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Discovery of novel markers in allergic lung inflammation through proteomic-based technologies

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"...knowledge of protein–protein and enzyme interaction will ultimately provide useful information that could be used for the treatment of allergic ailments."

Over the last several decades, allergic asthma and related atopic syndromes have emerged as major public health concerns. Studies from around the world suggest that the incidence and prevalence of asthma began to increase more than two decades ago, with no sign that these disturbing trends may be reversing [1]. Asthma is characterized by episodic dyspnea, airway obstruction, mucus cell metaplasia and T-helper cell type 2 (Th2)-dependent inflammation of the lung. The acute phase of allergic lung inflammation is marked by increased local proteolytic activity; particularly, the presence of activated matrix metalloproteinases (MMPs) that play a key role in progression of disease in asthma [2–4]. Local expression of Th2-type cytokines (IL-4, IL-5, IL-13) and chemokines (CCL7, CCL11, CCL17) direct much of the immune response to allergens. This includes recruitment to the lung parenchyma and airways of eosinophils, neutrophils, mast cells and IgE-secreting B cells, as well as alterations in lung connective tissues that lead to airway hyper-reactivity, goblet cell metaplasia and glycoprotein hypersecretion, which are the major causes of airway obstruction in asthma. Broad-spectrum immune-suppressive medications, such as glucocorticoids that target B and T cells, are used either to suppress existing inflammation or to prevent exacerbation of allergic inflammation in the setting of ongoing allergen exposure. However, undesirable side effects render glucocorticoids very unattractive for long-term use. Therefore, current research in asthma has focused on the discovery of novel, more specific pathways that coordinate airway-obstructive disease in the setting of allergic inflammation and narrow-spectrum modulators of pathways critical to the disease phenotype. Widely used as part of these endeavors are proteomics- and genomics-based approaches, which have indeed identified novel inflammatory biomarkers in human and experimental asthma [5–7].

"...only a few studies have attempted the far more difficult task of defining the biological role of the products of protein hydrolysis and nitration."

Bronchoalveolar lavage (BAL) fluid is perhaps the most widely used biological sample for the identification of novel asthma biomarkers through proteomics, although serum, whole lung and $CD3^+$ T cells have also been used [8–11]. Allergic inflammation affects essentially all structures within the lung, but the airway epithelium is perhaps most strikingly altered. Therefore, to discover how allergic inflammation affects the airway, proteomic analysis of airway epithelium isolated by laser-assisted microdissection in mice was performed. The microdissection demonstrated an increase in antioxidant protein 2, several heat-shock proteins, α_1-antitrypsin and the novel mammalian lectin Ym1 [12]. Proteomic analysis of both BAL fluid and airway tissues have successfully validated the role of some of the

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known cytokines and inflammatory mediators in allergic lung disease, but more importantly they led to the discovery of new candidate molecules much like genomics-based discovery studies. Further study of these molecules could lead to a better understanding of the pathophysiology of allergic lung inflammation.

"Future mechanistic studies in animal models of allergic lung disease will most likely seek to block the production or function of the altered, not native, proteins..."

Unlike genomics studies, several major issues limit the analysis of biological samples, such as BAL fluid, lung tissue or serum, through proteomics assays. First, these samples contain a large range of protein concentrations under different disease conditions and, although highly relevant to disease expression, may exist in concentrations below the limit detection of some proteomics assays. Second, large and highly abundant structural or carrier proteins, such as albumin, transferrin or haptoglobins, can easily overwhelm the ability of some assays to detect smaller, less abundant proteins in the same sample. Finally, depletion of overabundant proteins prior to proteomic analysis could potentially result in inadvertent removal of important but less abundant proteins, such as cytokines, in biological samples [13]. Mindful of these caveats, affinity depletion of a limited number of relatively high-abundance proteins has been used to determine protein profiles in BAL fluid of asthmatic and control subjects. For instance, BAL fluid collected from segmental lung challenge in human asthmatics revealed elevated levels of eosinophil-associated proteins, TARC (CCL17) and MMP-9, which are strongly linked to allergic inflammation [8]. In addition, upregulation of acute-phase proteins that are were not previously associated with asthma, such as S100A8, S100A9, amyloid A and amyloid P proteins, were also identified. Interestingly, using a proteomics approach, S100A8 and A9 proteins have also been detected in a mouse model of asthma, and inhibition of these proteins reduced inflammatory cell migration into the lung [14].

