Review

Present and future of the autoimmunity laboratory

José M. González-Buitrago a,*, Concepción González b

a Servicio de Bioquímica, Hospital Universitario, Salamanca, Spain and Departamento de Bioquímica y Biología Molecular, Universidad de Salamanca, 37007 Salamanca, Spain

b Servicio de Bioquímica Clínica, Hospital Universitario Virgen Macarena, Sevilla, Spain

Received 30 May 2005; received in revised form 4 July 2005; accepted 5 July 2005

Available online 26 August 2005

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.

© 2005 Elsevier B.V. All rights reserved.

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

Contents

1. Introduction ............................................................ 51
2. The role of the autoimmunity laboratory in autoimmune disease ................................ 51
3. Techniques used for the study of autoantibodies ......................................... 51
4. Indirect immunofluorescence ................................................... 52
5. Enzyme immunoassay ...................................................... 53
5.1. Solid phases ........................................................ 53
5.2. Antigens .......................................................... 53
5.3. Standardization ...................................................... 54
5.4. Applications ........................................................ 54

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.

* Corresponding author.

E-mail address: buitrago@usal.es (J.M. González-Buitrago).

0009-8981/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
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
most significant trend at the autoimmunity laboratory has
been the gradual replacement of microscopy (indirect
immunofluorescence, IIF) and other manual methods such
as double immunodiffusion and counterimmunoelectropho-
resis by immunoassay, mainly enzyme immunoassay (EIA),
which requires less skilled operators, is more objective, and
can easily be automated.

4. Indirect immunofluorescence

Indirect immunofluorescence has and continues to be a basic
technique in autoimmunity studies. In the first applications
of this technique, slices of mouse or rat tissue were employed.
For an assay, tissues are fixed by appropriate methods to slides. Then, they are incubated with serum samples and the binding of
serum autoantibodies to their corresponding antigens in the
tissues is detected by using fluorescent-labelled anti-immunoglo-
bulin antiserum. The most common fluorochromes used for
IIF in the autoimmunity laboratory are fluorescein isothioce-
nate, yielding green fluorescence, and rhodamine B, which
produces orange fluorescence.

Autoantibodies give characteristic fluorescence images
called fluorescence patterns. The staining pattern provides some
indication of the specificity of the antibodies in the sample. Background staining is a major drawback for the interpretation
of IIF patterns. For most IIF assays, the laboratory should select
a screening dilution, because undiluted serum gives a back-
ground staining due to the non-specific binding of clinically
non-significant levels of circulating autoantibodies. The choice
of the screening dilution depends on several factors, among
them the recommendations of the reagent manufacturer, its
actual performance in the laboratory, the population analysed,
and the needs of clinicians. Moreover, the screening dilution
performs a critical function in determining assay sensitivity
and specificity. Increasing the screening dilution produces a
less sensitive and more specific assay.

The possibility of discriminating between several patterns
and the fact that it is a good screening method are the main
advantages of IIF and mean that it is still an indispensable
technique in the autoimmunity laboratory. Subjective inter-
pretation, the wide variability of results, high intra-laboratory
and inter-laboratory variance, semiquantitative results, and
the fact that the technique cannot be completely automated
are the main disadvantages.

Use of HEp-2 cells instead of tissue slides for the assay of
antinuclear antibodies in IIF represented a significant
improvement in the study of autoantibodies [6]. HEp-2 are
cells from a human larynx epithelium cell line that have
larger nuclei and nucleoli than rodent tissue cells. This
facilitates their microscopic observation and provides greater
sensitivity in the detection of small quantities of antibodies
and a better resolution of some antigens. Moreover, cells in
different phases of mitosis or the cell cycle allow the
observation of antigens whose expression varies in the
different stages of cell cycle. HEp-2 cells allow the
recognition of more than 30 different nuclear and cytoplasmic
patterns, which are given by upwards of 100 different
autoantibodies [7,8]. However, the greater sensitivity of
ANA assays with HEp-2 cells as compared to rodent tissues is
associated with lower specificity. Thus, more patients with
diseases other than systemic lupus erythematosus and healthy
people show positive results. To overcome this problem a 1 /
160 titer has been recommended as the most acceptable cut-
off to separate normal from abnormal sera [9].

