GROWTH HORMONE CELL ANTIBODIES AND PARTIAL GROWTH HORMONE DEFICIENCY IN A GIRL WITH TURNER'S SYNDROME

G. F. BOTTAZZO, C. McINTOSH, W. STANFORD AND M. PREECE

Department of Immunology, Middlesex Hospital Medical School, London W1P 9PG,
Queen Mary's Hospital, Roehampton, London SW15, Paediatric Research Unit, Prince Philip Research Laboratories, Guy's Hospital Medical School, London SE1 and Institute of Child Health, University of London, London WC1

(Received 12 March 1979; revised 7 June 1979; accepted 25 June 1979)

SUMMARY

Growth hormone (GH) cell auto-antibodies have been demonstrated in the serum of a girl with an iso-chromosome variant of Turner's syndrome. On two occasions she showed an abnormal response of GH release during insulin induced hypoglycaemia. Her response to GH treatment was poor, but this was commenced at a very late bone-age. It is possible that autoimmunity is a new aetiological factor for GH deficiency in some cases.

Antibodies, directed against the prolactin (PRL) cells of the human anterior pituitary gland have been described (Bottazzo et al., 1975). We now report the first case of a patient with antibodies directed against the GH producing cells and discuss the possible significance of our findings.

CASE REPORT

The patient, G.W., a 17-year-old girl, presented for investigation of short stature and primary amenorrhoea. She had previously been investigated at another centre when at the age of 7, her height was below the 3rd centile. Investigations for malabsorption and hypothyroidism proved negative, but pituitary function was not studied. It was concluded that she was a normal, but small, child. For the next 9 years she grew, but slowly, and at the age of 17 presented again after her younger sister had outgrown her and achieved a normal puberty.

The patient was 136.3 cm in height (—5.7 SDs) and 36 kg in weight. She was of normal intelligence and her facial features were small and fragile with slight micrognathia. Her chest was broad with widely spaced nipples and she had a rather wide carrying angle for her arms. There was no evidence of secondary sexual development and no evidence of
systemic disease. Her family were all above average height, mid-parent height score being
one standard deviation above the mean and her sisters being all above the 50th centile for
height. Her mother was known to suffer from the polyendocrine syndrome and was
having treatment for Addison’s disease and autoimmune thyroiditis. No other relatives
had symptoms suggestive of endocrine or autoimmune disorders.

LABORATORY INVESTIGATIONS

Routine investigations including haemoglobin, full blood picture, urinalysis and blood
urea and electrolytes were entirely normal. X-rays of lateral skull and pituitary fossa were
normal. Bone-age was 15 ‘years’.

Thyroid function tests showed a serum thyroxine of 141 nmol/l, and a Free Thyroxine
Index of 140. In the TRH test (200 μg) serum TSH rose from a basal value of 3-5 mU/l to
>25 mU/l at 20 and 60 min. Insulin hypoglycaemia was used twice as a stimulus to
pituitary GH and ACTH function (on the second occasion following 1 month of
oestrogen replacement) and the results are shown in Table 1.

<table>
<thead>
<tr>
<th>Time</th>
<th>Glucose (nmol/l)</th>
<th>Cortisol (nmol/l)</th>
<th>Growth hormone (mU/l)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.4</td>
<td>34</td>
<td>1</td>
</tr>
<tr>
<td>15</td>
<td>1.7</td>
<td>10</td>
<td>1.5</td>
</tr>
<tr>
<td>30</td>
<td>1.0</td>
<td>12</td>
<td>8.3</td>
</tr>
<tr>
<td>45</td>
<td>1.9</td>
<td>8.4</td>
<td>7.0</td>
</tr>
<tr>
<td>60</td>
<td>2.6</td>
<td>773</td>
<td>10.2</td>
</tr>
<tr>
<td>90</td>
<td>2.8</td>
<td>814</td>
<td>6.4</td>
</tr>
</tbody>
</table>

*A normal GH response is > 20 mU/l at anytime during the test.
**The second test was performed after 1 month of oestrogen replacement with ethinyl
oestradiol 20 μg/day.

