

Annual Review of Pathology: Mechanisms of Disease

DAMPs, PAMPs, and LAMPs in Immunity and Sterile Inflammation

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Annu. Rev. Pathol. Mech. Dis. 2020. 15:493–518

First published as a Review in Advance on
November 1, 2019

The *Annual Review of Pathology: Mechanisms of Disease*
is online at pathol.annualreviews.org

<https://doi.org/10.1146/annurev-pathmechdis-012419-032847>

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Keywords

inflammation, sterile injury, damage-associated molecular pattern, DAMP, leukocyte trafficking, leukocyte migration, reverse leukocyte migration

Abstract

Recognizing the importance of leukocyte trafficking in inflammation led to some therapeutic breakthroughs. However, many inflammatory pathologies remain without specific therapy. This review discusses leukocytes in the context of sterile inflammation, a process caused by sterile (non-microbial) molecules, comprising damage-associated molecular patterns (DAMPs). DAMPs bind specific receptors to activate inflammation and start a highly optimized sequence of immune cell recruitment of neutrophils and monocytes to initiate effective tissue repair. When DAMPs are cleared, the recruited leukocytes change from a proinflammatory to a reparative program, a switch that is locally supervised by invariant natural killer T cells. In addition, neutrophils exit the inflammatory site and reverse transmigrate back to the bloodstream. Inflammation persists when the program switch or reverse transmigration fails, or when the coordinated leukocyte effort cannot clear the immunostimulatory molecules. The latter causes inappropriate leukocyte activation, a driver of many pathologies associated with poor

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lifestyle choices. We discuss lifestyle-associated inflammatory diseases and their corresponding immunostimulatory lifestyle-associated molecular patterns (LAMPs) and distinguish them from DAMPs.

INTRODUCTION

Inflammation in the absence of pathogens and their products is referred to as sterile inflammation. Inflammation absolutely and categorically depends on the recruitment of leukocytes. The key role of leukocyte trafficking has been confirmed in many fields of pathology during the past 30 years (1) including (but not limited to) autoimmune disorders (2), organ transplantation (3), tumor immunology (4, 5), cardiovascular diseases (6), metabolic diseases (7, 8), and, of course, infectious diseases. Most of the mechanisms involved in leukocyte trafficking have been best characterized by using inflammation triggered by infectious microbes or their evolutionarily conserved molecular patterns (9). These conserved microbial products, such as lipopolysaccharide, are also referred to as pathogen-associated molecular patterns (PAMPs), and they activate pattern recognition receptors (PRRs). PRR signaling pathways have been well characterized as the initiators of cascades that eventually lead to the migration of leukocytes to the site of infection. Undoubtedly, 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 made using *in vivo* microscopy and transgenic mouse reporters permitted documentation of how the immune system rapidly reacts to sterile tissue injury. Initial inflammation, with its hallmark leukocyte recruitment, is a prerequisite for effective tissue repair (10). Inflammation in sterile injury is initiated by the same innate pattern recognition systems used to detect microbes. However, the immunostimulatory molecular patterns in sterile inflammation differ from microbial patterns and are canonically associated with damage; thus, they are called damage-associated molecular patterns (DAMPs). DAMPs are released during tissue damage (9) and initiate an inflammatory response. Sterile inflammation and subsequent tissue repair depend on a well-orchestrated migration sequence of leukocytes to and from the site of injury.

In this article, we start by reviewing the initiation of sterile inflammation and the recruitment of inflammatory leukocytes from the blood. Specific mechanisms ensure the recruitment of the appropriate leukocyte population, such as monocytes, neutrophils, or invariant natural killer T cells (iNKT cells), at the right time and to the right location. Some steps are interdependent; for example, the disruption of iNKT cell activation leads to impaired monocyte maturation and a consequent failure in tissue repair (11). Other steps occur apparently independently of each other; for example, iNKT cell recruitment is not linked to neutrophil recruitment. Recent publications have shown that leukocytes not only are recruited from the blood but also can be recruited from alternative routes, such as from adjacent serous body cavities. This recruitment can be equally important for successful tissue repair. We also discuss the crucial *in situ* switch from an inflammatory to a reparative program as another important step toward successful tissue repair. We discuss the concept of the reverse transmigration of neutrophils out of the injury and back into the vasculature, and its implication for the proper resolution of inflammation. We also highlight how leukocyte trafficking and tissue repair in response to simple sterile injury are highly optimized, whereas in some pathologies the coordinated effort by recruited leukocytes to remove the offending agent fails and inflammation persists. In these instances, recruited leukocytes may be more foe than friend. These pathologies are often associated with the twenty-first-century human lifestyle, and, in many instances, an eliciting immunostimulatory molecular pattern can be defined that is neither clearly pathogen nor damage associated. We discuss these molecular patterns and

their roles in immunopathology, and we propose classifying them as lifestyle-associated molecular patterns (LAMPs).

The understanding of sterile inflammation in many disease models has been vastly improved during the past decade, leading to the identification of novel therapeutic targets. However, many promising molecules proved to be non-beneficial in chronic inflammatory diseases (12), providing support for the need for further mechanistic insight. Here, we review the known cellular and molecular mechanisms underlying sterile inflammation and aim to highlight questions that need to be addressed in this field.

INITIATION OF STERILE INFLAMMATION

Sterile inflammation refers to an inflammatory reaction to offending agents other than pathogens and their products (that is, other than PAMPs). Classical experimental models of sterile inflammation include physical or chemical damage that leads to the release of DAMPs. DAMPs are recognized by PRRs, such as Toll-like receptors (TLRs) and cytoplasmic Nod-like receptors (NLRs), and also by non-PRRs, such as the receptor for advanced glycation end products (RAGE), CD44, integrins, and CD91 (**Table 1**). PRRs are expressed on sentinels that are immune cells, including (but certainly not limited to) mast cells, macrophages, dendritic cells, innate lymphoid cells, and basophils. In addition, many tissues have non-immune-tissue sentinel cells (13, 14), and one could argue that any viable cell could sense and react to DAMPs. Ligation of PRRs on sentinel cells leads to the production of proinflammatory cytokines [e.g., tumor necrosis factor (TNF)- α and interleukin (IL)-1], vasoactive amines (e.g., histamine and serotonin), nitric oxide (NO), reactive oxygen species (ROS), neuropeptides, and arachidonic acid metabolites [e.g., prostaglandins (PGs) and leukotrienes]. Fluid-phase inflammatory pathways and platelets also contribute early to responses in sterile injury (15, 16). Damage and inflammation lead to the disruption of macro- and microbarriers (17), with a consequent influx of plasma proteins and platelets. Serine proteases of the kinin, coagulation, and complement cascades can be activated by DAMPs (18–20), 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 PRRs on immune and nonimmune cells. Platelets are activated by coagulation factors, such as von Willebrand factor [through the glycoprotein (GP)-Ib receptor], fibrinogen and fibronectin (through the GPIIb–GPIIIa receptor), as well as by contact with other extracellular matrix (ECM) proteins, such as collagen (through the GPVI receptor) (21, 22), or by contact with cell surface proteins not normally present in the vasculature, such as podoplanin (through C-type lectin domain receptor-2; CLEC2) (15). While the main function of platelets certainly lies in ensuring hemostasis, it is interesting to note that GPVI and CLEC2 receptors signal through an immunoreceptor tyrosine-based activation motif, highlighting the tight link between hemostasis and inflammation from a phylogenetic perspective (15, 21, 22). In addition, platelets carry PRRs, such as TLR2 and TLR4, that can recognize PAMPs and DAMPs and contribute to inflammation (23). Upon activation, platelets can influence innate immunity by secreting cytokines, chemokines, and other inflammatory mediators (24). Moreover, platelets can recruit immune cells directly. An illustrative example is endothelial-bound platelets that express P-selectin, which binds P-selectin glycoprotein ligand-1 (PSGL1) on leukocytes and facilitates their recruitment to the site of inflammation (25).

