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An Autoinflammatory Disease with Deficiency of the Interleukin-1–Receptor Antagonist

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Abstract

Background—Autoinflammatory diseases manifest inflammation without evidence of infection, high-titer autoantibodies, or autoreactive T cells. We report a disorder caused by mutations of *IL1RN*, which encodes the interleukin-1–receptor antagonist, with prominent involvement of skin and bone.

Methods—We studied nine children from six families who had neonatal onset of sterile multifocal osteomyelitis, periostitis, and pustulosis. Response to empirical treatment with the recombinant interleukin-1–receptor antagonist anakinra in the first patient prompted us to test for the presence of mutations and changes in proteins and their function in interleukin-1–pathway genes including *IL1RN*.

Results—We identified homozygous mutations of *IL1RN* in nine affected children, from one family from Newfoundland, Canada, three families from the Netherlands, and one consanguineous family from Lebanon. A nonconsanguineous patient from Puerto Rico was homozygous for a genomic deletion that includes *IL1RN* and five other interleukin-1–family members. At least three of the mutations are founder mutations; heterozygous carriers were asymptomatic, with no cytokine abnormalities in vitro. The *IL1RN* mutations resulted in a truncated protein that is not secreted, thereby rendering cells hyperresponsive to interleukin-1 β stimulation. Patients treated with anakinra responded rapidly.

Conclusions—We propose the term deficiency of the interleukin-1–receptor antagonist, or DIRA, to denote this autosomal recessive autoinflammatory disease caused by mutations affecting *IL1RN*. The absence of interleukin-1–receptor antagonist allows unopposed action of interleukin-1, resulting in life-threatening systemic inflammation with skin and bone involvement. (ClinicalTrials.gov number, NCT00059748.)

Autoinflammatory diseases constitute a group of genetic disorders whose main clinical features are recurrent episodes of inflammatory lesions that can affect the skin, joints, bones, eyes, gastrointestinal tract, and nervous system, in association with signs of systemic inflammation.¹ Examples of these disorders are familial Mediterranean fever^{2,3}; the tumor necrosis factor receptor–associated periodic syndrome¹; the hyper-IgD syndrome¹; a syndrome of pyogenic arthritis, pyoderma gangrenosum, and acne⁴; the cryopyrin-associated periodic syndromes^{5–7}; and others. The cryopyrin-associated periodic syndromes are related disorders that arise from abnormalities in the control of the potent proinflammatory cytokine interleukin-1 β and are caused by mutations in *NLRP3*, the gene encoding the NALP3 protein (also called cryopyrin). This protein forms a complex that activates caspase 1, an enzyme that cleaves the inactive interleukin-1 β precursor (pro-interleukin-1 β) to its active form, interleukin-1 β , a cytokine with potent proinflammatory effects.^{8,9} Anakinra, a recombinant human interleukin-1–receptor antagonist that blocks the proinflammatory effects of interleukin-1 β , rapidly relieves the symptoms of systemic

inflammation in patients with the cryopyrin-associated periodic syndromes and prevents organ damage due to inflammation in this disorder.¹⁰

Some of the autoinflammatory disorders in children, adults, and animal models involve bone and skin and manifest with osteomyelitis and pustulosis.^{11–13} We describe an autoinflammatory syndrome of skin and bone caused by recessive mutations in *IL1RN*, the gene encoding the interleukin-1–receptor antagonist. We propose the term deficiency of the interleukin-1 receptor antagonist, or DIRA, to denote this illness.

Methods

Patients

All protocols were approved by institutional review boards, and written informed consent for genetic testing and participation was provided by the parents for their children, participating family members, and controls to the National Institutes of Health (NIH) or to the local site. Empirical treatment with anakinra was initiated in all patients, at local sites or at the NIH. Functional assays were conducted on blood samples from Patients 1, 3, and 9 and their siblings and parents. Population-control studies were performed with the use of anonymous DNA samples that had been collected in other studies.

