REVIEW / SYNTHÈSE

S100A8/A9: a mediator of severe asthma pathogenesis and morbidity?[1]

Andrew J. Halayko and Saeid Ghavami

Abstract: Nearly 12% of children and 6% of adults in Canada have been diagnosed with asthma. Although in most patients symptoms are controlled by inhaled steroids, a subpopulation ($\sim$10%) characterized by excessive airway neutrophilia, is refractory to treatment; these patients exhibit severe disease, and account for more than 50% of asthma health care costs. These numbers underscore the need to better understand the biology of severe asthma and identify pro-asthma mediators released by cells, such as neutrophils, that are unresponsive to common steroid therapy. This review focuses on a unique protein complex consisting of S100A8 and S100A9. These subunits belong to the large Ca^{2+}-binding S100 protein family and are some of the most abundant proteins in neutrophils and macrophages. S100A8/A9 is a damage-associated molecular pattern (DAMP) protein complex released in abundance in rheumatoid arthritis, inflammatory bowel disease, and cancer, but there are no definitive studies on its role in inflammation and obstructive airways disease. Two receptors for S100A8/A9, the multiligand receptor for advanced glycation end products (RAGE) and Toll-like receptor 4 (TLR4), are expressed in lung. TLR4 is linked with innate immunity that programs local airway inflammation, and RAGE participates in mediating fibroproliferative remodeling in idiopathic pulmonary fibrosis. S100A8/A9 can induce cell proliferation, or apoptosis, inflammation, collagen synthesis, and cell migration. We hypothesize that this capacity suggests S100A8/A9 could underpin chronic airway inflammation and airway remodeling in asthma by inducing effector responses of resident and infiltrating airway cells. This review highlights some key issues related to this hypothesis and provides a template for future research.

Key words: airway smooth muscle, airway remodeling, neutrophils, lung, inflammation, myeloid-related protein.

Résumé : Près de 12 % des enfants et 6 % des adultes au Canada souffrent d'asthme. Bien que pour la majorité des patients les symptômes sont contrôlés par l'inhalation de stéroïdes, il en demeure environ 10 % qui présentent une résistance au traitement; on observe chez ces derniers une hyperneutrophilie dans les voies respiratoires. Ces patients, gravement malades, grèvent plus de 50 % des coûts de santé reliés à l'asthme. Il est donc important de mieux comprendre la biologie de l'asthme sévère et d'identifier les médiateurs proasthmatiques sécrétés par les cellules, dont les neutrophiles, et qui ne répondent pas à la thérapie stéroïdienne standard. Cet article-synthèse porte sur un complexe protéique spécial formé de S100A8 et de S100A9. Ces sous-unités qui font partie de la famille des calciprotéines S100 sont les protéines les plus abondantes dans les neutrophiles et les macrophages. Le complexe protéique S100A8/A9 est un assemblage moléculaire lésionnel (en anglais « damage-associated molecular pattern » ou DAMP) synthétisé abondamment en présence de polyarthrite rhumatoïde, de maladie intestinale inflammatoire et de cancer. Il n'y a cependant pas d'études concluantes sur le rôle de ce complexe protéique sur le syndrome obstructif et inflammatoire. Deux récepteurs du complexe S100A8/A9 sont exprimés dans le poumon: le récepteur-multiligand pour produits terminaux de glycation avancée (RAGE) et le récepteur 4 de la famille Toll (TLR4). Le TLR4 est associé à l'immunité innée qui programme l'inflammation locale des voies respiratoires et le RAGE collabore au remodelage fibroprolifératif en présence de fibrose pulmonaire idiopathique. Le complexe S100A8/A9 peut susciter la prolifération des cellules ou l'apoptose, l'inflammation, la synthèse du collagène et la migration des cellules. Selon nous, le complexe S100A8/A9 serait à la base de l'inflammation chronique des voies respiratoires et du remodelage de ces voies en présence d'asthme, et ce, par le déclenchement de réponses des cellules en place

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et de cellules s'infiltrant dans les voies respiratoires. Cet article-synthèse soulève des questions-clés pour tester cette hypothèse et présente une matrice pour des études ultérieures.

Mots-clés : muscle lisse des voies respiratoires, remodelage des voies respiratoires, neutrophiles, poumon, inflammation, protéine myéloïde.

[Traduit par la Rédaction]