TRANSENDOTHELIAL MIGRATION OF LEUKOCYTES TO SITES OF STERILE INJURY

The hallmark of inflammation is the infiltration of leukocytes. After leukocytes are transported by the blood to the site of sterile injury, they must breach a specific combination of barriers to exit

Table 1 Noninfectious (or sterile) immunostimulatory molecular patterns^a

Molecular pattern	Receptor	Physiological purpose	Potential role in immune-mediated disease or treatment
Intracellular DAMPs			
cDAMPs (nucleus)			
Double-stranded DNA	TLR9 (128), AIM2 (129, 130)	Damage surveillance	TLR antagonists, deoxyribonucleases (131)
HMGB1	TLR2, TLR4, TLR9, RAGE, CD24 (132)		Neutralizing antibodies, TLR4 antagonists (133)
Histones	TLR2, TLR4 (134)		TLR antagonists
SAP130	CLEC4E (135)		
cDAMPs (mitochondria)			
Mitochondrial DNA (136)	TLR9 (64), NLRP3 (137)	Damage surveillance	TLR antagonists, deoxyribonucleases (131)
Mitochondrial <i>N</i> -formyl peptides	FPR1		Antibodies, honokiol (138)
Cytochrome c	Unknown		Potential marker for mitochondrial and cellular damage (139), γ -tocotrienol
cDAMPs (cytosol)			
ATP	P2 receptors	Damage surveillance	P2X receptor inhibitors (140)
S100 calcium-binding proteins	RAGE (141)		Antibodies
K ⁺ ions	K ⁺ channels		Secondary messenger
CIRBP	TLR4–MD2 complex (142)		Inflammatory response in shock and sepsis (142)
Thioredoxin	Many		Cancer, viral diseases, aging
iDAMPs			
Heat shock proteins: hsp60, hsp70, hsp90, gp96, calreticulin (143)	TLR2, TLR4 (144), CD91 (145), CD14 (146), CD40, CD24 (132)	Signal amplification, DAMP gradient	Antibodies, anticancer immune response (147)
Defensins	CCR6, TLR4		Antagonists, antibodies (131, 148)
Galectins	CD2		Cancer, fibrosis, chronic inflammation
IL-1 α	IL-1R		Sepsis
Extracellular DAMPs			
Short fragment hyaluronan	CD44–TLR4–MD2 complex (149), TLR2 (150)	Damage surveillance, DAMP gradient	TLR antagonists, hyaluronidase (151)
Biglycan	TLR2, TLR4 (152)		
Versican	TLR2 (153)		Enhances tumor metastasis (153)
Heparan sulfate	TLR4		Alzheimer's disease
Extracellular matrix fragments (matricryptins) from collagen, elastin, laminin	CD14, TLR4, SHAP		Reportedly involved in autoimmune diseases, potential antitumor properties

(Continued)

Table 1 (Continued)

Molecular pattern	Receptor	Physiological purpose	Potential role in immune-mediated disease or treatment
Endogenous LAMPs			
Cholesterol crystals	NLRP3, CD36	Unclear	Atherosclerosis, cardiovascular disease
Uric acid and monosodium urate crystals	NLRP3 (118)	May physiologically act as a cDAMP	Gout
Calcium pyrophosphate dihydrate crystals	NLRP3	Pseudogout	
Oxidized lipoproteins	TLR (112)	Atherosclerosis, cardiovascular disease	
Prions and prion-like protein danger signals (e.g., β -amyloid)	NLRP3, CD36, RAGE (132)	Physiological iDAMP?	Alzheimer's disease, Creutzfeldt-Jakob disease
Exogenous LAMPs			
Silica particles, asbestos particles	NLRP3 (115, 116)	None	Frustrated phagocytosis, granuloma, silicosis, asbestosis, systemic immune disease
Biomaterials	Plasma proteins, complement receptors (154, 155), TLR	None	Foreign-body reaction, granuloma

^aEmpty cells indicate that a physiological purpose or potential role is unknown.

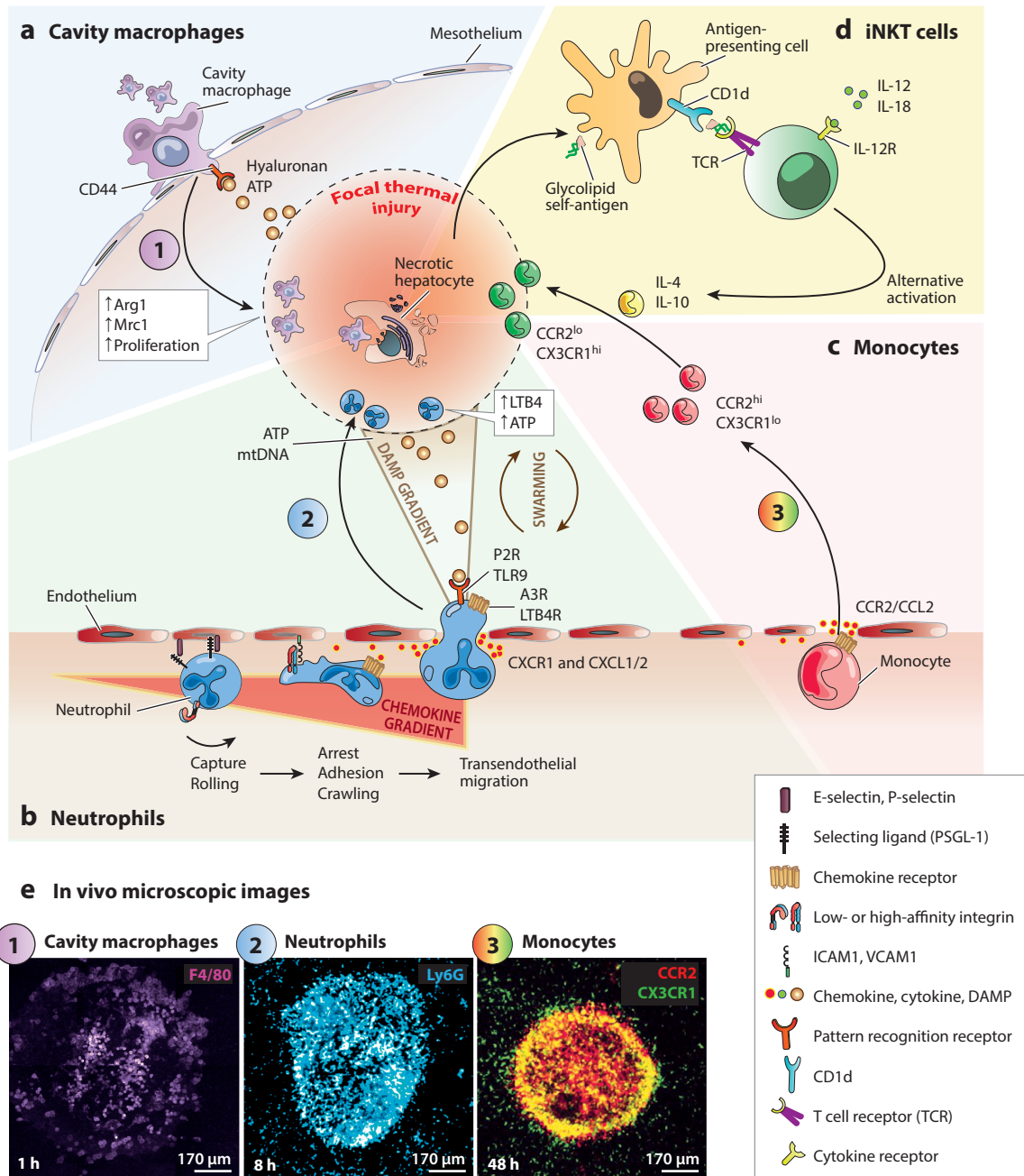
Data from References 9, 43, 110, 131, 132.