Genetic Analysis

Coding exons of *IL1RN* isoform 1 (accession number, NM_173842) were sequenced with the use of a BigDye Terminator kit (Applied Biosystems) on a DNA analyzer (ABI 3100 or 3730×1). We evaluated allele frequencies in DNA samples obtained from a panel of 364 white controls from the New York Cancer Project,¹⁴ 555 controls from Newfoundland, 351 Dutch controls, and 119 Puerto Rican controls, by using mass spectrometry (the homogeneous MassExtend assay, Sequenom). A high-density single-nucleotide–polymorphism bead-chip array (HumanCNV370-Quad, Illumina) was used to detect deletions. The deletion breakpoint was sequenced with the use of primers designed from each end of the boundaries of the deletion identified through the analysis of single-nucleotide polymorphisms.

Evaluation of Function

The Supplementary Appendix (available with the full text of this article at NEJM.org) describes the details of standard methods used for the quantitative polymerase-chain-reaction (PCR) assay, Western blotting of mononuclear-cell supernatants, leukocyte stimulation assays, functional analysis of mutant interleukin-1-receptor–antagonist proteins, and immunohistochemical analysis of skin-biopsy specimens.

Treatment with Anakinra

Anakinra (Biovitrum) was administered empirically at a dose of 1 mg per kilogram of body weight daily by means of subcutaneous injection. In patients with an incomplete response to anakinra, the dose was increased by 0.5 mg per kilogram per day at follow-up visits to achieve a C-reactive protein value of less than 0.5 mg per deciliter and an erythrocyte sedimentation rate of less than 15 mm per hour. The extent of rash, number of bone lesions, areas of periostitis, blood markers of inflammation (erythrocyte sedimentation rate, C-reactive protein), and a complete blood count before and after treatment with anakinra were either measured or obtained by means of a chart review.

Results

Clinical Phenotype

Table 1 summarizes the demographic characteristics and clinical presentation of the affected children. One similar case is reported in this issue of the *Journal* by Reddy et al.¹⁵ All patients presented at birth or by 2.5 weeks of age. Fetal distress, pustular rash, joint swelling, oral mucosal lesions, and pain with movement were the common manifesting features. Over time, cutaneous pustulosis, ranging from discrete crops of pustules to generalized severe pustulosis or ichthyosiform lesions, developed in the eight children for whom these data were known (Fig. 1A and 1B). Biopsies of skin lesions from two patients showed extensive infiltration of epidermis and dermis by neutrophils, pustule formation along hair follicles, acanthosis, and hyperkeratosis (Fig. 1A and 1B in the Supplementary Appendix). Histopathological evidence of vasculitis was observed in the connective and fat tissue adjacent to bone in one patient (Fig. 1C in the Supplementary Appendix). Nail changes were seen in four children (Fig. 1D in the Supplementary Appendix).

Pain and joint swelling led to an evaluation for bone lesions. One patient had extensive epiphyseal ballooning of the long bones (Fig. 1C, and Fig. 1E in the Supplementary Appendix). Characteristic radiographic findings were balloon-like widening of the anterior rib ends (in all nine patients) (Fig. 1D), periosteal elevation along multiple long bones (in eight patients) (Fig. 1E), and multifocal osteolytic lesions (in eight patients) (Fig. 1F). Less common were heterotopic ossification of the proximal femurs (in seven patients) (Fig. 1E), widening of the clavicles (in two patients) (Fig. 1D), metaphyseal erosions of the long bones (in two patients) (Fig. 1F in the Supplementary Appendix), and multiple osteolytic skull lesions (in one patient). Three patients had cervical vertebral fusion secondary to collapsing vertebral osteolytic lesions (Fig. 1G in the Supplementary Appendix). Bone-biopsy specimens were sterile; histologic analysis revealed purulent osteomyelitis, fibrosis, and sclerosis (Fig. 1H in the Supplementary Appendix). Cerebral vasculitis or vasculopathy was found in one patient on magnetic resonance imaging (Fig. 1I in the Supplementary Appendix).

