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Impaired Efferocytosis in Human Chronic Granulomatous Disease is Reversed by Pioglitazone Treatment

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To the Editor

Patients with Chronic Granulomatous Disease (CGD) suffer from infections and exaggerated inflammation ¹. Defects in efferocytosis, the process of clearing dying cells by macrophages, may contribute to the heightened, prolonged, and granulomatous inflammation in this disease. Impaired efferocytic capability has been shown for murine CGD macrophages *in vitro* and *in vivo* ^{2,3}, as well as human gp91^{phox}-deficient PLB-985 cells, and in one study, monocyte-derived macrophages from CGD patients ⁴. Efferocytosis generally leads to active suppression of inflammatory mediator production by macrophages, and this too, is reportedly deficient in CGD ^{2,5,6}.

Peroxisome proliferator-activated receptor γ (PPAR γ), a nuclear receptor activated by oxidized lipids, is a driver of metabolic programming of macrophages and enhances their ability to take up and digest dying cells ². PPAR γ is ordinarily expressed at low levels in circulating monocytes, but its expression and activation are upregulated once monocytes transit into oxidant-rich inflamed tissues and begin to differentiate into macrophages.

PPAR γ expression and activation are delayed and deficient in inflammatory macrophages derived from immigrant monocytes during inflammation in the murine model of X-linked CGD, but are restored by treatment of the animals with the PPAR γ agonist, pioglitazone, a drug approved for type 2 diabetes ². Pioglitazone treatment of CGD mice also restored the

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efferocytic capability of inflammatory macrophages and suppressed excessive inflammatory mediator production ².

In normal phagocytes PPAR γ activation links NADPH oxidase activity with enhanced mitochondrial ROS production ⁷. Accordingly, the lack of this upstream signaling by the NADPH oxidase and PPAR γ in CGD results in deficient mitochondrial ROS production in CGD phagocytes. Notably, mitochondrial ROS production was restored in murine and human CGD monocytes treated with pioglitazone, *in vivo* and *ex vivo*, respectively ⁷.

We hypothesized that human CGD blood monocytes, precursors of inflammatory macrophages, would show impaired efferocytosis in comparison to monocytes from normal human subjects, and that treatment with pioglitazone would restore CGD monocyte efferocytic capability. We investigated monocytes rather than monocyte-derived macrophages as preliminary experiments indicated that prolonged culturing required for derivation minimized differences between CGD and normal macrophages, and likely explains disparate reports of their efferocytic capability ^{4,5}. Monocytes were isolated from PBMCs obtained from CGD patients and healthy normal volunteers at the NIH Clinical Center (see online Supplement for Methods and Patient Demographics (Suppl. Table 1)). In a first set of experiments, freshly isolated monocytes were “fed” fluorescently labeled apoptotic Jurkat cells or carboxylated beads, mimicking apoptotic cells. Efferocytosis was then quantified either by flow cytometry as percent of monocytes taking up Jurkat cells or by visual inspection of internalized beads and expressed as an efferocytic index (Fig. 1A and Suppl. Fig. 1). As shown in Fig. 1A, freshly isolated monocytes from CGD subjects were significantly impaired in the ability to take up both apoptotic Jurkat cells and carboxylated beads in comparison to monocytes from normal healthy volunteers. Monocytes were provided with killed *Candida* to measure generalized phagocytic ability. No impairment was seen in the uptake of killed *Candida* by CGD monocytes (Suppl. Fig. 1B). These data are in keeping with murine CGD macrophage data and limited data in human CGD macrophages ^{2,4}.

In further experiments, PBMC from CGD and healthy subjects (Suppl. Table 1) were overnight shipped from the NIH to Denver where monocytes were isolated by plating and then cultured overnight with pioglitazone, 10 μ M. Culturing in this manner with pioglitazone mimics *in vivo* pioglitazone treatment of CGD mice as measured by restoration of stimulated mitochondrial ROS production ⁷. As hypothesized, overnight culture of CGD monocytes with pioglitazone significantly enhanced efferocytic capability restoring it to levels seen for healthy control monocytes (Fig. 1B). Pioglitazone treatment of normal monocytes had minimal effect on efferocytic capability, and also little effect on mitochondrial ROS production ⁷.

