Chitinase-like Proteins in Lung Injury, Repair, and Metastasis

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This report explains how our studies of asthma and Th2 inflammation led us to investigate the roles of chitinase-like proteins (CLPs) in lung injury and repair and puts forth an overall hypothesis that can explain the roles that these moieties play in biology and a hypothesis regarding the ways that dysregulated CLP expression may contribute to the pathogenesis of a variety of diseases. We test this hypothesis by assessing the contributions of the CLP breast regression protein (BRP)-39 in the pathogenesis of malignant melanoma metastasis to the lung.

Keywords: BRP-39/YKL-40; inflammation; injury; repair; metastasis

ASTHMA, TH2 INFLAMMATION, AND IL-13

The ability of asthma to cause episodic symptoms has been noted since antiquity, and a striking increase in asthma prevalence has been noted in western societies in recent decades. As a result of this “epidemic,” asthma now affects 300 million people globally and costs our collective societies billions of dollars annually. In keeping with its importance, we have entertained concepts of asthma pathogenesis that have extended from smooth muscle abnormalities to autonomic dysfunction to IgE-mediated responses. Studies using bronchoscopy and bronchoalveolar lavage have led to the contention that asthma is a chronic inflammatory disorder of the airway characterized by the excess production and consequences of type 2 cytokines. They also led to the appreciation that structural alterations, including fibrosis in the lamina reticularis and adventitia, mucus metaplasia, myocyte hypertrophy, and hyperplasia and neo-vascularization, are commonly seen in asthma and to the hypothesis that the inflammation causes these remodeling events, which, in turn, contribute to the pathogenesis and symptoms of the disorder. The importance of the Th1/Th2 paradigm for asthma was initially appreciated in studies that described the enhanced expression of Th2 cytokines at sites of asthmatic inflammation (1). This was followed by murine investigations using aeroallergen challenge and other modeling systems that demonstrated that IL-13 plays a critical role in the pathogenesis of Th2 inflammation (2, 3). Simultaneously, transgenic and other approaches demonstrated that IL-13 alone was able to induce striking asthma-like tissue and physiologic responses, including eosinophilic inflammation, mucus metaplasia, airways fibrosis, and airways hyperresponsiveness (4). In combination,

these murine studies demonstrated that IL-13 was necessary and sufficient to generate asthma-like Th2 responses in the mouse lung. Based on this solid foundation, human investigations were undertaken that highlighted the exaggerated expression of IL-13 in asthmatic tissues and BAL (5, 6), associations between polymorphisms of IL-13 and its receptor and asthma, and, most recently, the success of anti-IL-13-based interventions in human asthma (7). In combination, these studies demonstrated that asthma is associated with exaggerated Th2 inflammation and that IL-13 is a major effector at sites of Th2 inflammation and remodeling.

THE 18 GLYCOSYL HYDROLASE GENE FAMILY AND YKL-40/BRP-39

The 18 glycosyl hydrolase (GH18) gene family contains true chitinases (Cs) that bind and cleave chitin and chitinase-like proteins (CLP; also called chitolectins) that bind but do not cleave this polysaccharide. These C/CLP are found across species from lower life forms (archaea, prokaryotes, and eukaryotes) to humans. In addition, during speciation, selective pressures have caused gene duplication and loss and have changed the components of this family, increasing the number of Cs in species with chitin and decreasing the number of Cs and increasing the number of CLP in chitin-free animals (8, 9). In keeping with this impressive retention and modulation over species and time, many researchers have speculated that these moieties play essential roles in biology. The nature of their contributions have been enigmatic because chitin is the only documented substrate of Cs, chitin and chitin synthase do not exist in mammals, and higher life forms do not use chitin as a nutrient (8, 9). However, the fact that there is a prominent increase in CLP in the period preceding mammalian evolution has led to the speculation that CLP are important in mammalian development, biology, and physiology and the speculation that they sit at the interface of glyco- and protein biology (9). Their roles in these processes have only recently begun to be defined.