No patients had fever, but elevations of the erythrocyte sedimentation rate and C-reactive protein levels were marked. Therapy with disease-modifying antirheumatic drugs (Table 1) and high doses of corticosteroids only partially controlled symptoms and reduced acute-phase reactants. Two children died of multiorgan failure, secondary to the severe inflammatory response syndrome, at the ages of 2 months and 21 months; a third child died, at 9.5 years of age, of complications of pulmonary hemosiderosis with progressive interstitial fibrosis.

IL1RN Mutations

All nine patients were either homozygous for mutations affecting *IL1RN* (seven patients) or had parents who were heterozygous carriers (two patients) (Fig. 2A). Patient 1, from Newfoundland, was homozygous for a deletion of 2 bp (c.156_157delCA) (Fig. 2B) that caused a frame-shift mutation, N52KfsX25, followed by the incorporation of 24 aberrant amino acids and a termination codon. Both parents were heterozygous carriers of the same mutation. Patients 2 through 6 came from three unrelated families of Dutch ancestry; three were homozygous for a nonsense mutation affecting the amino acid at position 77 (nucleotide mutation, c.229G→T; resultant amino acid mutation, E77X) (Fig. 2B), and the other two, whose DNA was not available, had the same clinical phenotype and heterozygous parents. All the Dutch parents were carriers of the same mutation. Patients 7 and 8, from a consanguineous Lebanese family, were homozygous for a nonsense mutation (nucleotide mutation, c.160C→T; resultant amino acid mutation, Q54X) (Fig. 2B). Patient 9, from

Puerto Rico, was homozygous for a deletion of approximately 175 kb on chromosome 2q that includes six genes from a cluster of interleukin-1-related genes: *IL1RN* and the genes encoding interleukin-1 family, members 9 (*IL1F9*), 6 (*IL1F6*), 8 (*IL1F8*), 5 (*IL1F5*), and 10 (*IL1F10*) (Fig. 2C).

None of these mutations were found in DNA specimens obtained from a panel of 364 white controls from the New York Cancer Project. To evaluate the possibility of a founder effect, the frequency of each mutation, except that in the Lebanese family, was tested in DNA samples from controls from the patient's country of origin. In the panel of 555 controls from Newfoundland, 2 carried the N52KfsX25 mutation (allele frequency, 0.2%). No carriers of the E77X mutation were found in a panel of 351 Dutch controls, but this control group was not geographically matched with the Dutch patients, all of whom originated from a small enclave in the southern part of the country. However, the presence of the same mutation in the three unrelated Dutch families we studied strongly suggests a founder effect. The homozygous 175-kb deletion found in our patient, whose parent come from a genetically isolated population in the northwestern part of Puerto Rico, was also found in three unrelated carriers in a panel of 119 controls from geographically matched populations (allele frequency, 1.3%).

Functional Studies

The 3'-truncation mutants potentially encode proteins less than half the size of the secreted wild-type protein (Fig. 2A in the Supplementary Appendix). These mutants would likely bind less well than wild-type proteins to the type I interleukin-1 receptor (Fig. 2B in the Supplementary Appendix). Quantitative PCR revealed that interleukin-1-receptor antagonist messenger-RNA levels were greatly diminished in patients with truncating mutations and were absent in the patient with the genomic deletion (Fig. 3A). In assays measuring the amount of interleukin-1-receptor antagonist secreted by stimulated leukocytes, a band corresponding to glycosylated interleukin-1-receptor antagonist (Fig. 3B, arrow) was present in controls and, at reduced levels, in patients' relatives with heterozygous mutations but was absent in the three patients with homozygous mutations resulting in deficiency of the interleukin-1 receptor antagonist. Proteins corresponding to the predicted molecular weight of the truncation mutants were also not detected (Fig. 2C in the Supplementary Appendix). In cultured cells transfected with mutant *IL1RN*, the messenger RNA was overexpressed, but no interleukin-1-receptor antagonist protein was secreted. Instead, the protein accumulated in the cell, and the 25-amino-acid leader sequence that is cleaved during secretion was retained (Fig. 3C). The wild-type interleukin-1-receptor antagonist that was expressed in vitro suppressed the proliferation of an interleukin-1-dependent cell line, whereas supernatants from mutant transfectants did not suppress interleukin-1-dependent proliferation (Fig. 3C).