Introduction

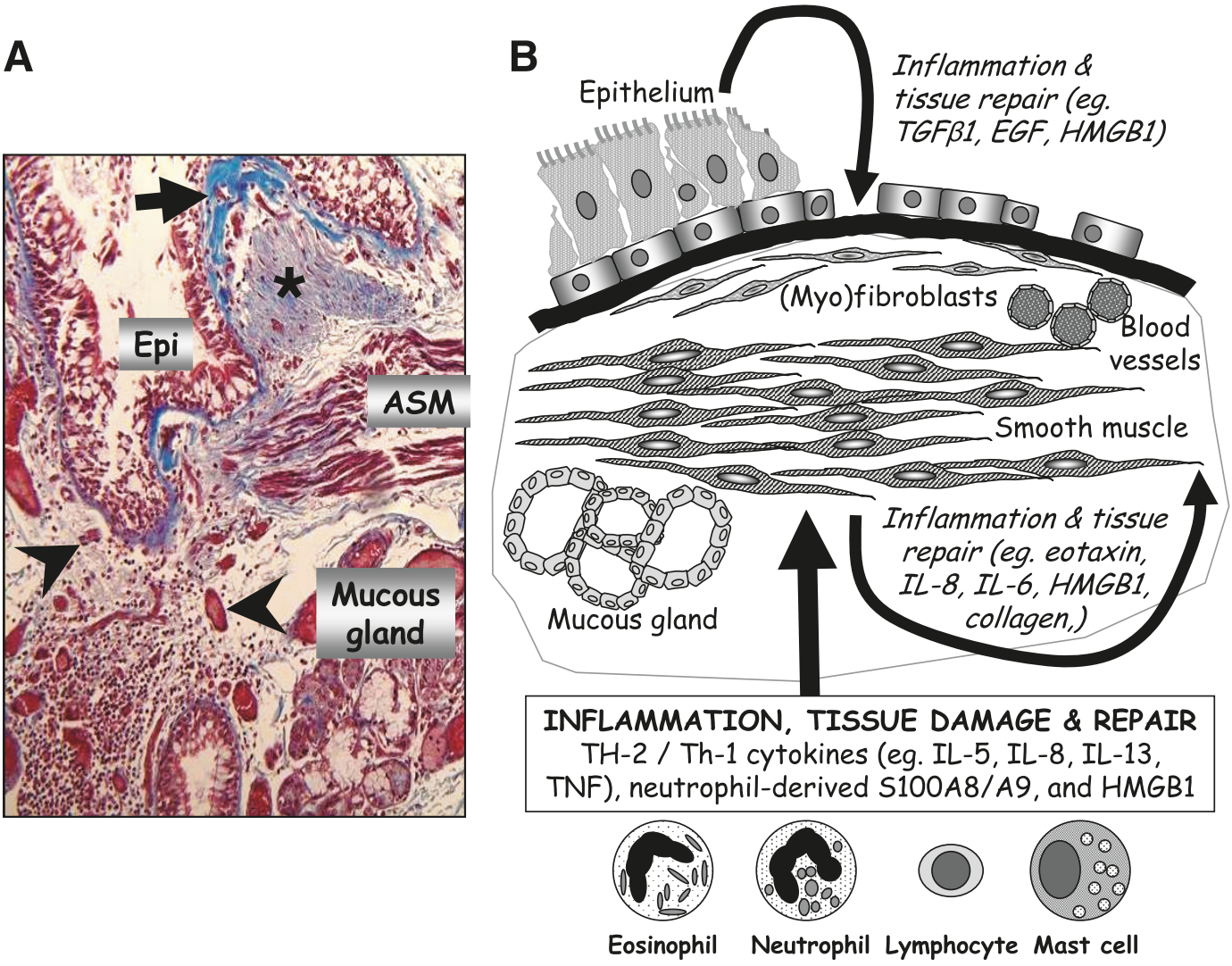
The World Health Organization estimates that over 300 million people have asthma worldwide, including 3 million Canadians — approximately 12% of the children and 6% of the adults in Canada. Although significant progress has been made in identifying disease risk factors, understanding the complex interplay among biological pathways that underpin development, maintenance, and progression of phenotypically diverse asthma in children and adults remains elusive (James and Wenzel 2007). That asthma is a chronic T helper type 2 (Th2)-polarized inflammatory disease of the airways is the principal guiding concept for first-line controller therapy using corticosteroids. Notwithstanding, inhaled steroid therapy is effective in only approximately 70% of patients with asthma (Holgate and Polosa 2006; Mjaanes et al. 2006). Steroid-refractory, 'difficult-to-treat' disease is the basis for a severe asthma subphenotype occurring in up to 10% of patients. This group requires substantial advanced clinical management, thus accounting for greater than 50% of all asthma-related health care costs, including an unacceptably low quality of life, increased frequency of emergency room visits, and higher level of asthma-related mortality (Accordini et al. 2006; Holgate 2006; Holgate and Polosa 2006). To meet the need for effective treatment of steroid-resistant severe asthma, a number of alternative treatment approaches, such as humanized monoclonal immunoglobulin E (IgE)-blocking antibodies, tumor necrosis factor (TNF)-blocking antibodies, or soluble TNF receptors, have been developed (Waserman et al. 2000; Feldmann et al. 2005; Howarth et al. 2005; Lanier 2005; Holgate et al. 2006); however, these therapies are expensive and not all patients benefit from their use. It is clear that to establish effective therapeutic strategies tailored to severe asthma patients, better understanding of biomarkers, novel biologically active mediators and their cellular sources, and the effects of novel mediators on airway inflammation and function is needed. This review outlines some biological aspects of severe asthma pathobiology, with focus on airway neutrophils and the highly abundant neutrophil-released protein complex S100A8/A9, which we propose could be a key contributor to the development and morbidity of severe asthma.

