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

Despina Moshous, MD, PhD,[a,b,c] Isabelle Meyts, MD, PhD,[d] Sylvie Fraitag, MD,[b,e] Carl E. I. Janssen, MBMS,[f] Marianne Debré, MD,[a,b] Felipe Suarez, MD,[b,g] Jaan Toelen, MD,[d] Kris De Boeck, MD, PhD,[d] Tania Roskams, MD, PhD,[f] Antoine Deschildre, MD,[h] Capucine Picard, MD, PhD,[a,b,i,k] Christine Bodemer, MD,[b,j] Carine Wouters, MD, PhD,[d] and Alain Fischer, MD, PhD[a,b,c] *Paris and Lille, France, and Leuven, Belgium*

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

From [a]the Department of Pediatric Immunology and Hematology, [c]the Department of Pathology, [g]the Department of Hematology, [i]Centre d'Etude des Déficits Immunitaires, and [j]the Department of Dermatology, Assistance Publique-Hôpitaux de Paris, Hôpital Necker Enfants-Malades, Paris; [b]Université Paris Descartes, Faculté de Médecine de l'Université René Descartes, Institut Fédératif de Recherche Necker Enfants-Malades (IFR94), Paris; [c]Institut National de la Santé et de la Recherche Médicale, Unité U768, Laboratoire du Développement Normal et Pathologique du Système Immunitaire, Paris; [d]Pediatric Immunology and Rheumatology, University Hospital Gasthuisberg, Leuven; [f]the Department of Pathology, University Hospital Gasthuisberg, Leuven; [h]Unité de pneumologie pédiatrique, Hôpital Jeanne de Flandre, Centre Hospitalier Régional, Universitaire, Lille; and [k]the Laboratory of Human Genetics of Infectious Diseases, INSERM U980, Paris.

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

Received for publication December 9, 2010; revised May 17, 2011; accepted for publication May 17, 2011.

Available online June 28, 2011.

Corresponding author: Despina Moshous, MD, PhD, Unité d'Immunologie et d'Hématologie Pédiatriques, Hôpital Necker-Enfants Malades, 149, rue de Sèvres, F-75743 Paris Cedex 15, France. E-mail: despina.moshous@nck.aphp.fr.

0091-6749/$36.00

doi:10.1016/j.jaci.2011.05.024

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,

TABLE I. Clinical manifestations in 4 patients with CHH with granulomatous inflammation

	Patient 1 (female)	Patient 2 (male)	Patient 3 (female)	Patient 4 (female)
Age at diagnosis of CHH	4 y, 10 mo	14 y	4 y, 4 mo	At birth
Clinical manifestations				
Short stature	Yes (−4 SD)	Yes (−6 SD)	Yes (−5 SD)	Yes (−6 SD)
Short limbs, metaphyseal dysplasia	Yes	Yes	Yes	Yes
Fine and sparse hair	Yes	Yes	Yes	Yes
Anemia	No	No	Aregenerative anemia at 1 mo of age	Autoimmune hemolytic anemia at the age of 17 y (IgG_3 autoantibodies)
Infectious complications				
Age at onset	3 mo	3 y	First year of life	2 y
Type of infections and complications	Upper airway infections, otitis, gastroenteritis Severe herpes simplex infection	Upper and lower airway infections Transient interstitial pneumonia Bronchiectasis at age 10 y	Upper airway infections Otitis complicated by tympanosclerosis and deafness Recurrent bronchopneumonia, bronchiectasis at age 11 y Pansinusitis	Upper airway and bronchopulmonary infections Bronchiectasis (onset at 10 y) Pansinusitis Recurrent otitis Conductive hearing loss necessitating hearing aids
Substitution by intravenous immunoglobulin	Since age 6 y, decreased frequency of infections	No	Since age 8 y without improvement of infections	Since age 5 y, decreased frequency of infections
Gut manifestations	Chronic diarrhea Duodenal biopsy at age 5 y: villous atrophy	Chronic diarrhea Duodenal biopsy at age 4 y: partial villous atrophy	Chronic diarrhea Duodenal biopsy at age 11 y: partial villous atrophy	Intermittent diarrhea No biopsy
Granulomas				
Age at onset/duration	12 mo/16 y	4 y/13 y	8 y/14 y up to now	4 y/14 y
Localization	Cutaneous and subcutaneous Persisted until age 17 y and then spontaneous regression	Cutaneous and subcutaneous	Cutaneous and subcutaneous	Cutaneous and subcutaneous Osteolytic lesion in calcaneus Nasal septum with saddle nose, larynx, supraclavicular lymph node, spleen
Response to anti–TNF mAb	Not applicable	Partial regression of the lesions	Partial regression of the lesions	Partial regression of the lesions
Side effects of anti–TNF mAb	Not applicable	None	None	JC virus PML, death (19 y)
HSCT (age at HSCT)	No	Yes (17 y)	No	Yes (18 y)

