



9.1 Introduction

The rediscovery of the innate immune system has led to many revolutionizing aspects of biology and medicine. One of the most exciting discoveries is that the innate host defense system consists not only of mobile and versatile immune cells such as DCs, macrophages, ILCs, and unconventional T cells but also of a large variety of resident sessile cells. Resident innate immune cells are predominantly located at the interface between the environment and the host, where pathogens can enter the body. There are several types of sessile cells: innate defense responses use mucosal EpCs such as bronchial, intestinal, tubular, gastric, pancreatic, and uterine EpCs as well as vascular cells such as ECs and VSMCs. Also, other cell types such as adipocytes and osteoblasts are found in the innate immune system. A few different cell types, as extracted from the rapidly growing literature, are presented in the following.

9.2 Epithelial Cells

9.2.1 Introductory Remarks

A large variety of resident cutaneous EpCs in the skin and mucosal duct EpCs of different organs are at the service of our innate immune system. Because EpCs cover the body surfaces, they represent a primary site of host ↔ microbe interaction. Of importance are mucosal surfaces prone to contact with pathogenic and non-pathogenic microbes, which requires that immune recognition principles have to be tightly controlled to avoid uncontrolled permanent activation. To be an effective physical first barrier of defense against invading infectious pathogens, EpCs, all intensely equipped with PRMs such as TLRs and NLRs, have evolved innate immune antimicrobial functions able to secrete local antimicrobial

peptides such as defensins and cathelicidins [1] (for antimicrobial peptides, see Part VI, Sect. 23.4).

Epithelial barriers are not only responsible for the sequestration of microorganisms and their inactivation but also exert homeostatic functions. Remarkably, EpCs possess an intrinsic capacity to survive despite continuous exposure to considerable amounts of microorganisms in the environment that are potentially toxic to host cells, suggesting that some homeostatic forces link antimicrobial strategies to mechanisms that control tissue integrity. In fact, evidence has already been reviewed in support of microbial factors in maintaining the structural integrity of epithelial tissues [2].

Besides operating as barriers, EpCs critically contribute to tissue repair via the phenomenon of re-epithelialization [3]. In this subchapter, it is not possible to describe all the interesting details of the innate biology of these cells; nevertheless, some selected epithelial barriers and a few examples of their potency for re-epithelialization are briefly mentioned.

9.2.2 Skin Cells: Keratinocytes

The skin, the largest organ of the body, is an essential barrier that under innate immunity-controlled homeostatic conditions efficiently prevents or minimizes damage from both environmental (e.g., microorganisms, physical trauma, UV irradiation) and endogenous (e.g., cancers, inflammation) factors. Clearly, the innate immune system of the skin is the first line of defense against invasion by microorganisms which gain entry after the skin is damaged. Pattern recognition molecules are expressed on a variety of cell types found in the skin including but not limited to LCs in the epidermis; resident and trafficking immune system cells such as macrophages, DCs, and T and B cells; mast cells in the dermis; ECs of the skin microvasculature; and skin stromal cells such as fibroblasts and adipocytes [4].

In particular, keratinocytes, the main constituent of the epidermis, play a leading role in cutaneous host defense responses. They not only form a passive barrier between external environment and internal organs but also produce various cytokines and chemokines upon recognition of MAMPs and/or DAMPs derived from trauma, bacterial and viral infections, toxins, chemical substances, or UV irradiation. Recognition of these infective or sterile insults by keratinocytes is provided by a variety of PRMs such as TLRs, NLRs, RLRs, and ALRs [4–7]. In addition to their defending role, keratinocytes contribute to tissue repair via the process of re-epithelialization by using several mechanisms including stimulation of mesenchymal stem cells [8] and secretion of the DAMP HSP90 [9].

As with all PRM-driven cellular innate immune responses, when uncontrolled they may cause pathologies. Thus, as reported, putative PRM dysfunction is associated with numerous immune disorders that affect the skin, such as SLE, cryopyrin-associated periodic syndrome (CAPS), and primary inflammatory skin diseases including psoriasis and atopic dermatitis [10]. In Volume 2, these disorders will be covered in detail.

9.2.3 Oral Gingival Epithelial Cells

Oral gingival epithelium appears to be the first barrier against periodontopathic bacteria found in periodontal pockets and tissue. In fact, there is growing evidence suggesting that innate immune mechanisms in cells of the oral mucosa represent an effective first-line defense against pathogens that enter and contaminate the oral cavity on a daily basis. Gingival epithelium accounts for a robust innate immune defense system that works in many ways for the protection of underlying connective tissue. First, it provides a physical barrier which does not allow microbes to invade while enabling selective transaction with the oral environment. Second, upon interaction with microbes, it secretes cytokines as well as chemokines to activate the influx of neutrophils and other immune cells into the sulcular area [11, 12]. Third, the gingival epithelium has been found to be a source of antimicrobial peptides which are crucial for an effective innate immune humoral defense response [13]. Cells of the gingival epithelium are equipped with various PRMs including TLRs [12, 14] and NLRs such as NLRP3 that forms the inflammasome [15, 16]. Again, when uncontrolled, MAMP/DAMP-induced, PRM-mediated innate immune responses of cells of the oral cavity may cause pathologies in the form of gingivitis and periodontitis which may result in chronic periodontal diseases [17].

9.2.4 Airway Epithelium: Tracheal/Bronchial and Alveolar Epithelial Cells

Characteristically, the airway epithelium is localized at the interface between the environment and the host. The cell layer not only forms a sizeable mechanical barrier but is also predisposed as a sentinel system to detect pathogens and non-pathogenic toxic agents entering via the airways. To rapidly initiate an acute innate immune inflammatory defense response in these circumstances, the airway epithelium is equipped with a variety of PRMs, in particular, TLRs that are able to recognize MAMPs and/or DAMPs [18–21].