Asthma and severe asthma pathobiology

Asthma is a chronic inflammatory disease of the airways that is characterized by persistent paroxysmal dyspnea and airway hyperresponsiveness (AHR) to inhaled spasmogens. It appears that chronic intermittent airway inflammation promotes asthma pathogenesis and compounds the functional consequences of structural remodeling of the airway wall (Bousquet et al. 2000; Holgate et al. 2000). Airway inflammation is primarily characterized by elevated levels of Th2-polarized lymphocytes and eosinophilia, but there are also increased numbers of airway mast cells and neutrophils in most asthmatic patients (Cowburn et al. 2008) (Fig. 1). Airway inflammation appears to drive disease exacerbation and progression, in particular the development of AHR and structural changes in the airways, called airway remodeling, that in part likely effects fixed airway obstruction and severe disease, possibly because of changes in structure–function properties of the airways within the lung (An et al. 2007; James and Wenzel 2007). Airway remodeling arises from rounds of tissue damage and repair and its key features include, but are not limited to, increased numbers of submucosal myofibroblasts (Gizycki et al. 1997; Holgate et al. 2000; Halayko and Amrani 2003; Halayko et al. 2006; Miller et al. 2006; Nihlberg et al. 2006), thickened airway smooth muscle (ASM) mass due to cell hyperplasia and hypertrophy (Holgate et al. 2000; Halayko et al. 2006), accumulation of extracellular matrix proteins (Bousquet et al. 2000; Bergeron et al. 2003; Chakir et al. 2003), increased number and size of bronchial blood vessels (Vrugt et al. 2000; Hoshino et al. 2001; Barbato et al. 2006), and goblet cell/mucous gland hyperplasia (Aikawa et al. 1992; Rogers 2004). Resident airway cells, including the epithelium and mesenchymal cells, including ASM cells and fibroblasts, contribute to local inflammation and tissue repair, thus promoting the progression of airway remodeling; for example, mesenchymal cells have the ability to proliferate, serve an immunomodulator function (by releasing proinflammatory cytokines and chemokines), and deposit collagen in the airway wall (Halayko and Stephens 1994; Halayko et al. 2006) (Fig. 1). Indeed, considerable research is focused on the potential of ASM cells and myofibroblasts as therapeutic targets for asthma control (Hirst and Lee 1998; Gosens et al. 2006; Halayko et al. 2006; Janssen and Killian 2006; Solway and Irvin 2007; Baroffio et al. 2008). Moreover, airway mesenchymal cells from asthmatic subjects exhibit unique proliferative and profibrotic features (Dubé et al. 1998; Johnson et al. 2001; Laliberté et al. 2001; Westergren-Thorsson et al. 2002; Chambers et al. 2003; Burgess et al. 2004, 2008; Johnson et al. 2004). Asthma is a complex inflammatory disease involving both resident and recruited effector cells that promote intermittent and persistent lung dysfunction due to acute and chronic inflammation and structural remodeling.

Asthma control refers to the response to treatment, that is, the extent to which manifestations of disease can be limited by anti-asthma therapy. In patients with mild to moderate disease, symptoms are generally well controlled with standard treatment that involves the regular use of inhaled corticosteroids and long-acting β2-adrenoceptor agonists. Conversely, patients with severe asthma are refractory even

Fig. 1. Fundamental features of airway remodeling and inflammation in chronic asthma. (A) Micrograph shows a portion of the cross section of a 3rd-generation bronchus obtained postmortem from an individual with moderate asthma (cause of death was not asthma related). The section has been stained with Masson's trichrome to identify fibrotic tissue (blue), airway smooth muscle (red, denoted as ASM), and the epithelium (red, denoted as Epi). The airway lumen, with residual mucous, appears in the top left above the epithelial layer, which is characterized by numerous goblet cells (clear/non-staining cells). A substantially thickened epithelial basement membrane (stained blue and denoted with an arrow) is evident, as well as marked accumulation of extracellular matrix in the submucosal compartment (asterisk). Numerous engorged blood vessels are visible in the airway wall (arrowheads), along with evidence of mucous gland hyperplasia. Accumulation of inflammatory cells in the airway wall is also evident in the lower left of the image, appearing as numerous darkly stained nuclei. (B) Schematic representation of the pathobiological processes and cells involved with the development of airway remodeling. A key local driving force is thought to be the cytokines, chemokines, and growth factors released by the epithelium that act on the underlying airway wall (myo)fibroblasts and airway smooth muscle cells. Airway smooth muscle and fibroblasts also release trophic and profibrotic factors that contribute to local inflammation and tissue repair. Central to the initiation and modulation of inflammation, tissue damage, and repair is recruitment of active inflammatory cells including Th2- and Th1-polarized lymphocytes, eosinophils, neutrophils, and mast cells. We speculate that neutrophils are a principal source for extracellular S100A8/A9, that HMGB1 (likely induced by S100A8/A9) is released by inflammatory cells and resident cells (epithelium, smooth muscle, and fibroblasts), and that both proteins promote inflammation and tissue repair leading to airway remodeling. Th1 and Th2, T helper cells; S100A8 and S100A9, damage-associated molecular pattern protein complex; HMGB1, high-mobility group box 1 protein; TGF, transforming growth factor; EGF, epidermal growth factor; IL, interleukin; TNF, tumour necrosis factor.



to high doses of inhaled anti-asthma therapy and exhibit oral corticosteroid dependency or resistance (Holgate et al. 2006; Holgate and Polosa 2006; Humbert et al. 2007). Defining asthma severity involves consideration of both the magnitude and frequency of the underlying disease and its response to treatment. Severe asthma is a heterogeneous disease, as classified by a number of criteria such as health status, airway obstruction (variable or partially fixed), AHR, and exacerbation frequency and severity (Chanez et al. 2007; Humbert et al. 2007). Severe asthma and its best treat-

ment options remain poorly understood. Notably, and of importance to the current review, strong associations have been established between airway neutrophilia and severe asthma, steroid-resistant asthma, frequency of asthma exacerbations, and acute fatal asthma (Kariyawasam et al. 2007; Macdowell and Peters 2007; Cowburn et al. 2008). Indeed, neutrophilic airway inflammation is believed to be a principal effector of airway damage leading to the development of fixed airflow obstruction characteristic of chronic and severe asthma (Shaw et al. 2007). Moreover, corticosteroid-refractory asthma is a disease subphenotype characterized by predominant neutrophilic airway inflammation, in the presence or absence of eosinophils, and significant airway remodeling (Holgate and Polosa 2006). This underscores a need to better understand the biology of severe asthma and identify pro-asthma mediators released by cells, such as neutrophils, that are unresponsive to steroid therapy.

