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Hyaluronan fragments as mediators of inflammation in allergic pulmonary disease

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Abstract

Asthma is frequently caused and/or exacerbated by sensitization to allergens, which are ubiquitous in many indoor and outdoor environments. Severe asthma is characterized by airway hyperresponsiveness and bronchial constriction in response to an inhaled allergen, leading to a disease course that is often very difficult to treat with standard asthma therapies. As a result of interactions among inflammatory cells, structural cells, and the intercellular matrix of the allergic lung, patients with sensitization to allergens may experience a greater degree of tissue injury followed by airway wall remodeling and progressive, accumulated pulmonary dysfunction as part of the disease sequela. In addition, turnover of extracellular matrix (ECM) components is a hallmark of tissue injury and repair. This review focuses on the role of the glycosaminoglycan hyaluronan (HA), a component of the ECM, in pulmonary injury and repair with an emphasis on allergic asthma. Both the synthesis and degradation of the ECM are critical contributors to tissue repair and remodeling. Fragmented HA accumulates during tissue injury and functions in ways distinct from the larger native polymer. There is gathering evidence that HA degradation products are active participants in stimulating the expression of inflammatory genes in a variety of immune cells at the injury site. In this review, we will consider recent advances in the understanding of the mechanisms that are associated with HA accumulation and inflammatory cell recruitment in the asthmatic lung.

Keywords

Asthma; extracellular matrix; hyaluronan

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CONFLICTS OF INTEREST

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INTRODUCTION

Allergic airway diseases such as rhinitis and bronchial asthma are complex disorders that are thought to arise as a result of aberrant immune cell responses to non-infectious environmental antigens (Nassenstein et al., 2005; Umetsu and DeKruyff, 2006). Recent statistics (2011) show that asthma afflicts 8.2% of adults and children in the United States, nearly 26 million people (Knutsen et al., 2012). In patients with moderate or severe persistent asthma, there is increased morbidity and significantly increased use of health care support and costs (Knutsen et al., 2012). Epidemiological studies in the U.S. and Europe have associated mold sensitivity, particularly to *Alternaria alternate* and *Cladosporium herbarum*, with the development, persistence, and severity of asthma (O'Driscoll et al., 2005; Knutsen et al., 2012). In addition, sensitivity to *Aspergillus fumigatus* has been associated with severe persistent asthma in adults (O'Driscoll et al., 2005; Chaudhary and Marr, 2011; Knutsen et al., 2012).

Sensitization to fungus is a particular risk factor for severe asthma that is difficult to treat with standard therapies and, as a result, can lead to considerable morbidity that may require multiple hospitalizations (O'Driscoll et al., 2005). The airway hyperresponsiveness (AHR) and peribronchial inflammation that result from the inhalation of conidia narrows the caliber of the airway lumen, limiting air exchange. Fungal spores (conidia) are ubiquitous in the atmosphere, and the persistent perturbation of the lung that results from repeated inhalations of conidia causes chronic remodeling of the allergic airway (Hogaboam et al., 2000; Hernandez et al., 2004; Hoselton et al., 2010; Ghosh et al., 2012b), consisting of peribronchial fibrosis with excessive accumulation of ECM (Murdock et al., 2012; Ghosh et al., 2014b), smooth muscle hypertrophy (Murdock et al., 2012; Shreiner et al., 2012), and goblet cell metaplasia that further restrict airflow (Hogaboam et al., 2000; Hoselton et al., 2010; Ghosh et al., 2012a; Ghosh et al., 2014a). As a consequence of repeated and persistent inflammation due to constant exposure to fungus, lung morphology eventually becomes altered even in mild forms of the disease, causing chronic dysfunction of the lung (Dagenais and Keller, 2009; Murdock et al., 2012; Pandey et al., 2013).

In the lung, the ECM was once considered to be inert scaffolding with a mechanical role in supporting and maintaining tissue structure. However, recent findings indicate that the role of ECM components is much broader than previously thought (Jiang et al., 2007; 2011). It is now known that the ECM has roles in cell attachment (Oharazawa et al., 1999), movement (Noble and Jiang, 2006), activation (McKee et al., 1996), tissue growth and repair (Noble, 2002), proliferation (Paez-Pereda et al., 2005), and differentiation (Lu et al., 2011), and thereby can play a role in mediating inflammation. Clearly, it functions in processes of both health and disease (Noble and Jiang, 2006; Lennon and Singleton, 2011), and understanding the contribution of ECM components to asthma pathogenesis may lead to new therapeutics for patients with asthma.

The ECM comprises a diverse group of proteins and glycoproteins, which can be divided into three groups: i) structural proteins, such as collagen and elastin, ii) specialized adhesion proteins, such as fibronectin, fibrilin, tenascin, and laminin, iii) glycosaminoglycans (GAGs) and proteoglycans (Olczyk et al., 2014). All of these components of the ECM provide

structural integrity and mechanical support for tissues and provides a meshwork for cell adhesion and motility (Derya et al., 2014; Olczyk et al., 2014). Collagen (type I, III, and V on the airway wall and type IV and VIII under the basement membrane) and elastin account for approximately 2/3 of the dry weight of the ECM, while the remainder is made up of glycoproteins (fibronectin, tenascin, laminin) and other matrix components (heparin sulfate, HA) (Liang et al., 2011; Derya et al., 2014). HA is a major component of the ECM that undergoes dynamic regulation under conditions of inflammation.

HA is widely distributed, from lower organisms such as bacteria to complex eukaryotes (Lowther and Rogers, 1955). HA was first isolated from vitreous humor of bovine eyes in 1934 by Meyer and Palmer (Meyer and Palmer, 1934). It is a non-sulfated GAG polymer consisting of repeating disaccharide units of D-glucuronic acid and D-N-acetylglucosamine that is synthesized by a variety of cell types, including stromal cells (Laurent and Fraser, 1992), fibroblasts (Smith and Ghosh, 1986), epithelial cells (Lauer et al., 2008), and smooth muscle cells (Lauer et al., 2009). The total normal systemic kinetics of HA are well established in several species including humans. The average 70 kg (154 lb.) person has roughly 15 grams of HA in the body and the normal turnover of HA is 10–100 mg/day in the adult (Laurent and Fraser, 1992; Stern, 2004). Similarly, the removal of HA from the circulation is very efficient, with a half-life of 2–6 min (Laurent and Fraser, 1992).

The association of increased HA deposition into the ECM after tissue injury and during inflammatory disease has been recognized for over 25 years (Hallgren et al., 1989). In the lung, HA expression increases after ozone damage and in the progression of a number of pulmonary diseases, including chronic obstructive pulmonary disease (COPD) (Sahu and Lynn, 1978; Dentener et al., 2005; Li et al., 2011), idiopathic arterial pulmonary hypertension (Papakonstantinou et al., 2008), acute respiratory distress syndrome (ARDS) (Hallgren et al., 1989), and allergic asthma (Sahu and Lynn, 1978; Cheng et al., 2011; 2013; Ghosh et al., 2014b), suggesting a role in pulmonary disease mediation. The successful repair of tissue injury requires a well-coordinated host response to limit the extent of tissue damage. The physical, mechanical, and cellular innate immune responses are the first line of defense against the insult caused by inhaled organisms and inform the adaptive inflammatory response. The host must also minimize the extent of structural cell damage. The ultimate outcome depends on the balance between the containment of the perceived pathogen (defense) and the cellular and molecular mechanisms that are associated with the breakdown or turnover of ECM components to maintain homeostasis (tissue integrity). This review focuses on the current understanding of molecular mechanisms that are associated with HA synthesis/accumulation/degradation and its role in mediating inflammation in allergic pulmonary disease.

