Damage Associated Molecular Patterns from the extracellular matrix in Chronic Obstructive Pulmonary Disease

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Abstract

Chronic Obstructive Pulmonary Disease (COPD) is a lung disease characterized by obstruction of the airways as a result of chronic inflammation often caused by noxious particles or gasses such as cigarette smoke. This chronic inflammatory milieu leads to a cascade of changes within lung tissue, such as extra cellular matrix (ECM) remodeling. Damage associated molecular patterns (DAMPs) have been of particular interest for the pathogenesis and pathology of COPD and have been found to play a significant role in inflammatory processes induced by noxious stimuli in epithelial cells. DAMPs released from necrotic epithelial cells lead to immune activation and leukocyte infiltration, especially innate immune cells such as neutrophils and macrophages, in lung parenchyma. In this review we focus on ECM proteins that act as DAMPs when released from the degraded ECM and how they are involved in the pathophysiology of COPD.

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1. Introduction

As of 2016 chronic obstructive pulmonary disease (COPD) has become the third most common cause of death worldwide. The main characteristics of this disease are airway obstruction and a loss of lung functionality. It is associated with chronic inflammation of the peripheral airways and lung parenchyma and can result in tissue destruction. A major risk factor for the development of COPD is the inhalation of irritants such as cigarette smoke, coalmining dust and other noxious particles or gasses. [1,2] Chronic inflammation is caused by continuous infiltration of activated innate immune cells such as neutrophils, marcophages, natural killer cells and mature dendritic cells and activation of the adaptive immune system.[3,4] COPD patients have an increased infiltration of B cells, CD8+ and CD4+ T-cell and a decrease in regulatory T-cells in lung tissue. [4–6]

So far, little is known about the underlying mechanisms for the initiation of innate and adaptive immune responses in COPD. Pouwels et al. suggested a key role for Polly Matzinger's 'Danger Theory' in the development of chronic lung inflammation.[1] Their working hypothesis was that immune activation in COPD is caused by increased levels of damage associated molecular patterns (DAMPs) as a consequence of immunogenic cell death, which can activate the immune system via Pattern Recognition Receptors (PRRs).[1] Excessive DAMP release may lead to downstream maturation of dendritic cells and infiltration of macrophages and subsequent adaptive immune activation leading to auto inflammatory disease if tolerance fails.[4]

DAMPs are mainly released via primary or secondary necrosis or necroptosis in lung epithelium.[7] Epithelial cells, which are the barrier between the environment and lung tissue, are directly exposed to environmental factors such as cigarette smoke and other toxic particles. This may result in apoptosis, necrosis or necroptosis in these cells. Research has shown that cigarette smoke induced epithelial cell damage causes a shift from apoptotic to necrotic cell death, resulting in increased DAMP and TGF-β release in these tissues.[8–10] The secretion of DAMPs such as heat-shock proteins (HSPs), S100 proteins, LL-37, galectin-3 (Gal-3) and high-mobility group box 1 (HMGB1) into the extracellular fluids can result in an increased immune response via activation of PRRs on immune cells, endothelial and epithelial cells.[3] An increase of these DAMPs has been shown in asthma and COPD patient sputum when compared to 'healthy smokers', suggesting a role for DAMPs in the pathology of these diseases.[3,9,11] These DAMPs elicit an immune response by binding specific PRR such as Toll-Like Receptors (TLRs), NOD-Like receptors (NLRs) and the Receptor for Advanced Glycation End products (RAGE), resulting in downstream immune activation via e.g. nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling. This results in increased chemokine (e.g. IL-6, IL-8 and TNF-α) cytokine production and secretion and leukocyte recruitment to the site of damage.[1,12–14] Some DAMPs can induce maturation of dendritic cells which can elicit an adaptive immune response when immunosuppressive functions are inadequate and (auto)-antigens are presented.[15]

However, not all DAMPs are derived from intracellular peptides. In the past decade there has been a growing interest in the extracellular matrix (ECM) and its interactions with cells in lung tissues. Research has shown that the ECM does not only provide structural compartmentalization, rigidity and elasticity to lung tissues, but may also play an important role in cell differentiation, signaling

and immune responses.[16] Furthermore, fragments of the key components such as proteoglycans (PGs), versican, fibronectin and hyaluronan may act as DAMPs under stress conditions (Figure 1.). In this review we discuss the role of the ECM peptides in damage signaling associated with lung inflammation diseases such as COPD.

