ARTHRITIS & RHEUMATISM
Vol. 50, No. 2, February 2004, pp 607–612
DOI 10.1002/art.20033

Spectrum of Clinical Features in Muckle-Wells Syndrome and Response to Anakinra

Philip N. Hawkins,[1] Helen J. Lachmann,[1] Ebun Aganna,[2] and Michael F. McDermott[2]

Objective. **Mutations in the *NALP3*/*CIAS1*/*PYPAF1* gene are associated with the autoinflammatory diseases Muckle-Wells syndrome (MWS), familial cold autoinflammatory syndrome (FCAS), and neonatal-onset multisystem inflammatory disease (NOMID), which is also known as chronic infantile neurologic, cutaneous, articular (CINCA) syndrome. Molecular studies suggest that *NALP3* is involved in the processing of interleukin-1β (IL-1β), prompting us to investigate whether IL-1 blockade may be therapeutic in patients with MWS.**

Methods. **We reviewed the clinical features of 3 members of a family, all of whom had MWS associated with the *NALP3* variant V200M (also designated V198M), and evaluated the response of their inflammatory disease to treatment with the recombinant human IL-1 receptor antagonist anakinra. The subjects kept a diary of symptoms and underwent fortnightly clinical and laboratory assessments, including measurement of the serum amyloid A protein concentration.**

Results. **Each subject had fever, rashes, arthralgia, conjunctivitis, sensorineural deafness, and an intense acute-phase response characteristic of MWS. However, additional features were identified, including exacerbation of their disease by cold and neurologic manifestations, that have hitherto been described only in FCAS and NOMID, respectively. Clinical and serologic evidence of active inflammatory disease resolved rapidly and completely during treatment with anakinra.**

Conclusion. **The remarkable response of MWS to anakinra suggests that IL-1β has a fundamental role in the pathogenesis of inflammation associated with mutations in the *NALP3* gene, and supports study of IL-1 inhibition in patients with NOMID/CINCA syndrome or FCAS. The clinical features of the various syndromes associated with mutations in the *NALP3* gene may overlap to a greater extent than has previously been recognized.**

Muckle-Wells syndrome (MWS; MIM no. 191900) is a dominantly inherited autoinflammatory disease characterized by rashes, fever, arthralgia, progressive sensorineural deafness, and the frequent development of systemic AA amyloidosis. MWS is associated with heterozygous mutations in a gene variously named *NALP3*, *CIAS1*, and *PYPAF1* that encodes a protein known as NALP3 or cryopyrin, which is a member of the recently characterized pyrin superfamily of death domain–fold proteins (1–3). *NALP3* mutations were initially identified in patients with familial cold urticaria/familial cold autoinflammatory syndrome (FCAS) (1) and are also responsible for neonatal-onset multisystem inflammatory disease (NOMID), which is otherwise known as chronic infantile neurologic, cutaneous, articular (CINCA) syndrome (4–6). FCAS is characterized by urticarial skin lesions, swollen and painful joints, conjunctivitis, and fever following exposure to cold, whereas NOMID/CINCA syndrome presents very early in life with severe dermatologic, rheumatologic, and neurologic manifestations associated with intense multisystem inflammation. More than 20 *NALP3* mutations have now been identified, some of which are associated with different phenotypes in different families (1,3,7).

Studies in hereditary autoinflammatory disorders have elucidated new proteins and pathways that are involved in inflammation and apoptosis generally. Pyrin/

Supported in part by the Medical Research Council, NHS Research and Development funds, and the Special Trustees of St. Bartholomew's and the Royal London Joint Research Board.

[1]Philip N. Hawkins, PhD, FRCP, Helen J. Lachmann, MRCP: Royal Free and University College Medical School, Royal Free Hospital, London, UK; [2]Ebun Aganna, BSc, Michael F. McDermott, MRCPI: St. Bartholomew's and the Royal London School of Medicine, London, UK.

Address correspondence and reprint requests to Philip N. Hawkins, PhD, FRCP, Centre for Amyloidosis and Acute Phase Proteins, Department of Medicine, Royal Free and University College Medical School, Royal Free Hospital, Rowland Hill Street, London NW3 2PF, UK. E-mail: p.n.hawkins@rfc.ucl.ac.uk.

