Phenotype, Genotype, and Sustained Response to Anakinra in 22 Patients With Autoinflammatory Disease Associated With CIAS-1/NALP3 Mutations

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Objective: To characterize the multisystem chronic inflammatory phenotype, dermatopathologic features, and response to therapy with interleukin 1 receptor antagonist (anakinra) in patients with mutations in the CIAS-1/NALP3 gene.

Design: Retrospective review of medical records and evaluation of histologic findings.


Patients: Twenty-two individuals from 13 families with autoinflammatory disease associated with CIAS-1/NALP3 mutations.

Main Outcome Measures: Phenotype, genotype, skin histologic findings, and response to treatment with anakinra.

Results: Five heterozygous missense mutations were identified in CIAS-1/NALP3. Skin histologic findings revealed marked vascular dilatation and neutrophilic infiltration involving small vessels and eccrine glands. Serologic evidence of intense inflammation was present in untreated patients, with median serum amyloid A protein and C-reactive protein levels of 141 and 38 mg/L, respectively. Fifteen patients received anakinra for up to 39 months, all of whom achieved serologic remission and complete resolution of fever, rash, conjunctivitis, and rheumatic symptoms, without any adverse effects. Six patients had AA (reactive systemic) amyloidosis, 2 of whom died of renal failure complications before interleukin-1–inhibiting therapy was available; 1 patient underwent renal transplantation and remains clinically well taking anakinra, and in the remaining 3 patients, anakinra therapy resulted in remission of their nephrotic syndrome.

Conclusions: Anakinra therapy was well tolerated and has sustained efficacy on dermatologic and rheumatic manifestations in these patients with CIAS-1/NALP3 mutations. This treatment also resulted in resolution of AA amyloidosis–associated nephrotic syndrome in all affected patients.

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THE INHERITED PERIODIC FEVER syndromes are characterized by recurrent attacks of fever with systemic inflammation that often includes skin manifestations. They can be complicated by AA (reactive systemic) amyloidosis, which typically presents as progressive renal dysfunction. Characterized syndromes include familial Mediterranean fever, tumor necrosis factor receptor–associated periodic syndrome, and hyperimmunoglobulin D and periodic fever syndrome. In 2001, the 3 separately described syndromes of familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS), and neonatal-onset multisystem inflammatory disorder (NOMID) were attributed to mutations in the gene encoding NALP3, a protein that is also known as cryopyrin.1,2 Familial cold autoinflammatory syndrome was first described in 19403 and is an autosomal dominant disorder characterized by cold-induced rash, fever, and distal arthralgia. Muckle-Wells syndrome, described in 1962,4 is another autosomal dominant disorder characterized by the triad of deafness, urticarial rashes, and reactive AA amyloidosis. Neonatal-onset multisystem inflammatory disorder, the most severe disorder, is usually sporadic and manifests at birth with inflammation affecting many organ systems, including skin, joints, and central nervous system.5 It is apparent that FCAS, MWS, and NOMID represent a spectrum of disease intensity with overlapping features.6,2

The NALP3 gene (also known as CIAS1) is expressed in peripheral blood leukocytes8 and chondrocytes.9 The NALP proteins are members of the death domain superfamily, and their nomenclature derives...
from their several motifs.\textsuperscript{10-12} NALP3 associates with other members of the death domain superfamily to form a multimeric cytosolic assembly that has been called the inflammation.\textsuperscript{11} This pathway results in activation of caspase 1, which processes prointerleukin 1 (pro-IL-1) and pro-IL-18 into their active forms, and it also up-regulates expression of nuclear factor κB, resulting in increased IL-1 gene expression. Up-regulation of IL-1 production has been reported in monocytes obtained from patients with FCAS\textsuperscript{13} and NOMID.\textsuperscript{6} Interleukin 1 is a key proinflammatory cytokine,\textsuperscript{14} and its major physiological regulator is IL-1 receptor antagonist, which binds to the IL-1 receptor and blocks further signaling. A recombinant form of IL-1 receptor antagonist (anakinra) has been developed and used with modest success in treating rheumatoid arthritis. Reports of complete remission of MWS following treatment with anakinra support a pivotal role for IL-1 in the treatment of clinical manifestations in patients with CIAS-1/NALP3 mutations.\textsuperscript{15}

We herein report the genotype, clinical phenotype, dermatopathologic features, and response to prolonged treatment with anakinra in 22 patients with NALP3-associated periodic fever syndromes. To our knowledge, this represents the largest series to date.

METHODS

PATIENTS

The subjects were 22 patients from 13 unrelated families who had symptoms consistent with a periodic fever syndrome in association with a mutation in exon 3 of CIAS-1/NALP3, representing all such patients in the National Amyloidosis Centre (London) database. Clinical features were recorded, and monthly blood samples were obtained for measurement of the acute-phase reactants C-reactive protein (CRP) and serum amyloid A protein (SAA) levels. Nineteen patients underwent iodine 123–labeled serum amyloid P component (SAP) scintigraphy, which is an imaging method for identifying and quantifying visceral amyloid deposits. Two children and 1 adult with clinically mild disease did not undergo scintigraphy. Fifteen patients were treated with anakinra (Kineret; Amgen Inc, Thousand Oaks, Calif), and their response was assessed clinically and by serial acute-phase protein assays. Of the other 7 patients, 2 died before therapy was available, 2 had mild disease and declined therapy, and 3 were participating in trials of experimental therapies. Skin biopsy specimens were available for review in 7 patients, which were reexamined by routine light microscopy and immunohistochemistry.

MEASUREMENT OF CRP AND SAA LEVELS

Samples were obtained monthly according to our routine clinical protocol for treatment of patients with periodic fever syndromes. Serum CRP level was determined using a highsensitivity automated microparticle-enhanced latex turbidimetric immunonassay (COBAS MIRA; Roche Diagnostics, Rotkreuz, Switzerland). The lower limit of detection was 0.2 mg/L, with interassay coefficients of variation of 4.2% at 4 mg/L and 6.3% at 1 mg/L. The SAA level was measured by latex nephelometry (BNII autoanalyzer; Dade Behring, Marburg, Germany).\textsuperscript{16} The lower limit of detection was 0.7 mg/L, with interassay coefficients of variation of 2.6% at 15 mg/L and 3.7% at 80 mg/L. Both assays were standardized using the appropriate World Health Organization criteria.\textsuperscript{17,18}

RESULTS

GENOTYPE ANALYSIS

All patients had a mutation in exon 3 of CIAS-1/NALP3. The most common variants were T348M in 7 individuals from 5 families, R260W in 9 individuals from 4 families, V198M in 3 individuals from 1 family, A439V in 2 individuals from 2 families, and G569R in 1 individual.

CLINICAL FEATURES

The clinical findings among 22 patients are summarized in Table 1. Twenty patients reported daily symptoms with a circadian rhythm that was worse in the evening. Typically, the patients would be symptom free in the morning and would develop progressive rash, fever, and arthralgia in the late afternoon to evening, accompanied by severe lethargy. Of 2 patients who denied daily symptoms, patient 20 had an episodic urticarial rash, and patient 11 had episodic symptoms of rash, leg pain, and febrile symptoms during the winter months. Nineteen patients had evidence of disease from birth; patients 7 and 12, both with NALP3 R260W, developed symptoms in adolescence; and patient 20 noted episodic urticaria in early adulthood.

Three patients had a mild rash consisting only of evanescent macules (Figure 1A), 3 patients had evanescent macules and sometimes urticaria, and the remaining 16 patients had urticarial rashes (Figure 1B and C). Most patients' rashes initially appeared as a faint macular eruption on the trunk and distal limbs, with the face tending to be spared; more typical urticarial papules and plaques developed during the course of the day. Individual lesions never lasted more than 24 hours. The rashes were minimally pruritic in 9 patients, although none showed excoriation. Severity of the rash did not correlate with other features. Patient 5 with NALP3 R260W developed a second distinct rash in her early 30s, which was clinically and histopathologically consistent with acute neutrophilic dermatosis (Sweet syndrome). This occurred on 2 occasions and resolved quickly with oral corticosteroids, which did not benefit her urticarial rash.