2. DAMPs in the extracellular matrix

With the developments in the field of matrices that have been made in the past decade, peptides in the ECM have been found to play a bigger role in matrix-cell interactions than previously thought. The pulmonary ECM consists of two main types of structures: 1) the basement membrane, a layer of non-fibrillar collagen and PGs that is connected to the epithelial or endothelial layer in the lungs and 2) the interstitial matrix, which is an acellular network of predominantly fibrous proteins (such as collagen and elastin), fibronectin and PGs that forms the parenchyma.[17–19] The components of the ECM, which provide lung tissue with their rigidity and elasticity, are produced by fibroblasts, epithelial cells and smooth muscle cells[20] and the negative charges of some of these components (e.g. PGs) allow them to bind environmental molecules such as growth factors and chemokines, thereby influencing morphological organization and physiological function. [17,19–21] The ECM and its environment undergoes continuous remodeling via synthesis and degradation of components and post-translational modification. This is regulated by enzymes, growth factors and inhibitors such as CD147, neutrophil elastase, transforming growth factor beta (TGF-β), matrix metalloproteinases (MMPs), A disintegrin and metalloproteases (ADAMs), a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) and tissue inhibitors of metalloproteinases.[19,22,23] The balance between ECM-cleaving MMPs and their inhibitors is crucial for the homeostasis of the ECM.[24]

One of the key characteristics of COPD is the chronic inflammation of lung tissue, which is known to lead to extensive ECM remodeling. This is associated with increased activity of proteolytic enzymes such as MMP-2, MMP-9, MMP-12 and neutrophil elastase, which are upregulated due to oxidative stress and inflammation. [2,17] Furthermore, extensive degradation of the lung ECM is known as emphysema which is a common phenotype in COPD. The degradation of alveolar tissue in emphysema may result in further DAMP release and subsequent inflammation causing a seemingly vicious circle.[25] This is due to the fact that matrix degradation by proteases such as MMP leads to the release of ECM peptide fragments that are normally confined within the ECM, which may act as DAMPs by ligating PRRs**.** As shown in Table 1, a plethora of matrix proteins have been described as potential DAMPs. Although findings support that proteins such as Aggrecan and Tenascin-C[26] or their derivates are DAMPs, to our knowledge they have not been found to be dysregulated or otherwise involved in COPD and will therefore not be discussed herein. However, some ECM peptide fragments, such as biglycan, decorin, versican, fibronectin, heparan sulfate and hyaluronan, have been implicated in the pathogenesis of COPD by acting as DAMPs through inducing inflammation. These will be discussed in more detail in the next paragraphs.

