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Review

Present and future of the autoimmunity laboratory

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Abstract

At present, autoimmunity laboratories are very dynamic owing to the constant and increasing availability of new tests, mainly due to the detection of new autoantibodies. The main characteristic of the autoimmunity laboratory and the one that differentiates it from other laboratories that use immunoassays as basic techniques is that it determines antibodies (autoantibodies) and not antigens. For this reason, immunoassay techniques must employ antigens as reagents. Indirect immunofluorescence has and continues to be a basic technique in autoimmunity studies. However, over the last few years, a significant trend at autoimmunity laboratories has been the gradual replacement of immunofluorescence microscopy by immunoassay. Of the several different forms of immunoassay, the enzyme-linked immunosorbent assay (ELISA) format is the one most used in autoimmunity laboratories. Recombinant DNA technology has allowed the production of large quantities of antigens for autoantibody analysis. Flow cytometry for the analysis of microsphere-based immunoassays allows the simultaneous measurement of several autoantibodies. Likewise, autoantigen microarrays provide a practical means to analyse biological fluids in the search for a high number of autoantibodies. We are now at the beginning of an era of multiplexed analysis, with a high capacity of autoantibody specificities. Future trends in this field include immunoassays with greater analytical sensitivity, simultaneous multiplexed capability, the use of protein microarrays, and the use of other technologies such as microfluidics.

Keywords: Autoimmune diseases; Autoimmunity laboratory; Enzyme immunoassay; Flow cytometry; Indirect immunofluorescence; Protein microarray

Contents

Abbreviations: AMA, anti-mitochondrial antibodies; ANA, Antinuclear antibodies; ANCA, anti-neutrophil cytoplasmic antibodies; ARA, American College of Rheumatology; DNA, deoxyribonucleic acid; EIA, Enzyme immunoassay; ELISA, Enzyme-linked immunosorbent assay; IIF, Indirect immunofluorescence; MPO, myeloperoxidase; mRNA, messenger RNA; SLE, Systemic Lupus Erythematosus; SS, Sjögren's syndrome; TPO, thyroid peroxidase.

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1. Introduction

Autoimmune diseases form a heterogeneous group of illnesses characterized by humoral or cell-mediated immune reactions against one or more of the body's own constituents. Clinicians classify autoimmune diseases as systemic or organ-specific. Although this classification is clinically useful, it does not indicate the cause or causes of the disease [1,2]. Systemic autoimmune diseases display autoantibodies directed against nuclear or cytoplasmic molecules that participate in DNA replication, DNA transcription, and the translation of messenger RNA. Organ-specific autoimmune diseases exhibit autoantibodies directed against an organ or related organs.

In several systemic autoimmune diseases the presence of certain autoantibodies in serum is one of the diagnostic criteria used for their classification. Antinuclear antibodies (ANA) are included among the classification criteria for both systemic lupus erythematosus (SLE) and Sjögren's syndrome (SS). In organ-specific autoimmune diseases, the detection of autoantibodies is a fundamental datum for diagnosing them as autoimmune diseases. In the case of some autoantibodies, apart from their usefulness for diagnosis they are important for establishing a prognosis. In any case, autoantibodies should be considered to be no more than markers of disease. They are commonly found in normal individuals in the absence of any definable disease and with increasing prevalence in ageing populations. Furthermore, the presence of autoantibodies may be detected several years before the onset of disease, and in this context they are markers of future disease in presently healthy individuals [3]. Such identification might allow immunological treatment to prevent disease, and when disease cannot be prevented, life-threatening but treatable conditions could be avoided [3].

2. The role of the autoimmunity laboratory in autoimmune diseases

The clinical laboratory offers a very important tool for clinicians to diagnose and treat autoimmune diseases. Autoimmunity laboratories analyse and measure an increasing number of autoantibodies, employing a broad spectrum of techniques and methods. The main characteristic of the autoimmunity laboratory, and indeed the one that differentiates it from other laboratories that use immunoassays as basic techniques, is that it determines antibodies (autoantibodies) and not antigens. For this reason, immunoassay techniques must employ antigens as reagents.

At present, autoimmunity laboratories are in a very dynamic situation owing to the constant and increasing availability of new tests, mainly due to the detection of new autoantibodies and demonstration of their clinical usefulness. Improved biochemical and molecular methods have allowed rapid dissection of the autoantigens associated with specific autoimmune diseases. Likewise, the spectacular development of serology analyses of autoantibodies over the last two decades can be attributed directly to the explosive growth of molecular biology.

As has been pointed out recently, new serologic technologies and assays for autoimmune diseases are currently being developed by laboratory scientists and commercial industries at a very fast pace and may eventually lead to better reproducibility in confirming diagnosis and estimating prognosis, ultimately improving the quality of clinical care [4].

Inadequate use of laboratory tests is one of the most frequent problems in autoimmunity, leading to incorrect diagnoses, inadequate treatment and unnecessary costs. Accordingly, different committees and task forces from the scientific community have developed guidelines for the adequate use of laboratory tests for the study of autoimmune diseases. Such guidelines are the result of the analysis and evaluation by these task forces of published results. One of the most active societies is the American College of Rheumatology (ACR). An introductory document on the guidelines for immunological laboratory tests for rheumatic diseases was published in 2002 [5]. The principal objective of these guidelines is to improve patient care through rational use of laboratory tests.

3. Techniques used for the study of autoantibodies

The main analytical techniques used in the autoimmunity laboratory are shown in Table 1. Over the last few years, the

Table 1
Analytical techniques used in the autoimmunity laboratory

Agglutination
Immunoprecipitation
Turbidimetry
Nephelometry
Double immunodiffusion
Counterimmunoelectrophoresis
Immunofluorescence
Immunoassays
ELISA
IRMA
Immunoblot
Western blot
Dot blot