Nineteen patients reported frequent diffuse flulike limb aches, which sometimes localized to joints. Knees and ankles were affected most commonly, while wrists, elbows, and small joints of the hands and feet were involved infrequently. Patient 22 with NALP3 G569R had a deforming arthropathy of the knees characterized by bilateral sterile pycarthrosis. No patients had erosive arthropathy. Four patients had finger clubbing.

Twenty-one patients experienced frequent conjunctivitis (Figure 1D). Patient 22 had significant visual impair-
ment, which was partly due to glaucoma. Headaches consistent with elevated intracranial pressure were reported by 9 patients, 7 of whom had chronic papilledema.

Sensorineural deafness was present in 13 patients and was associated with all NALP3 variants except A439V. Hearing impairment was progressive and began in childhood or early adolescence; audiograms showed high-tone loss, and 8 patients used hearing aids.

The overall severity of the clinical phenotype varied from mild, intermittent, and largely cold-precipitated symptoms (ie, FCAS) in 2 patients to NOMID features in 4 patients. The latter 4 patients showed minimal abnormalities consisting of mild venular dilatation in the papillary and upper reticular dermis along with scant perivascular histiocytic and T-lymphocytic exudates identified by CD3 immunoperoxidase staining (Figure 3A). All lesional biopsy specimens showed varying degrees of vascular dilatation and swelling of endothelial cells, with margination and emigration of neutrophils together with extension of neutrophils into the interstitium in the full thickness of the dermis (Figure 3B and C). In addition, pavementing and margination of polymorphs could be seen in small vessels in the subcutis, particularly around sweat glands (Figure 3D). There was a discernible increase in perivascular T lymphocytes and histiocytes, but mast cells did not seem to be increased in numbers in lesional vs nonlesional skin. No amyloid deposition or fibrinoid necrosis was seen.

DERMATOPATHOLOGIC FEATURES

Seven patients had undergone skin biopsies, including samples from lesional and clinically uninvolved sites in 4 patients. The latter 4 patients showed minimal abnormalities consisting of mild venular dilatation in the papillary and upper reticular dermis along with scant perivascular histiocytic and T-lymphocytic exudates identified by CD3 immunoperoxidase staining (Figure 3A). All lesional biopsy specimens showed varying degrees of vascular dilatation and swelling of endothelial cells, with margination and emigration of neutrophils together with extension of neutrophils into the interstitium in the full thickness of the dermis (Figure 3B and C). In addition, pavementing and margination of polymorphs could be seen in small vessels in the subcutis, particularly around sweat glands (Figure 3D). There was a discernible increase in perivascular T lymphocytes and histiocytes, but mast cells did not seem to be increased in numbers in lesional vs nonlesional skin. No amyloid deposition or fibrinoid necrosis was seen.

RESPONSE TO ANAKINRA

Fifteen patients were treated with anakinra; 6 had received therapy for 3 years (Table 2 and Table 3). Patients were started on a regimen of 100 mg/d by subcu-
taneous injection, and the dosages were progressively reduced according to response to a maintenance schedule of 20 mg/d to 50 mg/d in all adults. The 2 children receiving treatment (patients 8 and 9) continued taking a dosage of 100 mg/d in adherence with the pediatric prescribing protocol in our institution. Serum acute-phase markers were measured serially in all patients; before anakinra therapy, the median SAA level was 99.5 mg/L (interquartile range, 24-282 mg/L), and the median CRP level was 41 mg/L (interquartile range, 29-65 mg/L) (the reference range for both proteins is <10 mg/L) for the subgroup of 15 patients who received anakinra therapy. Remarkably, all 15 treated patients achieved complete remission from rash, fever, conjunctivitis, and rheumatic aches within 12 hours of starting anakinra, 6 patients within 4 hours. Serum SAA and CRP levels normalized within 1 week in all patients, with median values of 5 mg/L and 2 mg/L, respectively. In some patients, the dosage of anakinra was progressively reduced to 50, 33, 25, and 20 mg/d every second month according to symptoms and acute-phase protein values. Clinical disease recurred in 36 to 48 hours of anakinra injections in 5 patients who briefly stopped treatment, demonstrating the necessity of daily maintenance therapy. Nephrotic syndrome remitted within 8 to 33 months in 3 patients who had AA amyloidosis, associated with regression of amyloid on serial SAP scintigraphy. A fourth treated patient with amyloidosis began taking anakinra 18 months after undergoing renal transplantation; SAP scintigraphy showed regression of amyloid from his spleen and no involvement of the graft, which has functioned well throughout. The 15 patients were treated for a median of 17.7 months (range, 1-39.1 months). There have been no adverse effects from anakinra apart from minor local stinging and erythema at the injection sites, which gradually diminished in most patients. Three adults gained substantial weight, presumably reflecting resolution of their former lifelong chronic inflammatory catabolic state.