Historically, clinicians have relied on fluorescence pat-
terns as an aid in diagnosis. The main ANA-fluorescence
patterns observed are homogeneous, speckled, nucleolar and
centromeric [7]. The most frequent ANA-fluorescence pat-
terns and clinical associations are shown in Table 2. With the
advent of specific autoantibody tests, the clinical usefulness
of traditional fluorescence patterns has been reduced signifi-
cantly, except perhaps for anti-centromere and anti-nucleolar
patterns. Fluorescence patterns are now used mainly to help
guide which specific autoantibody is to be measured.

IIF has also been used to measure anti-dsDNA antibodies with
*Crithidia luciliae* [10,11]. The *Crithidia* assay detects
the binding of anti-dsDNA to the kinetoplast of the organism,
which contains circular dsDNA unassociated with histone
proteins. It can be used to detect IgG anti-dsDNA, IgM anti-
dsDNA, or all isotypes of anti-dsDNA. However, at present
the use of *Crithidia* assays in the autoimmunity laboratory
has been reduced and replaced by enzyme immunoassay.

Antineutrophil cytoplasmic antibodies (ANCA) are
autoantibodies directed against antigens found in cytoplas-
ic granules of neutrophils and monocytes [12]. ANCAs
are closely associated with Wegener granulomatosis, micro-
scopic polyangiitis, and Churg–Strauss syndrome [13].
Determination of ANCA by indirect immunofluorescence
using neutrophil preparations yields three patterns: cyto-
plasmic (C-ANCA), perinuclear (P-ANCA) and atypical (A-
ANCA) [14,15]. C-ANCA is associated with anti-proteinase
3 (PR3) antibodies, and P-ANCA with anti-myeloperox-
idase (MPO) antibodies. A-ANCA is a perinuclear pattern
obtained with ethanol-fixed neutrophils, but negative with
formalin-fixed neutrophils. In A-ANCA, the main target

<table>
<thead>
<tr>
<th>Fluorescence pattern</th>
<th>Target</th>
<th>Clinical association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogeneous</td>
<td>DNA, histones</td>
<td>SLE, RA, JRA</td>
</tr>
<tr>
<td>Speckled Coarse</td>
<td>Proteins that bind nucleic acids and ribonucleoproteins (U-RNP, Sm)</td>
<td>SLE, MCTD</td>
</tr>
<tr>
<td>Fine</td>
<td>Proteins that bind nucleic acids and ribonucleoproteins (SS-A, SSB)</td>
<td>SS</td>
</tr>
<tr>
<td>Nucleolar</td>
<td>Nucleolar components</td>
<td>Systemic sclerosis</td>
</tr>
<tr>
<td>Centromeric</td>
<td>Centromeric proteins</td>
<td>Limited systemic sclerosis</td>
</tr>
</tbody>
</table>
antigens are lactoferrin, elastase and cathepsin G. The A-ANCA pattern is mainly observed in non-vasculitic diseases, such as rheumatoid arthritis, intestinal bowel diseases and infectious bacterial or viral diseases [14].

Other autoantibodies measured by IIF are anti-mitochondrial antibodies (AMA), anti-smooth muscle antibodies, anti-liver and kidney microsomal (LKM) antibodies, antiparietal cell antibodies, anti-adrenal cell antibodies and other organ-specific autoantibodies.

As noted above, two of the objections to IIF are its subjectivity and the fact that it cannot be completely automated. Recently, a computer-assisted system for the classification of interphase HEp-2 cell immunofluorescence patterns for autoimmune diagnoses has been described [16]. Designed as a system for assistance, representative patterns are acquired by the operator with a digital microscope camera and transferred to a computer. By use of a software package based on image analysis, feature extraction, and machine learning algorithms, relevant characteristics describing the patterns can be detected. The system can be used to discriminate between positive and negative sera for a previous classification of the most important patterns [16].

As regards automation, in the last few years robot systems for microplate management have incorporated the capacity to prepare slides with cell or tissue preparations. These systems automatically add serum samples, incubate, wash, and add fluorescent conjugates. Thus, the slides are already prepared for microscopic observation.

5. Enzyme immunoassay

Today, enzyme immunoassays offer a basic technique in clinical laboratories. Of the several different forms of enzyme immunoassay, the enzyme-linked immunosorbent assay (ELISA) format is the one most used. Two of the main components of an ELISA for autoantibodies are the solid phase and the antigen.