Mononuclear cells from patients, carriers, and controls were stimulated with recombinant human interleukin-1 β , and 50 chemokines and cytokines were measured (Table 1 in the Supplementary Appendix). Five chemokines or cytokines (interleukin-1 α , macrophage inflammatory protein 1 α , tumor necrosis factor α , interleukin-8, and interleukin-6) were significantly overproduced after stimulation by interleukin-1 β of mononuclear cells from patients lacking functional interleukin-1-receptor antagonist (Fig. 4A). More interleukin-17-secreting cells were found in biopsy samples of inflamed skin from patients with deficiency of the interleukin-1-receptor antagonist than from controls (Fig. 4B). A higher percentage of type 17 helper T cells were found in three of the patients (Patients 1, 3, and 9) than in their siblings (Fig. 3 in the Supplementary Appendix).

Response to Anakinra

At the time of diagnosis, empirical anakinra therapy had already been started in two patients and was initiated in the other four who were alive. All six patients had a rapid response to treatment. The length of therapy varied between 2 weeks and 4.5 years. All but the Puerto Rican patient, who carried the chromosomal deletion, had clinical remission and acute-phase reactant levels and complete-blood-cell counts that became normal (Fig. 5A). The skin and bone manifestations (Fig. 5B) resolved within days and weeks, respectively, and after 4 years of treatment with anakinra, the disease in the living Dutch patient (Patient 3) remained suppressed. A trial of discontinuation of anakinra led to a relapse within 36 hours. Resumption of anakinra reinduced the remission within 72 hours. The Puerto Rican patient (Patient 9) had a rapid clinical response, but despite an increase in the dose of anakinra, inflammatory markers (erythrocyte sedimentation rate and C-reactive protein) remained elevated (Fig. 5A). Corticosteroids were discontinued in all patients except Patient 9, in whom the dose was able to be reduced. Anakinra-related adverse events were transient injection-site reactions in three patients and an anaphylactic reaction on day 9 of treatment in Patient 7. The subsequent discontinuation of anakinra caused a flare-up of his disease.

Discussion

We describe an autosomal recessive autoinflammatory syndrome, deficiency of the interleukin-1-receptor antagonist, which begins around birth with multifocal osteomyelitis, periostitis, and pustulosis. We identified homozygous truncating mutations in the *IL1RN* gene in six patients and, by inference, in two additional patients in families in which both parents were carriers of the mutation. A ninth patient has a 175-kb deletion in chromosome 2q that includes *IL1RN* and five other genes, all members of the interleukin-1 gene family. As a result of these mutations, no interleukin-1-receptor antagonist protein is secreted, which inhibits the proinflammatory cytokines interleukin-1 α and interleukin-1 β . In vitro studies of leukocytes from these patients with unopposed interleukin-1 signaling showed that interleukin-1 β drives overproduction of proinflammatory cytokines and chemokines. The dramatic clinical phenotype of our patients underscores the importance of tight regulation of interleukin-1 in skin and bone. Our molecular and functional findings were corroborated by the rapid clinical response of patients to treatment with a recombinant interleukin-1-receptor antagonist.

