Journal of Molecular Medicine
https://doi.org/10.1007/s00109-022-02206-2

JMolMed

ORIGINAL ARTICLE

Leukocyte cytokine responses in adult patients with mitochondrial DNA defects

Kalpita R. Karan[1] · Caroline Trumpff[1] · Marissa Cross[1] · Kristin M. Engelstad[2] · Anna L. Marsland[3] · Peter J. McGuire[4] · Michio Hirano[2] · Martin Picard[1,2,5]

Received: 21 December 2021 / Revised: 11 April 2022 / Accepted: 6 May 2022

Abstract

Patients with oxidative phosphorylation (OxPhos) defects causing mitochondrial diseases appear particularly vulnerable to infections. Although OxPhos defects modulate cytokine production in vitro and in animal models, little is known about how circulating leukocytes of patients with inherited mitochondrial DNA (mtDNA) defects respond to acute immune challenges. In a small cohort of healthy controls ($n = 21$) and patients ($n = 12$) with either the m.3243A > G mutation or single, large-scale mtDNA deletions, we examined (i) cytokine responses (IL-6, TNF-α, IL-1β) in response to acute lipopolysaccharide (LPS) exposure and (ii) sensitivity to the immunosuppressive effects of glucocorticoid signaling (dexamethasone) on cytokine production. In dose–response experiments to determine the half-maximal effective LPS concentration (EC_{50}), relative to controls, leukocytes from patients with mtDNA deletions showed 74–79% lower responses for IL-6 and IL-1β ($p_{IL\text{-}6} = 0.031$, $p_{IL\text{-}1\beta} = 0.009$). Moreover, whole blood from patients with mtDNA deletions ($p_{IL\text{-}6} = 0.006$), but not patients with the m.3243A > G mutation, showed greater sensitivity to the immunosuppressive effects of dexamethasone. Together, these ex vivo data provide preliminary evidence that some systemic OxPhos defects may compromise immune cytokine responses and increase the sensitivity to immune cytokine suppression by glucocorticoids. Further work in larger cohorts is needed to define the nature of immune dysregulation in patients with mitochondrial disease, and their potential implications for disease phenotypes.

Key messages

- Little is known about leukocyte cytokine responses in patients with mitochondrial diseases.
- Leukocytes of patients with mtDNA deletions show blunted LPS sensitivity and cytokine responses.
- Leukocytes of patients with mtDNA deletions are more sensitive to glucocorticoid-mediated IL-6 suppression.
- Work in larger cohorts is needed to delineate potential immune alterations in mitochondrial diseases.

Keywords Mitochondrial disease · mtDNA deletion · 3243A > G · Cytokine · Interleukin · Inflammation · Inflammation Suppression · Glucocorticoid

✉ Martin Picard
martin.picard@columbia.edu

Kristin M. Engelstad
ke4@cumc.columbia.edu

Michio Hirano
mh29@columbia.edu

1 Department of Psychiatry, Division of Behavioral Medicine, Columbia University Irving Medical Center, New York, NY, USA

2 Department of Neurology, H. Houston Merritt Center and Columbia Translational Neuroscience Initiative, Columbia University Irving Medical Center, New York, NY, USA

3 Department of Psychology, University of Pittsburgh, Pittsburgh, PA, USA

4 National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA

5 New York State Psychiatric Institute, New York, NY 10032, USA

Published online: 30 May 2022

Introduction

Mitochondria regulate innate and adaptive immune responses by orchestrating metabolic signals required for immune cell activation, differentiation, and survival [1]. During infections, host immunometabolic responses contribute to normal leukocyte activation [2, 3], such as monocyte polarization into pro- and anti-inflammatory states linked to distinct cytokine secretory profiles [4]. T lymphocyte activation also depends on major bioenergetic recalibrations [5]. Furthermore, we and others have shown that during a targeted pro-inflammatory challenge with the bacterial cell wall molecule lipopolysaccharide (LPS), acutely perturbing mitochondrial oxidative phosphorylation (OxPhos) with pharmacological inhibitors alters cytokine production [6, 7]. This demonstrated in healthy individuals that OxPhos function modulates leukocyte cytokine responses.

In patients with mtDNA disorders, infections are frequent causes of death [8–10]. This could arise from the deleterious effects of immune processes on metabolic capacity, or the effects of metabolic capacity on immune regulation [11]. One potential factor that may contribute to increased vulnerability to infections is an impaired ability of circulating leukocytes to effectively mount the required innate and adaptive immune responses. During pathogen exposure, the robust activation of immune cells requires effective cell–cell signaling via cytokines [12]. Therefore, cytokine responses represent a critical aspect of normal immune function. A systematic review of the literature on mitochondrial diseases confirmed that little is known about cytokine production in affected patients, and that no study has thus far characterized the leukocyte cytokine responses to targeted immune challenges in patients with mtDNA defects.

