In vivo regulation of interleukin 1β in patients with cryopyrin-associated periodic syndromes

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IL-1α and β, which were originally described as leukocytic pyrogens (1), are important regulators of the response to tissue damage and infections and mediate symptoms of fever, fatigue, pain, arthritis, and the hepatic acute phase responses including synthesis of C-reactive protein (CRP) and serum amyloid A protein (SAA) (2). Although studies using recombinant IL-1 in cancer patients confirmed the causative role of IL-1 for many of these symptoms (3), its direct investigation in man is hampered by the inability to detect IL-1 in biological fluids. cryopyrin-associated periodic syndromes (CAPS) is a clinical disease syndrome resulting from heterozygous gain-of-function mutations in NLRP3, the gene encoding cryopyrin. These mutations are supposed to promote release of IL-1, thereby providing an excellent paradigm for studying human IL-1 in vivo (4, 5). CAPS patients present with a spectrum of autoinflammatory diseases (6–11) involving almost all organ systems. NLRP3 mutations result in overactivation of caspase 1, the enzyme which cleaves the precursors of IL-1β, IL-18, and IL-33, members of the IL-1 family of cytokines, into their active forms (12). Although pro–IL-1α is not a substrate of caspase 1, recent studies in mice indicate that secretion of biologically active IL-1β requires functional NLRP3 (13) and activated caspase-1 (14). The recombinant IL-1 receptor antagonist (IL-1Ra) anakinra and the IL-1 receptor type I (IL-1RI) fusion protein rilonacept (IL-1 trap) have both induced clinical response in patients with CAPS. The investigation of interleukin 1β (IL-1β) in human inflammatory diseases is hampered by the fact that it is virtually undetectable in human plasma. We demonstrate that by administering the anti–human IL-1β antibody canakinumab (ACZ885) to humans, the resulting formation of IL-1β–antibody complexes allowed the detection of in vivo–produced IL-1β. A two-compartment mathematical model was generated that predicted a constitutive production rate of 6 ng/d IL-1β in healthy subjects. In contrast, patients with cryopyrin–associated periodic syndromes (CAPS), a rare monogenetic disease driven by uncontrolled caspase-1 activity and IL-1 production, produced a mean of 31 ng/d. Treatment with canakinumab not only induced long-lasting complete clinical response but also reduced the production rate of IL-1β to normal levels within 8 wk of treatment, suggesting that IL-1β production in these patients was mainly IL-1β driven. The model further indicated that IL-1β is the only cytokine driving disease severity and duration of response to canakinumab. A correction for natural IL-1 antagonists was not required to fit the data. Together, the study allowed new insights into the production and regulation of IL-1β in man. It also indicated that CAPS is entirely mediated by IL-1β and that canakinumab treatment restores physiological IL-1β production.

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response in CAPS, demonstrating that signaling via the IL-1RI is crucial for the pathogenesis of CAPS (15–17). This strongly implies that neither IL-18 nor IL-33 plays significant roles in the disease, as neither of these two cytokines signals via the IL-1RI, and suggests that the disease is caused by overproduction of IL-1. By administering the human anti–IL-1β antibody canakinumab to CAPS patients, we provide evidence in this paper that IL-1β is pivotal in the pathogenesis of CAPS. Treatment with the antibody allowed the detection of IL-1β and the creation of a mathematical model, which indicates that the in vivo production rate of IL-1β is fivefold higher in CAPS as compared with healthy subjects. Furthermore, in vivo IL-β production could be completely restored in these patients after canakinumab treatment.

RESULTS AND DISCUSSION

Four patients with active disease each received an i.v. dose of 10 mg/kg canakinumab. Within 1 d, their urticarial rashes had disappeared, and a complete clinical response was achieved within 1 wk. CRP and SAA (Fig. 1 A), as well as plasma levels of IL-6 and IL-1Ra, returned to their normal ranges (Fig. 1 B), whereas levels of IL-1α and TNF-α did not change (Fig. 1 C). Sensitive markers for neutrophil (S100A12) and monocyte/macrophage activation (S100A8/9) (18) showed a rapid decline (Fig. 1 D) along with a normalization of neutrophil counts (Fig. 1 F). Levels of soluble IL-1RII in serum were within the normal range and showed no change after treatment (Fig. 1 E). Canakinumab induced long-lasting complete clinical response; the median time until redosing after relapse was 185 d (Table I). Patients were retreated i.v. with 1 mg/kg canakinumab, resulting in clinical remissions for a median of 90.5 d. Three further patients were enrolled into the study, and all seven patients received a fixed 150-mg s.c. dose with repeat treatment on clinical disease flare. The median treatment duration in the study was 26.5 mo (range 13.5–28.5) and the median duration of clinical remission after each 150-mg s.c. dose was 127 d (range 55–230). Together, these data indicate that CAPS is solely driven by IL-1β.

