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The Diluted TSHR Antibody Titer in Untreated Graves' Disease May Predict Who Will Respond to Six Month's Treatment with Methimazole, and Mc4-Expressing CHO Cells Can Detect Both Blocking and Stimulating TSHR Antibodies

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Background

Antibodies that bind to the TSH receptor (TSHR) can cause hyperthyroidism or, more rarely, hypothyroidism. However most commercially available TSHR antibody measurements are not very useful for making a pretreatment prediction about the efficacy of antithyroid drug treatment. Furthermore, the absolute values of antibody determinations often do not correlate between assays, despite the use of an "international standard." Nonetheless, several third-generation assays are sufficiently sensitive and specific to use as affirmation of one's clinical diagnosis in the bulk of patients with Graves' disease. (The tests do require some technical proficiency, but their cost is only 5% to 10% of a thyroid scan and uptake.) Here, I review two recent papers that use CHO cells expressing Mc4, a TSHR/luteinizing hormone (LH)/chorionic gonadotropin receptor (CGR) chimera. One study assesses the prognostic value of measurements of TSHR-stimulating activity in serial dilutions of serum samples, while the other assesses their use for measuring TSHR-blocking antibodies.

STUDY 1

Leschik JJ, Diana T, Olivo PD, König J, Krahn U, Li Y, Kanitz M, Kahaly GJ. Analytical performance and clinical utility of a bioassay for thyroid-stimulating immunoglobulins. Am J Clin Pathol 2013;139:192-200.

Methods

First, two third-generation assays were compared for detection, quantitation and cutoff limits, as well as for half-maximal effective concentrations in response to stimulation with the human monoclonal M22 TSHR antibody. The results from the Thyretain bioassay, which uses cryopreserved Chinese hamster ovary (CHO) cells that stably express the Mc4 chimera plus a cAMP-induced luciferase reporter gene, were compared to the results from the Roche Elecsys assay, which measures antibody binding to wells coated with the porcine TSHR, followed by ruthenium-labeled M22 that binds to the unoccupied sites remaining on the porcine TSHR, and is detected by electrochemiluminescence.Second, a small clinical study compared the Thyretain and Roche assays prospectively on 40 German patients with thyrotoxic Graves' disease to determine whether pretreatment and intratreatment anti-TSHR titers were of use in predicting the likelihood that a patient would be in remission after 6 months of treatment with MMI. Sera were obtained before and during a 24-week course of MMI and after 12 weeks of follow-up; 20 of the 40 remained in remission at 36 weeks. Undiluted sera and threefold dilution up to at least 1:81 were assayed, and the number of samples becoming undetectable at each dilution were determined. The Thyretain assay was positive on more samples ($P<0.0001$) (e.g., at the 1:9 dilution, two thirds of samples remained positive in

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the Thyretain assay, while only one fourth remained positive in the Elecsys assay). The dilution at which a sample that first fell below the cutoff threshold was called the "dilution titer."

Results

The Thyretain assay responded to M22 over a range of 0.012 to 0.4 ng/ml, whereas the Elecsys assay responded between 20 and120 ng/ml. The Elecsys assay limits of detection and of quantitation were about 250 times less, and cutoff and half-maximal effective concentration were about 800 times less when M22 was used, but this difference in sensitivity was much less dramatic when patient sera were used. Twenty of the 40 patients remained in remission for at least 3 months after MMI was discontinued. Assays on the undiluted pre-MMI samples did not distinguish between those who went on to relapse from those who remained in remission. However, when the samples were diluted with normal serum, the Thyretain assay on the pretreatment samples of the 20 nonresponders was 4.0±0.39, whereas it was only 2.9±0.25 (mean ±SD) in the 20 who remained in remission (P = 0.018), and after 12 weeks of MMI, the difference between mean dilution titers increased twofold (P<0.00012). Using the Elecsys assay, the mean dilution titer in the baseline samples was 2.65±0.29 for the nonresponders versus 1.65±0.16 for the responders (P = 0.003), and after 24 weeks on MMI, the difference between the mean titers was 1.85 (P<0.0001). Thus, with either assay, patients whose dilution titer was lower at baseline—and those in whom the titer decreased after 12 weeks of MMI—achieved normal thyroid hormone levels by 24 weeks and remained euthyroid clinically and biochemically for at least 12 more weeks off MMI.

Conclusions

The Thyretain bioassay is much more sensitive to M22 and slightly more sensitive to low-but-positive concentrations of TSHR-stimulating antibodies, as compared with the Roche Elecsys automated TSHR binding assay. However, both assays had similar abilities to predict at least a 12-week remission on pretreatment samples and on samples drawn after 12 weeks of MMI treatment, but only if the titer of TSHR-stimulating activity was measured on serial dilutions.

STUDY 2

Li Y, Kim J, Diana T, Klasen R, Olivo PD, Kahaly GJ. A novel bioassay for anti-TSH receptor autoantibodies detects both thyroid-blocking and stimulating activity. Clin Exp Immunol. May 7, 2013 [Epub ahead of print].