In addition to TLRs, cytosolic NLRs have also been shown to be involved in airway epithelium activation. For example, in human lung epithelium, expression of NOD1 and lower expression of NOD2 was detected in resting human BEAS-2B cells (an immortalized bronchial epithelial cell line), suggesting that PRM-bearing airway EpCs are essential contributors to innate mucosal immunity and provide a variety of antimicrobial effectors [22]. Of particular relevance is the NLRP3 receptor that upon activation by DAMPs is capable of forming an inflammasome, thereby contributing to lung inflammation. For example, novel data have shown the presence and functional activation of the NLRP3 inflammasome by the DAMP *crystalite silica* in human lung EpCs that may contribute to lung diseases [23] (for silica particles as exogenous DAMPs, see Part IV, Sect. 15.2.4). Thus, via recognition of MAMPs and/or DAMPs by PRMs, bronchial EpCs play a critical role in the regulation of pulmonary tissue homeostasis by the modulation of numerous molecules, from antioxidants and lipid mediators to growth factors, cytokines, and chemokines [24].

Again, while controlled innate immune responses executed by airway EpCs guarantee maintenance of pulmonary homeostasis, uncontrolled/aberrant reactions are involved in the pathogenesis of human lung diseases. For example, there is a crucial role of allergen-induced innate immune responses by TLR-bearing airway EpCs in the pathogenesis of asthma and allergic rhinitis [25, 26]. Besides allergic disorders, DAMP-induced innate immune responses of the airway epithelium are also involved in the pathogenesis or consequences of acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) as well as chronic inflammatory lung diseases, including chronic obstructive pulmonary disease (COPD), and cystic fibrosis (CF).

9.2.5 Gastrointestinal Epithelial Cells

Gastrointestinal EpCs have a unique feature: the gastrointestinal (GI) tract is, like the skin or the lung, a major interface organ between the environment and interior milieu by providing the site with the largest population of microorganisms, the microbiota. As outlined by Lallès and Wang et al. [27, 28], the mammalian intestinal lumen is immediately colonized by trillions of microorganisms after the initiation of postnatal enteral feeding. This community of commensal microorganisms lives in a symbiotic relationship with the host by engaging in food digestion and vitamin production. In return, host cells provide the microbiota with the essential surviving niches and nutrients. Of note, however, growing evidence suggests the implication of the gut microbiota not only in various facets of health but also several diseases (for microbiota, also compare Part II, Sect. 4.3 and Part VIII, Chap. 34).

Quite obviously, gut EpCs are the first cells to be exposed to nutrients and the microbiota, with complementary functions between the small intestine aiming at digestion and nutrient absorption and the large intestine specialized in the fermentation of undigested materials. The gut epithelium is also the first line of the GI tract defense and protection. Its action is complementary to that of the associated mucosal immune system whose development and maintenance are induced by the microbiota [28].

The structure of the intestinal mucosa is of interest. As the innermost layer of the GI tract, it is responsible for most digestive, absorptive, and secretory processes. The mucosa is lined with highly polarized epithelial absorptive cells with their apical plasma membrane domain facing the lumen while the basolateral surfaces are associating with lamina propria where professional immune cells reside. A small population of intestinal epithelial stem cells is located at the bottom of crypts, from which functionally distinct epithelial cell types differentiate and mature. These cells operate as critical drivers of epithelial homeostasis and regeneration [29, 30].

In contrast to enterocytes, that is, the mucin glycoprotein-synthesizing goblet cells, the hormone-producing enteroendocrine cells, the mysterious tuft cells that are located in the villus epithelia, and the mature Paneth cells reside at the crypt bottom. These specialized cells are a significant source of antimicrobial peptides in the intestine. Also, under steady-state conditions, ~10% of the intestinal EpCs

within the follicle-associated epithelia that overlie gut-associated lymphoid tissues are so-called microfold cells. These cells are highly specialized for the phagocytosis and transcytosis of gut lumen macromolecules, particulate antigens, and pathogenic or commensal microorganisms across epithelium [31].

The abundance of innate and adaptive immune cells that reside together with trillions of beneficial commensal microorganisms in the human GI tract requires barrier and regulatory mechanisms. Such a critical barrier is formed by intestinal EpCs via tight junctions that physically segregate the microbiota from host tissues, thereby constituting one of the most important mechanisms to support mucosal innate immunity aimed at conserving tissue homeostasis [32, 33]. In addition to the barrier function, endocrine cells, goblet cells, and enterocytes of the intestinal epithelium express a range of PRMs to sense the presence of MAMPs and/or DAMPs, thereby contributing to the homeostatic interplay between the intestinal epithelia on one hand and nutrients, microbiota, intestinal injury-inducing pathogens, and toxins on the other hand. These PRMs include TLRs [34], NLRs including inflammasome-forming NLRP3 [35, 36], RLRs [37], and ALRs [38].

In sum, all innate immune mechanisms driven by the various PRMs converge to the molecular basis of the intestinal homeostasis at the epithelial level. Any perturbation of this homeostasis (i.e., “dysbiosis”) may lead to severe intestinal diseases including but not limited to IBD such as Crohn’s disease and ulcerative colitis as well as intestinal malignancies [39–42].

9.2.6 Intrahepatic Biliary Epithelial Cells (Cholangiocytes)

Intrahepatic biliary EpCs, also called cholangiocytes lining bile ducts, are a heterogeneous, highly dynamic population of EpCs. While these cells comprise a small fraction of the total cellular component of the liver, they perform the essential role of bile modification and transport of biliary and blood constituents. In addition to these functions, cholangiocytes, as sentinel cells of the innate immune system, are equipped with PRMs that actively sense MAMPs translocated from intestinal microbes and DAMPs released from injured organs, thereby modifying cholangiocyte function and/or phenotype. After recognition of MAMPs and/or DAMPs, PRM-triggered signalling pathways have been shown to induce cellular responses affecting repair and remodelling of the biliary epithelium [43]. Cholangiocytes vary in size, shape, and function along the biliary tree and, characteristically, the nature of the biliary insult reportedly determines which subpopulation of cholangiocytes is affected [43]. For example, as reviewed [44], in response to acute injury, large cholangiocytes proliferate and maintain normal biliary homeostasis, whereas chronic injury promotes increased proliferation of both large and small ducts and induces a reparative process.