It appears that time until relapse is a function of the duration of IL-1β neutralization. To study this hypothesis, a mathematical model was developed with the aim of gaining insights into the production of IL-1β in vivo and to predict the time until disease relapse. This was possible because IL-1β, which was undetectable in sera of patients at baseline (assay detection

![Figure 1](https://example.com/figure1.png)

Figure 1. Acute phase response after treatment with canakinumab. (A–E) Data for four patients treated i.v. with 10 mg/kg canakinumab as mean ± SEM. A, SAA and CRP; B, IL-6 and IL-1Ra; C, TNF-α and IL-1α; D, S100 A12 and A8/9; E, soluble IL-1RII (individual data and mean ± SEM). (F) Neutrophil (squares) and lymphocyte (triangle) counts for these patients for the treatment periods at 10 mg/kg i.v. (a), 1 mg/kg i.v. (b), and 150 mg s.c. (c) for treatment days 1 (predose), 2 (24 h after dose), 3 (48 h after dose), and 8 (268 h after dose).
Table I. Time until redosing in days after different doses and routes of administration of canakinumab

| Patient | 10 mg/kg i.v. | 1 mg/kg i.v. | First 150 mg | Second 150 mg | Third 150 mg | Fourth 150 mg | Fifth 150 mg | Sixth 150 mg |
|---------|---------------|--------------|--------------|---------------|--------------|---------------|--------------|--------------|
|         | s.c.          | s.c.         | s.c.         | s.c.          | s.c.         | s.c.          | s.c.         | s.c.         |
| 1       | 168           | 90           | 120          | 119           | 112          | 133           | 113a         | n.a.         |
| 2       | 189           | 91           | 98           | 133           | 126          | 132           | 90a          | n.a.         |
| 3       | 203           | 77           | 133          | 140           | 132          | 134a          | n.a.         | n.a.         |
| 4       | 182           | 98           | 118          | 141           | 132          | 127a          | n.a.         | n.a.         |
| 5       | n.a.          | n.a.         | 187          | 156           | 161a         | n.a.          | n.a.         | n.a.         |
| 6       | n.a.          | n.a.         | 91           | 84            | 98           | 78            | 55           | 85           |
| 7       | n.a.          | n.a.         | 168          | 230a          | n.a.         | n.a.          | n.a.         | n.a.         |

Patients 1–4 were initially treated with 10 mg/kg and 1 mg/kg i.v. and subsequently with fixed doses of 150 mg s.c. Patients 5–7 did not receive i.v. dosing but received fixed doses of 150 mg s.c. Because of difficulties being compliant with study visit requests, patient 6 withdrew consent after the sixth dose of 150 mg canakinumab s.c. The patient was subsequently treated with anakinra. n.a., not applicable.

aAfter this cycle, patients were rolled over into the subsequent study, in which they were treated with a fixed dosing regimen of 150 mg s.c. every 8 wk.
membrane bound, in the plasma or in the tissues. Collectively, the amount of IL-1β production in vivo alone appears responsible for the severity of the disease and variable duration of remission after administration of canakinumab treatment.

IL-1β has been shown to stimulate its own production in vitro (21). We looked for a similar positive-feedback loop in vivo by comparing the production of IL-1β in CAPS with healthy subjects and found that complexed IL-1β levels were much higher for CAPS patients over the first 8 wk after canakinumab treatment (Fig. 4 A); thereafter they were similar. This feedback mechanism was modeled by including a control loop whereby IL-1β stimulates its own production. Canakinumab, by suppressing free IL-1β, appears to disrupt this feedback mechanism, reducing IL-1β production to a normal and constitutive and, hence, IL-1β-independent rate (Fig. 4 B). This constitutive production of IL-1β was calculated to be 6 ng/d for both diseased and healthy subjects, whereas CAPS patients produced an additional 25 ng/d of IL-1β. Thus, the in vivo production of IL-1β in CAPS of 31 ng/d is about fivefold higher than in healthy subjects and is IL-1β dependent. This finding is consistent with the observations that anakinra treatment decreased in vitro–produced IL-1β in CAPS patients (16, 21) and that baseline IL-1β messenger RNA (mRNA) levels were higher in CAPS than in healthy subjects (Fig. 4 C). Furthermore, IL-1β mRNA expression in CAPS, but not in healthy subjects, declined within