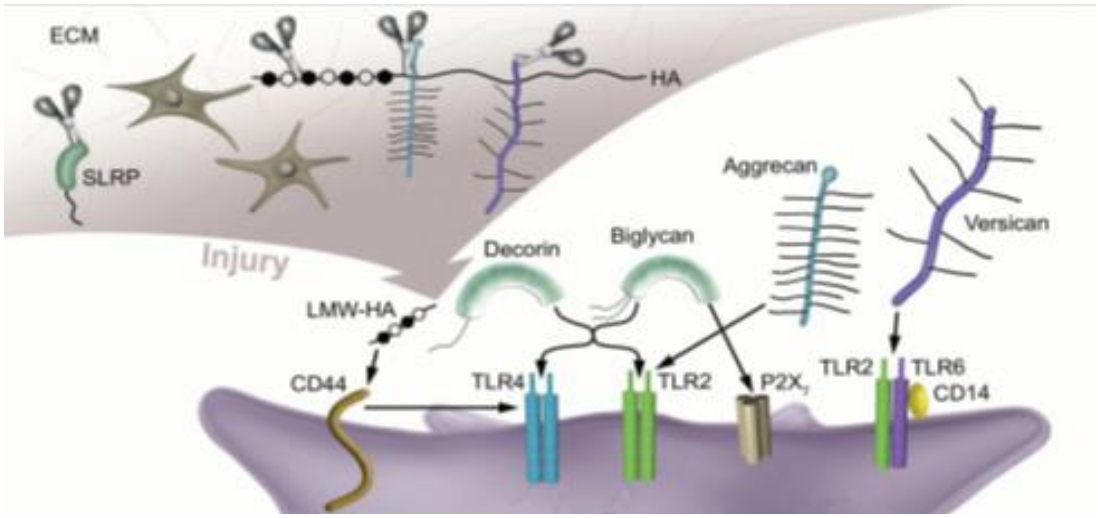

Figure 1. Fragments released from the ECM via proteolysis of the ECM components after injury induced increase in MMPs and other proteolytic enzymes. These fragments are DAMPs that can induce downstream inflammatory response via signaling of e.g. TLRs, CD44 or CD14. Abbreviations: ECM, extracellular matrix; SLRP, small leucine rich proteoglycans; HA, hyaluronan; LMW-HA, low molecular weight hyaluronan; CD, cluster of differentiation; TLR, toll like receptor; DAMPs, damage associated molecular patterns.

(Adapted from Frevert at al., 2018) [13]

Table 1. DAMPs of the extracellular matrix

Extracellular DAMPs	Release Mechanism	Target receptors	References
Aggrecan	MMP cleavage, ADAMs	TLR2	27
Biglycan	MMP cleavage, de novo synthesis	TLR2, TLR4, CD14	28–31
Decorin	MMP cleavage, de novo synthesis	TLR2, TLR4	13,32,33
lmw-HA	HYAL cleavage, ROS	TLR2, TLR4, CD44	31,34–37
Versican	Secretion, Proteolysis	TLR2, TLR6, CD14, CD44	38–40
HS	Heparanase	TLR2, TLR4, RAGE	41–43
Tenascin C	De Novo synthesis	TLR4	44
Fibrinogen	Extravasation	TLR4	13,45,46
Fibronectin	MMP cleavage, alternative splicing, unfolding	TLR2, TLR4	47–49

Abbreviations: MMP, matrix metalloprotease; TLR, toll like receptor; CD, cluster of differentiation; lmw-HA, low molecular weight hyaluronan; HYAL, hyaluronidase; ROS, reactive oxygen species; HS, heparan sulfate.

(Adapted from L. Schaefer, 2014)[31]

Versican

Versican is a large proteoglycan with a chondroitin sulfate region that is expressed by fibroblasts and plays an important role in cell-matrix interactions. It can interact directly with hyaluronan and has several isoforms with distinct biological functions, depending on the alternative splicing of the α- or β-glycosaminoglycan (GAG) regions[38,39,50]. Versican is involved in cell adhesion, cell proliferation and migration.[40] Its binding of hyaluronan in the ECM creates a aqueous environment to which leukocytes can bind.[51] In lung disease such as Asthma it has been shown to be involved in remodeling of the ECM, and aggregation of versican can be observed in the large airways.[51,52] Once detached from the ECM via proteolysis versican can induce inflammation, either via the direct interaction of versican with TLR2, TLR6, CD44, CD14 or other PRRs, or via proteolytic degradation of versican by MMPs (1,2,3,7 and 9) or ADAMTS at the β-GAG domain, creating a 70kDa versikine fragment which acts as a DAMP, also activating immune cells such as macrophages.[13,38–40] Besides its role in asthma, versican has been implicated as a driver of several inflammatory lung diseases such as COPD where significantly elevated levels of versican can be observed in lung tissue and sputum.[3] Studies have shown an increased versican production by fibroblasts in the distal airway, where versican production exceeded versican breakdown.[53] Furthermore, lung tissue of versican knockout mice showed significantly lower inflammatory response in response to a viral mimetic.[39,51] Although it is known that versican can act as a DAMP and is suggested to play a key role in inflammation in COPD, knowledge regarding mechanisms involved in versican and versikine release, PRR binding and signaling is still limited.

Small leucine-rich proteoglycans

Biglycan and decorin are examples of small leucine-rich proteoglycans (SLRPs) included in the ECM. In normal conditions these SLRPs are bound to the ECM, but under stress or injury they are released from the ECM via bone morphogenetic protein-1, MMP-2, MMP-3, MMP-13, and granzyme B mediated proteolysis.[29,31] When released from the ECM, Biglycan and Decorin act as DAMPs that induce sterile or pathogen induced inflammation via TLR2 on dendritic cells or TLR4 and CD14 on macrophages, albeit via different downstream pathways.[14,28–31] Activated macrophages can also produce biglycan, thereby increasing the inflammation signaling and inflammatory environment.[29] Biglycan was also shown to have an

