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IL-Converting Enzyme/Caspase-1 Inhibitor VX-765 Blocks the Hypersensitive Response to an Inflammatory Stimulus in Monocytes from Familial Cold Autoinflammatory Syndrome Patients

Jeffrey H. Stack,* Kevin Beaumont,[1]* Paul D. Larsen,* Kimberly S. Straley,* Greg W. Henkel,* John C. R. Randle,* and Hal M. Hoffman[†]

Familial cold autoinflammatory syndrome (FCAS) and the related autoinflammatory disorders, Muckle-Wells syndrome and neonatal onset multisystem inflammatory disease, are characterized by mutations in the CIAS1 gene that encodes cryopyrin, an adaptor protein involved in activation of IL-converting enzyme/caspase-1. Mutations in cryopyrin are hypothesized to result in abnormal secretion of caspase-1-dependent proinflammatory cytokines, IL-1β and IL-18. In this study, we examined cytokine secretion in PBMCs from FCAS patients and found a marked hyperresponsiveness of both IL-1β and IL-18 secretion to LPS stimulation, but no evidence of increased basal secretion of these cytokines, or alterations in basal or stimulated pro-IL-1β levels. VX-765, an orally active IL-converting enzyme/caspase-1 inhibitor, blocked IL-1β secretion with equal potency in LPS-stimulated cells from FCAS and control subjects. These results further link mutations in cryopyrin with abnormal caspase-1 activation, and support the clinical testing of caspase-1 inhibitors such as VX-765 in autoinflammatory disorders. *The Journal of Immunology,* 2005, 175: 2630–2634.

Mutations in the CIAS1 gene (also known as NALP3 and PYPAF1) have been identified as the basis for a group of related autoinflammatory syndromes that include familial cold autoinflammatory syndrome (FCAS),[2] Muckle-Wells syndrome (MWS), and neonatal onset multisystem inflammatory disease (NOMID), also known as chronic infantile neurologic cutaneous articular syndrome (1–5). Although these diseases vary in severity, all are characterized by the appearance of inflammatory symptoms without evidence of autoantibodies or Ag-specific T cells. FCAS, the mildest of these diseases, is characterized by episodic arthralgia, rash, and fever in response to cold exposure (6). MWS patients have similar inflammatory episodes that are more frequent and often unprovoked, and frequently develop progressive sensorineural hearing loss and systemic amyloidosis. NOMID, the most severe of the three phenotypes, is associated with more chronic inflammation involving joint, skin, and the nervous system. These patients commonly have severe neurologic symptoms due to chronic aseptic meningitis, as well as areas of bony overgrowth (7).

CIAS1 encodes cryopryin, an adaptor protein expressed in immune cells that contains at least three well-known protein-protein interaction domains, including a pyrin domain, a nucleotide binding site (NBS) domain, and a leucine-rich repeat domain. The pyrin domain and NBS domain associate with two adaptor proteins, apoptosis-associated speck-like protein containing a CARD (ASC) and CARDINAL, respectively. These proteins, in turn, bind the IL-converting enzyme (ICE)/caspase-1 through caspase recruitment domain (CARD)-domain interactions (8). A multiprotein inflammasome, formed by the association of all of these proteins, is involved in the proteolytic activation of ICE/caspase-1 in monocytes/macrophages, resulting in IL-1β and IL-18 production and subsequent activation of immune responses (9).

The recently reported finding of alterations in IL-1β production in monocytes from NOMID and MWS patients is consistent with the proposed role for cryopyrin in ICE/caspase-1 activation. In one study of CIAS1 mutations in NOMID (2), pro-IL-1β levels were elevated in unstimulated monocytes from a patient with a D303N mutation, although the response to LPS treatment was blunted compared with controls. In a more recent study of a different NOMID patient with the D303N mutation (10), IL-1β and IL-18 secretion were elevated in both unstimulated as well as LPS-stimulated whole blood cells. Adherent monocytes from a MWS patient with a R260W mutation also had elevated basal and LPS-stimulated IL-1β secretion (8).

