Parathyroid and autoimmunity

C. Betterle

Chair of Clinical Immunology, Endocrine Unit, Department of Medical and Surgical Sciences University of Padova, Padova, Italy.

Reprint request: Pr C. Betterle, MD, Chair of Clinical Immunology and Allergology, Endocrine Unit, Department of Medical and Surgical Sciences, Via Ospedale Civile 105, 35100, Padova, Italy.
e-mail : corrado.betterle@unipd.it

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Les arguments en faveur d’une origine autoimmunitaire à l’hypoparathyroïdie chronique sont revus de façon systématique. Nous revoymos également les différentes présentations cliniques, les aspects génétiques, le rôle de l’immunité cellulaire, des autoanticorps circulants, la nature des autoantigènes, les manifestations cliniques, les aspects diagnostiques et thérapeutiques. De plus, nous présentons les données de 71 patients italiens attend d’hypoparathyroïdie chronique.

Mots-clés : Hypoparathyroïdie, parathyroïdite lymphocytique, hypoparathyroïdie, hyperparathyroïdie autoimmune, récepteur sensible au calcium, maladies autoimmunes, syndromes autoimmune polyglandulaires.

Parathyroid and autoimmunity

Data in favor of chronic hypoparathyroidism as an autoimmune disease are examined. The article takes into consideration the different clinical forms, genetic patterns, histopathology, animal models, cellular immunity, circulating autoantibodies, target autoantigens, clinical manifestations, laboratory diagnosis and therapy. Furthermore, data on 71 Italian patients with chronic hypoparathyroidism are presented.

Key words: Parathyroid insufficiency, lymphocytic parathyroiditis, hypoparathyroidism, autoimmune hyperparathyroidism, calcium-sensing receptor, autoimmune diseases, autoimmune polyglandular syndromes.

EMBRYOLOGY AND ANATOMY OF PARATHYROID GLANDS

In humans, two pairs of parathyroid glands are generally present. The upper pair develops from the fourth branchial pouch and is associated with the thyroid gland, and the lower pair develops from the third branchial pouch with the thymus. The total weight of the parathyroids is about 120±3.5 mg in men and 142±5.2 mg in women [12]. The adult parathyroid glands contain two types of cells and a stroma largely consisting of fat cells: the chief cells which synthesize and secrete parathyroid hormone (PTH), and the oxyphilic cells which are rich in mitochondria. Both types of cells are arranged in cords and sheets adjacent to capillaries [12].

PHYSIOLOGY OF PARATHYROID HORMONE

The homeostasis of calcium and phosphate concentrations in extracellular fluid is maintained by parathyroid hormone (PTH) produced by the chief cells and by 1,25-dihydroxyvitamin D (calcitriol), synthesized by the kidney under the action of PTH. The chief cells synthesize and secrete a pre-pro-hormone (115 aminoacids, AA) that is converted to a pro-hormone (90 AA) which is then transported across the rough endoplasmic reticulum and stored in secretory granules (84 AA) [23]. PTH secretion is inversely proportional to the serum concentration of ionized calcium.
PTH has a direct effect on bone to regulate calcium exchange at osteocytic sites and to enhance osteoclast-mediated bone reabsorption. In the distal tubules of the kidney, PTH increases calcium reabsorption, decreases the reabsorption of phosphate, and stimulates the metabolic conversion of 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D, the active metabolite. Active vitamin D stimulates bone reabsorption and acts on the gastrointestinal mucosa to increase absorption of dietary calcium.

The chief cells have a calcium sensing receptor (Ca-SR) on their surface. The Ca-SR is a member of the G-protein-coupled receptor family and has characteristic membrane spanning domains: it consists of 1085 AA, with the first 613 N-terminal AA forming the extracellular domain. Ca-SR is expressed not only on parathyroid cells but also on renal cortex and medulla cells, in thyroid C cells, pituitary, hypothalamus and other regions of the brain [17]. Ca-SR is able to detect variations in extracellular ionized calcium concentrations and, in response to a fall in ionized calcium, it induces changes in phosphoinositide turnover and cytosolic Ca$^{2+}$ to increase the production and secretion of PTH. The integrated action of PTH and calcitriol on their target tissues regulate physiological levels of calcium [23].

