

DESIGNER ANTIGENS

MILESTONES IN DETERMINING AUTOIMMUNE DIAGNOSTICS

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▷ INTRODUCTION

“Designer antigens” are at the vanguard of modern serological diagnostics. As immune targets become better characterised, antigenic substrates used in immunoassays such as ELISA can be adapted to maximise diagnostic performance. Using cutting-edge biomolecular techniques, irrelevant or disease-unspecific epitopes can be removed and disease-relevant epitopes optimised with regard to number,

accessibility and reactivity.

ELISA systems based on artificially designed proteins offer in many cases vast improvements in performance over those utilizing naturally occurring antigens. By focusing only on relevant epitopes, antibody binding is increased and unspecific reactions are reduced to a minimum. Many designer-antigen-based test systems used today offer unprecedented diagnostic sensitivity and

specificity compared to their conventional counterparts.

One area that has profited particularly from advances in antigen design is autoimmune disease diagnostics. For many autoimmune diseases the detection of disease-associated autoantibodies is, alongside clinical symptoms, a cornerstone of diagnosis. Titer measurements are also frequently employed to monitor disease activity and assess the effectiveness

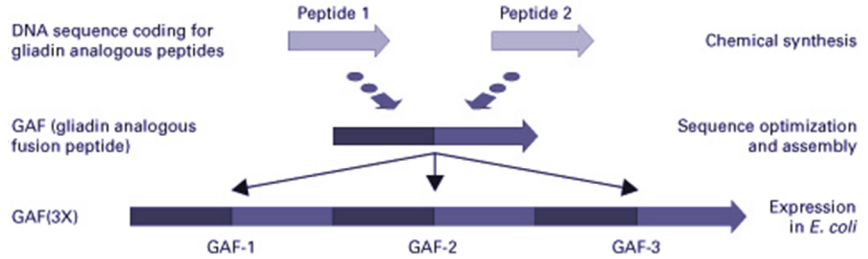
of treatment. Thus, efficient antibody detection is crucial for both initial diagnosis and subsequent patient follow-up. Below are some examples of how designer antigens have enriched the serological diagnosis of various autoimmune diseases.

▶ WEGENER'S GRANULOMATOSIS

Autoantibodies against proteinase 3 (PR3) are an established marker for the diagnosis of Wegener's granulomatosis, a febrile, chronic granulomatous autoimmune disease affecting predominantly the nasopharynx, lungs and kidneys. Anti-PR3 antibodies are detected by indirect immunofluorescence (cANCA pattern) combined with monospecific immunoassays. However, ambiguous results in IIFT, for example when other antibodies are simultaneously present, cannot always be confirmed with ELISA due to its insufficient sensitivity.

This limitation was addressed by the development of a novel target substrate (Figure 1). In the modified protein the proteolytically active centre of PR3 has been artificially inactivated by the exchange of one amino acid, resulting in higher protein stability without a reduction in antigen capacity. In cell culture the synthesised PR3 is no longer able to self-digest or interfere with cell metabolism, and can accumulate in cells in high concentrations. This means that the protein can, for the first time, be produced on large-scale in a human cell line where it undergoes authentic posttranslational modification, in contrast to conventionally used heterologous expression systems such as insect cells or *E. coli*.

This human expressed recombinant (hr) PR3 was combined with human native (hn) PR3 in a new immunoassay, the Anti-PR3-hn-hr ELISA, to provide an



▶ FIGURE 2: Gliadin-analogue fusion peptide (GAF-3X)

antigen spectrum that is unsurpassable. In a clinical study the new ELISA demonstrated impressive diagnostic capabilities - even by far surpassing the established anti-PR3 capture ELISA. With respect to indirect immunofluorescence, 94% of cANCA reactive sera could be confirmed as anti-PR3 positive, compared to 78% for a conventional ELISA and 88% for the capture ELISA.

With its exceptional sensitivity this designer antigen-based ELISA refines the diagnosis of antineutrophil cytoplasmic antibody (ANCA)-associated vasculitides, and is also suitable for subsequent long-term evaluation of patients to identify clinical relapses.