Neutrophils are myeloid granulocytes that play a fundamental role in the inflammatory response associated with chronic obstructive pulmonary disease (COPD), bronchiectasis, bronchiolitis, cystic fibrosis, and acute lung injury. Neutrophils are also recruited to the lung during acute asthma exacerbation, being readily evident in endobronchial biopsies and sputum (Green et al. 2002; Maneechotesuwan et al. 2005; Kariyawasam et al. 2007; Maneechotesuwan et al. 2007; Shaw et al. 2007; Cowburn et al. 2008). The existence of airway neutrophilic inflammation is linked to a predilection for asthma exacerbation and loss of asthma control after withdrawal of inhaled corticosteroids (Maneechotesuwan et al. 2007). Like eosinophils, neutrophils are among the first cells recruited to the site of tissue damage after inflammation. Consistent with the concept that neutrophils hold an important determinant role in asthma, the classical neutrophil chemoattractant and activator CXCL-8 (interleukin (IL)-8) is markedly elevated during asthma exacerbations and acute severe asthma (Ordoñez et al. 2000; Maneechotesuwan et al. 2007). Neutrophils are 'primed' by binding to the endothelium or extracellular matrix, or by exposure to lipopolysaccharide (LPS), TNF-α, or IL-6. Subsequent activation promotes their cytotoxic effects including the generation of reactive oxygen species (ROS), degranulation, and release of cytokines (IL-1β, -6, -8, TNF-α, and granulocyte macrophage-colony stimulating factor (GM-CSF)), lipid mediators (LTB4 and cysteinyl leukotrienes), and neutrophil elastase. In addition, upon activation that leads to the elevation of intracellular Ca^{2+}, neutrophils release a number of other proteins, including a heterodimer consisting of S100A8 and S100A9, which belong to the alarmin or damage-associated molecular pattern (DAMP) family of danger signals that initiate and modulate local inflammation and innate immune responses (Bhardwaj et al. 1992; Burwinkel et al. 1994; Foell et al. 2007; Vogl et al. 2007; Cheng et al. 2008). Of note, S100A8 and S100A9 are among the most abundant cytoplasmic proteins in neutrophils and macrophages (Odink et al. 1987; Roth et al. 1993, 2003; Foell and Roth 2004*b*), and the heterodimer is released in significant abundance in sepsis, rheumatoid arthritis, inflammatory bowel disease, and cancer (Hessian et al. 1993; Roth et al. 2003; Wulffraat et al. 2003; Sunahori et al. 2006; Cheng et al. 2008; Foell et al. 2008). To date there has been little focus on the possible role of myeloid cell-derived S100A8/A9 and its consequences in allergic asthma, which could be of particular relevance in neutrophil-dependent severe asthma. Thus in the remainder of this review we outline key features of this protein complex, its receptor-mediated cell signaling and biological activity, and its possible relevance to airway inflammation and remodeling.

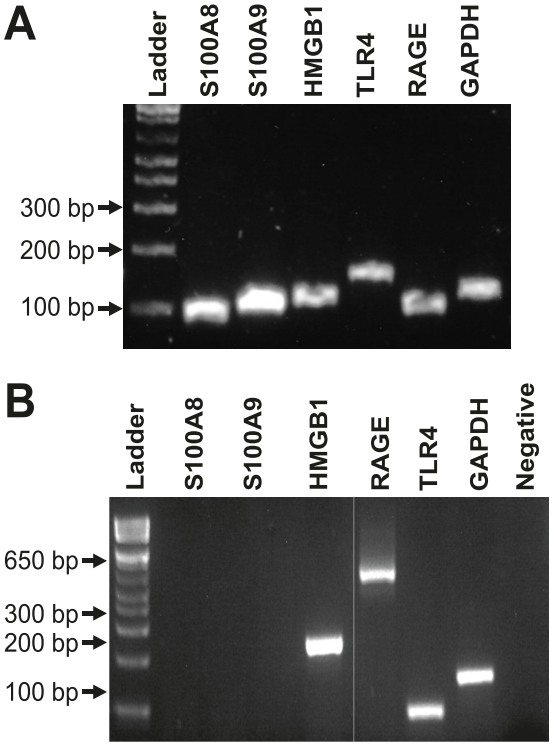
The S100 family

S100 protein family members are a specialized group of cell- and tissue-specific EF-hand Ca^{2+}-binding proteins that are found in humans and other vertebrate animals, but are apparently absent in invertebrates (Marenholz et al. 2004; Heizmann et al. 2007). Several S100 proteins may also bind Zn^{2+} in a physiologically relevant range (Heizmann and Cox 1998), as well as Mn^{2+} and Cu^{2+}, although the functional role for binding these ions is not fully established. Notably, S100 proteins have both an intracellular and an extracellular biological role. Intracellular S100 proteins mediate Ca^{2+}-dependent responses and can be associated with other partners; for example, S100A8/A9 binds annexin 6 until each is mobilized to the cell membrane (Bode et al. 2008). S100 protein dimers are also expressed on the cell surface and released by cells through a Ca^{2+}-dependent mechanism (Roth et al. 1993), where the complexes can induce receptor-mediated cell responses involved in inflammation and tissue injury and repair.

Since the initial discovery of S100A1 and S100B in bovine brain (Moore 1965), 20 *S100* genes have been identified, most of the genes being clustered on human chromosome 1q21 (S100A1–S100A16) (Marenholz et al. 2004). Because of an overabundance of names given to the members of the S100 family, a universal nomenclature has now been adopted (Schäfer et al. 1995; Marenholz et al. 2004, 2006). Although all S100 proteins form noncovalent homodimers in vitro and in vivo, some, including S100A8 and S100A9, form heterodimers (Santamaria-Kisiel et al. 2006). It is possible that S100 proteins form covalent dimers in the extracellular milieu; for example, oxidation of S100B into a disulphide cross-linked form has been shown to promote neurotropic effects in the extracellular space (Barger et al. 1992). S100 proteins can also form a variety of higher-order oligomers in vitro and in vivo, such as S100B tetramers (Ostendorp et al. 2007), and although these appear to have some physiological relevance, this issue is not yet resolved.