**CLASSIFICATION OF HYPOPARATHYROIDISM**

The term “functional hypoparathyroidism” defines a heterogeneous group of rare disorders characterized by hypocalcemia and hyperphosphatemia as a consequence either of destruction of the parathyroid glands with decreased level of PTH (true hypoparathyroidism) or an inability of PTH to elicit its appropriate biologic actions on target tissues (pseudohypoparathyroidism) [23]. The most frequent cause of acquired hypoparathyroidism is due to thyroid surgery. Table I summarizes the main causes of hypoparathyroidism.

**AUTOIMMUNE PARATHYROID DISEASE**

In 1956, it was discovered that Hashimoto’s thyroiditis is the consequence of an autoimmune destruction of normal thyroid tissue [36, 37]. The following year, the criteria for defining autoimmune diseases were established. These criteria are: a) demonstration of serum autoantibodies, b) demonstration of lympho-plasmacellular infiltration in the target organs, c) disease experimentally induced by immunization with autoantigens and passive transfer by serum or lymphocytes [50]. Over the next 50 years, more than 60 human diseases previously defined as “idiopathic” are now included in the group of autoimmune diseases on the basis of these criteria.

The following sections review chronic hypoparathyroidism (CHP) to determine if there are sufficient data to consider CHP as an autoimmune disorder.

CHP can present in various clinical forms: either isolated, or associated with autoimmune diseases, particularly autoimmune polyglandular syndromes.

**Clinical presentations**

*CHP associated with chronic candidiasis and/or Addison’s disease*

Historically, a child with chronic tetany due to “idiopathic hypoparathyroidism” and chronic candidiasis was described by Torpe in 1929 [44] but only in 1943 was the association between CHP, chronic candidiasis and Addison’s disease recognized [42]. In 1956, Withaker added Addison’s disease to the syndrome described earlier by Torpe [46]. This combination of clinical findings was defined as autoimmune polyglandular syndrome (APS) type 1 by Neufeld [29]. In 1981, 71 patients with...
APS type 1 were reviewed by Neufeld [30], and by 2002, more than 256 cases were described [6]. In general, APS type 1 is a disease that develops in children. The first manifestation is chronic candidiasis developing in the majority of the cases before the age of 5, followed by hypoparathyroidism developing before the age of 10, and Addison’s disease developing before the age of 14 marked by the presence of adrenal cortex antibodies [7].

In patients with CHP, parathyroid tissue is infiltrated by lymphocytes and Ca-SRAbs can also be present (see below). This triad is frequently associated with other autoimmune diseases such as primary hypogonadism, vitiligo, alopecia, gastritis, pernicious anemia, malabsorption, type 1 diabetes, and thyroid diseases [2, 5]. APS type 1 has also been called APECED (autoimmune-polycendocrine-candidiasis-ectodermal-dystrophy) because ectodermal dystrophy can also be present [2].

APS type 1 is a recessive inherited disease associated with a mutation in the autoimmune regulatory (AIRE) gene present on chromosome 21, and more than 48 mutations have been described linked with this syndrome [33].

CHP that develops during the neonatal period needs to be distinguish from DiGeorge’s syndrome [45, 47], which is characterized by defective development of organs dependent on cells of embryonic neural crest origin and includes: congenital cardiac defects, mainly involving the great vessels; hypocalcemic tetany due to failure of development of parathyroid tissue; and isolated T cell defects due to the absence of a normal thymus [13]. Other clinical syndromes associated with similar chromosome errors include Kenney-Caffey disease (locus mapped to chromosome 1q42-q43) [14], and Barakat-Syndrome (caused by GATA3 haplo-insufficiency) [3, 45]. Finally, hypoparathyroidism can occur as an isolated familial disease with different patterns of inheritance (autosomal dominant, autosomal recessive, or X-linked recessive [1, 32, 43].

**CHP associated with other autoimmune diseases**

Two additional subtypes of CHP can be described in association with other autoimmune diseases: APS type 3 occurs in association with thyroid autoimmune diseases (clinical or latent), whereas APS type 4 occurs in association with vitiligo, alopecia, type 1 diabetes or celiac disease. In these cases, CHP appears at a more advanced age, does not reveal any mutation in the gene AIRE, and is correlated with class II HLA genes [18]. Ca-SRAbs are frequently present, and parathyroid tissue is infiltrated by lymphocytes (see below).

**Isolated CHP**

Isolated CHP then occurs in the absence of other clinical autoimmune diseases and of other main autoantibodies. This disease appears in adult age, is related to Class II genes, and Ca-SRAbs are frequently present [18]. Table II summarizes the main clinical forms and features of CHP.

