The Promising Future of Proteomics in Sarcoidosis

Proteomic technologies are widely used to identify biomarkers for the early detection of human diseases, to develop predictive models of classification, and for monitoring the therapeutic efficacy of drugs. In this issue of the *AJRCCM* (pp. 1145–1154), Kriegova and colleagues (1) used an innovative proteomic technique, surface-enhanced laser desorption ionization–time-of-flight mass spectrometry (SELDI-TOF MS) to examine patterns of protein expression in sarcoidosis.

Mass spectrometry–based techniques may be considered a direct derivation of the original studies on proteomics by Hutchens and Yip (2). Combining chromatography and mass spectrometry, this platform is capable of detecting tens of thousands of data points in a short time from very small volumes (microliters) of biofluids containing a complex mixture of peptides or proteins, such as urine, serum, plasma, or bronchoalveolar lavage (BAL). The methodology resolves proteins, present in mixtures even at extremely low concentrations (femto- to attomoles), through a high-throughput approach (300 samples/day in batches of 100) (3). The principle behind the technique is very simple (Figure 1), with proteins of interest, such as those contained in the BAL, captured on an immobilized stationary-phase chip in an array format. The chip is then inserted into a vacuum chamber and bound proteins are irradiated with a laser that results in the molecules liberating gaseous ions. The ions are rapidly accelerated into a "flight tube" and the time of flight, which is related to the mass-to-charge ratio (m/z) of each protein/peptide, allows for their separation. An ion trap analyzer functions as a detector, which, when capturing the ions, starts to oscillate and transforms ion cyclotron resonance into signals. Detected proteins and peptides are then shown as a series of corresponding peaks. Since a typical mass spectrometry platform may generate a proteomic profile of up to 400,000 data points, sophisticated software packages are needed for the statistical analysis of large numbers of samples.

The on-chip capture and detection of proteins have evident advantages; it allows small sample sizes and limited sample manipulation, and produces high-throughput results (3, 4). Taking advantage of these advances, mass spectrometry platforms have been used for the discovery of biomarkers in a large variety of diseases, including cancer and autoimmune disorders, as well as in neurologic and metabolic disorders (3). The tool is also beginning to be used in respiratory medicine. Zhukov and colleagues (5) utilized SELDI-TOF MS to generate specific protein peak patterns for malignant lung tumors, whereas Merkel and colleagues identified specific protein patterns in BAL fluid samples of patients with chronic obstructive pulmonary disease (6).

The article published in this issue of the *Journal* represents an example of the potential utility of SELDI-TOF MS. The authors used this technique to examine the BAL proteome in patients with sarcoidosis. Data analysis identified a panel of 40 proteins that were altered in samples with this disorder versus those from healthy controls. Distinct protein patterns specific for patients with pulmonary sarcoidosis were identified and, more specifically, profiles were characterized from patients with sarcoidosis and Löfgren's syndrome. The authors also found distinguishing peaks on SELDI-TOF MS that correlated with the radiologic stages of the disease. After reverse-phase fractionation, peptide fingerprint mapping, and immunodepletion, a novel protocadherin-2 precursor was identified in the context of the sarcoidosis proteome.

The potential clinical utilities of such an approach are clear. Refinement of the characterization of the sarcoid proteome is likely to help to identify molecules expressed in different phases of the disease, which might, at least in theory, represent putative molecular targets. Furthermore, a proteomic profile could be designed for the clinical evaluation of patients with sarcoidosis. As protein expression data become available, the integration of this technology into protocols for disease diagnosis as well as outcome prediction should soon take place in sarcoidosis. Finally, the application of proteomics could be used in verifying the *in vitro* effect of molecules, a crucial step in the development of therapeutics. For instance, levels of immunosuppressive or immunomodulatory molecules could be measured directly from a tissue biopsy to assess adequacy of drug delivery to a particular organ site involved by the sarcoid process.