Submitted for publication May 14, 2003; accepted in revised form October 21, 2003.

marenostrin, the prototypic member of a new superfamily of proteins containing a characteristic 6–alpha helix death domain–related structure, now known as the pyrin domain, was identified as the product of the gene responsible for familial Mediterranean fever (FMF). The *NALP3* gene is expressed in peripheral blood leukocytes (6) and chondrocytes (4), and NALP proteins contain several distinct motifs including a nucleotide-binding site (NACHT domain), a C-terminal domain containing 7 leucine-rich repeats (LRR domain), as well as an N-terminal pyrin domain (PyD) (8–10). This nomenclature underlies the NALP terminology (NACHT, LRR, PyD). The pyrin domain of *NALP3* is thought to interact with ASC, leading to signaling of NF-κB and increased interleukin-1β (IL-1β) production (9). Up-regulation of IL-1β was reported in unstimulated monocytes obtained from a patient with NOMID/CINCA syndrome (6), and the very favorable response to an empiric therapeutic trial of a recombinant human IL-1 receptor antagonist (rHuIL-1Ra) in 2 patients under our care who had MWS complicated by AA amyloidosis further implicates IL-1 as an important mediator of inflammation in patients with *NALP3* mutations (11).

We report here a British kindred in which the *NALP3* variant V200M was associated with sustained and intense chronic inflammatory disease that had overlapping features of MWS, FCAS, and NOMID/CINCA syndrome and that responded rapidly and completely to treatment with rHuIL-1Ra.

PATIENTS AND METHODS

Patients and genotyping. The 3 patients were members of a previously described British family comprising a 42-year-old woman, her 15-year-old daughter, and her 22-year-old son (3). The diagnosis of MWS had been made many years previously, based on clinical criteria.

Genomic DNA was extracted from the blood of each subject, using Qiagen (Crawley, UK) kits (96-well format). Total RNA was extracted from the proband's daughter, using PureScript RNA isolation kits (Gentra Systems, Minneapolis, MN), with subsequent synthesis of complementary DNA (cDNA) by priming with random hexamers according to the manufacturer's instructions. Amplification of *NALP3* cDNA was performed using 9 primer pairs, and the polymerase chain reaction products were purified and sequenced on an ABI 3100 sequencer (Applied Biosystems, Foster City, CA), as previously described (3). The V200M variant detected in the proband's daughter was sought in the 2 other subjects using *Nla* III restriction fragment length polymorphism analysis (3).

Therapeutic trial of anakinra. The 3 subjects consented to undergo a 3-month therapeutic trial of anakinra, a rHuIL-1Ra (Kineret; Amgen, Cambridge, UK). The drug was self-administered by subcutaneous injection using an initial dosing schedule of 100 mg once daily, which is the dosage recommended for the treatment of rheumatoid arthritis (RA).

The response to treatment was documented by a handwritten diary of symptoms, weekly clinical consultations, and by monitoring of the serum C-reactive protein (CRP) and serum amyloid A (SAA) protein concentrations, first at weekly intervals and then at fortnightly intervals. CRP was measured using a high-sensitivity automated microparticle-enhanced latex turbidimetric immunoassay (Cobas Mira; Roche Diagnostics, Mannheim, Germany) (12). The lower limit of detection was 0.2 mg/liter, with an interassay coefficient of variation (CV) of 4.2% at 4 mg/liter and 6.3% at 1 mg/liter. SAA was measured by latex nephelometry (BNII autoanalyzer; Dade Behring Marburg, Marburg, Germany) (13). The lower limit of detection was 0.7 mg/liter, with an interassay CV of 2.6% at 15 mg/liter and 3.7% at 80 mg/liter. Both assays were standardized with the appropriate World Health Organization standards. A full blood cell count and biochemical profile were obtained at baseline and monthly thereafter. Potential adverse effects of anakinra were monitored according to the manufacturer's recommendations for patients with rheumatoid arthritis.

RESULTS

Genotype and phenotype. As reported previously (3), all 3 subjects were heterozygous for the *NALP3* variant V200M (also designated V198M) (1). An analysis of the daughter's complete cDNA identified no other sequence variants. The predominant clinical features in the 3 patients were rashes, arthralgia, fever, severe fatigue, and deafness. None of the patients had evidence of AA amyloidosis, including negative results on serum amyloid P component scintigraphy, but the phenotype was otherwise completely characteristic of MWS. Rashes resembling urticaria had occurred daily, beginning the first few weeks of life, mainly involved the trunk and limbs (Figure 1), and were frequently accompanied by conjunctivitis. Arthralgia associated with effusions was most marked in the knees and was readily exacerbated by minor trauma and exercise; the 2 adult subjects reported much less arthralgia after skeletal growth had ceased. Deafness was noted in each subject before 5 years of age and had become profound in the mother and son. The 15-year-old daughter had impaired hearing but preferred not to use hearing aids.