Methods

Serum antibodies that block the binding of TSH to the TSHR or that block the stimulation of cAMP production have been detected in patients with a variety of autoimmune thyroid diseases. Mc4-expressing CHO cells, as well as CHO cells expressing wild-type TSHR, were incubated with bovine TSH (bTSH) for 3 hours along with increasing concentrations of K1-70, a potent TSHR-blocking monoclonal antibody, to compare the percent inhibition of the cAMP response in the two cell types. After determining the assay cutoff level for blocking activity, sera from 300 euthyroid subjects were used to establish the mean percent inhibition produced by normal serum. Assay reproducibility was assessed on sera known to contain low, medium, and high levels of blocking antibodies. Samples from 171 patients with Graves' disease were then assayed for both stimulatory and blocking activity in the two CHO-cell assays. The two CHO-cell assays were tested on 50 normal sera and on sera from 50 patients with various autoimmune thyroid disorders.

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Results

CHO cells expressing wild-type TSHR were more sensitive to bTSH and recombinant human TSH (rhTSH), but Mc4-expressing CHO cells were 5 times more sensitive to inhibition by K1-70 when stimulated with 100 mIU/L of bTSH. The cAMP responses to TSH or M22 were inhibited by 50% using similar levels of K1-70. The CHO-Mc4 bioassay was estimated to be about 20 times more sensitive to the inhibitory action of K1-70 than the Kronus TRAb ELISA. Sera from 300 euthyroid controls produced a mean percent inhibition of –4% with a standard deviation of 21%, so the 2 SD cutoff limit for inhibition was about 40%, while for negative inhibition it was 46%. Sera with low, medium, and high levels of blocking antibody produced about 45%, 65%, and 95% inhibition, with coefficients of variation of about 4%, 9%, and 25%, respectively. Sera with high titers of blocking antibody could be diluted as much as 700-fold before they fell below the cutoff level. Sera from 50 tightly selected healthy euthyroid controls had blocking activity ranging from –16% to +37% inhibition. Sera from 50 patients with various autoimmune diseases had blocking activity ranging from –157% to +108% inhibition. Sera from 15 of the 50 patients displayed significant blocking activity: 2 were from patients with Graves' disease and 13 from patients with Hashimoto's thyroiditis (7 had hypothyroidism, including 2 with TSH >100). The Roche Elecsys assay detected activity in 10 of these 15 samples, which could have been interpreted as a TSHR-stimulating antibody. The assay of sera from 171 patients with Graves' disease revealed that the stimulatory activity in the CHO-Mc4 bioassay correlated closely with negative inhibition in the blocking assay, although sera with low but detectable stimulatory activity did not display significant negative blocking activity.

Conclusions

Assaying sera for blocking activity with CHO-Mc4 cells is about 20 times more sensitive than a commercial TSHR-binding assay that does not discriminate between stimulatory and blocking activity. The CHO-Mc4 blocking assay not only detects blocking antibodies, but it also indicates the presence of stimulatory antibodies, reporting them by their negative blocking activity, although it is not as sensitive as the regular CHO-Mc4 stimulatory assay.

ANALYSIS AND COMMENTARY

It is currently believed that patients with Graves' disease who are treated with antithyroid drugs are more likely to have a remission if their thyroid-stimulating antibody levels fall smoothly during treatment (1). Presumably, patients whose MMI dose needed to be adjusted downward had a better prognosis, although this is not mentioned in the article by Leschik et al. The new finding in their article is that pretreatment sera can be used to predict whether a patient will have a remission after 6 month's treatment with MMI, based on the serial dilution titer. Assuming the findings are confirmed in larger series of patients—and in laboratories not involving the assay's developers—serial dilution seems to be a promising way to predict which patients with Graves' disease will have a remission (which one would hope would last for more than 3 months). Serial dilution produced very similar results when the Roche Elecsys assay was used.

Li et al. report that CHO-Mc4 cells can be used to detect TSHR-blocking activity, while at the same time detecting TSHR-stimulating activity as negative

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inhibition. There is some irony to this report, since the TSHR/LH/CGR chimera was initially believed to be insensitive to TSHR-blocking antibodies, based on cAMP release from Cos-7 cells incubated with purified IgGs in the presence of isobutylmethylxanthine (2). The availability of the potent monoclonal antibodies M22 and K1-70 now provide a solid starting point for developing better TSHR antibody assays, but the pathophysiology of Graves' disease still seems to involve multiple binding sites and multiple antibodies. Indeed, studies using batteries of monoclonal antibodies directed at limited targets seem to indicate that the hinge–transmembrane region in the native LH receptor may have a more open structure than the native TSH receptor (3), which could be one reason the Mc4 chimeric receptor seems to be more responsive to some TSHR-stimulating antibodies when compared to the responsiveness of the wild-type TSH receptor. The likelihood that a patient with Graves' disease will have both blocking and stimulating antibodies is unknown, although in the 50 patients with autoimmune thyroid diseases, 2 such patients were found. One clinical situation that begs to be addressed with the blocking assay is the patient with Graves' disease who goes into remission after a course of antithyroid drug treatment, but then relapses. How commonly does this reflect a blocking antibody appearing during the "remission," which then disappears, while the stimulating antibody does not?

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