The allele frequencies of the founder mutations in Newfoundland and Puerto Rico are estimated to be 0.2% and 1.3%, respectively. The incidence of the deficiency of interleukin-1-receptor antagonist in some regions of Puerto Rico might be as high as 1 in 6300 births. Although we did not find the Dutch mutation in any of the 351 Dutch controls, the occurrence of the mutation in three independent families, one residing in Canada, suggests a founder effect. Screening of newborns may be warranted in these three high-risk populations. We had no DNA samples from Lebanese controls, but the homozygosity for the Q54X nonsense mutation in the family studied could simply be the result of consanguinity; the parents of the two affected children, who are cousins, could each have inherited the mutant copy of the gene from a common grandparent who carried a de novo mutation. Case descriptions of severe infantile chronic recurrent multifocal osteomyelitis and pustulosis raise the possibility of undiagnosed deficiency of the interleukin-1-receptor antagonist.^{16,17}

Deficiency of the interleukin-1-receptor antagonist resembles not only bacterial osteomyelitis but also the syndrome of infantile cortical hyperostosis, a self-limited disease caused by an autosomal dominant mutation in *COL1A1*, which encodes the major component of type 1 collagen.¹⁸ Another neonatal autoinflammatory disease, neonatal-onset multisystem inflammatory disease (also known as the chronic infantile neurologic cutaneous articular syndrome), is caused by gain-of-function mutations in *NLRP3* (the gene that

encodes cryopyrin) that causes constitutive activation and hypersecretion of interleukin-1 β .⁷⁻¹⁹ There are a number of clinical differences that can distinguish deficiency of the interleukin-1-receptor antagonist from neonatal-onset multisystem inflammatory disease. The absence of interleukin-1-receptor antagonist in patients with deficiency of the interleukin-1-receptor antagonist would permit overactivity of interleukin-1 α , a related proinflammatory cytokine that also signals through the interleukin-1 receptor. Interleukin-1 α is expressed in skin and is a potent osteoclast activator; in addition to its proinflammatory effects, it also acts as an auto-crine growth factor²⁰; its expression profile in skin and bone differs from that of interleukin-1 β .²¹

The homozygous genomic deletion on chromosome 2q in the Puerto Rican patient (Patient 9), which includes *IL1RN* and five other members of the interleukin-1 gene family, raises the question of whether the other deleted genes contribute to this phenotype that is more refractory to anakinra treatment than the phenotype of the patients with the truncating mutations affecting only *IL1RN*. The deleted genes encode relatively unknown interleukin-1-family agonists (interleukin-1F6, interleukin-1F8, and interleukin-1F9)²² and antagonists (interleukin-1F5 and interleukin-1F10) that share structural homology with the interleukin-1-receptor antagonist.²³ Except for interleukin-1F10, all these agonists and antagonists act on the interleukin-1-receptor-related protein 2 receptor, which is homologous with the interleukin-1 receptor.

The effect of absence of interleukin-1-receptor antagonist has been studied in knockout animal models. Arthritis and psoriasis-like skin lesions have been shown to develop in one such mouse model²⁴ and arteritis in another.²⁵ Although the osteolytic lesions and periostitis are not recapitulated in these models, unopposed interleukin-1 signaling could drive the differentiation of type 17 helper T cells²⁶ that contribute to the inflammation in the animal model and may be instructive in understanding inflammatory processes in human disease.

The clinical manifestations of deficiency of the interleukin-1-receptor antagonist resemble those of other inflammatory diseases with multiorgan involvement. The prominent role of interleukin-1 cytokines in the development of skin and bone manifestations in affected patients suggests a role for interleukin-1 in the pathophysiology of other autoinflammatory bone disorders such as chronic recurrent multifocal osteomyelitis and the syndrome with synovitis, acne, pustulosis, hyperostosis, and osteitis. A role for interleukin-1 β in Behçet's disease has also been suggested.²⁷ Elevated levels of interleukin-1 β have been associated with preterm labor²⁸; deficiency of interleukin-1-receptor antagonist may therefore explain the premature birth of some infants with the disease. Interleukin-1 β -driven inflammation may also be involved in carcinogen-induced skin cancer.²⁹