Additional features were present that have not previously been regarded as part of the MWS phenotype. Each subject acknowledged substantial lifelong provocation of fever, rash, conjunctivitis, and arthralgia following exposure to reduced ambient temperature, which is characteristic in FCAS, and all 3 subjects displayed short stature, mild frontal bossing of the skull,

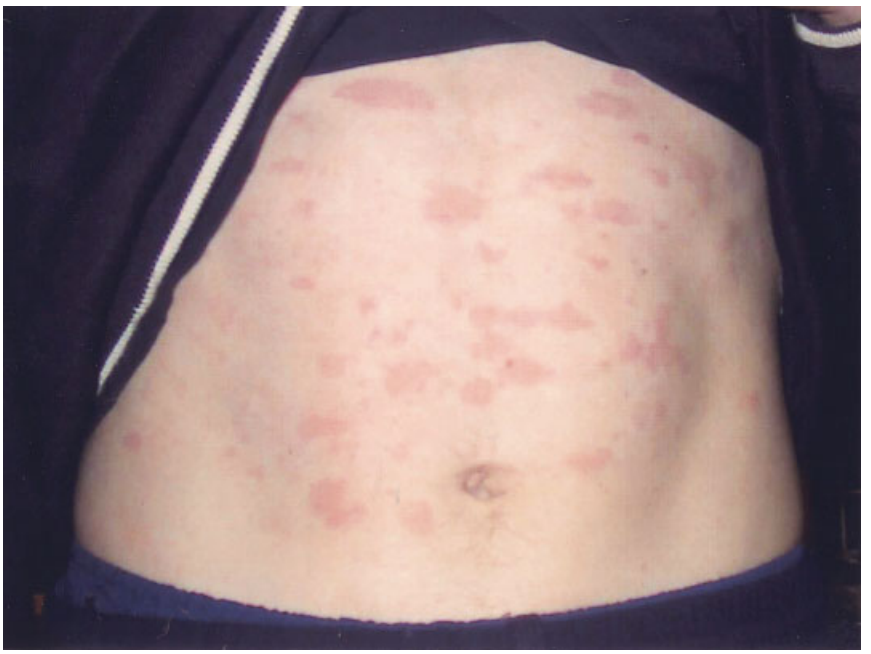

Figure 1. Rash in a 22-year-old man with Muckle-Wells syndrome associated with *NALP3* V200M variant. The rash, which had been present virtually every day of his life, resolved completely within 1 day of commencing therapy with recombinant human interleukin-1 receptor antagonist.

flattening of the nasal bridge, and papilledema, all of which are typical in NOMID/CINCA syndrome. The son had frequent early-morning headaches, consistent with raised intracranial pressure. Another intriguing feature was provocation of the inflammatory symptoms by emotional or physical stress.

Prior to treatment with anakinra, none of the subjects had received any antiinflammatory drug therapy for at least 6 months. The only abnormal laboratory findings were an intense acute-phase response, a moderate persistent neutrophilia, and anemia characteristic of chronic disease (Table 1). During the year prior to treatment, the median SAA (CRP) concentrations in the mother, son, and daughter were 141 mg/liter (58 mg/liter), 161 mg/liter (89 mg/liter), and 148 mg/liter (42 mg/liter), respectively (normal concentration <10 mg/liter for both SAA and CRP). Investigations that were performed before starting anakinra and for which results were normal or negative included biochemical profiles, serum immunoglobulin and autoantibody profiles, urinalysis, and plain radiography of the knees.