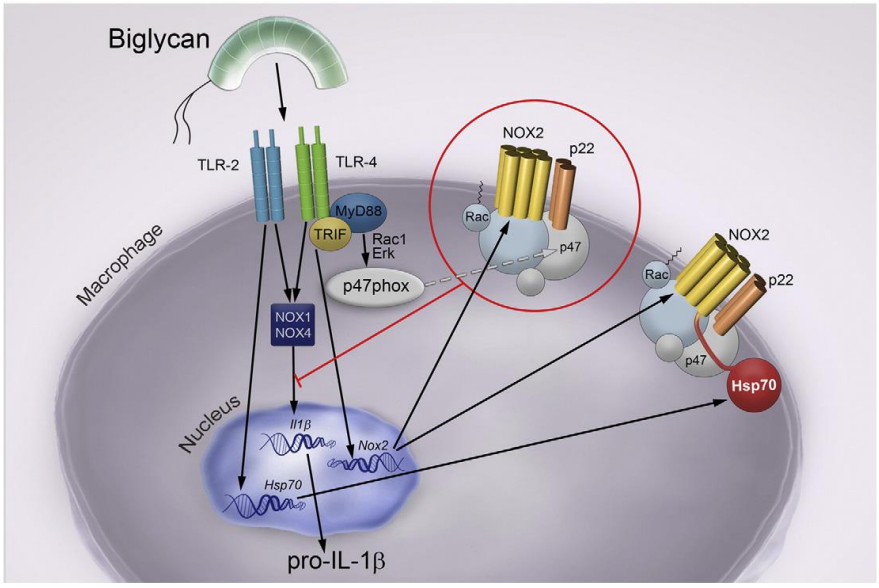

Figure 2. Soluble Biglycan induces pro inflammatory responses via interaction with TLR2 and TLR4. Signaling through NOX1/4 induce IL-1β production in macrophages. However, *NOX2* mRNA expression induced by TLR4 can prevent IL-1β maturation via NOX2 mediated inhibition of NOX1/4 signaling. Increased Hsp70levels, which can be a result of TLR2 signaling, can inhibit NOX2 leading to increased inflammatory signaling. Abbreviations: TLR, toll like receptor; IL, interleukin; NOX, NADPH oxidase; Hsp, Heat shock protein;

(Hsieh et al., 2016)[54]

anti-inflammatory function via a NOX-2 mediated downregulation of IL-1β. NOX-2, however, is inhibited via (biglycan induced production of) HSP70 (Figure 2).[54] This underlines the interplay of several DAMPs with inflammatory and anti-inflammatory pathways. Excess levels of DAMPs in the extracellular space tilt the scale toward inflammation signaling, resulting in a cascade of inflammatory responses. Although both decorin and biglycan were found to be significantly upregulated in asthma patients, in COPD SLRPs were found to be lower in peribronchiolar tissues compared to healthy controls.[55,56] This may be due to an increase of MMP activity, creating a larger fraction of soluble SLRPs in the extracellular fluids. To our knowledge, the levels of SLRPs in the extracellular fluids of COPD lung tissue has not been measured to date, as one explanation for decreased tissue levels of SLRP would be a larger soluble fraction of SLRP fragments that act as DAMPs.

Hyaluronan

Hyaluronan (HA) is a non-sulfated GAG, with extremely hydrophilic properties, that is a key component of the ECM. Its presence in the ECM alongside versican allows leukocyte binding and differentiation. HA is the only GAG that is produced on the inner surfaces of the plasma membrane and synthesis is mediated by HA synthase (HAS) 1-3. HASs produce distinct subtypes of HA and *HAS* knock-out combinations give a wide range of phenotypical changes in mice such as lethality in *HAS2* knockout or decreased cell migration in *HAS3* knockout.[57] Hyaluronidases (HYALs) are the main enzymes responsible for HA proteolysis, causing detachment from the ECM and fragmentation of HA. These HA fragments have different bioactive functions and are divided in two categories based on their size and function; high molecular weight HA (hmw-HA)which is >1000 kDa, and low molecular weight or short fragment HA (lmw-HA) which is <~500-700 kDa.[58,59] Hmw-HA has anti-inflammatory and immunosuppressive properties and is involved in cell survival.[59,60] On the other hand, lmw-HA has pro-inflammatory properties and is found to be upregulated in both bronchoalveolar lavage fluid and parenchyma of COPD patients and study shows that circulating HA levels are directly associated with COPD disease outcome.[59,60] Both HYAL and lmw-HA concentrations were found to be significantly higher in BAL of patients with acute exacerbations of COPD compared to healthy subjects and are linked directly to quality of life and survival rates in COPD patients.[60,61]