S100 proteins are believed to have evolved in complex organisms in such a way as to enable direct intracellular activation of specific Ca^{2+}-sensitive biochemical pathways. All members of the family except S100G assemble into a distinctive dimeric architecture first elucidated for S100A6 (Potts et al. 1995). The distinct S100 dimer architecture implies that their mode of interaction with effectors is fundamentally different from archetypal Ca^{2+} sensors such as calmodulin (Sastry et al. 1998). EF-hand proteins are characterized by a helix–loop–helix structural motif comprising entry (E) and exiting (F) helices connected by a conserved 12-residue loop that chelates one Ca^{2+} ion (Kretsinger and Nockolds 1973; Bode et al. 2008). The functional unit is a pair of EF hands capable of binding 2 Ca^{2+} ions. The ability of EF-hand proteins to transduce signals stems from struc-

Fig. 2. RT-PCR analysis of S100A8, S100A9, HMGB1, TLR4, and RAGE in whole lung and primary cultured human airway smooth muscle cells. Ethidium bromide-stained agarose gel of PCR products from mRNA originally isolated from (A) murine (BALB/c) whole lung, and (B) primary cultured human airway smooth muscle cells. Arrows on the left indicate the approximate base pair of the cDNA size markers shown on the Ladder. Negative refers to PCR reaction conducted using water instead of sample. TLR4, Toll-like receptor 4; RAGE, receptor for advanced glycation end products.



tural changes that occur upon binding Ca^{2+}, whereby the EF-hand domains transition from a closed state, typically seen at basal levels of cell Ca^{2+}, to an open and active state, induced when intracellular Ca^{2+} levels rise, which enables interaction of the protein with downstream effector proteins of a range of cell responses (Donato 2001). Specific functions identified for S100 proteins include the modulation of cell growth and differentiation, cell cycle progression, cell attachment and motility, specific signal transduction pathways, and transcription (Donato 2003; Heizmann et al. 2007).

The S100 class of EF-hand proteins is also characterized by the ability to be secreted into the extracellular space at sites of acute and chronic inflammation involving activation of phagocytes such as macrophages and neutrophils. Thus, their expression on the cell surface and release is associated with a broad range of disorders. As S100 proteins lack a so-called leader sequence, they are secreted via a nonclassic pathway, although the precise mechanism for export is not yet fully established. It does appear that mobilization of S100 dimers to the plasma membrane relies in part on the formation of complexes with intracellular annexins, which bind phospholipids and bring different membranes into proximity and promote their fusion (Davey et al. 2001). A recent report, for example, revealed a requirement for annexin 6 in cell surface exposition of S100A8/A9 in cytokine-exposed breast cancer cells (Bode et al. 2008). Intracellular Ca^{2+} mobilization, and possibly also the presence of Zn^{2+}, is an essential trigger for S100 dimer release from a number of cell types, including those of myeloid origin (Roth et al. 1993; Davey et al. 2001). Once secreted, S100 proteins can induce a number of biological effects associated with inflammation and tissue injury/repair including a unique endothelial inflammatory profile leading to vascular leakage and thrombosis, but also cell growth and apoptosis, neurite outgrowth, innate immunity responses, inflammatory cell recruitment, and release of cytokines and other inflammation-associated mediators, such as high-mobility group box 1 (HMGB1) protein, perhaps the best-studied member of the DAMP family to date (Bianchi 2007; Klune et al. 2008). Interestingly, these activities appear to be tied, in part, to the ability of S100 proteins to bind metals such as Zn^{2+} and Mn^{2+} rather than Ca^{2+}. Indeed, the binding of Zn^{2+} appears to provide both a direct mechanism for generating antimicrobial activity and a means for triggering S100 proteins to interact with certain cell surface receptors (Ghavami et al. 2004; Corbin et al. 2008; Ghavami et al. 2008*a*). When complexed with arachidonic acid, S100A8/A9 binds to the scavenger receptor CD36 on endothelial cells and facilitates fatty acid uptake (Kerkhoff et al. 2001). In addition, S100A8/A9 expressed on the surface of leukocytes binds to carboxylated endothelial glycoproteins, an interaction required for leukocyte adhesion (Srikrishna et al. 2001). On a range of cells, S100 proteins, including S100A8/A9 heterodimers, bind to and activate responses mediated by 2 widely expressed but divergent receptor subtypes: the receptor for advanced glycation end products (RAGE) and Toll-like receptor 4 (TLR4) (Hofmann et al. 1999; Huttunen et al. 2000; Hermani et al. 2006; Vogl et al. 2007; Ghavami et al. 2008*b*; Turovskaya et al. 2008). Of central importance to the focus of the current review is that both RAGE and TLR4 are abundantly expressed in the lung and airway mesenchymal cells (Fig. 2), where they are respectively involved in fibroproliferative pulmonary disease and in establishing acute and chronic inflammatory profiles in the lung.

The S100A8 and S100A9 heterodimer: role in inflammation

S100A8 and S100A9 subunits are Ca^{2+}- and Zn^{2+}-binding EF-hand proteins of the S100 family that are released from activated phagocytes as a heterodimeric complex in which S100A8 is the active component and S100A9 holds a modulator role (Dale et al. 1983; Schäfer and Heizmann 1996; Vogl et al. 2007). Each subunit is encoded by a separate gene, *Mrp8* for S100A8, and *Mrp14* for S100A9. The smaller 8 kDa component, S100A8, is also called myeloid-related protein (MRP) 8 and calgranulin A. The larger 14 kDa component, S100A9, is also called MRP14 and calgranulin B (Odink et al. 1987; Wilkinson et al. 1988). S100A8 and S100A9 monomers demonstrate a strong preference to form a heterodimer complex, which is regarded as the functionally relevant form (Hunter and Chazin 1998; Vogl et al. 2006). The S100A8/A9 complex has been suggested as a biomarker for inflammation, and of use for mon-

