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High serum corticotropin-releasing hormone (CRH) and bone marrow mast cell CRH receptor expression in a mastocytosis patient

To the Editor:

A 36-year-old white female patient presented with flushing; contact dermatitis (Fig 1, *A*); salmon-colored macules on her legs (Fig 1, *B*); itchy, sensitive skin; rashes; nasal congestion; chronic postnasal drip; sinus infections; musculoskeletal aches; fatigue; shortness of breath; chest tightness; lightheadedness; difficulty with concentration and memory; headaches; partial blackouts; tachycardia; anxiety; insomnia; depression; lower pelvic discomfort; diarrhea; bone pain; and osteopenia. Most of these worsened perimenstrually and with exercise, pressure, heat, cold, vibration, mold, pollen, certain foods and preservatives, scents, and especially physical and emotional stress. She was also sensitive to many medications, including fluoxetine, sertraline, bupropion, meperidine, clarithromycin, amoxicillin, and corticosteroids. Results of laboratory tests, including serum immunoglobulins, were within normal limits, except for elevated cortisol. No symptoms or laboratory tests were indicative of atopic dermatitis or any autoimmune diseases.

Bone marrow (BM) biopsy revealed clusters of mast cells (MC) positive for c-kit and tryptase (Fig 2). In addition, genetic analysis showed that MC were positive for the codon D816V self-activating mutation of *KIT*, the receptor for stem cell factor; over 50% of MC expressed CD2, while 70% expressed CD117 (c-kit) and CD25; serum tryptase was elevated at 20 ng/mL); the patient had mastocytosis in the skin; and she responded to H1 and H2 receptor antagonists. These findings fulfilled the World Health Organization criteria for systemic mastocytosis (SM) as well as those for a mast cell activation disorder[1,2]; the subcategory of SM was indolent SM.

Many of the MC in the BM were degranulated, as judged by their "ruffled" surface and reduced tryptase staining (Fig 2, *A*). Given the BM findings and the sensitivity of this patient to stress, MC were counterstained with an antibody against corticotropin-releasing hormone (CRH) receptor 1 (CRHR-1), the main receptor for the first hormone secreted under stress, and showed strong expression (Fig 2, *A*).

Fluorescence-activated cell sorting analysis of human umbilical cord blood-derived cultured MC showed that most of them were positive for both c-kit and CRHR-1 (Fig 2, *B*). Serum levels of CRH were measured and were extremely elevated (42.6 times) at 3.11 $\pm$ 0.056 ng/mL, as compared to 0.073 $\pm$ 0.054 ng/mL found in an age- and sex-matched healthy control. We also measured serum neurotensin, which was elevated at 0.097 $\pm$ 0.02 ng/mL as compared to 0.03 $\pm$ 0.09 ng/mL in normal females (CRH and neurotensin were measured with ELISA kits from Phoenix Pharmaceuticals, Burlingame, Calif).

This patient did respond to a number of drugs introduced and increased over time to the dosages listed below. Although it is difficult to describe which symptoms responded to which drugs, this patient was careful in adding drugs sequentially and kept a journal to note when each drug was added. Thus, it is reasonable to state the following: ibuprofen (400 mg orally 4 times a day) helped with musculoskeletal pain and flushing; fexofenadine (180 mg orally twice a day) helped with itching and gastrointestinal cramping; ranitidine (150 to 300 mg orally 4 times a day) reduced gastroesophageal reflux; montelukast (10 mg orally twice a day) eased chest congestion and tightness; cromolyn (400 mg orally 4 times a day) helped with gastrointestinal cramping; alprazolam (1 mg extended release orally twice a day) reduced anxiety; and liposomal luteolin and quercetin dietary supplement (2 softgel capsules 3 times a day) eased gastrointestinal cramping and musculoskeletal pain as well as itching due to seasonal allergens.