In vitro, LPS triggers inflammatory cascades that lead to the production of multiple cytokines [13, 14] (reviewed in [15]). This includes the pro-inflammatory cytokines interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and interleukin 1 beta (IL-1β), which are also physiologically induced by acute physical [16, 17] and psychological stress [18, 19], indicating their broad physiological significance and relative lack of specificity. Moreover, we note that cytokine responses are physiologically regulated by glucocorticoid signaling, which, at nanomolar concentration, potently suppresses pro-inflammatory cytokines, particularly IL-6 [20–22]. Experimentally, applying the glucocorticoid mimetic dexamethasone (Dex) in parallel with LPS to human blood leukocytes therefore allows quantitative assessment of glucocorticoid sensitivity.

Here, we report LPS-induced pro-inflammatory cytokine responses in blood from patients with a pathogenic mtDNA point mutation (m.3243A > G, hereafter "mutation"), or with a single, large-scale mtDNA deletion (hereafter "deletion"). We also quantify leukocyte glucocorticoid sensitivity, providing converging preliminary evidence for potential immune alterations in mitochondrial disorders.

Methods

Participant recruitment

Informed consent was obtained in compliance with guidelines of the Institutional Review Board of the New York State Psychiatric Institute IRB#7424. All participants provided informed consent for the study procedures and publication of data. Participants between the ages of 18 and 55 years were recruited between June 2018 and March 2020 as part of the larger Mitochondrial Stress, Brain Imaging, and Epigenetics (MiSBIE) study cohort. The first 21 healthy controls and 12 patients with mitochondrial diseases (Table 1) were included in this sub-study of whole blood cytokine responses.

Participants were recruited from our local clinic at the Columbia University Irving Medical Center and nationally in the USA and Canada. Patients with a genetic diagnosis of mitochondrial disease were eligible for inclusion if they have a molecularly defined genetic diagnosis for either (i) the m.3243A > G point mutation, with or without mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS), or (ii) a single, large-scale mtDNA deletion-associated chronic progressive external ophthalmoplegia (CPEO) or Kearns-Sayre syndrome (KSS). Exclusion criteria were severe cognitive deficit or inability to provide informed consent, neoplastic disease, symptoms of flu or other seasonal infection (acute febrile or infectious disease) 4 weeks preceding the study visit, Raynaud's syndrome, involvement in any therapeutic or exercise trial listed on ClinicalTrials.gov, steroid therapy (e.g., oral dexamethasone, prednisone, or similar), other immunosuppressive treatment, and metal inside or outside the body or claustrophobia precluding magnetic resonance imaging (MRI). All participants completed a brief questionnaire to collect information on their sex, age, ethnicity, health condition, and medication use. Overall disease severity and symptomatology was measured using the Newcastle Mitochondrial Disease Adult Scale (NMDAS) administered by a clinician [23].

Differential blood counts

Complete blood counts (CBCs) were performed on all participants and included proportions of white blood cells (WBCs), red blood cells, platelets, and differential WBC

Table 1 Participant characteristics

Variable[a]	Controls	m.3243A > G carriers	Single, large-scale deletion
N	21	7	5
Age, years	32.9 (9.5)	32.1 (9.5)	33.1(9.9)
Sex, female	15 (72%)	3 (42%)	4 (80%)
BMI	25.8 (6.9)	24.2 (5.0)	26.1 (4.1)
Race/ethnicity			
White	15 (71.4%)	7 (100%)	5 (100%)
Black	4 (19%)	–	–
Asian	1 (4.7%)	–	–
Hispanic or Latino	1 (4.7%)	–	–
Multiple	–	–	–
MELAS diagnosis	–	1	–
CPEO diagnosis	–	1	2
CPEO "Plus" diagnosis			2
Multi-systemic syndrome		1	1
NMDAS score	1.71 (2.7)	4.14 (4.0)	24 (10.8)
CNS score	72 (2.1)	70.2 (1.3)	62.25 (7.6)

BMI body mass index, *CNS* Columbia Neurological Scale, *NMDAS* Newcastle Mitochondrial Disease Assessment Scale, *MELAS* Mitochondrial Encephalopathy, Lactic Acidosis, Stroke-like Episodes, *CPEO* Chronic Progressive External Opthalmoplegia

[a]Data presented as means (standard deviation) or *n* (%)

counts using an automated hematologic analyzer (XN-9000 Sysmex systems). This yielded absolute cell counts and proportions (%) of total WBC that are neutrophils, lymphocytes, monocytes, eosinophils, and basophils.