BRP-39, the prototypic CLP, was originally discovered in mouse breast cancer cells (10). Subsequently, a variety of homologs with different names were described, including human YKL-40, porcine 38-kD heparin-binding glycoprotein, bovine 39-kD whey protein, and drosophila Imaginal Disc Growth Factors. BRP-39 and YKL-40 are produced by the *chitinase 3-like-1* (*Chi3l1*) gene on chromosome 1 in mouse and human as 39-kD proteins that lack chitinase activity. YKL-40/BRP-39 are expressed in and produced by a variety of cells, including macrophages; neutrophils; chondrocytes; fibroblasts; vascular smooth muscle cells; endothelial cells; hepatic stellate cells; and colonic, ductal, and airway epithelial cells (11, 12). YKL-40 is not expressed in monocytes and is marginally expressed in monocyte-derived dendritic cells but is strongly induced during specific stages of human macrophage differentiation. Thus, YKL-40 is regarded as a macrophage

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differentiation marker (13, 14). Studies from our laboratory and others have demonstrated that YKL-40/BRP-39 is stimulated by IL-13, IL-6, IFN- γ , TNF- α , IL-1 β , vasopressin, and parathyroid hormone-related protein in a variety of tissues (12, 15, 16). The contributions of these mechanisms to the dysregulation of YKL-40 that is seen during injury and repair and health and disease (see below) are poorly understood.

CLP IN HUMAN DISEASE

In recent years, elevated levels of YKL-40 have been noted in a wide variety of human diseases. Studies from our laboratory and others demonstrated that the levels of YKL-40 are increased in asthma, where they correlate with disease severity and the degree of airway fibrosis (17). Our studies of Caucasian and Asian populations also demonstrated that *Chi3l1* is a risk factor gene with promoter polymorphisms that correlate with the levels of circulating YKL-40, asthma prevalence, abnormal lung function, and atopy and allow for differential transcription factor binding (18, 19). Elevated levels of YKL-40 have also been noted in infectious diseases (meningitis and pneumonia), chronic inflammatory and remodeling diseases (rheumatoid arthritis, osteoarthritis, systemic lupus, inflammatory bowel disease, and chronic obstructive lung disease), hepatic disorders (alcoholic hepatitis and cirrhosis, hepatitis C virus-mediated fibrosis, autoimmune hepatitis-induced cirrhosis, and primary biliary cirrhosis), diabetes (type 1 and type 2), atherosclerosis, giant cell arteritis, and a variety of malignancies (20–39). Elevated levels of YKL-40 are also seen in fibrotic disorders, including sarcoidosis (26), asthmatic airway remodeling (17), and hepatic fibrosis (37, 40). In many of these diseases, the levels of YKL-40 correlate with disease severity, as noted in asthma.

THE BIOLOGY OF BRP-39/YKL-40

To address the biology of CLP, we initiated murine studies of BRP-39/YKL-40. These studies used genetically modified mice

generated in our laboratory, including BRP-39 null ($^{-/-}$) mice, mice in which YKL-40 is expressed in an inducible transgenic fashion in lung airway epithelium (YKL-40 Tg), and mice that lack BRP-39 and express YKL-40 only in lung airway epithelium (YKL-40Tg/BRP-39 $^{-/-}$) (12). These mice were studied in *in vivo* models of lung inflammation and repair. The respiratory models included aeroallergen-induced Th2 inflammation, IL-13-induced inflammation and fibrosis, hyperoxia-induced oxidant lung injury (12, 41), and pneumococcal lung infection. *In vitro* studies with murine and human cells and tissues were also undertaken.

Aeroallergen-induced Th2 Inflammation

These studies demonstrated that BRP-39 is induced in macrophages and epithelial cells at sites of aeroallergen-induced pulmonary inflammation and that this inflammation is significantly decreased in the BRP-39 $^{-/-}$ mice. This decrease was due, at least in part, to accelerated apoptosis of M2 macrophages and CD4 $^{+}$ T cells and exaggerated expression of Fas in BRP-39 $^{-/-}$ animals. BRP-39 was also a potent stimulator of M2 macrophage differentiation (comparable to IL-4) and inhibited Fas ligand- and TNF-induced macrophage and T-cell apoptosis *in vitro* (12). It also increased dendritic cell accumulation and activation (Figure 1).

