News Release

Title

Immune recognition of lysyl-tRNA synthetase and isoleucyl-tRNA synthetase by anti-OJ antibody-positive sera

Key Points

- *In vitro* translated recombinant isoleucyl/lysyl tRNA synthetases were used in ELISA for the dection of anti-OJ antibodies.
- The ELISA results correlated well with the anti-OJ results obtained by immunoprecipitation as a gold standard method.
- The main advantages of our ELISAs are 1) no need for radioisotopes, 2) saving time and labor, 3) easy interpretation of the results by non-experts.

Summary

Assoc. Prof. Yoshinao Muro (first and corresponding author), Prof. Masashi Akiyama at Department of Dermatology, Nagoya University Graduate School of Medicine (Dean: Kenji Kadomatsu, MD, PhD), Prof. Minoru Satoh at Department of Clinical Nursing, University of Occupational and Environmental Health, and co-investigators discovered that *in vitro* translated recombinants of isoleucyl/lysyl tRNA synthetases were useful for detecting anti-OJ antibodies.

Anti-aminoacyl-tRNA synthetase (anti-ARS) antibodies are useful for identifying a clinical subset of patients with idiopathic inflammatory myopathies (IIMs). Anti-OJ antibodies, which recognize multi-enzyme synthetase complexes including isoleucyl-tRNA synthetase (IARS) and lysyl-tRNA synthetase (KARS), are among the anti-ARS antibodies. Although testing antibodies to other ARSs have been used clinically, no validated immunoassays for detecting anti-OJ antibodies are available. Serum samples were collected from 279 patients with IIMs and 22 patients with idiopathic interstitial pneumonia (IIP). Sixty-four of the samples that had been confirmed to be negative for anti-OJ by standard immunoprecipitation were used as the negative control, and 12 anti-OJ-positive reference sera were used as the positive control. Antibodies to IARS and KARS were assayed by ELISA using biotinylated recombinant proteins generated by *in vitro* transcription/translation. The anti-OJ-positive sera strongly reacted with the KARS and IARS recombinant proteins in ELISA. Although all 12 reference sera were positive in the anti-KARS ELISA, 4 of the 64 anti-OJ-negative sera were also weakly positive. The sensitivity and the specificity were 100% and 93.8%, respectively. Since our anti-KARS ELISA performed well, showing a high agreement with the results for immunoprecipitation (Cohen's $\kappa > 0.8$), the remaining 237 samples were also tested. Thirteen anti-KARS-positive sera were newly found by ELISA, all of which were anti-OJ positive by immunoprecipitation. Our ELISAs can be applied to the first reliable, easy-to-use

measurement assays for anti-OJ antibodies.

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Research Background

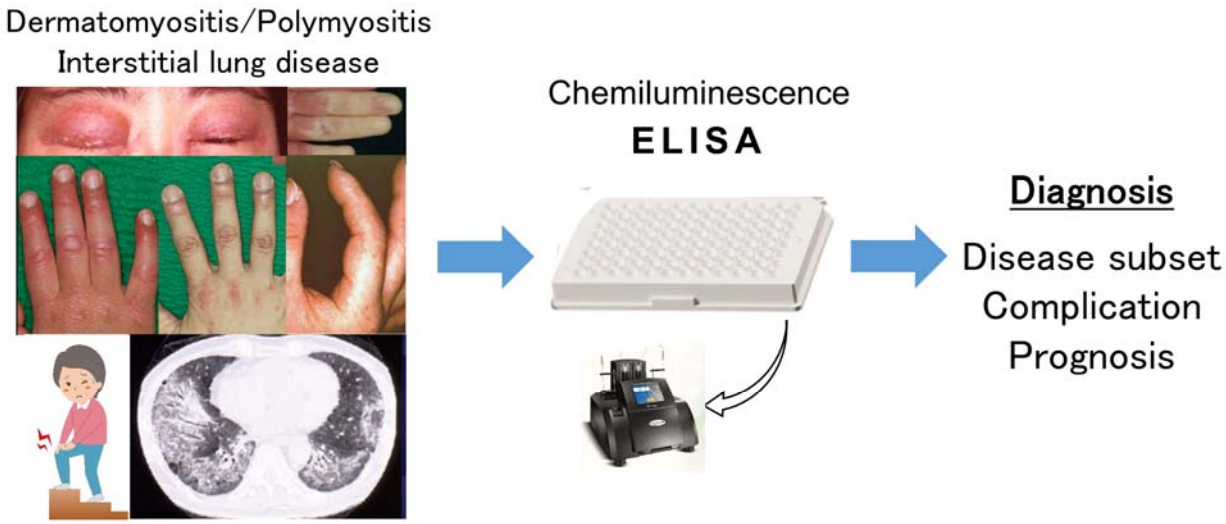
Anti-ARS antibodies help to diagnose, subset, and predict complications and prognoses in IIMs) Since the myositis of anti-ARS-positive patients is characterized by interstitial lung disease (ILD), fever, Raynaud's phenomenon, mechanic's hands, and arthralgia, the patients are classified as having anti-synthetase syndrome. To date, autoantibodies have been identified to 8 kinds of ARSs, including Jo-1, PL-7, PL-12, EJ, OJ, KS, Zo, and Ha. Although immunoprecipitation assays are recognized as the gold standard for detecting each anti-ARS, they are time- and labor-intensive and require specialists to interpret the results. Recently, a number of diverse commercial immunoassays have been used for identifying anti-ARS antibodies. However, their actual performances are not favorable for anti-OJ specificities. An anti-ARS antibody ELISA using a mixture of five recombinant ARSs (Jo-1, PL-7, PL-12, EJ, and KS) was developed in Japan. Although attempts were made to include the OJ antigen (IARS) in the anti-ARS assay, the recombinant IARS protein could not be included because it was not well recognized by the anti-OJ positive sera. Anti-OJ antibodies recognize a multi-enzyme synthetase complex that contains 9 synthetases and 3 non-catalytic components. The epitope(s) of anti-OJ antibodies has been thought to be dependent on the conformation of the protein complex.