Although deficiency of the interleukin-1-receptor antagonist is a rare disease, it may point to clues about the mechanisms of more common illnesses that affect the balance between interleukin-1 and interleukin-1-receptor antagonist,³⁰ such as those associated with polymorphisms in the interleukin-1 gene cluster, including sero-negative spondyloarthropathies, psoriasis, and osteoarthritis.³¹⁻³³ Interleukin-1 β is a known potent inflammatory mediator, and its expression, activation, and release are tightly controlled at multiple levels.³⁴ Diagnosis of deficiency of the interleukin-1-receptor antagonist presents an opportunity to study the effects of the removal of the natural antagonist that is the final barrier to interleukin-1 β function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Inflammatory Skin and Bone Manifestations in Patients with Deficiency of Interleukin-1-Receptor Antagonist

The skin manifestations range from groupings of small pustules (Panel A) to a generalized pustulosis (Panel B). The bone manifestations include epiphyseal ballooning of multiple distal and proximal long bones, in the single patient from Puerto Rico (Panel C); the more typical radiographic manifestations included widening of multiple ribs (with affected ribs indicated with asterisks) and the clavicle (arrows) (Panel D), heterotopic ossification or periosteal cloaking of the proximal femoral metaphysis (arrows) and periosteal elevation of the diaphysis (arrowheads) (Panel E), and an osteolytic lesion with a sclerotic rim (Panel F, arrow).

Figure 2. Mutations in the *IL1RN* Gene Encoding Interleukin-1-Receptor Antagonist and a Genomic Deletion in the Study Patients

The pedigrees of six families with deficiency of interleukin-1-receptor antagonist are presented in Panel A, according to the country or region of ancestry and the amino acid mutation. Solid symbols indicate Patients 1 through 9, open symbols unaffected relatives, squares male subjects, circles female subjects, and slashes deceased patients. For the subjects for whom the *IL1RN* genotype was determined, H denotes heterozygous mutant, W wild type, and M homozygous mutant. Panel B shows the structure of *IL1RN* (isoform 1) exons (black boxes) and the sequences of the homozygous mutations (which are named under the plots, along with the resultant amino acid mutation). A genetic isolate in northwestern Puerto Rico is a founder population for a 175-kb genomic deletion between bases 113,434,601 and 113,609,824 on chromosome 2. This deletes genes encoding six interleukin-1-related genes: *IL1RN* and the genes encoding interleukin-1 family, members 9 (*IL1F9*), 6 (*IL1F6*), 8 (*IL1F8*), 5 (*IL1F5*), and 10 (*IL1F10*) (Panel C). The centromere and the region of interest are shown in red.

Figure 3. Mechanism of Disease Caused by Deficiency of Interleukin-1-Receptor Antagonist Relative messenger RNA (mRNA) levels of *IL1RN*, encoding the interleukin-1-receptor antagonist, in whole blood were determined by means of quantitative polymerase-chain-reaction assay (Panel A). Levels are shown for seven controls, one Newfoundland subject heterozygous for the N52KfsX25 mutation, three Dutch subjects heterozygous for the E77X mutation, two Puerto Rican subjects heterozygous for the genomic deletion, and one patient homozygous for each of these three mutations. T bars indicate the standard deviations, where applicable. In Panel B, whole-blood specimens from homozygotes and heterozygotes for each of these three mutations, as well as controls (with the wild-type sequence) were stimulated ex vivo with lipopolysaccharide to induce secretion of glycosylated interleukin-1-receptor antagonist by leukocytes. Resultant protein levels, detected by means of Western blot using an antibody specific for the N-terminal of the protein, are shown. Panel C shows the results of in vitro analysis of cultured cells of the human embryonic kidney-cell line 293T, transfected with mutant or wild-type *IL1RN*. The 293T cells overexpressed the mRNA of both the wild-type and mutant *IL1RN* but secreted only the wild-type protein, as shown by analysis of the supernatant (top). The mutant proteins were detected within 293T-cell lysates at a molecular weight that suggests that the signal peptide mediating the secretion of the protein has not been cleaved (middle). Actin expression is shown as a control (bottom). The bar graph at the bottom of the panel shows the mean growth of an interleukin-1-responsive cell line in the presence of secreted interleukin-1-receptor antagonist. The wild-type protein efficiently inhibited proliferation, whereas the mutant proteins, which were not secreted, could not function in this capacity. T bars indicate the standard deviations.

Figure 4. Functional Consequences of Deficiency of Interleukin-1-Receptor Antagonist Peripheral-blood granulocytes and leukocytes were obtained from seven controls with wild-type interleukin-1-receptor antagonist, six heterozygous carriers of mutant interleukin-1-receptor antagonist, and three homozygotes with mutant interleukin-1-receptor antagonist. The monocytes were stimulated with recombinant interleukin-1 β for 18 hours. Panel A shows the mean production of five selected chemokines and cytokines, which were significantly up-regulated in samples from patients homozygous for mutations resulting in deficiency of interleukin-1-receptor antagonist as compared with those from heterozygotes and controls. (P values are shown for comparisons of patients with the wild-type controls for each chemokine or cytokine.) T bars indicate the standard deviations. MIP-1 α denotes macrophage inflammatory protein 1 α , and TNF- α tumor necrosis factor α . Panel B shows the results of cytohistochemical analysis of interleukin-17 expression in skin specimens (alkaline phosphatase stain). The interleukin was markedly up-regulated in a patient with deficiency of interleukin-1-receptor antagonist as compared with a control.

Figure 5. Clinical and Laboratory Response of Patients with Deficiency of Interleukin-1–Receptor Antagonist to Treatment with Anakinra

Laboratory values before and after treatment with the recombinant interleukin-1–receptor antagonist anakinra are shown in Panel A for each of the six patients who received the drug. Treatment with anakinra resulted in a rapid and sustained decline in the C-reactive protein level, erythrocyte sedimentation rate, and white-cell count; the platelet count normalized more slowly. The pink star in each plot indicates the time at which the Dutch Patient 6 had discontinuation of the therapy and a resultant flare-up of disease. Reinitiation of anakinra led to normalization of all four measures. Panel B shows an example of the clinical improvement in skin and bone manifestations after anakinra treatment was begun. Before

treatment there was extensive pustulosis, diffuse erythema, and crusting on the skin of an affected child that nearly completely resolved just days after initiation of anakinra, with exfoliation of the skin occurring 7 days after (shown here). Improvement in bone findings was similarly dramatic, as seen on radiography, with osteolytic lesions (arrows) present in the proximal and distal tibial metaphysis and periosteal elevation (asterisk) before treatment and resolution 5 months after treatment.