HYALURONAN METABOLISM IN THE LUNG

HA is a normal component of the basement membrane and makes approximately 10% of the proteoglycan content of the lung (Noble and Jiang, 2006; Liang et al., 2011). In the healthy lung, HA exists as a high molecular mass hyaluronan (HMM HA) polymer, in excess of 10^6 Da, found in the peribronchial and perialveolar spaces where it assists in structural integrity, cushioning and cell movement (Noble, 2002; Jiang et al., 2011).. However, during

inflammation, HA undergoes dynamic regulation and can be broken down into low molecular mass hyaluronan (LMM HA) fragments by the activity of hyaluronidases and reactive oxygen species (ROS) (Noble, 2002; Jiang et al., 2010; Monzon et al., 2010; Lennon and Singleton, 2011). HA differentially promote or inhibit lung pathology based on its molecular mass and accessibility to various HA binding proteins (Lennon and Singleton, 2011; Singleton and Lennon, 2011). Several studies have shown that LMM HA exhibits pronounced biological effects on cells and tissues (Noble and Jiang, 2006; Papakonstantinou and Karakiulakis, 2009; Jiang et al., 2011; Maharjan et al., 2011). Most importantly, LMM HA has multiple pro-inflammatory effects not observed for HMM HA (Noble, 2002; Yang et al., 2012). In fact, HMM HA can block the pro-inflammatory effects of LMM HA and help support tissue integrity (Jiang et al., 2011; Yang et al., 2012).

As HA regulates lung pathology, it is important to understand how cells regulate its metabolism. HA is synthesized by three different isozymes (HAS1-3) located in the plasma membrane, whereas HA degradation is mediated by a series of hyaluronidases (HYAL1-3) that produce HA fragments that can modulate inflammation and immune responses (Laurent, 1989; Lennon and Singleton, 2011; Vigetti et al., 2012). Therefore, regulation of HASes, together with HYALs are main control points to determine tissue HA content. Increased amounts of HA and its degradation products are observed in many allergy models of asthma (Cheng et al., 2011; Lennon and Singleton, 2011; Liang et al., 2011; Cheng et al., 2013; Ghosh et al., 2014b; Petrey and de la Motte, 2014a). Further, increased HA levels are observed in bronchoalveolar lavage (BAL) fluid and/or plasma from patients with lung disorders such as asthma (Soderberg et al., 1989; Lennon and Singleton, 2011; Liang et al., 2011; Eszes et al., 2014)

HYALURONAN SYNTHESIS IN THE LUNG

HA is synthesized by membrane-bound synthases on the inner surface of the plasma membrane, and the chains are extruded through pore-like structures into the extracellular space (Lowther and Rogers, 1955; Laurent, 1989; Noble and Jiang, 2006; Liang et al., 2011). This mode of HA synthesis is unusual, as it is made at the inner face of the plasma membrane and not inside the Golgi like other GAGs (Vigetti et al., 2012). The growing HA chain is extended at the reducing rather than the non-reducing terminus and, as the polymer grows, it is extruded into the extracellular space via the membrane spanning domains of the HASes (Toole, 2000; Singleton and Lennon, 2011). There are three mammalian hyaluronan synthases (HAS1, HAS2, and HAS3) that catalyze the same reaction (Laurent and Fraser, 1992; Watanabe and Yamaguchi, 1996; Lee and Spicer, 2000). The genomic location and structure of these HA synthases have been determined. HAS1, HAS2, and HAS3 are located on different chromosomes, suggesting that the HAS gene family may have arisen early in vertebrate evolution by sequential duplication of an ancestral HAS gene (O'Regan et al., 1994; Watanabe and Yamaguchi, 1996; Toole, 2004; Weigel and DeAngelis, 2007). All of the HAS isozymes are highly homologous in their amino acid sequences and have similar hydropathic features, suggesting that they are similarly organized within the membrane. Two types of membrane domains are present: transmembrane domains that span the membrane and membrane-associated domains that do not go all the way through the membrane (O'Regan et al., 1994; DeAngelis, 1999; Weigel and DeAngelis, 2007). There are

6–8 transmembrane domains and two membrane-associated domains. Over 60% of the protein (including the amino and carboxyl termini) is inside the cell (DeAngelis, 2002). Only 5% of the protein is exposed to the outside of the cell (O'Regan et al., 1994; DeAngelis, 1999).

The HAS enzymes use the two activated sugar precursors for HA biosynthesis: Uridine diphospho-glucuronic acid (UDP-GlcA) and Uridine diphospho-N-acetylglucosamine (UDP-GlcNAc) as substrates (Figure 1) (Sugahara et al., 1979; Vigetti et al., 2012). In order to make a disaccharide unit and extend the growing HA chain, hyaluronan synthases utilizes at least six distinct steps (Figure 1). These activities include two different sugar nucleotide binding sites, two different glycosyltransferase activities, one or more binding sites for the growing hyaluronan-UDP chain and the ability to transfer the HA chain, within the enzyme, to set up the next round of saccharide addition. The UDP-sugar substrates are produced and used by the hyaluronan synthase inside the cell, and the HA chain is continuously transferred (translocated) across the membrane so that it is extruded to the cell exterior (Sugahara et al., 1979; Toole, 2000). In the case of bacteria, this HA forms a capsule (Laurent and Fraser, 1992), whereas for many eukaryotic cells, the result is either that HA forms a pericellular coat surrounding the cell or is released into the surrounding ECM (Figure 1).

Of the three HA synthases, the most research has been done on HAS2 (Laurent and Fraser, 1992; Toole, 2004). Targeted deletion of HAS2 has yielded important insights into the function of HA (Lennon and Singleton, 2011), as the deletion of HAS2 causes major abnormalities in heart and blood vessel development (Jiang et al., 2007), which results in an embryonic lethal phenotype (Lennon and Singleton, 2011; Liang et al., 2011). HAS2 null embryos completely lack endocardial cushions, showing a critical role of the HA-ECM interaction in cardiac development (Camenisch et al., 2000; Liang et al., 2011). However, elucidating the role of HAS2 in lung injury/disease has been difficult because of the lethal phenotype in the HAS2 deficient mice. HAS1 and HAS3 have been reported to generate HA with broad size distributions (molecular masses of 2×10^5 to approximately 2×10^6 Da), whereas HAS2 generates HA with broad but extremely large sizes (average molecular mass of $> 2 \times 10^6$ Da) (Krasinski and Tchorzewski, 2007; Liang et al., 2011).

Dysregulation of HA synthases and their activities has been reported during tissue injury, consistent with the findings that HA accumulates during a number of injuries (Adamia et al., 2005; Jiang et al., 2011; Aya and Stern, 2014). Increased accumulation of HA and increased HAS expression have been reported in allergic asthma (Engstrom-Laurent, 1989; Soderberg et al., 1989; Cheng et al., 2011; 2013; Ghosh et al., 2014b). Although HA synthesis and total HA concentration are important in regulating lung function, we must also consider HA degradation products as they may directly or indirectly alter downstream signaling pathways.

HYALURONAN DEGRADATION IN THE LUNG

Enzymatic degradation of HA is initiated by hyaluronidases (also called hyaluronoglucosaminidases or HYALs), a family of endoglycosidases that hydrolyze the

hexosaminidic β (1-4) linkages between N-acetyl-D-glucosamine and D-glucuronic acid residues of HA and release HA fragments (Csoka et al., 2001; Chowdhury et al., 2013). Complete digestion of HA with hyaluronidase releases disaccharide D-glucuronic acid-N-acetyl-D-glucosamine. These enzymes also hydrolyze β (1-4) glycosidic linkages between N-acetyl-galactosamine or N-acetylgalactosamine sulfate and glucuronic acid in chondroitin, chondroitin 4-sulfate, chondroitin 6-sulfate, and dermatan (Lepperdinger et al., 2001; Gushulak et al., 2012). In humans, genes for six hyaluronidase family members have been identified to date: HYAL-1-4, PH-20, and HYALP1 (Lathrop et al., 1990; Csoka et al., 2001; Noble and Jiang, 2006). With the exception of the psuedogene HYALP1, each member encodes for protein products, and all but HYAL-3 have been shown to participate in HA catabolism (Laurent, 1989; Noble, 2002; Lennon and Singleton, 2011).