**Hypocalcemia or hypercalcemia due to parathyroid autoimmune disease**

Until 2003, there were no data about a pathogenetic role of Ca-SRAbs.

Recently, two cases with hypoparathyroidism and other autoimmune diseases with low levels of PTH but a normal parathyroid tissue have been described having Ca-S-RAbs able to activate the receptors and inhibit the secretion of PTH [21].

Recently members of 2 families affected by a syndrome simulating familial hypercalcemic hypocalciuria have been also reported [20]. These patients manifested other autoimmune diseases, and had Ca-SRAbs able to inhibit the receptor and consequently induce the secretion of PTH by dispersed human parathyroid cells [20].

**Table II**

<table>
<thead>
<tr>
<th>Form of CHP</th>
<th>APS type 1 (Clinical or latent)</th>
<th>APS Type 3 or 4 (Clinical or latent)</th>
<th>Isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Associated Diseases</strong></td>
<td>Chronic candidiasis and/or Addison’s disease</td>
<td>TAD*, vitiligo, alopecia, type 1 DM, celiac disease, pernicious anemia, etc.</td>
<td>None</td>
</tr>
<tr>
<td><strong>Mean age at onset, years</strong></td>
<td>7</td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td><strong>Female/Male ratio</strong></td>
<td>2/1</td>
<td>2/1</td>
<td>2/1</td>
</tr>
<tr>
<td><strong>AIRE Gene mutation</strong></td>
<td>Yes</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td><strong>Class II HLA</strong></td>
<td>DR5(?) or DR81<em>01 or DR81</em>09</td>
<td>DR81<em>01 or DR81</em>09</td>
<td>DR81<em>01 or DR81</em>09</td>
</tr>
<tr>
<td><strong>Ca-SRAbs</strong></td>
<td>17-29%</td>
<td>49%</td>
<td>35-100%</td>
</tr>
<tr>
<td><strong>PTH levels</strong></td>
<td>low</td>
<td>low</td>
<td>low</td>
</tr>
<tr>
<td><strong>Histopathology</strong></td>
<td>Lymphocytic parathyroiditis</td>
<td>Lymphocytic parathyroiditis/ normal parathyroid tissue</td>
<td>Lymphocytic parathyroiditis</td>
</tr>
</tbody>
</table>

* TAD = thyroid autoimmune diseases.
These data were confirmed in another patient who manifested hypercalcemic hypocalciuria associated with autoimmune diseases and who did not benefit from parathyroidectomy but improved after high doses of corticosteroids [31].

**Animal models**

Lymphocytic infiltration appears to be an important histological finding in the subsequent development of parathyroid autoimmunity syndromes. Rats immunized with thyroid tissue containing parathyroid tissue develop lymphocytic infiltration of parathyroid tissue [25]. When rats are immunized with parathyroid tissue in Freund’s adjuvant, they develop parathyroid atrophy, with disaggregation of the parathyroid glands, and hypoparathyroidism, although parathyroid autoantibodies are not detectable in this model [26].

A model of spontaneous hypoparathyroidism characterized by a lymphocytic infiltration of parathyroid glands was subsequently described in dogs [11], and in non-obese diabetic mouse [22]. In the mouse model, the majority of the lymphocytes infiltrating the parathyroid tissue possess a helper/inducer phenotype.

**Histopathology**

The few autopsy studies of parathyroid glands obtained from patients with CHP (either isolated CHP or associated with APS type 1) show mononuclear cell infiltration and parathyroid atrophy, but in some cases parathyroid tissue was undetectable [15, 28, 34, 39, 50]. Recently, a form of immunologically mediated CHP with normal parathyroid tissue was also documented associated with Ca-SRAbs and inhibition of PTH secretion [21].

**Cellular immunity**

Peripheral blood lymphocyte subset phenotypes in patients with adult-onset CHP show significantly higher frequencies of CD4 (helper T-cells), CD29/CD4 (inducer of Helper T cells), CD16 and CD56 (natural killer cells), and CD3/DR (activated T cells) as compared to normal controls [51]. In two of these patients, the mitogenic response of peripheral lymphocytes to parathyroid cell membranes was also determined, but increased proliferation could not be demonstrated. No data exist on the phenotype of lymphocytes infiltrating the parathyroid tissue in CHP.