Serum somatomedin-C was measured by porcine rib-cartilage bioassay (Spencer &
Taylor, 1978) before and three months after commencing GH-Therapy. The pre-treat-
ment concentration was 0-4 U/ml (normal range in our laboratory 0-6-1-5 U/ml). During
GH-therapy this rose to 1-1 U/ml. This change was comparable to that seen in the
majority of children with GH-deficiency. LH and FSH response to LHRH injection (100
μg) was exaggerated (15-2 rising to 57-8 U/l and 49-4 rising to 50 U/l respectively). Serum
prolactin was 238 U/l and ACTH < 7 ng/l.

Chromosome analysis showed three cell lines with 10/30 cells 45,X; 19/30 cells 46,X,
i(Xq); 1/30 cells 47,X, i(Xq),i(Xq).

Demonstration of GH-cell antibodies and their characteristics. Standard immunofluor-
escence (IFL) test with the patient’s serum on unfixed cryostat sections of human anterior
pituitary, obtained at hypophysectomy for alleviation of carcinoma of breast, showed a
granular cytoplasmic staining of isolated cells. The double fluorochrome IFL technique
was applied as for the demonstration of PRL-cell antibodies (Bottazzo & Doniach, 1978).
When this patient’s serum was tested in conjunction with rabbit h-PRL antiserum (kindly
donated by Prof. L. Rees) it was found to stain different cells from those reacting with the rabbit serum (Fig. 1A & B). In view of the clinical history, anti-h-GH serum (Hoechst Behringwerke AG) was applied in a second sandwich. This showed identity of the cells stained by the patient and the rabbit h-GH antiserum (Fig. 2A & B). In double exposure photographs all the cells appeared yellow, while in the test with anti-PRL serum separate green and red cells could be easily identified.

The GH-cell antibodies were detected at a titre of 1:8 and were of IgM class. The latter was determined using specific anti-human IgG, -IgM and -IgA sera (Wellcome Reagents). Follow up specimens showed a persistence of the positive reaction though the titre declined. Her serum did not contain any antibodies to circulating growth hormone as assessed by a modification of the double antibody method of Waldhausal & Rath (1971) and the IFL tests were performed before hGH injections were started.

A summary of these findings and the antibody profile in the patient and her family are given in Table 2. The patient and the mother showed thyroid antibodies and the latter also had adrenal antibodies. The father and three sisters were negative in all tests.

Sera from a further sixteen girls with various mosaic form of Turner's syndrome were also tested. Although in ten cases the sera were positive for thyroid microsomal and thyroglobulin antibodies, in no cases were antibodies to pituitary cells, including somatotrophs, detectable.

### Table 2. Autoantibodies study

<table>
<thead>
<tr>
<th>Type of antibody</th>
<th>Antibody titres</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pituitary GH-cell</td>
<td>Patient 8</td>
<td>Mother</td>
</tr>
<tr>
<td>Other pituitary cells</td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>Thyroglobulin (TGHA)</td>
<td>80</td>
<td>40</td>
</tr>
<tr>
<td>Thyroid microsome (MCHA)</td>
<td>—</td>
<td>1600</td>
</tr>
<tr>
<td>Gastric parietal cell</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Adrenal</td>
<td>—</td>
<td>8</td>
</tr>
<tr>
<td>Non-organ specific (nuclear,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>smooth muscle &amp; mitochondrial)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The father and the three sisters were negative in these tests. Thyroid antibodies detected by haemagglutination. Other organs by indirect IFL. — = negative result.

**PROGRESS**

With the results of the insulin hypoglycaemia tests suggesting a partial deficiency of growth hormone, a trial of replacement therapy was started when she was 17.5 years of age (bone-age 15.4 'years') and 137.5 cm in height (– 5.6 SDs).

In the 6 months prior to commencing growth hormone the growth velocity was 2.7 cm/yr. Over the next 12 months, receiving 5IU of human growth hormone three times a week the growth velocity remained unchanged at 2.7 cm/yr. At the end of this time, when she was 140.1 cm (– 5.2 SDs), growth hormone was discontinued and long term oestrogen replacement commenced.
Fig. 1 (A). Human anterior pituitary gland stained by the double (four-layer) immunofluorescence technique. Patient's serum containing antibodies to growth hormone secreting cells (original photo green; FITC conjugate).

