Granulomatous inflammation in cartilage-hair hypoplasia: Risks and benefits of anti–TNF-α mAbs

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Background: Cartilage-hair hypoplasia (CHH) is a rare autosomal recessive disorder characterized by short-limbed skeletal dysplasia. Some patients also have defects in cell-mediated immunity and antibody production. Granulomatous inflammation has been described in patients with various forms of primary immunodeficiencies but has not been reported in patients with CHH.

Objective: We sought to describe granulomatous inflammation as a novel feature in patients with CHH, assess associated immunodeficiency, and evaluate treatment options.

Methods: In a retrospective observational study we collected clinical data on 21 patients with CHH to identify and further characterize patients with granulomatous inflammation.

Results: Four unrelated patients with CHH (with variable degrees of combined immunodeficiency) had epithelioid cell granulomatous inflammation in the skin and visceral organs. Anti–TNF-α mAb therapy in 3 of these patients led to significant regression of granulomas. However, 1 treated patient had fatal progressive multifocal leukoencephalopathy caused by the JC polyomavirus. In 2 patients immune reconstitution after allogeneic hematopoietic stem cell transplantation led to the complete disappearance of granulomas.

Conclusion: To the best of our knowledge, this is the first report of granulomatous inflammation in patients with CHH. Although TNF-α antagonists can effectively suppress granulomas, the risk of severe infectious complications limits their use in immunodeficient patients. (J Allergy Clin Immunol 2011;128:847-53.)

Key words: *Cartilage-hair hypoplasia, primary immunodeficiency, granulomatous inflammation, anti–TNF-α mAb therapy, infliximab, progressive multifocal leukoencephalopathy*

Cartilage-hair hypoplasia (CHH; also known as McKusick-type metaphyseal chondrodysplasia [MIM#250250]) is an autosomal recessive disorder that was first recognized in the Old Order Amish population.[1] This condition shows an exceptionally high prevalence in Finland, but sporadic cases occur worldwide. CHH is characterized by short-limbed dwarfism, hypoplastic hair growth, ligamentous laxity, and impaired cell-mediated immunity.[2] An increased risk of cancer has also been reported.[3,4] The gene mutated in CHH encodes the untranslated RNA component of the mitochondrial RNA-processing ribonuclease (RNAse MRP)[5] and is called *RMRP*. Although a strong genotype-phenotype correlation has been found by means of *in vitro* testing of different mutations,[6] patients with the same genotype can show very variable degrees of immunodeficiency.[7] Immunodeficiency in patients with CHH affects predominantly T cell–mediated immunity, but antibody deficiencies have also been described.[8,9] An increase in mortality associated with defective immunity has been reported.[10] Other features of CHH include hypoplastic anemia.[9] Although several groups have reported successful immune reconstitution after allogeneic hematopoietic stem cell transplantation (HSCT),[11-14] this therapy does not change the course of skeletal dysplasia. The effect of HSCT on the increased risk of malignancy remains to be elucidated.

Granulomatous inflammation can be seen in patients with immunodeficiencies (especially chronic granulomatous disease,[15] common variable immunodeficiency [CVID],[16,17] and T-cell immunodeficiency caused by hypomorphic recombination-activating gene 1/2 mutations[18]) and inflammatory disorders (eg, Crohn disease[19]). Here we report on extensive granulomatous inflammation as a novel feature of CHH on the basis of clinical and histologic observations in 4 unrelated patients. The effect of anti–TNF-α mAb therapy on the course of the granulomatous disease is also described.

METHODS

This retrospective observational study was performed on a cohort of 21 patients with CHH from the Department of Pediatric Immunology at Necker Children's Hospital (Paris, France) and the Department of Pediatrics at the University Hospital Gasthuisberg (Leuven, Belgium). The diagnosis of CHH was based on clinical, radiologic, and immunologic characteristics. The records of all patients with CHH were reviewed for the presence of granulomatous inflammation. Granulomas were suspected on clinical examination and confirmed by means of biopsy. Routine hematoxylin and eosin histologic staining and Gram, Ziehl, Gomori-Grocott, Warthin-Starry, and periodic

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Abbreviations used
CHH: Cartilage-hair hypoplasia
CMV: Cytomegalovirus
CVID: Common variable immunodeficiency
EBER: EBV early RNA
HHV8: Human herpes virus 8
HSCT: Hematopoietic stem cell transplantation
PML: Progressive multifocal leukoencephalopathy
RMRP: RNA component of the mitochondrial RNA-processing ribonuclease (RNAse MRP)

acid–Schiff staining were performed in all cases. Additional immunohistochemical staining with specific antibodies (anti-cytomegalovirus [CMV] and anti–human herpes virus 8 [HHV8]) was performed in some cases. *In situ* hybridization with an EBV early RNA (EBER) probe was performed for several biopsy specimens. Extensive bacterial, viral, fungal, and mycobacterial cultures were done in all patients, with specific PCR testing for mycobacteria in patients 2, 3, and 4 and for *Bartonella henselae* and *Toxoplasma* species in patient 4. Immunohistochemical analysis was performed with the following murine mAbs: anti-CD4, anti-CD8, anti–HLA-DR (Becton Dickinson Biosciences, Erembodegem, Belgium), anti-CD68, anti–B-cell lymphoma 2, anti-CD4, anti-CD8, anti-CD20, anti-CD68 (Dako, Glostrup, Denmark), anti–TNF-α (PeproTech, Rocky Hill, NJ), anti-HHV8 (Novocastra laboratory, Newcastle upon Tyne, United Kingdom), and anti-CMV (Abcam, Cambridge, United Kingdom).

