflammatory ($CCR2^{hi}$) towards a reparative phenotype ($CXrCR1^{hi}$). This reprogramming depends on the activation and IL-4 production by iNKT cells that were presented with self-antigen on CD1d.

Trans-mesothelial migration of leukocytes to sites of sterile injury

Recent evidence challenges the dogma that all leukocytes are generically recruited from the bone marrow via blood circulation into the tissue. For many years it was well known that body cavities such as the pleural, pericardial and peritoneal cavities (Sidebar) are filled with macrophages, B cells and various other immune cells. In a focal thermal injury model trans-mesothelial recruitment of GATA6+ cavity macrophages from the peritoneum are among the first (within 1 hour) cells to arrive at the site of injury

(Wang and Kubes 2016)(Figure 1). More than 20% of these macrophages expressed the proliferation marker Ki67 and integrated bromodeoxyuridine (BrdU) at the injury site, suggesting local proliferation upon arrival. Local proliferation of tissue-resident macrophages has been associated with a type 2 immune response (Jenkins et al. 2011). A type 2 response in macrophages reflects alternative polarization (M2) promoting tissue repair. Indeed, arrival and local proliferation correlated with the upregulation of other characteristic M2 markers such as CD273, CD206 and Arginase-1 in cavity macrophages at the injury site (Wang and Kubes 2016). In vivo microscopy revealed that peritoneal macrophages clear debris and dismantle the nuclei of necrotic cells and their depletion or genetic ablation resulted in impaired tissue repair and re-vascularization in the liver (Wang and Kubes 2016).

In this non-canonical recruitment, the macrophages traveled through the abdominal cavity and then breached the liver capsule, consisting of the cellular mesothelial monolayer and a sub mesothelial connective tissue layer (Figure 1). Peritoneal macrophages were directed to this injury via various DAMPs including ATP and hyaluronan with the respective receptors of the P2X family and CD44 (Wang and Kubes 2016). Although it was unclear whether either of these molecules functioned as chemoattractants, they did contribute to the recruitment. Intriguingly, a deep liver injury was also capable of recruiting these cells suggesting that a chemotactic mechanism was actively recruiting these macrophages (Wang and Kubes 2016). The molecular details of trans-mesothelial cell migration remain to be elucidated. Mesothelial cells closely resemble endothelial cells in their structure and function and as such transmesothelial migration could employ similar mechanisms (Takahashi et al. 1991). Similar GATA6+ macrophage recruitment has been shown to be of importance in other solid organ pathologies such as lung and heart diseases (unpublished data). This opens a new perspective: in addition to tissue-resident cells and cells recruited from the blood, leukocyte traffic from associated body cavities may play an important role in immunopathology (Sidebar).

Macrophage have two other, better-known pathways by which they may affect a sterile injury site. First many macrophages are resident in tissues. It has been accepted that tissue macrophages (e.g. Kupffer cells, cardiac macrophages) (Epelman, Lavine, and Randolph 2014; Epelman, Liu, and Mann 2015) and certain dendritic cells (e.g. Langerhans cells)(Merad, Ginhoux, and Collin 2008) populate their respective organs during embryogenesis. Tissue-resident cells renew themselves under basal conditions and rapidly react to inflammatory challenge with local proliferation (Merad, Ginhoux, and Collin 2008; David et al. 2016). As such if a tissue is injured, the resident macrophage already within the injury site can affect repair if they themselves are not destroyed. However, we did not find Kupffer cells to invade into an adjacent focal thermal hepatic injury and contribute towards tissue repair (Wang and Kubes 2016). The other obvious pathway by which macrophage can arrive at an injury site is as monocytes and then differentiate into mature macrophage. This process has been reviewed elsewhere (Kratofil, Kubes, and Deniset 2017). In brief, proinflammatory Ly6C^{hi} monocytes recruited to a sterile cardiac injury, were shown to gradually differentiate into Ly6C^{low} macrophages depending on nuclear receptor subfamily 4, group a, member 1 (Nr4a1) (Hilgendorf et al. 2014). These macrophages started to produce transforming growth factor-beta (TGF-β) and vascular endothelial growth factor. This is consistent with a reparative phenotype and selective macrophage depletion between 3 and 8 days post injury lead to impaired wound healing in the skin (Lucas et al. 2010).