Abbreviations: AIM2, absent in melanoma-2; cDAMP, constitutively expressed DAMP; CIRBP, cold-inducible RNA binding protein; CLEC4E, C-type lectin domain family 4 member E (also called macrophage-inducible C-type lectin or Mincle); DAMP, damage-associated molecular pattern; HMGB1, high mobility group box-1; FPR1, formyl peptide receptor 1; HSP, heat shock protein; iDAMP, inducible DAMP; IL, interleukin; LAMP, lifestyle-associated molecular pattern; MD2, myeloid differentiation factor-2; PI3K, phosphatidylinositol 3-kinase; RAGE, receptor for advanced glycation end products; SAPI30, Sin 3A-associated protein 130; SHAP, serum-derived hyaluronan-associated protein; TLR, Toll-like receptor.

the bloodstream and enter the inflammatory site (**Figure 1**). First, leukocytes migrate through the endothelium, a process often described using the canonical steps of the leukocyte adhesion cascade: selectin-mediated rolling, chemokine-triggered activation, and integrin-dependent arrest (26). Early in vitro and in vivo microscopy studies have revealed the molecular mechanisms behind this multistep paradigm (27–30) that apply to the lymph nodes, muscle, brain, and other organs. However, more recent in vivo imaging has revealed some significant differences in the cascade in organs such as lung and liver, where recruitment occurs mostly in capillaries and rolling seems to be absent (31, 32). Leukocytes must subsequently transmigrate through multiple physical barriers, such as the venular wall, vessel basement membrane, and the pericyte sheath, before ultimately entering the interstitium. Increasing experimental evidence suggests that these steps are tightly regulated (33, 34). The leukocyte adhesion cascade and transendothelial migration have been revisited and reviewed most comprehensively by Nourshargh & Alon (33) and Nourshargh et al. (34). We summarize the key events important to understanding leukocyte trafficking in sterile injury (**Figure 1**).

The 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 & Sessa (35) and can be divided into a rapid (minutes), protein synthesis-independent type I activation, and a somewhat slower (hours) type II activation, which

depends on the synthesis of new proteins. In both types of activation, the blood vessels are locally stimulated for efficient leukocyte trafficking. Mechanistically, this leads to increased blood flow, vascular permeability, and leukocyte adhesion (35). The first two lead to the typical clinical signs of inflammation: rubor (redness), calor (warmth), and tumor (swelling). The fourth cardinal symptom, dolor (pain), is directly caused by inflammatory mediators on C-type sensory nerve fibers (35).



(Caption appears on following page)

Figure 1 (Figure appears on preceding page)

Leukocyte trafficking to and from sterile injury. (a) Transendothelial migration of F4/80⁺GATA6⁺ macrophages from the peritoneal cavity toward a hepatic sterile injury site. These cells recognize what we call a touch-me signal, consisting of ATP and hyaluronan at the liver capsule, in a CD44-dependent manner. At the injury site they undergo local proliferation and upregulate arginase-1 (Arg1) and mannose receptor type 1 (Mrc1) expression, signs of repair-type macrophages. Peritoneal macrophages promote tissue healing by dismantling the nuclei of dead cells and by removing necrotic DNA. (b) Neutrophils are also recruited rapidly to the site of sterile injury. They migrate through the vessel wall from the bloodstream, following the canonical leukocyte recruitment cascade. Initial capture and rolling are mediated by endothelial selectins and leukocyte integrins. Subsequent engagement of chemokine receptors on rolling leukocytes activates leukocytes and leads to a conformational change of integrins from low affinity to high affinity. This, in turn, strengthens leukocyte adhesion enough to resist shear stress, which leads to leukocyte arrest. Leukocytes next follow an intravascular chemokine gradient (mainly CXCR2 ligands for neutrophils) and exit mainly through the paracellular route, where the chemokine gradient is highest. After transendothelial migration, within the interstitial space neutrophils follow a gradient of damage-associated molecular patterns (DAMPs), such as *N*-formyl peptides, mitochondrial DNA (mtDNA), and ATP, using G protein-coupled formyl peptide receptor as well as P2 receptors (P2Rs) and Toll-like receptor 9 (TLR9). The chemotaxis along this DAMP gradient is also referred to as necrotaxis. The leading neutrophil is activated and secretes leukotriene B4 (LTB4) and ATP. These molecules are recognized by A3 receptors (A3Rs) and LTB receptors (LTB4Rs) on following neutrophils. This neutrophil–neutrophil signaling results in an autocorrelated behavior called swarming. At the site of injury, neutrophils contribute to tissue repair by clearing debris and releasing growth factors. (c) The third type of cells to arrive in hepatic sterile injury are classical proinflammatory monocytes (CCR2^{hi} and CX3CR1^{lo}). These cells transmigrate in a CCR2-dependent manner and must undergo *in situ* reprogramming into CCR2^{lo} and CX3CR1^{hi} alternative monocytes before they are able to enter the injury and promote tissue repair. (d) The *in situ* reprogramming of monocytes depends on interleukin (IL)-4 and IL-10. Invariant natural killer T (iNKT) cells play a key role in IL-4 and IL-10 production. Once iNKT cells are alternatively activated by glycolipid self-antigens presented by various cells on CD1d to their T cell receptor (TCR), they start to produce IL-4. In addition, iNKT cells induce the production of IL-10 in another yet unknown cell. Additional paracrine signals (such as IL-12, IL-18) are needed for persistent T helper 2–like iNKT activation. (e) *In vivo* microscopic imaging showing the infiltration of (1) cavity macrophages and (2) neutrophils and (3) the infiltration and reprogramming of monocytes. Images 1 and 2 kindly provided by M. Hossain of our lab. Image 3 used with permission from Reference 91.

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 Ca²⁺, arachidonic acid metabolism (involving COX1), arginine metabolism (leading to NO production), calcium-dependent vesicle exocytosis (involving release of P-selectin) and calcium-dependent modification of cell adhesion. These events result in the production of PGI₂ and NO, both potent vasodilators, an increase in surface expression of P-selectin by rapid vesicle exocytosis, and loosening of calcium-dependent tight and adherent junctions for leukocyte migration. The signals through the GPCRs last for 20–30 min, after which receptors become desensitized, thus limiting the power of an inflammatory response by type I activation alone (e.g., urticaria). A more sustained inflammatory reaction is provided by type II activation, and TNF- α and IL-1 are the typical mediators (35). TNF signaling in endothelial cells includes the signalosome transcription factors nuclear factor (NF)- κ B and activator protein 1 (AP1). IL-1 activates similar pathways, but this cytokine has been implicated more in sterile injury (36). Activation of these transcription factors leads to the induction of protein synthesis of E-selectin, ICAM1, VCAM1, chemokines, and COX2 (35). The synthesis of these molecules takes hours. The effects of type II activation are similar to type I activation and comprise vasodilation, increased endothelial leakiness, and leukocyte adhesion. In addition, type II activation leads to the synthesis of chemokines and other chemotactic cues needed for effective leukocyte recruitment of the right leukocyte subpopulation (35). Once established, type II activation is not only more sustained than type I activation but also evolves over time. For example, the expression of E-selectin gradually decreases over time and that of VCAM1, ICAM1, and CCL2 is prolonged, which leads to a transition from a neutrophil-rich to a mononuclear cell-rich infiltrate.