Like other hepatic cell types including the sinusoidal ECs, Kupffer cells, hepatocytes, HSCs, and DCs, cholangiocytes have been demonstrated to express multiple TLRs that upon MAMPs and/or DAMPs recognition initiate signalling pathways capable of initiating or perpetuating an innate immune response against potentially

pathogenic insults [43, 45, 46]. In case of uncontrolled overshooting responses, the PRM-triggered secretion of inflammatory and fibrogenic cytokines may initiate and prolong liver inflammation that—under the concomitant influence of genetically and epigenetically phenomena—may lead to cholangiopathies including liver fibrosis [43, 46].

9.2.7 Renal Tubular and Urinary Tract Epithelial Cells

The kidney is a complex organ, consisting of at least 12 functionally different epithelial cell types [47]. Since epithelial surfaces provide the first line of defense against infection, it is not surprising that the renal tubulus system together with the urinary tract mucosa constitutively expresses many PRMs. In humans, TLRs such as TLR1, TLR2, TLR3, TLR4, and TLR6, but mainly TLR2 and TLR4, are expressed on renal EpCs including the cells of Bowman's capsule [48], proximal and distal tubular cells [49, 50], and the lower urinary tract and bladder mucosa [51].

Systemic administration of exogenous agonists for TLR2, TLR3, TLR4, or TLR5, but not TLR9, in combination with IgFc receptor activation, was shown to induce glomerular inflammation [52] (for IgFc receptor, compare Part II, Sect. 5.3.8). On the other hand, endogenous DAMPs also have been shown to modulate renal inflammation. For example, HSPs such as HSP70, released after acute renal IRI, have been demonstrated to activate TLR2 and TLR4 and amplify renal injury [53, 54].

In addition to TLRs, human tubular EpCs have been found to express MDA5 and RIG-I as sensors of dsRNA and potent inducers of antiviral activity. Increased expression of these dsRNA sensors has been demonstrated in kidney transplant biopsies during CMV or polyomavirus 1 (BK) virus infection [55].

Activation of renal PRRs by DAMPs, mostly released during RCD from different cellular compartments (for RCD, see Part V, Chap. 19), leads to inflammation of the kidney. This occurs during acute kidney injury (AKI) together with renal cell necrosis (e.g., in thrombotic microangiopathy, necrotizing glomerulonephritis, or acute tubular necrosis {ATN}). In addition, inflammation is present in autoimmune and alloimmune kidney injury which includes all forms of glomerulonephritis, interstitial nephritis, and allograft rejection [56]. In this context, it is worth noting already here that there is a kidney-specific molecule, namely *Tamm-Horsfall protein*/uromodulin, that acts as a renal DAMP. Uromodulin is selectively produced by the cells of the thick ascending limb and secreted into the distal tubule. Uromodulin is an adhesive glycoprotein generating large aggregates that embed everything that is present in distal tubules such as cytokines, blood cells, or necrotic tubular cell debris bacteria [57].

In fact, a role of DAMPs in renal diseases should not be underestimated. As with injury-induced inflammatory processes in other organs, necrotic lesions inside the kidney usually are driven by a primary vascular necrosis (e.g., in crescentic glomerulonephritis or thrombotic microangiopathies). In addition, ischemic tubular necrosis has a critical component of peritubular vascular necrosis. In Volume 2, the

evidence for necrotic cell death-driving inflammation in these disease entities (nowadays called “necroinflammation”) is discussed in more detail.

9.2.8 Uterine and Cervico-Vaginal Epithelial Cells

Human uterine endometrium has evolved to orchestrate many essential functions for the host, ranging from fertilization, implantation, and pregnancy to defense from sexually transmitted diseases and other invading pathogens [58]. Known to be an effective physical barrier to infection, EpCs are in constant contact with the microbiota of the human female reproductive tract and, as with the intestine mentioned above, must discriminate between innocuous commensal microorganisms and pathogenic microbes [59]. To maintain the growth of commensal organisms on their external surface and defending the underlying tissues from invading pathogens, EpCs of the female genital tract have evolved innate immune antimicrobial functions as well as the capability to modulate the recruitment and activity of immune cells of both immune systems. In fact, PRM-bearing cervico-vaginal EpCs reportedly respond to MAMPs and/or DAMPs and produce an array of innate immune factors such as complement, pro-inflammatory mediators, adhesion molecules, and antiviral factors which allow these cells to communicate with the immune system [60–62]. Indeed, several studies have shown that uterine and cervico-vaginal EpCs—besides macrophages and DCs—execute immunological functions by expressing PRMs such as TLRs, RLRs, and NLRs. However, the mechanism by which these cells work together is still limited [63–65]. In fact, given the nascent state of knowledge concerning this important area, it is clear that more studies are needed to provide valuable insights into immunobiology of uterine and cervico-vaginal EpCs.

9.2.9 Résumé

As presented in this subchapter, EpCs of the innate immune system represent the first line of defense against invading microbial pathogens. They are significant contributors to innate mucosal immunity and generate various sophisticated antimicrobial defense mechanisms including the formation of a tight barrier and the secretion of antimicrobial substances. EpCs functionally express multiple PRMs to provide these active defense mechanisms. Notably, mucosal surfaces are prone to contact with pathogenic as well as commensal non-pathogenic microbes, and therefore, immune recognition principles must be strictly regulated to avoid uncontrolled permanent activation. Indeed, these balancing mechanisms are probably due to the pathogen-induced emission of DAMPs which are recognized by EpCs together with MAMPs on the one hand and a result of MAMP recognition alone in the presence of commensals on the other hand. But this has to be proven in future experiments. As also stressed elsewhere [66], deciphering these mechanisms will provide essential understanding to how mucosal tissues maintain health and activate immunity, as well as how pathogens promote disease.

9.3 Fibroblasts and Myofibroblasts

9.3.1 Introductory Remarks

Fibroblasts, equipped with a variety of PRMs, operate—similar to DCs—as classical sentinel cells of the innate immune system that respond to MAMPs and/or DAMPs when exposed, secreted, or released within an injured tissue milieu [67, 68]. Typically, fibroblasts synthesize most of the ECM of connective tissues, including interstitial collagens, proteoglycans, glycoproteins, cytokines, growth factors, and proteases, that is, compounds and molecules that are needed to maintain a structural framework for many tissues. Of note, persistently activated fibroblasts are called myofibroblasts (phenotypically characterized by increased alpha-smooth muscle actin { α -SMA}) that mediate the excessive secretion of collagen in chronically injured organs and tissues. Consequently, due to this extraordinary function, these cells play a crucial role in tissue repair processes such as wound healing.

