

BRIEF REPORT

Acquired Hypocalciuric Hypercalcemia Due to Autoantibodies against the Calcium-Sensing Receptor

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A COMPLEX HOMEOSTATIC SYSTEM INVOLVING THE INTERPLAY OF THE bones, the kidneys, and the intestines has evolved to maintain extracellular calcium concentrations within a relatively narrow range.¹ The primary regulator of this system is parathyroid hormone, the release of which is initiated by signals from the calcium-sensing receptor. Overproduction of parathyroid hormone gives rise to hypercalcemia by stimulating the efflux of calcium from bone, increasing the reabsorption of urinary calcium, and promoting the uptake of dietary calcium by means of the activation of vitamin D.¹ Parathyroid hormone–dependent hypercalcemia is commonly caused by parathyroid adenomas and hyperplasia.² Rarer causes of parathyroid hormone–mediated hypercalcemia include parathyroid carcinoma,² ectopic production of parathyroid hormone,^{3,4} and familial hypocalciuric hypercalcemia.^{5,6} Familial hypocalciuric hypercalcemia is typically due to inactivating mutations of the gene for the calcium-sensing receptor that result in inappropriate secretion of parathyroid hormone in the presence of hypercalcemia and in markedly enhanced reabsorption of urinary calcium through mechanisms that are both dependent on and independent of parathyroid hormone.^{7,8} We previously described two families with features of familial hypocalciuric hypercalcemia who had autoantibodies directed against the calcium-sensing receptor.⁹ In this report, we describe a woman with an acquired form of hypocalciuric hypercalcemia and a history of multiple autoimmune processes. The patient's hypercalcemia and elevated parathyroid hormone levels were responsive to the administration of glucocorticoids. Examination of the resected tissue after subtotal parathyroidectomy revealed patchy lymphocytic infiltration of otherwise normal glands; the procedure had little effect on her hyperparathyroidism. Subsequent testing showed that the patient's disorder was due to the presence of IgG4 autoantibodies directed against the calcium-sensing receptor.

CASE REPORT

A 66-year-old woman was admitted to the hospital in April 2003 with fatigue and parathyroid hormone–mediated hypercalcemia, which was marked by progressively worsening hypercalcemia and hypophosphatemia associated with frank elevation of parathyroid hormone levels. Her medical history provided strong evidence of immune dysregulation that included psoriasis, adult-onset asthma, Coombs'-positive hemagglutination, rheumatoid arthritis, uveitis, and autoimmune hypophysitis. The autoimmune hypophysitis was characterized by enhanced thickening of the pituitary stalk on magnetic resonance imaging, central diabetes insipidus, and central hypothyroidism (with negative anti-thyroid peroxidase antibodies) requiring maintenance therapy with desmopressin and thyroxine. When the patient was 58 years of age, bullous pemphi-

goid had been diagnosed after she presented with tense bullae covering approximately 85 percent of her body-surface area.

After three years of intermittent treatment with varying doses of glucocorticoids, she was started on daily glucocorticoid therapy in April 2001, owing to repeated flares of pemphigoid. Mycophenolate mofetil was eventually added to her regimen, with improved control of pemphigoid. Also during the spring of 2001, she was found to have a 3-cm mass at the head of the pancreas, and she underwent a Whipple procedure. A pathological evaluation after the procedure showed extensive fibrosis and lymphoplasmacytic pancreatitis consistent with the presence of sclerosing pancreatitis, an autoimmune variant of primary sclerosing cholangitis. The family history was notable: her mother had Raynaud's phenomenon, and a maternal cousin had scleroderma. There was no family history of disorders of calcium metabolism.