Capture of leukocytes by the endothelium of inflamed postcapillary venules under conditions of blood flow that produce shear forces is mediated by leukocytes expressing glycosylated selectin ligands (e.g., PSGL1, glycosylated CD44, E-selectin ligand-1) that bind endothelial E- and

P-selectin. This process is referred to as tethering or capture, and due to the transient nature of the adhesion, it does not completely resist blood flow, thus allowing leukocytes to roll along the vessel (26). Integrins are another important group of proteins that facilitates leukocyte recruitment. 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 the endothelium, further slowing leukocyte rolling (26, 37). The decreased velocity allows for activating signals presented on the vessel wall, such as chemoattractants and chemokines, to be transmitted through GPCRs on leukocytes (38). GPCR-mediated activation of leukocytes involves a complex intracellular network, happens within milliseconds, and has also been referred to as inside-out signaling (26). Leukocyte activation is needed to develop high integrin affinity, which, in turn, is necessary to establish firm, shear-resistant adhesion between leukocytes and the endothelium. Increased integrin binding (higher avidity) is the product of two events on a molecular level: first, a conformational change of individual integrin heterodimers (higher affinity) (39) and, second, an increased integrin density achieved by lateral mobility and increased expression (higher valency) (40). High-affinity neutrophil integrins, such as $\alpha_4\beta_7$, VLA4 ($\alpha_4\beta_1$), and LFA1 ($\alpha_L\beta_2$), bind their respective endothelial ligands MADCAM1, VCAM1, and ICAM1 (26). This firm binding leads to leukocyte arrest under flow conditions, a process also referred to as adhesion (26). It is well established that integrins act as signal transducers that regulate cell adhesion and motility (41, 42). This process, referred to as outside-in signaling, involves the formation of a signalosome and a plethora of intracellular signaling pathways, such as those involving SRC kinases and phosphatidylinositol 3-kinase (PI3K), and results in strengthening of leukocyte adhesion and adhesion spreading (26).

Migration through the vessel wall requires navigating three distinct barriers: the endothelium, the endothelial basement membrane, and the pericyte sheath (34). This process is regulated by endothelial cells and their associated pericytes, as well as by 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 (33). 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 the efficiency of leukocyte effector function and reduces collateral damage in healthy zones (43). Crawling is directed by chemokine and lipid chemoattractant gradients and the generation of multiple millipede-like contacts on the leukocytes that is integrin dependent (44–46) and potentiated by shear stress (47). On the molecular level, crawling is a tightly regulated process involving the canonical actin–myosin machinery (46). 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 (33). In the peripheral circulation, leukocytes mainly breach the endothelium between adjacent endothelial cells (i.e., the paracellular route, 80–90%), with transcellular migration being relatively rare (48). In addition to inflammatory type I and type II activation of the endothelium, endothelial cells further reduce barrier properties by leukocyte-driven molecular changes (33). To complete paracellular, transendothelial migration, a series of endothelial cell–cell junctions must be breached by the leukocyte. This tightly regulated process involves spatiotemporal and functional changes in adherent [e.g., vascular endothelial (VE)-cadherin] and tight junction proteins [e.g., junctional adhesion molecule (JAM) family, endothelial cell-selective adhesion molecule (ESAM), and claudin family]. 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 migration. It involves the formation of leukocyte podocytes, also

referred to as podosomes, and an endothelial cell organelle called a vesiculo-vacuolar organelle that forms transcellular pores through which leukocytes can migrate (49–51).

After crossing 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 to be the mechanism by which leukocytes breach this formidable barrier, similar to cancer cell invasion (52). A growing body of imaging evidence, however, supports the constitutive existence of regions within the basement membrane of the vascular beds of multiple tissues (e.g., 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 and were purported to act as gateways for leukocytes, allowing for emigration without having to cause massive proteolysis (53–55). Basement membrane crossing by leukocytes is thus becoming better understood, but additional physical (e.g., tractional force by pericytes) and enzymatic processes may be involved, and these provide interesting future research directions (56–58).

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 PRRs (e.g., TLRs and NLRs) and inflammatory signals such as TNF- α and IL-1 by TNF receptor-I (TNFR1), TNFR2, and IL-1R (53, 59, 60). Activated pericytes express key adhesion molecules (e.g., ICAM1, VCAM1) and chemokines [e.g., CXCL1, CXCL8, and macrophage migration inhibitory factor (MIF)] (53, 59, 60). Pericytes play a key role in subendothelial neutrophil motility (57), an ICAM1–Mac1- or –LFA-mediated process believed to prime leukocytes for optimized interstitial navigation and effector function (60, 61). 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 (62).

SEQUENTIAL RECRUITMENT AND IN SITU REPROGRAMMING OF LEUKOCYTES IN STERILE HEPATIC INJURY

With the greater availability of multiphoton intravital imaging and transgenic reporter mice expressing enhanced fluorescent proteins, researchers have begun to move their focus further to investigate the dynamic behavior of leukocytes after they have entered the site of injury. We begin with our own model of sterile injury, which has characterized leukocyte recruitment into a small (200 μ m) thermally destroyed area of liver tissue during the first 72 h after injury. The sterile damage is surgically induced by focally applying a thermal probe to the liver surface. The fact that this is a small nidus of injury allows us to visualize every immune cell that gets recruited and the entire temporal process. While there is much work to be done, this model has provided a good start to understanding the sequence of events that leads to complete repair, which we review here (**Figure 1**).

Neutrophils are the first leukocytes to be recruited to the inflammatory site from the bloodstream (within 30 min) (10, 63) (**Figure 1**). After adhering at a substantial distance from the injury, they must migrate toward the nidus of injury via the capillaries. Neutrophils show consistent behavior within the tissue vasculature, a process believed to be mediated mainly by chemoattractant mediators. Chemoattractants can be roughly grouped into four families (43): chemokines (primarily CXCR2 ligands for neutrophils), lipids (including leukotriene B₄, or LTB₄), complement anaphylatoxins (C5a and C3a), and DAMPs, with not all groups participating in any one model and at least two participating in the liver thermal injury. An intravascular chemokine gradient seems to be important for successful directional migration over the initial

distance within the vessel (10) (**Figure 1**), while a gradient of DAMPs emanating directly from the injury site provides the most potent chemotactic cue once the neutrophil is in the interstitium and close to the wound. This DAMP gradient is paramount for effective migration into the injury site (10, 64) (**Figure 1**). In this particular case, the DAMP appears to be an *N*-formyl peptide. These DAMPs are recognized by their respective receptors on neutrophils, including the formyl peptide receptor (FPR) (10, 64). It is worth mentioning that there is a limit to any chemotactic gradient, and so to travel, for example, a distance of 600 μm , which is approximately 60 cell lengths, multiple gradients need to be established. This requires that tiny amounts of the follow-on gradient need to be dominant over the previous gradient so that the neutrophils continue to move in the direction of injury. Indeed, hierarchical chemotaxis has been described (10).

Through a purinergic receptor, ATP also helps to direct neutrophils to the injury site, but this molecule has a short half-life, making the formation of a gradient quite unlikely. ATP may activate the endothelium or intravascular macrophage, or both, to induce the initial chemokine gradient, but this has yet to be formally demonstrated. Alternatively, ATP could be released from 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 (63, 65, 66). This autocrine signal has been reported to occur via neutrophil-derived ATP (a DAMP) and LTB₄ (a lipid mediator) acting on, respectively, neutrophil P2Y₂ and LTB₄ receptors (63, 67). Thus, the combination of an interstitial DAMP gradient and autocrine feedforward signal amplification rapidly directs neutrophils through the interstitial space toward the site of injury (**Figure 1**).

At the site of injury, neutrophils promote tissue healing. Although their canonical role is to battle microbes, a growing body of evidence suggests that they are imperative for timely restoration of tissue architecture in sterile injury (10, 68). 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 (10). Second, neutrophils are an important source of growth factors (68, 69). For example, they significantly contribute to neoangiogenesis in a vascular endothelial growth factor (VEGF)-dependent manner (69). Third, neutrophil apoptosis and subsequent clearance by macrophages contribute to a proresolution program that is characterized by an anti-inflammatory cytokine signature [e.g., transforming growth factor (TGF)- β , IL-10] (68). However, neutrophils can also significantly increase tissue damage by amplifying the inflammatory response and releasing toxic effectors (70). Main mechanisms include the release of ROS, proteolytic enzymes, and antimicrobial proteins, as well as neutrophil extracellular traps (NETs). These proteolytically coated chromatin entities are emerging as key regulators of neutrophil-mediated tissue injury, and they may contribute to the development of many noninfectious diseases (71). In a simple, focal hepatic injury, however, NETs are not produced, and neutrophils rapidly remove necrotic tissue, paving the road for the next step toward homeostasis.