Immunologic analysis of the T-, B-, and natural killer cell compartments was performed by means of flow cytometry with a FACScalibur (BD Biosciences, Le Pont de Claix, France) and mAbs against CD3, CD4, CD8, CD19, CD16, CD45RA and CD45RO, as described elsewhere.[20,21] Lymphocyte proliferation was determined *in vitro* based on the amount of tritiated thymidine incorporated into PBMCs, as described previously,[20,21] after stimulation with PHA or specific antigen after an appropriate immunization schedule. Each study was run in parallel with samples from healthy adult control subjects. Serum levels of IgG, IgA, and IgM were assessed by means of immunonephelometry. The presence or absence of serum antibodies to polioviruses, tetanus and diphtheria toxoids, *Streptococcus pneumoniae* and *Haemophilus influenzae* was determined by using commercial ELISAs, as established in the diagnostic laboratories of our centers.

This retrospective data collection was conducted according to the Helsinki Declaration, informed consent was obtained from the patient's families, especially before anti–TNF-α treatment (patients 2, 3, and 4) and before publication of clinical photographs and magnetic resonance images (patients 3 and 4).

RESULTS

Patients' and disease characteristics

Granulomas were present in 4 of the 21 patients. These patients' clinical characteristics are summarized in Table I. In brief, all patients had short stature, disproportionally short limbs with typical skeletal dysplasia, recurrent respiratory tract infections, and intermittent diarrhea. A small-bowel biopsy specimen showed villous atrophy in 3 patients. Genetic analysis was available for 2 patients and revealed biallelic mutations in the *RMRP* gene; a compound heterozygous C4T nucleotide change in the transcribed region was combined with a 13-bp duplication in the regulatory region in patient 2 and a compound heterozygous C63T and A70G mutation in patient 4.

Table II[22] shows the patients' immunologic features on first admission to our centers (ie, before any immunosuppressive treatment). Immunologic investigations showed severe lymphopenia in all cases (between 60 and 400 $CD3^+$ cells/μL); this predominantly affected $CD4^+$ lymphocytes, with a significant decrease in naive T-cell numbers (<5% of the $CD45RA^+$/$CD4^+$ cells in patients 1, 2, and 3; data not shown). Functional assays evidenced a reduction in or the absence of T-lymphocyte proliferation on stimulation with both mitogens and antigens. T-cell lymphopenia and impaired T-cell function persisted in all patients over time and were present in patients 3 and 4 before the onset of granulomatous inflammation.

Patients 2 and 3 had almost normal humoral immunity, with normal IgG and IgM but low IgA levels and, more importantly, protective postvaccination antibody levels for tetanus, diphtheria toxoid, poliovirus, *H influenzae*, and *S pneumoniae*, as assessed before any immunoglobulin substitution. Patients 1 and 4 presented with important hypogammaglobulinemia and variable specific antibody titers (Table II). Interestingly, there were no notable differences in the immune status of patients with CHH with (n = 4) and without (n = 17) granulomatous inflammation.

Immunoglobulin replacement therapy was initiated in patients 1, 3, and 4 but did not reduce the frequency of infectious complications in patient 3. Lymphopenic patients received standard trimethoprim-sulfamethoxazole prophylaxis. Patients 2 and 4 underwent allogeneic bone marrow transplantation from a matched unrelated donor after reduced-intensity conditioning with fludarabine, melphalan, and alemtuzumab (according to the guidelines published by the European Society for Immunodeficiencies and the European Group for Blood and Marrow Transplantation).[23]

Granulomas

Extensive granulomas were observed at an early age in all 4 patients. Skin lesions appeared at the age of 12 months in patient 1, 4 years in patients 2 and 4, and 8 years in patient 3. All patients had infiltrative skin lesions consisting of plaques, papules, and nodules with an erythematous and violaceous aspect. The latter were poorly delineated and were sometimes associated with skin atrophy, ulceration, or both. Variably sized lesions on the arms and legs (Fig 1) were observed in all patients. Some lesions remained small, whereas others progressed and became large confluent lesions with ulceration. The appearance, number, and spread of the lesions fluctuated over time; some persisted for many years in each patient, despite the use of various lines of therapy specified below. T-cell lymphopenia had been documented before the appearance of granulomas in patients 3 and 4; T-cell counts before the onset of granulomas were not available for patients 1 and 2. In patient 1 a regression of granulomatous skin lesions was observed after 17 years in the absence of specific treatment. Interestingly, the T-cell counts in this patient remained stable over time (at the age of 18 years: 448 T cells/mm^3 and 217 $CD4^+$ cells/mm^3, almost normal mitogen-induced lymphocyte proliferation, and normal tetanus toxoid–induced proliferation). Patient 4 had additional lesions in the bones, lymph nodes, spleen, sinuses, and cartilaginous laryngeal structures, with partial destruction of the bony palate and the nasal septum and the ensuing appearance of saddle-nose deformity.

In all cases histopathological analysis of biopsied tissue revealed granulomas with a variety of morphological characteristics. Interestingly, different types of granulomas were observed in the same patient, sometimes within 2 biopsy specimen and sometimes in biopsy specimens from distinct sites. Figs 2 and 3 depict the histopathology of the patients' cutaneous granulomas,