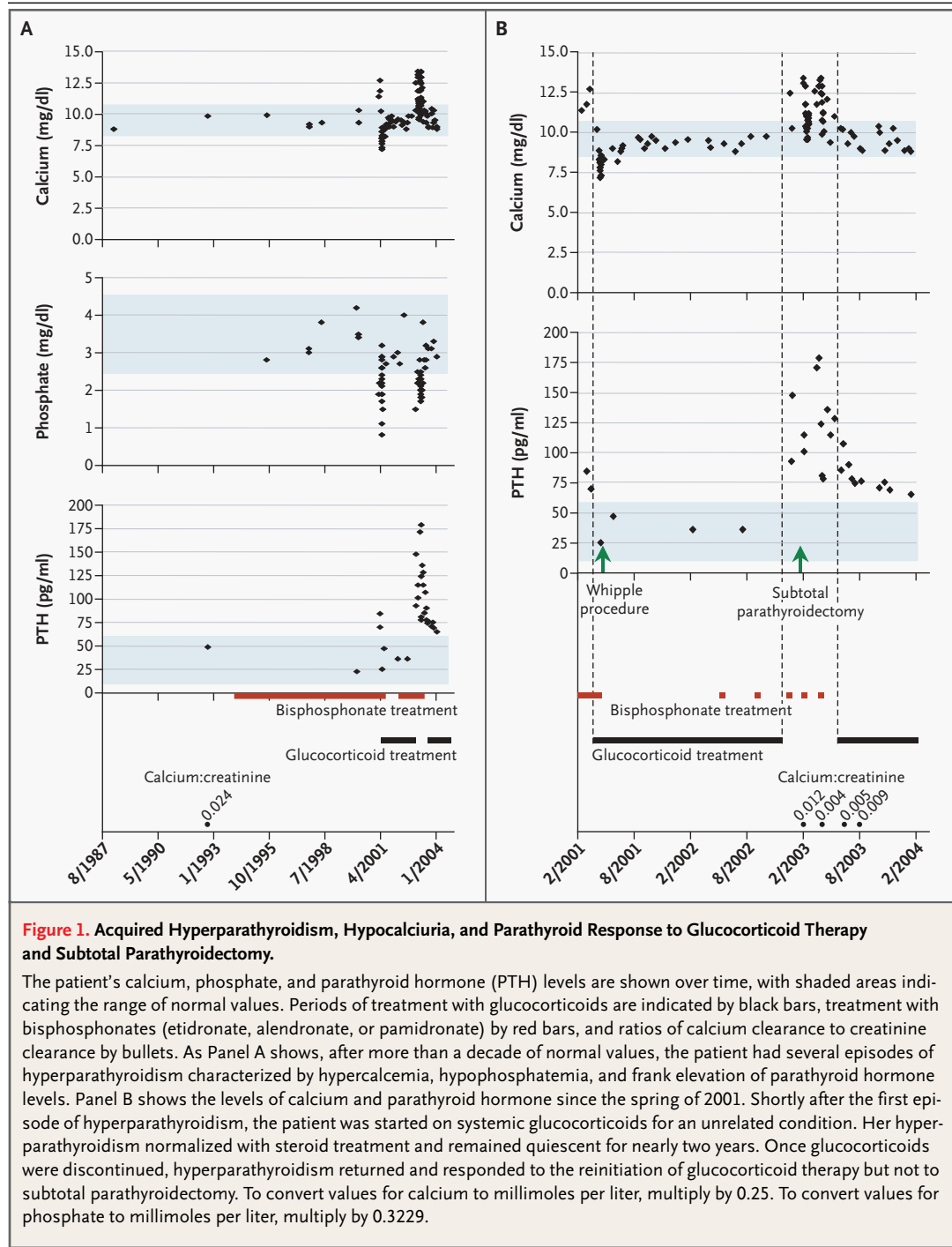
On examination, the patient was cachectic, weighed 34.5 kg, and measured 155 cm in height. Notable physical findings included two blisters on her right wrist, a well-healed scar at the base of her neck anteriorly, and mild abdominal discomfort on palpation. Laboratory analysis revealed normal electrolyte levels, a blood urea nitrogen level of 18 mg per deciliter (6.4 mmol per liter), a creatinine level of 0.8 mg per deciliter (70.7 μ mol per liter), and an elevated magnesium level (2.3 mg per deciliter [0.9 mmol per liter]). The serum calcium level was elevated, at 13.4 mg per deciliter (3.4 mmol per liter; normal range, 8.5 to 10.5 mg per deciliter [2.1 to 2.6 mmol per liter]), as was the serum level of ionized calcium (1.77 mmol per liter; normal range, 1.14 to 1.30 mmol per liter). The phosphate level was low, at 2.1 mg per deciliter (0.7 mmol per liter; normal range, 2.6 to 4.5 mg per deciliter [0.8 to 1.5 mmol per liter]), and the parathyroid hormone level was elevated, with values ranging from 81 to 128 pg per milliliter (normal range, 10 to 60 pg per milliliter) during the week before admission. The level of 25-hydroxyvitamin D was 13 ng per milliliter (normal range, 8.9 to 46.7 ng per milliliter), and the 1,25-dihydroxyvitamin D level was 32 pg per milliliter (normal range, 6 to 62 pg per milliliter). Cortisol levels increased from 12 to 26 μ g per deciliter after cosyntropin stimulation.