**Serological studies (parathyroid autoantibodies)**

The presence of parathyroid autoantibodies was described, as determined by indirect immunofluorescence (IIF) using human parathyroid adenomas removed at surgery or normal parathyroid tissue obtained at post-mortem: in 38% of patients with CHP, most of whom having APS type 1; in 26% of patients with autoimmune AD; in 12% of patients with Hashimoto’s disease, and in 6% of controls [8]. In this report, the parathyroid cells involved in the autoimmune reaction were not specified. Subsequently, an antibody to parathyroid oxyphil cells was found by IIF in 11% of the patients with CHP but also in 11% of the controls [19]. A later study revealed that the reactivity against human parathyroid in patients with CHP was due to a mitochondria autoantibody [41]. We confirmed that the reactivity against the parathyroid oxyphilic tissue was absorbed by human mitochondria and this reactivity was against a mitochondrial antigen of 46kD molecular weight [4].

In a subsequent study, autoantibodies reacting by IIF with the surface of human parathyroid cells (or parathyroid sections) which had the ability to inhibit PTH secretion were reported [9, 35]. These autoantibodies were able to mediate a complement-dependent cytotoxicity in cultured bovine parathyroid cells [9]. However, these autoantibodies also lost their reactivity after absorption with endothelial cells [16].

Autoantibodies against a membrane-associated antigen of 120-140kD (the size of the Ca-SR) of human parathyroid glands extracts (Ca-SRAbs) were investigated by immunoblot in 25 patients with CHP (17 cases with APS type 1, and 8 cases with chronic thyroiditis). They were found to be positive in 5 (20%) patients (2/17=12% of APS type 1 and 3/8=37.5% of CHP associated to thyroiditis) [24]. Using a membrane fraction of HEK-293 cells expressing the recombinant human Ca-SR, a positive reaction was found in 8/25 (32%) patients by immunoblot analysis. This increment of frequency was probably due to the fact that these cells overexpress the antigen [24]. In order to identify the autoepitopes involved, the Ca-SR cDNA was translated in vitro into two parts and 14/25 (56%) sera of patients with CHP (5/17=29% with APS type 1 and 8/8=100% with chronic thyroiditis) reacted with the extracellular domain of Ca-SR but none of the patients with CHP or normal controls reacted with the intracellular domain of the receptor [24]. Subsequently, 51 patients (45 with isolated CHP and 6 with CHP associated with other autoimmune disorders) were evaluated for Ca-SRAbs using Western Blot analysis on parathyroid adenoma tissues, and 25/51 (49%) sera were found to be positive, but these antibodies were found also in 13% of normal controls [18]. Ca-SRAbs were investigated in 31 patients with CHP (17 isolated, 6 APS type 1, 8 with other autoimmune diseases) and 7/31 sera (23%) (6/17 isolated=35% and 1/6 with APS type 1=17%) were found to be positive by immunoblotting.
assay using recombinant extracellular domain of the human Ca-SR [27]. However, Ca-S-RAbs could not be detected in any of 90 APS Type 1 patients, 73 of whom had hypoparathyroidism, in a more recent study using immunoprecipitation assay with $^{35}$S labelled cloned Ca-SR antigen [40].

Various antibodies to cell surface receptors can cause endocrine dysfunction by mimicking or blocking the action of their respective hormones or mediators (such as TSH-RAbs, Ach-RAbs, Insulin-RAbs) [48]. Until 2003 there were no data about a pathogenic effect of Ca-S-RAbs, but recent case reports suggest that, in some cases, Ca-S-RAbs can act by either blocking or stimulating the receptors.

Specifically, Ca-S-RAbs ability to activate the receptors and inhibit PTH secretion were described in 2004 in two cases of hypoparathyroid syndrome with low levels of PTH and a normal parathyroid tissue [21]. On the contrary, 2 families were described where some members were affected by a syndrome simulating familial hypercalcemic hypocalciuria [20]. These patients manifested other autoimmune diseases, and their blood contained Ca-S-RAbs able to inhibit the receptor and consequently induce the secretion of PTH on dispersed human parathyroid cells in vitro [20]. The presence of these Ca-S-RAbs was confirmed in another patient who manifested hypercalcemic hypocalciuria associated with autoimmune diseases and who did not benefit from parathyroidectomy [31]. Familial hypocalciuric hypercalcemia is an autosomal dominant inherited syndrome due to inactivating mutations in the extracellular Ca-SR gene on chromosome 3 and is characterized by mild to moderate hypercalcemia with relative hypocalciuria and normal or slightly elevated circulating PTH levels [10].