Figure 2. Structure of the PK–biomarker–symptom model. (A) canakinumab is injected into the plasma compartment and then permeates and distributes to a peripheral (tissue) compartment, where it can bind IL-1β. Unbound IL-1β in the tissue stimulates production of CRP and SAA and an increased probability of a disease flare. The model was fitted to data from the first four patients for canakinumab pharmacokinetics (B), total IL-1β in plasma (C), and the suppression profile for free IL-1β in the peripheral (black) and central (red, dashed) compartments (D). Also fitted were data for the clinical response (1, flare; 0, remission; E), CRP (F), and SAA (G). Observed data for each patient are shown as colored circles. Straight lines indicate model-derived data. Each of the four MWS patients initially received 10 mg/kg canakinumab i.v., inducing complete clinical remission. After the first relapse, they received 1 mg/kg i.v., followed at each subsequent relapse by 150-mg s.c. injections. Beyond the data, the model was used to predict the effect of an 8-wk regimen of s.c. doses of 150 mg.
The similarities in the kinetics of S100A8/9 and IL-1β suggest that S100A8/9 may serve as a sensitive marker for subclinical inflammation in CAPS (18). Whether IL-1β stimulates the production of S100A8/9 or S100A8/9 stimulates the production of IL-1β has not yet been fully elucidated. Although IL-1β has been shown to induce S100A8/9 production in human monocytes in vitro (22), engagement of TLR-4 by S100A8 stimulated the production of IL-1β (23).

A simpler nonlinear mixed effect pharmacokinetic (PK) flare probability model was created (Table S2) to identify a dosing regimen that should keep IL-1β production below the threshold associated with clinical evidence of CAPS. This model established an effective inhibition constant, $K_i$, describing the concentration at which there is a 50:50 probability of a flare event, and a Hill coefficient. The Hill coefficient, through the logit transformation, approximates the inverse of

Figure 3. A prediction check of the model. Simulations were performed for each of the three patients enrolled to the study who were treated only with 150 mg canakinumab injected s.c. Predictions from the model were then compared with the observed data, taking account of their specific bodyweights. Two curves are presented in each case: the dashed line is the prediction check using the parameters that described the data for patients 1–4, and the solid line is that given by the model after adjustment to the new patient. The columns show the comparisons for each patient, with A, D, G, J, and M showing patient 5 and so on for patients 7 and 6. The top row (A–C) compares the model prediction of canakinumab pharmacokinetics with the observed concentrations. The next row (D–F) shows the total captured IL-1β. G–I shows CRP, J–L shows SAA, and the final row (M–O) shows the observed flare (1, flare; 0, remission) overlaid with the model-predicted probability of flare.
the variance of a Gaussian distribution of canakinumab concentrations over which patients transition from a remission to a flare state (a Hill coefficient of 4.22 is, therefore, a variance of 0.24 and a standard deviation of 0.49, such that the 5–95% probability interval for canakinumab concentrations runs from 0.56 to 2.3 µg/ml, with 50:50 at 1.13 µg/ml, for a typical average patient). The model fitted the time of flare and the need for redosing very well. Across all observed events, patients were retreated at a mean of 69% probability of flare. To deduce a dose and regimen for preventing flare, Monte Carlo simulations were run using 1,000 replicates of the seven-patient database. The most practical posology was suggested to be 150 mg s.c. every 8 wk to keep patients flare free. This regimen has been applied to a randomized placebo-controlled study with a total of 31 patients. Data show that within a period of 24 wk, all patients randomized

Figure 4. IL-1β production in CAPS patients as compared with healthy controls. (A) Total IL-1β levels in plasma for four CAPS patients (red) and six healthy volunteers (blue) after 10 mg/kg canakinumab i.v. (B) Model-derived estimation of IL-1β production per day in those subjects. (C) Quantitative mRNA expression for IL-1β in whole blood of seven patients and seven healthy subjects before canakinumab treatment. +, P = 0.0498. Horizontal lines indicate means of seven data points. (D) mRNA expression levels for IL-1β in four CAPS patients (left) and seven healthy volunteers (right) at baseline and 24 h after infusion of 10 mg/kg canakinumab. +, P = 0.0144 as compared with baseline (paired Student’s t test). (E) mRNA IL-1β expression as a function of doses of 10 mg/kg i.v., 1 mg/kg i.v., and 150 mg s.c. shown for each cycle before dose and 24 h after dose. Note that only four CAPS patients underwent the first two cycles, whereas data show all seven patients for the 150-mg s.c. cycle. +, first cycle, P = 0.0144; *, second cycle, P = 0.0088; **, third cycle, P = 0.0359 (paired Student’s t test for 24 h vs. before dose).
to canakinumab remained disease free, whereas 81% of patients on placebo flared (24).

