Mammalian target of rapamycin inhibition counterbalances the inflammatory status of immune cells in patients with chronic granulomatous disease

Aurélie Gabrion, MSc,[a] Isabelle Hmitou, PhD,[a] Despina Moshous, MD, PhD,[b,c,d] Bénédicte Neven, MD, PhD,[b,c,d] Alain Lefèvre-Utile, MD, MSc,[b] Jean-Sébastien Diana, MD, MSc,[b] Félipe Suarez, MD, PhD,[c,d,e] Capucine Picard, MD, PhD,[c,d,f] Stéphane Blanche, MD,[b,c] Alain Fischer, MD, PhD,[b,c,d,g] Marina Cavazzana, MD, PhD,[a,c,d] and Fabien Touzot, MD, PhD[a,c,d] *Paris, France*

Background: Chronic granulomatous disease (CGD) is a primary immunodeficiency caused by defective production of reactive oxygen species in phagocytic cells that results in life-threatening infections and severe inflammatory manifestations. The treatment of inflammatory manifestations remains challenging because it can be associated with an increased risk of infections. Previous studies have shown that phagocytes from patients with CGD display a defect in autophagy and a reactive oxygen species–independent activation of the inflammasome.
Objective: Because the intersections between autophagy and the inflammasome have been observed in patients with various diseases and microbial infections, we investigated the possible benefit of restoring the autophagy defect through rapamycin, a potent autophagy inducer, in the setting of CGD.
Methods: We studied 15 patients given a diagnosis of CGD and followed in our institution. All patients were free of any active infection at the time of the study.
Results: We show that patients with CGD present a consistent inflammatory phenotype defined by (1) increased nonclassical and intermediate monocytes, (2) a proinflammatory state of mononuclear phagocytes with increased IL-1β and TNF-α content, (3) a T_H17 bias of $CD4^+$ T cells, (4) and an increase in IL-17A–secreting neutrophil numbers. We document the reversion of CGD inflammatory status by the mammalian target of rapamycin inhibitor rapamycin on the different immune cell subsets. We also provide evidence for the enhancement of rapamycin's inhibitory effect on IL-1β secretion by the IL-1 receptor antagonist anakinra in phagocytes of patients with CGD.
Conclusion: Altogether, these data open new therapeutic approaches for CGD-related inflammatory manifestations. (J Allergy Clin Immunol 2017;139:1641-9.)

Key words: *Chronic granulomatous disease, inflammatory manifestations, mammalian target of rapamycin inhibition, autophagy, inflammasome, IL-17A, IL-1β*

Chronic granulomatous disease (CGD) is a primary immunodeficiency of innate immunity caused by defects in phagocyte nicotinamide adenine dinucleotide phosphate (NADPH) oxidase subunits. Loss-of-function mutations in the NADPH oxidase components (ie, gp91phox, p22phox, p67phox, or p47phox) abrogate oxidase activity and compromise host immunity against certain bacteria and fungi.[1] Because a patient's life expectancy increases thanks to progress in diagnosis and management, the burden of inflammatory manifestations occurring independently of infectious agents has become more evident. Patients with CGD have granulomatous disorders, notably colitis, as a consequence of dysregulated inflammatory response.[2] The treatment of such manifestations remains challenging because it can be associated with an increased risk of infections.[3] Previous studies have shown that NADPH complex–deficient phagocytes display a defect in autophagy and reactive oxygen species–independent activation of the inflammasome.[4,5] Because IL-1β blockade decreases the activation of inflammasome and restores autophagy in mice with CGD *in vivo* and in human cells *in vitro*, clinicians have been tempted to treat patients with CGD with the IL-1β receptor antagonist anakinra, with mixed results.[6,7] Rapamycin is a macrolide naturally produced by the bacterium *Streptomyces hygroscopicus*, which was initially developed as an antifungal agent.[8] However, rapamycin, a potent mammalian target of rapamycin (mTOR) inhibitor, has mainly been used in clinical care for its immunosuppressive properties.[9]

Through mammalian target of rapamycin complex 1 (mTORC1) inhibition, rapamycin has also been shown to act as an autophagy inducer.[10] Because intersections between autophagy and the inflammasome have been observed in patients with various diseases and microbial infections, we investigated the possible benefit of restoring the autophagy defect

From [a]the Biotherapy Department, Necker-Enfants Malades Hospital, Assistance Publique-Hôpitaux de Paris, AP-HP, Paris; the Departments of [b]Pediatric Immunology, Hematology and Rheumatology and [e]Hematology and [f]Centre d'Etude des Déficits Immunitaires, Necker-Enfants Malades Hospital, AP-HP; [c]University Paris Descartes Sorbonne Paris Cité, Institut Imagine, Paris; [d]Inserm UMR 1163, Paris; and [g]Collège de France, Paris.

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Corresponding author: Fabien Touzot, MD, PhD, Biotherapy Department, Necker-Enfants Malades Hospital, 149 rue de Sèvres 75015 Paris, France. E-mail: fabien.touzot@aphp.fr.