5.1. Solid phases

Microplates, beads, and magnetic particles are the main solid phases employed in ELISA. Of these three, microplates are the most used. This solid support is very useful for developing the techniques and runs well with a number of samples that is not too high. Microplate handling of ELISA tests has been automated with programmed Cartesian robots. In recent years, systems that use beads or magnetic particles as the solid phase have been introduced for autoantibody measurements [17–21].

5.2. Antigens

The specificity of ELISAs for autoantibody measurements is strongly dependent on the quality of the antigens used. In ELISAs for autoantibody measurements, it is of the utmost importance that the antigen should have exactly the same sequence, conformation and post-translational modifications as the human antigen.

The main sources of antigenic material are shown in Table 3. In the production of antigens, one crucial step is their purification, which is accomplished by affinity chromatography of either human or animal tissues. During the purification process, the post-translational modifications must be maintained as well as the tertiary and quaternary structures of the antigens. Likewise, conformational epitopes must be conserved. If during the purification process a contaminant antigen is present, cross-reactivity may occur during the later ELISA assay.

Recombinant technology has allowed the production of large quantities of defined proteins. In the field of autoimmunity, recombinant DNA technology has been used for antigen production in bacteria and eukaryotic cell cultures [22,23]. The advantages of recombinant antigens are that the immunodominant antigens are chosen selectively; they combine optimally in the solid phase coating; they provide excellent reproducibility, with good precision and quantification, and they provide highly sensitive assays.

To obtain complementary DNA (cDNA) that will code for an autoantigen, several approaches have been developed. All the procedures begin with the isolation of messenger RNA (mRNA) from human tissue, the target of the autoantibodies. Total mRNAs are converted into cDNAs by reverse transcriptase. The cDNAs are then inserted into bacteriophage vectors and a cDNA library is constructed. The cDNAs are cultured and allowed to express the corresponding proteins. These are purified and their antigenicity is analysed using antibodies.

The system most frequently used in recombinant technology is E. coli. However, for antigen production in autoantibody analysis bacteria are not very suitable. The size of the proteins expressed is very limited; there are no post-translational modifications (glycosylation, phosphorylation, acylation), which may severely disturb immunological recognition by the autoantibody. Finally, foreign proteins expressed in bacteria tend to form insoluble aggregates. Alternatives to bacteria are yeast and insect cell/baculovirus systems [22,23]. The main disadvantages of yeast are the problems encountered when extracting cellular proteins, and the additional carbohydrate attached to the secreted proteins, which reduces their immunoreactivity [23].

Table 3. Sources of antigenic material

<table>
<thead>
<tr>
<th>Source of antigenic material</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native</td>
</tr>
<tr>
<td>Human tissues and blood products</td>
</tr>
<tr>
<td>Animal tissues</td>
</tr>
<tr>
<td>Recombinant</td>
</tr>
<tr>
<td>Prokaryotic (E. coli)</td>
</tr>
<tr>
<td>Eukaryotic</td>
</tr>
<tr>
<td>Mammalian cells</td>
</tr>
<tr>
<td>Yeasts</td>
</tr>
<tr>
<td>Insect cells/baculovirus</td>
</tr>
</tbody>
</table>
During the past decade, the insect cell/baculovirus system has allowed the expression of many human autoantigens [23].

When developing an ELISA for autoantibody measurement, for the antigen that will be bound to solid phases approaches involving cell extracts, purified antigens from animal tissues, recombinant antigens, mixtures of cell extracts and purified antigens, mixtures of cell extracts and recombinant antigens, and mixtures of purified and recombinant antigens can be used. All of these approaches have been used in commercial kits for autoantibody determination.

5.3. Standardization

One of the main problems with commercial EIA kits for autoantibody measurements is that of standardization. In 1982 the Arthritis Foundation (AF) in collaboration with the Centers for Disease Control (CDC) prepared a panel of five AF-CDC reference sera that includes specificities for ANA, dsDNA, SS-B, RNP, and Sm antibodies [24]. The AF-CDC standards were later reevaluated to define their usefulness [25]. The AF-CDC serum panel was used to evaluate the performance of EIA kits from different manufacturers [26]. Likewise, the Association of Medical Laboratory Immunologists has developed a panel of antinuclear and anti-cytoplasmic antibody consensus sera that could be useful in the evaluation of newly available EIA methods for the detection of autoantibodies [27].