Recent clinical studies in patients with cryopyrin-associated inflammatory disorders have suggested that IL-1 dysregulation is the key pathogenic mechanism involved in all three disorders. MWS patients showed a remarkable response to treatment with the human rIL-1 receptor antagonist, anakinra, with a rapid reduction in inflammatory symptoms and normalization of serum amyloid A levels (11, 12). Rapid amelioration of symptoms upon treatment with anakinra has also been reported recently in NOMID (13, 14). Additionally, anakinra treatment before cold exposure prevented clinical symptoms and laboratory signs of inflammation in FCAS patients (15). These rapid positive clinical responses to treatment

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[2] Abbreviations used in this paper: FCAS, familial cold autoinflammatory syndrome; ICE, IL-converting enzyme; MWS, Muckle-Wells syndrome; NBS, nucleotide binding site; NOMID, neonatal onset multisystem inflammatory disease; ASC, apoptosis-associated speck-like protein containing a CARD.

with anakinra strongly indicate that IL-1 is a central mediator of disease in these autoinflammatory syndromes.

The positive clinical results with anakinra in these inflammatory disorders, along with the potential role of cryopyrin in ICE/caspase-1 activation, provide the rationale for a clinical study to test caspase-1 inhibition as a means of treating the autoinflammatory disorders. VX-765 is the orally available prodrug of a potent and selective competitive inhibitor of ICE/caspase-1. VX-765 is currently under clinical development for the treatment of inflammatory and autoimmune conditions. In preparation for clinical trials of VX-765 in autoinflammatory disorders, we examined whether the ICE/caspase-1-mediated release of IL-1β and IL-18 is indeed enhanced in PBMCs from FCAS patients compared with normal subjects, and whether the efficacy and potency of VX-765 to inhibit this response are altered by mutations in cryopyrin.

Materials and Methods

Patients

The study received the approval of the University of California Human Research Protection Program committee, and informed consent was obtained from the subjects before the study. Eight patients with FCAS were included in the study, seven female and one male, with ages ranging from 21 to 86 years and mean age of 56 ± 20 (SD) years. Control subjects were five age-matched male spouses and one unaffected son, ages ranging from 19 to 83 and mean age of 60 ± 24 (SD) years. All of the FCAS patients had a classic clinical presentation and met diagnostic criteria, as described previously (6). None of the FCAS or control subjects were experiencing significant inflammatory symptoms at the time of study, and none were on regular anti-inflammatory medications. Three of the FCAS patients in this study were treated previously with 2 doses of anakinra >6 mo before this study. None of the FCAS subjects had evidence of neurologic symptoms, skeletal abnormalities, hearing loss, or amyloidosis.

The FCAS patients were from three families, and expressed three different CIAS1 gene mutations. Five related patients (mother, two daughters, granddaughter, and a distant male relative) possessed the common L353P ancestral mutation in cryopyrin that has been described previously (16). The other patients possessed novel mutations that have not been reported. Two patients (mother and daughter) had a T-to-A transition at nt 1976 in the CIAS1 gene, leading to a M659K substitution in cryopyrin. One patient had a G-to-A transition at nt 1573 in the CIAS1 gene, generating a E525K amino acid substitution that has not been reported, as well as the V198M variation described previously in several patients with diverse phenotypes, but also in normal controls. All of the mutations are located in exon 3, which encodes the NBS (NACHT) domain or NBS-associated domain, and all are within 3 aa of other reported mutations, consistent with studies suggesting mutational hot spots (17).

VX-765

VX-765 (synthesized by Vertex Pharmaceuticals) was prepared as a DMSO stock solution and diluted to appropriate concentrations in medium, as described below. VX-765 is a prodrug that is converted to its active metabolite, VRT-043198, both in vivo and in vitro by enzymatic and hydrolytic cleavage (to be reported separately). VRT-043198 binds to the catalytic site and competitively inhibits ICE/caspase-1 with an inhibition constant (K_i) = 0.8 nM. VX-765 inhibits the release of LPS-induced IL-1β and IL-18 by human PBMCs with an IC_{50} of ~0.7 μM and reduces inflammatory response in murine models of inflammatory disease (W. Wannamaker, R. Davies, M. Namchuk, J. Pollard, P. Ford, G. Ku, C. Decker, K. Kuida, and J. Randle, manuscript in preparation).

