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A chip for the detection of antibodies in autoimmune diseases

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Serum	Diagnosis	Nuclei	DNA	SmD1	U1snRNP	Ro60	Ro52	La	histone	Scl70	CENP B	Jo-1	RA 33	PR3	MPO
Kä	Vasculitis	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Bo	Scleroderma	+	-	-	-	+	-	-	-	+	-	-	+	-	-
He	Sjoegren Syndrome	+	-	-	-	+	+	+	-	-	-	-	-	-	-
CO	Myositis	+	-	-	-	+	+	+	-	-	+	+	+		-
Fö	SLE	+	+	+	+	-	-	-	-	-	-	-	-	+	-
733206	Reference serum	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Donor sera															
H2	Healthy	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L23	Healthy	-	-	-	-	-	-	-	-	-	-	-	-	-	-
N27	Healthy	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 1. Microarray results using sera from patients with rheumatic diseases.

Biochip technology has rapidly developed into a booming sector in life sciences over the last few years. The several thousand articles dedicated to the topic of microarrays reflect the significance of the potential impact of this technology on the biosciences [1]. Most of the research has been carried out on gene expression analysis, where DNA microarrays make it possible to generate a vast amount of information from only a few experiments. Newly developed protein microarrays are designed for the detection, quantification and functional analysis of proteins (e.g., antibodies) [2]. Applications of protein microarrays include assessment of DNA-protein and protein-protein interactions. However, progress has been slow, in part because of the challenges posed by the natural differences between proteins and DNA molecules. Proteins are highly diverse conformational structures commonly consisting of twenty different amino acids, whereas DNA, apart from its sequence, has a relatively uniform structure. Proteins may be totally or partially hydrophilic, hydrophobic, acidic or basic. Furthermore, they may undergo post-translational modifications such as glycosylation, acetylation and/or phosphorylation.

Up to now, only a relatively small number of scientists have used protein array technology to directly investigate autoimmune diseases. Protein microarrays are technically more sophisticated than DNA arrays due to the heterogeneity of protein molecules as well as the need to preserve the complex three-dimensional structure (conformational epitopes) and function of proteins after immobilisation on the chip surface [3]. The main challenge is to find uniform incubation conditions (buffers, etc.) and chip surfaces that allow simultaneous detection of all target antigens on a single chip despite the biochemical and immunochemical differences in the antigens [4, 5].

Autoantigen microarrays that reveal antibody profiles characteristic of autoimmune diseases would be a powerful tool for studying the specificity and pathogenesis of autoantibody responses in autoimmune diseases [1, 6]. One of the main problems in the development of protein biochips is the measurement of spot intensities. Nowadays, most biochip or protein chip microarrays are modelled on DNA microarray technology systems where the fluorescence of conjugates is measured with a laser scanner [7, 8]. The use of this technology for the determination of autoantibodies is not only expensive, but also pointless in some cases. Because they were originally designed for comparative studies (e.g., parallel measurement of two different expression profiles), DNA microarrays require the measurement of more than one signal per spot.

Here, we describe a sensitive and reliable miniaturised biochip with 14 different autoantigens (more will be available soon) suitable for the simultaneous determination of the most significant autoantibodies associated with rheumatic diseases. Our RheumaChip uses a patented technique to measure spot intensities, comprising a radical difference in design. We simplified the technique by replacing laser-based scanning systems with a simple desktop photo scanner. By carefully optimising the reagents and incubation procedures, we achieved excellent sensitivity and specificity without the strong background signals which are still a major problem with conventional protein biochips.

Materials and methods

The autoantigens SS-SA/Ro52, Jo1, RA33, Scl70, U1snRNP-68-A,-C, SS-B/La and CENP B were expressed in either SF9 insect cells or *E. coli*. SS-A Ro60, dsDNA, PR3, MPO, histones and HeLa nuclei were prepared and purified from natural sources. The main epitope of SmD1 - a 45 amino acid (AA) peptide - was chemically synthesised. An NP-1.2 piezo-spotting device (GESIM, Germany) was used to produce ordered autoantigen arrays (4 replicates of each). Antigens were spotted (2 nL per spot) on membrane carrier foils. Each chip contained spotted human immunoglobulin as an internal functional control. Biochips were blocked to reduce non-specific binding and were subsequently preserved with a stabilising buffer. Serum samples were diluted 1/100 and incubated at room temperature. The membrane chips were incubated with HRP-labelled secondary antibodies and visualised with precipitating TMB substrate. The microarrays were scanned with a commercially available desktop scanner (Canon). Array software was used to measure spot intensities using the median feature pixel intensity minus median background pixel intensity. The patient sera had been characterised by immunoblotting (IB), indirect immunofluorescence (IIF), Line Immunoassay (LIA) and ELISA for the presence of the respective autoantibodies.

Results

By using optimised microarray spotting and incubation parameters, we were able to simultaneously detect various autoantibodies to biochemically different antigens (DNA, phospholipids, ribonucleoprotein complexes, peptides, proteins, enzymes and cell nuclei) on a single chip. Autoimmune serum samples from patients suffering from different autoimmune diseases with known autoantibody profiles were serially diluted to validate the sensitivity of the method. Specificity was tested using sera from healthy volunteers and autoantibody-negative patients. The microarray results were in agreement with those obtained by IIF, ELISA, LIA and blotting methods [Table 1].