A review of previous laboratory data provided evidence of an acquired hypocalciuric hypercalcemia. In March 2001, the patient had her first epi-

sode of clinical hyperparathyroidism while undergoing evaluation of the mass in her pancreas. This episode was characterized by hypercalcemia (serum calcium level, 12.7 mg per deciliter [3.2 mmol per liter]), hypophosphatemia (phosphate level, 2.2 mg per deciliter [0.7 mmol per liter]), and elevation of the parathyroid hormone level (70 pg per milliliter). Until then, multiple measurements of the patient's calcium, phosphate, and parathyroid hormone levels had been normal (Fig. 1A). Specifically, in October 1992, during an evaluation for osteoporosis, her serum calcium level was 9.8 mg per deciliter (2.4 mmol per liter), and her parathyroid hormone level was 49 pg per milliliter. A 24-hour urine collection at that time showed normal calcium excretion of 220 mg per 24 hours, with a ratio of calcium clearance to creatinine clearance of 0.024 (Fig. 1A). The majority of patients with familial hypocalciuric hypercalcemia have ratios below 0.010.⁶ Since the patient's initial episode of hyperparathyroidism in 2001, she has been admitted to the hospital twice because of parathyroid hormone-mediated hypercalcemia. Analysis of 24-hour urine specimens for calcium at the time of those hospitalizations, in February and April 2003, showed marked hypocalciuria (65 and 19 mg of calcium per 24 hours) with a decrease in the ratio of calcium clearance to creatinine clearance (0.012 and 0.004) (Fig. 1B).

METHODS

We used a human embryonic-kidney-cell line (HEK293) that had been transfected with the human calcium-sensing receptor, as previously described.¹⁰ Serum samples from the patient and pooled samples from normal controls were incubated with transfected and nontransfected HEK293 cells. For detecting bound autoantibodies, we used a peroxidase-conjugated goat polyclonal antihuman antibody specific for the IgG- γ chain.⁹ In enzyme-linked immunosorbent assays, the same antibody was used to detect autoantibodies specific for peptides 4641, 4637, and LRG (leucine, arginine, and glycine are the first three amino acids of this peptide), corresponding to amino acids 214 through 238, 344 through 358, and 374 through 391, respectively, within the extracellular N-terminal domain of the calcium-sensing receptor.⁹ Determination of IgG subclasses for the autoantibodies was performed with sheep monoclonal antihuman antibodies (Binding Site). Serum samples



collected at different times and stored at -80°C were used to determine autoantibody titers. The results reflect at least triplicate measurements. Pooled serum samples from normal controls were used as references in all these studies. The patient provid-

ed written informed consent for the studies, which were approved by the institutional review board of Partners HealthCare (covering Massachusetts General Hospital and Brigham and Women's Hospital).