As discussed above, neutrophils play an important role in tissue repair by clearing debris, releasing growth factors (68), 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 toward 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 (68). The prevailing view is that 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 α -defensins (72). The ingestion of apoptotic cells or apoptotic bodies induces an anti-inflammatory tissue-repair polarization in macrophages toward an M2 repair phenotype, a state that includes the expression of TGF- β , IL-10, PGE₂, and VEGF (68, 73). M2 macrophages, and also neutrophils and other cells, produce lipoxin A₄, resolvins and protectins, which inhibit further neutrophil recruitment and enhance the clearance of apoptotic

neutrophils by phagocytosis (73). This process is referred to as efferocytosis (74). Additional anti-inflammatory proresolution signaling from neutrophils to macrophages includes (but is not limited to) phosphatidylserine-containing microvesicles being shed by neutrophils (75), chemokines being sequestered through CCR5 modulation by neutrophils (76), the release of annexin A1 from neutrophil granules or in microvesicles (77), and IL-10 release (78). The latter has been consistently observed in mice (78), while the IL-10 locus in human neutrophils seems to be inactive (79, 80). These proresolution signals create a resolving feedforward loop, leading to an exponential decrease in neutrophil numbers by terminating immigration and increasing efferocytosis (74).

However, several *in vivo* microscopic observations are hard to reconcile with monocytic phagocytosis being the main mechanism of neutrophil clearance (81). First, in several sterile injury models, neutrophils entered and disappeared well before any monocytes or macrophages 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) (82). rTEM describes the process of neutrophils migrating back to the vasculature, and it was discovered through microscopic observations in zebrafish and *in vitro* models that used human endothelial cells and leukocytes (83, 84). 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 to the blood via rTEM (84).

More recently, rTEM was visualized in mammals using *in vivo* microscopy, and endothelial JAM-C was identified as an important regulator of rTEM (85). Blockade or genetic deletion of endothelial JAM-C led to increased neutrophil rTEM (85). Similarly, local LTB₄-induced proteolytic cleavage of JAM-C by endothelial neutrophil elastase promoted rTEM into the circulation and may lead to dissemination of these activated neutrophils, giving rise to systemic inflammation (86). However, these results seemed to be more consistent with aborted forward TEM rather than with neutrophils entering tissues, completing their effector function in the interstitial space, and then reverse transmigrating. In 2017, Wang et al. (81) formally demonstrated rTEM from the interstitial space back to the bloodstream by using photoactivatable green fluorescent protein (GFP). They showed that neutrophils reverse transmigrated 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 upregulated CXCR4, which presumably enabled them to home back to the bone marrow, where they underwent apoptosis (**Figure 2**). Notably, reverse (but not forward) migration depended on cathepsin C, an enzyme needed to activate various proteases. Impairment of proteolytic capacity resulted in neutrophil persistence at the injury site, which was associated with no revascularization or tissue repair. In zebrafish embryos, rTEM was regulated by hypoxia-inducible factor 1 α (HIF-1 α) and CXCL8/CXCR2 signaling. HIF-1 α -activated neutrophils continued to patrol the injury site during the resolution phase, when neutrophils would normally migrate back into the vasculature (87). CXCL8/CXCR2 has been identified as a specific ligand–receptor pair that orchestrates chemotaxis through the interstitial space back toward blood vessels (88). Collectively, these studies suggest that rTEM is necessary for resolution. However, some authors have suggested that rTEM could also be a means by which sterile local inflammation (e.g., pancreatitis) may spread to distant sites (e.g., lung) or become systemic (89). Indeed, in a murine model of acute pancreatitis JAM-C was downregulated, which led to increased rTEM and, thus, more activated neutrophils being present in the lung vasculature. This, in turn, was correlated with more severe lung interstitial damage (89), which could become clinically apparent as acute respiratory distress

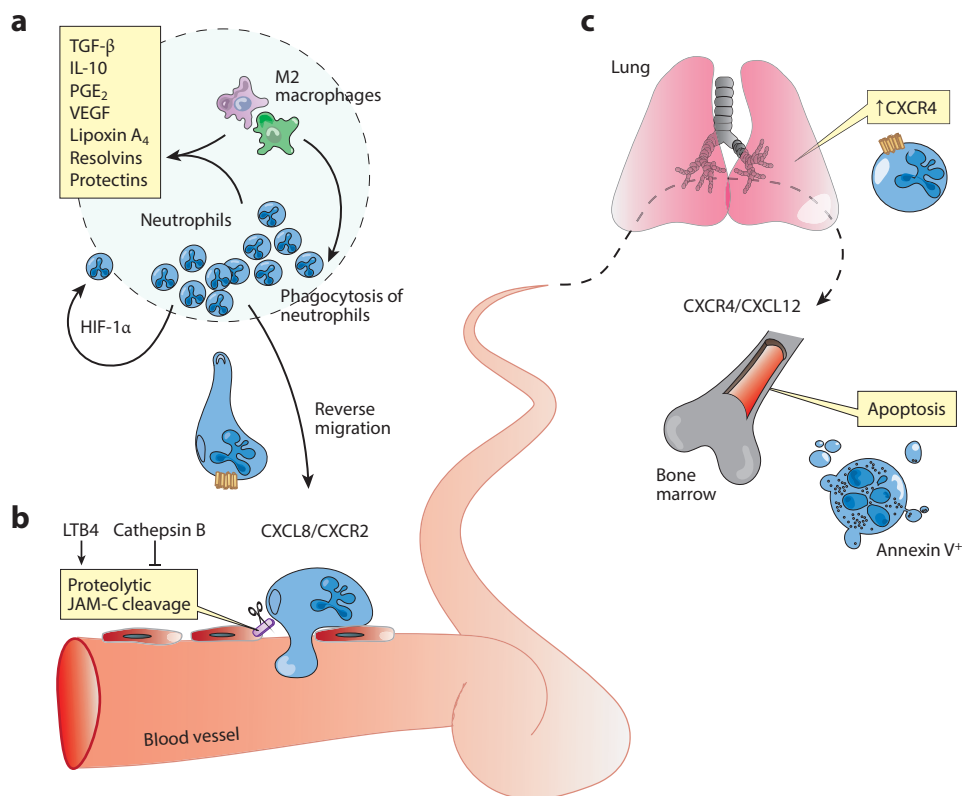


Figure 2

Reverse migration of leukocytes. (a) As DAMPs are cleared from the site of injury, neutrophils and macrophages start producing anti-inflammatory proresolution mediators. Some neutrophils may be phagocytosed by macrophages. However, the majority engage in a reverse migratory process that is required for effective restoration of homeostasis. Reverse chemotaxis through the interstitial space depends on the CXCL8/CXCR2 pathway. (b) Reverse transendothelial migration back into the vessel lumen depends on the proteolytic cleavage of JAM-C, a process induced by LTB₄. (c) Then, neutrophils circulate into the lung capillaries where they upregulate CXCR4, a receptor needed for subsequent homing into the bone marrow, where the neutrophils undergo apoptosis and thus become positive for annexin V. Abbreviations: DAMPs, damage-associated molecular patterns; HIF-1α, hypoxia-inducible factor 1α; JAM-C, junctional adhesion molecule C; LTB₄, leukotriene B₄.

syndrome. Therefore, both the molecular mechanisms of rTEM and its specific role in many inflammatory pathologies need to be further elucidated, and this may open up therapeutic avenues for the future. In addition, it is worth mentioning that these processes (rTEM and efferocytosis) are not mutually exclusive, and, in fact, both may be required for proper resolution.

