Measurement of autoantibodies against human eye muscle plasma membranes in Graves' ophthalmopathy

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Abstract

Antibodies that reacted with plasma membranes of human eye muscle but showed no binding to plasma membranes of human skeletal muscle were identified in serum of patients with Graves' ophthalmopathy. Rabbit antithyroglobulin serum at a dilution of 1×10^{-3} to 20×10^{-3} had no effect on the binding of these antibodies to eye muscle membrane antigens. There was no correlation between antihuman eye muscle plasma membrane antibodies and antihuman thyroid membrane antibodies or antibodies against thyroglobulin.

It is suggested that specific antibodies against eye muscle membranes are present in Graves' ophthalmopathy and that this disease might represent a distinct autoimmune disorder.

Introduction

The aetiology of Graves' ophthalmopathy is not fully understood, but substantial evidence indicates that it is a separate organ specific autoimmune disorder. Though cell mediated immunity seems to be well documented,[1 2] the presence of specific autoantibodies directed against retro-orbital antigens has only recently been reported. Kodama *et al* showed serum autoantibodies reacting with soluble antigens isolated from human eye muscle cytosol.[3] Kendall-Taylor *et al* also showed autoantibodies in the serum of patients with severe infiltrating ophthalmopathy that specifically react with antigens localised in porcine eye muscle membranes.[4] In the present study we measured antihuman eye muscle plasma membrane antibodies in the serum of patients with Graves' ophthalmopathy, using the ability of protein A from *Staphylococcus aureus* to interact with the Fc fragment of IgG. We also attempted to study the possible correlation between these autoantibodies and antihuman thyroid membrane antibodies and antithyroglobulin antibodies.

Patients and methods

PATIENTS

We studied 20 patients (seven men, 13 women) aged 22-70 with infiltrating Graves' ophthalmopathy. The severity of the eye signs ranged from class 2 to class 6 in the classification of the American Thyroid Association.[5 6] At the time of the study five patients were hyperthyroid, nine were euthyroid but had been hyperthyroid in the past, and six had no signs of thyroid disease. Nineteen healthy blood donors served as controls.

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ESTIMATION OF ANTIEYE MUSCLE PLASMA MEMBRANE ANTIBODIES

Protein A (Pharmacia Fine Chemicals, Sweden) was labelled with iodine-125 by means of iodogen (1, 3, 4, 6-tetrachloro, 3α, 6α, diphenylglycouril; Pierce Co, USA) and purified as described by Gardas *et al.*[7]

Crude human eye muscle or skeletal muscle plasma membranes were prepared by the method of Amir *et al.*[8] Both eye muscles and skeletal muscles were obtained at necropsy from subjects without a history of autoimmune disease within 24 hours of death. Antieye muscle membrane antibodies were estimated by a slight modification of the method of Gardas *et al.*[7] The crude fraction of human eye muscle membrane (0·5 mg protein) diluted in 250 μl 10 mM phosphate buffer, pH 7·4, containing 50 mM sodium chloride and ovine albumin (1 g/l) was first preincubated with 50 μl serum for two hours at 37°C and then left overnight at 4°C. After preincubation 1 ml phosphate buffer was added and the tubes mixed and centrifuged at 10 000 *g* for 10 minutes at 4°C. The supernatant was discarded and the pellet washed with 1 ml cold buffer. Eye muscle plasma membranes preincubated with serum (100 μg protein) were then incubated with ^{125}I protein A (50 000 cpm) for two hours at 4°C. The reaction was stopped by adding 1 ml cold phosphate buffer, and samples were centrifuged at 10 000 *g* for 10 minutes at 4°C. The supernatant was discarded and the radioactivity in the resulting pellet counted in a 80 000 Wallac-LKB counter.

The final results were expressed as an antieye muscle membrane antibody index. The index was calculated by dividing the percentage binding of ^{125}I protein A to plasma membranes preincubated with test serum minus non-specific binding by the percentage binding of ^{125}I protein A to plasma membranes preincubated with normal serum minus non-specific binding. Non-specific binding was the binding of ^{125}I protein A to membranes preincubated with buffer only.

OTHER PROCEDURES

Antithyroid plasma membrane antibodies were estimated by the method of Gardas *et al.*[7] Further studies have shown that an antithyroid plasma membrane antibody index above 1·6 should be considered to be positive for the presence of antithyroid plasma membrane antibodies.[9]

Antithyroglobulin antibodies were estimated by radioimmunoassay as described by Kielczynski.[10] Antithyroglobulin antibodies with a titre of $1/4 \times 10^6$ were obtained by immunisation of rabbits with highly purified human thyroglobulin.[10] The Cochran-Cox test was used to evaluate the significance of the results.

Results

Values of the antihuman eye muscle plasma membrane antibody index in patients with Graves' ophthalmopathy varied from 0·81 to 2·17 and in controls from 0·53 to 1·55 (fig 1). The upper limit of normal for the index was therefore chosen as 1·55. The mean index in patients and controls was significantly different (1·66 (SE 0·33) *v* 1·00 (0·29), $p < 0.001$).

When human skeletal muscle plasma membranes were used as a source of antigen no significant difference in antihuman eye muscle plasma membrane antibody index was found between the patients with Graves' ophthalmopathy and the controls (1·04 (0·22) *v* 1·00 (0·11)).

To exclude a possible role of thyroglobulin in the binding of antihuman eye muscle plasma membrane antibodies to putative antigens, preincubation mixtures of pooled serum from patients and from

controls were enriched with rabbit antithyroglobulin antibodies at dilutions from 1×10^{-3} to 20×10^{-3}. At these dilutions the thyroglobulin antibodies had no effect on ^{125}I protein A binding to membranes preincubated with serum from either the patients or the controls (fig 2).