Proteomics-based studies to discover protein–enzyme interactions during the acute phase of allergic lung inflammation

Several members of the MMP family are upregulated in the lungs of humans and mice that exhibit the asthma phenotype. Additional experimental studies have established that upregulation of MMPs is at least partly coordinated by cytokines, illustrating the cross-talk between newly arrived inflammatory cells and the resident lung parenchymal cells that is essential for the coordination of the allergic lung disease phenotype [15]. Although neither the expression nor biological activity of MMPs is required for the development of Th2 cells in response to allergen challenge, the importance of upregulation of MMPs in allergic lung disease is underscored by recent reports demonstrating that mice deficient in MMP2, MMP9 or both show enhanced accumulation of lung inflammatory cells and are susceptible to asphyxiation when exposed to allergens [16,17]. The *in vivo* action of MMPs in normal or pathological conditions and the substrates that are cleaved by various MMPs *in vivo* are of immense interest in further understanding the inflammatory mechanisms underlying asthma. MMPs are readily detected in BAL fluid, but many questions remain unanswered. Why are MMPs secreted into the airway? Are secreted MMPs bound to their substrates and, if so, what is the biological function of this interaction? Proteomics-based analyses of BAL in single and multiple *MMP* gene-deficient mice have been used to determine the role of these enzymes in allergic inflammatory cell clearance from the lungs. Using this knowledge, combined with high-throughput methods for global protein discovery, the *in vivo* interaction of endogenous proteins and MMPs during allergic lung inflammation was recently studied [14].

2D-DIGE analysis of BAL fluid from allergen-challenged MMP2 and MMP9 double-null mice (MMP2/9$^{-/-}$) demonstrated significant differences in the biochemical properties (molecular size and isoelectric points) of several proteins compared with wild-type mice [14]. These findings suggested alterations and/or other post-translational modifications of these proteins were occurring that could affect their function. Quantification of proteins in BAL fluid, based on protein labeling with distinct fluorescent dyes, to rigorously define the differences in the resolved proteins showed an increased abundance of calgranulin B, superoxide dismutase, and Ym1/Ym2 in the BAL fluid of MMP2/9$^{-/-}$ compared with wild-type mice. The distinct differences in the proteome composition between these samples were consistent with the different phenotypes of wild-type and MMP2/9$^{-/-}$ mice with respect to allergic inflammatory cell clearance [17]. Two of these newly identified proteins, calgranulin B and Ym1, have putative chemotactic properties and were proved to be *in vivo* substrates of MMP2 and MMP9 [14]. Ym1 and Ym2 are enzymatically inactive members of the chitinase family of proteins that has eosinophil chemoattractant properties [18]. Immunohistochemistry confirmed the presence of Ym1 in macrophages of saline-challenged mice and in macrophages and airway epithelium of allergen-challenged animals and in humans with asthma [14]. Analysis of BAL fluid of wild-type and MMP2/9$^{-/-}$ mice confirmed the presence of Ym1, but only in allergen-challenged animals. Although the parent molecule is 44 kDa, a single molecular species of 37 kDa was found in both mouse genotypes, indicating that neither MMP2 nor MMP9 were required to generate this cleavage product. Intriguingly, much more Ym1 was found in MMP2/9$^{-/-}$ BAL fluid, suggesting that these MMPs were required for the further processing and or clearance of Ym1.

Proteomics-based discovery of post-translational modification of proteins *in vivo*

In addition to proteomics-based studies to discover *in vivo* interaction of proteins and enzymes during the acute phase of allergic lung inflammation and detection of new biomarkers of

disease, innovative proteomics-based methods have been used to identify other types of post-translational modification of proteins *in vivo*. Although not specific to models of allergic lung inflammation, proteomics-based discovery of proteins that undergo nitration of their tyrosine residues in response to inflammation was recently described [19,20]. Tyrosine nitration of proteins requires nitric oxide (NO) that, in turn, relies on the function of NO synthases during inflammatory conditions. Nitration of proteins *in vivo* is a powerful means of post-translational modification that could render gain or loss of biological function *in vivo*. For instance, a combination of 2D gel protein separation followed by western blot detection of nitrotyrosine proteins and spot matching has revealed a large number of mitochondrial and cytosolic proteins, such as voltage-dependent anion channels, ATP synthase and glutathione *S*-transferase, that are critical in cell metabolism and survival [21]. This approach identified proteins that are nitrated *in vivo* during NO-dependent inflammation and could provide a new level of complex molecular interaction *in vivo*.

Conclusions & future outlook

In the last 7 years, a number of proteomics-based studies have focused on allergic lung inflammation and, not surprisingly, a large number of novel biomarkers that are associated with asthma have been reported. Logically, the next phase of this proteome-based discovery initiative should address the biological significance of these findings. Currently, however, only a few studies have attempted the far more difficult task of defining the biological role of the products of protein hydrolysis and nitration. Future mechanistic studies in animal models of allergic lung disease will most likely seek to block the production or function of the altered, not native, proteins to determine whether this affects the expression of airway inflammation. In the case of MMPs, inhibition of these enzymes during acute inflammation was found to be deleterious; however, antibodies neutralizing S100A8 and A9 (two proteins that are found to act as *in vivo* substrates for MMPs) attenuated migration of allergic inflammatory cells into the alveolar space. Therefore, a comprehensive approach that will include knowledge of protein–protein and enzyme interaction will ultimately provide useful information that could be used for the treatment of allergic ailments.