Monocyte recruitment is the next critical step in the sequence for successful wound healing. Monocytes generally follow neutrophils in the recruitment sequence for 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 (90). This neutrophil–monocyte cross talk depends on the neutrophil-derived heparin binding protein azurocidin and antimicrobial peptide LL-37, which act via FPR on monocytes (90). Other models, however, such as that of focal sterile hepatic injury, have failed to demonstrate this causal link and have suggested that neutrophils and monocytes have independent programs of recruitment (91). This is consistent

with the fact that they are recruited to different locations in the injury. In focal sterile hepatic injury, classical proinflammatory CCR2^{hi}CX3CR1^{low} monocytes are recruited from the blood at 8–12 h postinjury in a CCR2/CCL2-dependent manner, but they are recruited entirely independently of neutrophils (91). In addition to the classical proinflammatory CCR2^{hi}CX3CR1^{low} monocytes, another CCR2^{low}CX3CR1^{hi} monocyte population, referred to as alternative monocytes, gradually appears during the 48 h after injury. While classical proinflammatory monocytes form a ring-like structure around the necrotic area, this second alternative monocyte population forms an identical ring around the necrotic area before entering the injury site to promote tissue repair (91). However, recruitment of alternative monocytes from the blood could not be demonstrated. Using a combined CCR2^{RFP/+} and CX3CR1^{GFP/+} reporter and a rainbow hue analysis revealed there is a continuum of monocytes, ranging from classical proinflammatory monocytes (CCR2^{RFP}) to alternative green monocytes (CX3CR1^{GFP}), as well as many intermediate populations, including various shades of orange, yellow, and light green. This suggested a switching from inflammatory to alternative monocytes at the site surrounding the injury (91). Long-term in vivo imaging showed that, indeed, monocytes are recruited as CCR2^{hi} cells and gradually change their phenotype toward CX3CR1^{hi} while clustered within the ring-like structure around the focal damage. This reflects phenotypic conversion in situ, from proinflammatory to alternative (91). 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?

iNKT cells, a specialized subset of innate T lymphocytes, have been described as the orchestrators of immunity as they exhibit both potent immunostimulatory and immunoregulatory roles. iNKT cells express a restricted T cell receptor repertoire that allows them to recognize exogenous glycolipid antigens (from pathogens) presented by CD1d, a molecule similar to major histocompatibility complex class I molecules (92, 93). In addition, iNKT cells have been demonstrated to recognize self-antigen glycolipids (94). Depending on whether an iNKT cell is activated by a foreign or self-antigen, it rapidly secretes the proinflammatory type 1 cytokine interferon (IFN)- γ during infection or secretes type 2 cytokines, such as IL-4 and IL-10, during tissue repair (95, 96). It remains unclear whether a single cell has the capacity to do both or whether there are two subsets of iNKT cells, each responsible for producing different cytokines; however, the latter is the prevailing view. Under baseline conditions, CXCR6^{GFP/+} iNKT cells constantly crawled within the liver sinusoids, patrolling for perturbations (95, 97, 98). Within 8 h after focal liver injury, they arrested their crawling and accumulated in a concentric circle around the injury zone, where the inflammatory monocytes accumulated (11). Several studies have shown that arrested iNKT cells—that is, cells that paused their patrolling behavior—ligated their T cell receptor with an antigenic ligand presented by a CD1d molecule on an antigen-presenting cell (97, 99). This CD1d-dependent antigen presentation activated the iNKT cells (i.e., increased their CD69 expression), and they began producing the appropriate cytokines (11, 97). Liver sinusoidal endothelial cells as well as Kupffer cells were the main (but not exclusive) cells that presented self-antigen to iNKT cells in the ring surrounding the sterile hepatic injury. In addition, IL-12 and IL-18 also helped to activate and retain the iNKT cells in their prescribed location (11). During liver infections, iNKT cells were activated to produce type 1 cytokines, such as IFN- γ (97). In contrast, a hepatic focal sterile injury led to the production of type 2 cytokines, such as IL-4. The accumulation and activation (i.e., crawling arrest, CD69 expression) of iNKT cells and the production of type 2 cytokines coincided with the switch from recruited inflammatory CCR2^{hi}CX3CR1^{low} monocytes to alternative CCR2^{low}CX3CR1^{hi} repair monocytes, suggesting cross talk between these two cell types (91). Indeed, when iNKT cell activation was inhibited by genetic ablation of CD1d or through combination of anti-CD1d, anti-IL-12, and anti-IL-18

treatment, CCR2^{hi}CX3CR1^{low} cells accumulated around the injury but failed to convert to a CCR2^{low}CX3CR1^{hi} polarization, and, thus, tissue repair was impaired (11).

In summary, successful wound healing in sterile hepatic injury requires a well-timed sequence of events and specific localization of specific cells. 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 the bloodstream and accumulate around the injury. In order to enter the injury and promote tissue repair, they need to be reprogrammed in situ from an inflammatory (CCR2^{hi}) to a reparative (CX3CR1^{hi}) phenotype. This reprogramming depends on the activation of and IL-4 production by iNKT cells that were presented with self-antigen on CD1d.

INVASION OF LEUKOCYTES FROM CAVITIES 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 has been known that body cavities, such as the pleural, pericardial, and peritoneal cavities (see the sidebar, Perspective: Body Cavities Harbor Conserved Entities of the Mammalian Immune System), are filled with macrophages, B cells, and other immune cells. In a model of focal thermal liver injury, GATA6⁺ cavity macrophages from the peritoneum adhered within 1 h to the surface of the site of injury (100) (**Figure 1**). More than 20% of these macrophages expressed the proliferation marker Ki67 and integrated bromodeoxyuridine at the injury site, both clear signs of proliferation. Local proliferation of tissue-resident macrophages has been associated with a type 2 immune response (101). A type 2 response in macrophages reflects alternative polarization (M2), promoting tissue repair. Indeed, arrival and local proliferation of macrophages correlated with the upregulation of other characteristic M2 markers, such as CD273, CD206, and arginase-1, in cavity macrophages at the injury site (100). In vivo microscopy revealed that peritoneal macrophages cleared debris

PERSPECTIVE: BODY CAVITIES HARBOR CONSERVED ENTITIES OF THE MAMMALIAN IMMUNE SYSTEM

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 that later becomes the dorsal body cavity. The dorsal cavity is lined by meninges, with its upper part (the cranial cavity) containing the brain and its lower part (the vertebral canal) containing 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, respectively, the heart, lungs, and the digestive, reproductive, and urinary organs. The mesothelium ensures free organ movement and maintains a serous fluid named after each compartment—that is, pericardial, pleural, or 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 (126). We have revealed the role of serous body cavity macrophages in several diseases (100, 102). In addition, attention to meningeal leukocyte populations has been increasing, with leukocyte subpopulations reported to exert neuromodulatory effects deep within the brain (recently reviewed in Reference 127). In this review, we challenge the field to think about other potential body cavity–resident leukocyte populations, alternative migration routes, and their implications in pathology.

and dismantled necrotic cells, and their depletion or genetic ablation resulted in impaired tissue repair and delayed revascularization in the liver (100).

In this type of recruitment, the macrophages traveled through the abdominal cavity and then breached the liver capsule, which consists of the cellular mesothelial monolayer and a submesothelial layer of connective tissue (**Figure 1**). In the thermal injury model, these layers were destroyed, but in other models of injury, including carbon tetrachloride–induced injury, the intact mesothelium was crossed by the macrophages. Peritoneal macrophages were localized to this injury via various DAMPs, including ATP binding to the P2X7 receptor, and then adhered to hyaluronan with CD44 (100). Although it was unclear whether either of these molecules functioned as chemoattractants, they did contribute to recruitment. In fact, it remains unclear whether a chemoattractant is involved. Setting up a chemokine gradient within a cavity is difficult to conceive, and pertussis toxin, which inhibits all G α i-associated chemokine receptors, did not inhibit the recruitment of cavity macrophages to the injury. Intriguingly, a deep liver injury was also capable of recruiting these cells, suggesting that a chemotactic mechanism was actively recruiting these macrophages in this scenario (100). The molecular details of transmesothelial 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 (103). Similarly, GATA6⁺ macrophage recruitment has been shown to be of importance in other solid organ pathologies, such as lung and heart diseases (102). 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 (see the sidebar, Perspective: Body Cavities Harbor Conserved Entities of the Mammalian Immune System).

