Indian J.Sci.Res. 15 (1): 97-99, 2017 ISSN: 0976-2876 (Print)
ISSN: 2250-0138 (Online)

RECENT CONCEPTS OF IMMUNOFLUORESCENCE IN ORAL MUCOCUTANEOUS DISEASES

KANUPRIYA GUPTA[1]

MDS, Oral and Maxillofacial Pathology, Senior Research Fellow, Faculty of Dental Sciences, IMS, BHU, Varanasi Uttar Pradesh, India

ABSTRACT

The investigation of autoimmunity provides an interest challenge in "omics" research and, particularly, proteome research, as autoimmune diseases are common disorders of unsolved etiology that occur in a wide range of manifestations, in all of which tissues and organs are attacked by the body's own immune system. Autoantibodies are a hallmark of many autoimmune diseases and the presence of autoantibodies is a distinctive and key characteristic of autoimmune diseases. Conventionally, the study of autoimmune response has always been conducted by analysing the presence and/or concentration of individual antibodies in biological fluids. New proteomic techniques allow the simultaneous identification/measurement of different autoantibodies in sera of patients suffering from autoimmune diseases.

KEYWORDS: Autoantibodies, Etiology, Omics

An explosion of knowledge of biology, coupled with development of powerful measurements tools such as the polymerase chain reaction (PCR), mass spectrometry (MS) and microarray assays (MA), offers enormous opportunity to learn about etiology, diagnosis, and prognosis in multiple "-omics" fields. " Fields with names like "genomics" (genetic complement),"transcriptomics" (gene expression), "proteomics" (protein synthesis and signalling), "metabolomics" (concentration and fluxes of cellular metabolites), "metabonomics" (systemic profiling through the analysis of biological fluids) have been introduced in medicine with an increasing emphasis [Plebani, 2005].

The investigation of autoimmunity provides an interest challenge in "omics" and, particularly, proteome research, as autoimmune diseases are common disorders of unsolved etiology that occur in a wide range of manifestations, in all of which tissues and organs are attacked by the body's own immune system.

AUTOIMMUNE DISEASES AND AUTOANTIBODIES

Autoimmune diseases are a heterogeneous group of diseases characterized by immune reactions against one or more components of the body itself, causing inflammation and damage to tissues and organs [Davidson and Diamond, 2001]. Autoantibodies are a hallmark of many autoimmune diseases and the presence of autoantibodies is a distinctive and key characteristic of autoimmune diseases. For some systemic autoimmune dis- eases, moreover, the presence in serum of certain autoanti- bodies represents one of the classification criteria. It should be underlined that autoantibodies in autoimmune diseases do not appear individually, associating a single, specific autoanti- body with a certain well-defined disease; in general, auto- immune diseases are characterized by the presence of several autoantibodies. As an example, Sherer et al. described more than 100 different autoantibodies in systemic lupus erythe- matosus (SLE) patients [Sherer et.al., 2004].

Laboratory medicine plays a fundamental role in the diagnosis, monitoring, and prediction of the outcome of auto- immune diseases; in addition, autoantibodies can be used as predictive markers of ongoing diseases in apparently healthy subjects.

Historically, the detection and analysis of autoantibodies has relied on a number of different technologies such as immunodiffusion, (ID), indirect immunofluorescence (IIF), particle aggregation (PA), complement fixation (CF), hemagglutination,counter immunoelectrophoresis (CIE), radioimunoassay (RIA), enzyme immunoassays (EIA). For some decades, namely between 1957 and 1997, conventional immunoassay methods were the main tool used in clinical laboratories for the diagnosis of autoimmune diseases [Tozzoli, 2007].

During the 1990s, the application of immunometric methods to instruments increasingly advanced in terms of analytical reliability and automation led to widespread use of antibody testing, with increase in the volumes of tests per- formed at each clinical laboratory, and improvements in turn- around time [Tozzoli, 2007]. In the last years the advent of the

[1]Corresponding author

"proteomic revolution" has opened up new horizons in the diagnosis of autoimmune diseases [Villalta et.al., 2007]. The goals of proteomic analyses include the elucidation of the molecular mechanisms that regulate cellular processes, the characterization of complex protein interacting networks and their perturbations, the discovery of biomarkers useful in the diagnosis and monitor- ing of disease, and the identification of therapeutic targets. Therefore, the investigation of autoimmunity provides an interesting challenge in proteomic research, as autoimmune diseases are common disorders of unresolved etiology that occur in a wide variety of manifestations [Dotzlaw et.al., 2006].

Among the numerous systems developed and used in the last few years for proteomic analyses, line-blot assays, immunoassays with bar-coded particles, bead-based assays with flow cytometry detection, antigen microarrays and mass spectrometry, namely, surface-enhanced laser desorption ionization-time of flight (SELDI-TOF), seem to be potentially interesting [Gonzàles-Buitrago, 2006]. In particular, antigen microarrays for autoanti- body profiling have been developed and evaluated for a possible utilization in clinical practice.

MICROARRAY APPROACHES TO AUTOANTIBODY PROfiLING

Microarrays are miniaturized arrays of immobilized sub- stances used to detect binding events [Astle and Kodadek, 2008]. The concept of a microarray was first published in the late 1980s and was termed a "multianalyte immunoassay" for analyzing an array of antibody-antigen interactions [Ekins, 1989]. The first scientific publication of microarrays that were not solely immunoas- say-based was in 1991 and it presented a method on-spot synthesis of diverse chemicals, including peptides and a dinucleotide, using light-directed solid-phase chemistry with photolabile protecting groups [Fodor et.al., 1991]. Soon after, the use of microarrays was fueled by the development of DNA micro- arrays and the sequencing of various genomes, leading to a momentous increase in the number of publications per year that include "microarray" as a key term.

