CASE REPORT

Hyperparathyroidism in a Patient with Autoimmune Polyglandular Syndrome

Lorraine Pelletier-Morel¹, Nicole Fabien¹, Yamina Mouhoub¹, Christian Boitard¹,³ and Etienne Larger¹

Abstract

Autoimmune hypercalcemia has been reported in only a few cases, and never in the context of autoimmune polyglandular syndrome.

A patient with type 1, insulin-dependent diabetes mellitus, Graves’ disease, and antiparietal cell antibodies presented with persistent hypercalcemia with inappropriate PTH secretion. Other causes of hypercalcemia were excluded.

In this context of two associated organ-specific autoimmune diseases we searched for autoantibodies directed to parathyroid tissue and to calcium-sensing receptor. Anti-parathyroid autoantibodies were detected by indirect immunofluorescence on parathyroid adenomas, and autoantibody against a peptide of the extracellular domain of the calcium-sensing receptor were detected by immunoblotting.

Autoimmune hypercalcemia may be another organ-specific feature of autoimmune polyglandular syndrome.

Key words: hypercalcemia, calcium-sensing receptor autoantibodies, autoimmune polyglandular syndrome, diabetes, type 1, thyroiditis

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Introduction

Diseases associated with the calcium-sensing receptor include both loss-of-function and gain-of-function disorders. Loss-of-function disorders are associated with hypercalcemia, while gain-of-function disorders are associated with hypocalcemia (1). The best characterized disease that is associated with loss-of-function of the calcium-sensing receptor is familial benign hypercalcemia, an autosomal dominant disorder characterized by lifelong hypercalcemia (2, 3). Disorders of the calcium-sensing receptor also include autoimmune diseases. In this last context the disorders of calcium metabolism are more frequently an autoimmune acquired hypoparathyroidism than hyperparathyroidism. In the autoimmune context, hypoparathyroidism can be transient unless when associated with features of autoimmune polyglandular syndrome type 1 (4-6). Considering autoimmune hypoparathyroidism, the role of anti-calcium sensor autoimmunity has been challenged (7), but it has also been shown that detection of antibodies against the receptor is influenced by the assay system used (6). Only a few cases of autoimmune acquired hypercalcemia have been reported, and never in the context of well characterized autoimmune polyglandular syndrome (8-10). Here, we report a case of hyperparathyroidism associated with anti-calcium-sensing receptor autoantibodies in a patient with type 1 diabetes and Graves’ disease.

Case Report

A man patient aged 46 years, originating from Algeria, who had migrated to France one year previously, presented first in the year 2001 with insulin-dependent diabetes mellitus. Except for an early greying of a wick of scalp hair, a feature that has been associated with autoimmune polyglandular syndromes and that is considered as an equivalent of vitiligo, he had no personal medical history, and his parents

¹Hotel Dieu, APHP, Université Rene Descartes, Paris, France, ²Hospices Civils de Lyon, University of Lyon, Department of Immunology and INSERM U851, Centre Hospitalier Lyon-Sud, F-69495, Pierre-Benite, France and ³INSERM U561, Paris, France
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Correspondence to Dr. Etienne Larger, etienne.larger@htd.aphp.fr
were unrelated.

Diabetes was first diagnosed in the context of diabetic ketoacidosis in a patient who had lost 17 kg over the three previous weeks, to a final weight of 47 kg. Both islet cells antibodies (ICA) and anti-glutamate decarboxylase autoantibodies (GADA) were detected at diagnosis (Table 1). GADA persisted until the last determination, in 2008.

At the onset of diabetes, the massive weight loss led to a diagnosis of Graves’ disease with overt hyperthyroidism. The thyroid scintigram showed diffuse, homogenous uptake and the serum TSH was undetectable. Free-T4 serum concentration was 87 pmol/L (normal values: 12-25 pmol/L) and free-T3 serum concentration was 18.7 pmol/L (normal values: 2.8-7.1 pmol/L). Anti-TSH receptor autoantibodies were detected on the first analysis four years later, as well as anti-thyroid peroxypase (TPO)- and anti-thyroglobulin autoantibodies (Table 1). Hyperthyroidism was controlled by tapering doses of carbimazole, but relapsed in the year 2005 and he was again treated with carbimazole. A low dose, 5 mg/d, carbimazole was maintained for two years. At the time of writing of this manuscript, one year after discontinuation of carbimazole, the TSH concentration was 3.1 mU/L, with undetectable anti-TSH-receptor antibodies.