▶ COELIAC DISEASE

Antibodies against gliadin occur in gluten-sensitive enteropathy (coeliac disease), a gastrointestinal autoimmune disease triggered in predisposed individuals by the consumption of gluten-containing cereal products. However, up until now these antibodies were of limited use in diagnostics, since, using conventional ELISA based on native gliadin, they are also frequently detected in healthy individuals. Recently, areas of gliadin have been identified against which only patients with coeliac disease and not healthy persons develop antibodies. Based on these findings a recombinant gliadin-analogue fusion peptide (GAF) consisting of two

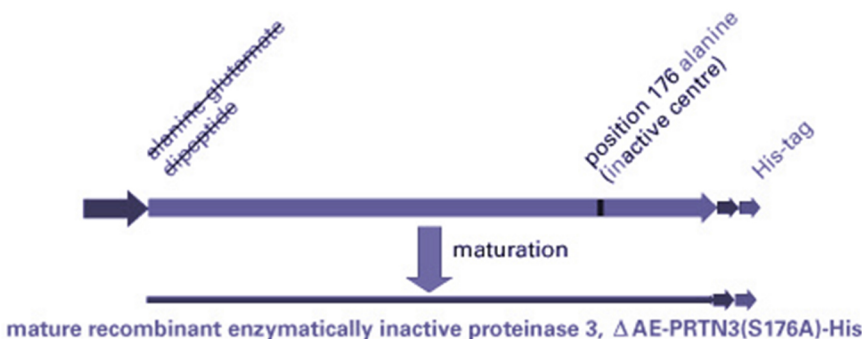
nonapeptide components expressed in trimeric form (3X) was created (Figure 2). The remaining 90% of the gliadin molecule is not used in the target antigen — this is only immunological ballast, which serves predominantly as a target for unspecific reactions.

The engineered antigen was subsequently employed in a new immunoassay, the Anti-Gliadin (GAF-3X) ELISA, which yields significantly higher sensitivity and specificity than conventional anti-gliadin ELISAs. In a multicentre study using a total of over 900 sera the new test yielded a sensitivity (at 95% specificity) of 83%/94% (IgA/IgG), compared to 54%/31% for a conventional ELISA. This represents an increase of 29% for IgA and 63% for IgG.

Used alongside established tests for antibodies against tissue transglutaminase (IgA; endomysium), the new immunoassay significantly enhances the diagnosis of coeliac disease. The Anti-Gliadin (GAF-3X) ELISA (IgG) is especially suited for the identification of patients with an IgA deficiency, which is frequently associated with coeliac disease. It can also be used for assessing disease activity or monitoring a gluten-free diet or a gluten-load test.

▶ PRIMARY BILIARY CIRRHOSIS

Autoantibodies against the mitochondrial antigen M2 (AMA M2) are determined in the diagnosis of primary biliary cirrhosis (PBC), an immune-mediated chronic inflammatory cholestatic liver disease. The M2 antigen is a complex of several enzymes, of which the following units are important as autoantigens: pyruvate dehydrogenase, branched-chain 2-oxoacid dehydrogenase and oxoglutarate dehydrogenase. Up to 90% of M2-specific autoantibodies are directed against pyruvate dehydrogenase, and many commercial ELISAs are based solely on this main target antigen. But 5-10% of PBC →



▶ FIGURE 1: Modified PR3 antigen

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patients exhibit only antibodies against epitopes of the two other antigens and are therefore not detected with conventional tests.

To address this shortcoming a recombinant fusion protein was created containing the immunogenic domains of all three relevant enzyme complexes (3E) of the M2 antigen in a defined stoichiometric relationship (Figure 3). An ELISA based on an optimised mixture of this designer antigen and native pyruvate dehydrogenase (Anti-M2-3E ELISA) demonstrated a previously unattained sensitivity of 93% in a study with 170 sera from clinically characterised patients. A conventional anti-M2 ELISA, in contrast, showed a sensitivity of only 79% with same patient panel. This markedly increased sensitivity combined with a high specificity (98%) makes the Anti-M2-3E ELISA a powerful new tool for detection of AMA M2 in autoimmune liver disease diagnostics.

▷ BULLOUS PEMPHIGOID

Bullous pemphigoid is an acquired subepidermal blistering autoimmune disease, which is characterised by autoantibodies against the hemidesmosomal proteins BP180 and PB230. The vast majority of patients demonstrate autoantibody binding to epitopes clustered within the 16th noncollagenous domain NC16A of BP180. Based on this observation a

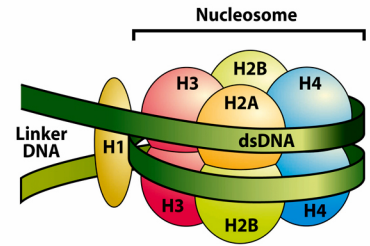
new recombinant designer antigen was developed which is much better suited for diagnostic purposes than the natural version (Figure 4). From the huge BP180 molecule it contains only the pathogenetically relevant target structure (NC16A). This, moreover, is present as a tetramer, increasing the number of available antibody binding sites and optimising the immunoreactivity.