The hyaluronidase 1 (HYAL1) gene encodes a hyaluronidase found in the major parenchymal organs such as the liver, kidney, spleen, lung, and heart (Csoka et al., 2001; Stern, 2003). HYAL1 is also present in the human serum and urine (Ikegami-Kawai et al., 2004). The enzyme degrades HA within the cell, is active at an acidic pH, and is the major hyaluronidase in plasma (Ikegami-Kawai et al., 2004). HYAL2 is a glycosylphosphatidylinositol-anchored cell-surface receptor in all tissue types except the brain (Noble and Jiang, 2006; Singleton and Lennon, 2011). HYAL2 has very low hyaluronidase activity in comparison to serum hyaluronidase HYAL1 (Lepperdinger et al., 2001). Furthermore, unlike HYAL1, the HYAL2 enzyme hydrolyzes only HA of high molecular mass, yielding intermediate-sized HA fragments of approximately 20 kDa (Lepperdinger et al., 1998; Lepperdinger et al., 2001). HYAL3 transcripts show the strongest expression of this enzyme in testis and bone marrow with relatively weak expression in other organs (Reitinger et al., 2007; Atmuri et al., 2008). The role of HYAL3 in the degradation of HA is not clear, and there are currently no studies that demonstrate HYAL3 activity (Csoka et al., 2001; Noble and Jiang, 2006). PH-20 is a testicular enzyme encoded by the sperm adhesion molecule 1 (SPAM1) gene, which plays a role in fertilization (Lathrop et al., 1990).

Expression and activity of hyaluronidases have long been observed in diseases such as rheumatoid arthritis and periodontal disease (Soltes et al., 2006). Increased accumulation of HA and increased HYAL expression have also been reported in mouse models of allergic asthma (Jiang et al., 2011; Cheng et al., 2013; Ghosh et al., 2014b). However, hyaluronidases are not the only HA-degrading moiety in the lung, and other factors like ROS that accumulate at sites of tissue injury also provide a mechanism for generating HA fragments (Liang et al., 2011; Petrey and de la Motte, 2014b). ROS have been reported to degrade ECM components such as collagen, laminin, and HA (Papakonstantinou and Karakiulakis, 2009; Petrey and de la Motte, 2014b). Accumulation of ROS in chronic inflammatory conditions has been noted, and direct depolymerization of HA by ROS has been illustrated largely *in vitro* (Agren et al., 1997). In an epithelial airway culture system, xanthine/xanthine oxidase generation of ROS led to HA degradation and tissue kallikrein-mediated epidermal growth factor (EGF) receptor activation (Casalino-Matsuda et al., 2004). ROS species are also capable of stimulating HYAL2 gene expression via p39 mitogen-activated protein kinases (p39 MAPK) (Monzon et al., 2010). However, whether

HA fragments generated directly by ROS species are functionally equivalent to enzymatic products is currently not known in allergic pulmonary disease.

THE HYALADHERINS - HYALURONAN BINDING PROTEINS

HA exists in the ECM in soluble form and can covalently bind to a variety of proteins to influence their functions (Jiang et al., 2011). These binding proteins have been grouped together and are called hyaladherins (Day and Prestwich, 2002). The differential activities of HA are regulated in the lung, in part, through interactions with HA binding proteins (hyaladherins) including CD44, receptor for hyaluronan mediated motility (RHAMM), tumor necrosis factor-α-stimulated glycoprotein-6 (TSG-6), lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1), and many other proteins (Lesley et al., 1993; Hall et al., 1994; Milner and Day, 2003; Toole, 2004). The role of these proteins in lung injury is discussed below (**also see** Table 1). Some HA binding proteins are associated with cell membranes, whereas others are found in the ECM. Structurally, the link module and the B(X_7) B motif (where B is arginine and X is any nonacidic amino acid) are thought to constitute the HA-binding region (Toole, 1990; 2004).

CD44

CD44 is the best characterized cell-surface HA binding protein. It is a structurally variable and multifunctional glycoprotein whose expression may be induced on most cell types (Lesley et al., 1993). It participates in many cellular processes including regulation of cell division (Lesley et al., 1993), survival (Baaten et al., 2010b), migration (Peck and Isacke, 1998), and adhesion (Kawakami et al., 1999) through the binding of its major ligand, HA. The human CD44 gene is located on chromosome 11p13 and consists of 19 coding exons of which 9, residing between constitutive exons 5 and 6, can be alternatively spliced into many different isoforms (Ponta et al., 2003; Weidle et al., 2011). The CD44 family consists of a standard form (CD44s) and variant isoforms (CD44v) (Entwistle et al., 1996). The standard form of CD44 (CD44s) contains none of the 9 variable exons, whereas the variant isoforms (CD44v2 - v10) includes them all (exon v1 is not expressed in humans) (Entwistle et al., 1996; Ponta et al., 2003). The CD44v3 - v10 isoform has one less exon and the CD44v8-10 isoform includes only the last three of the variable exons (Entwistle et al., 1996; Naor et al., 1997). Additional isoforms formed by alternative splicing, and various posttranslational modifications further increase the heterogeneity of the CD44 protein products. The standard form (CD44s) is ubiquitously expressed in vertebrates, mostly in quiescent cells (Entwistle et al., 1996; Naor et al., 1997). On the other hand the CD44 variants (CD44v) are expressed in a tissue-specific and cycle-specific manner (Entwistle et al., 1996). The different expression of CD44s and CD44v is probably correlated to the fact that variant isoforms have specific function, most likely different from that of CD44s (Entwistle et al., 1996; Naor et al., 1997).

CD44 binds HA via the link domain encoded by exon 2 (Teriete et al., 2004). This link domain, which is well conserved and similar to the link domains found in other hyaladherins, is ~100 amino acids long, has two antiparallel β-sheets crossed over by two α-helices, and is held together by two sulfide bridges (Teriete et al., 2004). The receptor on resting leukocytes does not bind HA until it is “activated” by proinflammatory cytokines,

which results in the modification of the N-glycans on CD44 (Gee et al., 2003). It is thought that tumor necrosis factor-alpha (TNF-α) induces sialidase activity to remove sialic acid from the N-glycans, which then allows HA-binding to the receptor (Gee et al., 2003).

The most significant feature that distinguishes CD44 from other HA binding proteins is that CD44 binding to HA takes place at the cell surface where multiple, closely arrayed CD44-receptor molecules interact with the highly multivalent repeating disaccharide chain of HA (Lesley et al., 2000). Although glycosaminoglycan side chains associated with some CD44 isoforms can bind a subset of heparin-binding growth factors, cytokines, and ECM proteins such as fibronectin, most of the function ascribed to CD44 thus far can be attributed to its ability to bind and internalize HA (Rothenberg, 2003; Banerji et al., 2007). N-glycosylation regulates CD44 structure by altering both the affinity and avidity of CD44-HA binding (Skelton et al., 1998). Most cells express the standard isoform, which is approximately 85 kDa protein that undergoes posttranslational modification (Skelton et al., 1998; Banerji et al., 2007). Some examples of signaling pathways and molecules activated by HA binding of CD44 include Rac activation, ERM and merlin proteins, SRC, and ROCK (Do et al., 2004; Banerji et al., 2007).