including (1) sarcoidosis-like granulomas (mainly composed of epithelioid cells associated with few lymphocytes and giant cells without any necrosis and arranged in well-circumscribed nodules [Fig 2, *A*]); (2) poorly defined tuberculoid granulomas (with numerous giant multinucleated cells associated with some lymphocytes and few epithelioid cells [Fig 2, *B*]); and (3) histiocytic palisading granulomas with a central necrobiotic area, mimicking a granuloma annulare (Fig 2, *C*). These granulomas were mainly located in the dermis and upper subcutaneous tissue. Immunohistologic staining showed $CD68^+$ macrophages and epithelioid cells (Fig 3, *A*). $CD4^+$ and (predominantly) $CD8^+$ T lymphocytes were found in the granulomas (Fig 3, *B* and *C*). Investigation of biopsy specimens taken at different time points revealed a fluctuation in the CD4/CD8 ratio over time. In addition, substantial infiltration by $CD20^+$ lymphocytes was observed in initial biopsy specimens. HLA-DR staining test results were positive, especially in macrophages in the interstitium and the granulomatous infiltrate (Fig 3, *D*). We observed positive anti–TNF-α antibody staining in all granulomas (Fig 3, *E*) and variably intense B-cell lymphoma 2 expression (Fig 3, *F*).

Gram, Ziehl, Gomori-Grocott, Warthin-Starry, and periodic acid–Schiff staining results were negative for all samples. There was no histologic evidence of viral infection in the biopsy specimens, and the results of immunohistochemistry were negative for CMV and HHV8. Specific cultures for bacteria, fungi, and mycobacteria (performed for all available biopsy specimens) did not identify any infectious agents. The same was true of specific PCR tests for mycobacterial species in several patients' samples. In patient 2 results of *in situ* hybridization with EBER probe were positive for the inflammatory cells within 1 skin biopsy specimen (EBV blood load at the time of the biopsy). EBV screening results with the EBER probe had been repeatedly negative in skin biopsy specimens collected 2, 3, and 5 years before, as well as 5 years after, this unique positive result. Furthermore, the histologic features of patient 2's granulomas did not differ from those seen in the other patients. Results of *in situ* hybridizations with the EBER probe performed in several biopsy specimens from patient 3 were negative. We therefore concluded that this finding was coincidental and unrelated to the pathogenesis of the granulomas.

TABLE II. Immunologic features at first admission

	Patient 1 (female)	Patient 2 (male)	Patient 3 (female)	Patient 4 (female)
Age at first admission to our centers	5 y, 7 mo	10 y	7 y, 6 mo	2 y
Absolute lymphocyte count/mm^3	800 (1100-5900)	800 (1000-5300)	700 (1100-5900)	1752 (1700-6900)
CD3$^+$ cells/mm^3	60 (700-4200)	408 (800-3500)	238 (700-4200)	357 (900-4500)
CD4$^+$ cells/mm^3	ND	200 (400-2100)	189 (300-2000)	168 (500-2400)
CD8$^+$ cells/mm^3	ND	128 (200-1200)	7 (300-1800)	102 (300-1600)
CD19$^+$ cells/mm^3	352 (200-1600)	250 (200-600)	308 (200-1600)	390 (200-2100)
NK cells/mm^3	388 (90-900)	142 (70-1200)	154 (90-900)	1005 (100-1000)
Proliferation: mitogens (cpm $\times$ 10^{-3})	21 (>50)	9 (>50)	9 (>50)	31 (>50)
Proliferation: tetanus (cpm $\times$ 10^{-3})	Absent	Absent	38 (>10)	Absent
IgG (g/L)	3.16 (6.8-11.8)	9.8 (8.3-14.9)	13.3 (7-12.6)	1.43 (5.3-10.1)
IgA (g/L)	0.41 (0.66-1.34)	0.6 (1.02-1.94)	0.48 (0.78-1.62)	0.1 (0.34-0.78)
IgM (g/L)	0.16 (0.5-1.14)	1.0 (0.68-1.28)	1.58 (0.66-1.18)	0.22 (0.54-1.06)
IgE	In normal range	In normal range	In normal range	In normal range
Anti-tetanus antibodies	Absent	Protective	Protective	Not done
Anti–polio 1, 2, or 3 antibodies	Protective	Protective	Protective	Protective
Anti-diphtheria antibodies	Absent	Protective	Protective	Not done
Anti–*S pneumoniae* antibodies	Not done	Protective	Protective	Absent
Anti–*H influenzae* B antibodies	Not done	Protective	Protective	Not done
Isohemagglutinins	Absent	1/256 anti-A	Not done	Not done

In brackets are age-matched normal values for lymphocyte counts (according to Comans-Bitter et al[22]), proliferation assays, and immunoglobulin levels (as established by the performing laboratory). Proliferation assays included routinely the patients' cells without stimulation as well as positive controls of healthy donors.
ND, Not done; *NK*, natural killer.

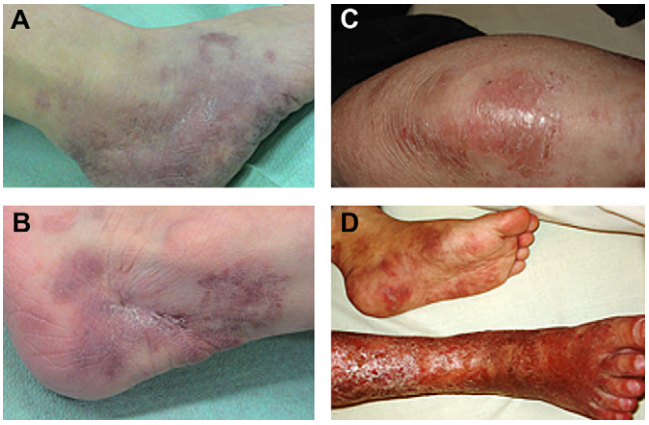

FIG 1. Cutaneous manifestations in patient 4 (foot, **A** and **B**) and patient 3 (knee, **C**; foot, **D**).

Anti–TNF-α therapy

Despite several courses of broad-spectrum antibiotic treatment, which also targeted atypical intracellular microorganisms, no significant improvement of the granulomatous lesions was observed in any of the patients. Only a modest transient response was observed in patients 1 and 2 after the local application of steroids. Oral steroids led to partial transient improvement of granulomas in patient 4 but also produced severe side effects (a Cushingoid aspect and multiple vertebral collapse). No significant benefit was observed after 15 months of local application of tacrolimus in patient 2. Patient 3 received hydroxychloroquine sulfate for 15 months at the age of 11 years and subcutaneous IFN-α 2a for 3 months at the age of 12.5 years; again, the results were not satisfactory.

Intravenous anti–TNF-α mAb (infliximab [Remicade; Centocor Ortho Biotech, Inc, Horsham, Pa]) treatment was initiated in 3 of the 4 patients (patients 2, 3, and 4) at a dose of 5 mg/kg on days 0 and 14, followed by 1 injection every 4 weeks in patient 2 and every 6 to 8 weeks in patients 3 and 4. In patient 4 infliximab was discontinued after 7 months because of severe neurologic side effects (described below). In patients 2 and 3 cyclosporine was combined with infliximab.

After as few as 2 months of infliximab treatment, we observed notable improvement of the granulomatous lesions in patient 4 and partial regression in patients 2 and 3. In patient 2 the granulomas had regressed by approximately 50% after 4 months of combination therapy with anti–TNF-α mAb and cyclosporine. In this patient infliximab was maintained at 5 mg/kg every 4 weeks for a total duration of 15 months and was withdrawn at the time of HSCT. Cyclosporin was continued for 12 months after HSCT as prophylaxis for graft-versus-host disease. Fourteen months after HSCT, the skin lesions had resolved completely. At the last follow-up (3 years after HSCT), patient 2 is alive and well, with his immune function completely restored. In patient 4 similar regression of the granulomas was observed as early as 3 months after HSCT. This patient also received cyclosporine as prophylaxis for acute graft-versus-host disease.

Adverse effects of anti–TNF-α mAb therapy

A fine intentional tremor was observed in patient 4 at month 3 after initiation of anti–TNF-α mAb therapy. At month 6, major neurologic deterioration was noted; the patient presented with severe headaches, had lost the ability to write, and had slowed speech, dysarthria, and ataxia. Magnetic resonance imaging showed hyperintense lesions in the cerebellum and frontal and temporal subcortical areas (Fig 4), suggesting the diagnosis of progressive multifocal leukoencephalopathy (PML). PCR analysis of the cerebrospinal fluid revealed the presence of JC virus. Infliximab was immediately discontinued (after 7 months of treatment), and steroids were tapered. Despite treatment with cidofovir, high-dose intravenous immunoglobulins, leflunomide, mirtazapine, and cytarabine, only a slight neurologic improvement was observed. Phenoidentical HSCT was performed to

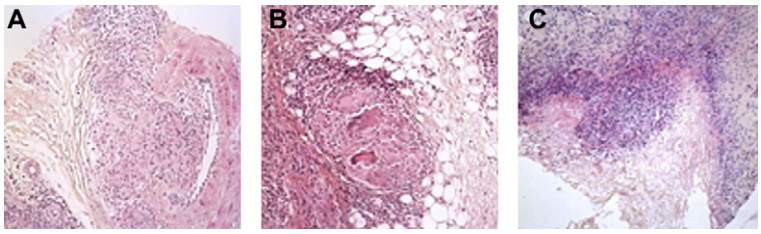

FIG 2. Histologic staining of skin biopsy specimens in patients with CHH. **A**, Sarcoidosis-like granuloma mainly composed of epithelioid cells, with a few lymphocytes and giant cells but no necrosis, located in the deep dermis next to a small venule (hematoxylin and eosin staining, original magnification ×250). **B**, Tuberculoid granuloma located in the upper part of the subcutis, showing numerous giant multinucleated cells resembling Langerhans cells. There are some lymphocytes, few epithelioid cells, and no necrosis (hematoxylin and eosin staining, original magnification ×250). **C**, Palisading histiocytic granuloma surrounding a large area of eosinophilic necrosis, with many lymphocytes but no epithelioid or giant cells (hematoxylin and eosin staining, original magnification ×100).

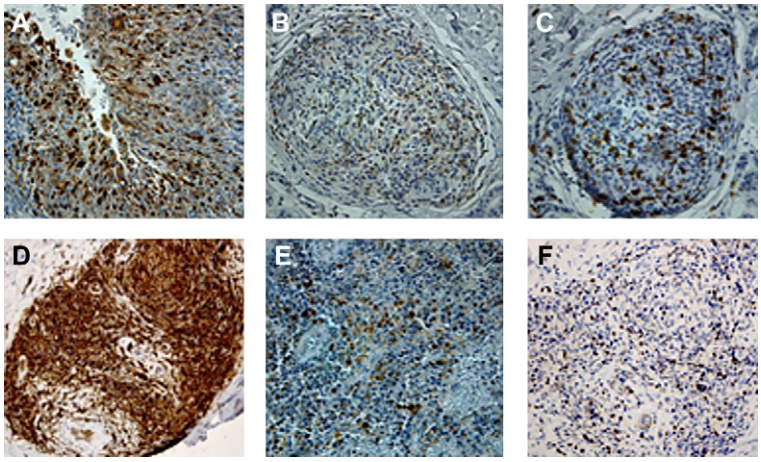

FIG 3. Immunohistochemical staining of epithelioid and palisading granulomas (original magnification ×200). **A**, CD68 staining highlighted cells from the mononuclear phagocyte system. Here a palisading granuloma complex is depicted. **B**, A small proportion of $CD4^+$ T lymphocytes was found in and around the epithelioid granulomas. **C**, Predominance of $CD8^+$ T lymphocytes in the granulomas. **D**, HLA-DR staining was positive (especially in the macrophages) in both the interstitium and the granulomatous infiltrate. **E**, TNF-α staining was strongest in the palisading granulomas. **F**, B-cell lymphoma 2 expression.

restore T-cell function (9 months after the onset of the first discrete neurologic signs and 5 months after the diagnosis of PML and discontinuation of infliximab). The patient died 15 months after PML onset (ie, 6 months after an otherwise successful HSCT). Immune reconstitution inflammatory syndrome might have contributed to the neurologic degradation that ultimately led to the patient's death.

DISCUSSION

Here we present 4 patients with CHH immunodeficiency and granulomatous inflammation, a previously undescribed combination. Noncaseating granulomas without an identifiable infectious cause can occasionally be seen in patients with primary humoral immunodeficiencies, cellular immunodeficiencies, or both[24] (namely CVID,[16,25-27] ataxia-telangiectasia,[28,29] and chronic granulomatous diseases[15]). Anecdotal cases of granulomas have been reported in patients with X-linked hypogammaglobulinemia,[30] hypomorphic recombination-activating gene 1/2[18,31] or Artemis deficiency,[32] and Nijmegen breakage syndrome.[33] Granulomas can also be identified in patients with incomplete immune reconstitution after HSCT for immunodeficiencies (our unpublished observation).

Granulomas are most frequently seen in the skin but can also be found in the liver, lung, bone marrow, spleen, and gastrointestinal tract.[16,18,31,32] They are thought to be a manifestation of immune dysregulation because no specific infectious causes have been identified to date. Nevertheless, infection by as yet unidentified microorganisms cannot be ruled out, emphasizing the need for novel microbiological techniques.[34,35]

The morphology of the granulomas in our patients was polymorphic and variable. Immunohistochemical studies showed a predominance of $CD8^+$ T cells, although the cell populations fluctuated over time. A similar $CD8^+$ predominance has previously been reported in skin granulomas in patients with other primary immunodeficiencies.[36]

Granuloma formation typically occurs in the context of chronic inflammation and increased release of proinflammatory cytokines and chemokines. The polypeptide cytokine TNF-α has important roles in inflammatory, immunoregulatory, and proliferative responses[37,38] and is thought to be necessary for the formation and maintenance of granulomas.[39] Significantly increased serum

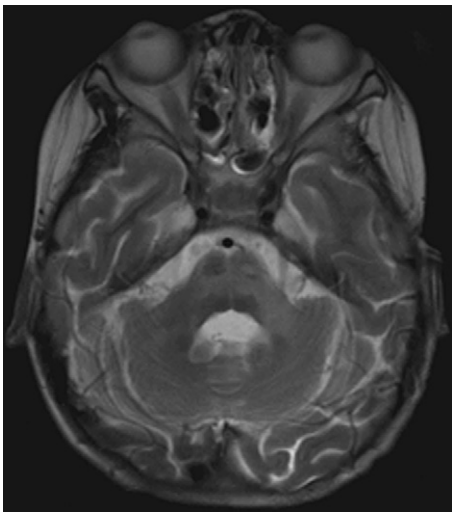

FIG 4. Radiologic manifestations in patient 4. Brain magnetic resonance imaging revealed demyelination in the left hemisphere (more than the right), as well as in the pons and the left pedunculus cerebellaris and dentate nucleus.

levels of TNF-α and soluble TNF receptors in patients with CVID with granulomatous inflammation also argue in favor of a role for TNF-α.[40,41] Accordingly, we observed strong TNF-α expression in biopsy specimens of several patients at different time points during the disease.

The severity of granulomatous lesions, their progression, and the resulting major handicap might require aggressive treatment. Topical steroids, systemic steroids, or both can be effective but sometimes produce severe side effects and significant relapse on withdrawal.[42] Infliximab's efficacy has been demonstrated in a variety of autoimmune and granulomatous inflammatory disorders, including Crohn disease[43] and sarcoidosis,[44] as well as in patients with Blau syndrome.[45] Furthermore, some patients with CVID with severe granulomatous disease who did not respond to corticosteroids showed an improvement on treatment with infliximab[46,47] or etanercept (a soluble TNF-α receptor inhibitor),[48] whereas 2 other patients with CVID with lung granulomas did not respond to TNF-α inhibitors.[17] However, no controlled trials have been performed in this field.

In our patients with CHH, several courses of broad-spectrum antibiotics and antivirals and anti-inflammatory therapy (including local and systemic corticosteroids) did not consistently suppress the granulomatous lesions. Conversely, a significant improvement was observed after initiation of anti–TNF-α mAb in all 3 treated patients. Although patient 3's granulomatous skin lesions persisted during anti–TNF-α mAb treatment, withdrawal led to significant deterioration, suggesting that an underlying treatment-related improvement was nevertheless present. We cannot rule out an additional beneficial effect of cyclosporine in patients 2 and 3, although the evidence for its efficacy in patients with granulomatous inflammation is extremely limited.[49] Furthermore, patient 4 (who showed the most rapid and significant response to infliximab) had not received cyclosporine concurrently. This patient was not completely weaned off steroids when infliximab was introduced but had not responded satisfactorily to steroids. Our observation of TNF-α overexpression in the granulomatous lesion might also be in agreement with a positive effect of anti–TNF-α treatment.

Post-HSCT immune reconstitution led to complete regression of the residual skin lesions in both patients undergoing transplantation, demonstrating that HSCT presents the only curative treatment. Because the recovery of immune function might both resolve unidentified infections and correct immune dysregulation, this observation does not prove (or disprove) either hypothesis. However, our observation that the granulomatous lesions did not worsen throughout anti–TNF-α treatment or during the profound immunosuppression caused by HSCT conditioning suggests a noninfectious cause. Because TNF-α is involved in host defense, TNF blockade might result in unexpected infections.[50] Patient 4 had PML caused by a JC virus infection, a known complication of immunodeficiency. Although no such cases had been reported until recently in large cohorts of patients with Crohn disease[43,51] or sarcoidosis[44,52] treated with anti-TNF reagents, it is likely that this complication is favored in patients with an inherited immunodeficiency disorder. It was only very recently that a first case of PML was reported in a patient treated with infliximab, prednisone, and methotrexate during 3 years.[53] PML did not occur in any other patients with CHH of our cohort, nor are we aware of PML among the published patients with CHH. Thus far, we did not observe any other serious adverse events with infliximab in our immunodeficient patients, but the infectious risk has to be carefully evaluated before considering infliximab in the treatment of granulomas in patients with CHH or other primary immunodeficiencies.

In conclusion, this is the first report (to the best of our knowledge) of cutaneous and visceral granulomatous inflammation in patients with CHH. Our findings demonstrate that although infliximab might be an effective treatment, the infectious risk is a major concern, particularly in the setting of underlying primary immunodeficiency, and warrants close clinical and biological surveillance.

We thank the patients and their families for cooperation, and we thank the nurses and clinicians for the care of the patients.

Clinical implications: Although granulomatous inflammation (a new clinical feature in patients with CHH with immunodeficiency) can be treated effectively with TNF-α antagonists, there is a risk of severe infectious complications.

REFERENCES

1. McKusick VA, Eldridge R, Hostetler JA, Ruangwit U, Egeland JA. Dwarfism in the Amish. Ii. Cartilage-hair hypoplasia. Bull Johns Hopkins Hosp 1965;116:285-326.
2. Makitie O, Sulisalo T, de la Chapelle A, Kaitila I. Cartilage-hair hypoplasia. J Med Genet 1995;32:39-43.
3. Makitie O, Pukkala E, Teppo L, Kaitila I. Increased incidence of cancer in patients with cartilage-hair hypoplasia. J Pediatr 1999;134:315-8.
4. Taskinen M, Ranki A, Pukkala E, Jeskanen L, Kaitila I, Makitie O. Extended follow-up of the Finnish cartilage-hair hypoplasia cohort confirms high incidence of non-Hodgkin lymphoma and basal cell carcinoma. Am J Med Genet A 2008; 146A:2370-5.
5. Thiel CT, Horn D, Zabel B, Ekici AB, Salinas K, Gebhart E, et al. Severely incapacitating mutations in patients with extreme short stature identify RNA-processing endoribonuclease RMRP as an essential cell growth regulator. Am J Hum Genet 2005;77:795-806.
6. Thiel CT, Mortier G, Kaitila I, Reis A, Rauch A. Type and level of RMRP functional impairment predicts phenotype in the cartilage hair hypoplasia-anauxetic dysplasia spectrum. Am J Hum Genet 2007;81:519-29.
7. Rider NL, Morton DH, Puffenberger E, Hendrickson CL, Robinson DL, Strauss KA. Immunologic and clinical features of 25 Amish patients with RMRP 70 A→G cartilage hair hypoplasia. Clin Immunol 2009;131:119-28.