Fibroblasts have classically been viewed as a uniform cell type with equivalent functions regardless of the origins of tissue. This view has been challenged by data illustrating extensive phenotypic heterogeneity among fibroblasts from different tissues and a given tissue under various physiologic conditions. For example, as reviewed [69], lung fibroblasts have been demonstrated to be heterogeneous in cell surface marker expression as well as in their levels of collagen production. Cardiac fibroblasts were shown to be well-established as key regulators of ECM turnover in the context of myocardial remodelling and fibrosis. Also, periodontal fibroblasts also are characterized by heterogeneity based on morphology, glycogen pools, and collagen production. Further, fibroblasts from different anatomic sites have been found to possess distinct transcriptional patterns. Under appropriate stimulation, for example, mediated by DAMPs, quiescent fibroblasts can acquire an active synthetic, contractile phenotype and express several SMC markers (i.e., myofibroblasts) which are not exclusive to fibroblasts. On the other hand, human hypertrophic scar-derived fibroblasts show the biologic characteristics of mesenchymal stem cells [69, 70].

9.3.2 Origin of Fibroblasts and Myofibroblasts

The possible origin of the fibroblasts has been intensely discussed in the literature, and current notions hold that fibroblasts/myofibroblasts may arise from a variety of sources (Fig. 9.1). Traditionally, fibroblasts are defined as local resident cells of mesenchymal origin. The mesenchymal cells that form the fibroblast population are believed to be derived from a process called epithelial-to-mesenchymal transition (EMT). This transition process is a form of cell plasticity in which EpCs lose their special molecular markers and acquire mesenchymal phenotypes associated with expression of proteins of fibroblasts [70, 71]. A similar process, called endothelial-mesenchymal transition (EndMT) has also been reported. The EndMT is a fundamental cellular mechanism that regulates embryonic development and diseases such as cancer and fibrosis. Recent developments in biomedical research have shown

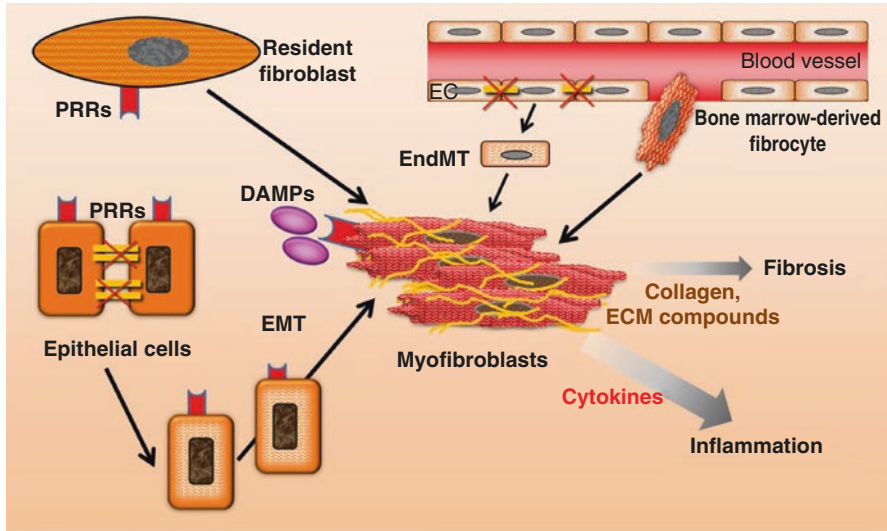


Fig. 9.1 Schematic diagram: sources of myfibroblast formation. Traditionally, fibroblasts are local resident cells of mesenchymal origin. The mesenchymal cells that form the fibroblast population derive from processes called epithelial-to-mesenchymal and endothelial-to-mesenchymal transition. The processes are discussed to result from a collective series of DAMP-induced transcriptional and post-translational events that cause epithelial and endothelial cells to take on mesenchymal features, thus allowing the cells to separate from the tissue context and gain motility. In addition, fibroblasts may originate from circulating fibrocytes derived from bone marrow stem cells. DAMP-activated myfibroblasts then may contribute to inflammation and fibrosis. *EC* endothelial cell, *ECM* extracellular matrix, *EMT* epithelial-to-mesenchymal transition, *EndMT* endothelial-to-mesenchymal transition, *PRRs* pattern recognition molecules. Sources: Refs. [70–74]

remarkable potential to harness the EMT process for tissue engineering and regeneration [72, 73]. In addition, another source of fibroblasts refers to circulating fibroblast-like cells called fibrocytes that are derived from bone marrow stem cells [74]. Finally, so-called pericytes have been suggested to contribute to the adult interstitial fibroblast population able to synthesize collagen as well [75].

9.3.3 MAMP/DAMP-Induced Activation of Fibroblasts and Myfibroblasts

Fibroblasts/myofibroblasts are equipped with a range of PRMs including TLRs, NLRs (plus NLRP3), and ALRs (plus AIM2) that sense various MAMPs and/or DAMPs associated with infectious or sterile tissue injury [68, 76–78]. For example, receptor activation on cardiac fibroblasts by DAMPs has been shown to be coupled to altered cellular function including changes in proliferation, migration, myofibroblast transdifferentiation, ECM turnover, and production of fibrotic and inflammatory paracrine factors which directly impact on the heart's ability to respond to injury [68]. Moreover, investigations on human gingival fibroblasts revealed clear

mRNA expression of TLRs and NLRs which were found to be activated by chemically synthesized agonists mimicking microbial products for these receptors [79]. Also of interest in this context are studies on synovial fibroblasts that showed the DAMP HMGB1 mixed with the MAMP LPS to synergistically up-regulate TLR4 and RAGE expression on the surface of these cells [80].