Finally, PTH can also be the target of an autoimmune reaction as demonstrated for others hormones [48]. This finding was documented in a patient with apparently high levels of PTH by RIA that resulted in an unnecessary neck exploration [49]. The autoantibodies and autoantigen profiles against parathyroid tissue are summarized in the table III.

### Personal experience

We have followed 71 Italian patients with CHP. Their main clinical features are summarized in table IV.

### Clinical features of CHP

The clinical manifestations of CHP vary greatly from latent to clinical manifestations of tetany. The manifestations of latent tetania are characterized by Chvostek and Trousseau signs. The first sign is elicited by tapping the facial nerve anterior to the ear to produce homolateral contraction of the facial muscles. The second sign

<table>
<thead>
<tr>
<th>Forms of CHP</th>
<th>APS type 1 (Clinical or latent)</th>
<th>APS Type 3 or 4 (Clinical or latent)</th>
<th>Isolated</th>
<th>Pseudo-hypoparathyroidism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases, N</td>
<td>46</td>
<td>4</td>
<td>19</td>
<td>2</td>
</tr>
<tr>
<td>Associated diseases</td>
<td>Chronic candidiasis and/or Addison's disease</td>
<td>TAD, vitiligo, alopecia, type 1 DM, celiac disease, pernicious anemia, etc.</td>
<td>None</td>
<td>No</td>
</tr>
<tr>
<td>Mean age at onset</td>
<td>10 years</td>
<td>20 years</td>
<td>25 years</td>
<td>Adult age</td>
</tr>
<tr>
<td>Female/Male ratio</td>
<td>2/1</td>
<td>4/1</td>
<td>2/1</td>
<td>nd</td>
</tr>
<tr>
<td>AIRE gene Mutations</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>nd</td>
</tr>
</tbody>
</table>
is characterized by adduction of the fingers, flexion of the wrist and of the metacarpophalangeal joints after inducing limb ischemia by insufflation of a blood pressure cuff on the upper arm to 20 mm Hg above the systolic blood pressure for 3-5 minutes [23]. Cataracts and calcifications of the basal ganglia are also common in patients with all forms of CHP. Clinical manifestations of hypoparathyroidism are summarized in the table V.

Laboratory diagnosis of CHP

The diagnosis of chronic hypoparathyroidism can be made when hypocalcemia is accompanied by hyperphosphoremia and the plasma PTH concentration is low or normal [23].

Imaging

Calcifications of the basal ganglia can occur in all forms of hypoparathyroidism and can be detected by computed tomographic scanning even when routine skull radiographs do not demonstrate intracranial calcification. Rarely, these calcifications may be associated with neurologic signs or symptoms [23].

Therapy of CHP

In patients with severe hypocalcemia, therapy consists of intravenous infusion of calcium: to obtain the desired serum calcium levels, it may be necessary to administer 1-3 g of calcium gluconate (10 to 30 mL of 10% calcium gluconate) in 10 minutes followed by a continuous infusion of calcium using, for example, a solution of 5% dextrose in water containing 100 mL of 10% calcium gluconate per liter.

Oral calcium and vitamin D therapy should be started as soon as possible. Calcitriol, the active form of vitamin D, is a physiologic treatment and the doses required vary from 0.25 μg twice a day to 0.5 μg 3-4 times a day. In addition, 1-3 g of oral calcium citrate or calcium carbonate per day in divided doses should be given.

The goal of therapy is to maintain serum ionized calcium levels in the low limit of the normal range in order to avoid hypercalciuria [23].

CONCLUDING REMARKS

In conclusion, as previously supposed by Rose [37], chronic hypoparathyroidism can be viewed as an autoimmune disorder because it satisfies many of the criteria proposed by Witebsky [50]. Specifically, these criteria are: a) the presence of lymphocytic infiltration of the parathyroid glands, b) the disease can be reproduced in animal by injection of parathyroid extracts, c) Ca-SRAbs have been documented and the extracellular domain of the CaSR is the putative autoantigen, d) CHP can be associated with other autoimmune diseases, and e) there is a genetic predisposition linked to AIRE gene mutations or to class II HLA genes.

Regarding the mechanism of the damage, there are experimental and histopathological data suggesting that the majority of cases are due to cell-mediated immunity against parathyroid tissue and that circulating Ca-SRAbs, in general, do not seem to have a pathogenetic effect.

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