CLINICAL RESEARCH

Antibody that blocks stimulation of cortisol secretion by adrenocorticotrophic hormone in Addison's disease

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Abstract

To investigate whether Addison's disease may in some cases be due to the blocking of adrenocorticotrophic 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 adrenocorticotrophic 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 adrenocorticotrophic hormone.

These results suggest that the IgG from the patient acted against the receptor for adrenocorticotrophic hormone, and its presence may explain the patient's raised concentrations of adrenocorticotrophic hormone, failure to respond to exogenous adrenocorticotrophic 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 adrenalitis. The histological changes are well documented,¹ and in most patients antibodies to cells that contain steroids can be identified.² Evidence that these antibodies are the cause of the adrenalitis 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.³ 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 adrenocorticotrophic hormone, and no cortisol response to exogenous adrenocorticotrophic 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 adrenocorticotrophic 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, loin 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 nmol/l) basally but did not change with stimulation (after 30 minutes the value was still 425 nmol/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 adrenocorticotrophic 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 microsomes 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 assays—Plasma cortisol concentration was measured by radioimmunoassay with a commercial kit (Amerlex). 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 macrolog. 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 ion exchange 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 radioassay⁷ before lyophilisation.

Assessment of *in vitro* biopotency and site of action of IgG—Guinea pig adrenal cells were dispersed and cultured as described.⁶ Aliquots (40 µl) of cell suspension were dispensed into a tissue culture plate of 96 wells and stimulated for 90 minutes at 37°C with adrenocorticotrophic hormone (1-24) 11 pmol/l either alone or with increasing concentrations of the IgG (0.05-0.5 g/l). The total volume incubated in each well was 80 µl and the final cell concentration 0.75×10^6 cells/l. After 90 minutes' incubation duplicate 10 µl samples were taken and assayed for cortisol concentration by radioimmunoassay. Duplicate cultures were set up and two separate experiments performed. In a second set of experiments the cells were stimulated with the steroid precursors of cortisol pregnenolone, 17α-hydroxypregnenolone, progesterone, and 11-deoxycortisol (all at 10^{-5} mol/l), dibutyl cyclic adenosine monophosphate (10^{-3} mol/l), or adrenocorticotrophic hormone (22 pmol/l) with and without IgG (2 g/l) from the patient or human IgG from Sigma Chemical Co (the steroids and dibutyl cyclic adenosine monophosphate were also obtained from Sigma).

Assay for antibodies to adrenocorticotrophic hormone was based on the method described for detecting insulin antibodies.⁷ Adrenocorticotrophic hormone (1-24) (tetracosactrin) was used initially and adrenocorticotrophic hormone (1-39) in repeat experiments for iodination by the chloramine T method. Guinea pig antibodies to human adrenocorticotrophic hormone were used as a positive standard in serial dilutions. Assay buffer was 0.1 M phosphate buffer containing 0.5% bovine serum albumin, 0.01% bacitracin, and 0.01 M edetic acid and aprotinin. Serum samples (25 µl) were incubated in assay buffer with iodinated (iodine-125) adrenocorticotrophic hormone with gentle mixing overnight at 4°C. IgG bound to iodinated adrenocorticotrophic hormone was precipitated by protein A and the pellet counted.

Results

Table I shows the effect of increasing concentrations of IgG prepared from the patient's serum on cortisol secretion stimulated by adrenocorticotrophic hormone. Adrenocorticotrophic hormone (11 pmol/l) increased cortisol secretion more than 10-fold compared with that found in the unstimulated control samples. The patient's IgG before treatment inhibited the stimulation of cortisol secretion by adrenocorticotrophic hormone, by 77 (SD 2)% and 57 (12)% at concentrations of 0.5 and 0.05 g/l, respectively. In contrast, the commercial human IgG preparation and IgG preparations from three

TABLE I—*In vitro* biopotency of IgG prepared commercially, IgG from three normal subjects, and IgG from patient with Addison's disease in presence of adrenocorticotrophic hormone 11 pmol/l

IgG (g/l)	Mean (SD) response (%) ^a	No of samples
Commercially prepared	0.5 96 (8)	6
	0.05 90 (2)	6
From normal subjects	0.5 85 (6)	12
	0.05 83 (6)	11
From patient with Addison's disease	0.5 23 (2)	8
	0.05 43 (12)	8

^aCortisol 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)%.

TABLE II—Mean (SD) cortisol secretion (nmol/l) from adrenal cells under different stimulatory conditions with and without IgG from patient with Addison's disease

	Without IgG	With patient's IgG (2 g/l)
No adrenocorticotrophic hormone	526 (16)	588 (9)
Adrenocorticotrophic hormone (22 pmol/l)	15 295 (343)	4 970 (282)
Dibutyl cyclic adenosine monophosphate	9 118 (614)	9 963 (706)
Pregnenolone	5 155 (747)	4 575 (628)
17α-Hydroxypregnenolone	4 310 (234)	3 540 (55)
Progesterone	6 140 (553)	6 505 (555)
11-Deoxycortisol	6 498 (203)	6 660 (181)

normal subjects were without effect on cortisol output at any of the concentrations used. IgG from the patient taken five months after she had started treatment with replacement steroids inhibited the stimulation of cortisol secretion by adrenocorticotrophic hormone by 74 (1)% (at 0.5 g IgG/l) and 51 (15)% (at 0.05 g IgG/l).

Further studies were designed to show the site of action of this blockade. Table II shows cortisol secretion from adrenal cells stimulated by adrenocorticotrophic hormone, the steroid precursors of cortisol (pregnenolone, 17α-hydroxypregnenolone, progesterone, and 11-deoxycortisol), and dibutyl cyclic adenosine monophosphate with and without the patient's IgG. All these stimulators gave rise to more than an eightfold increase in cortisol output compared with that seen without them. Cortisol secretion stimulated by adrenocorticotrophic hormone was inhibited by more than 68% by the patient's IgG. In contrast, cortisol secretion stimulated by either dibutyl cyclic adenosine monophosphate or precursor steroids was not inhibited. Commercial IgG was without effect on any of the substances tested.

Iodinated adrenocorticotrophic hormone incubated with increasing dilutions of guinea pig antiserum showed precipitation by protein A that was related to dose (42% with antiserum dilution 1/20, 19% at dilution 1/200); when iodinated adrenocorticotrophic hormone was incubated with serum from the patient precipitation by protein A was minimal (5%) and did not differ from that obtained in a series of control serum samples (4(1)%).

Discussion

Addison's disease is an autoimmune disease characterised by lymphocytic infiltration,¹ in which antibodies to cells producing steroids can usually be detected.^{2,8} 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,^{3,9} in which there are multiple immunological abnormalities with evidence for deficient immunoregulation¹⁰ but no linkage with HLA.¹¹

IgG from our patient blocked the stimulation of cortisol production by adrenocorticotrophic hormone; the blockade occurred 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 disease¹²; although it may merely reflect the short duration of the disease, it 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 hormone¹³ and gastrin,¹⁴ and the presence of others has been hypothesised.¹⁵ Receptor antibodies have not yet been described in adrenal disease, but from what is known of receptors 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.¹⁶ Furthermore, IgG from

patients with nodular adrenal dysplasia stimulates adrenal cell growth in vitro, thus apparently mimicking the trophic effect of adrenocorticotrophic hormone.¹⁷

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.^{4,18,19} 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.

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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.^{2,5} In one year 65 000 patients were seen by 600 healers in The Netherlands, resulting in some 2 million patient consultations.⁶ 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

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