In contrast to earlier experiments carried out using silane-activated glass slides, developed with fluorescent conjugates and measured with an Agilent laser scanner (data not shown), the RheumaChip arrays achieved excellent positive/negative ratios and signal/background ratios. Microarray spot intensities were measured using a conventional desktop scanner

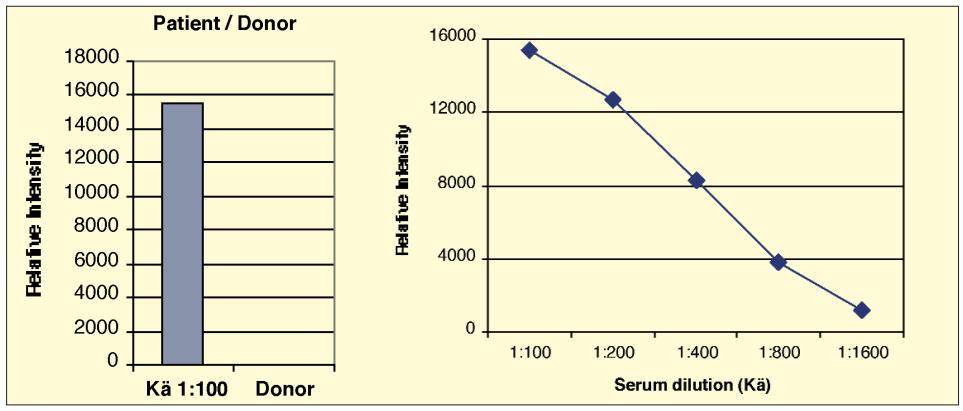

Figure 1. Signal intensities of an anti-PR3 antibody-positive serum vs. a negative control serum from a healthy blood donor and signal intensities of a serum dilution as detected using the RheumaChip.

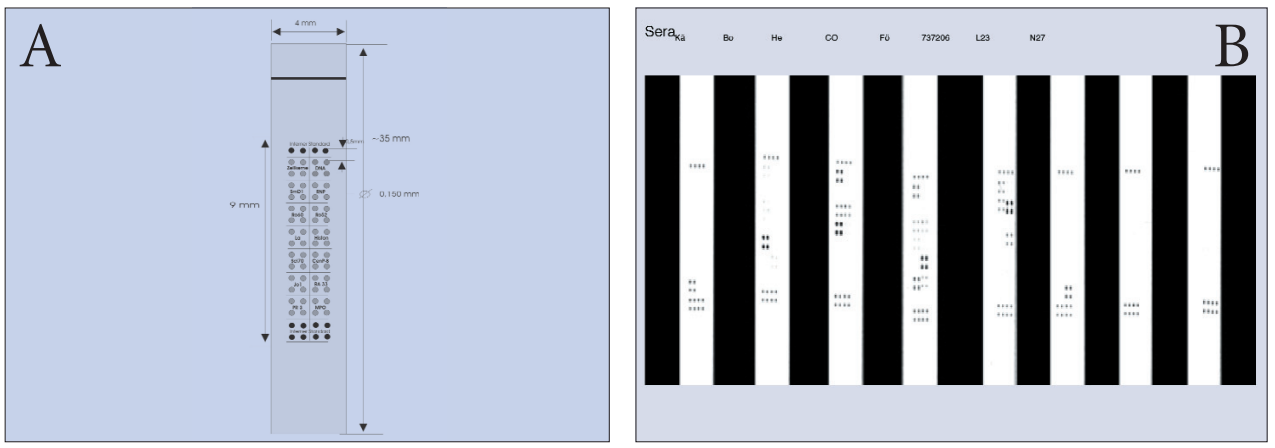

Figure 2. (A) Schematic design of the RheumaChip. (B) Autoantibody profiles from six patients with rheumatic diseases and two healthy blood donors as detected with the RheumaChip.

equipped with a low-cost polychromatic light source. All sera from healthy volunteers tested negative on the RheumaChips.

Figure 1 shows the signal intensities measured in individuals positive and negative for the autoantigen PR3. Representative autoantibody profiles of various patients with different rheumatic diseases [Table 1] are presented in Figure 2. The intra- and inter-chip variation coefficients remained below 15%. The special coating procedure ensures a shelf-life of more than one year at 2 to 8 °C.

Conclusion

Assays for multiplex determination of antibodies with different specificities have a wide range of applications in autoimmune disease diagnosis. Although ELISA-based tests are well-suited for this purpose, they are expensive, time-consuming and require relatively large quantities of antigens and sera, which limits their feasibility for mass screening. Since microarray formats incorporate true parallelism, miniaturisation and high throughput, they may overcome most of the limitations of ELISA technology.

With the RheumaChip, we have developed the first biochip for simultaneous detection of 14 diagnostically relevant autoantibodies associated with rheumatic diseases. The number of different antigens on the RheumaChip is however not limited by the compatibility of the antigens: we successfully tested 30 different proteins under identical conditions. Our findings indicate that this novel method can achieve excellent sensitivity and specificity, even when used in combination with an inexpensive, non-laser-based, commercially available desktop scanner (patent pending). We attained an excellent signal-to-noise ratio with no non-specific reactions, and all sera from healthy volunteers tested negative (n>200).

The RheumaChip provides an ordered chip-based autoantigen microarray system for simple, multiplex characterisation of human autoantibody profiles in patients with autoimmune diseases. Our microarrays contain biochemically different autoantigens, including DNA, phospholipids, ribonucleoprotein complexes, peptides, proteins, enzymes and cell nuclei. We demonstrated that the new test system is able to sensitively and specifically recognise characteristic autoantibody profiles in sera from patients with autoimmune diseases.

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