Macrophages 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) (6, 104) and certain dendritic cells (e.g., Langerhans cells) (105) populate their respective organs during embryogenesis. Tissue-resident cells renew themselves under basal conditions and rapidly react to inflammatory challenge with local proliferation (105, 106). As such, if a tissue is injured, the resident macrophages already within the injury site can effect repair if they are not destroyed. However, we found that if they are destroyed, Kupffer cells did not invade an adjacent focal thermal hepatic injury and contribute to tissue repair (100). The other pathway by which macrophages can arrive at an injury site is as monocytes that then differentiate into mature macrophages. This process has been reviewed elsewhere (107). In brief, proinflammatory Ly6C^{hi} monocytes recruited to a sterile cardiac injury were shown to gradually differentiate into Ly6C^{low} macrophages. This transdifferentiation depends on nuclear receptor subfamily 4 group 1 member 1 (Nr4a1) (108). These macrophages start to produce TGF- β and VEGF. This is consistent with a reparative phenotype, and selective macrophage depletion between 3 and 8 days postinjury led to impaired wound healing in the skin (109). It is worth mentioning that in many of the inflammatory conditions that are discussed in the next section, monocytes become inflammatory macrophages and contribute to injury (107–109).

INEFFECTIVELY CLEARED IMMUNOSTIMULATORY MOLECULAR PATTERNS IN THE CONTEXT OF LIFESTYLE-ASSOCIATED IMMUNOPATHOLOGIES

Sterile injury leads to a sequence of leukocyte-mediated responses 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 the associated leukocyte recruitment may be responsible for persistent tissue injury and may contribute

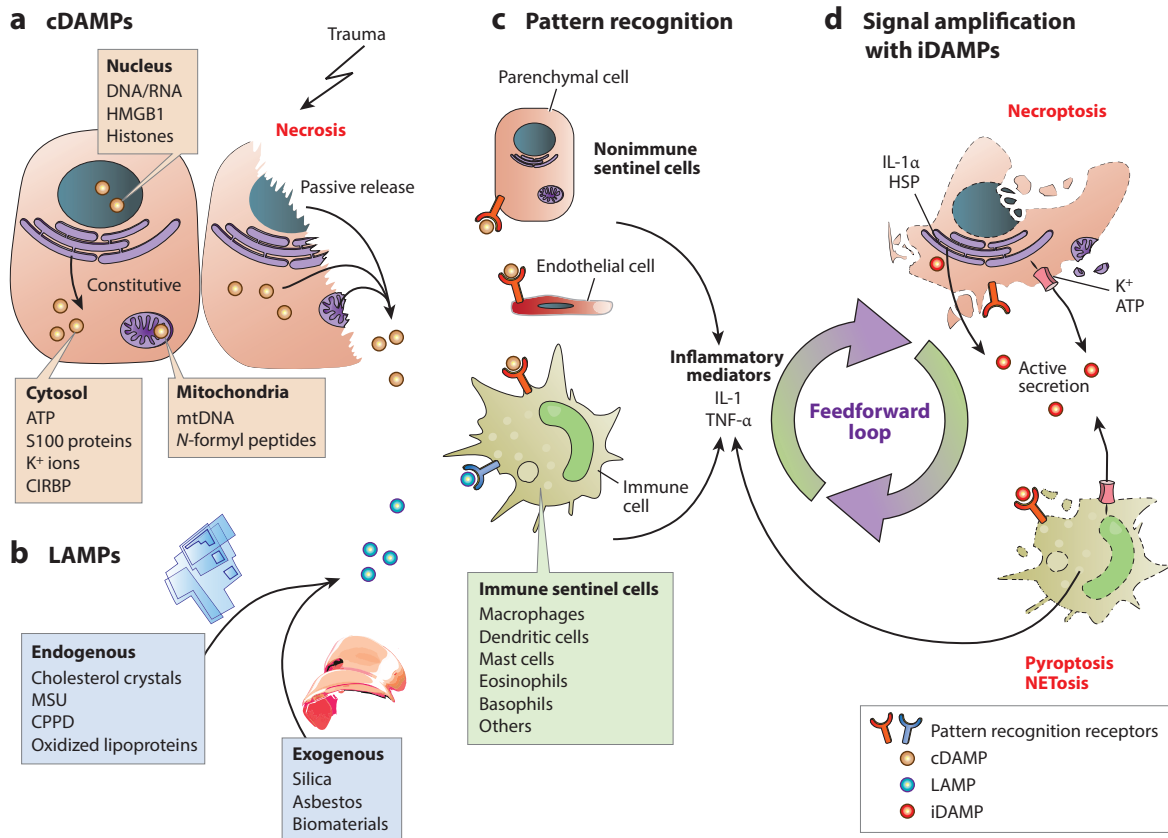


Figure 3

Sterile immunostimulatory patterns. (a) Mechanical, thermal, or chemical trauma leads to the release of cDAMPs from their respective intracellular compartment (extracellular DAMPs are not shown). (b) Alternatively, sterile inflammation may be started by LAMPs. (c) Immunostimulatory patterns are recognized by PRRs on both nonimmune cells and specialized immune sentinels. Ligation of PRRs leads to the secretion of proinflammatory cytokines (e.g., TNF- α and IL-1) as well as other inflammatory mediators. (d) Independent of initial induction, once inflammation is mounted, proinflammatory signals, such as IL-1 and TNF- α , induce programmed nonapoptotic cell death in parenchymal cells (necroptosis) as well as in leukocytes (referred to as pyroptosis, or NETosis in neutrophils). This leads to the induction and active (noncanonical) secretion of iDAMPs. Abbreviations: cDAMP, constitutively expressed DAMP; CIRBP, cold-inducible RNA binding protein; CPPD, calcium pyrophosphate dihydrate; DAMP, damage-associated molecular pattern; HSP, heat shock protein; iDAMP, inducible DAMP; HMGB1, high mobility group box 1; IL, interleukin; LAMP, lifestyle-associated molecular pattern; MSU, monosodium urate; mtDNA, mitochondrial DNA; NET, neutrophil extracellular trap; PRR, pattern recognition receptor; TNF, tumor necrosis factor.

to morbidity (17, 43). On a molecular level, the initial physical, chemical, or ischemic damage is associated with cell death and the release of self-DAMPs (110). These initial molecular danger signals are constitutively expressed and compartmentalized in the nucleus, mitochondria, and cytosol and are invisible to the immune system (**Figure 3**). Thus, they are termed constitutively expressed DAMPs, or cDAMPs (110). The cDAMPs include, for example, double-stranded DNA, which is usually limited to the nucleus, and ATP, which usually exists in the cytosol. Also, cDAMPs comprise many molecules found in high concentrations only within mitochondria. In addition to the release of molecules normally compartmentalized in cells, damage may also include modified components of the ECM, such as oxidized or structurally modified hyaluronan, collagen, laminin,

and elastin. The ligand domain of a damaged ECM protein may be cryptic—that is, it may be exposed only after the ECM is damaged—and the bioactive cryptic domains are referred to as matricryptins (111). Alternatively, modification of the ECM structure by secreted proteins—for example, proteolytically modified collagen—activates immunostimulatory receptors. These described cDAMPs are recognized by PRRs on sentinel cells.