5.4. Applications

In the late 1980s, commercial enzyme immunoassay kits were developed for ANA screening to overcome the drawbacks of IIF [28]. These kits used HEp-2 cell extracts, attempting to mimic the IIF technique. However, they did not gain popularity because they were unable to produce results equivalent to those possible with ANA-IIF [29]. In recent years, new commercial ANA-EIA kits that use HEp-2 nuclear extracts plus purified or recombinant antigens have been developed. The objective is to replace ANA-IIF by ANA-EIA as a screening test. Comparative studies between ANA-IIF and ANA-EIA have been performed, but with discordant results [17,20,26,30–38].

In general, ANA-ELISAs show good sensitivity and a high negative predictive value, so they can be used to eliminate samples without ANA, although because of their low positive predictive value, positive samples must be analysed by IIF to confirm the presence of ANA and to determine the final result. To conclude our discussion on ANA, it should be noted that new horizons are continually being found for EIA in the autoimmunity laboratory, raising the question of whether this new era of ANA screening might mean farewell to the microscope [39].

ELISA is also used to measure anti-dsDNA antibodies [26,40,41]. These assays use purified dsDNA as antigen. However, during the physical process of dsDNA binding to the solid phase, the double-helix structure may become distorted, exposing hidden epitopes, and modifying conformational epitopes, which could contribute to the discrepancies observed on comparing commercial ELISAs for anti-dsDNA. In general, anti-dsDNA ELISAs have very good diagnostic sensitivity, but low diagnostic specificity, because they detect high- and low-avidity antibodies; the latter considered of low clinical relevance. When using ELISA as a screening method for SLE, another test should be used to increase diagnostic specificity, such as IIF and the Farr assay, which only detect high-avidity antibodies.

Nucleosomes have been considered a major autoantigen in SLE and the measurement of anti-nucleosome antibodies has been proposed as more sensitive and specific than that of anti-dsDNA antibodies for SLE and drug-induced lupus [42,43]. Antinucleosome autoantibodies react with epitopes comprised of the native DNA structure found in chromatin, the native histone epitopes exposed in chromatin, and epitopes made up of the histone–DNA complex found in chromatin. Specifically excluded are reactivities to non-histone proteins such as centromere and DNA topoisomerase I [43]. However, in our experience anti-nucleosome antibodies and anti-dsDNA antibodies provide similar information in established SLE [44]. ELISA is also currently being used for the measurement of anticentromere antibodies using recombinant antigen [45].

Several ELISA methods have been described for the detection of anti-neutrophil cytoplasm antibodies, using purified and recombinant antigens. Attempts to obtain recombinant PR3 have not been successful for obtaining a protein reactive to human antibodies. Immobilisation of native PR3 by coating plastic plates can provide partial denaturation, with changes to conformational epitopes, which can produce a loss of reactivity with autoantibodies. This phenomenon has been observed in some patients treated with anti-PR3 antibodies and could be overcome by using a capture monoclonal antibody to immobilize PR3 protein [46].

6. Flow cytometry

Flow cytometry for the analysis of microsphere-based immunoassays has been developed for the simultaneous determination of different substances [47,48]. Among the applications of this technology is the simultaneous measurement of several autoantibodies. The system uses polystyrene microspheres labelled internally with different ratios of two different fluorochromes. Each fluorochrome can have any of 10 possible levels of fluorescence intensity, thereby creating a family of 100 spectrally addressed bead sets. The antigens are bound to the microspheres. Each of the 100 spectrally addressed bead sets can contain an antigen specific for a unique target antibody.

In brief, the assay is as follows. Serum is incubated with the microspheres for around 30 min at room temperature in a filter-membrane microtitre plate. After incubation, the microspheres are washed and a solution of antihuman IgG.
conjugated with another fluorochrome is added and the microspheres are incubated again for 30 min at room temperature. The reactions are then analysed directly by the flow cytometer, which measures the fluorescence of the internal fluorochromes, allowing the classification of microspheres in an array of the 100 sets. Simultaneously, the fluorescence associated with autoantibody binding is measured.

At present, several commercial kits have been introduced for the simultaneous measurement of different autoantibodies by flow cytometry. The evaluation of a kit (Biomedical Diagnostics, Marne la Vallée, France) for the simultaneous quantitative determination in the same sample of 9 antinuclear antibody specificities (dsDNA, SSA, SSB, Sm, Sm/RNP, Scl70, Jo-1, ribosome and centromere B) has yielded good results; the concordances with other established methods were between 99.1% and 100.0% [49]. The system is reliable (coefficient of variation <10%) and accurate (correlation coefficient with ELISA between 0.90 and 0.97) over a large measurement range. Other kits for the simultaneous determination of antinuclear antibody specificities have also been evaluated, excellent correlation with ELISA methods being reported [50–52].