In summary, a targeted medicine approach using the anti–IL-1β antibody canakinumab in the rare monogenetic disease CAPS, along with data generated in healthy human subjects, has generated novel insights into in vivo IL-1β regulation. Modeling and simulation data indicated that the majority of elevated IL-1β in CAPS is produced by its own production and is completely restored after canakinumab treatment. Constitutive IL-1β in healthy subjects is IL-1β independent and not targeted by canakinumab. Further evolution of this mechanism-based concept should also allow new insights into more heterogeneous and complex chronic inflammatory diseases.

MATERIALS AND METHODS

Patients and clinical study. Canakinumab was produced and initially characterized as previously described (25). An open-label study (CACZ885A2102, an open-label single-center phase I/IIa dose titration study of canakinumab [IL-1β monoclonal antibody] to assess the clinical efficacy, safety, pharmacokinetics, and pharmacodynamics in patients with NALP3 mutations) was conducted at the National Amyloidosis Centre (Royal Free and University College Medical School, London, UK) and approved by the local Ethics Committee and UK Health Authority (ClinicalTrials.gov Identifier: NCT00487708). Patients with proven NLRP3 mutation, clinical symptoms consistent with CAPS, and a need for treatment were enrolled. Patient details are given in the supplemental text. Canakinumab was administered as 10 mg/kg i.v. to the first four patients, and time until redosing after clinical relapse was recorded. At relapse, patients were retreated with 1 mg/kg i.v. and again monitored until the next flare became evident. From then on, patients received 150 mg canakinumab s.c. at each relapse. Three more patients were enrolled and were treated from the start with 150 mg canakinumab s.c. and redosed at each relapse. Complete remission was defined as absence of symptoms of skin rash, absence of joint or muscle pain, improvement of arthralgia, normal serum values of CRP and SAA (<10 mg/liter), normal temperature (<37.5°C), and normal leukocyte count. Relapse or complete remission was defined as reappearance of symptoms of skin rash, reappearance of joint or muscle pain, reappearance of eye discomfort or redness, reappearance of fatigue or malaise, or reappearance of fever or chills (at least two symptoms were required along with biotin-labeled canakinumab. Bound biotinylated canakinumab was captured from human serum by affinity chromatography using a Protein G column. The mean retention of IgG was calculated to be 97% of the total load in five experiments. Eluted free IL-1β was then measured using the Quantikine immunoassay.

Determination of canakinumab and IL-1β in plasma. Levels of canakinumab in human serum were determined using a competitive ELISA. A purified sheep antidiotypic anti–canakinumab antibody, generated in house, was immobilized on microtiter plates and serum samples were incubated in parallel with biotinylated canakinumab. Bound biotinylated canakinumab was detected by horseradish peroxidase–conjugated streptavidin with O-phenylenediamine dihydrochloride as substrate. The lowest level of detection was 42.6 pg/ml, the intraday accuracy was 89.1–117%, and the interday accuracy was 32.5–39%. The limit of detection was 0.1 pg/ml in human serum. The lower limit of detection was 0.7 mg/liter, with an interassay coefficient of variation of 4.2% at 4 mg/liter and 6.3% at 1 mg/liter. SAA was measured in serum by latex nephelometry (BNII analyzer; Dade Behring). The lower limit of detection was 0.7 mg/liter, with an interassay coefficient of variation of 2.6% at 15 mg/liter and 3.7% at 80 mg/liter. Standardization of both CRP and SAA assays is based on the respective World Health Organization International Reference Standards.

Mathematical models. Two mathematical models were used to analyze the data from the Muckle-Wells patients, full details of which are given in the supplemental text. In the mechanism-based model, two binding reactions were written for canakinumab binding IL-1β, one for the plasma compartment and the other for the peripheral tissue interstitium. These were represented as differential equations. Hyperbolic equations of the Hill form were used for CRP, SAA, absolute neutrophil count, and the probability of flare, parameterized with stimulation constants, Ks, and Hill coefficients. They were all controlled by the concentration of IL-1β in the peripheral compartment. A first-order rate constant kD was used to describe the absorption of drug from the subcutaneous injection site. PS was the permeability–surface area coefficient for exchange of monoclonal antibody and IL-1β–antibody complex between the plasma and tissue. PSs was the same for the ligand. Vr and Vo were the volumes for the systemic circulation and peripheral compartments. CLD, CLs, and CLI were the clearances for drug, ligand, and drug–ligand complex. Rsadj was that part of the rate of ligand input (i.e., the expression level of IL-1β that was independent of IL-1β–stimulated positive feedback (i.e., was dependent on other processes). Rsadj was the rate of IL-1β input dependent on IL-1β receptor modulation. KIr was the stimulation constant for the concentration of free IL-1β in the peripheral compartment affecting the modulator system. ki was the turnover rate constant for the modulator system, explaining delays between changes in the driving force, IL-1β, and observed responses. Although implemented in this model as a direct feedback of IL-1β stimulating its own production, it is realized that there may be a chain or network of interlinked responses resulting in the up-regulation of the cytokine, perhaps including some of those measured. However, without serial data on network entities, it is not possible to deduce the structure and dependencies; therefore, the most parsimonious model representation was chosen.