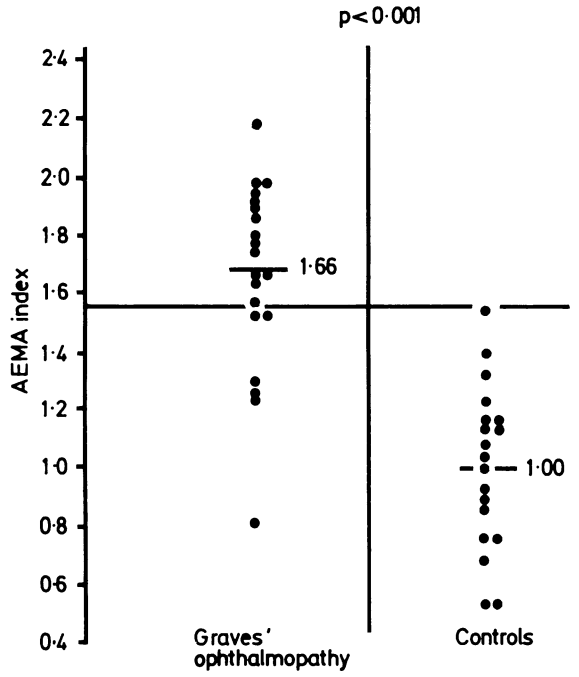

FIG 1—Antihuman eye muscle plasma membrane antibody (AEMA) index in patients with Graves' ophthalmopathy and controls.

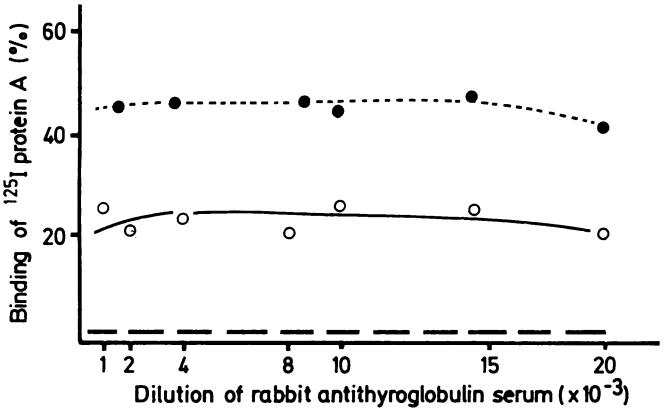

FIG 2—Influence of antithyroglobulin antibodies on ^{125}I protein A binding to eye muscle plasma membranes preincubated with pooled serum from patients with Graves' ophthalmopathy (●----- ●), pooled serum from healthy blood donors (O——O), or buffer alone (— —).

To investigate whether antibodies against eye muscle plasma membranes and thyroid plasma membranes represent the same type of IgG the antihuman eye muscle plasma membrane antibody and antihuman thyroid membrane antibody indexes obtained in the same patients were compared, but no correlation could be demonstrated ($r = -0{\cdot}07$). Similarly, there was no correlation between the antihuman eye muscle plasma membrane antibody index and titres of thyroglobulin antibodies in the same patients ($r = 0{\cdot}22$).

Discussion

Using the ability of protein A to interact with Fc fragment of IgG we were able to show the presence of antibodies that react with human eye muscle plasma membranes in most patients with Graves' ophthalmopathy. We also showed that these antibodies are specific as they did not react with human skeletal muscle plasma membranes. The presence of such antibodies was recently reported by Kendall-Taylor *et al*, who also emphasised their high specificity.[4] We detected these antibodies in 14 (70%) of patients with Graves' ophthalmopathy. Similar results were obtained by Kodama *et al*, who used soluble human eye muscle antigens to investigate specific antibodies and obtained positive results in 75% of patients studied.[3] Two possibilities might explain the "negative" values of the antihuman eye muscle plasma membrane antibody index in some of our patients. As some of the patients had a long history of eye disease they may possibly have achieved an immunological remission. Alternatively, a negative index might be due to the relatively low sensitivity of the assay as a result of using tissues obtained at necropsy.

In previous studies Konishi *et al* reported an affinity of eye muscle for thyroglobulin.[11] Mullin *et al* also suggested that putative antigens present in retro-orbital tissues contain either thyroglobulin or material like thyroglobulin.[12] Using specific antibodies against human thyroglobulin in a wide range of concentrations we were unable to alter the binding of IgG from patients or controls to human eye muscle plasma membranes. These results exclude the presence of thyroglobulin in human eye muscle membranes as well as the possibility that thyroglobulin, within the eye muscle membrane, could be a component of the antigen or antigens that react with autoantibodies present in the serum of patients with Graves' ophthalmopathy.

The studies of Kodama *et al* and Kendall-Taylor *et al* strongly suggest that Graves' ophthalmopathy is a separate organ specific autoimmune disorder that for reasons yet unknown commonly occurs in association with hyperthyroidism.[3 4] In our previous studies we have shown that antithyroid plasma membrane antibodies are present in almost all patients with Graves' disease.[13] The lack of correlation between indexes of antihuman eye muscle plasma membrane antibodies and antihuman thyroid membrane antibodies in individual patients with Graves' ophthalmopathy also provides support for the possibility that ophthalmopathy is an independent autoimmune disorder. Further studies are necessary to investigate the possible correlations of the level of orbital antibodies with the duration of the eye disease and with the severity of the disease.

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