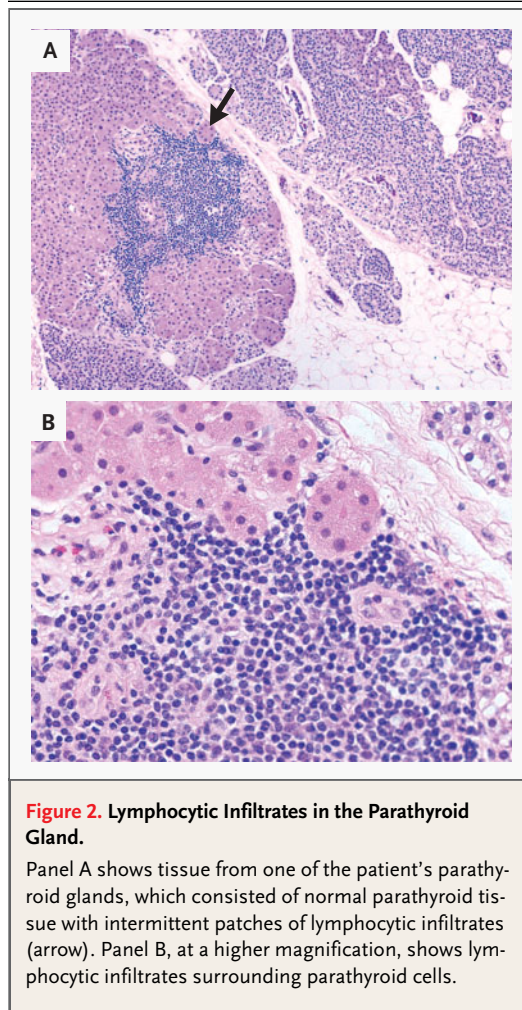
RESULTS

RESPONSE OF HYPERPARATHYROIDISM TO GLUCOCORTICOID THERAPY

In March 2001, laboratory tests showed elevated calcium and parathyroid hormone levels (11.8 mg per deciliter [3.0 mmol per liter] and 84 pg per milliliter, respectively), documenting the patient's first episode of hyperparathyroidism (Fig. 1B). Systemic glucocorticoid therapy, starting at a dose of 70 mg of prednisone per day, was subsequently initiated for a flare of bullous pemphigoid. The patient's calcium and parathyroid hormone levels both normalized while she was receiving this therapy. After the Whipple procedure, in mid-April 2001, the patient continued to receive prednisone therapy for 18 months (dosage range, 5 to 70 mg per day) because she had flares of bullous pemphigoid and uveitis. Although cushingoid features developed in the patient while she was receiving this regimen, the calcium, phosphate, and parathyroid hormone levels remained within normal limits. Mycophenolate mofetil was added to the glucocorticoid regimen in August 2001. As the dosage of mycophenolate was increased from 500 mg once a day to 500 mg three times a day, the doses of prednisone were reduced and finally discontinued in December 2002. By mid-January 2003, the patient's calcium and parathyroid hormone levels had risen, after having remained normal for more than 18 months during glucocorticoid therapy (Fig. 1B). In contrast to the response to glucocorticoids, treatment with bisphosphonates lowered serum calcium levels but increased parathyroid hormone secretion.

PARATHYROID HORMONE LEVELS AFTER SUBTOTAL PARATHYROIDECTOMY

In February 2003, shortly after prednisone was discontinued, the patient was admitted to the hospital for fatigue. Serum calcium and parathyroid hormone levels measured 13.4 mg per deciliter (3.3 mmol per liter) and 115 pg per milliliter, respectively. Serum calcium levels normalized after treatment with intravenous hydration and pamidronate. Imaging of the parathyroid glands showed no definite evidence of a parathyroid adenoma, but given the severe calcium elevation, the patient underwent removal of three and a half of the four parathyroid glands. Intraoperatively, all glands appeared normal. Microscopical evaluation of resected tissue showed patches of lymphocytic infiltration in otherwise normal parathyroid tissue (Fig. 2). With-



in three weeks, calcium and parathyroid hormone levels started to rise again, and hypercalcemia necessitated another hospitalization.

AUTOANTIBODIES TO THE CALCIUM-SENSING RECEPTOR

Incubation of the patient's serum with HEK293 cells transfected with the human calcium-sensing receptor and subsequent analysis demonstrated that the patient had circulating autoantibodies targeting this receptor (Fig. 3). Enzyme-linked immunosorbent assays showed that the cognate epitopes for these autoantibodies corresponded to regions in the extracellular domain of the receptor (Fig. 4A). Further analysis showed that the autoantibodies were predominantly of the IgG4 subtype (Fig. 4B). Evaluation of the patient's autoantibody titers showed a strong correlation with hypercalcemia and elevated parathyroid hormone levels (Fig. 4C).