A growing body of evidence indicates that HA/CD44 binding regulates both lymphoid and myeloid cells (Rafi et al., 1997; Do et al., 2004; Katoh et al., 2007; Ruffell and Johnson, 2008; Jiang et al., 2011; Cheng et al., 2013). HA-CD44 interactions play an important role in development (Graham et al., 2007), inflammation (Pure and Cuff, 2001), T cell recruitment and activation (Kawakami et al., 1999), and tumor growth and metastasis (Baaten et al., 2010a). More recently, CD44 has been shown to play a role in inflammatory cell recruitment in the allergic airway (Pure and Cuff, 2001; Rothenberg, 2003; Li et al., 2009) and CD44 expression is increased in the lungs of rats with experimental asthma (Girodet et al., 2010). CD44v5 and CD44v6 expression has also been shown to positively correlate with IgE levels in asthmatic patients and CD44v6 is up regulated in bronchial smooth muscle of asthmatic patients (Li et al., 2009; Girodet et al., 2010; Lennon and Singleton, 2011). Furthermore, antibody blockage of CD44 decreases mast cell adhesion to human bronchial smooth muscle cells *in vitro*, a process associated with AHR and remodeling (Girodet et al., 2010; Lennon and Singleton, 2011). While CD44 is regarded as the primary cell-surface receptor for HA in many cell types, investigation into the mechanisms underlying HA fragment signaling has demonstrated that HA fragments are capable of signaling independently of CD44 (Jiang et al., 2005; Hill et al., 2012).

RHAMM

RHAMM is present as both a plasma membrane GPI-anchored HA receptor and intracellular HA-binding protein lacking both transmembrane and cytoplasmic signal sequences (Turley et al., 1987; Entwistle et al., 1995). Consequently, it is also called intracellular HA binding protein (IHABP) or CD168 (Underhill, 1989). The human gene encoding RHAMM is located on chromosome 5q33.3 and contains 18 exons, which therefore makes it a likely candidate for alternative splicing like CD44 (Turley et al., 1987; Underhill, 1989; Entwistle et al., 1995). RHAMM was originally identified as a hyaladherin derived from chick embryonic heart fibroblasts, while its expression was later found on the surface of chick

heart fibroblasts in the presence of HA (Turley, 1982; Turley et al., 1985). Following this initial discovery, the masses reported for RHAMM have been quite variable, ranging from 58 kDa on B cells to 120 kDa in fibroblasts from *src* null mice, based on immunoreactivity against anti-RHAMM antibodies (Turley et al., 1987; Underhill, 1989). The wide variety of molecular masses could be due to proteolysis, different glycosylation states of the receptor, or the presence of splice variants. The full-length human RHAMM, which is about 85% homologous to murine RHAMM, is identified as a 725 amino acid protein that migrates at ~84 kDa by sodium dodecyl sulfate polyacrylamide gel electrophoresis (Yang et al., 1994).

Although RHAMM lacks both a signal sequence and transmembrane domain, it is thought to be exported via an unconventional transport pathway (Turley, 1982; Turley et al., 1985). Unconventionally, exported RHAMM can exhibit extracellular function by coupling with integral cell surface receptor proteins such as CD44 or platelet-derived growth factor (Vigetti et al., 2014). Interestingly, RHAMM is not only located on the cell surface, but also released from the surface, and soluble forms have been shown to attenuate cell cycle progression n (Turley et al., 2002). RHAMM is a functional receptor in many cell types including endothelial cells (Savani et al., 2001), smooth muscle cells (Evanko et al., 2004), fibroblasts (Tolg et al., 2006), neuronal cells (Nagy et al., 1995), and thymocytes (Pilarski et al., 1994).

RHAMM is believed to be a HA receptor involved in cell locomotion. RHAMM is alternatively spliced like CD44 and it activates extracellular signal-regulated kinases (ERKs) and regulates mitotic-spindle integrity (Lokeshwar and Selzer, 2000). RHAMM binds to HA and has been shown to promote inflammatory cell recruitment and cell growth by a complex network of signal transduction events and interaction with the cytoskeleton (Jiang et al., 2011; Solis et al., 2012). Transforming growth factor-β1 (TGF-β1), which is a potent stimulator of cell motility, elicits the synthesis and expression of RHAMM and HA, and thus initiates locomotion (Samuel et al., 1993). RHAMM expression is increased on macrophages after bleomycin injury and HA-stimulated macrophage chemotaxis is inhibited by anti-RHAMM antibody (Zaman et al., 2005). Furthermore, daily administration of anti-RHAMM antibody to injured animals resulted in a 40% decrease in macrophage accumulation and lung architecture was improved suggesting a role of RHAMM in tissue injury and repair (Zaman et al., 2005). However, there are no studies regarding RHAMM-mediated inflammation in the allergic airways.

TSG-6

TSG-6, the gene product of tumor necrosis factor (TNF)-stimulated gene-6, is a ~35 kDa secreted protein comprised almost entirely of a Link module and a CUB module (Wisniewski and Vilcek, 1997; Milner and Day, 2003). Its amino acid sequence is very highly conserved between species, with the mouse and human proteins being >94% identical (Milner and Day, 2003). TSG-6 is not constitutively produced in healthy adult tissues, but is rapidly induced in a wide variety of cell types (e.g. monocytes (Bayliss et al., 2001), fibroblasts (Klampfer et al., 1994), vascular smooth muscle cells (Ye et al., 1997), cervical smooth muscle cells (Fujimoto et al., 2002), synoviocytes (Bayliss et al., 2001), chondrocytes (Maier et al., 1996), and epithelial cells (Janssen et al., 2001)) under

conditions of inflammation (Milner and Day, 2003). It has been found to be associated with inflammatory conditions such as arthritis and bacterial sepsis (Dias et al., 2001; Getting et al., 2002; Kehlen et al., 2003; Milner and Day, 2003). Additionally, it is also produced during certain normal physiological processes that can be defined as 'inflammation-like' such as ovulation and cervical ripening (Mukhopadhyay et al., 2001; Fujimoto et al., 2002; Wisniewski et al., 2005). Recent studies have revealed that TSG-6 protects against cartilage matrix destruction and has potent anti-inflammatory effects in mouse models of arthritis (Getting et al., 2002; Glant et al., 2002). These studies suggest that TSG-6 is part of a negative feedback loop capable of down-regulating the inflammatory response and initiating tissue repair process. Similarly, Swaidani and colleagues have implicated a role of TSG-6 in an experimental model of allergic asthma (Swaidani et al., 2013). They demonstrated that endogenous TSG-6 is dispensable for the induction of Th2 immunity, robust increase in pulmonary HA deposition, eosinophilic inflammation, and AHR (Swaidani et al., 2013). This suggests that TSG-6 may have an important role in the initiation and maintenance of allergic asthma and that future studies are warranted to delineate the downstream signaling pathways activated by TSG-6-HA interaction.

LYVE-1

LYVE-1 is a type I, single-pass ~60 kDa plasma membrane glycoprotein containing 322 amino acids (Banerji et al., 1999; Prevo et al., 2001). A link domain, which comprises the bulk of the receptor ectodomain, binds only HA, not other GAGs. The cysteine's (Cys) of this link domain are highly conserved, but only three other residues (Lys^{46}, Try^{87}, and Asn^{109}) are conserved in the link family and known to be involved in HA binding (Banerji et al., 1999; Prevo et al., 2001). LYVE-1 has an overall homology of 43% with CD44 and binds both soluble and immobilized HA (Jiang et al., 2011; Wu et al., 2014). Expression of mouse LYVE1 remains restricted to the lymphatic system, and it has been implicated in the trafficking of cells within lymphatic vessels and lymph nodes (Prevo et al., 2001). However, to date, no study has reported a role for this receptor in allergen-induced asthma.