Preparation of PBMCs

Venous blood was drawn from donors into EDTA/Vacutainer tubes at 10–11 a.m., the time of the day when FCAS patients are generally least symptomatic. Blood (25 ml) was layered over 10 ml of Ficoll in 50-ml conical tubes, and centrifuged for 30 min at 1500 rpm at 22°C. The PBMC fractions were collected and pooled for each patient; the volume was adjusted to 50 ml with room temperature PBS; and samples were centrifuged for 10 min at 1200 rpm. Cell pellets were resuspended in 50 ml of PBS/tube and centrifuged a second time for 5 min at 1200 rpm. Supernatants were aspirated, and cell pellets were resuspended in prewarmed RPMI 1640 complete medium containing 10% FBS, nonessential amino acids, 50 μM 2-ME, 1 mM Na pyruvate, and penicillin/streptomycin. Cells were counted

with hemacytometer, and cell density was adjusted to 2 million cells/ml by addition of RPMI 1640 complete medium.

Assay for secreted cytokines

A total of 2×10^5 cells/well (100 μl cell suspension) was distributed in triplicate in flat-bottom 96-well plates. Either 50 μl of VX-765 (40 μM in RPMI 1640 complete medium containing 0.2% DMSO) or vehicle control was added to appropriate wells. Following a 30-min incubation at 37°C, 50 μl of LPS (*Escherichia coli* O111:B4; Calbiochem) diluted in RPMI 1640 complete medium was added at final concentrations varying from 0.001 to 10 ng/ml. Cells were returned to a 37°C incubator. At 4 h after LPS addition, 75 μl of supernatant was removed from wells, cleared by centrifugation for 5 min at 1500 rpm, and stored at 4°C until assayed. Cells were returned to a 37°C incubator until 24 h after LPS addition, at which time 100 μl of supernatant was removed, cleared by centrifugation, and stored at 4°C. Supernatants were tested using ELISA kits for IL-1β (BD Pharmingen; catalogue 558848), IL-6 (eBioscience; catalogue 88-7066), IL-18 (R&D Systems; catalogue 7620), and IL-1α (R&D Systems; catalogue DLA-50), according to the manufacturers' instruction.

Immunoblotting

A total of 10^6 cells/well (500 μl cell suspension) was distributed in 24-well plates. Cells were treated with VX-765 and LPS, as described above, adjusted for 1 ml final volume. At 4 h after addition of LPS, all of the medium was transferred to 1.5-ml tubes and centrifuged at $1000 \times g$ for 5 min at 4°C to pellet the suspended cells, and supernatants were removed. To lyse attached cells, 100 μl of 1× NuPage sample buffer (NOVEX) plus 2-ME were added to each well, and plates were placed on an orbital shaker. The 100 μl of sample buffer in the wells was transferred to corresponding pellets and boiled for 15 min, then frozen at −20°C. Samples were boiled again before loading onto a 4–12% Bis-Tris NuPage gel and run with NuPage MES running buffer. Gels were transferred to nitrocellulose filters. Filters were blotted in TBST (0.05% Tween 20) + 5% dry milk. For IL-1β immunoblotting, blots were incubated overnight at 4°C with 1:2,000 mouse anti-IL-1β (Sigma-Aldrich) primary Ab, then incubated with 1:10,000 HRP goat anti-mouse secondary Ab for 1 h, and developed with ECL (Pierce SuperSignal Dura Western Substrate).
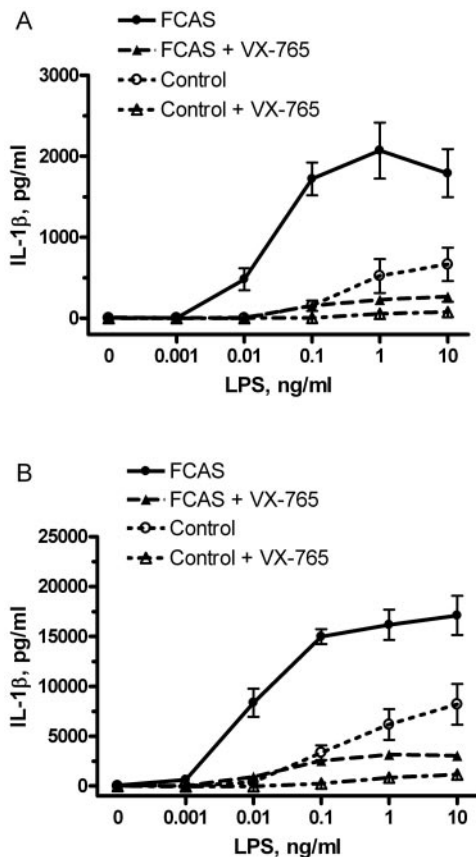
Results