An increase in lmw-HA may be achieved via upregulation of HAS3 lmw-HA production although this has not been conclusively proven to produce more lmw-HA than HAS1-2.[62] However, HAS3 upregulation has been associated with chronic inflammation and tumorigenesis, suggesting a role in chemokine and cytokine expression, likely mediated by the production of lmw-HA. Furthermore, lmw-Ha can also be formed via HYALs and/or reactive oxygen species (ROS), which degrade hmw-HA into smaller lmw-HA or HA oligosaccharides. The inflammatory effect of lmw-HA and HA oligosaccharides are a result of ligation of hyaluronan to hyaluronan receptor for endocytosis, lymphatic vessel endothelial hyaluronan receptor-1, layilin, TLR2, TLR4, RHAMM and CD44[37], resulting in proinflammatory signaling, cell migration and survival, leukocyte recruitment and cyto- and chemokine release by macrophages.[35,36] This balance between hmw-HA and lmw-HA is important for homeostasis in lungs and research has shown that cigarette smoke may directly tilt the scales towards proinflammatory responses via the vast amount of ROS and upregulation of HYALs and HASs.[34,63] However, a recent clinical study on the effect of HA on COPD found no

evidence for lmw-HA induced inflammation.[64] Furthermore, binding of hmw-HA to RHAMM or CD44 induces an anti-inflammatory response and promotes cell survival.[37,65] Although this clinical study where HA was used to treat COPD showed a decrease in elastin degradation[64], the mechanisms regarding HA in relation to COPD are still not fully understood. The ambiguous role of HA depending on size and localization emphasizes the need for further research into the role of ECM-HA in the pathology of COPD.

Fibronectin

Fibronectin (Fn) is a high MW glycoprotein that is produced by fibroblasts and provides the ECM with its tensile strength.[66] Fn is a dimer of two almost identical subunits and has many alternative splice variants, all encoded by the same gene, with different functions in plasma and ECM. The Fn molecule consists of three domains representing Fn subunits, labelled Type I-III, of which Type I and II are structurally stable. Fn found in the ECM has 17 Type III repeats as opposed to the 15 Type III repeats of plasma Fn. The Type III subunits are not stabilized by disulfide bonds and are therefore susceptible to chemical or mechanical unfolding or other conformational changes.[47,48] Research has found that the insoluble Fn found in the ECM known as Fn extra domain A (EDA), which is increased under inflammatory conditions, can activate TLR2 on fibroblasts and TLR4 on macrophages via its first Type III domain (III-1).[47–49] FnIII-1c, the partially unfolded fragment of FnEDA found to be responsible for immune activation via these TLRs is generated via cellular contractile force or MMPs, which play a role in proteolysis of a plethora of DAMPs.[47–49] Fn fragments have therefore been implicated as an important DAMP for the pathophysiology of COPD, which is known for its constant ECM remodeling in an inflammatory environment with increased MMP levels.[2]

Krimmer et al. showed increased Fn deposition in COPD patient tissue compared to healthy smokers as a result of cigarette or biomass smoke exposure.[67,68] This was supported by studies showing upregulation of fibronectin production *in vitro* and *in vivo* by fibroblasts stimulated with nicotine.[26,69] However, A study where the effect of cigarette smoke on extracellular fibronectin levels in healthy subjects were measured showed a significant decrease in soluble Fn. This may be explained by the fact that this study focused on extracellular soluble Fn and measured the effect of cigarette smoke without COPD.[70] It is therefore possible that increased levels of Fn as a result of cigarette smoke is caused by COPD or that the decrease in soluble Fn is due to Fn deposition.

Heparan Sulfate

Heparan and its sulfated isoform Heparan Sulfate (HS) are GAGs that bind ECM Fn and are thought to directly affect matrix assembly via promotion or inhibition of Fn matrix formation depending on extracellular HS levels.[71] HS can be incorporated in Heparan Sulfate Proteoglycans (HSPGs) Syndecans and Glypicans on the cell surface and Perlecan in the ECM.[72] Furthermore, HS binds a wide range of proteins (e.g. growth factors and chemokines), thereby regulating signaling within the ECM.[42,73] HSPGs can activate CD14 and TLR4 in the presence of LPS. Furthermore, when cleaved by heparanase, HS fragments can regulate inflammatory processes by binding TLR1,TLR2, TLR4 and TLR6, implicating HS fragments as important DAMPs.[13,42,43] HS is also essential for the RAGE receptor signal transduction and DAMP ligation to RAGE.[41] Research into pathology of COPD found that heparanase is upregulated in lung tissue of COPD patients. An increase in heparanase activity decreases ECM stability and allows for more cell infiltration.[73,74] This ECM breakdown leads to the