itoring disease activity and response to treatment (Foell et al. 2004*a*). The proteins were first discovered in children with cystic fibrosis, but elevated serum levels have also been reported for patients with rheumatoid arthritis, chronic bronchitis, AIDS, diabetes, and cancer (Sorg 1992; Lügering et al. 1995; Strasser et al. 1997). Local tissue concentration is also markedly increased in association with rheumatoid arthritis (Berntzen et al. 1991), inflammatory bowel disease (Røseth et al. 1992), viral or microbial infections (Sander et al. 1984; Müller et al. 1994), tumor growth (Stulík et al. 1999), and a number of other inflammatory states (Zwadlo et al. 1988; Johne et al. 1997; Stulík et al. 1997; Nacken et al. 2003; Foell et al. 2004*a*; Fessatou et al. 2005; Gebhardt et al. 2006).

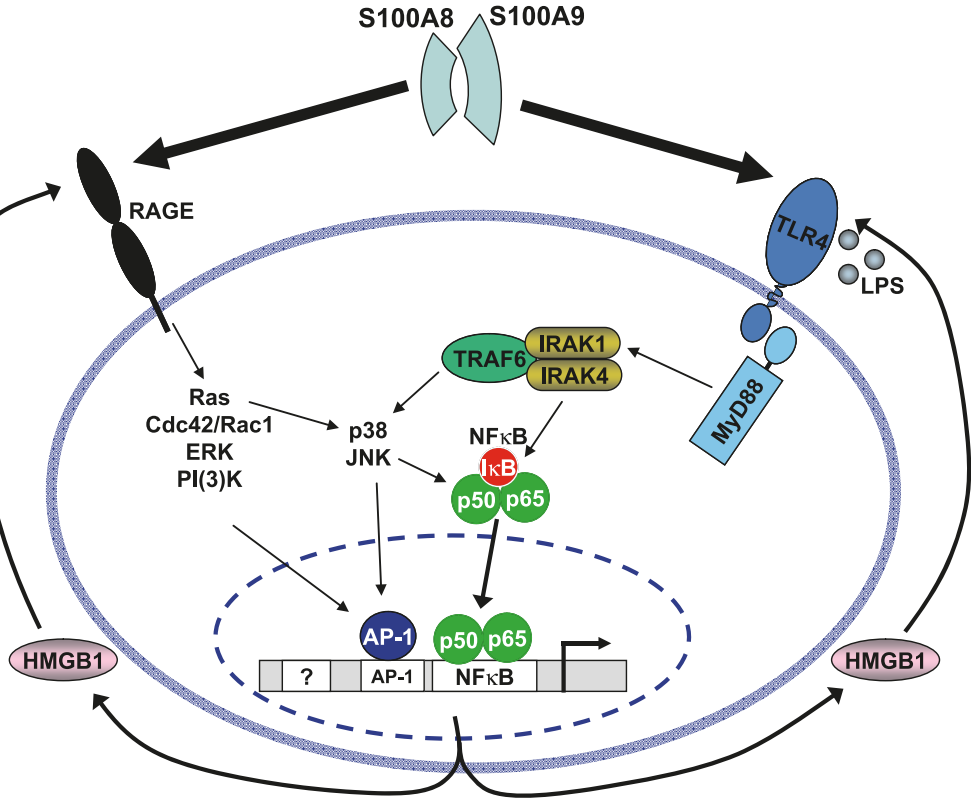
S100A8 and S100A9 are expressed in great abundance by neutrophils, activated monocytes, and macrophages, contributing to 40%–50% of the soluble, cytosolic content of granulocytes (Dale et al. 1983). The proteins have diverse intracellular functions in myeloid cells and are associated with cytoskeletal rearrangements, arachidonic acid metabolism, and the regulation of neutrophilic NADPH oxidase (Doussiere et al. 2002; Nacken et al. 2003; Vogl et al. 2004; Tugizov et al. 2005). Nonetheless, most biological functions relevant to infectious and sterile inflammation are associated with plasma membrane expression and the release of the S100A8/A9 heterodimer into the extracellular space (Katz and Taichman 1999). S100A8/A9 is best characterized in relation to neutrophils and macrophages as being a component of the innate immune system, particularly considering recent reports that the secreted complex is a direct ligand for TLR4 and a modulator of dendritic cell differentiation (Rammes et al. 1997; Ghavami et al. 2004; Vogl et al. 2007; Cheng et al. 2008). Notably, S100A8/A9-positive myeloid cells are the first cells to infiltrate inflammatory lesions and it appears that S100A8/A9 promotes further leukocyte recruitment (Roth et al. 2003; Ghavami et al. 2008*b*), as the complex reportedly regulates leukocyte adhesion and chemotaxis, phagocytosis, exocytosis, and ROS generation (Klempt et al. 1997; Rammes et al. 1997; Yui et al. 1997). Monocytes and macrophages that express S100A8/A9 also secret abundant TNF-α (Kerkhoff et al. 1999), and S100A8/A9 can induce TNF-α expression in human and mouse macrophages and microvascular endothelial cells (Xu and Geczy 2000; Viemann et al. 2005). S100A8/A9 can bind Zn^{2+} and Mn^{2+} (Corbin et al. 2008), and the binding of these divalent ions is thought to be an important mechanism for their antimicrobial activity; for example, tissue abscesses can contain more than 1 mg/mL of the protein complex (Clohessy and Golden 1995; Corbin et al. 2008). At high concentrations, the capacity for S100A8/A9 to sequester Zn^{2+} appears to be associated with an ability to induce cell death in some human cells (Ghavami et al. 2004; Nakatani et al. 2005; Ghavami et al. 2008*a*). In contrast, and consistent with the effects of S100B (Huttunen et al. 2000), at low concentrations S100A8/A9 can promote cell proliferation through mechanisms mediated by RAGE expressed on the surface of target cells (Ghavami et al. 2004, 2008*a*, 2008*b*). Thus, it is now evident that S100A8/A9 has diverse effects and multiple functions in infection, tissue injury, cell growth and survival, and immune responses.