Ineffectively cleared immunostimulatory molecular patterns in the context of lifestyle-associated immunopathologies

Sterile injury leads to a sequence of leukocyte-mediated responses in order to clear damaged tissue and engage tissue repair, with the ultimate goal of restoring the injured tissue to homeostasis. Acute inflammation is necessary to initiate this sequence. However, acute inflammation and associated

leukocyte recruitment, may be responsible for persistent tissue injury and may contribute to morbidity (Huber-Lang, Lambris, and Ward 2018; McDonald and Kubes 2011). On a molecular level physical, chemical or ischemic damage is associated with cell death and the release of 'self' damage associated molecular patterns (DAMPs)(Yatim, Cullen, and Albert 2017). These initial molecular danger signals are constitutively expressed and compartmentalized in the nucleus, mitochondria and cytostol and invisible to the immune system (Figure 3). They are thus termed constitutively expressed damage associated molecular patterns (cDAMP) (Yatim, Cullen, and Albert 2017). The cDAMPs include for example double stranded DNA (dsDNA) which is usually limited to the nucleus or ATP which usually exists in the cytosol. Also, cDAMPs comprise many molecules found in high concentrations only within mitochrondria. In addition to the release of molecules normally compartmentalized in cells, damage may also include modified extracellular matrix (ECM) components such as oxidized or structurally modified hyaluronan, collagen, laminin and elastin. The ligand domain of a damaged ECM protein may be cryptic, that is, exposed only after the ECM is damaged and the bioactive cryptic domains are referred to as 'matrycryptins' (Adair-Kirk and Senior 2008). Alternatively, modification of ECM structure by secreted proteins, e.g. proteolytically modified collagen, or serum derived hyaluronan associated protein binding of hyaluronan activates immunostimulatory receptors. The described cDAMPs are recognized by PRR on sentinel cells. Inflammation induces non-apoptotic programmed cell death scenarios such as necroptosis, pyroptosis and NETosis. This in turn leads to the induction and release of a group of damage associated molecular patterns that are inducible and have thus been termed iDAMPs (Yatim, Cullen, and Albert 2017)(Figure 3). The latter may involve non-canonical protein secretion which includes Golgi-Bypassing, microvesicle formation and membrane pore formation (Medzhitov 2008). In Table 1 we provide a comprehensive overview and classification of sterile immunostimulatory patterns consisting of cDAMPs, iDAMPs and LAMPs.

In the modern world, the immune system is challenged with molecular patterns that were not present during the evolution of the host pattern recognition system. For example, it is now widely accepted that atherosclerosis, the most important cause of death in the industrialized world, has most characteristics of a classic inflammatory response (Miller et al. 2003; Gisterå and Hansson 2017) without resolution. The consumption of 'western-type diets can lead to hypercholesterinemia and, especially in genetically predisposed individuals, to the accumulation of the principal atherogenic factor low density lipoprotein (LDL). Sound biochemical and epidemiological data link modified LDL with binding and activation of innate macrophage pattern recognition receptors including TLRs (Miller et al. 2003; Robbins et al. 2013). The uptake of LDL by macrophages then leads to the formation of intracellular cholesterol crystals which activate the NLRP3 inflammasome (Hornung et al. 2008; Dostert et al. 2008). The interplay between cholesterol, inflammation and innate immunity is well established but rather than DAMPs or PAMPs being the key driver of inflammation this is a lifestyle disease driven by the lifestyle associated molecular pattern LDL. While the immune system recognizes LDL as an excessive atherogenic toxin, no processes exist to effectively eliminate or detoxify the toxin and inappropriate inflammatory plaques form.

Another well described example is monosodium urate (MSU), the causative agent of gouty arthritis (Dalbeth, Merriman, and Stamp 2016). Upon contact with a host cell MSU induces alone or after binding antibodies or complement, a set of membrane events that signal through Syk and PI3K activation and lead to phagocytosis and cytokine production (Shi, Mucsi, and Ng 2010). The activation of the NLRP3 inflammasome and induction of IL-1β production by phagocytosed MSU has attracted significant attention and the molecular details of how MSU interacts with events controlled by NALP3/ASC/Caspase-1 have now been partially elucidated (Shi, Mucsi, and Ng 2010; Martinon, Mayor, and Tschopp 2009). The immunological mechanisms in Calcium Pyrophosphate Deposition Disease (CPDD, also called Pseudogout) are considered to be very similar (Martinon, Mayor, and Tschopp 2009) and are another example of excessive inflammation due to a lack of timely resolution. Asbestos and silica particles also are strong