8. Notarangelo LD, Roifman CM, Giliani S. Cartilage-hair hypoplasia: molecular basis and heterogeneity of the immunological phenotype. Curr Opin Allergy Clin Immunol 2008;8:534-9.
9. Makitie O, Kaitila I. Cartilage-hair hypoplasia—clinical manifestations in 108 Finnish patients. Eur J Pediatr 1993;152:211-7.
10. Makitie O, Pukkala E, Kaitila I. Increased mortality in cartilage-hair hypoplasia. Arch Dis Child 2001;84:65-7.
11. Berthet F, Siegrist CA, Ozsahin H, Tuchschmid P, Eich G, Superti-Furga A, et al. Bone marrow transplantation in cartilage-hair hypoplasia: correction of the immunodeficiency but not of the chondrodysplasia. Eur J Pediatr 1996;155:286-90.
12. Gallimore CI, Lewis D, Taylor C, Cant A, Gennery A, Gray JJ. Chronic excretion of a norovirus in a child with cartilage hair hypoplasia (CHH). J Clin Virol 2004; 30:196-204.
13. Guggenheim R, Somech R, Grunebaum E, Atkinson A, Roifman CM. Bone marrow transplantation for cartilage-hair-hypoplasia. Bone Marrow Transplant 2006; 38:751-6.
14. Bordon V, Gennery AR, Slatter MA, Vandecruys E, Laureys G, Veys P, et al. Clinical and immunological outcome of patients with cartilage hair hypoplasia after hematopoietic stem cell transplantation. Blood 2010;116:27-35.
15. van den Berg JM, van Koppen E, Ahlin A, Belohradsky BH, Bernatowska E, Corbeel L, et al. Chronic granulomatous disease: the European experience. PLoS One 2009;4:e5234.
16. Ardeniz O, Cunningham-Rundles C. Granulomatous disease in common variable immunodeficiency. Clin Immunol 2009;133:198-207.
17. Cunningham-Rundles C. How I treat common variable immune deficiency. Blood 2010;116:7-15.
18. Schuetz C, Huck K, Gudowius S, Megahed M, Feyen O, Hubner B, et al. An immunodeficiency disease with RAG mutations and granulomas. N Engl J Med 2008; 358:2030-8.
19. Sewell GW, Marks DJ, Segal AW. The immunopathogenesis of Crohn's disease: a three-stage model. Curr Opin Immunol 2009;21:506-13.
20. de Villartay JP, Lim A, Al-Mousa H, Dupont S, Dechanet-Merville J, Coumau-Gatbois E, et al. A novel immunodeficiency associated with hypomorphic RAG1 mutations and CMV infection. J Clin Invest 2005;115:3291-9.
21. Morelon E, Dautry-Varsat A, Le Deist F, Hacein-Bay S, Fischer A, de Saint Basile G. T-lymphocyte differentiation and proliferation in the absence of the cytoplasmic tail of the common cytokine receptor gamma c chain in a severe combined immune deficiency X1 patient. Blood 1996;88:1708-17.
22. Comans-Bitter WM, de Groot R, van den Beemd R, Neijens HJ, Hop WC, Groeneveld K, et al. Immunophenotyping of blood lymphocytes in childhood. Reference values for lymphocyte subpopulations. J Pediatr 1997;130:388-93.
23. EBMT/ESID guidelines for haematopoeitic stem cell transplantation for primary immunodeficiencies. Available at: http://www.esid.org/bone-marrow-transplantation-ebmt-esid-guidelines-for-haematopoietic-stem-cell-transplantation-for-pi-350-0. Accessed June 15, 2011.
24. Arbiser JL. Genetic immunodeficiencies: cutaneous manifestations and recent progress. J Am Acad Dermatol 1995;33:82-9.
25. Fernandez-Ruiz M, Guerra-Vales JM, Francisco-Javier CF, Yolanda RG, Miguel-Ingel MG, Cristina GC, et al. Fever of unknown origin in a patient with common variable immunodeficiency associated with multisystemic granulomatous disease. Intern Med 2007;46:1197-202.
26. Morimoto Y, Routes JM. Granulomatous disease in common variable immunodeficiency. Curr Allergy Asthma Rep 2005;5:370-5.
27. Torrelo A, Mediero IG, Zambrano A. Caseating cutaneous granulomas in a child with common variable immunodeficiency. Pediatr Dermatol 1995;12:170-3.
28. Drolet BA, Drolet B, Zvulunov A, Jacobsen R, Troy J, Esterly NB. Cutaneous granulomas as a presenting sign in ataxia-telangiectasia. Dermatology 1997;194:273-5.
29. Paller AS, Massey RB, Curtis MA, Pelachyk JM, Dombrowski HC, Leickly FE, et al. Cutaneous granulomatous lesions in patients with ataxia-telangiectasia. J Pediatr 1991;119:917-22.
30. Fleming MG, Gewurz AT, Pearson RW. Caseating cutaneous granulomas in a patient with X-linked infantile hypogammaglobulinemia. J Am Acad Dermatol 1991; 24:629-33.