9.3.4 Résumé

The fact that DAMPs are capable of activating fibroblasts and myofibroblasts represents the basis of tissue repair and, thus, is a prerequisite of successful wound healing after traumatic lesions. This is good news, but there is again bad news: uncontrolled excessive and/or permanent activation of myofibroblasts by DAMPs may lead to excessive ECM and collagen production during their proliferation, thereby replacing regular organ structure (see also Part VIII, Sect. 36.4). These events result in functional impairment and scar formation, which may further trigger persistent fibrogenesis and fibrosis. In fact, myofibroblasts are regarded as a significant type of effector cells in organ fibrosis that, aside from secreting copious amounts of ECM proteins, provide signalling molecules to drive fibrosis. Myofibroblasts also mediate the mechano-regulation of fibrotic matrix remodelling via contraction of their stress fibers [70, 81]. As a matter of fact, several life-threatening/lethal chronic human diseases are due to such an overshooting organ fibrosis—as described in more detail in Volume 2.

9.4 Vascular Cells

9.4.1 Introductory Remarks

The role of vascular cells of the innate immune system can be seen in an evolutionarily developed first-line defense against any inciting insult hitting the vessel walls including bacteria, viruses, microbial toxins, and chemical noxa such as nicotine. Probably during every minute, these cells protect us from those various insults mediated by the bloodstream. To handle such challenges, the sessile cells such as ECs and VSMCs but also adventitial cells, equipped with various PRMs to sense MAMPs and/or DAMPs, are capable of mounting robust innate immune responses and also recruiting mobile cells such as macrophages and DCs to a site of attack. This results in the creation of an acute inflammatory milieu in the vessel wall aimed at curing the vascular injury concerned. In the following, a few remarks are made on innate vascular cells, that is, vessel wall-residing cells and mobile vascular macrophages and DCs.

9.4.2 Endothelial Cells

Endothelial cells serve as the interface between circulating blood and surrounding tissues. As estimated, the average human adult apparently contains over one trillion

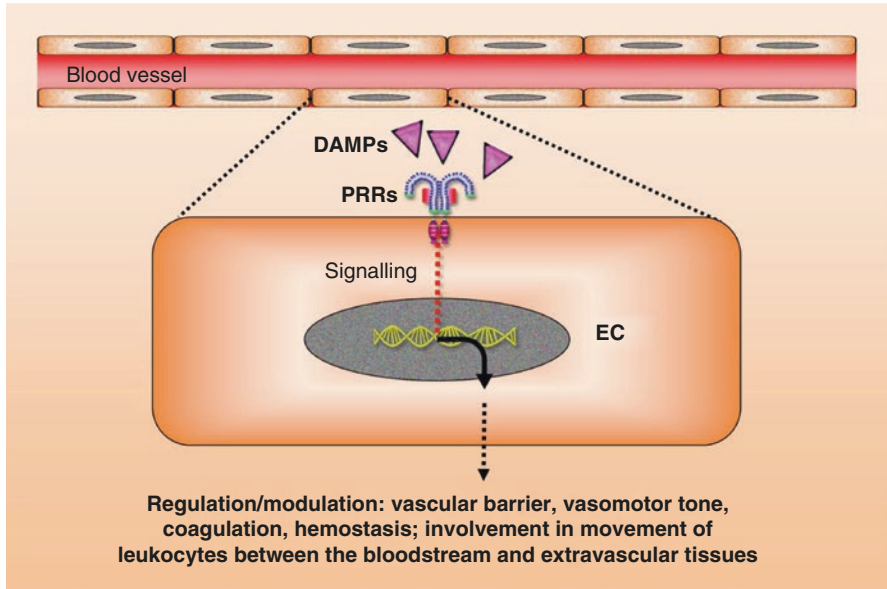


Fig. 9.2 Schematic diagram: endothelial cells. DAMP-activated, PRR-bearing endothelial cells regulate the vascular barrier, modulate vasomotor tone, play central roles in coagulation and hemostasis, and are critically involved in the movement of leukocytes between the bloodstream and extravascular tissues (compare also Part VI, Fig. 22.3). *EC* endothelial cell, *PRRs* pattern recognition molecules. Sources: Refs. [84, 85]

ECs that cover a surface area in excess of 1 million cm^2 and altogether weigh ~ 1 kg [82, 83]. Endothelial cells dynamically regulate the vascular barrier, modulate vasomotor tone, play central roles in coagulation and hemostasis, and, in addition, are critically involved in the movement of leukocytes between the bloodstream and extravascular tissues (Fig. 9.2) [84, 85]. Moreover, ECs—as VSMCs—play a dominant role in DAMP-induced angiogenesis following vascular injury but unfortunately, on the other hand, are also required for the growth of some tumors and atherosclerosis, among other pathologies.

Of note, ECs are increasingly appreciated as key members of the whole family of cells of innate immune cells that are actively involved in defense responses of healthy arteries to infectious and sterile injury [86, 87]. Accordingly, ECs typically express PRRs including members of the TLR family (Refs. [84, 88–93]) as well as the NLR and RLR families (Refs. [84, 94–100]). Engagement of endothelial PRRs with MAMPs and/or DAMPs was shown to up-regulate the secretion of specific cytokines, chemokines, and adhesion molecules and, in addition, to increase the binding of neutrophils to the endothelium (Refs. [84, 90, 101–107]).

Interestingly, histones, operating as DAMPs, reportedly induce the release of *von Willebrand factor* (VWF) from Weibel-Palade bodies (WPBs) and cause thrombocytopenia which impaired arterial thrombus formation in mice [108]. Weibel-Palade bodies are dynamic EC organelles that contain pro-coagulant and pro-inflammatory mediators and are released in response to cell stresses. Other injury-induced

constitutive DAMPs and WPB-released proteins—operating as inducible DAMPs—were shown to be elevated during inflammation and were positively correlated with scenarios of chronic inflammation. These studies revealed that DAMPs can regulate VWF levels and function by inducing its release from endothelial WPBs. Thus, this constitutive DAMPs → inducible DAMPs (WPB) axis may propagate immuno-thrombosis associated with inflammation (this intriguing issue is resumed in Part IV, Sect. 14.2.5.3).