purported activators of the NLRP3 inflammasome (Otsuki et al. 2007), particularly in the lung. Unlike MSU or CPDD these particles cannot be dissolved by macrophages and even the smallest amounts of inhaled crystalline silica or asbestos dust can lead to chronic inflammation and cause chronic lung diseases including silicosis and asbestosis, respectively (Wagner 1997). In these cases, frustrated phagocytosis, an event that does not lead to productive digestion of the ingested agent induces cell death of the phagocyte, followed by further recruitment followed by further cell death wherein scarring is the eventual endpoint with a significantly increased risk for local malignancies (Wagner 1997). Many of these inert particles enter the body due to lifestyle, ie., working in mines etc., and lead to persistent inflammation.

In recent decades, there has been a tremendously increased use of bioengineered implantable devices such as prosthetic joint replacements, blood vessel composite grafts, hernia mesh materials, heart valves, coronary artery stents, heart pacemaker, aesthetic and reconstructive implants, and artificial organs such as ventricular assist devices or insulin pumps (Mariani et al. 2019). Biomaterials comprise a broad range of molecular patterns that can stimulate innate immunity. They range from naturally occurring to fully synthetic macromolecules; and implant success and implant survival is linearly dependent on the severity of the inflammatory reaction and consequent fibrosis (Mariani et al. 2019). For example, the inflammatory response to an implanted surgical mesh in hernia repair surgery is well recognized. Polypropylene (PP) is the most commonly used material for the manufacturing of synthetic surgical mesh, with more than one million prosthetics implanted worldwide (Cobb, Kercher, and Heniford 2005). PP is chemically inert to enzymes released by leukocytes and therefore another example of excessive inflammation due to a lack of timely resolution resulting in a strong scar formation. This can be desirable when contained, because it provides additional stability to the abdominal wall and prevents hernia recurrence, however when the scars extend beyond the abdominal wall into the abdominal cavity this causes severe intraperitoneal adhesion formation with significant morbidity.

As this list grows larger it becomes more difficult to reconcile these molecular patterns with the classical "danger model" , which comprises self-molecules associated with distress, damage or danger that activate an immune response to alienate the danger and help in restoration of homeostasis (Matzinger 1994). Furthermore, the above examples are clearly not microbe associated. Therefore, these lifestyle molecules are neither damage nor pathogen associated and the categories DAMP and PAMP both fall short. Since the occurrence of these patterns is typically lifestyle associated, they are referred to as LAMPs. Moreover, these immunostimulatory patterns did not co-evolve with the innate immunity's dual function of first line defense against pathogens and tissue repair after damage but rather are an attempt to deal with a foreign substance adapting mechanisms that work for pathogen or trauma. The single commodity among LAMPs is thus persistence which induces ongoing or chronic inflammation. The list of LAMPs provided here (Table 1) is not exhaustive and the LAMPs of many inflammatory conditions, potentially including some putative autoimmune disorders, need yet to be identified.

It is intriguing that more and more patterns are purported to trigger inflammation through a finite number of soluble proteins and cellular receptors. However, it has been difficult for example, to find sequences or structural homologies among ligands activating the same PRR (Seong and Matzinger 2004). Seong and Matzinger proposed a rather simple hypothesis to reconcile these features, namely that many innate immune receptors, whether to battle pathogens, repair damage or cause a fibrotic reaction to asbestos, have the capacity to be activated by the hydrophobic portions (hyppos) of molecules that are inadvertently exposed (Seong and Matzinger 2004).

Concluding remarks

The importance of an appropriate inflammatory response and tissue repair following sterile injury is indisputable. The response includes recognition of damage and rapid recruitment of neutrophils followed by monocytes together with iNKT cells and the latter immunoregulate the phenotype of the monocytes. In addition, serous cavity macrophage also invade sterile injuries, switch to a repair

phenotype and help affect repair. Certainly, a switch from inflammation towards resolution is the key to successful repair. However, in association with an industrialized western life style, damage may occur in a manner our pattern recognition system has not evolved to deal with. LAMPs such as asbestos, prosthetic materials or cholesterol crystals cannot be cleared by immunity and the single commonality among LAMPs is thus persistence which induces ongoing or chronic inflammation. Ideally, homeostasis can be re-established after unsuccessful clearing of an insult by compartmentalization of the sterile injury in chronic inflammatory aggregates or granulomas (reviewed by (Pagán and Ramakrishnan 2018)). In other instances, compartmentalization cannot be achieved, and the LAMP prevents the switch from a proinflammatory to repair program due to a persistence of the immunostimulatory molecule. This leads to unresolved chronic inflammation and differs from PAMP or DAMP induced healthy return to homeostasis.