TOLL-LIKE RECEPTORS

HA is a component of the cell coat of groups A and C *Streptococcus* and *Pasteurella multocida* (DeAngelis, 2002; Noble, 2002; Boeriu et al., 2013). The repeating disaccharide structure of HA has features characteristic of pathogen-associated molecular patterns (PAMPs) (Jiang et al., 2006; Lafferty et al., 2010; Jiang et al., 2011; Black et al., 2013; Ebid et al., 2014). Many PAMPs on pathogens trigger innate immune responses via toll-like receptors (TLRs). Similarly, HA that has been modified by the inflammatory milieu attains functions similar to those of a PAMP. While an increasing number of studies have shown that TLRs are involved in HA signaling, the underlying mechanisms remain unclear. TLR4, the primary signaling receptor for lipopolysaccharides, and TLR2, a recognition receptor for mycoplasma and gram-positive bacteria, are both involved in the recognition of fragmented HA (Chaudhuri et al., 2005; Jiang et al., 2011; Bezemer et al., 2012). In dendritic cells, TLR4 is required for recognizing LMM HA, and this recognition is independent of CD44, TLR2, and RHAMM (Termeer et al., 2000; Termeer et al., 2002). However, studies by Noble and colleagues in a non-infectious lung injury model have shown that macrophages isolated from either TLR2 or TLR4 knockout mice are still capable of chemokine gene

expression induced by HA fragments, while macrophages from TLR2/4 double knockout mice are not (Jiang et al., 2005; Jiang et al., 2011). They also demonstrated that HA-TLR signaling was abolished in myeloid differentiation primary response gene (MyD88)-deficient macrophages (Jiang et al., 2005). Similarly, in an ozone-induced lung injury model, Hollingsworth and colleagues have shown a role of the HA-TLR4-MyD88-TIRAP signaling pathway in mediating AHR and cytokine production (Li et al., 2011). However, a recent study indicates that HA fragments of ~200 kDa are capable of inducing type 1 interferons by a TLR4 MyD88-independent pathway (Black et al., 2013). These differences observed in signaling complex requirements may depend upon the size of HA used (135 vs 200 kDa) or the receptors present at the cell surface during experiments. Although data support CD44-independent TLR signaling by HA fragments in certain cell types, it is not clear if there is competition between these two receptors for HA or if they work in a cooperative fashion. These findings offer a new target for the diagnosis and treatment of asthma and may also provide insights into the mechanisms of asthma development. Future studies are needed to define the signaling pathways activated by different sizes of HA fragments and the receptors utilized.

HYALURONAN FRAGMENTS AS SIGNALING MOLECULES

The trafficking of inflammatory cells in allergic asthma is dependent on integrins, selectins, and cytokine/chemokine gradients. However, HA-based ECM defines where the problem is locally and subsequently promotes adhesion and likely subsequent activation of inflammatory cells (Figure 2). In its native state, HA exists as HMM HA, usually in excess of 10^6 Da (Laurent, 1989). However, under conditions of inflammation and tissue injury, HA is more polydisperse and contains a variety of HA polymers with overlapping lengths and functions, with a preponderance of low-molecular mass forms (Figure 2) (Laurent, 1989). A number of studies have shown that low molecular mass forms of HA (< 500 kDa, but not the native form >1000 kDa), induce inflammatory responses in various cell types, supporting the concept that HA fragments generated at sites of inflammation, but not native HMM HA, can stimulate the production of inflammatory mediators by many cell types (Ohkawara et al., 2000; Jiang et al., 2007; Krasinski and Tchorzewski, 2007). One possible explanation for these different functions of HA is that LMM HA may bind more firmly to cells to induce receptor cross-linking than the HMM HA, although this possibility needs further investigation. Additionally, the indicated molecular mass of HA used in different studies, represent the average molecular mass of HA of polydisperse distribution, and the preparations of HA used are not homogenous with respect to size. As such, care must be taken when considering the effects of different polymer sizes. A summary of the genes induced by LMM HA in different cell types is shown in Table 2.

HYALURONAN AS AN IMMUNE REGULATOR IN THE ALLERGIC LUNG

HA is present in low concentrations in the bronchoalveolar lavage (BAL) fluid from healthy individuals, while increased concentrations have been reported in the BAL fluid from patients with allergic asthma (Noble, 2002; Jiang et al., 2011; Eszes et al., 2014). Furthermore, HA levels in BAL fluids are significantly elevated in patients with chronic persistent asthma, in comparison with patients with intermittent asthma (Liang et al., 2011).

The increase in the concentrations of HA in the BAL fluid of asthma patients is a result of either increased HA synthesis or HA breakdown by inflammatory cells (Figure 2B). While it is unclear whether the generation of HA fragments is the result of HA catabolic enzymes (HYAL1-4), reactive oxygen species (ROS), a truncated product of the HA biosynthetic enzymes (HAS1-3), or a combination of these mechanisms, it is clear that HA polymers of specific sizes contain distinct biological properties (Figure 2). Similarly, an increase in TGF-β levels in asthma has been associated with the increase in GAGs by smooth muscle cells (Papakonstantinou and Karakiulakis, 2009). IL-11β and TNF-α have a synergistic effect on LMM HA accumulation in lung fibroblasts (Ellis et al., 1992). Fibroblasts from subjects with the most hyperresponsive airways in asthma produced significantly more total proteoglycans, such as HA, than cells from subjects with less reactive or normal airways (Todorova et al., 2010). Some of the cell types that are known to interact with HA are T cells, eosinophils, macrophages, fibroblasts, smooth muscle cells, and B lymphocytes.

T CELLS

CD44, one of HA's major receptors, undergoes dynamic regulation on T lymphocytes (Do et al., 2004; Ruffell and Johnson, 2008; Jiang et al., 2011). The interaction of cell-surface HA and CD44 on T cells is manifested by polarization, spreading, and co-localization of cell-surface CD44 with a rearranged actin cytoskeleton (Ariel et al., 2000). Thus, cytokines and chemokines present in the vicinities of blood vessels or present intravascularly in tissues where immune reactions take place can rapidly activate the CD44 molecules expressed on T cells (Liang et al., 2011; Maeshima et al., 2011). HA binding requires the activation of T cells, and their activation is associated with the increased surface levels of CD44. In addition, binding of CD44 on activated T lymphocytes to endothelial HA can promote extravasation and egress of T lymphocytes on inflamed vascular beds by mediating a primary adhesive interaction under shear stress (Katoh et al., 2010; Winkler et al., 2012). $CD4^+$ T cells expressing high levels of CD44 are recruited to the allergic lung after antigen administration (Kennedy et al., 1995). Recently, Cheng and colleagues reported a correlation between HA deposition and lymphocytes in a cockroach-induced model of asthma (Cheng et al., 2011; 2013).

HA binding to CD44 on T cells has also been correlated to superior suppressor activity, suggesting that CD44 is more than a cell surface marker and plays a role in regulating regulatory T cell (Treg) function (Bollyky et al., 2007; Bollyky et al., 2009). HMM HA has been shown to enhance human $CD4^+CD25^+$ regulatory T cell functional suppression of responder cell proliferation, whereas LMM HA does not (Bollyky et al., 2007; Bollyky et al., 2009). In addition, HMM HA has been reported to up-regulate the transcription factor FOXP3 and $CD4^+CD25^+$ regulatory T cells (Bollyky et al., 2007; Bollyky et al., 2009).