**COMMENT**

To our knowledge, this is the largest series of patients with NALP3-associated autoinflammatory disease yet re-
ported. Remarkable findings included the spectrum of
disease from mild (FCAS type) to severe (NOMID type)
and identification of 2 patients with mild symptoms in
whom NALP3-associated autoinflammatory disease was
diagnosed only during the course of specialized investi-
gation of AA amyloidosis of uncertain origin. The his-
tological findings concur with previous reports show-
ing predominant neutrophil involvement, in contrast to
the typical lymphocytic or eosinophilic infiltrate of clas-
cic urticaria. A characteristic feature was the presence of
pavementing and emigration of neutrophils not just in
the dermis but also in the subcutis, particularly around
the capillary plexus associated with eccrine glands. The
development of Sweet syndrome in 1 patient and the de-
velopment of pyoderma gangrenosum in another pa-
tient with an
NALP3 mutation (K.S.L., unpublished data, February 2006)
places these conditions in the spectrum of
neutrophilic dermatoses, in which there is an abnor-
mally exaggerated neutrophilic response to IL-1. How-
ever, the most remarkable finding was the uniformly rapid,
complete, and sustained response to treatment with small
dosages of anakinra and the ability for this to facilitate
resolution of AA amyloidosis–associated renal dysfunc-
tion. The small dosages of anakinra required to main-
tain complete remission, as little as 0.3 mg/kg daily, fur-
ther highlight the pivotal role of excessive IL-1 production
in the pathogenesis of NALP3-associated autoinflamma-
tory disease.

Different diagnostic criteria for NALP3-associated au-
toinflammatory disease have been considered, and al-
though FCAS and NOMID in their typical forms are char-
acteristic, the daily but varying features of MWS (the clinical
diagnosis of most of the patients described herein) are less
so. The key clinical features in the present series were re-
current fever and urticarial rash, which were present most
days and were usually worst in the evenings. The rash was
not pruritic in approximately 60% of patients and was only
mildly pruritic in the remainder. Common associated symp-
toms were conjunctivitis, cold precipitation, headache that
was often worse in the morning and consistent with raised
intracranial pressure, and diffuse aching in the limbs that
localized poorly to specific structures and was often de-
scribed as flulike. Deafness was present in approximately
50% of patients, and finger clubbing was present in just
under 20% of patients. There seemed to be a dispro-
portionate number of patients with reduced fertility. The some-
times mild and nonspecific nature of symptoms is illus-
trated by the 2 patients who were initially seen with AA
amyloidosis, in whom NALP3-associated autoinflamma-
tory disease was not suspected, despite our familiarity with
it, the clinical symptoms were only recognized in retro-
spect when an NALP3 variant was identified by direct DNA
sequencing. Although it is possible that the frequency and spectrum of clinical features and severity of NALP3-associated autoinflammatory disease may ultimately be shown to be greater than presently recognized, we speculatively sequenced \textit{CIAS-1}/\textit{NALP3} with wildtype findings in 243 individuals with unexplained fevers and rashes (H.J.L., unpublished data, 2006), with the results suggesting that it is truly rare. Although there are reports of \textit{CIAS-1}/\textit{NALP3} mutation–negative children with NOMID, we have not encountered an adult patient with characteristic clinical features of MWS who does not have a mutation in this gene.