Since the first assay, the serum calcium level has been moderately elevated, or in the high-normal range: 2.39-2.72 mmol/L (Table 2). The serum albumin level was 45 g/L. Serum phosphorus levels were in the low-normal range, whereas the serum PTH levels using an ELISA assay for 1-84, intact, PTH, (ELISA-PTH®, Cisbio International, Bagnoles/Cèze, France) were elevated and clearly inappropriate for the calcium level. Urinary calcium excretion was 5.6-7.3 mmol/24 h, and the urinary calcium/creatinine ratio was 0.63-0.78, above the threshold defining hypercalciuria. The results were unchanged at last evaluation: serum calcium 2.65 and 2.47, serum phosphorus level 0.91 and 0.81 and urinary calcium/creatinine ratio: 0.93. The calcium/creatinine clearance ratio (CCCR) calculated as: CCCR = (24 h-U-calcium/P-calcium, total)/(24 h-U-creatinine/P-creatinine) with variables entered as mmol or mmol/L, was 0.028.

Despite this long-term hypercalcemia, there was no history of lithiasis. The serum creatinine level was normal, 72 μmol/L (creatinine clearance 82 mL/min/1.73 m², using the Cockroft formula normalized for body surface). Bone mineral density evaluated by dual energy X-ray absorptiometry was normal: z score -0.1, t score -0.9 at the femoral neck, and -0.1 and -0.3 at the first lumbar vertebra (Lunar Prodigy®, General Electric Company, Diegem, Belgium).

At last evaluation in 2008, seven years after the first finding of hypercalcaemia, a sesta-MIBI parathyroid scintigram showed no evidence of a parathyroid adenoma, while parathyroid ultrasonography had shown a hypoechoic nodule, that was suggestive of an adenopathy, as there was no retention of MIBI at this location.

There was no clinical evidence of Addison’s disease, the serum cortisol level was 167 ng/mL and increased to 269 ng/mL one hour after an i.v. injection of 0.25 mg cosyntropin. No anti-21-hydroxylase autoantibodies were detected. Analyses for other organ-specific autoantibodies were carried out; the results are summarized in Table 1. HLA typing was performed by molecular techniques, as previously described (11). HLA class II alleles were 03 and 04 at the DRB1 locus and 0201/0302 at the DQB1 locus. Informed

### Table 1. Autoantibodies

<table>
<thead>
<tr>
<th>AutoAbs directed to:</th>
<th>2001</th>
<th>2005</th>
<th>Threshold of positivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAD</td>
<td>1691</td>
<td>42</td>
<td>&gt;1 IU/mL</td>
</tr>
<tr>
<td>ICA</td>
<td>20</td>
<td></td>
<td>&gt;5 JDF-U</td>
</tr>
<tr>
<td>IA2</td>
<td>0.45</td>
<td></td>
<td>&gt;0.5 IU/mL</td>
</tr>
<tr>
<td>TPO</td>
<td>2400</td>
<td></td>
<td>&gt;60 IU/L</td>
</tr>
<tr>
<td>TSH-R</td>
<td>1.5</td>
<td></td>
<td>&gt;1.5 IU/L</td>
</tr>
<tr>
<td>Tg</td>
<td>115</td>
<td></td>
<td>&gt;60 IU/L</td>
</tr>
<tr>
<td>Parietal cells</td>
<td>1/1280</td>
<td>1/40</td>
<td></td>
</tr>
<tr>
<td>Endomysium IgA</td>
<td>0</td>
<td>&gt;1/5</td>
<td></td>
</tr>
<tr>
<td>21-hydroxylase</td>
<td>0</td>
<td>&gt;1 U/mL</td>
<td></td>
</tr>
<tr>
<td>LKM-1</td>
<td>0</td>
<td>1/40</td>
<td></td>
</tr>
<tr>
<td>Actin</td>
<td>0</td>
<td>1/40</td>
<td></td>
</tr>
<tr>
<td>Mitochondria</td>
<td>0</td>
<td>1/40</td>
<td></td>
</tr>
</tbody>
</table>

GAD: glutamate decarboxylase, IA2: tyrosine phosphatase, ICA: islet cells antibodies, TPO: thyroperoxydase, TSH-R: receptor to thyrotropin, Tg: thyroglobulin, LKM-1: liver kidney microsome type 1.
Table 2. Biological Characteristics of the Patient