→ = cells fluorescent with human serum but negative with rabbit antiprolactin.

↗ = cells fluorescent with rabbit antiprolactin but negative with human autoantibodies.
Fig. 1 (B). Human anterior pituitary gland stained by the double (four-layer) immunofluorescence technique. Same section stained with rabbit antiprolactin serum (original photo red; rhodamine conjugate):

→ = cells fluorescent with human serum but negative with rabbit antiprolactin.

□ = cells fluorescent with rabbit antiprolactin but negative with human autoantibodies.
Fig. 2(A). Human pituitary stained as in Fig. 1. Patient's serum containing antibodies to GH cells (original photo green).
Fig. 2 (B) Human pituitary stained as in Fig. 1. Same section stained with rabbit anti-GH serum (original photo red). Identical cells react with the human autoantibodies and with the rabbit antiserum.
DISCUSSION

The patient presented here had at least one good reason for being short; she had Turner’s syndrome. She was, however, unusually short for this single diagnosis. Using the data reported by Brook et al. (1974) and the patient’s parental heights, her mature stature would be expected to be 146 cm or -2.8 SDs. In reality, she was 140 cm by 18–60 years (−5.2 SDs) with a growth velocity near zero. This is just within the range to be expected but suspicious of a complicating, extra cause for her short stature.

The GH data suggest a partial deficiency, at least in terms of response to hypoglycaemia (Rimoin, 1976; Preece & Tanner, 1976). Confirmation of this would have come if she had responded to GH replacement. This did not occur, but on the other hand, treatment was commenced at a very late bone-age. A significant increase in growth velocity would have been meaningful, the negative response does not really help.

Her thyroid hormone levels were normal and TRH test produced an excessive TSH response; LH and FSH were also elevated. This demonstrates that her thyrotrophs and gonadotrophs were functioning normally but that her thyroid gland had a low reserve compatible with a degree of thyroiditis. In view of this, and her low titre of thyroid antibodies, follow up studies will be necessary to see if this is a progressive lesion giving rise to thyroid deficiency in later life.

The importance of the GH-cell antibodies in the patient’s growth failure remains uncertain. By analogy with other organ-specific autoimmune diseases these antibodies might represent serological markers for GH-cell destruction. This concept is reinforced by the possible coexistent autoimmune thyroid disease in the patient and the autoimmune polyendocrinopathy in her mother. We should emphasize that the antibodies demonstrated here were directed against the cytoplasmic antigens and not to the hormone itself.

The association of Turner’s syndrome, especially of the isochromosome variety, with antibodies to endocrine organs has been described, in particular with reference to thyroid and other organ specific antibodies (Doniach et al., 1968). The relative importance of the chromosome anomaly and the family history in this patient are uncertain. We are left, however, with the unequivocal finding we have reported here and the need to pursue similar studies in patients with unexplained growth defects, especially in girls with Turner’s syndrome who seem ‘too small’. Similarly, it is important to investigate patients with short stature and a positive personal or family history of autoimmune disease.

The aetiology of GH deficiency, other than when associated with pituitary or hypothalamic tumours, is still obscure. Birth trauma has been implicated as a major factor (Rona & Tanner, 1977), and congenital rubella as a rare cause (Preece et al., 1977). Autoimmunity should now be added to this list as another, but hitherto unrecognized, possible aetiological factor.

The real incidence of such antibodies in growth hormone deficient children is as yet unknown, but extensive screening is now in progress to evaluate this.

ACKNOWLEDGEMENTS

We would like to thank Prof. Lesley Rees for anti-PRL serum and for helpful suggestions; Dr. H. C. Grant and Mr. R. A. Williams for pituitary glands; Professor Deborah Doniach for helpful discussion and Professor I. M. Reit for his support.
The skilful technical assistance of Maura O’Kane is gratefully acknowledged. We thank Phillipa Peck and Glynis Orrell for help with the manuscript.

The Medical Research Council made available the growth hormone for the therapeutic trial. Work in the Department of Immunology is supported by the M.R.C. and W.H.O.

REFERENCES