Monocytes from two CGD patients (gp91^{phox} and p47^{phox} deficient, respectively) who were treated with pioglitazone (15 mg/day) for severe inflammatory bowel disease ⁸ were also available for investigation. Blood from both patients was obtained before pioglitazone treatment and at two time points during treatment and shipped along with healthy control monocytes overnight to Denver for investigation. Monocytes were plated, rested for 2 hours, and then investigated for efferocytic capability. At baseline, monocytes from CGD patient 1

showed efferocytic capability of approximately 60% of that for healthy control monocytes (Fig. 2A). While no enhancement was noted at 15 days of pioglitazone treatment, after 30 days treatment, efferocytosis by monocytes was enhanced and comparable to the healthy controls. For CGD patient 2, baseline efferocytosis prior to pioglitazone treatment was similarly diminished as compared to normal monocytes. Efferocytosis was enhanced by day 45 and day 120 of pioglitazone treatment. As predicted, stimulated production of mitochondrial ROS was also enhanced in monocytes from these patients following their treatment with pioglitazone (Fig. 2B) and mirrored monocyte efferocytic capability.

PPAR γ agonists such as pioglitazone given over weeks alter cellular metabolism of liver and muscle cells as well as inflammatory macrophages⁹. In addition, PPAR γ agonists have many anti-inflammatory effects including suppression of Th1 and Th17-induced inflammation, inhibition of NF κ B and upregulation of IL-10^{9, 10}. As such, PPAR γ agonists are being trialed for a number of inflammatory diseases (www.clinicaltrials.gov). We have shown that PPAR γ activation occurs downstream of the activated NADPH oxidase and is lacking in CGD phagocytes, but can be restored by pioglitazone^{2, 7}. PPAR γ agonism restores efferocytosis by macrophages in murine CGD, and as shown here, in human CGD monocytes, as well. This was demonstrated following both *ex vivo* treatment of human CGD monocytes, and treatment of 2 CGD patients with pioglitazone. As in our earlier studies, PPAR γ agonism was also accompanied by enhanced phagocyte mitochondrial ROS production⁷. Whether mitochondrial ROS contributes to efferocytic capability is an area for future study. Whether pioglitazone will decrease inflammation in CGD, and whether pioglitazone-enhanced mitochondrial ROS will bolster host defense in human CGD, as it did in murine models⁷, are key unanswered questions. If human data are similar to the preclinical models, pioglitazone, an on-the-shelf therapy, may ameliorate both immunodeficiency and inflammatory aspects of CGD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CGD	Chronic Granulomatous Disease
CPT	Cell preparation tube
PBMC	Peripheral blood mononuclear cell
PPARγ	Peroxisome proliferator-activated receptor gamma
PLB	PLB-985 cells derived from a patient with acute myeloid leukemia

ROS Reactive oxygen species

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Fig. 1.

Impaired efferocytosis by CGD monocytes is restored by pioglitazone treatment *ex vivo*. A. Freshly isolated monocytes from CGD and normal subjects were “fed” fluorescently labeled apoptotic Jurkat cells (*left*) or carboxylated beads (*right*) N=9 subjects/group. B. Overnight-shipped CGD and normal monocytes, plated and treated for 24h with pioglitazone or vehicle, were then “fed” carboxylated beads as in A. N=5 subjects/group.

Fig. 2.

Efferocytosis is restored and mitochondrial oxidant production enhanced in monocytes isolated from CGD patients during treatment with pioglitazone. A. Efferocytosis by CGD monocytes appears to be restored (compared to normal monocytes) following pioglitazone treatment of at least 30 days in 2 CGD subjects. Patient data are shown as mean $\pm$ range for duplicate wells at each time point. B. Mitochondrial oxidant production by stimulated CGD monocytes was enhanced during treatment with pioglitazone.