EOSINOPHILS

The recruitment, activation, and degranulation of eosinophils in the lung is a characteristic hallmark of allergic asthma (Schmekel and Venge, 1993; Wardlaw et al., 2000; Venge, 2010; Ghosh et al., 2013; Acharya and Ackerman, 2014). HA stimulates the growth of $CD34^+$ progenitor cells into specifically differentiated, mature eosinophils (Hamann et al., 1995). Both TNF-α and HA contribute to the long-term survival of eosinophils *in vivo* by

enhancing the production of granulocyte macrophage colony stimulating factor (GM-CSF) and TGF-β in asthma (Esnault and Malter, 2001; 2003). Ohkawara and colleagues have reported that the LMM HA has a pronounced effect on eosinophil survival in both patients with asthma and healthy subjects in a dose-dependent fashion (Ohkawara et al., 2000; Esnault and Malter, 2001). The HMM HA had a smaller effect on eosinophil survival than did the LMM HA (Ohkawara et al., 2000). Furthermore, they demonstrated that the LMM HA results in morphologic changes in eosinophils such as transforming from a round to a spindle shape and in homotypic aggregation, up regulates intercellular adhesion molecule-1 expression, and increases TGF-β mRNA expression and protein secretion by eosinophils (Ohkawara et al., 2000). We and other research groups have also reported a correlation between LMM HA deposition and eosinophils in allergic asthma (Cheng et al., 2011; 2013; Ghosh et al., 2014b). Other groups have suggested a role of CD44 in eosinophil migration in the allergic lung as administration of anti-CD44 monoclonal Ab prevents eosinophil accumulation in the lung and blocked elevation of Th2 cytokines/chemokines (Katoh et al., 2003). These observations suggest previously unforeseen interactions between eosinophils and LMM HA, and thus, novel pathways by which eosinophils may contribute to the regulation of airway inflammation and airway remodeling in allergic asthma.

MACROPHAGES

Elevated concentrations of HA are associated with the accumulation of macrophages in the lung after injury (Savani et al., 2000; Noble and Jiang, 2006). Similarly, HA has been reported in macrophage migration through interactions with RHAMM, TLR2, and CD44 (Noble, 2002; Turley et al., 2002; Shi et al., 2006; Foley et al., 2012). CD44-deficient mice succumb to unremitting inflammation following lung injury, characterized by persistent accumulation of HA fragments at the site of tissue injury, and impaired activation of TGF-β1 (Liang et al., 2007). This phenotype was partially reversed by reconstitution with $CD44^+$ macrophages, thus demonstrating a critical role for this receptor and macrophages in resolving lung inflammation (Liang et al., 2007). In a LPS-induced lung injury model, alveolar macrophages have been shown to promote HA synthesis in a TLR4 dependent manner to further modulate the inflammatory response (Chang et al., 2014). LMM HA has been reported to activate cytosolic Phospholipase A2α and promote eicosanoid production in a TLR4 dependent pathway in macrophages (Sokolowska et al., 2014). Similarly, LMM HA is able to polarize human macrophages to a M1 phenotype, and CD44 has been shown to regulate phagocytosis of apoptotic neutrophils by human macrophages (McCutcheon et al., 1998; Sokolowska et al., 2014). A recent study also demonstrates that Interferon-β (IFN-β) is induced in murine macrophages by LMM HA fragments by a novel pathway independent of MyD88 but dependent on TLR4 via TIR-domain-containing adapter-inducing interferon-β (TRIF) and Interferon Regulatory Factor-3 (IRF-3) (Black et al., 2013). Additionally, Feng and colleagues have demonstrated a role of HA in cleavage of macrophage-derived caspase1 and IL-1β, suggesting a role for alveolar macrophages in Nlrp3-dependent AHR (Feng et al., 2012).

FIBROBLASTS

Fibroblasts are the main cell that synthesizes HA (Smith and Ghosh, 1987; Noble, 2002; Liang et al., 2011; Maharjan et al., 2011). Fibroblasts express all three isoforms of HA

synthases and release HA upon tissue injury when stimulated by inflammatory factors such as IL-1β and TNF-α. LMM HA stimulates fibroblasts to release cytokines and chemokines and regulate inflammatory responses (Prosdocimi and Bevilacqua, 2012; Tolg et al., 2014). Moreover, HA modulates TGF-β dependent myofibroblast differentiation (Thannickal et al., 2003).

HA-binding proteins regulate fibroblast functions through their interactions with HA. CD44 has been shown to play a role in fibroblast migration and injury in acute lung injury (Acharya et al., 2008). RHAMM deficient fibroblasts are defective in CD44-mediated ERK signaling, demonstrating a role of RHAMM in CD44 signaling and fibroblast migration (Tolg et al., 2006). A recent study also reported a role of HMM HA in promoting fibroblast invasion by increasing snail2 expression (Craig et al., 2009).

SMOOTH MUSCLE CELLS

GAGs, especially HA, regulate tissue flexibility, cell motility, and inflammation. Airway smooth muscle cells (ASMCs) of patients with asthma exhibit abnormal HA metabolism, which contributes to inflammation and remodeling (Papakonstantinou et al., 2012). Growth factors and cytokines at sites of inflammation or tissue injury regulate HA production by smooth muscle cells (Lauer et al., 2009). For example, Platelet-derived growth factor (PDGF) stimulates HA production in vascular smooth muscle cells, while IL-15 inhibits HA production and smooth muscle cell migration (Goueffic et al., 2006; Iwasaki et al., 2007; Lauer et al., 2009). Furthermore, HA binding proteins regulate smooth muscle proliferation and migration during lung injury (Boudreau et al., 1991). For example, RHAMM is necessary for the migration of smooth muscle cells, which is mediated by the PI3K-dependent Rac activation pathway (Goueffic et al., 2006). Other studies have reported a decrease in secretion of HA by ASMCs from patients with asthma or COPD (Klagas et al., 2009). This decrease was associated with a significant reduction in the expression of HAS1 and HAS2 and a significant increase of HYAL1. Furthermore, the expression of the HA receptor, CD44, was significantly decreased, whereas RHAMM was not expressed in asthma or COPD. This suggests that a decreased expression of HA by ASMC in asthma and COPD is associated with a synergistic regulation of HA metabolizing enzymes that may regulate the pathological airway remodeling in these lung diseases. It is also possible that reduced CD44 and RHAMM expression are responsible for impaired clearance of LMM HA from the lung, resulting in persistent inflammation.

A recent report suggests a role of glucocorticoids and long-acting beta agonists (LABAs) in inhibiting the increased secretion and deposition of total GAGs by asthmatic ASMCs (Papakonstantinou et al., 2012). However, these ASMCs secreted and deposited HA of high molecular mass (Papakonstantinou et al., 2012). This effect was attributed to increased mRNA and protein expression of HAS1 and to the reduced expression of HYAL1. Furthermore, drug treatment stimulated the expression of CD44 receptors in asthmatic ASMCs indicating that the combination of glucocorticoids with long-acting beta agonists (LABAs) counteracts the pathologic degradation of HA, and may reduce the proinflammatory potential of asthmatic ASMCs (Papakonstantinou et al., 2012).

B LYMPHOCYTES

The interaction of CD44 with its ligand, HA, plays a vital role in lymphopoiesis and lymphocyte homing, and there is growing evidence that HA/CD44 binding may play a role in the regulation of both lymphoid and myeloid cells (Rafi et al., 1997; Kryworuchko et al., 1999; Do et al., 2004; Katoh et al., 2007; Ruffell and Johnson, 2008; Jiang et al., 2011). *In vitro*, HA promotes survival, differentiation, and IgM production by B lymphocytes (Rafi et al., 1997). A recent report by Cheng and colleagues shows HA co-localization with B lymphocytes in the allergic lung (Cheng et al., 2013). Although CD44 helps to facilitate B lymphocyte adhesion to HA and proliferation in the spleen (Rafi et al., 1997; Vasconcellos et al., 2010), the contribution of HA/CD44 to B lymphocyte migration and activation in the context of allergic asthma has not been investigated and remains a focus for future studies.