Hypothesis: receptor-mediated mechanisms for S100A8/A9 in airway inflammation and asthma

Despite its abundance in conditions such as rheumatoid arthritis and inflammatory bowel disease, which more-or-less mimic the profile of inflammation in asthma, a link between S100 proteins and the risk for asthma remains largely unresolved. Eosinophil-derived S100A12 has been suggested to potentiate both asthma and innate immunity via RAGE by promoting airway inflammation, monocyte recruitment, and mast cell degranulation in vitro and in vivo (Yang et al. 2007). There is a recent report that polymorphisms in a 0.5 Mb region of human chromosome 1q21, which harbors the *S100* family gene cluster that includes S100A8 and S100A9, show significant association with elevated serum IgE levels in populations that include atopic asthma patients (Sharma et al. 2007). This is consistent with previous studies linking this chromosomal location to atopic dermatitis and other autoimmune pathologies (Mischke et al. 1996). Using a proteomics approach, Greenlee and colleagues (2006) determined that matrix metalloproteinase-2 and -9 promote egress of lung inflammatory cells through the airways by virtue of the ability to cleave specific bioactive proteins in the airways, including S100A8 and S100A9. Moreover, they confirmed that function-blocking antibodies to S100 proteins significantly modulated allergic inflammatory cell migration into the alveolar space. Of particular note, S100A8 has also been identified as a corticosteroid-refractory protein induced by transnasal LPS exposure leading to profound neutrophilic lung inflammation in mice (Bozinovski et al. 2005). Steroid-refractory S100A8 expression was highly abundant, transcriptionally regulated, secreted into lung lavage fluid, and localized to tissue neutrophils. Collectively, these observations suggest S100A8/A9 may be associated with chronic allergic airway and lung inflammation that may be insensitive to steroid therapy.

The paradigm we propose for a role for S100A8/A9, using both RAGE and TLR4, expressed in the lung and by airway mesenchymal cells, is based in part on preliminary evidence from our laboratory for the expression of S100A8/A9 in the lung, and its receptors (RAGE and TLR4) by airway mesenchymal cells (Fig. 2). We have not detected any evidence for direct expression of S100A8 or S100A9 by human ASM or fibroblasts (Fig. 2B), but there is ample transcript expressed by other lung cells, most likely alveolar macrophages and neutrophils. In total, this suggests the potential for the existence of an effector pathway to promote local inflammation and tissue repair involving airway mesenchymal cells that would support the progression of airway remodeling. In support of this idea, we have also reported novel mechanisms for the extracellular activity of S100A8/A9 on tumor and normal somatic cells, including promoting their proliferation at low concentration (<20 μg/mL) and apoptosis at high concentration (>25 μg/mL) via intracellular signaling pathways triggered by the binding of S100A8/A9 with RAGE (Ghavami et al. 2004, 2008*a*, 2008*b*). A key role for S100A8/A9 in initiation and modulation of inflammation is strongly supported by recent reports that tumor-derived S100A8/A9 can induce formation of myeloid-derived suppressor cells that inhibit dendritic cell

Fig. 3. Schematic representation of the proposed major receptor-mediated signaling pathways induced by S100A8/A9 in airway mesenchymal cells. S100A8/A9 can independently activate intracellular signaling pathways via RAGE and TLR4. S100A8/A9 can induce secretion of HMGB1, which is also a ligand for RAGE, and can work in concert with LPS to potentiate activation of TLR4. RAGE can induce multiple signaling pathways including Ras/mitogen-activated protein kinases (ERK, p38, JNK), PI3K, and Cdc42/Rac1 small GTPases. These pathways have been linked, directly or indirectly, with the activation of NF-κB and AP-1 (c-Fos/c-Jun)-regulated transcription. TLR4 activates a canonical transduction pathway, in which the MyD88 adaptor protein facilitates IRAK-1 phosphorylation and signaling through TRAF6, and a protein kinase cascade to activate NF-κB and AP-1. LPS, lipopolysaccharide; PI3K, phosphatidylinositol 3-kinase; NF-κB, nuclear factor κB; AP-1, activator protein-1; IRAK, IL-1 receptor-associated kinase; TRAF6, TNF receptor-associated factor 6.



differentiation (Cheng et al. 2008). Dendritic cells are essential for antigen presentation that leads to the subsequent activation and phenotype of T lymphocytes. This response is modulated by Toll-like receptors, critical determinants of innate immunity, and thus it is significant that S100A8/A9 can amplify the endotoxin-triggered inflammatory responses of phagocytes by direct activation of TLR4 (Vogl et al. 2007). Moreover, S100A8/A9 also promotes the release of the well-characterized proinflammatory alarmin HMGB1 from tumor, somatic, and myeloid cells (Hamada et al. 2008). Collectively these observations and those described hereafter, underpin the development our hypothesis linking S100A8/A9 with asthma pathogenesis (Figs. 1B and 3).