Transgenic IL-13

To define the role of BRP-39 in the pathogenesis of the inflammatory and remodeling responses that are induced by IL-13, we crossed mice in which IL-13 was expressed in a transgenic manner in airway epithelial cells and the BRP-39 $^{-/-}$ animals. These studies demonstrated that IL-13 is a potent and chronic stimulator of BRP-39 and that IL-13-induced inflammation and fibrosis are markedly decreased in the absence of BRP-39. They also demonstrate that IL-13 induces TGF- β 1 via a BRP-39-dependent mechanism(s) (12) (Figure 1).

Hyperoxic Acute Lung Injury

The roles of BRP-39 in pulmonary oxidant injury were assessed by comparing the responses in wild-type (WT) and null mice in

Figure 1. Proposed roles of BRP-39/YKL-40 in Th2 inflammation and remodeling. Antigen interacts with antigen-presenting cells (APCs) in an appropriate host tissue compartment in the presence of BRP-39/YKL-40. The BRP-39/YKL-40 contributes to Th2 responses by augmenting sensitization and inducing effector responses. The former includes the stimulation of dendritic cell (DC) accumulation and activation. The latter includes the inhibition of CD4 cell, macrophage (macro) and eosinophil (Eos) cell death, the induction of M2 macrophage differentiation, and the stimulation of TGF- β 1 production. In combination, these responses contribute to Th2 inflammation, mucus metaplasia, and tissue fibrosis.

Figure 2. BRP-39/YKL-40 in the idealized antipathogen response. A successful antipathogen response would include macrophage (MO) phagocytosis and pathogen killing, appropriately controlled innate immune and oxidant responses that kill the pathogen without causing major bystander tissue injury, the generation of antigen-specific adaptive immunity, and the induction of responses that wall off the offending agent or induce tissue repair and healing. BRP-39/YKL-40 may be a major contributor to antipathogen responses because it keeps macrophages alive long enough to allow them to kill pathogens like bacteria, controls innate and oxidant responses, augments the generation of adaptive immunity, and contributes to the fibrosis that is seen in abscess formation and tissue healing and repair by participating in the induction of TGF- β 1.

TABLE 1. BRP-39/YKL-40/CHI3L1 AXIS HYPOTHESES

1. The BRP-39/YKL-40/CHI3L1 system is designed to contribute to prereproductive or reproductive age antipathogen responses.
2. Chronic elevation of YKL-40 that is out of proportion to or independent of pathogen infection contributes to pathologic responses by

a. Inhibition of innate immunity

b. Loss of physiologic apoptosis (cancer, inflammation, atherosclerosis)

c. Exaggerated adaptive immunity

d. Stimulation of tissue fibrosis

100% oxygen. In these studies, epithelial injury and apoptosis and lung permeability were exaggerated in BRP-39^{-/-} mice (41).

Pneumococcal Pneumonia

The roles of BRP-39 in pneumococcal pneumonia have recently begun to be investigated. This was done by comparing the pneumococcus-induced responses in BRP-39 null and WT mice. These studies demonstrated that BRP-39 plays a critical role in the antipneumococcal response, with null mice manifesting exaggerated inflammation and injury, augmented innate immune activation, decreased bacterial clearance, and exaggerated mortality compared with WT control mice. Comparisons of macrophages from null and WT mice demonstrated that the decrease in bacterial clearance was associated with normal levels of bacterial phagocytosis, decreased bacterial killing, and enhanced bacteria-induced macrophage cell death.

When viewed in combination, these studies demonstrate that BRP-39/YKL-40 contributes to the pathogenesis of adaptive Th2 immunity and mediate tissue effects of IL-13 while protecting against nonspecific oxidant injury. They also suggest that BRP-39/YKL-40 contributes to the pathogenesis of fibrosis via its ability to stimulate TGF-β1.