THERAPEUTIC POTENTIAL OF HYALURONAN

Although HMM HA (≥1 million Da) is produced endogenously and is an integral component of the ECM, synovial fluid and vitreous humor, recent attention has been focused on the use of exogenously administered HMM HA in a variety of diseases including lung diseases (Noble and Jiang, 2006; Bollyky et al., 2007; Gaffney et al., 2010). *In vitro*, exogenous administration of HMM HA inhibits ROS, nitrotyrosine and inflammatory cytokine production as well as promotes immune tolerance (Garantziotis et al., 2010; Miki et al., 2010). Excess production of endogenous HMM HA in mice overexpressing HAS2 in airway epithelia protects against bleomycin-induced lung injury and ozone-induced AHR (McKee et al., 1996; Liang et al., 2011). Singleton and colleagues have demonstrated that intravenous administration of HMM HA (~1 million Da) four hours after intratracheal administration of LPS provides protection against lung injury in mice (Singleton et al., 2010). Nadkarni also demonstrated that pretreatment of hamsters with aerosolized HMM HA protects against endotoxin-induced lung injury (Nadkarni et al., 2005). Interestingly, treatment with aerosolized HMM HA after endotoxin treatment actually enhanced lung inflammation, indicating the timing and route of administration are important determinants of HMM HA's effectiveness (Cantor, 2007; Singleton and Lennon, 2011).

Smoking is a well-recognized cause of lung injury that can lead to the development of emphysema and COPD (Cantor et al., 1999; Cantor et al., 2005). Cigarette smoke is believed to induce an imbalance in the protease-antiprotease levels in the lung (Shen et al., 2011). This imbalance, which develops due to increased inflammatory cell recruitment, activation and release of protease enzymes (including elastase), leads to the proteolytic breakdown of the ECM and the elastin fibers. Breakdown of the elastin fibers can lead to alveolar distention and rupture, a prominent feature of emphysema and COPD (Cantor et al., 2011; Shen et al., 2011). Although HA itself does not inhibit protease activity, studies by Cantor and Turino have shown that it may be protective against elastin fiber breakdown (Cantor et al., 1997; Cantor et al., 2011). They have shown that aerosolized LMM HA (150kDa) binds or closely associates with the elastin fibers and may physically protect them from degradation by proteases (Cantor et al., 2011; Shen et al., 2011). A clinical trial is currently underway to determine the use of HA as a treatment in COPD.

In relation to asthma, very few studies have examined the role of exogenous HMM HA on airway hyperreactivity. Aerosolized HMM HA has been shown to reduce neutrophil elastase induced bronchoconstriction in sheep (Cantor et al., 1998; Scuri and Abraham, 2003). In addition, oropharyngeal administration of exogenous HMM HA either before or after ozone exposure significantly attenuates airway hyperreactivity in mice (Garantziotis et al., 2009). Further, pre-treatment of aerosolized HMM HA protects asthmatic patients from exercise-induced bronchoconstriction (Petrigni and Allegra, 2006).

HA may be a double-edged sword with regard to its therapeutic efficacy. In the absence of existing inflammation, HMM HA may exert a beneficial effect, as both an anti-inflammatory agent and a shielding barrier against the degradative activities of various inflammatory products. Conversely, the presence of a pre-existing inflammatory milieu has the potential to convert HMM HA into a LMM HA proinflammatory mediator and thereby counteract its therapeutic activity. However, all these studies suggest a possible therapeutic role of HA in mediating lung injury and similar studies in mouse models of allergic asthma are needed to fully understand the specific molecular interactions of HA fragments with inflammatory cells.

CONCLUSIONS

The synthesis and degradation of the ECM are fundamental components of lung injury and repair. HA appears to have important functions in this biological process. Observations from experimental animals and asthmatic patients suggest a direct participation of HA in mediating the pathophysiology associated with allergic pulmonary disease, although the mechanisms by which HA contributes to the pathogenesis are rather complicated. Emerging evidence suggests that HA has a broad range of functions beyond that of a basic structural support in allergic asthma. The initiation and maintenance of allergic asthma relies on the balance between the low and high molecular mass HA accumulation. More extensive studies are needed to fully understand the specific molecular interactions between HA and HA-binding proteins in the initiation and resolution of inflammatory response in allergic asthma. Future studies should focus on the various molecular mass products of HA and their role in regulating the allergic phenotype in response to allergens. Selective interference with HA production or interaction with receptors may present a therapeutic target that can be exploited to minimize long-term damage associated with excessive collagen deposition in severe asthma. Additionally, studies involving the recognition of epigenetic factors in regulating the HA turnover in allergic disease may lead to new therapeutic approaches in the future.

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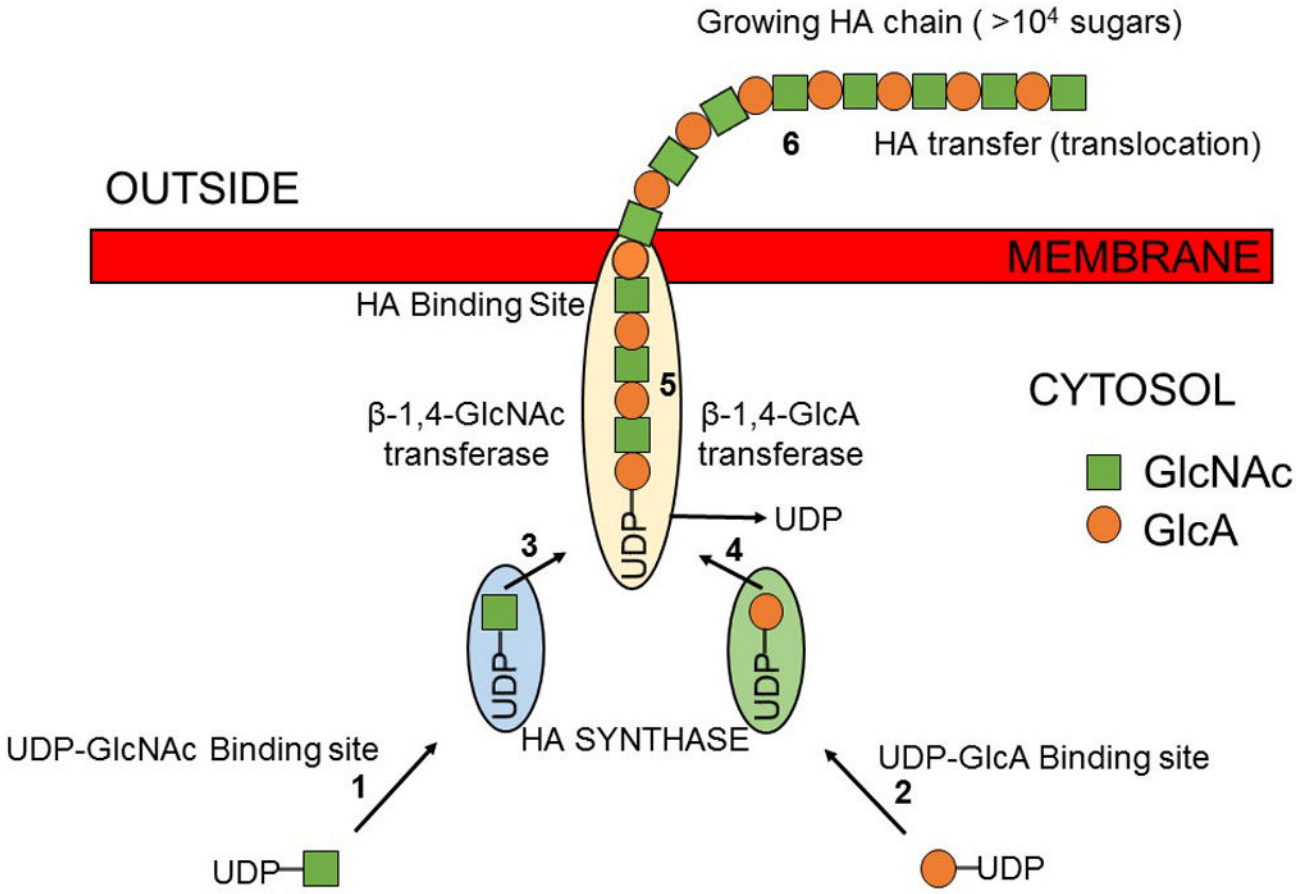