Cytokine release

Because of the evidence suggesting the involvement of cryopyrin in IL-1β production, we measured IL-1β secretion from PBMCs obtained from FCAS patients and controls. Levels of IL-1β secreted after 4- or 24-h exposure to LPS were markedly greater for PBMCs from FCAS patients than unaffected donors (Fig. 1). IL-1β released in the absence of LPS treatment was below the level of detection in PBMCs from patients and controls. Exposure to as little as 0.01 ng/ml LPS for 4 h, which produced negligible IL-1β secretion from control cells, stimulated robust secretion from PBMCs of FCAS patients. IL-1β released at the highest concentration of LPS (10 ng/ml) was 2.1- to 2.7-fold higher in PBMCs from FCAS patients. Consistent with the involvement of ICE/caspase-1 in the processing of pro-IL-1β, the caspase-1 inhibitor VX-765 (10 μM) markedly (>80%) inhibited release of IL-1β from both FCAS and control cells (Fig. 1).

Before its release from monocytes, IL-18, like IL-1β, undergoes proteolytic maturation by ICE/caspase-1, so levels of this cytokine in cell medium were also measured. The level of IL-18 release was ~100-fold lower than that of IL-1β. As with IL-1β, LPS-stimulated release of IL-18 was strikingly higher in PBMCs from FCAS patients compared with control subjects and was markedly inhibited by VX-765 at 10 μM (Fig. 2). Also like IL-1β, there was no detectable constitutive release of IL-18 in the absence of LPS exposure, while there was significant IL-18 release from FCAS cells at low LPS concentrations that did not produce IL-18 release in normal cells.

To determine whether the abnormality in FCAS PBMCs is simply a general hyperinflammatory state or a specific defect in caspase-1 regulation, we also measured IL-6 and IL-1α in the cell medium after 24-h exposure to LPS (Fig. 3). In marked contrast to

FIGURE 1. Release of IL-1β by PBMCs from FCAS patients or control subjects after exposure to LPS for 4 (*A*) or 24 (*B*) h in the absence or presence of 10 μM VX-765. Results are mean $\pm$ SEM for seven FCAS patients and five controls.

IL-1β and IL-18, secretion of IL-6 was not increased in PBMCs from FCAS compared with control subjects. Secretion of IL-1α appeared to be lower in FCAS than in controls (Fig. 3), although results were available from only two control subjects. At the highest concentration of LPS (10 ng/ml), IL-1α secretion was reduced by ~50% in the presence of VX-765 (10 μM). Interestingly, IL-1α secretion is reduced in macrophages from ICE/caspase-1 knockout mice (18), suggesting that IL-1α secretion is partially dependent on ICE/caspase-1 or IL-1β levels.

Pro-IL-1β and ICE/caspase-1 expression

It is possible that the increased cytokine secretion in FCAS patients is due to increased biosynthesis of precursors. To determine whether the increased IL-1β and IL-18 secretion in FCAS is due to dysregulation at the transcriptional or posttranslation level, the effects of LPS on pro-IL-1β expression were determined. In the absence of LPS stimulation, pro-IL-1β was not detectable in either FCAS or control PBMCs (Fig. 4). Pro-IL-1β expression increased markedly in response to LPS, but with no notable difference between FCAS and control PBMCs (Fig. 4), even at low LPS concentrations. This suggests that the difference observed in cytokine secretion between FCAS and control secretion was not due to differences in synthesis of the cytokine precursors.

Inhibitory potency of VX-765

Because the mutations in FCAS appear to affect the activity state of ICE/caspase-1, we tested whether the potency of VX-765 to inhibit ICE/caspase-1-mediated IL-1β production is altered by the

mutations. The ability of VX-765 to inhibit IL-1β release was determined using PBMCs from three FCAS and three control subjects, measured after 24-h exposure to 10 ng/ml LPS. VX-765 inhibited IL-1β release with similar potency in PBMCs from FCAS (IC$_{50}$ = 0.99 $\pm$ 0.29 μM) and control (IC$_{50}$ = 1.10 $\pm$ 0.61 μM) subjects (Fig. 5).

Discussion

The identification of the genetic basis of several inherited autoinflammatory syndromes has led to rapid advances in understanding their pathophysiology, with significant implications for the development of novel targeted therapies. Specifically, members of the pyrin family of proteins are altered in FCAS, MWS, NOMID, familial Mediterranean fever, pyogenic arthritis, pyoderma gangrenosum, and acne syndrome. The role of these proteins in the regulation of IL-1 is supported by experimental data and the efficacy of IL-1-directed treatment (11–13). This translational study bridges mechanistic cytokine data and novel ICE/caspase-1-directed therapeutics using PBMCs from FCAS patients with three different CIAS1 mutations. Our results indicate that altered ICE/caspase-1-dependent posttranslational processing of IL-1β, rather than altered precursor biosynthesis, results from the cryopyrin mutations in FCAS.

Recent in vitro data suggest that disease-related mutations in cryopyrin are gain of function mutations. THP-1 cells (a monocytic cell line) transfected to overexpress mutant cryopyrin spontaneously secrete IL-1β (19). However, it is unclear whether this effect is due to increased transcription of pro-IL-1β, posttranslational cleavage of pro-IL-1β to its active secretable form, or a combination of both functions. Using a luciferase reporter assay, HEK293T cells transfected with mutant cryopyrin and ASC, a pyrin domain-containing adaptor protein that has been shown to interact with cryopyrin, have significantly increased NF-κB activity compared with cells with wild-type cryopyrin, supporting a transcriptional effect (19). However, immunoprecipitation experiments have shown that cryopyrin and ASC interact with other proteins, including CARDINAL and ICE/caspase-1, in a molecular platform called the inflammasome that is responsible for pro-IL-1β processing (8). Follow-up studies have also implicated cryopyrin as an intracellular sensor of muramyl peptides, the primary immunologically active component of peptidoglycan, and a common contaminant of most preparations of LPS (20), suggesting that the biosynthesis and release of IL-1β in response to LPS may have both TLR 4-dependent and independent components. The
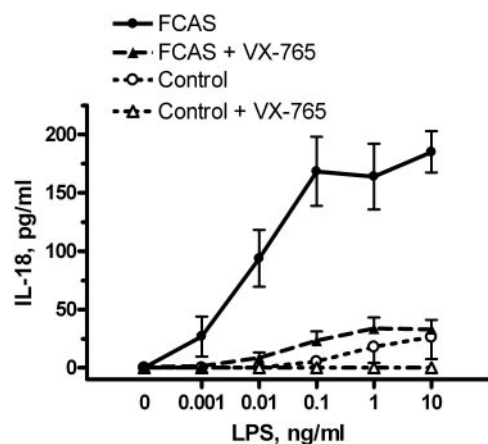
FIGURE 2. Release of IL-18 by PBMCs from FCAS patients or control subjects after 24-h exposure to LPS in the absence or presence of 10 μM VX-765. Results are mean $\pm$ SEM for three FCAS and three controls.
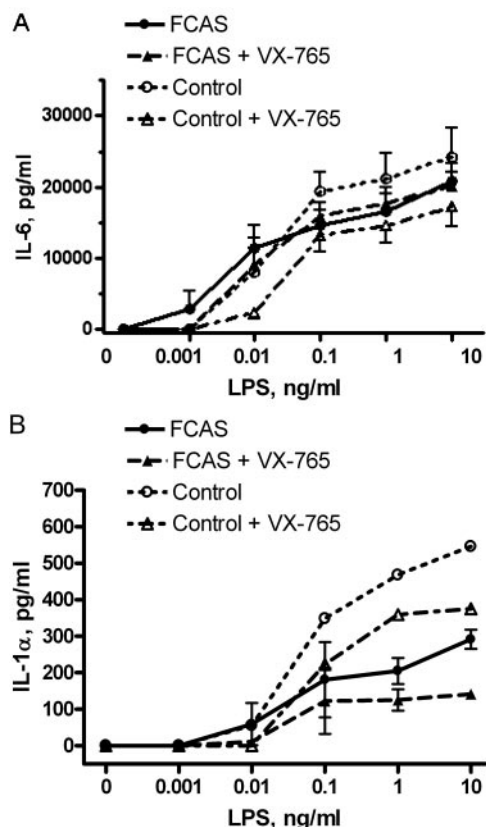
FIGURE 3. Release of IL-6 (*A*) and IL-1α (*B*) by PBMCs from FCAS patients or controls after 24-h exposure to LPS in the absence or presence of 10 μM VX-765. For IL-6, results are from seven FCAS and five control subjects (mean ± SEM). For IL-1α, results are from four FCAS patients (mean ± SEM) and two control subjects (mean of $n = 2$).