<table>
<thead>
<tr>
<th>2001</th>
<th>2002</th>
<th>2005</th>
<th>normal values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Serum Calcium (mmol/L)</td>
<td>2.66</td>
<td>2.72</td>
<td>2.66</td>
</tr>
<tr>
<td>Phosphorus (mmol/L)</td>
<td>1.12</td>
<td>0.80</td>
<td>0.82</td>
</tr>
<tr>
<td>Urinary Calcium (mmol/24h)</td>
<td>7.3</td>
<td>5.6</td>
<td></td>
</tr>
<tr>
<td>Calcium/creatininuria (mmol/mmol)</td>
<td>0.63</td>
<td>0.78</td>
<td>0.64</td>
</tr>
<tr>
<td>serum PTH concentration (pg/mL)</td>
<td>117</td>
<td>114</td>
<td>115</td>
</tr>
<tr>
<td>serum 25-hydroxyvitamin D3 (μg/L)</td>
<td>5.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>serum 1,25-dihydroxyvitamin D (pg/mL)</td>
<td>48</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Serum PTH, to convert to SI unit (pmol/l): \( \times 0.105 \); Serum 25-hydroxyvitamin D3, to convert to SI unit (nmol/L): \( \times 2.599 \). Serum 1,25-dihydroxyvitamin D to convert to SI unit (pmol/L): \( \times 2.599 \).

Materials and Methods

Detection of autoantibodies against parathyroid tissue

The indirect immunofluorescence (IIF) technique has been described elsewhere (5). Briefly sera, at a dilution of 1:10, were incubated on frozen sections of human parathyroid adenoma for 30 min at room temperature. After 3 washes, the sections were incubated with a fluorescein-conjugated mouse anti-human IgG (Biorad, Marnes la Coquette, France) for 30 min at room temperature.

Detection of autoantibodies against the calcium sensing receptor

The method has been described elsewhere (5, 12). Briefly the immunoblotting test used a recombinant peptide corresponding to the extracellular domain of the protein, i.e. amino acids 1 to 603 (SWISS-PROT nr. P41180). The antigen was loaded (20 μg per lane) onto a 10%-polyacrylamide gel containing 0.1% SDS. Human sera or mouse monoclonal antibody to human CaSR (ADD), donated by Dr S. De Vries, Dr K. Rogers, Dr J. Garrett (NPS Pharmaceuticals, Salt Lake City, UT, USA), Dr A. Spiegel and Dr P. Goldsmith (Metabolic Diseases Branch NIDDK/NIH), were diluted at 1:100 or 1:10,000 with 1% non-fat milk in PBS, respectively, for 2 hours. After 3 washes the membranes were probed with peroxidase-conjugated anti-human-IgG, IgA, IgM (H+L) (1:400, Biorad) or anti-mouse IgG (1/5,000, Jackson ImmunoResearch Laboratories Inc., West Grove, PA, USA) with 1% non-fat milk in PBS, respectively. After three washes, the antigen-antibody complexes were revealed with the peroxidase substrate (hydrogen peroxide and 4-chloro alpha-naphtol).

Results

As shown in Fig. 1, anti-parathyroid autoantibodies were detected by IIF. Two different adenomas were used for this detection. The first serum reacted with both adenomas, whereas the second serum, obtained 2 years later, was negative. The specificity of the technique was assessed by a negative reaction with a serum from a patient with no hypoparathyroidism.

The immunoblotting using the extracellular domain of the protein, allowed the detection of autoantibodies against the calcium-sensing receptor (Fig. 2). This reactivity was detected in both sera obtained at 2 years of interval. The specificity of the technique was assessed by a negative reaction using sera obtained from a patient with no hypoparathyroidism and from a patient suffering from an autoimmune polyglandular syndrome type II with no hypoparathyroidism.

Discussion

In this patient primary hyperparathyroidism was diagnosed on the basis of persistent, modest, hypercalcemia associated with inappropriate serum PTH concentration and hypercalciuria (13, 14). Familial hypercalcemia was ruled out, in the absence of a family history, by the consistently high calcium/creatinine urinary ratio and a calcium/creatinine clearance ratio above 0.02 (15). There was no evidence of a parathyroid adenoma at last evaluation, seven years after the finding of hypercalcemia. There was no indi-
cation for parathyroid surgery in this patient, according to the criteria proposed by an expert panel in 2002 (16). This patient had an autoimmune polyglandular syndrome (17), as evidenced by the association of type 1, autoantibody-positive, diabetes mellitus, autoimmune thyroiditis and anti-parietal cell autoantibodies. There was no evidence of Addison’s disease, or candidiasis. The HLA-DRB1 genotype was DR3 and DR4.