Whole blood LPS-stimulation and glucocorticoid suppression

Fasting whole blood was collected between 9 and 10 a.m. in heparin vacutainer tubes (BD #367,878). Blood was diluted with phenol red-free 1 × RPMI without serum or glutamine (Thermofisher #11,835,055) in a 1:1 ratio, and 200 µL of RPMI-diluted blood was incubated with the bacterial endotoxin lipopolysaccharide (LPS) from *Escherichia coli* (Sigma-Aldrich #L2880) at increasing concentrations ranging from 3.2 pg/mL to 50 ng/mL, in 96-well tissue culture plates (Eppendorf #30,730,127) at 37 °C with 5% CO_2 for 6 h, as described in [7]. LPS is a potent toll-like receptor (TLR) 4 agonist derived from outer membrane of Gram-negative bacteria [24, 25]. Relative to isolated cell preparations, the whole blood preparation imposes less stress on leukocytes, preserves physiologically relevant interactions between circulating leukocytes, and preserves the influence of potential circulating humoral factors on immune responses [26].

In glucocorticoid suppression experiments, the cortisol-mimetic dexamethasone (Dex, Sigma-Aldrich #D4902) was added at a final concentration of 100 nM [27] to all LPS concentrations (3.2 pg/mL–50 ng/mL). Each plate contained an untreated 'blood-only' control sample for baseline measures. Plasma was collected from each well of the plate by centrifuging the plate, first at 1000 g for 5 min. The plasma was then centrifuged at 2000 g for 10 min at 4 °C to obtain clean, cell-free plasma. Plasma was stored at −80 °C for cytokine measures.

Three plasma cytokines: interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and interleukin-1beta (IL-1β), were measured using a broad-range fluorescence-based detection method (Human Catchpoint Simple Step ELISA kits, Abcam #ab229434, ab229399, and ab229384), following the manufacturer's instructions. Briefly, standards were prepared by serial dilutions to generate 12–14-point standard curves. Plasma samples were diluted to 1:4 ratio using a diluent reagent for all cytokine measures. Fifty microliters of standards and samples were added to appropriate wells in the 96-well strip plate followed by the addition of 50 µL of capture- and detection Ab cocktail to all the wells which was then incubated on a shaker at RT for 1 h. Post incubation, the wells were aspirated and washed 3 × times with 1 × wash buffer. After the final wash, 100 µL of prepared CatchPoint HRP Development Solution was added to the wells and incubated for 10 min at RT. The plates were read for fluorescence per well at an Ex/Cutoff/Em 530/570/590 nm in in a micro-plate reader (Molecular Devices, SpectraMax M2). Two plasma samples with known cytokine levels were used as internal standards per plate to account for inter-assay variations. A background correction was applied to all RFU values in a run based on 'no-sample' blank values. The

cytokine concentrations were interpolated from respective standard curves, and the final concentration was obtained after correcting for dilution factor.

LPS-induced cytokine sensitivity

To determine cytokine sensitivity of each participant, a 4-parameter (bottom plateau, top plateau, the EC_{50}, and the slope factor) logistic regression was fitted to the levels of IL-6, TNF-α, and IL-1β increasing LPS concentrations (3.2 pg/mL–50 ng/mL). Half-maximal effective concentrations of LPS (EC_{50}) for each cytokine response were derived for each participant using best-fit regression values on the dose–response curve. The maximal cytokine response was obtained at 50 ng/mL of LPS (EC_{max}) for all participants. Mean EC_{50} and mean max cytokine responses were obtained for both control and disease (mutation and deletion) groups for downstream statistical analyses.

Statistical analyses

One-way ANOVAs with Dunnett's multiple comparisons were used to test group differences in LPS- and LPS + Dex-treated cytokine responses. Paired *t*-tests were used to examine intra-individual differences from pre- to post-Dex treatment. The effect size estimate Hedge's *g* [28], which is derived from the variance within groups relative to the group differences, and includes a correction to guard against inflation from small sample sizes, was calculated to obtain a standardized estimate of the magnitude of the effect independent of sample size. Spearman rank correlations were used to quantify the strength of the association between cytokine responses and leukocyte cell counts. All statistical analyses were performed using GraphPad Prism v8.2. $p < 0.05$ was considered statistically significant.

Results

LPS-stimulated cytokine responses

A total of 21 healthy controls and 12 patients (7 3243A > G mutation, 5 single, large-scale deletion) were recruited for this project (see participant characteristics in Table 1). The mutation group generally presented with mild disease severity (NMDAS score = 4.1 ± 4.0 (SD)) whereas patients with deletions were more severely affected (NMDAS score = 24.0 ± 10.8: Table 1). Cytokine responses were quantified as (i) cytokine levels at maximal LPS concentration (EC_{max}) and (ii) the sensitivity of cytokine responses, calculated as the half-maximal effective concentration of LPS required to elicit 50% of the maximal response (EC_{50}) for each cytokine (Fig. 1c).