SM involves BM and extramedullary proliferation and activation of MC leading to multiple symptoms, including

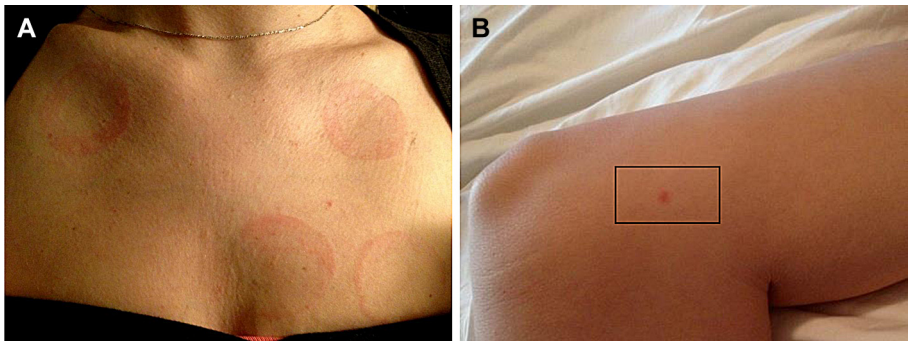

FIG 1. Photograph of the chest of this patient showing contact/pressure dermatitis at the sites of the adhesive lead placement 6 hours after electrocardiography monitoring (Fig 1, *A*); photograph of her left leg showing one urticaria pigmentosa lesion (Fig 1, *B*, within the *rectangular box*).

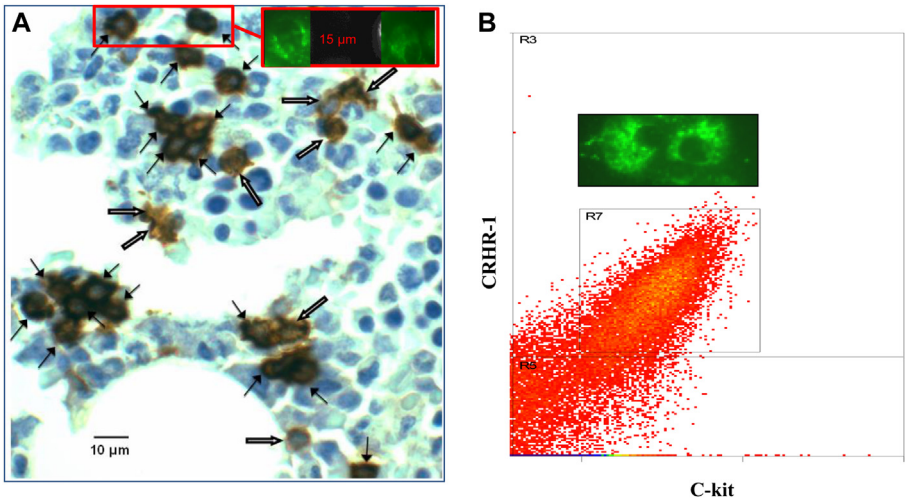

FIG 2. Photomicrograph (Fig 2, *A*) of a BM biopsy from this patient showing clusters of MC *(solid arrow)* and some degranulated MC *(open arrow)*. The insert *(red rectangle)* shows 2 MC with positive CRHR-1 fluorescent staining *(green)*. The slide was deparaffinized and reprobed with a fluorescent-tagged polyclonal goat anti-human CRHR-1 antibody (V-14-sc-12381, Santa Cruz, Calif); staining with a nonspecific anti-IgG antibody produced no fluorescent staining. Fluorescence-activated cell sorting analysis (Fig 2, *B*) of human umbilical cord blood–derived cultured MC. The *x* axis corresponds to FITC-conjugated c-kit, and the *y axis* corresponds to phycoerythrin-conjugated CRHR-1. The cells labeled in quadrant R3/R7 *(box)* are positive for both c-kit and CRHR-1; the insert shows 2 such fluorescent cells. Quadrant R5 shows nonspecific fluorescence.

neurologic concerns.[2] However, there has never been a report, to our knowledge, of MC activation within the BM, or of either CRHR-positive MC in the BM or elevated serum CRH levels in SM.

Degranulated MC in the BM is an important finding since no environmental triggers are expected to reach there. Activation of these MC may possibly occur by stress-related triggers such as CRH, acting alone or together with other peptides such as neurotensin, which was elevated in the serum of this patient, especially because stress uniquely worsened her symptoms. We had previously reported that both leukemic HMC-1 cells and human umbilical cord blood-derived cultured MC express CRHR-1.[3] Increased skin MC expression of CRHR was associated with severe worsening in response to acute stress in a patient with cutaneous mastocytosis.[4] Moreover, CRH augmented allergic stimulation of human cultured MC and increased FcεRI expression on these cells.[5]

We had previously reported increased serum CRH and neurotensin in atopic dermatitis,[6] but those levels were much lower (0.031 ± 0.0195 ng/mL)[3] than those reported for the patient discussed here (3.1 ± 0.056 ng/mL for CRH and 0.097 ± 0.02 ng/mL for neurotensin). Moreover, this patient did not have atopic dermatitis. CRH could act synergistically with the neurotensin, because we had shown they have synergistic action to increase skin vascular permeability that could promote inflammation.[7] Moreover, neurotensin can induce CRHR-1 expression on human MC, creating a cyclic reaction.[8] We did not have the opportunity to measure serum CRH or tryptase during different times of symptom exacerbation in this patient.

The increased serum CRH could derive from the skin, which is known to have the equivalent of a hypothalamic-pituitary-adrenal axis,[9] or from MC themselves.[10] This latter possibility suggests that CRH may have autocrine actions on the MC.

Our findings do not constitute a cause-and-effect relationship. However, they provide an intriguing possibility wherein high serum or skin CRH, along with other triggers, could stimulate MC to secrete vascular endothelial growth factor and/or other mediators, thus increasing vascular permeability and promoting tissue inflammation. It would be important to document in patients with SM not only the number and shape of MC, but also the extent of MC activation within the BM and in other tissues. Even though tryptase content of MC may provide some index of degranulation, MC can also release many mediators without degranulation,[2] making it necessary to analyze tissue biopsy samples also for gene expression of the target mediators.

It would also be useful to investigate BM and skin expression, as well as serum levels of CRH and neurotensin, as predictors of disease severity. Moreover, serum levels should be measured both during quiet periods and during acute exacerbations. Future treatment options should focus on inhibitors of MC stimulation by CRH and neurotensin. The possible use of CRH and neurotensin receptor antagonists should also be considered.

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Haploidentical T-cell alpha beta receptor and CD19–depleted stem cell transplant for Wiskott-Aldrich syndrome

To the Editor:

Hematopoietic stem cell transplant (HSCT) is curative for patients with Wiskott-Aldrich syndrome (WAS), a severe X-linked disorder characterized by microthrombocytopenia, eczema, and immunodeficiency. Nontransplanted patients often develop autoimmune and inflammatorycomplications, and there is an increased risk of hematological malignancies. Median life expectancy without HSCT is 15 years.[1] HSCT using a HLA-identical sibling is highly successful, with above 90% 5-year survival. Results of matched unrelated donor HSCT for treatment of WAS have improved over time, with recent studies showing above 80% survival.[2] However, there remains a small number of children for whom no suitably matched family or unrelated donor can be found. The use of HLA-haploidentical family donors requires T-cell depletion to avoid graft-versus-host disease (GVHD), which increases the risk of graft rejection, particularly for patients with forms of primary immunodeficiency other than severe combined immune deficiency.[3] Various T-cell depletion strategies have been used to minimize GVHD and maximize sustained engraftment and early immune reconstitution, such as enrichment of $CD34^+$ cells or depletion of $CD3^+$ and $CD19^+$ cells to prevent post-transplant lymphoproliferative disorder. γδ T cells normally represent 1% to 10% of peripheral blood lymphocytes and have various functions, including against intra- and extracellular pathogens. They are not MHC-restricted and are therefore unlikely to cause GVHD. Depletion of T-cell alpha beta receptor (TCR αβ) and $CD19^+$ cells, with selection of TCR γδ T cells, is a new technique with promising results in haploidentical HSCT.[4,5] We report a child with WAS treated successfully using this method.

A 1-year-old Nigerian boy with WAS (c.7781G>A mutation in intron 8) and previous disseminated cytomegalovirus (CMV) infection with pneumonitis requiring ventilation at the age of 4 months was admitted for haploidentical paternal (CMV-positive) HSCT in the absence of a suitably matched family member or unrelated donor. He was pretreated with ganciclovir, and on admission, a CMV PCR test result was negative. Prophylactic foscarnet was given through the transplant. He was conditioned using ATG-Fresenius, busulfan, fludarabine, and thioTEPA. Cyclosporine and mycophenolate mofetil were given for additional GVHD prophylaxis, as GVHD carries no advantage in nonmalignant disease. Donor stem cells were mobilized using granulocyte colony-stimulating factor, and peripheral blood stem cells were harvested. T-cell manipulation was performed using a Miltenyi Biotec CliniMACS system.

Transplant characteristics are shown in Table I. Modified conditioning for haploidentical HSCT was given according