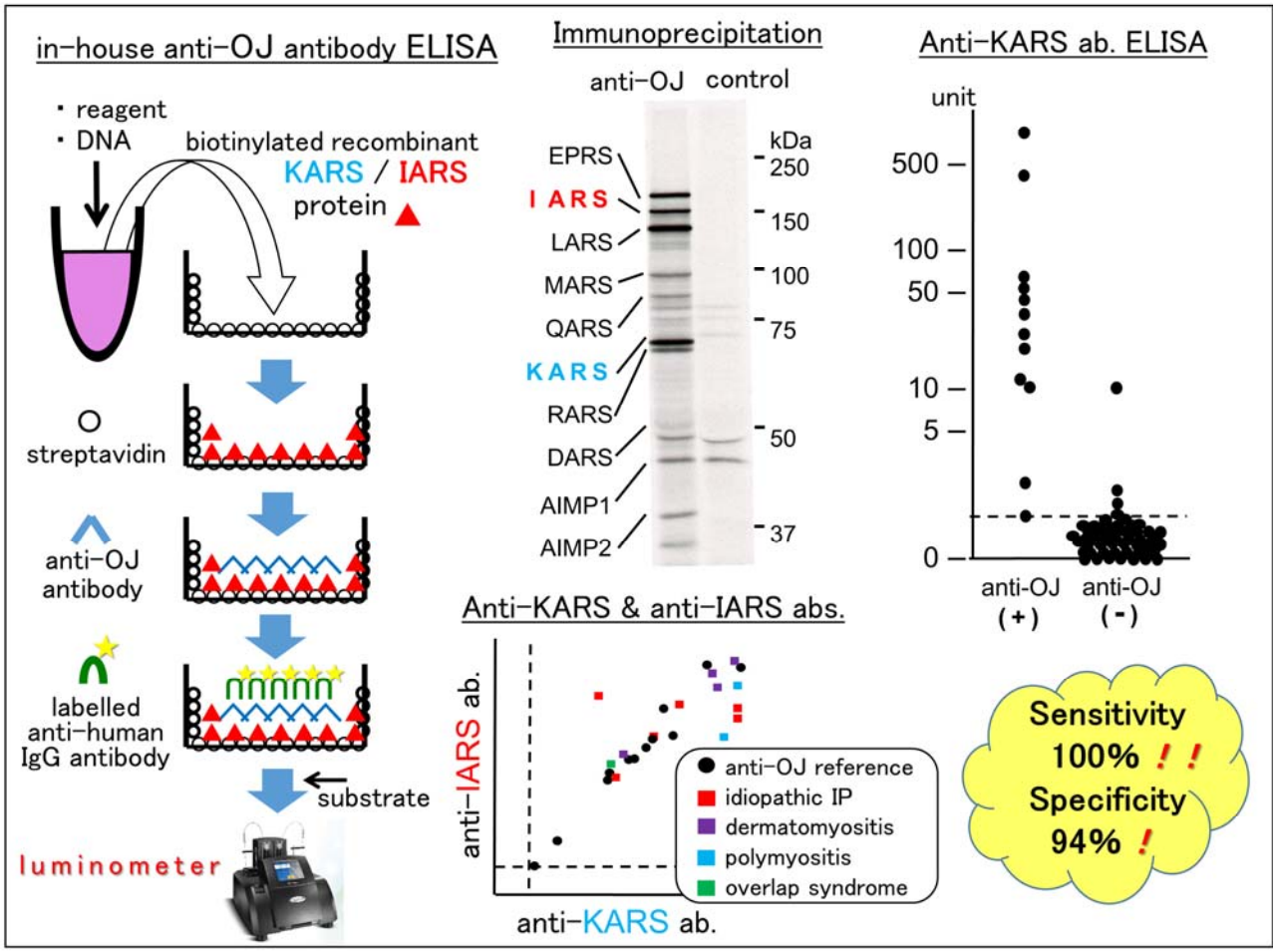
Research Results

Since we previously reported an in-house ELISA to detect various myositis-specific autoantibodies using *in vitro* transcription/translation (TnT) products, we tested the anti-OJ positive sera using IARS and lysyl-tRNA synthetase (KARS), which was shown to be reactive with anti-OJ antibodies by immunoblotting, TnT recombinant proteins. Twelve anti-OJ-positive reference sera strongly reacted with the KARS and IARS recombinant proteins in ELISA. Using the ELISA unit of the 76 samples that were confirmed to be positive or negative for anti-OJ, the best cutoff value was calculated based on the ROC curve. The sensitivity and specificity of the anti-KARS ELISA for anti-OJ antibodies was 100% (12/12) and 93.8% (60/64), respectively. We examined anti-OJ antibodies in sera from 279 patients with IIM and 22 patients with IIP by anti-KARS ELISA. The anti-KARS ELISA was selected instead of the anti-IARS ELISA for the cost-benefit: KARS requires smaller amounts of recombinant proteins than IARS requires. Thirteen serum samples exceeded the cutoff value and these sera were also tested by anti-IARS ELISA, which showed all these samples also to be positive for anti-IARS antibodies. They were confirmed to have anti-OJ antibodies also by standard immunoprecipitation. Cohen's κ value was 0.89 (95% CI; 0.79 – 1.00), which suggests that the inter-rater agreement between our anti-KARS and anti-IARS ELISA and standard immunoprecipitation was excellent. The clinical and laboratory profiles of these 13 patients with anti-OJ antibodies were very compatible with anti-synthetase syndrome. Notably, 4 (31%) patients were complicated with malignancy (OR = 3.43, 95% CI: 1.06–11.18).

Research Summary and Future Perspective

Immunoassays for detecting anti-OJ antibodies using KARS and IARS recombinant proteins were developed. The main advantages of our ELISAs when compared with immunoprecipitation are 1) they do not need any radioisotopes, 2) they can save time and labor, 3) the results can be interpreted by non-experts. Moreover, our ELISAs are semi-quantitative assay systems that can detect changes in serum levels of autoantibodies and may be useful for monitoring disease activity or the effects of treatment. If an automated apparatus for producing KARS and IARS TnT recombinants from the plasmid vector were to become available, it would also facilitate the commercialization of the ELISA kits.





Publication

Yoshinao Muro[a,*], Yasuhiko Yamano[b], Ken Yoshida[c], Yohsuke Oto[c], Kimiko Nakajima[d], Teruyuki Mitsuma[e], Shiori Kikuchi[f], Akihiro Matsumae[g], Mariko Ogawa-Momohara[a], Takuya Takeichi[a], Yasuhiro Kondoh[b], Masao Katayama[g], Yasuyuki Todoroki[h], Yoshiya Tanaka[h], Minoru Satoh[i], Masashi Akiyama[a]

[a]Department of Dermatology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya, Aichi 466-8550, Japan

[b]Department of Respiratory Medicine and Allergy, Tosei General Hospital, Seto 489-8642, Japan

[c]Division of Rheumatology, Department of Internal Medicine, The Jikei University School of Medicine, Tokyo 105-8461, Japan

[d]Department of Dermatology, Kochi Medical School, Kochi University, Kochi 783-8505, Japan

[e]Department of Dermatology, Ichinomiya Municipal Hospital, Ichinomiya 491-8558, Japan

[f]Division of Neurology, First Department of Internal Medicine, Asahikawa Medical University, Asahikawa 078-8510, Japan

[g]Department of Internal Medicine, Nagoya Medical Center, National Hospital Organization, Nagoya 460-0001, Japan

[h]The First Department of Internal Medicine, University of Occupational and Environmental

Health, Kitakyushu 807-8555, Japan
[i]Department of Clinical Nursing, University of Occupational and Environmental Health, Kitakyushu 807-8555, Japan
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