Together, as also discussed elsewhere [84], the combination of inflammation, activation of coagulation pathways, and increased vascular permeability—created via the DAMPs → endothelial PRRs axis—may serve to create a physical barrier that limits the spread of infection into the bloodstream. The hypothetical concept of “hemostatic containment” postulates that leukocyte adhesion to vessel walls and microvascular thrombosis directly obstruct vessels draining sites of infectious/sterile inflammation and that tissue edema resulting from increased vascular permeability further limits blood outflow by externally compressing vessels [109, 110].

Nevertheless, the inappropriate endothelial activation, also known as endothelial dysfunction—for example, occurring through uncontrolled DAMP ↔ PRR interaction—is known to contribute to further tissue damage, as observed in certain autoimmune and inflammatory cardiovascular diseases promoted by atherosclerosis, hypertension, or diabetes.

9.4.3 Vascular Smooth Muscle Cells

The capability of the vascular machinery of innate immune recognition can be extended to PRR-bearing VSMCs representing the second line of innate immune vascular cells. This category of sessile cells in the vessel wall is also equipped with PRRs including distinct TLRs [111, 112], NLRs (NLRP3) [113], RLRs [114], and ALRs (AIM2) [115]. Activation of VSMCs by DAMPs such as HSP60 [116], dsRNA [111], or HMGB1 [117] has been found to instigate both efferent pro-inflammatory and proliferative responses.

As will be pointed out in Volume 2, VSMCs, like ECs, play a crucial role in angiogenesis, a process that is beneficial for tissue regeneration following tissue injury, for example, critical limb ischemia, but, on the other hand, may be detrimental when associated with tumor growth. Moreover, in response to vascular injury, DAMP-promoted activation of PRM-bearing VSMCs results in their proliferation and differentiation to myofibroblasts aimed at repairing the injured vascular wall. However, when becoming overshooting and exaggerated, this DAMP-induced process may lead to atherosclerosis as characterized by intimal thickening [118].

9.4.4 Vascular Macrophages and Dendritic Cells

During the past decade, the myeloid compartment of the vessels wall structure including macrophages and DCs has received particular attention in the contributing

role to atherogenesis. In fact, both key cells of the innate immune system work in close collaboration in different stages and the progression of this chronic complex vessel disease [119], an observation that deserves a few more words.

9.4.4.1 Vascular Macrophages

Macrophages play multiple crucial roles in vascular inflammation. Their plasticity allows them to serve various functions adapted for the state of the tissue. Having accessed the subendothelial space, recruited vascular monocytes differentiate into macrophages, a process that is driven by interactions with the ECM and cytokines including *macrophage colony-stimulating factor* (M-CSF) and members of the TNF family. In fact, macrophages represent a major component of vessel wall infiltrates. Several distinct subsets of macrophages such as inflammation-promoting M1-like and inflammation-resolving regulatory M2-like cells have been identified in atherosclerotic plaques, including subsets that are specific to atherosclerosis itself (reviewed in [120–124]).

As with macrophages located everywhere in the body, vascular macrophages are equipped with all categories of PRMs. As reviewed [125, 126], activated by DAMPs emitted during vascular injury, the pathogenic roles of macrophages in vascular inflammation range from secretion of soluble factors such as cytokines, growth factors, and enzymes to the production of ROS. As executed at various locations of the body, phagocytosing vascular macrophages can participate in debris removal and efferocytosis (see Part VI, Sect. 22.6.3), and evidence has been presented that they can mediate cytotoxic functions in the vessel wall as well. Notably, defective efferocytosis in advanced atherosclerosis is regarded as a significant promoter of atherosclerotic plaques progression [121]. Importantly, there is another mechanism of atherosclerosis progression: macrophages engulf the deposited normal and modified lipoproteins, transforming them into cholesterol-laden foam cells. Foam cells, known as characteristic cells involved in atherosclerosis, persist in plaques and promote disease progression through several mechanisms including contribution to plaque instability [127].

9.4.4.2 Vascular Dendritic Cells

Vascular DCs are present within healthy but also “atheroprone” regions of the vasculature, such as bifurcations and curvatures, and accumulate in large numbers in atherosclerotic plaques [128]. Like vascular macrophages, vascular DCs are known to play an intricate role in the potentiation and control of vascular inflammation and atherosclerosis. Sessile resident vascular DCs have been observed in the intima of atherosclerosis-prone vascular regions exposed to disturbed blood flow patterns. In experiments on mice, several phenotypically and functionally distinct vascular DC subsets including cDCs and pDCs have been described [128]. Similar to mice, in humans, DCs can be detected in the arterial intima of healthy young individuals, and increased numbers of DCs have been observed in atherosclerotic lesions [129]. Nevertheless, a detailed analysis of the different DC subsets awaits further investigations. Human vascular DCs are mainly found in the plaque shoulder and rupture-prone regions as well as in marginal parts of the plaque core. The majority of DCs

in advanced plaques appears to be activated as revealed by the expression of costimulatory molecules which allow them to stimulate autoreactive T cells [129]. In fact, the contribution of vascular DCs in propagation and progression of atherosclerosis via promotion of autoimmune processes should not be underestimated.

9.4.5 Résumé

For physicians and clinicians, vascular cells of the innate immune system are of high interest owing to the recent exciting recognition that they are critically involved in atherogenesis. Ironically, when the vascular insults turn out to be too severe or occur chronic-repetitively and persistently, this primarily beneficial protective function of vascular cells shifts to the contrary, namely, chronic inflammation associated with overshooting repair mechanisms (VSMC proliferation!), which drive life-threatening atherosclerotic processes. In fact, atherosclerosis as a chronic disease is characterized by two fundamental hallmarks: innate immune-mediated inflammation and lipid retention. Accumulating evidence suggests that ECs, VSMCs, adventitial cells, and—perhaps most importantly—vascular DCs and macrophages are predominantly involved in atherogenesis. Pattern recognition molecules such as TLRs, NLRs, and RLHs have been identified as an essential link between the immune system and cardiovascular disease development. In fact, there is rapidly growing evidence indicating that DAMPs ↔ PRMs interactions contribute to the development and progression of atherosclerosis and its concomitant diseases. Although much has been discovered about TLR activation in cellular components of the cardiovascular system, the roles that individual members of the TLR, NLR, and RLH family have in the pathophysiology of cardiovascular diseases and associated clinical practice remain undefined. The scenario will be described in more detail in Volume 2.