Maximal responses As expected across all groups, LPS substantially increased IL-6, TNF-α and IL-1β cytokine levels (range 30 to 4190-fold, $ps < 0.0001$: Fig. 1b, c). There was no significant difference between control and disease groups in the mean IL-6 levels at EC_{max} (one-way ANOVA, $p = 0.94$). However, though not significant due to high variance, relative to healthy controls, deletion patients tended to produce 45% less TNF-α ($p = 0.35$, Hedge's $g = -0.79$) and 21% less IL-1β ($p = 0.78$, Hedge's $g = -0.42$) at EC_{max} (Supplemental Fig. S1). The cytokine responses were not associated with the proportions of lymphocytes, monocytes, or neutrophils (Supplemental Fig. S2). The deletion group had similar absolute blood counts as the controls for lymphocytes, monocytes, and neutrophils. However, the mutation group had a higher number of circulating lymphocytes (2.18×10^9 cells/mL, $p = 0.047$) and monocytes (0.89×10^9 cells/mL, $p = 0.002$) relative to controls (1.72×10^9 lymphocytes/mL, 0.47×10^9 monocytes/mL).

LPS sensitivity Patients with mitochondrial disease showed a 2.8-fold higher EC_{50} for IL-6 response relative to controls (Hedge's $g = 0.71$) translating to 79% reduction in IL-6 response. Consistent with the trend towards reduced peak responses, relative to controls, deletion patients exhibited 1.5- and 2.7-fold higher EC_{50} for TNF-α and IL-1β, respectively (Fig. 1d). This translates into 48% and 74% lower sensitivities for TNF-α and IL-1β, respectively, meaning that deletion patients required higher LPS doses to elicit comparable TNF-α and IL-1β responses as controls. The diverging cytokine response phenotypes among groups are illustrated in bi-variate plots in Fig. 1e.

Because the small sample sizes increase the probability that a true difference may be missed (i.e., false negatives), we also computed standardized effect sizes (Hedges' *g*) [28] of the maximal responses and EC_{50}-based sensitivity (Fig. 1f). These results indicate that blood leukocytes from deletion patients when compared with the control group exhibit blunted sensitivity of moderate ($g > 0.5$) to large ($g > 0.8$) effect sizes, whereas potential alterations in mutations patients are generally in the same direction but are small-to-moderate in magnitude.

Glucocorticoid sensitivity

Resistance to glucocorticoid-mediated cytokine suppression indicates impaired immune regulation (reviewed in [22, 29]). To examine glucocorticoid sensitivity in mitochondrial diseases, we repeated the LPS dose–response curves in the presence of the glucocorticoid receptor agonist dexamethasone (Dex, 100 nM) (Fig. 2a). All study groups showed the expected anti-inflammatory response to GC administration, illustrated by decreased IL-6, TNF-α, and IL-1β responses at EC_{max} (Fig. 2b). Supplementary Fig. S3 shows the paired pre- and post-Dex individual-level cytokine levels.

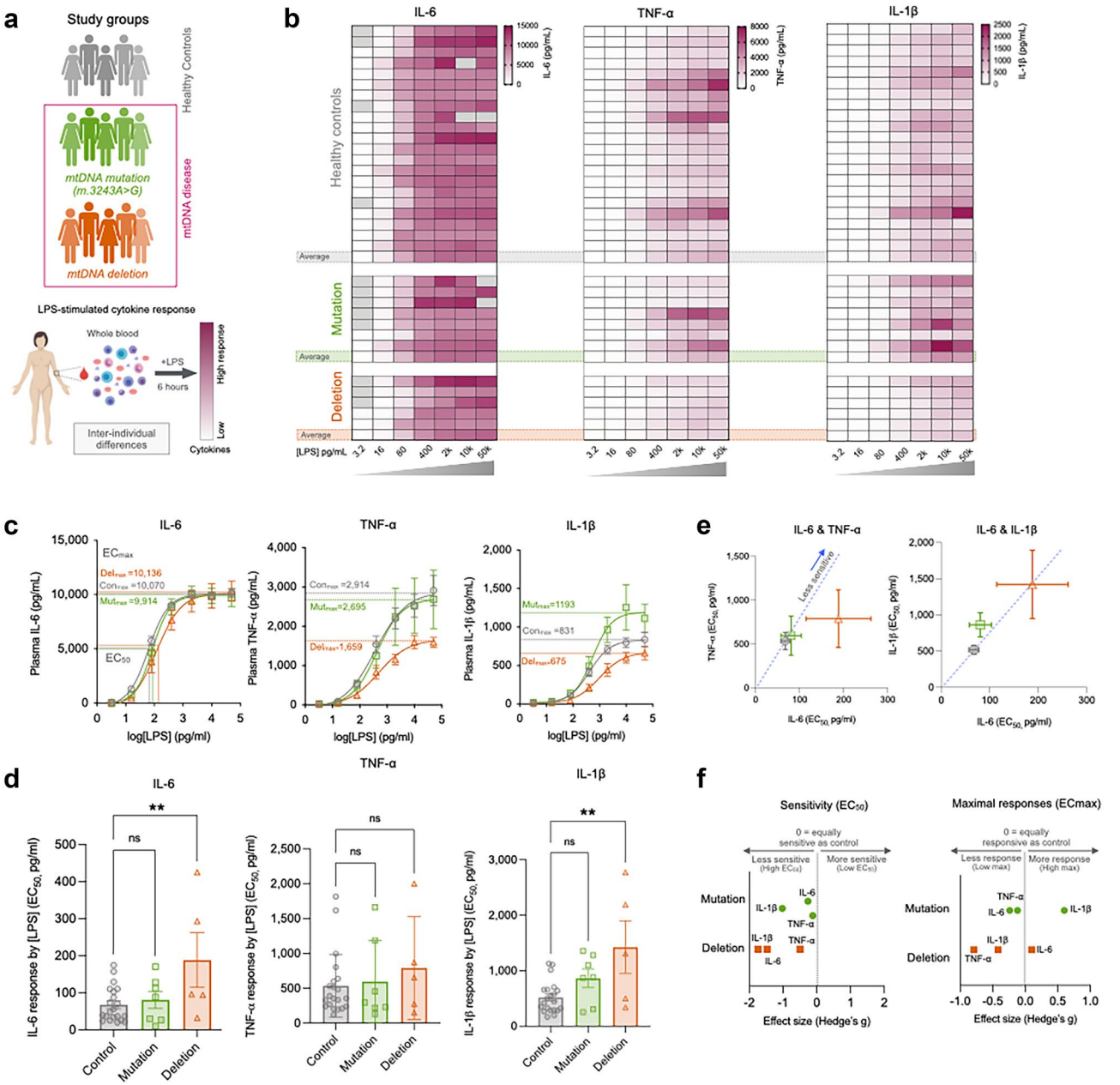

Fig. 1 Inter-individual differences in stimulated cytokine responses in patients with mitochondrial disease. **a** Schematic of study groups included in whole blood LPS experiments. **b** Pro-inflammatory cytokine (IL-6, TNF-α, and IL-1β) levels from healthy controls, patients with 3243A > G mutation and single deletions after 6 h of exposure to increasing concentrations of LPS. The grey boxes indicate missing/undetermined data. **c** Dose-dependent cytokine responses and fitted models used to determine the maximal (ECmax) and half maximal concentration of LPS (EC_{50}) in controls (grey) and in patients with mitochondrial disease (green = mutation, orange = deletion). **d** Inter-individual differences in cytokine sensitivity among patients with mitochondrial diseases and healthy controls. **e** Cytokine sensitivity bi-plots based on LPS EC_{50} values. The dotted line crosses the origin and average of the control group. **f** Standardized effect sizes (Hedge's *g*) of cytokine response (EC_{50}, *left*; max response, *right*) in mitochondrial disease groups relative to healthy controls. $g > 0.5$ is a medium effect size, and $g > 0.8$ is a large effect size. Non-linear regression analysis was used in dose–response curves presented in **c**, and one-way ANOVA with Dunnett's multiple comparison post hoc analysis was used in **d**. $*p < 0.05$; $**p < 0.01$; ns not significant. Assays were performed once in technical duplicates. Error bars indicate SEM in all panels. Controls $n = 21$, Mutation $n = 7$, Deletion $n = 5$

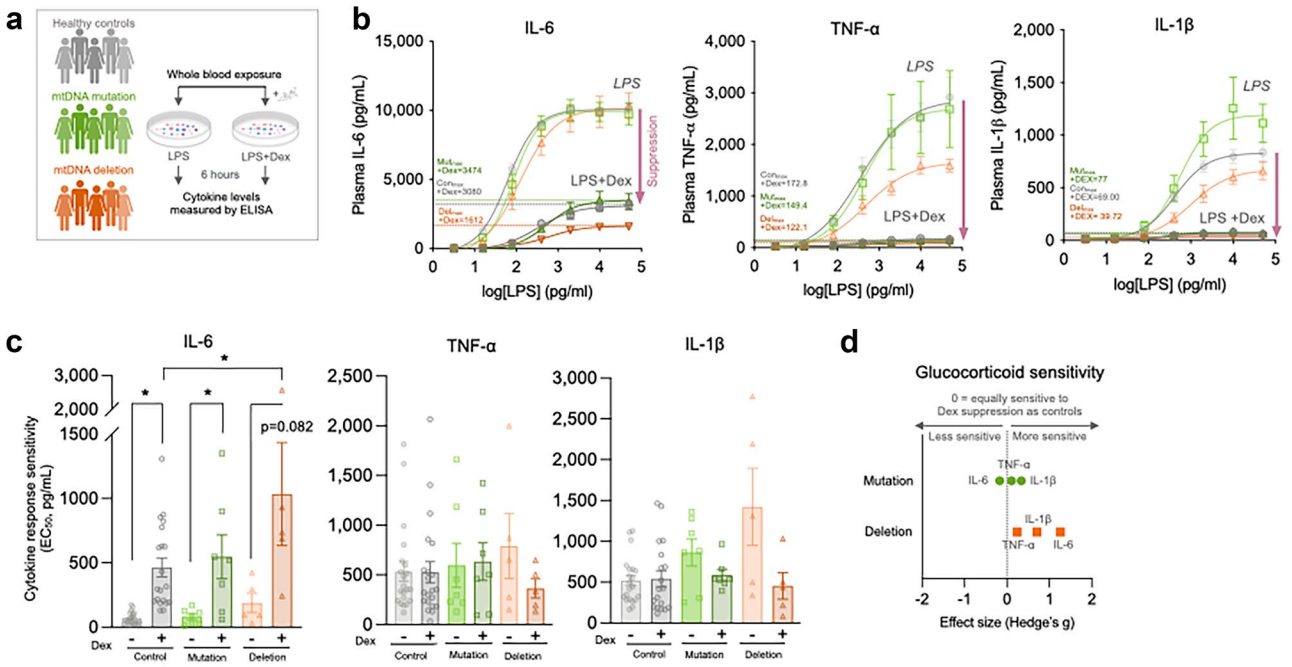

Fig. 2 Glucocorticoid sensitivity to LPS-stimulated cytokine responses in mitochondrial diseases. **a** Overview of the dexamethasone (Dex) sensitivity assay in whole blood. **b** LPS dose–response curves for IL-6, TNF-α, and IL-1β without Dex (*top traces*, same as in Fig. 1) and with Dex (*bottom traces*). The dotted lines mark the maximal cytokine responses (EC_{max}) in healthy participants (*grey*) and patients with the m.3243A > G mutation (*green*) and single, large-scale mtDNA deletions (*orange*). **c** Effect of Dex on EC_{50} LPS sensitivity; note that higher EC50 values represent lower sensitivity (greater dose required to elicit half of the maximal cytokine response). **d** Summary of effect sizes (Hedge'*g*) for deletion and mutation groups relative to control. $g > 0.5$ is a medium effect size, $g > 0.8$ is a large effect size. One-way ANOVA with Dunnett's multiple comparison test was used in **c** to test for group differences. Pre- to post-Dex differences were tested with paired *t*-tests. $*p < 0.05$. Assays were performed once in technical duplicates. Error bars indicate SEM. Controls $n = 21$, mutation $n = 7$, deletion $n = 5$

Compared to controls, the leukocytes of deletion patients were more sensitive to the glucocorticoid suppression of IL-6. Compared to the 3.8-fold increase in EC_{50} with Dex in controls, Dex increased EC_{50} by 6.6-fold in the deletion group ($p = 0.006$: Fig. 2c). Dex did not significantly alter EC_{50} for TNF-α or IL-1β, indicating that the sensitization to glucocorticoid signaling was cytokine specific. The effect sizes for Dex-induced cytokine suppression (Fig. 2d) were small ($g > 0.1$) to moderate ($g > 0.5$) in the deletion group and mostly small ($g > 0.2$) in mutation group. Furthermore, relative to controls, the Dex-induced suppression of EC_{max} IL-6 levels was 12.7% more potent in deletion patients ($p = 0.026$) (Supplemental Fig. S3c), again indicating that leukocyte IL-6 production is more easily suppressed (i.e., less robust) in the blood of deletion patients. No significant effects were observed in the 3243A > G mutation group.

Discussion

In this study, we have quantified LPS-induced leukocyte cytokine responses and glucocorticoid sensitivity in the blood of patients with two different primary mitochondrial DNA defects. Patients with single, large-scale mtDNA deletions showed significantly reduced pro-inflammatory IL-6 and IL-1β responses to LPS, along with an exaggerated glucocorticoid-mediated IL-6 suppression, indicating a less robust cytokine production capacity. These preliminary results in a small patient cohort converge to suggest that subgroups of patients with mtDNA defects may exhibit deficient cytokine production capacity and regulation. If confirmed in larger studies, the blunted leukocyte cytokine responses to an acute immune agonist could contribute to poor immune responses, and thus to the vulnerability of patients with mitochondrial diseases to infectious conditions [8, 30].

The mechanism for the blunted cytokine response remains unclear. Using blood from healthy individuals, we previously showed that acutely inhibiting individual OxPhos complexes reduced both maximal cytokine levels and sensitivity (EC_{50}) to LPS [7]. These pharmacological findings align with our current results in genetically defined mitochondrial diseases. Moreover, our previous acute pharmacological design where OxPhos inhibition was initiated at the same time as the LPS challenge implicates direct intracellular processes in this response, such as energy deficiency, redox alterations, or other intracellular signal. However, in patient leukocytes

where the mtDNA defect exists since conception or during development, or where mtDNA defects trigger systemic, cell non-autonomous effects that influence leukocytes, we cannot rule out other potential factors such as reduced receptor expression (i.e., toll-like receptor 4, for LPS), variable kinetics of cytokine production, or other humoral factors that may exert secondary effects on immune cells [31, 32].

Our report adds to recent preclinical studies implicating immune dysregulation in the pathogenesis of mitochondrial disorders. Compared to wild-type mice, Polg mutator mice (with increased somatic mtDNA mutation burden) injected intraperitoneally with LPS exhibited exaggerated TNF-α and IL-6 responses and increased mortality [33]. In the Ndusf4$^{-/-}$ mouse model of Leigh syndrome, a study noted leukocyte hyperproliferation, while experimentally depleting leukocytes markedly improved survival, suggesting an immune contribution to disease progression [34]. Loss of Ndusf4 in the mouse model of optic neuropathy also elevated innate immune response causing retinal ganglion cell death concurrent with vision loss suggesting mitochondrial complex I (CI) as a modulator of immune signaling in retina [35]. Moreover, global Ndusf4 deletion in mice was associated with systemic inflammation and osteoporosis [36], consistent with a role of mitochondrial OxPhos regulation of inflammatory pathways either in immune or non-immune cells. Additionally, mice lacking a cytochrome c oxidase subtype (*Cox10*) in T lymphocytes also demonstrated immunodeficiency due to compromised T cell proliferation and apoptotic phenotype [37]. Thus, available data in animal models converge on a general role of mitochondria on immune regulation, and cytokine production, although the specific manifestations and magnitude of the reported effects vary between studies and models. Considering potential discrepancies between rodent and human immune characteristics and regulatory features [38], our data add to these and other studies to document abnormal cytokine responses in human mitochondrial diseases. Additional work in affected patients is necessary to understand the basis for these effects.

An essential aspect of immune function is the regulation of cytokines by secondary neuroendocrine factors, such as glucocorticoids [39]. Natural differences in GC-sensitivity attributable to glucocorticoid receptor subtypes have been previously documented [40] and clinically may underlie the resistance to anti-inflammatory glucocorticoid treatment, i.e., glucocorticoid resistance [41]. To our knowledge, immune glucocorticoid sensitivity has not previously been investigated in mitochondrial diseases. Our finding that deletion patients are more sensitive to GC-induced IL-6 suppression suggests the opposite to glucocorticoid resistance, possibly representing a form of glucocorticoid "hypersensitivity" as it relates to IL-6 cytokine downregulation. One study showed that the immunosuppressed phenotype of mice with high corticosterone was partially rescued by pyruvate supplementation [42], suggesting that mitochondrial metabolism may directly contribute to leukocyte glucocorticoid sensitivity. This mitochondrial regulation of glucocorticoid sensitivity is also in line with previous work where we showed in the context of whole blood LPS stimulation that inhibiting complex I increased leukocyte GC sensitivity, or Dex-mediated IL-6 suppression, by 12.3% [7]. Thus, in the absence of a normal OxPhos system, or possibly to secondary systemic signals of OxPhos dysfunction, stimulated leukocyte cytokine production is less robust, and more easily suppressed. If these effects are confirmed, increased glucocorticoid sensitivity in subgroups of patients with mitochondrial diseases could have implications for their clinical care, possibly warranting additional caution for the use of steroid therapy in this population. We also note that the immunosuppressive glucocorticoid hormone cortisol is endogenously produced by the hypothalamic–pituitary–adrenal (HPA) axis in response to physiological and psychosocial stressors [43]. Animal studies have shown that mitochondrial OxPhos and redox defects can influence HPA axis function [44, 45]. If also present in patients with mtDNA defects, neuroendocrine dysregulations could interact with immune cytokine production and possibly influence both the immune phenotype and psychophysiological resilience of patients with mitochondrial diseases.

Some limitation of this study should be noted. First, the sample size is small, which precludes generalization to other patient groups, and calls for replication. Nevertheless, a strength of our study is the homogeneity of our disease groups, which either harbor the same mtDNA point mutation (m.3243A > G) or a single, large-scale mtDNA deletion, established by clinical genetic testing. Leukocyte heteroplasmy could not be determined in this study and represents a major factor that future adequately powered studies need to address, in multiple tissues other than blood. Large-scale mtDNA deletions are depleted from immune cell lineages, and thus are rarely detectable in blood [46–48] possibly due to purifying selection in blood leukocytes and bone marrow [49]. In comparison, the m.3243A > G mutation and other point mutations are typically present in immune cells and may segregate between different immune lineages [50]. Hence, our main observation regarding blunted cytokine response in circulating leukocytes of deletion patients is particularly intriguing. The specificity of this finding for deletion, but not 3243A > G carriers, could suggest cell non-autonomous effects mediated by unknown humoral factors arising from somatic tissues harboring mtDNA deletions [46, 51–53]. We also note that the greater disease severity of the deletion group could contribute to explain the group differences, independently from the specific genetic defects. Although no patient was on steroid hormone, we also cannot rule out the possibility that medications may non-specifically influence cytokine responses,

which our sample did not allow to control. Finally, extracellular cytokine levels were measured at a single time-point (6 h post-stimulation) which may limit the detection of cytokines peaking at earlier or later time-points. Further time-resolved data could refine our understanding of the dynamics and magnitude of cytokine response mitochondrial diseases.

In summary, our ex vivo results in a small cohort of patients with two different mtDNA defects provide preliminary evidence that immune sensitivity and cytokine responses are blunted in patients with single, large-scale mtDNA deletions. Thus, this work provides initial evidence in need of validation in larger studies, and if confirmed could help to understand why adult patients with mitochondrial diseases are at increased risk to die of infectious conditions.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s00109-022-02206-2.

Acknowledgements We thank the patients and healthy volunteers who participated in this study, Johanne Fortune for assistance with phlebotomy, Logan Beharry for assistance with the systematic literature review, and the rest of the MiSBIE Team.

Author contribution K.R.K. and M.P. designed the study. C.T., M.H., M.P. developed the clinical protocol. K.E. and M.H. provided the clinical diagnosis and recruited the patients. M.C. coordinated the study activities and collected the data. K.R.K. performed the LPS stimulation and cytokine measurements. K.R.K. analyzed the data and prepared the figures. K.R.K. and M.P. drafted the manuscript with P.M. All the authors reviewed and edited the final version of this manuscript.

Funding This work was supported by the Wharton Fund, the Irving Scholars Program, the Baszucki Brain Research Fund, and the National Center for Advancing Translational Sciences through grant numbers UL1TR001873, P30CA013696, and NIH grants R21MH113011 and R01MH119336 to M.P. and M.H. The content of this article is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Data availability Requests for additional information and data will be fulfilled by the corresponding author.

Declarations

Consent to participate and publish Informed consent was obtained in compliance with guidelines of the Institutional Review Board of the New York State Psychiatric Institute IRB#7424. All the participants provided informed consent for the study procedures and publication of data.

Competing interests The authors declare no competing interests.

References

1. Angajala A et al (2018) Diverse roles of mitochondria in immune responses: novel insights into immuno-metabolism. Front Immunol 9:1605
2. Shi L et al (2019) Biphasic dynamics of macrophage immunometabolism during mycobacterium tuberculosis infection. mBio 10(2)
3. Gleeson LE et al (2016) Cutting edge: mycobacterium tuberculosis induces aerobic glycolysis in human alveolar macrophages that is required for control of intracellular bacillary replication. J Immunol 196(6):2444–2449
4. Van den Bossche J et al (2016) Mitochondrial dysfunction prevents repolarization of inflammatory macrophages. Cell Rep 17(3):684–696
5. Dumitru C, Kabat AM, Maloy KJ (2018) Metabolic adaptations of CD4(+) T cells in inflammatory disease. Front Immunol 9:540
6. Weiss SL et al (2019) Mitochondrial dysfunction is associated with an immune paralysis phenotype in pediatric sepsis. Shock
7. Karan KR et al (2020) Mitochondrial respiratory capacity modulates LPS-induced inflammatory signatures in human blood. Brain Behav Immun Health 5
8. Walker MA et al (2014) Predisposition to infection and SIRS in mitochondrial disorders: 8 years' experience in an academic center. J Allergy Clin Immunol Pract 2(4):465–468, 468 e1
9. Barends M et al (2016) Causes of death in adults with mitochondrial disease, in JIMD Reports, Volume 26, E. Morava, et al., Editors. Springer Berlin Heidelberg: Berlin, Heidelberg 103–113
10. Eom S et al (2017) Cause of death in children with mitochondrial diseases. Pediatr Neurol 66:82–88
11. Kapnick SM, Pacheco SE, McGuire PJ (2018) The emerging role of immune dysfunction in mitochondrial diseases as a paradigm for understanding immunometabolism. Metabolism 81:97–112
12. Albrecht LJ et al (2016) Lack of proinflammatory cytokine interleukin-6 or tumor necrosis factor receptor-1 results in a failure of the innate immune response after bacterial meningitis. Mediators Inflamm 2016:7678542
13. Manderson AP et al (2007) Subcompartments of the macrophage recycling endosome direct the differential secretion of IL-6 and TNFalpha. J Cell Biol 178(1):57–69
14. Netea MG et al (2009) Differential requirement for the activation of the inflammasome for processing and release of IL-1beta in monocytes and macrophages. Blood 113(10):2324–2335
15. