Antibody that blocks stimulation of cortisol secretion by adrenocorticotropic hormone in Addison’s disease

PAT KENDALL-TAYLOR, ANN LAMBERT, ROBERT MITCHELL, WILLIAM R ROBERTSON

Abstract
To investigate whether Addison’s disease may in some cases be due to the blocking of adrenocorticotropic hormone’s action at the adrenal cortex by antibodies IgG isolated from a woman with Addison’s disease associated with the autoimmune polyglandular syndrome type I was studied. Its effects on guinea pig adrenal cells in vitro were investigated and compared with those of IgG from three normal subjects and IgG obtained commercially. IgG from the patient inhibited the stimulation of cortisol secretion by adrenocorticotropic hormone by 77 (SD 2)% and 57 (12)% at concentrations of 0.5 and 0.05 g/l, respectively; IgG prepared five months after she had started treatment with replacement steroids inhibited cortisol secretion by 74 (1)% (0.5 g/l) and 51 (15)% (0.05 g/l). The other IgGs had no inhibitory effects. The IgG from the patient and that obtained commercially did not inhibit the stimulation of cortisol secretion by dibutyryl cyclic adenosine monophosphate or precursors of cortisol. None of the IgGs bound to adrenocorticotropic hormone.

These results suggest that the IgG from the patient acted against the receptor for adrenocorticotropic hormone, and its presence may explain the patient’s raised concentrations of adrenocorticotropic hormone, failure to respond to exogenous adrenocorticotropic hormone, and normal basal cortisol concentrations. Addison’s disease may thus in some instances be a receptor antibody disease.

Introduction
Addison’s disease in Western countries occurs most commonly as an autoimmune condition associated with chronic lymphocytic adrenitis. The histological changes are well documented, and in most patients antibodies to cells that contain steroids can be identified. Evidence that these antibodies are the cause of the adrenitis and clinical syndrome is lacking, and, indeed, the pathogenesis of the disease is not known, though it is assumed to be a destructive process leading to adrenal atrophy. Addison’s disease occurs sometimes as part of an autoimmune polyglandular syndrome. Our study resulted from observations made in a patient with autoimmune polyglandular syndrome type I. Hypoparathyroidism had been diagnosed earlier, and routine screening tests for associated Addison’s disease showed that the patient, who had light skin, had normal basal plasma cortisol concentrations, extremely high plasma concentrations of adrenocorticotropic hormone, and no cortisol response to exogenous adrenocorticotropic hormone. These findings were similar to those that we had observed in some patients with subclinical hypothyroidism associated with antibodies that blocked the effect of thyroid stimulating hormone, and they suggested the presence of antibodies that block the stimulation of the adrenal cortex by adrenocorticotropic hormone.

Patient and methods
Case report—The patient was a woman with the candida endocrinopathy syndrome who had presented at the age of 3 with hypoparathyroidism. She had attended the paediatric clinic for supervision of treatment with vitamin D and management of her candidiasis until at the age of 18 she was transferred to the endocrine clinic. There detailed assessment was possible, including screening for Addison’s disease. Her symptoms included tiredness, loss of weight, joint pain, and irregular menses. On examination she was thin, weighing 45 kg and being 1.67 m tall. Her blood pressure was 70/40 mm Hg; her skin was not abnormally or excessively pigmented. Serum electrolyte concentrations were normal (sodium 140 mmol/l, potassium 4.4 mmol/l, chloride 105 mmol/l, and bicarbonate 27 mmol/l). A short test with tetracosactrin showed that plasma cortisol concentrations were normal (425 mmol/l) basally but did not change with stimulation (after 30 minutes the value was still 425 mmol/l). A five hour test with 1 mg tetracosactrin acetate was therefore performed. At the start plasma cortisol concentration was 335 nmol/l and plasma adrenocorticotropic hormone 150 pmol/l; cortisol

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concentrations in response to stimulation were 330 nmol/l after 30 minutes, 320 nmol/l after one hour, 305 nmol/l after two hours, 305 nmol/l after three, 320 nmol/l after four, and 335 nmol/l after five. Antibodies to adrenal and ovarian tissue and thyroid microes were detected. Her younger sister also had the candida endocrinopathy syndrome but without Addison’s disease. The patient was positive for HLA-A9, A19, B14, B18, DR1, and DR7. Steroid replacement treatment was started.