HMGB1, originally identified as a transcription factor, is released from necrotic cells or actively secreted from macrophages, dendritic cells, and natural killer cells during infectious and sterile inflammation (Lotze and Tracey 2005; Ellerman et al. 2007). The protein activates cells involved in the immune process, and recent evidence suggests it has a central role as a proinflammatory mediator in autoimmune disorders, cancer, trauma, and ischemic reperfusion injury (Bianchi 2007; Klune et al. 2008). Of note for a possible role in the airway remodeling process, HMGB1 is released from airway epithelial cells and inflammatory cells and is significantly elevated in lung lavage from patients with idiopathic pulmonary fibrosis; inflammation and fibrosis in bleomycin-exposed mice are suppressed by HMGB1-blocking antibodies (Hamada et al. 2008). In addition, like S100A8/A9, HMGB1 can induce somatic cell proliferation, most notably in lung fibroblasts (Hamada et al. 2008). HMGB1 shares an additional important similarity with S100A8/A9: it also binds to and activates RAGE and TLR4 (Park et al. 2004; He et al. 2007), although HMGB1 also has affinity for additional Toll-like receptor subtypes (Park et al. 2006). Collectively these data suggest neutrophil-derived S100A8/A9 could induce effects that promote airway inflammation and remodeling through effector cells that express RAGE and TLR4. Furthermore, this likely involves induction and release of HMGB1, either in parallel with S100A8/A9 or induced by S100A8/A9, which is capable of promoting inflammation and tissue repair via a similar repertoire of receptors expressed by resident effector cells (Figs. 1B and 3).

RAGE is a member of the immunoglobulin super family of cell surface receptors (Schmidt et al. 1992). In most healthy adult tissues RAGE is expressed at low to undetectable levels; however, it is relatively highly expressed in normal adult lungs (Brett et al. 1993; Hanford et al. 2003; Demling et al. 2006) (Fig. 2A). Within the lung, our early

studies demonstrated that RAGE is expressed abundantly by airway mesenchymal cells (Fig. 2B). Activation of RAGE by its multiple ligands, which include S100 proteins, advanced glycation end products, HMGB1, and amyloid-β peptide, often leads to proinflammatory signaling as well as marked auto-upregulation of RAGE expression (Schraml et al. 1997). RAGE can act as an endothelial adhesion receptor promoting leukocyte recruitment to inflamed environments, which appear to require direct contact of RAGE with the β_2-integrin Mac-1 (Chavakis et al. 2003). Intracellular signaling mediated via RAGE includes Ras/mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K), the Janus kinases (JAKs), monomeric GTPases such as Cdc42/Rac, and transcription factors including signal transducer and activator of transcription protein (STAT) 3, nuclear factor (NF)-κB, and activator protein (AP)-1 (Huttunen et al. 1999; Taguchi et al. 2000; Donato 2001; Ghavami et al. 2008*b*) (Fig. 3). Proximal signaling mechanisms, which probably involve the cytosolic tail of the receptor, have not been characterized. Interestingly, RAGE-mediated NF-κB activation is of prolonged time course, which appears to overwrite endogenous autoregulatory feedback loops. Owing to its ability to sustain cellular activation, RAGE can convert a transient proinflammatory response into sustained cellular dysfunction (Bierhaus et al. 2005). RAGE appears to be involved with epithelial–mesenchymal cell transdifferentiation that is essential for pulmonary reepithelialization and repair (Fehrenbach et al. 1998; Dahlin et al. 2004; Shirasawa et al. 2004). Moreover RAGE appears to be essential for mediating lung fibrosis induced by bleomycin, which induces S100A8/A9 and HMGB1, as RAGE knockout mice exhibit markedly suppressed pulmonary fibrosis. As airways fibrosis is a hallmark of airway remodeling, RAGE expressed by resident cells (Fig. 2B) is likely to be a mediator of this process. To date this possibility has not been tested, but it is clear that future studies are warranted on the role of RAGE and S100A8/A9 in airway remodeling.

TLR4 is a member of the 10-member TLR family characterized by an intracellular Toll/IL-1 receptor (TIR) domain from which originates cytoplasmic signaling (Takeda and Akira 2004). TLR4 is expressed in widely divergent cell types in the lung, including airway mesenchymal cells (Fig. 2B). The canonical TLR4 signaling pathway includes association with a TIR-containing adaptor protein, MyD88, which recruits IL-1 receptor-associated kinase (IRAK)-4 and -1. IRAK-4 phosphorylates IRAK-1 and then associates with TNF receptor-associated factor (TRAF) 6, leading to activation of distinct pathways that activate c-Jun N-terminal kinase (JNK) and its downstream targets AP-1 or NF-κB, and thus promoting the expression of inflammatory cytokines (Takeda and Akira 2004) (Fig. 3). TLR4 binds LPS and is important in regulating innate immune responses. More recent evidence shows that TLR4 binds S100A8/A9, inducing canonical signaling, and that HMGB1 can also augment activation of TLR4 signaling triggered by LPS (Cheng et al. 2008).

Conclusions and future directions

Severe asthma, unresponsive to common steroid treatment, presents a frustrating challenge. Biological evidence indicates that steroid-refractory severe asthma is associated with high numbers of airway neutrophils, thus suggesting that treatment strategies targeting inflammatory processes associated with neutrophils may provide improvement in treatment response. As S100A8/A9 is one of the most highly expressed proinflammatory proteins expressed by neutrophils, and growing evidence reveals that this protein complex has proinflammatory properties and promotes tissue damage and repair, better understanding of the role of this mediator in asthma pathogenesis is clearly needed. In this review we have provided an overview of current knowledge about S100A8/A9, its biological effects, and the receptor-mediated responses that can occur in target cells. Collectively, this information suggests a complex regulatory system, involving multiple receptors (i.e., RAGE and TLR4) and secondary proinflammatory mediators (i.e., HMGB1), that recruits and integrates multiple intracellular signaling responses in a number of cell types, including inflammatory cells and resident structural cells (i.e., airway smooth muscle, fibroblasts, and epithelial cells). In total, this platform could subserve asthma pathogenesis in a steroid-refractory manner. This is a key question to be addressed in the near future, as insight will provide opportunity for developing more effective treatment options for patients with severe asthma.

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