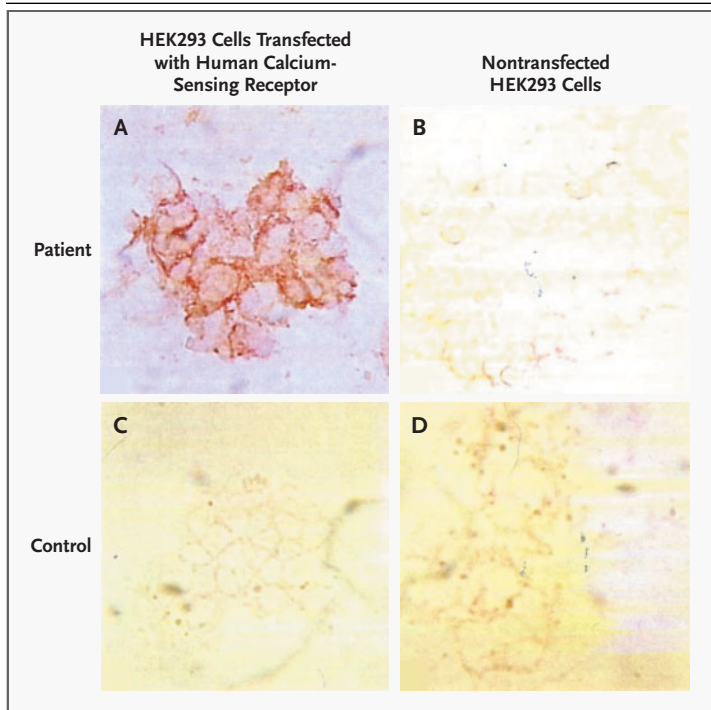


Figure 3. Autoantibodies to the Calcium-Sensing Receptor.

A sample of the patient's serum was incubated with HEK293 cells transfected with the human calcium-sensing receptor (Panel A) and with nontransfected HEK293 cells (Panel B). A peroxidase-conjugated goat polyclonal antihuman antibody specific for the human IgG- γ chain was used to detect autoantibodies. Only the transfected cells showed significant binding of autoantibodies in the patient's serum. Similarly, serum samples from normal control patients were also incubated with HEK293 cells transfected with the human calcium-sensing receptor (Panel C) and with nontransfected HEK293 cells (Panel D); no significant binding was observed.

Moreover, analysis of serum from 1995, before hyperparathyroidism developed in the patient, did not show the presence of autoantibodies against the calcium-sensing receptor, with levels similar to those in controls, thus confirming the acquired nature of the disorder.

TREATMENT

While testing for autoantibodies was under way, during her hospitalization in April 2003, the patient was again treated with intravenous hydration and pamidronate, which transiently normalized serum calcium levels. Three weeks after discharge, however, the calcium and parathyroid hormone levels rose to 12.1 mg per deciliter (3.0 mmol per liter) and 136 pg per milliliter, respectively. Prednisone therapy (40 mg a day) was initiated and was gradually decreased to 5 mg a day over several months,