Routine assay—Plasma cortisol concentration was measured by radioimmunoassay with a commercial kit (Amersham). Plasma adrenocorticotrophic hormone concentration was measured by radioimmunoassay with the Immunonuclear kit, with which separation of bound and unbound hormone was achieved by a goat antirabbit precipitating complex with macrogol. Antibodies to adrenal tissue were detected by immunofluorescence.

Serum samples—Three samples were collected from the patient, two before and one five months after the start of steroid replacement treatment. Serum was also collected from three normal subjects.

Preparation of IgG—IgG fractions from the serum samples were prepared by diethylaminoethanol Sephadex column chromatography: 2 ml serum at pH 6-8 was passed through a column of diethylaminoethanol Sephadex A50 and eluted with 0.0275 M phosphate buffer, pH 6.8. The 0.5 ml fractions were monitored at 280 nm; the fractions rich in protein were pooled and subjected to dialysis against distilled water, and IgG concentrations were measured by radioassay before lyophilisation.

Assessment of in vitro bioposity and site of action of IgG—Guinea pig adrenal cells were dispersed and cultured as described. Aliquots (40 µl) of cell suspension were dispensed into a tissue culture plate of 96 wells and 10 µl assay buffer with iodinated (iodine-125) adrenocorticotrophic hormone. Adrenocorticotrophic hormone (11ß-hydroxypregnenolone, progesterone, and 11-deoxycorticosterone) were used as a positive standard in serial dilutions. Assay buffer was 0.01 M phosphate buffer containing 0.5% bovine serum albumin, 0.01% bacitracin, and 0.05 M sodium fluoride.

Results

Table I shows the effect of increasing concentrations of IgG prepared from the patient’s serum on cortisol secretion stimulated by adrenocorticotrophic hormone.

**Table I**—Mean (SD) cortisol secretion (nmol/l) from adrenal cells under different stimulatory conditions with and without IgG from patient with Addison’s disease.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Without IgG</th>
<th>With patient’s IgG</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD) response (%)*</td>
<td>No of samples</td>
</tr>
<tr>
<td><strong>IgG (g/l)</strong></td>
<td>*<em>response (%)</em></td>
<td><strong>No of samples</strong></td>
</tr>
<tr>
<td>Commercially prepared</td>
<td>0-05</td>
<td>96 (8)</td>
</tr>
<tr>
<td>From normal subjects</td>
<td>0-05</td>
<td>90 (2)</td>
</tr>
<tr>
<td>From patient with Addison’s disease</td>
<td>0-05</td>
<td>85 (6)</td>
</tr>
<tr>
<td></td>
<td>0-05</td>
<td>83 (6)</td>
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<td></td>
<td>0-05</td>
<td>83 (6)</td>
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<td></td>
<td>0-05</td>
<td>43 (12)</td>
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* Cortisol secretion stimulated by adrenocorticotrophic hormone 11 pmol/l alone was taken as 100% response; when no adrenocorticotrophic hormone or IgG was added the response was 3 (2%).

Discussion

Addison’s disease is an autoimmune disease characterised by lymphocytic infiltration, in which antibodies to cells producing steroids can usually be detected. The pathogenic mechanisms leading to adrenocortical insufficiency are not known and have been inadequately studied. Our patient had Addison’s disease associated with the familial candida endocrinopathy syndrome or polyglandular syndrome type I, in which there are multiple immunological abnormalities with evidence for deficient immunoregulation but no linkage with HLA.

