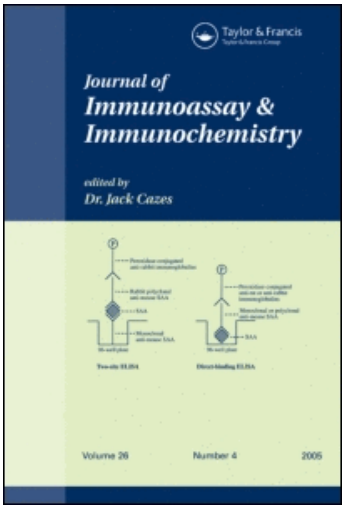

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SENSITIVE THYROID STIMULATING ANTIBODY (TSAb) ASSAY USING POLYETHYLENE GLYCOL (PEG)—A REVIEW

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INTRODUCTION

Thyroid stimulating antibody (TSAb) causes hyperthyroidism of Graves' disease. TSAb has been used as the diagnosis for Graves' disease and also in the index of clinical management of Graves' patients.[1,2] TSAb activity is measured as cAMP responses using porcine thyroid cells (PTC),[3] FRTL5-cells,[4] and CHO cells transfected by TSH receptor (R).[5] These bioassays are able to distinguish between stimulating type antibody (TSAb) and blocking type antibody [thyroid blocking antibody (TBAb)], which blocks TSH-stimulated cAMP production.

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The measurement of TSAb activity is essentially based on two separate assays. The first step is the incubation of serum or IgG with thyroid cells in culture. The second step is the measurement of cAMP in the culture medium by radioimmunoassay (RIA).[3] Recently, Watson et al.[6] and Evans et al.[7] developed new bioassays based on the luciferase receptor gene which was introduced into TSH-receptor expressing cells.

We found that polyethylene glycol (PEG) augmented TSAb activities.[8] Based on this PEG-augmentation of TSAb activities, we developed a highly sensitive TSAb assay system.[8–13] We review this sensitive TSAb bioassay method and its clinical usefulness.

RESULTS

PEG Augmented TSAb-Stimulated cAMP Responses; Sensitive TSAb Assay Using Porcine Thyroid Cells (PTC)

Shewring and Smith[14] first used PEG (4000) (final 15%) instead of ammonium sulfate for the precipitation of crude IgG fractions in an assay for TSH binding inhibitory immunoglobulin (TBII). Kasagi et al.[3] added PEG-precipitated IgG fractions into the porcine thyroid cells (PTC) culture medium in the TSAb assay.

In 1998, we demonstrated that more than 12.5% PEG precipitated fraction (PF) from Graves' serum augmented cAMP production in PTC assay. When the PEG 12.5, 15.0, 17.5, 20.0, and 22.5% PF from Graves' sera were incubated, higher concentrations of PEG augmented significantly TSAb-stimulated cAMP productions in many sera. Both the PEG 12.5% PF and the PEG 22.5% PF retained more than 85% of serum IgG. Thus, the augmentation of TSAb-stimulated cAMP production by PEG 22.5% PF is not dependent on the difference in the recovered IgG concentrations. PEG 22.5% PF from normal serum augmented the purified TSAb-IgG-stimulated cAMP productions (TSAb-IgG was purified with Protein A).[8] PEG (5%) augmented not only purified TSAb-IgG-stimulated cAMP productions but also TSAb-positive whole serum ($\leq$0.05 mL)-stimulated cAMP productions. However, the augmentative effect of PEG was not found in TSH-stimulated production or control-IgG.[8]

The augmentative effect of PEG was specific for TSAb-stimulated cAMP productions. Determined TSAb activities in 32 Graves' sera with four different methods; serum method (0.05 mL), serum (0.05 mL) plus 5% PEG, PEG 12.5% PF from serum (0.2 mL), and PEG 22.5% PF from serum (0.2 mL) are shown in Fig. 1.[9] In many Graves' sera with positive TBII, cAMP produced by crude IgG using PEG 22.5% PF was significantly higher