relative contributions of these cryopyrin functions have not been elucidated.

Our ex vivo data with FCAS PBMCs support a primarily post-translational role for cryopyrin in IL-1β responses. The increased IL-1β and IL-18 release from the FCAS cells is evidence for ICE/caspase-1 dysregulation, as both of these cytokines are processed by ICE/caspase-1 to be released from the cell. In contrast, IL-6 and IL-1α levels in cell supernatants are either unchanged or somewhat reduced in FCAS, indicating that there is no increase in transcription or translation of other inflammatory cytokines not processed by ICE/caspase-1. The Western blot data (Fig. 4) also show no measurable difference between pro-IL-1β protein levels in cells from patients and controls, suggesting that mutant cryopyrin exerts its effect at a later step in IL-1 release.

The limitations of this study include sample size, differences in sex between patients and controls, and use of mixed PBMCs instead of monocytes. Sample size was limited by the number of available local patients and controls, as fresh blood samples are necessary for these types of studies. We made a significant attempt to match age of patients and controls by choosing spouses and siblings. Unfortunately, the number of available FCAS-affected males and unaffected females was limited in this study. However, we believe that differences in IL-1β responses due to sex are unlikely, given our previous unpublished data of healthy subjects in which IL-1β release from whole blood in response to 5 ng/ml LPS did not significantly differ between male and female subjects: males = 403.1 ± 201.5 pg/ml ($n = 16$); females = 403.3 ± 212.1 pg/ml ($n = 3$) (mean ± SD). We chose to use PBMCs instead of

monocytes in this study because of the limited amount of blood available by venipuncture and the large number of conditions required to study the LPS dose response at two time points. Minimal amounts of the cytokines measured in this study are released by cells other than monocytes.

The finding of altered IL-1β secretion in PBMCs from FCAS patients is consistent with studies reported previously using monocytes or whole blood from single NOMID (2, 10) and MWS (8) patients. However, our results in FCAS differ from the previous results in MWS and NOMID in that no constitutive pro-IL-1β biosynthesis or IL-1β release was observed in any of the eight FCAS patients studied. This difference may be a reflection or even a contributing factor to the less severe and persistent nature of FCAS relative to MWS or NOMID. It is possible that FCAS cells have constitutively active ICE/caspase-1, and that the lack of IL-1β release at baseline is due to the absence of pro-IL-1β. It is also possible that increased pro-IL-1β biosynthesis seen in monocytes from a NOMID patient was due to IL-1R-mediated NF-κB activation. Like monocytes from a MWS and one NOMID patient, the FCAS cells showed increased IL-1β release with LPS. There are clear differences in IL-1β release between patients and controls at low LPS doses at both 4 and 24 h, suggesting a state of hyper-responsiveness. We did observe some differences in IL-1β responses between individual FCAS patients, but the lack of significant variation in clinical severity among these patients and the limited number of patients with different mutations did not allow for adequate power to study this. It seems likely that the differences between monocyte IL-1β responses among the cryopyrin-associated diseases correlate with clinical phenotype; however, additional studies with larger numbers of NOMID, MWS, and FCAS patients with additional mutations need to be performed to confirm this.

Although the exact role of cryopyrin in the activation of ICE/caspase-1 has not been determined, our data clearly show that FCAS mutations in cryopyrin result in enhanced processing and release of the ICE/caspase-1-dependent cytokines, IL-1β and IL-18, in the presence of LPS. VX-765, via its active metabolite VRT-043198, inhibited LPS-induced IL-1β and IL-18 release by PBMCs from FCAS patient and healthy volunteers with very similar efficacy and potency. Therefore, cryopyrin mutations do not alter the
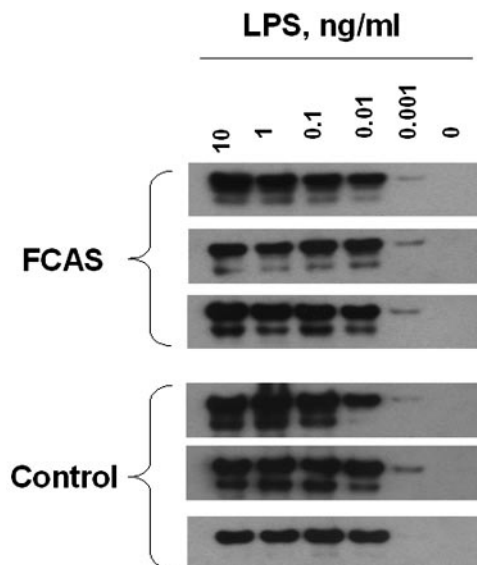
FIGURE 4. Western blot analysis for pro-IL-1β expression in PBMCs stimulated for 4 h with LPS. Pro-IL-1β band at 31 kDa probed with anti-IL-1β is shown. PBMCs were from three FCAS and three control subjects.
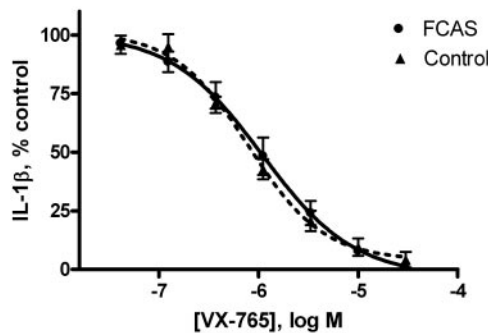
FIGURE 5. Inhibition by VX-765 of LPS-stimulated IL-1β release from PBMCs. Control and FCAS PBMCs were incubated with 10 ng/ml LPS in the presence of increasing concentrations of VX-765 for 24 h, and then IL-1β in cell medium was measured by ELISA. Results for each subject were normalized to the level of IL-1β release in the absence of VX-765. Mean $\pm$ SEM, $n = 3$ control and 3 FCAS subjects.

ability of VX-765 to interact with and inhibit ICE/caspase-1. The effectiveness of the orally active ICE/caspase-1 inhibitor, VX-765, in this ex vivo model supports the testing of this agent in autoinflammatory disorders.