Table 1
Characteristics of Study Patients and Their Clinical Disease*

Characteristic	Patient 1	Patient 2	Patient 3	Patient 4
Age at diagnosis	13 Mo	Deceased	7.2 Yr	Deceased
Sex	Male	Male	Female	Male
Country or region of origin†	Newfoundland, Canada	Netherlands	Netherlands	Netherlands (residing in Canada)
Relation to other patients		Brother of Patient 3	Sister of Patient 2	
Gestational age (wk)	37.5	35, with fetal distress	38, with fetal distress	36, with fetal distress
Birth weight (g)	4640	Not known	2880	2880
Clinical outcome	Alive and well	Deceased at 2 mo, from SIRS	Alive and well	Deceased at 9.5 yr
Age or time of clinical presentation	2 Wk	Birth	Birth	Birth
Symptoms at initial presentation	Finger swelling, vesicular stomatitis	Respiratory distress	Aspiration pneumonia, rash at 2 weeks	Rash on forehead, joint swelling (proximal interphalangeal joint and wrist), stomatitis
Presentation of rash	Mild-to-severe pustulosis, pathergy	Mild pustulosis	Mild pustulosis	Severe pustulosis
Nail changes	Yes (pits)	No	No	Yes
Skeletal abnormalities on radiography‡	Yes	Yes	Yes	Yes
Widening of ribs	Yes	Yes	Yes	Yes
Periosteal cloaking	Yes	Yes	Yes	Yes
Periosteal elevation	Yes	Yes	Yes	Yes
Multifocal osteolytic lesions	Yes	Yes	Yes	Yes
Cervical vertebral fusion	No	No	Yes	Yes
Findings of bone-tissue culture	Negative	Negative	Negative	Negative
Hepatosplenomegaly	No	Yes	Yes	Yes
Other manifestations	Vasculitis (on biopsy), central nervous system vasculitis or vasculopathy			Interstitial lung disease, hypotonia, developmental delay
Treatments before anakinra§	Antibiotics, indomethacin, prednisolone, IV immune globulin	Antibiotics	Antibiotics, indomethacin, prednisolone, methotrexate, cyclosporine, azathioprine, etanercept, thalidomide	Antibiotics, prednisone, methotrexate, cyclosporine
Maximal prednisone dose	2 mg/kg/day	Not known	2 mg/kg/day	2 mg/kg/day

Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
Deceased	2 Mo	1.8 Yr	4 Mo	9.5 Yr
Female	Female	Male	Male	Male
Netherlands	Netherlands	Lebanon (residing in Sweden)	Lebanon (residing in Sweden)	Puerto Rico (residing on U.S. mainland)
Sister of Patient 6	Sister of Patient 5	Brother of Patient 8	Brother of Patient 7	
36, with fetal distress	37	38	38	34, with fetal distress
3000	3780	3220	2815	1930
Deceased at 21 mo, from SIRS	Alive	Alive but failure to thrive	Alive with vertebral collapse	Alive with skeletal deformities
2 Days	2.5 Wk	5 Days	2 Days	8 Days
Fever, multifocal osteomyelitis, pathergy	Pustular dermatitis on cheeks, oral candidiasis	Pustular dermatitis, mouth ulcers	Respiratory distress, mouth ulcers	Chorioamnionitis, swelling of right foot and ankle
Not known	Severe pustulosis	Severe pustulosis, pathergy	Mild pustulosis	Mild pustulosis, pyoderma gangrenosum
Not known	Yes	Not known	Yes	No
Yes	Yes	Yes	Yes	Yes
Yes	Yes	Yes	Yes	Yes
Yes	Yes	No	Yes	No
Yes	No	Yes	Yes	Yes
Yes	No	Yes	Yes	Yes
No	No	Not known	Yes	Yes
Negative	None (tissue culture not performed; blood cultures negative)	None (tissue culture not performed; blood cultures negative)	Negative	Negative
Yes	Yes	No	No	Not known
		Conjunctival injection	Conjunctival injection	
Antibiotics, indomethacin, prednisolone	None	Antibiotics, antiviral and antifungal agents, ibuprofen, methylprednisolone sodium succinate, prednisolone	Antibiotics, ibuprofen, prednisolone	Antibiotics, prednisone, interferon-γ
2 mg/kg/day		2 mg/kg/day	0.25 mg/kg/day	2 mg/kg/day

* IV denotes intravenous, and SIRS the severe inflammatory response syndrome.

† Country of origin was reported by the parents of the patients.

‡ Examples of radiographic features are depicted in Figure 1D, 1E, and 1F, and in Figure 1E, 1F, and 1G in the Supplementary Appendix.

§ Patient 9 had an incomplete response to anakinra at a dose of 4 mg per kilogram per day. His symptoms have improved but he continues to have elevated acute-phase reactant levels 6 months after treatment with anakinra.