9.5 Chondrocytes, Osteoblasts, and Osteoclasts

9.5.1 Introductory Remarks

Osteoimmunology is a new area of research focusing on the associations between the innate immune and cartilage/bone systems, the aim being to uncover injury-promoted inflammatory and regenerative processes specific for these systems, known to be prone to bone disorders.

Chondrocytes are the resident cell of the cartilage ECM and are regarded as the primary targets of cartilage injury. In particular, articular cartilage is exposed to heavy compression forces associated with considerable damage. However, due to the lack of access to blood supply, lack of lymphatic vessels, and lack of nerves, hyaline articular cartilage has a limited capacity for self-heal and full repair of defects and, thus, may be the origin of a developing osteoarthritis. This all the more so as cartilage cannot readily recruit circulating mesenchymal stem cells but is

dependent on the repair capacity of the sessile mature chondrocytes and chondrocyte progenitors [130–133].

Apart from chondrocytes, osteoblasts and osteoclasts represent other vital cells of the skeletal system. In fact, bone remodelling during development and bone integrity throughout life are typically regulated by a balance between bone formation, performed by osteoblasts, and bone resorption, performed by osteoclasts. Pathological bone loss occurs when this homeostatic relationship is disturbed. Thus, under normal physiological conditions, osteoblasts are responsible for the formation of new bone in the developing skeleton and during the process of bone remodelling. On the other hand, overactivation of osteoclasts is directly accountable for the resorptive bone loss evident, for example, in osteoporosis.

9.5.2 Chondrocytes

Chondrocytes are specialized cells located in healthy cartilage connective tissue. They are characterized by production and maintenance of the cartilaginous matrix, which consists to a large extent of collagen and proteoglycans. *In vivo*, it has to resist to very high compressive loads, and that is explicable in terms of the physicochemical properties of cartilage-specific macromolecules and with the movement of water and ions within the matrix [133, 134]. Chronic mechanical injury or excessive levels of ROS may damage chondrocytes or even result in their death associated with the release of DAMPs [135, 136]. So it is not surprising that these cells are also equipped with various PRMs including TLRs that can sense DAMPs to initiate inflammatory responses [136, 137]. In fact, mature chondrocytes express TLR1 and TLR2 and may react to cartilage matrix/chondrocyte-derived DAMPs. This interaction may lead to secretion of pro-inflammatory cytokines which stimulate further TLRs and expression of inducible DAMPs such as cytokines, thereby establishing a vicious circle. Apart from a role of TLRs, there is first evidence suggesting that the recognition receptor RAGE when stimulated by the DAMP S100A4 (for S100 proteins, also compare Part IV, Sects. 12.2.4.2 and 14.2.2.4) can activate human articular chondrocytes, thereby promoting joint inflammation [138, 139].

This new recognition of involvement of innate immune responses has led to the notion that osteoarthritis reflects a sterile autoinflammatory disease and should not be regarded any more like a mechanical wear-and-tear disorder. Apparently, this modern DAMP-based concept provides strong evidence suggesting that the chondrocyte itself is the earliest and most important inflammatory cell in the onset and long-term course of osteoarthritis [132].

9.5.3 Osteoblasts

The proliferation and migration of osteoblasts are essential for both skeletal development and bone fracture healing, whereby osteoblasts differentiate to osteocytes in bone or to bone-lining cells on bone surfaces. In this way, old bone areas are

regenerated as new bone [140]. Osteoblasts are derived from multipotential mesenchymal progenitor cells that also differentiate into marrow stromal cells and adipocytes [141]. However, the regulatory signals that drive the progenitor cells to an osteoblast fate have not been fully elucidated [142].

Although the data on the function of osteoblasts derived from innate immune research is still sparse, a limited notation can already be made. Thus, osteoblasts mediate their bone-repairing and bone-regenerating functions through PRMs including TLRs; also, a role of DAMPs (e.g., HMGB1) has already been shown to drive osteoblast migration and proliferation [143, 144]. In this context, it is also worthwhile to mention that human osteoblasts have been shown to possess the NLRP3 inflammasome that, for example, is capable of promoting autophagy of the DAMP urate crystals phagocytized by these cells [145]. Thus, further, more targeted studies on the role of the NLRP3 inflammasome in osteoblasts can be expected.

9.5.4 Osteoclasts

Osteoclasts which form as multinucleated cells following fusion between mononuclear precursor cells are unique in their capacity to efficiently resorb bone. As mentioned above, as vital bone-resorbing cells, they play a pivotal role in skeletal development and adult bone remodelling. They also participate in the pathogenesis of various human bone diseases. Osteoclasts differentiate from cells of the monocyte/macrophage lineage upon stimulation of two essential factors, the M-CSF and *receptor activation of NF- κ B ligand* (RANKL) [146]. The close relationship of osteoclast function to responses of the innate immune system has already been recognized. Thus, there is growing evidence suggesting that DAMP-induced activation of PRM-bearing cells of the innate immune system may be involved in osteoclast formation [147, 148]. In particular, DAMP-triggered activation of TLR5 has been shown to activate RANKL and osteoclast formation and therefore represents a potential key factor in inflammation-induced bone erosions in diseases like RA, reactive arthritis, and periodontitis [149]. These data are of interest regarding the observation that TLR5 can be activated by endogenous ligands produced in inflamed RA synovium and, thus, may reflect a first clue that TLR5 may play a crucial role in inflammatory bone loss seen in a wide range of autoimmune and infectious diseases [149, 150].

Furthermore, as discussed elsewhere [151], the recognition receptor RAGE and its ligands appear to be also implicated in RANKL-induced osteoclast activation and bone remodelling. Further supporting this view is that blockade of cellular RAGE using the soluble RAGE diminished alveolar bone loss in a murine diabetic periodontal disease model, and that elevated level of auto-antibodies for RAGE is correlated with less erosive course of RA [151]. As one of the cognate “osteoclastogenic” DAMPs sensed by RAGE, HMGB1 has to be taken into account, as HMGB1 function-blocking antibody was shown to inhibit RANKL-induced in vitro and in vivo osteoclastogenesis [152].