Disclosures

J. H. Stack, K. Beaumont, K. S. Straley, G. W. Henkel, and J. C. R. Randle are employees of Vertex Pharmaceuticals.

References

1. Hoffman, H. M., J. L. Mueller, D. H. Broide, A. A. Wanderer, and R. D. Kolodner. 2001. Mutation of a new gene encoding a putative pyrin-like protein causes familial cold autoinflammatory syndrome and Muckle-Wells syndrome. *Nat. Genet.* 29: 301–305.
2. Aksentijevich, I., M. Nowak, M. Mallah, J. J. Chae, W. T. Watford, S. R. Hofmann, L. Stein, R. Russo, D. Goldsmith, P. Dent, et al. 2002. 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.* 46: 3340–3338.
3. Dode, C., N. Le Du, L. Cuisset, F. Letourneur, J. M. Berthelot, G. Vaudour, A. Meyrier, R. A. Watts, D. G. Scott, A. Nicholls, et al. 2002. 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.* 70: 1498–1506.
4. Aganna, E., F. Martinon, P. N. Hawkins, J. B Ross, D. C. Swan, D. R Booth, H. J. Lachmann, A. Bybee, R. Gaudet, P. Woo, et al. 2002. 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.* 46: 2445–2452.
5. Feldmann, J., A. M. Prieur, P. Quartier, P. Berquin, S. Certain, E. Cortis, D. Teillac-Hamel, A. Fischer, and G. de Saint Basile. 2002. 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.* 71: 198–203.
6. Hoffman, H. M., A. A. Wanderer, and D. H. Broide. 2001. Familial cold autoinflammatory syndrome: phenotype and genotype of an autosomal dominant periodic fever. *J. Allergy Clin. Immunol.* 108: 615–620.
7. McDermott, M. F., and I. Aksentijevich. 2002. The autoinflammatory syndromes. *Curr. Opin. Allergy Clin. Immunol.* 2: 511–516.
8. Agostini, L., F. Martinon, K. Burns, M. F. McDermott, P. N. Hawkins, and J. Tschopp. 2004. NALP3 forms an IL-1β-processing inflammasome with increased activity in Muckle-Wells autoinflammatory disorder. *Immunity* 20: 319–325.
9. Martinon, F., and J. Tschopp. 2004. Inflammatory caspases: linking an intracellular innate immune system to autoinflammatory diseases. *Cell* 117: 561–574.
10. Janssen, R., E. Verhard, A. Lankester, R. Ten Cate, and J. T. van Dissel. 2004. Enhanced interleukin-1β and interleukin-18 release in a patient with chronic infantile neurologic, cutaneous, articular syndrome. *Arthritis Rheum.* 50: 3329–3333.
11. Hawkins, P. N., H. J. Lachmann, and M. F. McDermott. 2003. Interleukin-1-receptor antagonist in the Muckle-Wells syndrome. *N. Engl. J. Med.* 348: 2583–254.
12. Hawkins, P. N., H. J. Lachmann, E. Aganna, and M. F. McDermott. 2004. Spectrum of clinical features in Muckle-Wells syndrome and response to anakinra. *Arthritis Rheum.* 50: 607–612.
13. Hawkins, P. N., A Bybee, E. Aganna, and M. F. McDermott. 2004. Response to anakinra in a de novo case of neonatal-onset multisystem inflammatory disease. *Arthritis Rheum.* 50: 2708–279.
14. Frenkel, J., N. M. Wulffraat, and W. Kuis. 2004. Anakinra in mutation-negative NOMID-CINCA syndrome. *Arthritis Rheum.* 50: 3738–379.
15. Hoffman, H. M., S. Rosengren, D. L. Boyle, J. Y. Cho, J. Nayar, J. L. Mueller, J. P. Anderson, A. A. Wanderer, and G. S. Firestein. 2004. Prevention of cold-associated acute inflammation in familial cold autoinflammatory syndrome by interleukin-1 receptor antagonist. *Lancet* 364: 1779–1785.
16. Johnstone, R. F., W. K. Dolen, and H. M. Hoffman. 2003. A large kindred with familial cold autoinflammatory syndrome. *Ann. Allergy Asthma Immunol.* 90: 233–237.
17. Neven, B., I. Callebaut, A. M. Prieur, J. Feldmann, C. Bodemer, L. Lepore, B. Derfalvi, S. Benjaponpitak, R. Vesely, M. J. Sauvain, et al. 2004. Molecular basis of the spectral expression of CIAS1 mutations associated with phagocytic cell-mediated autoinflammatory disorders CINCA/NOMID, MWS, and FCU. *Blood* 103: 2809–2815.
18. Kuida, K., J. A. Lippke, G. Ku, M. W. Harding, D. J. Livingston, M. S. Su, and R. A. Flavell. 1995. Altered cytokine export and apoptosis in mice deficient in interleukin-1β converting enzyme. *Science* 267: 2000–203.
19. Dowds, T. A., J. Masumoto, L. Zhu, N. Inohara, and G. Nunez. 2004. Cryopyrin-induced interleukin 1β secretion in monocytic cells: enhanced activity of disease-associated mutants and requirement for ASC. *J. Biol. Chem.* 279: 21924–21928.
20. Martinon, F., L. Agostini, E. Meylan, and J. Tschopp. 2004. Identification of bacterial muramyl dipeptide as activator of the NALP3/cryopyrin inflammasome. *Curr. Biol.* 14: 1929–1934.