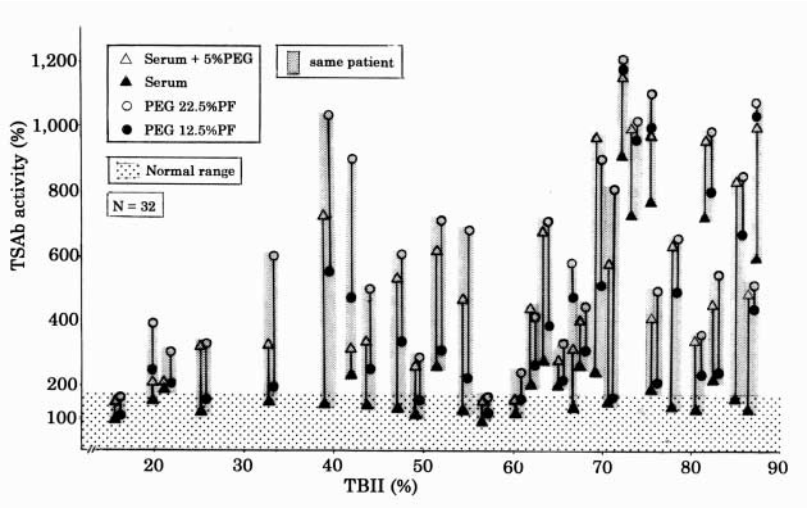

Figure 1. TSAb activities determined by 4 different assay methods in untreated Graves' serum. PEG, PEG 12.5% PF from serum (0.2 mL), and PEG 22.5% PF from serum (0.2 mL) were performed. Normal range shows mean of each individual normal range which was determined by 4 different assays using normal pooled serum (10 cases). Values are a mean of duplicate determinations.

than that by crude IgG using PEG 12.5% PF. The amount of cAMP produced by the serum (0.05 mL) plus 5% PEG was significantly higher than those by the serum (0.05 mL). The amount of cAMP produced by the serum plus 5% PEG was higher than those by the PEG 12.5% PF, but lower than those by PEG 22.5% PF in many sera, although a few discrepancies were observed.

The augmentative effect of PEG (5%) was observed by not only the whole molecule of TSAb-IgG, but also the protease-digested TSAb-IgG [$F(ab')_2$, Fab and smaller molecular component (Mr. 20 KD)].[10]

TSAb-Stimulated cAMP Responses by Polyvinyl Alcohol and Dextran, Non-ionic Hydrophilic Polymers

The augmentative effect on TSAb activity was also observed by other non-ionic hydrophilic polymers, such as polyvinyl alcohol and dextran. However, polyvinyl alcohol and dextran produced a minimal or barely detectable effect on TSH-stimulated cAMP responses. The augmentative

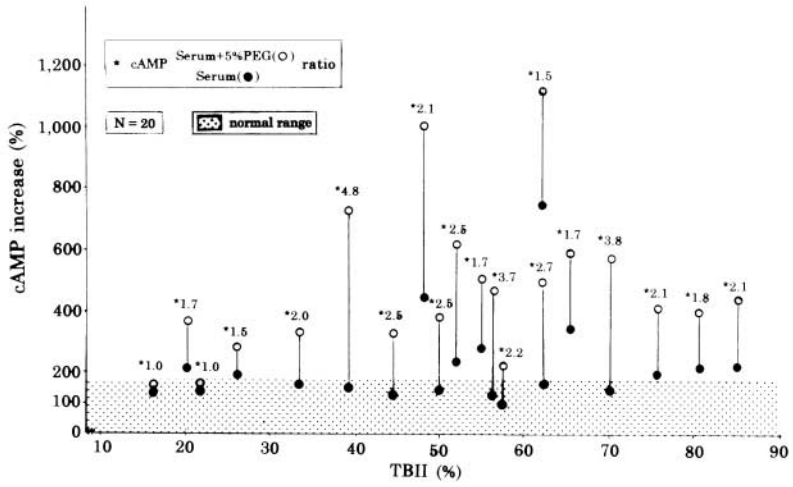

Figure 2. Augmentative effects of 5% PEG on cAMP production by whole serum from untreated Graves' patients in CHO-hTSH R cells. In the normal control, the mean cAMP by normal serum was almost the same the presence or absence of 5% PEG. Values are shown as the mean of duplicate determinations.