Thus, the particle-based flow cytometric assay of autoantibodies has great potential. The multiplexing ability and reasonable cost suggest that this technique will have a significant impact in the autoimmunity laboratory in coming years.

7. Microarrays

Microarray technology allows the simultaneous analysis of thousands of molecular parameters. Microarrays are made using either on-chip synthesis strategies or with an arrayer based on contact-printing or ink-jet technology. Antigens (proteins, nucleic acid fragments) are deposited as microspots at defined positions on the chip. These capture molecules, termed ligands or probes, are immobilized in rows and columns on the solid support. The chips are then exposed to samples containing autoantibodies, which bind to their corresponding immobilized antigens in the microarray. The concentration of the analyte in the sample can be determined by detecting the resulting antigen–antibody complex in the microspots of the chip. The recognition of autoantibodies may be achieved using fluorescence or radioactive labelling, chemiluminescence, mass spectrometry, or electrochemical methods [53].

Several reports employing protein microarray technology for the analysis of autoantibodies have been published. The first group describing the development of antigen arrays for the specific purpose of analysing autoantibodies was that of Joos et al. [53]. This group used microarrays for the simultaneous determination of 18 known autoantigens used as serologic markers in prominent autoimmune diseases such as autoimmuno thyroiditis, systemic rheumatic diseases, systemic vasculitis and antiphospholipid syndrome. The antigen corresponding to autoimmuno thyroiditis is thyroid peroxidase (TPO), and the antigen to systemic vasculitis is myeloperoxidase (MPO). Antinuclear antigens are important markers for systemic rheumatic diseases: dsDNA, Sm, SmB’histone, U1snRNP A y C for SLE; U1snRNP 68 kDa for MCTD, SsA and SSB for Sjögren’s syndrome, Scl-70 for scleroderma; CENP B for Crest syndrome, Jo1 for polymyositis, Pm-Scl 100 for an overlapping syndrome, and β2-glycoprotein I for the antiphospholipid syndrome.

Microarrays contain serial dilutions of the various antigens, thereby allowing accurate determination of autoantibody titres but using minimum amounts of serum. For detection, rabbit anti-human peroxidase-conjugated antibody was used [53]. Detection was accomplished using luminol as substrate, and chemiluminescence measurements were performed. Images were acquired with a charge-coupled device (CCD) camera. The authors reported that the assay was very sensitive and highly specific, and the signal intensities observed from serial dilutions of the immobilized antigen correlated well with serial dilutions of autoimmune sera [53].

Another study used microarrays with 196 different biomolecules, representing major autoantigens targeted by autoantibodies from patients with autoimmune rheumatic diseases [54]. The authors used a robotic arrayer to print 1152-feature arrays containing 196 different biomolecules. The arrays were incubated with human sera followed by secondary anti-human antibody conjugated to the Cy-3 fluorochrome. The arrays were scanned and the images were analysed to determine the level of autoantibody binding. The authors reported that their antigen arrays were four- to eight-fold more sensitive than conventional ELISAs. They concluded that autoantigen microarrays represent a powerful tool for studying the specificity and pathogenesis of autoantibody responses, and for identifying and defining relevant autoantigens in human autoimmune diseases.

More recently, another study used microarrays with 15 autoantigens for the detection of autoantibodies in autoimmune rheumatic diseases [55]. The authors indicated that the use of microarrays combined with artificial intelligence analysis might provide additional improvements in throughput, cost-effectiveness, and accuracy for the molecular diagnosis of autoimmune diseases.

Several reviews and opinion analyses and theoretical developments concerning the use of microarrays for the study of autoimmune diseases have been published [56–60]. As yet, arrays for autoantibodies have not been commercialised, but in the coming future this technology should be accessible to clinical autoimmune laboratories.

8. Conclusions

Present-day clinical laboratories have at their disposal immunoassay techniques and methods that are more and more sensitive and specific for the measurement of an increasing number of different autoantibodies. To a large extent, this improvement has been due to the production of recombinant antigens. 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 autoantibodies directed against thousands of different autoantigens with a low-cost and low-volume format. 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 multiplex analysis, the use of protein microarrays and the use of other nanotechnologies such as microfluidics.

References