Summary points

- Inflammation starts with the recognition of damage associated molecular patterns (DAMPs).
- Inflammation leads to the canonical trans endothelial recruitment of Leukocytes from the blood.
- In addition, leukocytes from serous body cavities are recruited trans-mesothelial.
- Leukocyte traffic in focal sterile hepatic injury follows a well-defined sequence of body cavity macrophage recruitment, neutrophil recruitment and recruitment of inflammatory monocytes
- Inflammatory monocytes must undergo an in-situ reprogramming from inflammatory to alternative (tissue repair) before they can enter the focal hepatic injury zone and promote healing.
- Monocyte in-situ reprogramming is mediated by IL-4 and IL-10 production by iNKT cells which are alternatively activated by self-antigen presenting $CD1d^{+}$ antigen presenting cells and cytokines (IL-12, IL-18).

- After debris clearance neutrophils reversely migrate from the site of sterile injury into the lung capillaries where they upregulate CXCR4, a chemokine receptor needed for a consecutive homing to the bone marrow where they undergo apoptosis.
- LAMPs are lifestyle associated molecular patterns that cannot be cleared by the immune system and lead to a failure in the switch from inflammation to resolution with chronic inflammation, leukocyte-mediated damage and its associated morbidity.

Future Issues list

- The importance of alternative migration routes (e.g. trans-mesothelial from serous body cavities) to the site of sterile injury must be highlighted and may be of importance even in solid organ pathologies.
- Reverse leukocyte migration may have implications at the site of emigration as well as distant and/or systemically in many pathologies.
- Further study of leukocyte trafficking in sterile injury, in particular the in-situ switch of recruited proinflammatory to reparative phenotypes, may reveal therapeutic approaches to alleviate leukocyte-mediated damage in the context of LAMPs.

Sidebar

At the third embryological week a sequence of folding and fusion events leads to the creation of several body cavities. The neuroectoderm elevates and closes dorsally, creating a neural tube which later becomes the ‘dorsal body cavity’. The dorsal cavity is later lined by meninges, its upper part (cranial cavity) contains the brain, and its lower part (vertebral canal) the spinal cord. The fluid circulating through the dorsal cavity is called cerebrospinal fluid. Similarly, the lateral mesoderm folds up and fuses ventrally in the midline to create the coelom cavity. The coelom is lined by the mesothelium, an epithelium derived from the mesoderm. The coelom is later divided into the pericardial, thoracic (pleural) and abdominopelvic (peritoneal) cavities containing the heart, lungs and digestive/

reproductive/urinary organs respectively. The mesothelium ensures free organ movement and maintains a serous fluid respectively named after its compartment: pericardial, pleural and peritoneal fluid. Therefore, coelom-derived cavities are also referred to as serous body cavities. Additional body cavities comprise the connective tissue cavities formed around true joints. Joint cavities are filled with synovial fluid and lined by the synovial membrane (SEER Training Modules). We have shown the role of serous body cavity macrophages in several diseases. In addition, attention for meningeal Leukocyte populations has been increasing with leukocyte subpopulations reported exert neuro-modulatory effects deep within the brain (recently reviewed here (Rua and McGavern 2018)). Here, we challenge the field to think about other potential body cavity-resident leukocyte populations, alternative migration routes and their implications in pathology.