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References

1 Lawson JA, Senthilselvan A. Asthma epidemiology: has the crisis passed? *Curr. Opin. Pulm. Med.* 11(1), 79–84 (2005).

2 Cohn L, Elias JA, Chupp GL. Asthma: mechanisms of disease persistence and progression. *Annu. Rev. Immunol.* 22, 789–815 (2004).

3 Greenlee KJ, Werb Z, Kheradmand F. Matrix metalloproteinases in lung: multiple, multifarious, and multifaceted. *Physiol. Rev.* 87(1), 69–98 (2007).

4 Maisi P, Prikk K, Sepper R *et al.* Soluble membrane-type 1 matrix metalloproteinase (MT1-MMP) and gelatinase A (MMP-2) in induced sputum and bronchoalveolar lavage fluid of human bronchial asthma and bronchiectasis. *APMIS* 110(11), 771–782 (2002).

5 Hakonarson H, Bjornsdottir US, Halapi E *et al.* Profiling of genes expressed in peripheral blood mononuclear cells predicts glucocorticoid sensitivity in asthma patients. *Proc. Natl Acad. Sci. USA* 102(41), 14789–14794 (2005).

6 de Torre C, Ying SX, Munson PJ *et al.* Proteomic analysis of inflammatory biomarkers in bronchoalveolar lavage. *Proteomics* 6(13), 3949–3957 (2006).

7 Kiss A, Montes M, Susarla S *et al.* A new mechanism regulating the initiation of allergic airway inflammation. *J. Allergy Clin. Immunol.* 120(2), 334–342 (2007).

8 Wu J, Kobayashi M, Sousa EA *et al.* Differential proteomic analysis of bronchoalveolar lavage fluid in asthmatics following segmental antigen challenge. *Mol. Cell Proteomics* 4(9), 1251–1264 (2005).

9 Ghosh S, Janocha AJ, Aronica MA *et al.* Nitrotyrosine proteome survey in asthma identifies oxidative mechanism of catalase inactivation. *J. Immunol.* 176(9), 5587–5597 (2006).

10 Jeong H, Lee SY, Lee EJ *et al.* Proteomic analysis of peripheral T-lymphocytes in patients with asthma. *Chest* 132, 489–496 (2007).

11 Murphy VE, Johnson RF, Wang YC *et al.* The effect of maternal asthma on placental and cord blood protein profiles. *J. Soc. Gynecol. Investig.* 12(5), 349–355 (2005).

12 Marko-Varga GA, Fehniger TE. Microscale protein expression profiling during disease evolvement. *J. Chromatogr. A* 1053(1–2), 279–290 (2004).

13 Granger J, Siddiqui J, Copeland S *et al.* Albumin depletion of human plasma also removes low abundance proteins including the cytokines. *Proteomics* 5(18), 4713–4718 (2005).

14 Greenlee KJ, Corry DB, Engler DA *et al.* Proteomic identification of *in vivo* substrates for matrix metalloproteinases 2 and 9 reveals a mechanism for resolution of inflammation. *J. Immunol.* 177(10), 7312–7321 (2006).

15 Zheng T, Zhu Z, Wang Z *et al.* Inducible targeting of IL-13 to the adult lung causes matrix metalloproteinase- and cathepsin-dependent emphysema. *J. Clin. Invest.* 106(9), 1081–1093 (2000).

16 Corry DB, Rishi K, Kanellis J *et al.* Decreased allergic lung inflammatory cell egression and increased susceptibility to asphyxiation in MMP2-deficiency. *Nat. Immunol.* 3(4), 347–353 (2002).

17 Corry DB, Kiss A, Song LZ *et al.* Overlapping and independent contributions of MMP2 and MMP9 to lung allergic inflammatory cell egression through decreased CC chemokines. *FASEB J.* 18(9), 995–997 (2004).

18 Webb DC, McKenzie AN, Foster PS. Expression of the Ym2 lectin-binding protein is dependent on interleukin (IL)-4 and IL-13 signal transduction: identification of a novel allergy-associated protein. *J. Biol. Chem.* 276(45), 41969–41976 (2001).

19 Aulak KS, Koeck T, Crabb JW *et al.* Proteomic method for identification of tyrosine-nitrated proteins. *Methods Mol. Biol.* 279, 151–165 (2004).

20 Handy DE, Loscalzo J. Nitric oxide and posttranslational modification of the vascular proteome: S-nitrosation of reactive thiols. *Arterioscler. Thromb. Vasc. Biol.* 26(6), 1207–1214 (2006).

21 Aulak KS, Miyagi M, Yan L *et al.* Proteomic method identifies proteins nitrated *in vivo* during inflammatory challenge. *Proc. Natl Acad. Sci. USA* 98(21), 12056–12061 (2001).