In this context, we considered whether the hyperparathyroidism was related to the autoimmune polyglandular syndrome. Indirect immunofluorescence on frozen sections of human parathyroid adenomas was positive in the first serum sample and negative 2 years later. An immunoblotting test using the extracellular domain of the calcium-sensing receptor showed a reactivity of the sera with the protein, in both sera obtained at first encounter and two years later. The immunoblotting test is much more sensitive than the indirect immunofluorescence technique as previously described (5). The negative result of the second immunofluorescence should also be interpreted in the context of decreasing titers of autoantibodies as exemplified by decreased titers of anti-GAD and anti-TSH-receptor autoantibodies.

There have been only rare cases of hyperparathyroidism associated with anti calcium-sensing receptor autoantibodies (8-10). In those patients hyperparathyroidism was frequently associated with various immune diseases such as rheumatoid arthritis and autoimmune hypophysitis (9), thyroiditis or celiac disease in two families with multiple cases (8), but never in the context of well characterized autoimmune polyglandular syndrome, i.e., in association with diabetes, thyroiditis or anti-gastric parietal cells autoantibodies. In the patients who have been reported as having autoimmune hyperparathyroidism, the diagnosis was suspected based on the presence of abnormalities suggesting familial hypercalcemic-hypocalciuric syndrome, that was proved to be acquired in 2 patients (9, 10) and on the absence of mutation of the calcium-sensing receptor in one patient. In the other patients there was no definitive evidence that the hypercalcemia was acquired, but there was no mutation of the calcium-sensing receptor (8). In these last patients, the metabolic pattern was variable. All of the patients were moderately hypercalcemic (range 2.38-2.95 mmol/L, average 2.71 mmol/L). Among the 4 patients with available clinical data, only one was constantly hypocalciuric. The way by which anti-calcium-sensing receptor autoantibodies cause hypercalcemia and variable effects on calciuria remains unknown. In cases of hypoparathyroidism associated with anti-calcium-sensing receptor autoantibodies, it was shown that anti-calcium-sensing receptor autoantibodies modulate aspects of the function of both parathyroid and renal tubular cells in ways that provide strong evidence that they activate the receptor (18). In this work, it was suggested anti-calcium-sensing receptor autoantibodies exert greater effects

Figure 1. Detection of human anti-parathyroid aAbs by an indirect immunofluorescence technique using cryostat sections of human parathyroid gland. Homogeneous cytoplasmic staining of the parathyroid chief cells was observed with the serum of the patient (A). The control sera produced little or no staining (B) Photomicrographs ×200.

Figure 2. Immunoblots of sera using the extracellular domain of the CaSR. The main reactivity was observed with a 70 kDa protein corresponding to the extracellular domain of the CaSR. Mouse monoclonal antibody to the CaSR (ADD) (lane 1), positive sera from AH (lane 2), negative control sera (lane 3), negative sera from a patient suffering from an autoimmune polyglandular syndrome type II with no hypoparathyroidism (lane 4). Molecular markers are included on the right.
on the parathyroid than on the kidney. We can only speculate that anti-calcium-sensing receptor autoantibodies in the present patient had the reverse effect on the calcium-sensing receptor, directly causing hypercalcaemia by inactivation, and that the variable effect on calciumuria in the patients that have been reported are likewise due to differential effects on the parathyroid and the kidney.

We have no definitive evidence that the hyperparathyroidism is the consequence of the presence of anti-calcium-sensing receptor autoantibodies in the present patient. There was no clinical indication for parathyroid surgery according to previously reported recommendations (16). We therefore had no direct access to the parathyroid tissue. A lymphocytic infiltration was observed in one of the published cases (9). In the present patient there was also no indication for corticosteroids or other treatments targeting the immune system.

The prevalence of hyperparathyroidism is higher in patients with diabetes than in the general population (19), and hyperparathyroidism may alter glucose tolerance, as shown by the cure of diabetes in some patients after parathyroidectomy (20). However, autoantibodies to the calcium-sensing receptor are not usually looked for in patients with hyperparathyroidism and diabetes.

In conclusion, in patients with polyendocrine autoimmunity and hypercalcaemia, the possibility of an immune-mediated hyperparathyroidism should not be overlooked, to avoid unnecessary parathyroid surgery. In this context of hypercalcaemia and inappropriate serum PTH levels, the calciumuria may be either low or normal or in the high normal range. Clinical suspicion should be raised by using a test detecting the presence of anti-calcium-sensing receptor autoantibodies.

References