On the basis of this innovative antigenic construct an extremely efficient ELISA (Anti-BP180-NC16A-4X ELISA) was developed, which exhibits a sensitivity of 90% and a specificity of 98%, and detects 100% of patients with gestational pemphigoid. If autoantibodies against BP230 are additionally determined, 93% of patients with bullous pemphigoid can now be identified serologically. Since the serum level of anti-BP180 antibodies correlates with disease activity, this parameter is also relevant for monitoring the disease course and the response to therapy.

▷ SYSTEMIC LUPUS ERYTHEMATOSUS

Autoantibodies against double-stranded DNA (dsDNA) are found in 60-90% of patients with systemic lupus erythematosus (SLE) and are the most important serological marker for diagnosis of this disease. In conventional ELISA systems the target dsDNA is often linked to the solid phase by means of poly-L-lysine or protamine sulphate. However, these substances are prone to cause unspecific reactions.

In vivo, dsDNA is wrapped around a histone core, forming the nucleosome (Figure 4). In an innovative new ELISA highly purified nucleosomes (6) are



► FIGURE 5: Structure of a nucleosome: dsDNA wrapped around a core of histone proteins.

used as a novel linker substance. Since nucleosomes have a strong adhesive ability, even the smallest concentration is highly suited to coupling isolated dsDNA to the surface of a microplate well. The resulting Anti-dsDNA-NcX ELISA far exceeds the quality criteria of conventional anti-dsDNA ELISAs. Many false positive results are prevented, and the specificity of the ELISA equals that of the indirect immunofluorescence assay (IFA) using *Crithidia luciliae*. Due to the new coating technique, the Anti-dsDNA-NcX ELISA is much more sensitive than both conventional ELISA and Farr-RIA.

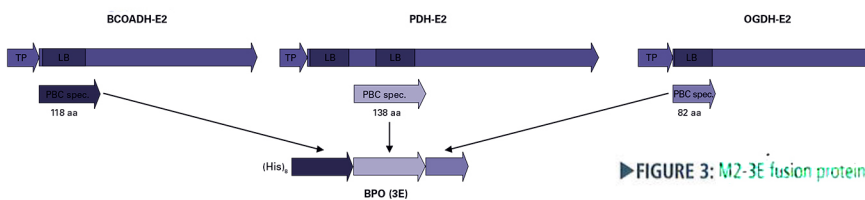
In a clinical comparison study with a large cohort of patients with SLE and other rheumatic diseases, the Anti-dsDNA-NcX ELISA demonstrated the highest sensitivity for the diagnosis of SLE. At a specificity of 98%, the sensitivity of the Anti-dsDNA-NcX ELISA amounted to 60.8%, clearly exceeding that of conventional ELISA (35.4%), of *C. luciliae* IFA (27.4%) and of Farr RIA (53.1%). The Anti-dsDNA-NcX ELISA has thus proven superior to Farr-RIA and *C. luciliae* IFA, and has the potential to replace them as gold standards in the serological diagnosis of SLE.

▷ CONCLUSIONS

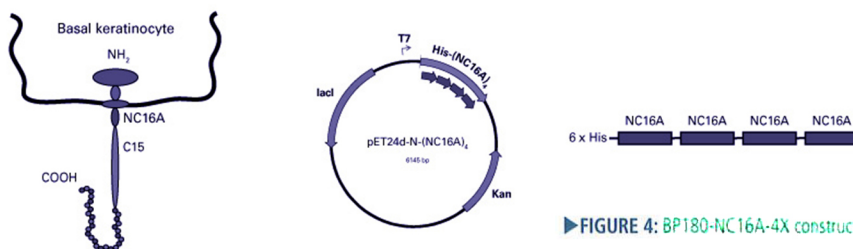
Biomolecular engineering of antigens with the explicit purpose of boosting their diagnostic performance is a growing trend and one with enormous future potential. State-of-the-art designer proteins have already substantially enhanced the diagnosis and/or monitoring of a host of autoimmune diseases. Since the possibilities for adapting proteins are potentially inexhaustible, further advancements in immunoassays based on designer antigens are to be anticipated.

▷ REFERENCES

References available on request (magazine@iirme.com)



► FIGURE 3: M2-3E fusion protein



► FIGURE 4: BP180-NC16A-4X construct