MASS SPECTROMETRY

Mass spectrometry (MS), a technique that measures the mass-to-charge ratio (m/z) of ions in the gas phase has assumed a key role in most of the proteomic workflows. In particular, the surface-enhanced laser desorption ionization – time of flight (SELDI-TOF) technology represents a very novel and promising approach combining chromatography and mass spectrometry. The combined use of a proteinchip technology allows the selective capture of proteins of interest (autoantibodies) directly from the original source material and without sample preparation. This, in turn, makes this technique very simple and suitable for clinical applications. In addition, the throughput is very high, and the technique requires far less biological material than conventional meth- ods. The search for biomarkers using SELDI-TOF can directly be used for diagnosis of diseases such as prostate, breast, ovarian carcinomas and many other diagnostic purposes, in- cluding autoimmune diseases.

Grus et al. have developed a SELDI-TOF approach to detect and analyze complex mixtures of natural autoantibodies in human sera and compared the results to those obtained by conventional Western blotting [Grus et.al., 2003]. They detected a compar- able number of peaks in SELDI-TOF and Western blots at higher molecular mass. At lower molecular mass (b 30 kDa), the sensitivity of SELDI-TOF far exceeded Western blotting. These investigations have resulted in the identification of putative biomarker proteins and signature protein expression patterns characteristic for a specific autoimmune disease, and provide insights into putative mechanisms involved in the development and pathogenesis of these disorders.

PROTEOMICS AS A PARADIGM OF TRANSLATIONAL MEDICINE

During the last years, a large number of scientists were able to identify other candidate protein disease biomarker profiles using patient research study sets and to achieve high diagnostic sensitivity and specificity in blinded test sets. Nevertheless, translating these research findings to useful and reliable clinical test has been the difficult part. Clinical translation of promising

ion fingerprints has been hampered by "sample collection bias, interfering substances, biomarker perishability, laboratory-to-laboratory variability, SELDI chip discontinuance and surface lot changes, and the stringent dependence of the ion signature on the subtleties of the reagent composition and incubation protocols" [Liotta and Petricoin, 2008].

CONCLUSION

Finally, the full range of clinical research, from translation to outcomes analysis, cannot be performed if clinicians are not involved in making possible the true impact of new diagnostic tests in clinical practice, with real patients in a real context.

However, the problem seems to be much more complex, involving not only practical considerations but, more inter- estingly, some philosophical reasoning. It has been stressed that the linear process of hypothesis-driven discovery that characterized past decades of science has recently been replaced by the hypothesis-generating power of high-throughput, array-based technologies that provide vast and complex datasets that may be mined in various ways using emerging tools of computational biology.

Currently, several multiplexed technologies for the simulta- neous measurement of several autoantibodies in a minimum volume of serum have been developed and evaluated. In par- ticular, antigen microarrays and SELDI-TOF mass spectrometry represent very promising approaches to the simultaneous identification of autoantibodies in autoimmune disease.

REFERENCES

Plebani M., 2005. Proteomics: the next revolution in laboratory medicine? Clin Chim Acta, **357**:113–22.

Davidson A. and Diamond B., 2001. Autoimmune disease. N. Engl. J. Med., **345**: 340–50.

Sherer Y., Gorstein A., Fritzler M.J. and Shoenfeld Y., 2004. Autoantibody explosion in systemic lupus erythematosus: more than 100 different antibodies found in SLE patients. Semin Arthritis Rheum, **34**:501–37.

Tozzoli R., 2007. Recent advances in diagnostic technologies and their impact in autoimmune diseases. Autoimmun Rev., **6**:334–40.

Villalta D., Tozzoli R., Tonutti E., Bizzaro N., 2007. The laboratory approach to the diagnosis of autoimmune diseases: it is time to change? Autoimmun Rev., **6**:359–65.

Dotzlaw H., Eggert M., Neeck G, Schulz M., 2006. Spots, blots, peaks and chips:proteomic approaches in autoimmune diseases. Curr. Pharmacol Design, **12**:3699–706.

Gonzàles-Buitrago J.M., 2006. Multiplexed testing in the autoimmunity laboratory. Clin. Chem. Lab Med., **44**:1169–74.

Astle J.M. and Kodadek T., 2008. Microarray approaches to autoantibody profiling. In: Van Eyk J, Dunn MJ, editors. Clinical Proteomics. From diagnosis to therapyWILEY-VCH Verlag: Weinheim, 511-132.

Ekins R.P., 1989. Multi-analyte immunoassay. J. Pharm. Biomed. Anal., **7**:155–68.

Fodor S.P., Read J.L., Pirrung M.C., Stryer L., Lu A.T. and Solas D., 1991. Light-directed, spatially addressable parallel chemical synthesis. Science, **251**:767–73.

Grus F.H., Joachim S.C. and Pfeiffer N., 2003. Analysis of complex autoantibody repertoires by surface-enhanced laser desorption/ionization-time of flight mass spectrometry. Proteomics, **3**:957–61.

Liotta L.A. and Petricoin E.F., 2008. Putting the "Bio" back into biomarkers: orienting proteomic discovery toward biology and away from the measurement platform. Clin. Chem., **54**:3–5.