release of ECM bound growth factors and the created HS fragments induce pro-inflammatory responses via TLR4 and possibly TLR2. [42,74] Although heparanase was shown to be an important mediator of neutrophil recruitment, findings in knockout models suggest a compensatory increase in MMPs although this has not been consistently shown.[73] Papakonstantinou et al. corroborate this proposed mechanism by showing a significant increase in MMP-2, MMP-9 and MMP-12 in acute exacerbations of COPD together with increased HS and other GAGs in BAL fluid.[75]

Mechanisms of release, receptor binding and downstream pathways

In COPD patients an increase in DAMP release can be seen in comparison to healthy smokers which may be related to a predisposition to release DAMPs and subsequent development of COPD.[9] The release of DAMPs such as HMGB1, S100s, HSPs, SLRPs, HA, HS, versican, fibronectin and galectins in response to tissue damage leads to a cascade of proinflammatory stimuli.[3,8,76,77] This results in increased extracellular levels of proteinases such as ADAMs and MMPs released by surrounding cells such as macrophages, which were shown to have a 3-fold increase of MMP release via extracellular vesicles after cigarette smoke stimulation[78,79]. Normally TIMPs, which can for example control MMP activity by interacting with sulfated GAGs[80], and MMPs maintain homeostasis within the ECM, but DAMP induced proinflammatory cyto- and chemokine release results in a surge in protease concentrations, leading to matrix degradation which is necessary for leukocyte infiltration.[52] In COPD patients the DAMP mediated response to tissue damage was shown to be significantly higher than in healthy subject, disrupting ECM homeostasis, leading to excessive ECM degradation.[9] Degradation of the ECM by proteases and ROS as a result of noxious stimuli further leads to the release of fragments from proteins such as Fn, versican, lmw-Ha, SLRPs and HS which can act as DAMPs when present in the extracellular space. The DAMPs released from the ECM can ligate several PRRs, most importantly TLR2 and TLR4 on leukocytes (Figure 3).[12] These TLRs are cell surface receptors with extracellular recognition domains that regulate inflammation by activating the MyD88 signaling pathway.[81] This leads to downstream NF-κB and mitogen-activated protein kinase (MAPK) pathway activation, inflammatory cytokine production and release. Futhermore TLR4 can activate these pathways and interferon type-1 (IFN-1) production via a MyD88 independent TRIF pathway via TRAM activation. For more detailed information regarding TLR2 and TLR4 signaling pathways and inhibitors refer to these comprehensive reviews ([81,82]). TLR activation leads downstream to cytokine production, via NF- κB, MAPK and IFN-1, and recruitment of innate and adaptive immune cells to tissues. Furthermore this cytokine release leads to further upregulation of MMP production by macrophages.[23,83] Although this paints a picture of a severe inflammation cascade induced by DAMPs, it is important to note that DAMPs, such as biglycan, may also have immunosuppressive functions by interacting with other receptors. Proteins treated as DAMPs are often only immunostimulating when present in high quantities.