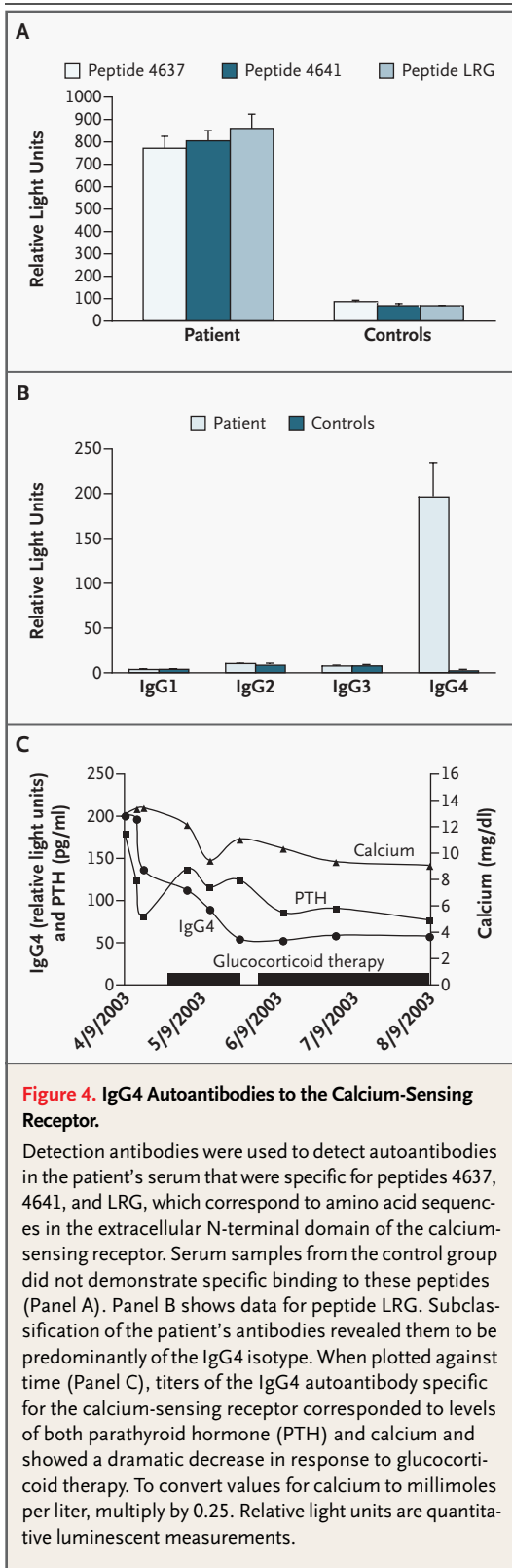
with only a brief period of interruption. With prednisone treatment, calcium, phosphate, and magnesium levels all normalized, and parathyroid hormone levels decreased to 65 pg per milliliter (Fig. 1B). With normalization of her serum calcium levels, the patient reported an increase in her energy level. Analysis of repeated 24-hour urinary calcium collections showed slight improvement in the ratio of the calcium clearance to creatinine clearance (April 2003, 0.004; June 2003, 0.005; July 2003, 0.009) (Fig. 1B), although the absolute levels of urinary calcium excretion remained low.

DISCUSSION

Familial hypocalciuric hypercalcemia is an autosomal dominant disorder with a high degree of penetrance that is characterized by mild parathyroid hormone-dependent hypercalcemia and low ratios of urinary calcium clearance to creatinine clearance. These features are present from infancy, and most cases are associated with inactivating mutations of the gene for the calcium-sensing receptor, which is present in the parathyroid glands and in the thick ascending limb of the loop of Henle.⁷ Homozygous mutations of this receptor give rise to neonatal severe hyperparathyroidism, which is an extreme form of hyperparathyroidism and hypercalcemia that can be fatal in infancy.^{7,11} Since the inappropriately normal or the frankly elevated parathyroid hormone levels in these conditions result from a defect in calcium sensing that affects all parathyroid tissue, subtotal parathyroidectomy does not usually lead to long-term normocalcemia.^{12,13}

Our patient had many of the features seen in familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism, including hypercalcemia, hypocalciuria, hyperparathyroidism, and recurrent hypercalcemia after subtotal parathyroidectomy. However, since the patient had no family history of calcium abnormalities and had a personal history of normal calcium and parathyroid hormone values, these inherited conditions were ruled out. The similarities between her condition and these genetic disorders suggested an acquired defect in the calcium-sensing mechanism. Her history of immune dysregulation led us to suspect that inactivating autoantibodies directed against the calcium-sensing receptor were responsible for her condition.

The correlation between the patient's history of waxing and waning hyperparathyroidism and



the intermittent glucocorticoid therapy further supported our theory that her condition had an immune-mediated mechanism. The initial episode of hyperparathyroidism was transient and seems to have been temporally related to glucocorticoid treatment for an unrelated problem. Not only was her hyperparathyroidism responsive to steroids but, after the withdrawal of exogenous glucocorticoids, she had repeated episodes of hyperparathyroidism that necessitated hospitalization for hypercalcemia. Although glucocorticoids can be used to treat hypercalcemia in disorders such as sarcoidosis and lymphoma,^{14,15} the administration of these drugs does not lower serum calcium levels in parathyroid hormone-mediated disorders.^{13,16}