Inflammation induces nonapoptotic programmed cell death scenarios, such as necroptosis, pyroptosis, and NETosis. This, in turn, leads to the induction and release of a group of DAMPs that are inducible and, thus, have been termed iDAMPs (110) (**Figure 3**). iDAMPs may involve noncanonical protein secretion, such as Golgi bypassing, microvesicle formation, and membrane pore formation (9). In **Table 1**, we provide a comprehensive overview and classification of sterile immunostimulatory patterns consisting of cDAMPs, iDAMPs, and a term we would like to introduce, lifestyle-associated molecular patterns, or 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. Our lifestyles have outpaced the genetic adaptation of the immune response, leading to inappropriate inflammation. For example, it is now widely accepted that atherosclerosis, the most important cause of death in the industrialized world, has most of the characteristics of a classic inflammatory response (112, 113) without resolution. The consumption of high-fat diets can lead to hypercholesterolemia 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 the binding and activation of innate macrophage PRRs, including TLRs (112, 114). The uptake of LDL by macrophages leads to the formation of intracellular cholesterol crystals, which activate the NLRP3 inflammasome (115, 116). The interplay between cholesterol, inflammation, and innate immunity is well established, but rather than DAMPs or PAMPs being the key drivers of inflammation, this is a lifestyle disease driven by the sterile molecule LDL. While the immune system recognizes LDL as an excessive atherogenic toxin, no processes exist to effectively eliminate or detoxify it, and inappropriate inflammatory plaques form.

Another well-described example is monosodium urate (MSU), the causative agent of gouty arthritis (117). 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 (118). The activation of the NLRP3 inflammasome and induction of IL-1 β production by phagocytosed MSU have attracted significant attention, and the molecular details of how MSU interacts with events controlled by NALP3–ASC–caspase-1 have now been partially elucidated (118, 119). The immunological mechanisms in calcium pyrophosphate deposition disease (also called pseudogout) are considered to be similar (119) and are another example of excessive inflammation due to a lack of timely resolution. Asbestos and silica particles also are strongly purported to be activators of the NLRP3 inflammasome (120), particularly in the lung. Unlike MSU or calcium pyrophosphate, asbestos and silica particles cannot be dissolved by macrophages, and even small amounts of inhaled crystalline silica or asbestos dust can lead to chronic inflammation and cause chronic lung diseases, including silicosis and asbestosis, respectively (121). In these cases, frustrated phagocytosis, an event that does not lead to productive digestion of the ingested agent, induces cell death of the phagocyte, and this is followed by further recruitment that is followed by further cell death in which scarring and organ dysfunction are the eventual end points, along with a significantly increased risk for local malignancies (121). Many of these inert particles enter the body due to lifestyle—for example, through work in mines—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 pacemakers, aesthetic and reconstructive implants, and artificial organs, such as ventricular assist devices and insulin pumps (122). Biomaterials comprise a broad range of molecular patterns that can stimulate innate immunity. Their composition ranges from naturally occurring to fully synthetic macromolecules; and implant success and implant survival are linearly dependent on the severity of the inflammatory reaction and consequent fibrosis (122). For example, the inflammatory response to implanted surgical mesh in hernia repair surgery is well recognized. Polypropylene is the most commonly used material for manufacturing synthetic surgical mesh, with more than 1 million prosthetics implanted worldwide (123). Polypropylene is chemically inert to enzymes released by leukocytes and, therefore, its use creates another example of excessive inflammation caused by a lack of timely elimination of the introduced foreign substance that results in 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, severe intraperitoneal adhesions are formed, causing significant morbidity.

As the list of sterile molecules with the ability to induce inflammation grows longer, it becomes more difficult to reconcile all underlying molecular patterns with the classical danger model that comprises self-molecules associated with distress, damage, or danger that activate an immune response to alienate the danger and help in restoring homeostasis (124). Furthermore, the above examples are clearly not microbe associated. Therefore, an increasing number of sterile immunostimulatory molecules are being identified that are neither damage nor pathogen associated, and the categories DAMP and PAMP both fall short. However, a large body of epidemiological evidence links some sterile immunostimulatory molecular patterns with the twenty-first-century lifestyle in industrialized countries. Therefore, we propose classifying known molecular patterns that have been identified as causing an inflammation-driven lifestyle-associated process that potentially progresses to disease as LAMPs. Moreover, these immunostimulatory patterns did not coevolve with the innate immune system's dual function of first-line defense against pathogens and tissue repair after damage, but rather they are an attempt to deal with a foreign substance, invoking improvised mechanisms that work for pathogen clearance or trauma. Another commonality among LAMPs is their persistence, which induces ongoing or chronic inflammation. The list of LAMPs provided here (**Table 1**) is but a first compilation of the most obvious molecules. The LAMPs of many inflammatory conditions, potentially including some putative autoimmune disorders, remain to be identified.

CONCLUDING REMARKS

The importance of an appropriate inflammatory response for tissue repair following sterile injury is indisputable. As discussed in this review, the response to a sterile, non-repetitive injury has been optimized through evolution and leads to effective, rapid repair. It involves the infiltration of the right leukocytes at the right time and requires that leukocytes are either reprogrammed in situ from inflammation to resolution or, alternatively, leave the site altogether by rTEM. However, when one step in this sequence goes awry, the resulting response may be harmful and lead to disrepair or disease, or both. Many diseases in which inflammation leads to persistent damage are associated with the twenty-first-century lifestyle in industrialized countries. Damage in these situations may occur in response to molecular patterns that are recognized as foreign but for which an eradication strategy has not evolved or is not possible. LAMPs such as asbestos, prosthetic materials, or cholesterol crystals cannot be cleared by the immune system. Ideally, homeostasis can be reestablished after unsuccessful clearing of an insult by compartmentalization of the sterile injury in chronic inflammatory aggregates or granulomas (reviewed in Reference 125). In other

instances, compartmentalization cannot be achieved, and the persisting LAMP prevents the local switch from a proinflammatory to a repair program. This leads to unresolved chronic inflammation and differs from the canonical PAMP- or DAMP-induced inflammation that is followed by a return to homeostasis.

SUMMARY POINTS

1. Sterile inflammation canonically starts with the recognition of damage-associated molecular patterns (DAMPs).
2. Sterile inflammation leads to the canonical transendothelial recruitment of leukocytes from the blood.
3. In addition, leukocytes from serous body cavities invade across a disrupted or an intact mesothelium.
4. Leukocyte traffic in focal sterile hepatic injury follows a well-defined sequence of body cavity macrophage recruitment, neutrophil recruitment, and recruitment of inflammatory monocytes.
5. Inflammatory monocytes must undergo in situ reprogramming, from inflammatory to alternative (tissue repair), before they can enter the focal hepatic injury zone and promote healing.
6. Monocyte reprogramming in situ is mediated by the production of interleukin (IL)-4 and IL-10 and by invariant natural killer T cells, which are alternatively activated by self-antigen-presenting CD1d⁺ antigen-presenting cells and cytokines (e.g., IL-12, IL-18).
7. After debris clearance, neutrophils reverse their migration away from the site of sterile injury into the lung capillaries, where they upregulate CXCR4, a chemokine receptor needed for subsequent homing to the bone marrow, where they undergo apoptosis.
8. 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, resulting in chronic inflammation and leukocyte-mediated damage and its associated morbidity.

FUTURE ISSUES

1. The importance of alternative migration routes (e.g., transmesothelial invasion) from body cavities to the site of sterile injury must be further investigated and may be of importance even in solid organ pathologies.
2. The lack of reverse leukocyte migration may be a defect that needs to be explored further as a contributor to persistent inflammation leading to pathologies.
3. Further study of leukocyte trafficking in sterile injury, in particular the in situ switch of recruited proinflammatory phenotypes to reparative phenotypes, may reveal potential therapeutic approaches that could be used to alleviate leukocyte-mediated damage in the context of LAMPs.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

The authors thank Dr. M. Hossain from the Kubes Lab, University of Calgary, for providing the microscope images used in **Figure 1**.

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