Beyond its clinical implications, proteomic approaches might yield novel information about the pathophysiology of the disease. For example, using SELDI-TOF MS, a number of differences in protein patterns have been demonstrated between alveolar macrophages (AMs) and their precursors or among AMs under different pathologic conditions (7). Characterization of macrophage-derived and Th1-derived proteins during sarcoidosis will offer a better understanding of its pathogenesis. Laser-capture microdissection permits the isolation of single cells or single populations of cells from thin tissue sections. In combination with SELDI-TOF MS, laser-capture microdissection promises to permit the collection of protein signatures from a single cell type within a heterogeneous sample obtained from a tissue involved by sarcoidosis (8). This information could significantly improve the understanding of molecular mechanisms underpinning granuloma formation and impact diagnostic pathology of the disease.

It is easy to anticipate that within 5 years the clinical application of proteomic-based techniques will have transformed our knowledge of most physiologic and pathologic pulmonary processes, including sarcoidosis. To use such methodology on a routine basis for a protein-based evaluation of patients with sarcoidosis, the clinical sensitivity and specificity of protein profiling of BAL should be validated by different groups. Of note, comparative analyses of proteomic data obtained from biofluids highlighted important discrepancies in the final results among laboratories, mostly due to the absence of standardized sample collection and processing, and in the use of different pre- and postanalytic strategies (9, 10). For these reasons, the international scientific community is adopting standardized protocols, which include the use of pooled reference standard samples applied on each protein array and standardized daily calibrations of the instruments, as the minimal essential conditions to validate the data produced by SELDI. There is also a clear need to develop international standards for archiving data obtained from the analysis of lung samples. The validation of standardized

Am J Respir Crit Care Med Vol 173. pp 1053–1055, 2006
Internet address: www.atsjournals.org

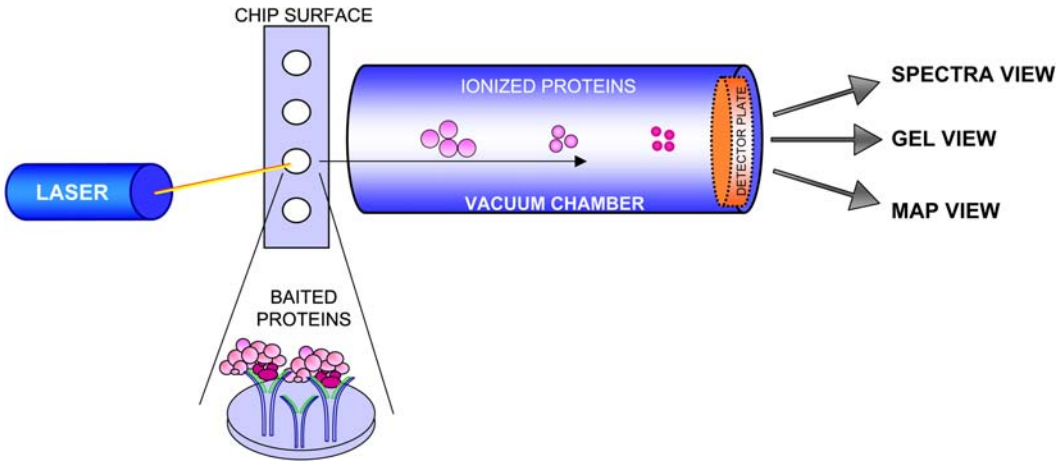

Figure 1. Surface-enhanced laser desorption ionization–time-of-flight mass spectrometry. The sample is applied to the surface of the chip. As described in the text, the sample is subjected to laser desorption ionization and the formed ions are measured using a time-of-flight mass analyzer

SELDI-TOF MS methodologies will perhaps require months or years of cooperative effort by the groups involved, but the result will be a rich source of information to aid the physician in patient management.

Conflict of Interest Statement: Neither author has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

Acknowledgment: The authors thank Dr. Martin Donach for his help in the preparation of the manuscript.