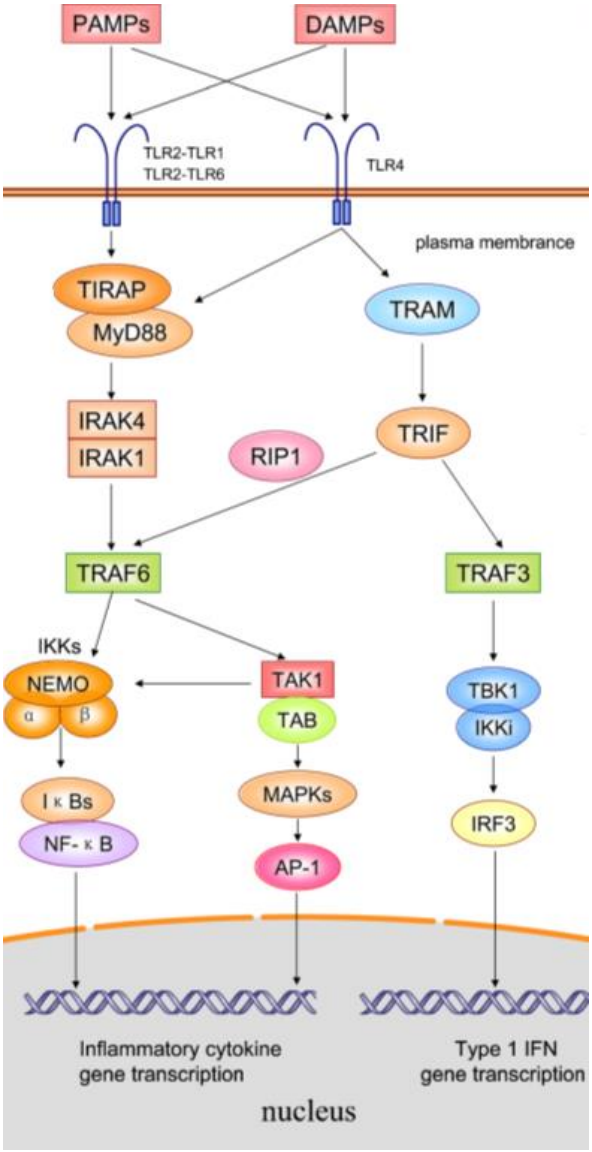

Figure 3. TLR2/4 signaling pathways activated via PAMPs or DAMPs. DAMPS induce TIRAP/myD88 signaling and its downstream kinases IRAK1/4 by binding TLR2-1 and TLR2-6 complexes or TLR4. Furthermore, activation of TLR4 leads to TRAM mediated activation of TRIF which interacts with TRAF 3/6. TRAF 3 activation via IRAK 1/4 and TRIF leads to IKKs complex and TAK1/TAB complex activation. Downstream this leads to NF-κB and MAPK mediated gene transcription for pro inflammatory cytokines. The MyD88 independent pathway results in IFN-1 gene transcription via TBK1/IKKi activation and the resulting translocation of IRF3.

(Liu et al., 2014) [81]

3. Summary and discussion

In this review we discussed various relevant ECM molecules that may act as DAMPs and have been shown to be associated with COPD. The role of DAMPs in COPD has gained substantial traction in the past few decades since the discovery of DAMPs and PRRs. These DAMPs are released as a result of immunogenic cell death and a subsequent cascade of proinflammatory signals.[83–85] In COPD, the amount of DAMPs (e.g. HMGB-1, Galectins and S100s) that are released due to immunogenic cell death in response to damage are significantly higher than in healthy subject.[1,8,9,76,84] Whether this is due to COPD pathology or a genetic variation/other variable has to our knowledge not been elucidated. DAMPs, growth factors and proteases released via (secondary) necrosis and necroptosis of damaged cells either directly or indirectly induce ECM remodeling although the precise pathway involved in damage response to noxious particles is not fully understood. With increased interest in matrix biology, the role of the ECM in disease pathology is slowly being unraveled.[16,66] In COPD, the remodeling of ECM in response to ROS and DAMPs can result in release of extracellular growth factors (e.g. TGF-β) and ECM components such as Fn, versican, HS, lmw-Ha and SLRPs, which were previously bound to the ECM.[13] Here we attempted to summarize how these ECM components induce inflammation via ligation of different PRRs and how soluble levels of these ECM DAMPs relate to COPD. Although studies have found association between these ECM DAMPs and COPD, the underlying mechanisms are not clearly described and to date no large scale study has assessed all DAMPs originating from the ECM in COPD patients.[3,34,55,56,60,61,63,73–75] Furthermore the complexity of the involved pathways regarding pro- and anti-inflammatory signaling and cross-talk between different DAMPs (from ECM or cells) makes it difficult to attribute inflammatory responses to any one DAMP. However, as ECM remodeling appears to play a key role in the chronic lung inflammation seen in COPD patients, preventing extensive matrix degradation may prove to be an effective therapeutic target. For the development of future

interventions, determining the exact interplay of DAMPs and COPD pathophysiology may prove important for finding targetable pathways. In conclusion, multiple studies showed increased levels of ECM related DAMPs in tissues and sputum of COPD patients after cigarette smoke exposure. Targeting these DAMPs or preventing matrix degradation in COPD may help protect patients from developing acute exacerbations or emphysema by protecting parenchyma from degradation.

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