Figure 1. Schematic diagram illustrating hyaluronan biosynthesis

A membrane-bound hyaluronan synthase utilizes uridine diphospho-glucuronic acid (UDP-GlcA) and uridine diphospho-N-acetylglucosamine (UDP-GlcNAc) as substrates for hyaluronan biosynthesis. The UDP-sugar substrates are used by the hyaluronan synthase inside the cell and the hyaluronan chain is continuously translocated across the membrane at the reducing end. Two enzymes are shown to illustrate how one enzyme could alternately make each of the two types of glycoside bonds in hyaluronan.

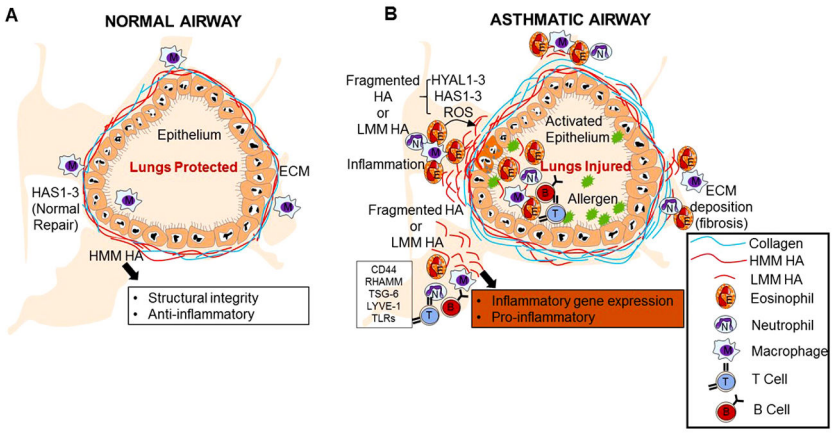

Figure 2. Hyaluronan metabolism in the allergic lung

(A) Under physiological conditions high molecular mass HA (HMM HA) protects the lung and helps in the repair process. **(B)** Under conditions of chronic inflammation or tissue injury hyaluronan synthases (HAS1, HAS2, and HAS3) and hyaluronidases (HYAL1-3) play a role in the generation of fragmented HA (LMM HA). If hyaluronan fragments are generated, the balance shifts from HMM HA to LMM HA. This LMM HA is pro-inflammatory and helps in cellular recruitment and inflammatory gene expression. The differential activities of LMM HA in the lung are regulated through interactions with HA binding proteins including CD44, RHAMM, TSG-6, LYVE-1, and TLRs. Altering the balance of LMM HA and HMM HA may have therapeutic utility in the treatment of lung inflammation.

Table 1
Hyaluronan binding proteins and their associated lung pathologies

Summary of the HA binding proteins discussed in this review, their association with various associated lung pathologies and corresponding references.

HA Binding Protein	Associated Lung Pathology	References
CD44	LPS-induced lung injury Non-infectious lung injury COPD Asthma	(Do et al., 2004; Krasinski and Tchorzewski, 2007; Li et al., 2009; Jiang et al., 2010; 2011)
RHAMM	Asthma LPS-induced lung injury COPD ARDS	(Tolg et al., 2006; Jiang et al., 2010; 2011; Foley et al., 2012)
TSG-6	Asthma Bleomycin-induced lung injury	(Swaidani et al., 2013; Foskett et al., 2014)
TLR2/TLR4	Asthma LPS-induced lung injury COPD Non-infectious lung injury	(Chaudhuri et al., 2005; Garantziotis et al., 2010; Jiang et al., 2010; Bezemer et al., 2012; Foley et al., 2012)

Table 2
Selected genes induced by HA fragments

Summary of the various genes that are induced by HA binding in different cell types discussed in this review, and corresponding references.

Category	Gene	Cell type	References
Cytokines	IL-12	Macrophages	(Hodge-Dufour et al., 1997)
	TNF-α	Macrophages	(Jiang et al., 2006)
	IL-1β	Macrophages	(Jiang et al., 2006; Feng et al., 2012)
	IL-6	Fibroblasts, Macrophages, B Cells	(Iwata et al., 2009; Yamawaki et al., 2009; Vistejnova et al., 2014)
	IL-8	Fibroblasts, Airway Epithelial Cells, Macrophages, Endothelial Cells	(McKee et al., 1996; Taylor et al., 2004; Boodoo et al., 2006; Vistejnova et al., 2014)
	IL-10	Regulatory T Cells, B Cells	(Bollyky et al., 2009; Iwata et al., 2009; Bollyky et al., 2011)
	IFNβ	Macrophages	(Black et al., 2013)
Chemokines	MIP-α	Macrophages	(Hodge-Dufour et al., 1997)
	KC	Macrophages	(Horton et al., 1998)
	MCP-1	Macrophages	(Yamawaki et al., 2009)
	CXCL2	Macrophages	(Jiang et al., 2005)
	CCL5	Macrophages	(McKee et al., 1996)
	CXCL9	Macrophages	(Horton et al., 1998)
	CXCL10	Macrophages	(McKee et al., 1996; Horton et al., 1998)
	CXCL1	Endothelial Cells	(Takahashi et al., 2005)
	CCL3	Macrophages	(McKee et al., 1996)
	CCL4	Macrophages	(McKee et al., 1996)
ECM	MMP-9	Fibroblasts, Macrophages, Dendritic Cells	(Fieber et al., 2004; Stern et al., 2006)
	MMP-13	Fibroblasts	(Fieber et al., 2004; Stern et al., 2006)
	MMP-10	Endothelial Cells	(Stern et al., 2006)
	PAI-1	Macrophages	(Horton et al., 2000)
	uPA	Macrophages	(Horton et al., 2000)
	MME	Macrophages	(Horton et al., 1999)
Transcription factors	NF-kappa B	Dendritic Cells	(Fieber et al., 2004)
	IκBα	Macrophages	(Noble et al., 1996)
	AP-1	Endothelial Cells	(Deed et al., 1997)
Growth factors	TGF-β1	Eosinophils, Macrophages, B cells	(Ohkawara et al., 2000; Iwata et al., 2009; Tolg et al., 2014)
	TGF-β2	Monocytes	(Taylor et al., 2007)
	IGF-1	Macrophages	(Noble et al., 1993)
Others	cPLA2α	Monocytes, Macrophages	(Sokolowska et al., 2014)
	c-jun	Endothelial Cells	(Wang et al., 2011)
	c-fos	Endothelial Cells	(Wang et al., 2011)
	Cdc2 (Cdk1)	Endothelial Cells	(Matou-Nasri et al., 2009)

Category	Gene	Cell type	References
	Src	Endothelial Cells	(Wang et al., 2011)
	iNOS	Hepatocytes, Endothelial Cells	(Rockey et al., 1998)
	MDR-1	Lymphocytes	(Tsujimura et al., 2006)