31. De Ravin SS, Cowen EW, Zarember KA, Whiting-Theobald NL, Kuhns DB, Sandler NG, et al. Hypomorphic Rag mutations can cause destructive midline granulomatous disease. Blood 2010;116:1263-71.
32. Rohr J, Pannicke U, Doring M, Schmitt-Graeff A, Wiech E, Busch A, et al. Chronic inflammatory bowel disease as key manifestation of atypical ARTEMIS deficiency. J Clin Immunol 2010;30:314-20.
33. Vogel CA, Stratman EJ, Reck SJ, Lund JJ. Chronic noninfectious necrotizing granulomas in a child with Nijmegen breakage syndrome. Pediatr Dermatol 2010;27: 285-9.
34. Clarridge JE 3rd. Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. Clin Microbiol Rev 2004;17:840-62.
35. Whitley R. The new age of molecular diagnostics for microbial agents. N Engl J Med 2008;358:988-9.
36. de Jager M, Blokx W, Warris A, Bergers M, Link M, Weemaes C, et al. Immunohistochemical features of cutaneous granulomas in primary immunodeficiency disorders: a comparison with cutaneous sarcoidosis. J Cutan Pathol 2008;35: 467-72.
37. Ruddle NH. Tumor necrosis factor (TNF-alpha) and lymphotoxin (TNF-beta). Curr Opin Immunol 1992;4:327-32.
38. Kruglov AA, Kuchmiy A, Grivennikov SI, Tumanov AV, Kuprash DV, Nedospasov SA. Physiological functions of tumor necrosis factor and the consequences of its pathologic overexpression or blockade: mouse models. Cytokine Growth Factor Rev 2008;19:231-44.
39. Kindler V, Sappino AP, Grau GE, Piguet PF, Vassalli P. The inducing role of tumor necrosis factor in the development of bactericidal granulomas during BCG infection. Cell 1989;56:731-40.
40. Aukrust P, Lien E, Kristoffersen AK, Muller F, Haug CJ, Espevik T, et al. Persistent activation of the tumor necrosis factor system in a subgroup of patients with common variable immunodeficiency—possible immunologic and clinical consequences. Blood 1996;87:674-81.
41. Mullighan CG, Fanning GC, Chapel HM, Welsh KI. TNF and lymphotoxin-alpha polymorphisms associated with common variable immunodeficiency: role in the pathogenesis of granulomatous disease. J Immunol 1997;159:6236-41.
42. Mitra A, Pollock B, Gooi J, Darling JC, Boon A, Newton-Bishop JA. Cutaneous granulomas associated with primary immunodeficiency disorders. Br J Dermatol 2005;153:194-9.
43. Peyrin-Biroulet L, Deltenre P, de Suray N, Branche J, Sandborn WJ, Colombel JF. Efficacy and safety of tumor necrosis factor antagonists in Crohn's disease: meta-analysis of placebo-controlled trials. Clin Gastroenterol Hepatol 2008;6: 644-53.
44. Sweiss NJ, Hushaw LL. Biologic agents for rheumatoid arthritis: 2008 and beyond. J Infus Nurs 2009;32(suppl):S4-24.
45. Milman N, Andersen CB, Hansen A, van Overeem Hansen T, Nielsen FC, Fledelius H, et al. Favourable effect of TNF-alpha inhibitor (infliximab) on Blau syndrome in monozygotic twins with a de novo CARD15 mutation. APMIS 2006; 114:912-9.
46. Thatayatikom A, Thatayatikom S, White AJ. Infliximab treatment for severe granulomatous disease in common variable immunodeficiency: a case report and review of the literature. Ann Allergy Asthma Immunol 2005;95:293-300.
47. Hatab AZ, Ballas ZK. Caseating granulomatous disease in common variable immunodeficiency treated with infliximab. J Allergy Clin Immunol 2005;116:1161-2.
48. Lin JH, Liebhaber M, Roberts RL, Dyer Z, Stiehm ER. Etanercept treatment of cutaneous granulomas in common variable immunodeficiency. J Allergy Clin Immunol 2006;117:878-82.
49. Paramothayan S, Lasserson T. Treatments for pulmonary sarcoidosis. Respir Med 2008;102:1-9.
50. Marodi L, Casanova JL. Primary immunodeficiencies may reveal potential infectious diseases associated with immune-targeting mAb treatments. J Allergy Clin Immunol 2010;126:910-7.
51. Lavagna A, Bergallo M, Daperno M, Sostegni R, Costa C, Leto R, et al. Infliximab and the risk of latent viruses reactivation in active Crohn's disease. Inflamm Bowel Dis 2007;13:896-902.
52. Thielen AM, Barde C, Saurat JH, Laffitte E. Refractory chronic cutaneous sarcoidosis responsive to dose escalation of TNF-alpha antagonists. Dermatology 2009; 219:59-62.
53. Kumar D, Bouldin TW, Berger RG. A case of progressive multifocal leukoencephalopathy in a patient treated with infliximab. Arthritis Rheum 2010;62: 3191-5.