IgG from our patient blocked the stimulation of cortisol production by adrenocorticotrophic hormone, the blockade occurring before the generation of cyclic adenosine monophosphate, and therefore the antibody may be a receptor antibody. Unfortunately, because of the lack of a receptor binding assay for adrenocorticotrophic hormone we were not able to show direct interaction of the IgG with the receptor for adrenocorticotrophic hormone. Our patient’s lack of hyperpigmentation was an interesting feature that has been noted occasionally in patients with Addison’s disease, although it may merely reflect the short duration of the disease, and might be due to blocking of the effect of adrenocorticotrophic hormone on melanocytes.

The phenomenon of receptor antibodies causing clinical syndromes is well known in myasthenia gravis, Graves’ disease, and some rare forms of insulin resistance, but, in addition, antibodies have recently been described that are directed against the receptors for follicle stimulating hormone and gastrin, and the presence of others has been hypothesised. Receptor antibodies have not yet been described in adrenal disease, but from what is known of receptor and receptor antibodies the presence of antibodies to the receptor for adrenocorticotrophic hormone may be predicted. Experimentally monoclonal antibodies have been produced against adrenal cells that stimulate, or in one case block, production of corticosterone and hence are presumably antibodies to the receptor for adrenocorticotrophic hormone. Furthermore, IgG from...
patients with nodular adrenal dysplasia stimulates adrenal cell growth in vitro, thus apparently mimicking the trophic effect of adrenocorticotropic hormone.27

Receptor antibodies in autoimmune diseases are frequently stimulatory, as in Graves' disease, in which they mimic the effect of the endogenous hormone thyroid stimulating hormone; antibodies that inhibit the biological stimulation of the cell are, however, now well documented in hypothyroidism.18,19 Our patient's antibody seems to be similar in its biological effect to these inhibitory antibodies that occur in primary hypothyroidism. We therefore suggest that in some cases at least Addison's disease may be yet another example of a receptor antibody disease.

We thank Ms M Holcombe for technical help and Dr C McMartin, Ciba Geigy, Harsham, West Sussex, for giving us the adrenocorticotropic hormone (1-24).

References

Paranormal healing and hypertension

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Abstract
A prospective randomised trial was carried out to see whether paranormal healing by laying on of hands might reduce blood pressure in essential hypertension and whether such an effect might be due to a paranormal, psychological, or placebo factor. Patients were randomised to three treatment groups: paranormal healing by laying on of hands (n=40), paranormal healing at a distance (n=37), and no paranormal healing (controls; n=38). Healing at a distance and no paranormal healing were investigated double blind. Systolic and diastolic blood pressures were significantly reduced in all three groups at week 15 (mean reduction (95% confidence interval) 17-1 (14-0 to 20-2)/8-3 (6-6 to 10-0) mm Hg). Only the successive reductions in diastolic blood pressures among the groups from week to week were significantly different. Each week diastolic pressure was consistently lower (average 1-9 mm Hg) after healing at a distance compared with control, but on paired comparison these differences were not significant. Probably week to week variations among the groups accounted for any differences noted.

In this study no treatment was consistently better than another and the data cannot therefore be taken as evidence of a paranormal effect on blood pressure. Probably the fall in blood pressure in all three groups either was caused by the psychosocial approach or was a placebo effect of the trial itself.

Introduction
Paranormal healing has been used in the treatment of various diseases for centuries and has a long tradition reaching back into Christianity and spiritualism.1 It has become increasingly popular in the United Kingdom and The Netherlands.2-3 In one year 65 000 patients were seen by 600 healers in The Netherlands, resulting in some 2 million patient consultations.2 Paranormal healing is performed in two main ways—by laying on of hands and by healing at a distance, in which the healer takes a patient in mind and healing occurs by thought projection. In a previous study most patients (85%) claimed improvement after paranormal treatment.2 To our knowledge, however, no data are available on whether paranormal healing results in measurable improvement. We have therefore investigated whether laying on of hands might reduce blood pressure and whether...