9.5.5 Résumé

Taken together, there is growing evidence in support of the notion that DAMPs—via recognition by PRM-bearing chondrocytes, osteoblasts, and osteoclasts—control and strictly regulate cartilage and bone integrity and remodelling, aimed at maintaining and restoring homeostasis of these parts of the skeletal system. However, as stressed many times when sketching the “Résumé” of a section in this monograph, there is also a dark side of those innate immune responses: when uncontrolled, they may lead to cartilage/bone-destructive pathologies such as RA, osteoarthritis, and osteoporosis. Understanding DAMP ↔ PRM-triggered signalling in bone remodelling may provide essential insights into those cartilage and bone pathologies. Further studies will be required to assess these issues.

9.6 Adipocytes

Adipose tissue has long been regarded as a mostly resting tissue dedicated solely to energy storage and release. However, in recent years, this view has dramatically changed following insight into the metabolic and immunological functions of pre-adipocytes and adipocytes. Today, it is well-established that adipose tissue is an active metabolic tissue that is composed of many functionally and developmentally distinct cell types, including the “stromal vascular fraction”—a heterogeneous mixture of mesenchymal, endothelial, and hematopoietic cell types, however, the metabolic core being the adipocyte. In obese individuals, adipose tissue consists of ~50% of total body mass and, thus, represents a significant compartment of the innate immune system capable of influencing systemic inflammation. Of note, the continuous extensive expansion of adipose tissue during obesity steadily increases its ability to act as an immunological tissue that can switch from controlled to uncontrolled systemic inflammation associated with considerable pathologies [153–155].

Modern classification of adipocytes encompasses three primary types—white, brown, and beige—with distinct origins, anatomic distributions, and homeostatic functions. White adipose tissue has long been recognized as the main energy reservoir derived from food intake. White adipose tissue, composed of adipocytes that are held together by a poorly vascularized and innervated connective tissue, stores dietary energy in a highly concentrated form as triglyceride, mostly in a single large lipid droplet. In periods of caloric need, these triglycerides can be rapidly hydrolyzed by lipases (a process known as lipolysis), and the resulting free fatty acids are then released and transported to other tissues to be oxidized in mitochondria as an energy source. However, the WAT is not only a site of an energy reservoir but also acts as an active metabolic organ that can secrete specific bioactive molecules that have endocrine, paracrine, and autocrine actions. Some of these secreted molecules function as a variety of adipokines as well as pro- and anti-inflammatory proteins (e.g., TNF, IL-1 β , and adiponectin and IL-10, respectively) [155–158].

By contrast, brown adipose tissue (BAT) is a critical site of heat production in mammals known as “thermogenesis.” The heat produced by these cells is

mandatory for the survival of small mammals in cold environments and arousal in hibernators. Brown adipocytes in BAT are packed with mitochondria (determining the brownish color) that contain uncoupling protein 1 (UCP1). This protein, when activated, short-circuits the electrochemical gradient that drives ATP synthesis, thereby stimulating respiratory chain activity [157, 158].

Clusters of UCP1-expressing adipocytes with thermogenic capacity also develop in WAT in response to various stimuli. These adipocytes have been denoted beige, “brite,” or white adipose BAT. Similar to adipocytes in BAT, beige cells in murine WAT are defined by their multilocular lipid droplet morphology, high mitochondrial content, and the expression of a core set of brown [157].

Of note, pre-adipocytes and adipocytes express a broad spectrum of functional TLRs, and the former can convert into macrophage-like cells. Studies have reported the expression of TLR2 and TLR4 in subsets of adipocytes, and LPS has been shown to signal through TLR4 (although not increasing its expression) and induce IL-6 and TNF secretion [165]. Also, it could be demonstrated that TLR1, TLR2, and TLR4 protein co-localized with adiponectin in human adipocytes, with TLR4 exhibiting the highest immunohistochemical expression [159–161]. It is also worthwhile to briefly mention here that the dual activation of TLR4 in adipocytes by LPS and fatty acids represents a molecular gate that connects innate immunity with metabolism [153]. Potential DAMPs in adipose tissue released by dying adipocytes or individual lipids are thus present in increased concentrations in obesity [162]. It is therefore of high interest to identify what PRRs mediate the link between obesity and adipose tissue inflammation. In sum, accumulating evidence indicates that obesity is closely associated with an increased risk of metabolic diseases such as insulin resistance, type 2 diabetes (T2D), dyslipidemia, and non-alcoholic fatty liver disease.

9.7 Outlook

As our knowledge about the function of PRM-bearing cells of the innate immune system continues to grow, it becomes increasingly more apparent that we have to redefine what we mean by immune defense. Previously, the term “immune cells” was restricted to mobile and versatile cells such as DCs, macrophages, granulocytes, and lymphocytes; however, presently, we are learning about an entire family of cells with different functions. We may distinguish cells of the innate and adaptive immune system, mobile circulating cells, sessile cells, resident tissue cells, and so on. Ultimately, a whole organism—a body—is designed as a “defense machinery” with different body parts and tissues having distinct functions and exerting different mechanisms of defense. In this context, Matzinger [163] concludes: “In their own defense, tissues send a panoply of signals that initiate immunity and guide the choice of effector class. Th1-Th2 and Treg is far too simple a representation of the breathtaking variety of the resulting responses.” Moreover, all those innate immune cells are committed to rapidly recognizing both infective and sterile inciting insults, aimed at mediating essential mechanisms of elimination of the inciting agent (e.g.,

pathogens) as well as—if necessary—facilitating and preparing subsequent acquired/adaptive immune responses. As also discussed elsewhere [164], numerous intricate interactions among the various innate cells take place that finally guarantee protective immunity. Thus, rather than acting as isolated sensory and effector cells, innate immune cells are in constant communication and collaboration with each other, locally and distally. These interactions are critically important for the efficient control of both infectious and sterile injuries.

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