Effect of anakinra therapy. On the day that anakinra therapy was commenced, all 3 subjects had typical clinical and serologic evidence of active inflammatory disease. This evidence objectively included rash, conjunctivitis, neutrophilia, and an intense acute-phase response as evidenced by SAA concentrations of 146 mg/liter, 264 mg/liter, and 193 mg/liter in the proband, her son, and her daughter, respectively (Table 1). Re-

Table 1. Laboratory parameters in patients with Muckle-Wells syndrome before and during treatment with recombinant human interleukin-1 receptor antagonist

Patient, parameter	Baseline	Anakinra treatment		
		1 week	4 weeks	12 weeks
Mother				
Serum amyloid A, mg/liter	146	<1.0	<1.0	1.3
C-reactive protein, mg/liter	56	2.2	1.1	1.3
Hemoglobin, gm/dl	11.5		12.3	14.4
Leukocyte count, 10^9/liter	13.8		8.5	9.9
Platelet count, 10^9/liter	312		154	198
Son				
Serum amyloid A, mg/liter	264	2.8	2.3	2.2
C-reactive protein, mg/liter	129	4.1	4.3	4.3
Hemoglobin, gm/dl	12.1		13.3	14.1
Leukocyte count, 10^9/liter	13.2		6.2	5.9
Platelet count, 10^9/liter	421		231	246
Daughter				
Serum amyloid A, mg/liter	193	2.9	1.9	1.8
C-reactive protein, mg/liter	59	1.7	0.9	0.9
Hemoglobin, gm/dl	7.3		9.4	11.5
Leukocyte count, 10^9/liter	14.5		10.5	9.8
Platelet count, 10^9/liter	659		327	331

markably, in each subject all features associated with active inflammation ceased within 4 hours of administration of the first subcutaneous injection of anakinra, including complete resolution of rash. After 7 days, the plasma SAA concentration fell to <3 mg/liter in all 3 subjects and remained below this level on fortnightly followup testing, for 3 months.

In view of the completeness of the clinical response, after 2 weeks the dosage of anakinra was reduced from 100 mg/day to 50 mg/day. None of the 3 subjects has had any rash, conjunctivitis, arthralgia, or fever since commencing treatment with anakinra. The headaches experienced by the son ceased within 1 week of initiation of therapy. The bilateral knee effusions in the daughter that had prevented physical training resolved completely. The full blood cell counts improved (Table 1). There were no adverse effects from the treatment other than mild inflammation at injection sites, which decreased over time. There was no subjective change in their hearing or results of their followup fundoscopic examinations. Each subject reported a marked improvement in general well-being and has tolerated exposure to cold ambient temperature without development of any symptoms. The 15-year-old daughter has undergone menarche.

DISCUSSION

The complete and rapid clinical and serologic response to rHuIL-1Ra in Muckle-Wells syndrome confirms that IL-1β has a fundamental role in the pathogenesis of inflammation associated with mutations in the *NALP3* gene. These findings support studies of anakinra in patients with FCAS and in children with the much more severe NOMID/CINCA syndrome phenotype, given the similar nature of the underlying molecular defect in all of these entities. Therapy with anakinra has the potential to be life saving in patients with Muckle-Wells syndrome complicated by AA amyloidosis, and the response to treatment also raises the possibility that inhibition of IL-1 might be beneficial in other pyrinopathies, potentially including acute attacks of FMF itself. These studies also illustrate the potential for elucidating disease processes, using highly specific biologic drugs.

Anakinra is a recombinant nonglycosylated homolog of human IL-1Ra that competitively inhibits binding of IL-1α and IL-1β to the IL-1 receptor type 1, which is expressed in a wide variety of tissues and organs (14). Anakinra has been evaluated most extensively in patients with rheumatoid arthritis, in whom it is safe and well tolerated (15,16). IL-1, originally named endogenous pyrogen, is a key proinflammatory cytokine that has many actions, including a contribution to increased synthesis of SAA by hepatocytes during the acute-phase response.

The availability of rHuIL-1Ra enabled us to undertake an empirical therapeutic trial of this agent in 2 patients with Muckle-Wells syndrome in whom nephrotic syndrome due to AA amyloidosis had developed, and in whom many drugs had been unable to suppress their inflammatory disease and abundant production of SAA. These unrelated patients both had the *NALP3* R262W variant (also annotated as R260W) (2) and had failed to respond to treatment with colchicine, corticosteroids, chlorambucil, antihistamines, dapsone, azathioprine, mycophenolate mofetil, and infliximab. In both patients, symptoms of inflammation ceased within hours of the first injection of rHuIL-1Ra, and plasma SAA concentrations normalized within 3 days and have remained normal for 6 months (11). Even during this relatively short treatment period, the amyloid-related proteinuria level fell from 11.2 gm/day to 4.9 gm/day in one of these patients, and from 10.2 gm/day to 2.3 gm/day in the other. The glomerular filtration rate has remained normal in both patients. Anakinra is modestly effective in rheumatoid arthritis (15,16), whereas its remarkable effect in small doses in patients with Muckle-Wells syndrome indicates that IL-1β has a pivotal role in the pathogenesis of this disorder.

AA amyloidosis develops in <5% of patients with chronic inflammatory diseases overall, but it eventually occurs in approximately one-fourth of patients with MWS, reflecting the very intense and prolonged acute-phase response in this particular disorder. AA amyloid fibrils are derived from the circulating acute-phase protein SAA. This is the most responsive and dynamic marker of the acute-phase response, with the plasma concentration of SAA increasing from normal values of <10 mg/liter up to as much as 2,000 mg/liter in response to a wide range of inflammatory stimuli (17). Frequent monitoring of the plasma SAA concentration is vital in patients with AA amyloidosis in order to guide antiinflammatory therapy in a rational manner (18), and SAA is also an extremely sensitive and objective marker of disease activity in MWS. AA amyloidosis cannot occur in the absence of an acute-phase SAA response, and the rapid normalization of the SAA concentration observed in members of this family reflects the completeness of their response to anakinra therapy.

Elucidation of the molecular basis of MWS, FCAS, and NOMID/CINCA syndrome is providing insights into some of the distinctive features of these

disorders. Approximately 20 *NALP3* mutations have been identified, mostly within the NACHT domain (6,9,10). This gene is expressed in peripheral blood leukocytes, and it is possible that *NALP3* mutations lead to defective apoptosis of neutrophils, leading to their persistence and inappropriate activation. The gene is also expressed in chondrocytes, which may account for the joint pain that occurs in MWS and FCAS, and the premature patellar and long bone ossification with resultant bony overgrowth in NOMID/CINCA syndrome (5). Expression of this gene in cartilage might also account for the deafness in patients with MWS. It remains to be determined whether early and prolonged treatment with rHuIL-1Ra may prevent deafness and abnormal bone development. Induction of systemic inflammation by exposure to cold in FCAS remains intriguing and unexplained at the present time.

The fact that *NALP3* mutations cause MWS, FCAS, and NOMID/CINCA syndrome—3 diseases previously regarded as distinct—prompted us to look for clinical features that characteristically occur in FCAS and NOMID/CINCA syndrome in our patients with MWS. Inflammatory disease activity was markedly exacerbated by exposure to cold in all 3 members of this family and in both of our patients with MWS associated with *NALP3* R262W, who also responded to anakinra. Neurologic manifestations, including chronic aseptic meningitis, hearing loss, and mental retardation, are features of children with NOMID/CINCA syndrome, many of whom die before reaching adulthood. Each member of the present family had papilledema, and one experienced daily headaches with features suggestive of raised intracranial pressure, which resolved after initiation of anakinra therapy.

We suspect that the clinical features of patients with *NALP3* mutations overlap much more than has previously been recognized, and that the correlation of phenotype and *NALP3* genotype may prove to be quite broad in terms of disease characteristics, penetrance, and severity. For example, in a previous study, we identified the V200M variant in 1 of 130 healthy Caucasian controls and in 2 of 47 healthy Indian controls, and a cousin in the present family who has this variant does not have clinical evidence of MWS (3). The same variant (annotated as V198M) was reported by Hoffman et al to be associated with FCAS (1). Certain variants such as D305N (also annotated as D303N) underlie NOMID/CINCA syndrome in some individuals but underlie MWS in others (2,4,6), and R262W (also annotated as R260W) can be associated with MWS and FCAS in different families. These diseases are sometimes caused by de novo mutations in the *NALP3* gene, and, indeed, this is usually the case among patients with NOMID/CINCA syndrome, who typically are too sick to parent families of their own. A lack of family history should therefore not discourage analysis of this gene in any patient with features that are suggestive of this spectrum of disease, nor should a lack of periodicity of such symptoms, which tend to be much more constant in MWS and NOMID/CINCA than in other inherited periodic fever syndromes such as FMF.