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Abbreviations used
APC: Allophycocyanin
CGD: Chronic granulomatous disease
DMSO: Dimethyl sulfoxide
FITC: Fluorescein isothiocyanate
HC: Healthy control subject
LC3: Microtubule-associated protein 1A/1B-light chain 3
MDM: Monocyte-derived macrophage
mTOR: Mammalian target of rapamycin
mTORC1: Mammalian target of rapamycin complex 1
NADPH: Nicotinamide adenine dinucleotide phosphate
PE: Phycoerythrin
PMA: Phorbol 12-myristate 13-acetate
RORγt: Retinoic acid–related orphan receptor γ

through rapamycin in the setting of CGD.[11] For this purpose, we investigated the biological inflammatory status of a French cohort of patients with CGD and performed an *in vitro* study of the effect of rapamycin treatment on immune cells from patients with CGD.

METHODS

Patients

We studied 15 patients given a diagnosis of CGD followed in our institution. Characteristics of the patients are detailed in Table I. Informed consent for participation in the study was obtained from the patient's parents or from the patient in accordance with French regulatory requirements, the approval of the Institutional Review Board of the Necker-Enfants Malades Hospital, and the Declaration of Helsinki.

Each patient's median age was 8 years (range, 1-22 years). No patients presented with active bacterial or fungal infection at the time of the study. Eight patients presented with inflammatory manifestations (colitis, n = 7; granulomatous cystitis, n = 1). The contribution of each patient with CGD to the biological study is detailed in Table E1 in this article's Online Repository at www.jacionline.org.

Cohort of healthy control subjects

The median age of the healthy control subjects (HCs) was 24 years (range, 2-56 years). The control cohort comprised 5 children (2, 4, 7, 10, and 14 years old). We did not observe any significant correlation between the age of the HCs and the different biological parameters evaluated. The contribution of each HC to the biological study is detailed in Table E2 in this article's Online Repository at www.jacionline.org.

Monocyte, $CD4^+$ T-cell, neutrophil, and monocyte-derived macrophage assays

All assays, including cell isolation, flow cytometric analysis, cell stimulation analysis, cytokine secretion assay, macrophage differentiation, caspase-1 activation assay, and immunofluorescence detection of microtubule-associated protein 1A/1B-light chain 3 (LC3), were performed according to standard protocols. Detailed methods are provided in the Methods section in this article's Online Repository at www.jacionline.org.

Statistical analysis

Statistical analysis was performed with GraphPad Prism software (version 6.0; GraphPad Software, La Jolla, Calif). The Mann-Whitney *U* test was used for comparison of unpaired values, whereas the Wilcoxon paired *t* test was used for comparison of paired values. Correlation between 2 variables was assessed by using nonparametric Spearman correlation.

RESULTS

Circulating monocytes in patients with CGD display an inflammatory profile

We first investigated the circulating monocytes from patients with CGD followed at the Necker-Enfants Malades Hospital. Each patient's median age was 8 years (range, 1-22 years). No patients presented with active bacterial or fungal infection at the time of the study. Eight patients presented with inflammatory manifestations (colitis, n = 7; granulomatous cystitis, n = 1). Characteristics of the patients are detailed in Table I. Absolute counts of circulating monocytes were higher in patients with CGD versus HCs (median, 750/μL vs 400 μL, respectively; $P = .006$). The analysis of monocyte subsets, which were defined as $CD14^+CD16^-$ classical, $CD14^+CD16^+$ intermediate, and $CD14^{low}CD16^+$ nonclassical monocytes, showed a significant increase in the absolute number of circulating nonclassical and intermediate monocytes in patients with CGD versus HCs (mean nonclassical and intermediate monocyte count of 18.5 ± 9.7/μL and 13.6 ± 6.5/μL for patients with CGD versus 6.7 ± 3.8/μL and 4.4 ± 2.4/μL, respectively, for HCs; $P < .0001$

TABLE I. Characteristics of patients with CGD

	Age at time of study (y)	Gene	Inflammatory manifestations	Anti-inflammatory therapy at the time of the study	Antimicrobial prophylaxis
P1	22	*CYBB*	Colitis	Prednisolone and mesalazine	Co-trimoxazole/itraconazole
P2	2	*CYBB*	None	None	Co-trimoxazole/itraconazole
P3	14	*CYBB*	None	None	Co-trimoxazole/itraconazole
P4	8	*CYBB*	Colitis	Budesonide	Co-trimoxazole/posaconazole
P5	17	*CYBB*	Granulomatous cystitis	Prednisolone	Co-trimoxazole/itraconazole
P6	1.8	*CYBB*	None	None	Co-trimoxazole/itraconazole
P7	4	*CYBB*	None	None	Co-trimoxazole/voriconazole
P8	1	*CYBB*	None	None	Co-trimoxazole/itraconazole
P9	6	*CYBB*	Colitis	Prednisolone/azathioprine	Co-trimoxazole/itraconazole
P10	10	*CYBB*	None	None	Co-trimoxazole/itraconazole
P11	12	*NCF1*	Colitis	Infliximab/methotrexate	Co-trimoxazole/itraconazole
P12	6	*CYBB*	Colitis	None	Co-trimoxazole/posaconazole
P13	15	*CYBB*	Colitis	Prednisolone	Co-trimoxazole/itraconazole
P14	17	*CYBB*	Colitis	Prednisone/azathioprine	Co-trimoxazole/itraconazole
P15	2	*CYBB*	None	None	Co-trimoxazole/posaconazole