The presence of antibodies against the calcium-sensing receptor was confirmed by the results of incubation of a sample of the patient's serum with a cell line transfected with the calcium-sensing receptor. Additional confirmation was obtained by enzyme-linked immunosorbent assays that showed the presence of antibodies that recognized several epitopes in the extracellular N-terminal domain of the calcium-sensing receptor. Several patients with autoantibodies targeting the calcium-sensing receptor have been described in the literature.¹⁷ However, these patients had hypoparathyroidism, probably associated with parathyroid-cell destruction.¹⁸ It is postulated that the damage to the glandular tissue may be attributable to direct complement fixation by these antibodies. Our patient's autoantibodies did not result in the destruction of parathyroid cells, perhaps because they did not have the capacity to activate complement.¹⁹ Because bullous pemphigoid and sclerosing pancreatitis are associated with IgG4 autoantibodies,^{20,21} which do not activate the complement cascade,²² we hypothesized that the patient had IgG4 autoantibodies against the calcium-sensing receptor. Subclassification of her autoantibodies revealed that this was indeed the case.

We previously described two families with hyperparathyroidism who had autoantibodies directed against the calcium-sensing receptor. In vitro assays showed that these autoantibodies could block signaling by the receptor and induce parathyroid hormone secretion from parathyroid tissue.⁹ However, since the hypercalcemia in these patients was familial, became apparent when the patients were young, and could not be shown to be acquired, familial hypocalciuric hypercalcemia could not be

completely eliminated as a cause of their mild parathyroid hormone–dependent hypercalcemia. In the woman in this report, however, hyperparathyroidism was clearly acquired and directly correlated with autoantibody titers. Treatment with glucocorticoids not only lowered her antibody titers but also successfully normalized serum calcium levels and lowered parathyroid hormone levels substantially.

Thus, the patient described here has evidence of autoimmune hyperparathyroidism caused by autoantibodies directed against the calcium-sensing receptor. The predominance of IgG4 autoantibodies seems to represent a novel mechanism resulting in inactivation of the calcium-sensing receptor without glandular destruction, which may explain the patient's hyperparathyroidism, in contrast to previously described autoimmune hypoparathyroidism. Finally, specific treatment with glucocorticoids lowered the parathyroid hormone levels and normalized the hypercalcemia.

This disorder should be considered in patients who have hyperparathyroidism in combination with other autoimmune disorders. If glucocorticoids are

used to treat an accompanying autoimmune disease, as in the case of this patient, the hyperparathyroidism may normalize intermittently, delaying or preventing recognition of this syndrome. Depending on the severity of the hyperparathyroidism, treatment with glucocorticoids can reverse severe hypercalcemia and possibly avert the need for parathyroid surgery. Another potential treatment is the use of calcimimetic drugs to sensitize the parathyroid glands to extracellular calcium.²³ However, it is unclear whether these pharmacologic agents can overcome the effects of the autoantibodies against the calcium-sensing receptor.

