Graves' autoantibodies to extrathyroidal TSH receptor: Their role in ophthalmopathy and pretibial myxedema

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Patients with Graves' disease often present extrathyroidal complications involving the connective tissue, i.e. exophthalmos and pretibial myxedema. This association, confirmed by studies on experimental exophthalmos (Wall 1984), has suggested that there were shared thyroid and non-thyroid antigens. On this basis two possibilities were raised: 1) that the shared antigens might be related to the TSH receptor; and 2) that subpopulations of the TSH receptor autoantibodies in the sera of Graves' patients might be inducing the extrathyroidal complications.

This subpopulation would presumably carry some target specific information in addition to TSH receptor determinants, since the complications are so unusually localized (Wall 1984). The possible role of a shared TSH receptor antigenic determinant in the expression of exophthalmos was, however, questioned, and that thyroglobulin might be the shared determinant was also considered (Wall 1984). The intent of the present report was to establish, using circulating IgG purified from Graves' sera or monoclonal antibodies to the TSH receptor, whether shared thyroid and non-thyroid antigens existed; whether TSH receptor determinants on the thyroid and on non-thyroid tissues existed; and whether these were related to the connective tissue complications of Graves' disease.

Graves' disease and other patients attending the University Thyroid Clinic were selected according to the standard protocol in use for the individual disease considered (Rotella et al. 1986). FRTL-5 thyroid cells and human skin fibroblasts were cultured according to the already described procedures (Rotella et al. 1986). The characteristics of the monoclonal anti-TSH receptor antibodies used in this report have been previously summarized (Kohn et al. 1985). Collagen biosynthesis in human skin cultured fibroblasts was evaluated by measuring the incorporation of tritiated proline into pepsin-digested high salt-precipitable material (Rotella et al. 1986). Glycosaminoglycan production by human skin cultured fibroblasts and by differentiated rat thyroid cells in culture (FRTL-5) was measured by evaluating the incorporation of tritiated glucosamine into pronase-digested cetylpiridinium low salt-precipitable material (Bukingham et al. 1983).

To determine whether the collagen biosynthesis assay was relevant to Graves' ophthalmopathy, IgGs obtained from the sera of 82 patients with and without ophthalmopathy have been studied. From results reported in Fig. 1, it is clear that the totality of Graves' patients with exophthalmos, independently from the presence of pretibial myxedema, were positive in this assay, whereas 13/14 IgGs from Graves' patients without ophthalmopathy behaved like normal IgG. No activity was found in patients with non-toxic diffuse...
Collagen biosynthesis, evaluated as tritiated proline incorporation into collagenous proteins synthetized by human skin fibroblasts induced by IgGs prepared from sera of Graves' patients, with or without extrathyroidal complications.

goitre, Hashimoto's thyroiditis or primary thyroid atrophy. The response of Graves' IgG in this assay was totally unrelated to that obtained in TSAb, TBIAb and TGPAb assays.

To verify if the activity of these IgG could be related to anti-TSH receptor autoantibodies we tested in the collagen-fibroblast assay the activity of a number of monoclonal antibodies to the TSH receptor, produced by clones either obtained by fusing spleen cells of mice, immunized with solubilized thyroid membranes, with mouse mieloma cells (Mouse IgG) or heterohybridomas obtained by fusing circulating lymphocytes of Graves' patients with mouse mieloma cells (Human IgG). 307H6 was the more potent, among the heterohybridoma produced IgG, in stimulating collagen biosynthesis, while 129H8, 122G3, 208F7 were weak or systemic lupus has been tested. All were inactive in the fibroblast collagen system, except IgGs from patients with type B insulin resistance, a form due to the development of autoantibodies to the insulin receptor. In the collagen assay 2/4 of the patient IgG preparations were positive. The activity of positive antibodies was more than additive with the 11E8 and 307H6 activities. It was noted that the positive antibodies (B7 and B8) were capable of interacting weakly with the insulin receptor, and more potently with the IGF-I receptor; in contrast, the 2 negative antibodies reacted only with the insulin receptor. Neither insulin nor IGF-I alone could enhance collagen biosynthesis in the fibroblasts nor could they, alone, inhibit the activity of the B7 and B8 antibodies or the 11E8 monoclonal to the TSH receptor, again raising the issue of multiple determinants.

Since modifications of glycosaminoglycan (GAG) synthesis have been also reported in connective tissue complications of Graves' disease (O'Brien 1984), the possibility that IgGs from Graves' patients can increase GAG production of human fibroblasts has been investigated. IgGs from the same series of Graves' patients studied in Fig. 1, either with or without pretibial myxedema, were not able to significantly affect GAG production of skin fibroblasts. As purified TSH increases the cellular synthesis of proteoglycans of a continuous line of differentiatate rat thyroid cells, FRTL-5 cells, as well as their release to the medium, the effect of polyclonal IgG preparations on the synthesis and release of GAGs has been studied.

Data reported in Fig. 2 indicate that IgG from
patients with non-toxic diffuse goitre were not able to significantly increase proteoglycan biosynthesis in FRTL-5 cells by comparison to IgG from normals. Patients with Graves' disease, but without pretibial myxedema, behaved, on average, as did normal subjects in this assay. On the other hand all of the 24 Graves' patients and pretibial myxedema, independent of the presence of exophthalmos, significantly increased proteoglycan biosynthesis in FRTL-5 cells.

Using the heterohybridoma technique, we have recently obtained clones from a patient with severe ophthalmopathy as well as pretibial myxedema. Of this fusion (the 800 series) 142 clones were able to produce antibodies. Of these, 13 produced IgG that acted only as stimulators of collagen synthesis in fibroblasts, 9 produced IgG that only stimulated GAG synthesis in FRTL-5 cells, and 4 produced IgG that stimulated in both the collagen and proteoglycan synthesis assays. These data suggest that distinct antibodies which are responsible for triggering the ophthalmic stimulators and a host of others were inactive. Several mouse IgG monoclonal autoantibodies (11E8, 22A6 and 13D11), were also potent stimulators of collagen synthesis by fibroblasts. Also in the case of monoclonal antibodies the activity in this assay is totally unrelated to that displayed in TSAb, TBIAb and TGPAb assays.

To further ascertain the specificity of this phenomenon, IgGs prepared from patients with autoimmune diseases in which the thyroid system was not involved, for example, patients with myastenia, reumatoid arthritis, pathy or the pretibial myxedema circulate in the sera of patients with Graves' disease and connective tissue complications. Thus far, only half of these antibodies fit criteria established for TSH receptor autoantibodies.

In conclusion, monoclonal antibodies to the TSH receptor can induce changes in non-thyroidal as well as thyroidal tissues which seem related to the expression of the non-thyroidal complications of Graves' disease, ophthalmopathy and pretibial myxedema. The data thus indicate that shared thyroidal and non-thyroidal antigenic determinants exist; that they include determinants on the TSH receptor structure; and that they appear related to the expression of the connective tissue complications of Graves' disease.

Fig. 2.
Glycosaminoglycan production by FRTL-5 rat thyroid cells induced by IgGs prepared from sera of Graves' patients, with or without pretibial myxedema.
References


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