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Tables

	Molecular pattern	Receptor	Physiological purpose	Potential role in disease/ treatment
Intracellular DAMPs				
cDAMPs				
	Nucleus			
	Double stranded DNA (dsDNA)	TLR9 (Boule et al. 2004) and AIM2 (Fernandes-Alnemri et al. 2009; Hornung et al. 2009)	**Damage surveillance**	TLR antagonists, Desoxyribonucleases (Kubes and Mehal 2012)
	High mobility group box 1 protein (HMGB-1)	TLR2, TLR4, TLR9, RAGE and CD24 (Chen and Nuñez 2010)		Neutralizing antibodies, TLR4 antagonists (Andersson and Tracey 2011)
	Histones	TLR2 and TLR4 (Xu et al. 2011)		TLR antagonists
	SAP130	CLEC4E (Yamasaki et al. 2008)		
	Mitochondria			
	Mitochondrial DNA (West and Shadel 2017)	TLR9 (Zhang et al. 2010) and NLRP3 (Shimada et al. 2012)		TLR antagonists, Desoxyribonucleases (Kubes and Mehal 2012)
	Mitochondrial N-formyl peptides (NFP)	FPR1		Antibodies, Honokiol (Liu et al. 2017)
	Cytochrome c	unknown		Potential marker for mitochrondiral and cellular damage (Eleftheriadis et al. 2016), γ-tocotrienol (GTT)
	Cytosol			
	ATP	P2 receptors		P2X receptor inhibitors (Romagnoli et al. 2008)
	S100 calcium-binding proteins	RAGE (Hofmann et al. 1999)		Antibodies
	K^+ Ions	K^+ channels		
	Cold inducible RNA binding protein (CIRBP)	TLR-4-MD2 complex (Qiang et al. 2013)		Inflammatory response in shock and sepsis (Qiang et al. 2013)
	Thioredoxin	many		
iDAMPs				
	Heat shock proteins: hsp60, hsp70, hsp90, gp 96, calreticulin (Sukkurwala et al. 2014)	TLR2, TLR4 (Ohashi et al. 2000), CD91 (Basu et al. 2001), CD14 (Asea et al. 2000), CD40, and CD24 (Chen and Nuñez 2010)	**Signal amplification, DAMP gradient**	Antibodies, anticancer immune response (Feng et al. 2003)
	Defensins	CCR6 and TLR4		Antagonists and antibodies (Kubes and Mehal 2012; Yang et al. 2004)
	Galectins	CD2		
	IL-1a	IL-1R		
Extracellular DAMPs				
	Short fragment Hyaluronan	CD44-TLR4-MD2 (Taylor et al. 2007) and TLR2 (Scheibner et al. 2006)	**Damage surveillancen,** DAMP gradient	TLR-Antagonists, Hyaluronidase (Jiang et al. 2005)

	Biglycan	TLR2 and TLR4 (Schaefer et al. 2005)		
	Versican	TLR2 (Kim et al. 2009)		Enhances tumor metastasis (Kim et al. 2009)
	Heparansufate	TLR4		
	ECM-fragments (matricryptins) from collagen, elastin, laminin	CD14 and TLR4, SHAP		
Endogenous LAMPs				
	Cholesterol crystals	NLRP3, CD36	?	Atherosclerosis, Cardiovascular Disease
	Uric acid and monosodium urate (MSU) crystals	NLRP3 (Shi, Mucsi, and Ng 2010)	May physiologically act as cDAMP	Gout
	calcium pyrophosphate dihydrate (CPPD) crystals	NLRP3	?	Pseudogout
	oxidized lipoproteins	TLR (Miller et al. 2003)	?	Atherosclerosis, Cardiovascular Disease
	Prions and prion-like protein danger signals (e.g. β-Amyloid)	NLRP3, CD36 and RAGE (Chen and Nuñez 2010)	Physiological iDAMP?	Alzheimer's disease, Creutzfeldt-Jakob disease
Exogenous LAMPs				
	Silica particles, Asbestos particles	NLRP3 (Hornung et al. 2008; Dostert et al. 2008)	None	Frustrated phagocytosis, Granuloma, Silicosis, Asbestosis, systemic immune disease
	Biomaterials	Plasma proteins, Complementreceptors (Szaba and Smiley 2002; Andersson et al. 2005), TLR	None	Foreign-body reaction, Granuloma

Table 1 – Non-infectious ("sterile") immunostimulatory molecular patterns. Adapted from (McDonald and Kubes 2011; Medzhitov 2008; Yatim, Cullen, and Albert 2017; Kubes and Mehal 2012; Chen and Nuñez 2010). TLR: toll like receptor, AIM2: absent in melanoma, RAGE: receptor for advanced glycation end products, CLEC4E: C-type lectin domain family 4 member, also called Macrophage-inducible C-type lectin (Mincle), FPR1: formyl peptide receptor 1, PI3K: phosphatidylinsolitol 3-kinase, SHAP: serum-derived hyaluronan associated protein, MD2: myeloid differentiation factor 2.