AA amyloidosis developed in more than one quarter of our cohort and has historically been a frequent cause of renal failure and death in patients with MWS. The incidence of amyloidosis in NALP3-associated autoinflammatory disease is in an order of magnitude higher than that in rheumatoid arthritis, the most common cause of AA amyloidosis in the Western world, presumably reflecting the intensity and the duration of acute-phase response in this inherited condition. AA amyloidosis deposits are derived from the circulating acute-phase reactant SAA, which is an apolipoprotein of high-density lipoprotein synthesized by hepatocytes under the transcriptional regulation of IL-1, IL-6, and tumor necrosis factor \(\alpha\). A sustained elevated circulating SAA level is a prerequisite for the development of AA amyloidosis, and although the degree by which SAA is elevated has not been formally studied (to our knowledge) in terms of its potency as a susceptibility factor, active NALP3-associated autoinflammatory disease is associated with high concentrations of this most sensitive and dynamic acute-phase protein. AA amyloidosis usually manifests as proteinuria or impaired renal function, typically leading to renal failure and death within about 10 years in the absence of effective therapy. This is illustrated by 3 of 6 patients in this series who developed end-stage renal failure before the advent of anakinra, leading to death in 2 of them. Quantitative monitoring of amyloid deposits was achieved in this cohort by serial iodine 123-

### Table 2. Response to Long-term Anakinra Therapy in 15 Patients Showing the Fall in Acute-Phase Response C-Reactive Protein (CRP) and Serum Amyloid A Protein (SAA)

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Median Pretreatment Level, mg/L</th>
<th>Maintenance Dosage of Anakinra, mg/d</th>
<th>Duration of Treatment, mo*</th>
<th>Median CRP Level on Treatment, mg/L</th>
<th>Median SAA Level on Treatment, mg/L</th>
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<td>90</td>
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<td>115</td>
<td>314</td>
<td>50</td>
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<td>11</td>
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<td>Total median</td>
<td>47</td>
<td>133</td>
<td>...</td>
<td>17.7</td>
<td>2</td>
</tr>
</tbody>
</table>

Abbreviation: Ellipses, not applicable.
*All patients had a complete clinical response.

### Table 3. Renal Function in Patients With AA Amyloidosis

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Before Anakinra Therapy</th>
<th>After Anakinra Therapy</th>
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<tbody>
<tr>
<td></td>
<td>Urinary Protein Leak, g/d</td>
<td>Serum Creatinine Level, mg/dL</td>
</tr>
<tr>
<td>4</td>
<td>10.21</td>
<td>1.10</td>
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<tr>
<td>11</td>
<td>4.60</td>
<td>0.81</td>
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<tr>
<td>12</td>
<td>10.35</td>
<td>0.90</td>
</tr>
<tr>
<td>17*</td>
<td>0.10</td>
<td>0.95</td>
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</table>

SI conversion factor: To convert serum creatinine to micromoles per liter, multiply by 88.4.
*Patient 17 underwent renal transplantation 18 months before starting therapy, and the graft function remains excellent. The other 3 patients demonstrate substantially improved proteinuria.
labeled SAP scintigraphy, and we observed regression of amyloid deposits and gradual resolution of amyloid-related nephrotic syndrome in each of 3 recent patients who received anakinra. The SAP scans also showed that graft deposits did not occur in the other anakinra-treated patient with amyloidosis who had undergone renal transplantation. Two hypospermic patients with amyloidosis fathered children after effective treatment with anakinra, possibly reflecting chance, regression of amyloid, remission of inflammation, or a direct toxic effect of abundant IL-1 on spermatogenesis. AA amyloidosis should routinely be sought in patients with inherited periodic fever syndromes by regular urinalysis, with a low threshold for proceeding to rectal or renal biopsy or, preferably, to SAP scintigraphy where available. Our findings provide compelling encouragement to offer IL-1 inhibiting therapy to patients with NALP3-associated autoinflammatory disease who develop AA amyloidosis, regardless of whether their inflammatory disease symptoms are troublesome in their own right.

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REFERENCES