A UNIFYING HYPOTHESIS

BRP-39/YKL-40 is an evolutionarily ancient moiety that plays complex roles in bacterial clearance, innate and adaptive immunity, and tissue healing and repair. Because the retention of this and related moieties over species and time would likely be related to their ability to confer a survival advantage for early humans, we speculated on how this might be happening. In keeping with the concept that the immune response is designed to control pathogens, we highlighted the features of an “idealized” antipathogen response. Specifically, we can see how a successful

Figure 4. Comparisons of the levels of circulating YKL-40 in patients with malignant melanoma. Patients with superficial lesions that were removed without evidence of metastasis (control subjects) are compared with patients with advanced stage 4 disease.

response would require (1) efficient phagocytosis and pathogen killing, (2) an appropriate innate and oxidant response that controls the pathogen while minimizing local tissue injury, (3) processes that augment the transition from nonspecific innate immunity to antigen-specific adaptive immunity (this transition would contribute to pathogen control while minimizing bystander tissue damage and contributing to long-term immunity), and (4) mechanisms that stimulate a healing to initiate repair and, if needed, wall off the offending agent.

The studies noted above give insights into how BRP-39/YKL-40 might contribute to each of these idealized responses because BRP-39/YKL-40 (1) controls the survival and augments the bacterial killing of macrophages that have ingested pneumococcus, (2) controls innate immune responses, (3) augments Th2 adaptive immunity, and (4) initiates healing by contributing to the induction of TGF-β1 (Figure 2). This allows for the hypothesis that the BRP-39/YKL-40/Chi3l1 axis (and possibly other CLPs) is designed to foster prereproductive or reproductive antipathogen responses. It also allows for the hypothesis that chronic elevations of YKL-40 that are out of proportion to or occur in the absence of pathogen infection contribute to disease pathology via exaggerated physiologic effector responses, including suppressed innate immunity, loss of physiologic cell death, exaggerated adaptive immunity, and augmented tissue fibrosis (Table 1).

ROLE OF BRP-39 IN MELANOMA METASTASIS

A number of studies have demonstrated strong correlations between the expression of YKL-40 and the development of primary and metastatic malignancies. This includes tumors of the breast, colon/rectum, ovary, lung, prostate, kidney, brain (glioblastoma), bone (osteosarcoma), and melanoma (for details, see References 21 and 25). However, it is not known if YKL-40 is a biomarker or contributes to disease pathogenesis in these settings. If the hypothesis we put forth (Table 1) is correct, elevated levels of YKL-40 should contribute to disease pathology. To test this hypothesis, we compared the magnitude of the metastatic response in WT mice and BRP-39^{-/-} mice treated with B-16-F10 melanoma cells via tail vein infusion. We also used VEGF transgenic (Tg) mice in which the CC10 promoter was used to target VEGF to the lung (42). Metastasis was seen in WT mice and was increased in VEGF Tg mice. In both cases, the levels of metastasis were decreased in the absence of BRP-39. This can be seen in Figure 3, which compares the metastatic burden in WT mice treated with preimmune serum and antiserum against BRP-39. In accord with our hypothesis, these

Figure 3. Role of BRP-39 in pulmonary melanoma metastasis. Wild-type mice were treated with malignant melanoma cells by tail vein injection and treated with antiserum against BRP-39 or preimmune control antiserum. The number of pleural surface melanoma colonies is illustrated.

studies demonstrate that host BRP-39 plays a critical role in the pathogenesis of melanoma metastasis to the lung. The levels of circulating YKL-40 were also significantly increased in patients with metastatic disease. This can be seen in Figure 4, which compares patients presenting with malignant melanoma to Yale/Yale New Haven Hospital with superficial lesions that were surgically removed without evidence of metastasis (control subjects) and patients with advanced stage 4 disease. In combination, these studies suggest that YKL-40 is a biomarker and a therapeutic target in this disorder.

Author disclosures are available with the text of this article at www.atsjournals.org.

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