effect of PEG, polyvinyl alcohol, and dextran on cAMP produced by GTP∂S, forscolin, and pituitary adenyl cyclase activating peptide (PACAP) was not observed.[8,11,13] The augmentative effect of PEG (5%), polyvinyl alcohol (10%), and dextran (10%) on whole serum (<0.05 mL) is available to establish a sensitive TSAb assay.[11]

Sensitive TSAb Assays Using FRTL5-Cells and Recombinant CHO Cells

The augmentative effect of these polymers was also observed in FRTL5-cells and CHO cells expressing human TSH R (CHO-hTSH R cells) similar to PTC.[13] The augmentative effect of 5% PEG on cAMP production by whole serum (0.05 mL) from untreated Graves' patients in CHO-hTSH R cells is shown in Fig. 2. This suggests that there is no apparent specificity among human, porcine, and rat thyroid cells. TSH R linked cAMP production is important for the PEG augmentation of TSAb-stimulated cAMP production. These non-ionic polymers are available to increase the bioassay sensitivity using PTC, FRTL5-cells and recombinant CHO cells.[13]

Sensitive TSAb Assay in Coexisted Serum of TSAb and TBAb

Kaagi and Nagata[15] and we[16,17] reported that the precise determination of TBAb activity was difficult in sera containing strongly positive TSAb (>600%), because the additional effect of bovine TSH on high cAMP produced by high TSAb was unclear. Lack of the augmentative effect of PEG on TBAb activity was observed. However, PEG augmented TSAb activity, even in the presence of TBAb, by two methods (incubation of crude IgG using PEG 22.5% PF from serum (0.2 mL) and co-incubation of 5% PEG with test serum (0.05 mL)).[16] The sensitive assay data by two methods in co-existed cases of both TSAb and TBAb activity are shown in Table 1. TBAb activities were expressed by two calculation methods, $A = (1 - (a - b)/(c - d) \times 100)$ and $B = (1 - (a - d)/(c - d) \times 100)$, where a is cAMP produced in the presence of bTSH and patient's IgG, b is cAMP produced in the presence of patient's IgG, c is cAMP produced in the presence of bTSH and normal IgG, and d is cAMP produced in the presence of normal IgG. Method-A is a re recise TBAb determination method, compared to method-B. In the presence of TSAb, the values of method-A were always higher than those of method-B, since TSAb stimulated cAMP synthesis.

Mechanism of the Augmentative Effect of PEG on TSAb Activity

PEG, one of commonly used non-ionic hydrophilic polymers, has been shown to induce fusion between cells[18] and the transfer of plasmids into cells.[19] PEG accelerates the DNA ligase reaction,[20] affects protein adsorption,[21] and induces precipitation of proteins.[22]

The augmentative effect of PEG on TSAb-stimulated cAMP production in the incubation medium was dependent on the increased cAMP content within thyroid cells.[23] Although the mechanism by which a high PEG concentrations specifically increase cAMP production by TSAb remains unclear, several factors are suggested: (a) increasing ligand affinity to the receptor, (b) improving signal transduction to adenylate cyclase, (c) inhibition of cAMP phosphodiesterase, and (d) inhibiting internalization of receptor ligand complexes. The augmentative effect by recombinant TSHR cells (non-thyroid cells) may be any factors which accelerate the interactions between TSAb and the TSH R. Further study is required to clarify this mechanism.

Table 1. TSAb Activity Determined by PEG PF from Test Serum and Whole Serum in the Presence of 5% PEG Using TSBAb-Positive Serum

