The T348M mutated form of cryopyrin is associated with defective lipopolysaccharide-induced interleukin 10 production in CINCA syndrome


The term autoinflammatory disease has been proposed to describe a group of disorders characterised by attacks of seemingly unprovoked inflammation without increased levels of autoantibodies or increased numbers of autoreactive T cells. Such inflammatory conditions are often associated with mutations of genes of the pyrin superfamily. For instance, mutations in cryopyrin (CIAS1, NALP3, PYPAF1) have been found in about 50% of patients with CINCA syndrome. These patients are characterised by neonatal onset of cutaneous symptoms, chronic meningitis, and joint manifestations with recurrent fever and inflammation. Despite the description of several mutations within the cryopyrin gene, it remains unclear how the resulting amino acid changes modify the function of this protein and why inflammation develops under these conditions. A recent study demonstrated increased spontaneous interleukin (IL) 1 production by macrophages expressing the R260W mutated form of cryopyrin.

METHODS AND RESULTS
We identified a patient with CINCA syndrome who had a T348M mutation of the cryopyrin gene using genomic DNA extracted from whole blood, as described previously. Because cryopyrin is largely expressed in monocytes and neutrophils, we performed functional in vitro tests using blood leucocytes of this patient (table 1) at three time points: A. medium inflammatory activity (9.59×10⁶ blood neutrophils/l); B. high inflammatory activity (14.95×10⁶/l); and C. low inflammatory activity (1.09×10⁵/l).
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Cytokines were measured in the PBMC culture supernatants using a Cytometric Bead Array Assay (BD Biosciences), according to the manufacturer's instructions. In these data suggest that LPS-mediated but not PHA-mediated inflammatory condition of the patient. Taken together, these data suggest that LPS-mediated but not PHA-mediated inflammatory condition of the patient (time points A, B, and C, see text). The horizontal dashed lines represent the mean cytokine levels generated by normal PBMCs after stimulation with LPS and PHA (n = 4; means (SEM) were as follows: LPS-induced IL10: 666 (142) pg/ml; PHA-induced IL10: 621 (157) pg/ml; LPS-induced IL1: 2108 (521) pg/ml; PHA-induced IL1: 40 (29) pg/ml).

Figure 1 T348M cryopyrin-expressing PBMCs generated IL1 but not IL10 upon LPS stimulation. The same cells, however, produced IL10 after PHA stimulation (for culture conditions, see text). The lack of IL10 production after LPS stimulation did not appear to be dependent on the inflammatory condition of the patient (time points A, B, and C, see text). T348M cryopyrin-expressing PBMCs, but not normal PBMCs, generate large amounts of IL1 upon PHA stimulation. The horizontal dashed lines represent the mean cytokine levels generated by normal PBMCs after stimulation with LPS and PHA (n = 4; means (SEM) were as follows: LPS-induced IL10: 666 (142) pg/ml; PHA-induced IL10: 621 (157) pg/ml; LPS-induced IL1: 2108 (521) pg/ml; PHA-induced IL1: 40 (29) pg/ml).

inflammatory activity (5.47 x 10^6/l). The patient did not receive immunosuppressive drug treatment except at time point C (300 mg infliximab).

Earlier work suggested a defect in apoptosis mechanisms associated with mutations of the cryopyrin gene.1 We excluded an intrinsic defect of spontaneous neutrophil apoptosis associated with the T348M mutation using methods previously described (data not shown).4,5 We then tested the production of cytokines of peripheral blood mononuclear cells (PBMCs, 1 x 10^6/ml) after phytohaemagglutinin (PHA, 10 μg/ml) and lipopoly saccharide (LPS, 10 ng/ml) stimulation (24 hour cultures, 96 well plates, triplicate cultures).4 Cytokines were measured in the PBMC culture supernatants using a Cytometric Bead Array Assay (BD Biosciences), according to the manufacturer's instructions. In normal PBMCs both stimuli induced high levels of IL10 (fig 1). In contrast, only PHA but not LPS induced significant levels of IL10 in T348M cryopyrin-expressing PBMC. The defect in LPS-induced IL10 production was not dependent on the inflammatory condition of the patient. Taken together, these data suggest that LPS-mediated but not PHA-mediated IL10 production requires functionally active cryopyrin.

The defect in LPS-induced IL10 production was associated with a particularly high susceptibility to generate IL1 after PHA activation (fig 1). Normal PBMCs did not generate significant amounts of IL1 in this system. Moreover, and in contrast with R260W macrophages,6 T348M PBMCs demonstrated no, or only marginal, IL1 production in the absence of stimulation. Thus, it is likely that increased IL1 generation particularly occurs after T cell activation, which may result from defective tolerance mechanisms due to decreased IL10 generation upon exposure to microbial antigens, in our patient. Based on these data, we successfully treated our patient with an IL1 receptor antagonist (data not shown). Similarly, IL1 antagonism proved to be useful in two patients with the R260W cryopyrin variant.7

DISCUSSION

As far as we know, this is the first study providing direct evidence that microbial antigens may trigger an abnormal inflammatory response in an autoinflammatory disease. In contrast, a primary defect of apoptosis, which has been suggested in earlier studies, was, at least in T348M cryopyrin-expressing neutrophils, not seen. Additional investigations should be performed to evaluate the functional consequences of other known mutations in the genes of the pyrin superfamily. Moreover, further work is required to define the exact hierarchy of signalling events after LPS stimulation to define the place of cryopyrin in this pathway, including its distal molecular targets.

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Authors’ affiliations

T Bihl, M K Boettger, M Seitz, P M Villiger, T Bihl, M K Boettger, M Seitz, P M Villiger, Department of Rheumatology/Clinical Immunology/Allergology, Inselspital, University of Bern, Bern, Switzerland

E Vassina, H U Simon, Department of Pharmacology, University of Bern, Bern, Switzerland

R Goldbach-Mansky, National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH, Bethesda, MD, USA

Correspondence to: Professor H U Simon, Department of Pharmacology, University of Bern, Friedbuehlstrasse 49, CH-3010 Bern, Switzerland, hus@pki.unibe.ch

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