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REFERENCES

1. Bringhurst FR, Demay MB, Kronenberg HM. Hormones and disorders of mineral metabolism. In: Larsen PR, Kronenberg HM, Melmed S, Polonsky KS, eds. *Williams textbook of endocrinology*. 10th ed. Philadelphia: W.B. Saunders, 2003:1303-71.
2. Marx SJ. Hyperparathyroid and hypoparathyroid disorders. *N Engl J Med* 2000; 343:1863-75.
3. Yoshimoto K, Yamasaki R, Sakai H, et al. Ectopic production of parathyroid hormone by small cell lung cancer in a patient with hypercalcemia. *J Clin Endocrinol Metab* 1989; 68:976-81.
4. Nussbaum SR, Gaz RD, Arnold A. Hypercalcemia and ectopic secretion of parathyroid hormone by an ovarian carcinoma with rearrangement of the gene for parathyroid hormone. *N Engl J Med* 1990;323: 1324-8.
5. Heath H III. Familial benign (hypocalcemic) hypercalcemia: a troublesome mimic of mild primary hyperparathyroidism. *Endocrinol Metab Clin North Am* 1989;18: 723-40.
6. Brown EM. Familial hypocalcemic hypercalcemia and other disorders with resistance to extracellular calcium. *Endocrinol Metab Clin North Am* 2000;29:503-22.
7. Attie MF, Gill JR Jr, Stock JL, et al. Urinary calcium excretion in familial hypocalcemic hypercalcemia: persistence of relative hypocalciuria after induction of hypoparathyroidism. *J Clin Invest* 1983;72:667-76.
8. Hebert SC, Brown EM, Harris HW. Role of the Ca(2+)-sensing receptor in divalent mineral ion homeostasis. *J Exp Biol* 1997; 200:295-302.
9. Kifor O, Moore FD Jr, Delaney M, et al. A syndrome of hypocalcemic hypercalcemia caused by autoantibodies directed at the calcium-sensing receptor. *J Clin Endocrinol Metab* 2003;88:60-72.
10. Kifor O, Diaz R, Butters R, Brown EM. The Ca2+-sensing receptor (CaR) activates phospholipases C, A2, and D in bovine parathyroid and CaR-transfected, human embryonic kidney (HEK293) cells. *J Bone Miner Res* 1997;12:715-25.
11. Marx SJ, Attie MF, Spiegel AM, Levine MA, Lasker RD, Fox M. An association between neonatal severe primary hyperparathyroidism and familial hypocalcemic hypercalcemia in three kindreds. *N Engl J Med* 1982; 306:257-64.
12. Marx SJ, Stock JL, Attie MF, et al. Familial hypocalcemic hypercalcemia: recognition among patients referred after unsuccessful parathyroid exploration. *Ann Intern Med* 1980;92:351-6.
13. Foley TP Jr, Harrison HC, Arnaud CD, Harrison HE. Familial benign hypercalcemia. *J Pediatr* 1972;81:1060-7.
14. Seymour JE, Gagel RF. Calcitriol: the major humoral mediator of hypercalcemia in Hodgkin's disease and non-Hodgkin's lymphomas. *Blood* 1993;82:1383-94.
15. Sandler LM, Winearls CG, Fraher LJ, Clemens TL, Smith R, O'Riordan JL. Studies of the hypercalcaemia of sarcoidosis: effect of steroids and exogenous vitamin D3 on the circulating concentrations of 1,25-dihydroxy vitamin D3. *Q J Med* 1984;53:165-80.
16. Dent CE, Watson L. The hydrocortisone test in primary and tertiary hyperparathyroidism. *Lancet* 1968;2:662-4.
17. Li Y, Song YH, Rais N, et al. Autoantibodies to the extracellular domain of the calcium-sensing receptor in patients with acquired hypoparathyroidism. *J Clin Invest* 1996;97:910-4.
18. Brandi ML, Aurbach GD, Fattorossi A, Quarto R, Marx SJ, Fitzpatrick LA. Antibodies cytotoxic to bovine parathyroid cells in autoimmune hypoparathyroidism. *Proc Natl Acad Sci U S A* 1986;83:8366-9.
19. Fattorossi A, Aurbach GD, Sakaguchi K, et al. Anti-endothelial cell antibodies: detection and characterization in sera from patients with autoimmune hypoparathyroidism. *Proc Natl Acad Sci U S A* 1988;85: 4015-9.
20. Hofmann S, Thoma-Uszynski S, Hunziker T, et al. Severity and phenotype of bullous pemphigoid relate to autoantibody profile against the NH2- and COOH-termi-

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nal regions of the BP180 ectodomain. *J Invest Dermatol* 2002;119:1065-73.

21. Hamano H, Kawa S, Horiuchi A, et al. High serum IgG4 concentrations in patients with sclerosing pancreatitis. *N Engl J Med* 2001;344:732-8.

22. Jefferis R, Pound J, Lund J, Goodall M. Effector mechanisms activated by human IgG subclass antibodies: clinical and molecular aspects. *Ann Biol Clin (Paris)* 1994;52:57-65.

23. Shoback DM, Bilezikian JP, Turner SA,

McCary LC, Guo MD, Peacock M. The calcimimetic cinacalcet normalizes serum calcium in subjects with primary hyperparathyroidism. *J Clin Endocrinol Metab* 2003;88:5644-9.

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