The simpler PK-flare model specified a two-compartment pharmacokinetic model for canakinumab, linked directly to a probability function for the reoccurrence of disease symptoms. The probability function was a transformation of the statistical logistic equation into the form familiar to pharmacologists, the hyperbolic Hill equation. Beyond the conventional pharmacokinetic parameters, which are the same as those for the drug in the mechanistic binding model, the Hill equation specifies an inhibition constant, Ki, which is the concentration of canakinumab where there is a 50/50 probability of a flare occurring. The variance describing the range of canakinumab concentrations over which the flare probability changes from zero to one is the inverse of the Hill coefficient.

Real-time RT-PCR of IL-1β. Peripheral blood samples were collected into Paxgene Blood RNA tubes (PreAnalytiX). Samples were stored at −80°C until analysis. Total RNA was extracted according to the manufacturer’s instructions (QIAGEN). The integrity of the RNA samples was confirmed by 1% agarose gel electrophoresis, and the RNA concentration was determined spectrophotometrically. Real-time PCR was conducted using a 7900HT Sequence Detection System (SDS) and TaqMan assays (Applied Biosystems; Table I). For each measurement, an aliquot of ~1 ng cDNA template was amplified. The following amplification conditions were used: 50°C for 2 min, 95°C for 10 min, and then 40 cycles of 95°C for 15 s, followed by 60°C for 1 min each. The fluorescence signals were

Normal ranges for healthy volunteers given by the manufacturer are the following: IL-6, 1.5 (0.4–9.9) pg/ml; sIL-1Ra, 230 (15–1,062) pg/ml; TNF-α, 1.0 (0.6–2.1) pg/ml in EDTA plasma; IL-1α, 3.9 (0.9–37.9) pg/ml (in serum); and sIL-1RII, 11 (6–21) ng/ml. S100 proteins were measured as previously described (26). The normal upper limit of A8/9 is 450 mg/ml and of S100A12 is 150 mg/ml in serum. CRP was measured in serum using a high-sensitivity automated microparticle–enhanced latex turbidimetric immunoassay (COBAS MIRA; Roche). The lower limit of detection was 0.2 mg/liter with an interassay coefficient of variant of 4.2% at 4 mg/liter and 6.3% at 1 mg/liter. SAA was measured in serum by latex nephelometry (BNII analyzer; Dade Behring). The lower limit of detection was 0.7 mg/liter, with an interassay coefficient of variant of 2.6% at 15 mg/liter and 3.7% at 80 mg/liter. Standardization of both CRP and SAA assays is based on the respective World Health Organization International Reference Standards.

Biomarkers. The proteins IL-6, sIL-1Ra, TNF-α, IL-1α, and sIL-1RII were measured in plasma samples using kits obtained from R&D Systems.
processed using the SDS software (version 2.2). The cycle at which the fluorescence from a sample crosses the threshold is called the cycle threshold (Ct). The measured Ct value of the IL-1β transcript (TaqMan Assay Identifier Hs01555413_m1) was subtracted by the geometric mean of the Ct values of three reference genes, ACTG1 (TaqMan Assay Identifier Hs02340971_gH), GAPDH (TaqMan Assay Identifier Hs99999905_m1), and TINF2 (TaqMan Assay Identifier Hs00173291_m1) in the same sample to normalize between different samples. The samples were measured in duplicates and the averaged results were expressed as marker abundance normalized to the geometric mean of the three reference genes 2^(-ΔCt).

Online supplemental material. Supplemental text shows patient histories and describes in detail the derivation of pharmacokinetic and pharmacodynamic equations to build the model. Fig. S1 shows quantitative mRNA data for IL-1B as a function of time over 1 yr for individual patients. Table S1 shows the parameter values for the mechanistic pharmacokinetic, biomarker, and clinical response model. Table S2 shows the population parameter values for the PK-flare model. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20082481/DC1.

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