			TSBAb Activity (%)		TSAb Activity (%)			TSAb Activity (%)		
Pt. No.	TSH MU/L	TBII (%)	B Method	A Method	PEG 2.5% PF	PEG 22.5% PF	Coexistence of TSAb and TSBAb	Serum	Serum + 5% PEG	Coexistence of TSAb and TSBAb
1	1.2	90.0	95.6	98.0*	115	133*	−	110	115*	−
2	0.1	69.7	94.0	98.8*	123	108*	−	137	158*	−
3	0.05	76.5	95.7	98.6*	106	110*	−	98	115*	−
4	0.8	60.2	92.1	97.2*	110	114*	−	126	146*	−
5	0.1	75.2	87.4	97.6*	113	116*	−	134	140*	−
6	1.7	77.7	95.7	98.2*	114	126*	−	103	122*	−
7	1.0	75.0	96.5	100.0*	100	105*	−	135	145*	−
8	0.2	76.5	80.2	90.0*	113	123*	−	145	148*	−
9	0.08	81.0	92.1	98.0*	150	190##	+	142	157*	−
10	1.6	76.2	71.4	72.6*	130	575#	+	118	273#	+
11	0.2	69.6	70.0	78.0*	153	370#	+	113	220#	+
12	1.0	65.8	68.5	87.7##	250	333##	+	128	158*	−
13	0.7	70.0	76.6	90.6##	221	382#	+	167	216##	+
14	0.1	79.5	30.5	89.8#	693	594¤	+	242	383#	+
15	0.1	82.1	52.0	89.1#	416	383ø	+	205	311#	+

Nos. 1–6; primary hypothyroidism during T_4 therapy; No. 7: Graves' disease treated with anti-thyroid drug; No. 8: Graves' disease treated with radioisotope; Nos. 9–13: Graves' disease treated with anti-thyroid drug; Nos. 14 and 15: Graves' disease treated with radioisotope; TSH: <2 mU/L was used to remove the effect of TSH on TSAb assay; TBII: All TSBAb-positive sera showed high TBII activity; TSBAb activity calculated by A and B methods; TSAb activity (PEG 12.5% PF and 22.5% PF) means TSAb activity by PEG 12.5% PF and PEG 22.5% PF method; TSAb activity (serum and serum +5% PEG) means TSAb activity by serum (0.05 mL) method and serum (0.05 mL) in the presence of 5% PEG; #($p < 0.01$), ##($p < 0.05$) and *($p > 0.05$): Significance of difference from TBAb activity by the B method, TSAb activity by PEG 12.5% PF method and TSAb activity by serum method; ¤($p < 0.05$), ø($p > 0.05$); Reduction of TSAb activity by PEG 22.5% PF method compared to PEG 12.5% PF method.

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Clinical Usefulness of Sensitive TSAb Assay

The sensitive TSAb assay kit using PEG 22.5% PF instead of PEG 13.5% PF has been introduced from the beginning of 2000 in Japan. Using this kit, Kamijo reported that the stimulatory effect of PEG occurred in 85% of TSAb-positive patients and a detectable TSAb activity was found in approximately 90% of TSAb-negative patients in spite of 62% positive in TBII activity by TSH R assay.[24] This sensitive TSAb assay kit has been used clinically as the routine test in Japan.

Effect of PEG on TBII Assay

Although PEG augmented TSAb-stimulated cAMP production, the effect of PEG on TBII activity of Graves' sera remained unchanged or slightly affected.[8,11] This means that PEG does not have a significant stimulating effect on binding of TSAb to isolated thyroid membranes, in spite of the strong thyroid-stimulating action to intact thyroid cells.

SUMMARY

We observed augmentation of PEG on TSAb-stimulated cAMP production, using porcine thyroid cells in two methods. One was the incubation of crude IgG using PEG 22.5% precipitated fraction (PF) instead of PEG 12.5% PF from Graves' serum; the other was co-incubation of 5% PEG to test serum. Other non-ionic hydrophilic polymers, such as polyvinyl alcohol and dextran, showed the similar augmentated TSAb-stimulated cAMP responses. The augmentation of these non-ionic hydrophilic polymers on TSAb-stimulated cAMP production was also observed by FRTL5-cells and CHO cells expressing human TSH receptor. The augmentative effect by high PEG concentrations on TSAb activity occurred in co-existence of both TSAb and TBAb.

CONCLUSION

Two sensitive methods to detect TSAb using PTC, FRTL-5 cells, and recombinat CHO cells are useful: (1) incubation of crude IgG using PEG 22.5% PF from serum (0.2 mL), and (2) co-incubation of 5% PEG with whole serum (50 μL). Although the latter assay is less sensitive than the former assay, the latter assay is also available as a routine TSAb assay

because of the direct assay using small amounts of serum. These sensitive methods cast some progress on the TSAb assay.

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