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References

1. Kriegova E, Melle C, Kolek V, Hutyrova B, Mrazeka F, Bleul A, du Bois RM, von Eggeling F, Petrek M. Protein profiles of bronchoalveolar lavage fluid from patients with pulmonary sarcoidosis. *Am J Respir Crit Care Med* 2006;173:1145–1154.
2. Hutchens TW, Yip TT. New desorption strategies for the mass spectrometric analysis of macromolecules. *Rapid Commun Mass Spectrom* 1993;7:576–580.
3. Petricoin EF, Liotta LA. SELDI-TOF-based serum proteomic pattern diagnostics for early detection of cancer. *Curr Opin Biotechnol* 2004;15:24–30.
4. Issaq HJ, Veenstra TD, Conrads TP, Felschow D. The SELDI-TOF MS approach to proteomics: protein profiling and biomarker identification. *Biochem Biophys Res Commun* 2002;292:587–592.
5. Zhukov TA, Johanson RA, Cantor AB, Clark RA, Tockman MS. Discovery of distinct protein profiles specific for lung tumors and premalignant lung lesions by SELDI mass spectrometry. *Lung Cancer* 2003;40:267–279.
6. Merkel D, Rist W, Seither P, Weith A, Lenter MC. Proteomic study of human bronchoalveolar lavage fluids from smokers with chronic obstructive pulmonary disease by combining surface-enhanced laser desorption/ionization-mass spectrometry profiling with mass spectrometric protein identification. *Proteomics* 2005;5:2972–2980.
7. Wu HM, Jin M, Marsh CB. Toward functional proteomics of alveolar macrophages. *Am J Physiol Lung Cell Mol Physiol* 2005;288:L585–L595.
8. Chaurand P, Sanders ME, Jensen RA, Caprioli RM. Proteomics in diagnostic pathology: profiling and imaging proteins directly in tissue sections. *Am J Pathol* 2004;165:1057–1068.
9. Karsan A, Eigl BJ, Flibotte S, Gelmon K, Switzer P, Hassell P, Harrison D, Law J, Hayes M, Stillwell M, *et al.* Analytical and preanalytical biases in serum proteomic pattern analysis for breast cancer diagnosis. *Clin Chem* 2005;51:1525–1528.
10. Semmes OJ, Feng Z, Adam BL, Banez LL, Bigbee WL, Campos D, Cazares LH, Chan DW, Grizzle WE, Izbicka E, *et al.* Evaluation of serum protein profiling by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry for the detection of prostate cancer: I. Assessment of platform reproducibility. *Clin Chem* 2005;51:102–112.

DOI: 10.1164/rccm.2602001

Who Are the Children with Asthma Most Susceptible to Air Pollution?

In this issue of the *AJRCCM* (pp. 1098–1105), Rabinovitch and colleagues (1) show a positive association between fine-particulate air pollution and a biomarker of airway inflammation, urinary leukotriene E_4 (LTE_4), in 73 schoolchildren with asthma. LTE_4 is a stable product of the cysteinyl leukotrienes C4 and D4, which are produced by inflammatory cells, including eosinophils, and have bronchoconstrictive and proinflammatory properties. Specifically, the authors of this article found that urinary LTE_4 was associated with morning hourly ambient exposure to particulate matter of less than 2.5 $\mu m/m^3$ in aerodynamic diameter ($PM_{2.5}$). They also found that as-needed bronchodilator use at school increased in relation to morning $PM_{2.5}$. LTE_4 was not significantly associated with 24-hour average $PM_{2.5}$. Daily peaks in $PM_{2.5}$ occurred in the morning, suggesting that both proximity of exposure measurement to the biomarker measurement as well as peak exposure were important. The availability of hourly $PM_{2.5}$ allowed investigators to assess exposures immediately preceding the measurement of outcome.

Many epidemiologic studies have shown associations of asthma hospital admissions and emergency department visits with 24-hour average PM levels below the current U.S. National Ambient Air Quality Standards (NAAQS) (2). This could be partly explained by unmeasured short-term $PM_{2.5}$ excursions above or even below the current 24-hour standard, which is 65 $\mu g/m^3$ (2). The Denver findings were for morning means, ranging up to only 30 $\mu g/m^3$, on days when 24-hour $PM_{2.5}$ concentrations were no greater than 12 $\mu g/m^3$. For more on the current controversy regarding the NAAQS, *see* Rom and Samet (3).

Other epidemiologic studies have shown associations between acute asthma outcomes in children and short-term peaks in $PM_{2.5}$ or $PM_{2.5}$ exposure in the hours immediately preceding the outcome measurements, including exhaled NO (4), asthma