Comparison of ELISA with CENP-A and CENP-B for the detection of anti-centromere antibody

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Autoantibodies against intracellular antigens are commonly found in a number of systemic autoimmune diseases. Although anti-centromere antibodies (ACA) are closely related to the limited form of scleroderma, they are also detected in patients with various autoimmune diseases (1). The indirect immunofluorescence (IIF) pattern of ACA is discrete speckled nucleoplasmic staining (2) and IIF is the most reliable assay. ACA reactivity is directed primarily against three major autoantigens: centromere protein (CENP)-A, CENP-B, and CENP-C (1, 3). Nearly all ACA-positive sera are detected by ELISA using the recombinant C-terminus of CENP-B, which is the major epitope detected by ACA (4).

We previously showed that the major epitopes of ACA are located not only in the C-terminus of CENP-B but also in the N-terminus of CENP-A (5-7). In this study, we compared the sensitivity of detection of ACA between a homemade anti-CENP-A ELISA and a commercial anti-CENP-B ELISA kit, and then evaluated the availability of ELISA with CENP-A or CENP-B.

From our department's serum bank, we used 70 ACA-containing sera from patients with autoimmune diseases, including 41 scleroderma (9 were with coexistent Sjögren's syndrome (SjS)), 11 systemic lupus erythematosus, 8 SjS, 3 dermatomyositis, and 3 Raynaud's disease. The existence of ACA was confirmed by IIF. Thirty normal sera were collected from healthy blood donors and used as normal controls.

Anti-CENP-B antibodies were assayed using a commercial ELISA kit for anti-CENP-B (MBL, Nagoya, Japan). This kit used recombinant protein of the C-terminus of CENP-B (covering amino acids 538-599) and a mixture of anti-human IgG, IgA, and IgM as the secondary antibodies. Of the 70 ACA-positive sera, 67 (96%) were positive, 2 (3%) were negative, and 1 (1%) was in the equivocal range (10-16 IU/ml). Anti-CENP-A antibodies were assayed by homemade ELISA with a mixture of two synthetic peptides conjugated to BSA (0.5 μg/ml in PBS), each of which corresponded to amino acid sequences 3-17 and 25-38 of CENP-A (6). As the secondary antibodies, peroxidase-conjugated rabbit anti-human IgG, IgM, or IgA (Dako, Glostrup, Denmark) was used. The absorbance with the synthetic peptides after subtracting the antibody reactivity to BSA alone was determined for each individual. Values greater than two standard deviation units above the mean of 30 normal controls were considered positive. Of the 70 ACA-positive sera, 65 (93%), 39 (56%), and 46 (66%) were positive for the IgG-, IgM-, and IgA-class antibodies, respectively. All five IgG-negative sera were also negative for IgM and IgA. The ELISA results are summarized in Table I.

Table I. Numbers of patients with elevated levels of anti-CENP-A and anti-CENP-B.

Autoantibody	positive	negative	equivocal
Anti-CENP-B (IgG+IgM+IgA)	67	2	1
Anti-CENP-A (IgG)	65	5	–
Anti-CENP-A (IgM)	39	31	–
Anti-CENP-A (IgA)	46	24	–

Of the 70 ACA-positive sera, 69 sera were at least reactive to CENP-A or CENP-B by ELISA. These results suggest that combining ELISAs with CENP-A and CENP-B can improve sensitivity, and ELISA kits mixing both CENP-A and CENP-B peptides are already available commercially (INOVA Diagnostics, San Diego, CA). The equivocal anti-CENP-B serum was negative for anti-CENP-A antibody by ELISA, as well as for CENP-A, -B, and -C by immunoblotting.

There was a statistically significant correlation among the levels of IgG-, IgM-, and IgA-anti-CENP-A antibodies. Although significant correlation between the levels of anti-CENP-A and anti-CENP-B by ELISA has been reported (8), our study showed no correlation. The isotype of the anti-CENP-B antibody detected by ELISA has been reported (9). However, the isotype of the anti-CENP-A antibody detected by ELISA has not yet been studied. We showed here that while the IgG isotype of anti-CENP-A antibody was the most common, the IgM and IgA isotypes were also commonly found.

In conclusion, this is the first report comparing isotypes of anti-CENP-A antibodies. Moreover, we suggest that combining the detection of CENP-A and CENP-B into a single ELISA might improve the sensitivity of ACA detection.

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