REFERENCES

1. Hoffman HM, Mueller JL, Broide DH, Wanderer AA, Kolodner RD. Mutation of a new gene encoding a putative pyrin-like protein causes familial cold autoinflammatory syndrome and Muckle-Wells syndrome. Nat Genet 2001;29:301–5.
2. Dode C, Le Du N, Cuisset L, Letourneur F, Berthelot JM, Vaudour G, et al. New mutations of CIAS1 that are responsible for Muckle-Wells syndrome and familial cold urticaria: a novel mutation underlies both syndromes. Am J Hum Genet 2002;70: 1498–506.
3. Aganna E, Martinon F, Hawkins PN, Ross JB, Swan DC, Booth DR, et al. Association of mutations in the NALP3/CIAS1/PYPAF1 gene with a broad phenotype including recurrent fever, cold sensitivity, sensorineural deafness, and AA amyloidosis. Arthritis Rheum 2002;46:2445–52.
4. Feldmann J, Prieur A-M, Quartier P, Berquin P, Certain S, Cortis E, et al. Chronic infantile neurological cutaneous and articular syndrome is caused by mutations in CIAS1, a gene highly expressed in polymorphonuclear cells and chondrocytes. Am J Hum Genet 2002;71:198–203.
5. Prieur AM, Griscelli C, Lampert F, Truckenbrodt H, Guggenheim MA, Lovell DJ, et al. A chronic, infantile, neurological, cutaneous and articular (CINCA) syndrome: a specific entity analysed in 30 patients. Scand J Rheumatol Suppl 1987;66:57–68.
6. Aksentijevich I, Nowak M, Mallah M, Chae JJ, Watford WT, Hofmann SR, et al. De novo CIAS1 mutations, cytokine activation, and evidence for genetic heterogeneity in patients with neonatal-onset multisystem inflammatory disease (NOMID): a new member of the expanding family of pyrin-associated autoinflammatory diseases. Arthritis Rheum 2002;46:3340–8.
7. Hull KM, Shoham N, Chae JJ, Aksentijevich I, Kastner DL. The expanding spectrum of systemic autoinflammatory disorders and their rheumatic manifestations. Curr Opin Rheumatol 2003;15: 61–9.
8. Martinon F, Hofmann K, Tschopp J. The pyrin domain: a possible member of the death domain-fold family implicated in apoptosis and inflammation. Curr Biol 2001;10:R118–20.
9. Tschopp J, Martinon F, Burns K. NALPs: a novel protein family involved in inflammation. Nat Rev Mol Cell Biol 2003;4:95–104.
10. Gumucio DL, Diaz A, Schaner P, Richards N, Babcock C, Schaller M, et al. Fire and ICE: the role of pyrin domain-containing proteins in inflammation and apoptosis. Clin Exp Rheumatol 2002;4 Suppl 26:S45–53.
11. Hawkins PN, Lachmann HJ, McDermott MF. Interleukin-1-receptor antagonist in Muckle-Wells syndrome. N Engl J Med 2003;348: 2583–4.
12. Eda S, Kaufmann J, Molwitz M, Vorberg E. A new method of

measuring C-reactive protein, with a low limit of detection, suitable for risk assessment of coronary heart disease. Scand J Clin Lab Invest 1999;230:32–5.
13. Ledue TB, Weiner DL, Sipe JD, Poulin SE, Collins MF, Rifai N. Analytical evaluation of particle-enhanced immunonephelometric assays for C-reactive protein, serum amyloid A and mannose-binding protein in human serum. Ann Clin Biochem 1998;35: 745–53.
14. Hannum CH, Wilcox CJ, Arend WP, Joslin FG, Dripps DJ, Heimdal PL, et al. Interleukin-1 receptor antagonist activity of a human interleukin-1 inhibitor. Nature 1990;343:336–40.
15. Nuki G, Bresnihan B, Bear MB, McCabe D. Long-term safety and maintenance of clinical improvement following treatment with anakinra (recombinant human interleukin-1 receptor antagonist) in patients with rheumatoid arthritis: extension phase of a randomized, double-blind, placebo-controlled trial. Arthritis Rheum 2002;46:2838–46.
16. Bresnihan B, Alvaro-Gracia JM, Cobby M, Doherty M, Domljan Z, Emery P, et al. Treatment of rheumatoid arthritis with recombinant human interleukin-1 receptor antagonist. Arthritis Rheum 1998;41:2196–204.
17. Wilkins J, Gallimore JR, Tennent GA, Hawkins PN, Limburg PC, van Rijswijk MH, et al. Rapid automated enzyme immunoassay of serum amyloid A. Clin Chem 1994;40:1284–90.
18. Gillmore JD, Lovat LB, Persey MR, Pepys MB, Hawkins PN. Amyloid load and clinical outcome in AA amyloidosis in relation to circulating concentration of serum amyloid A protein. Lancet 2001;358:24–9.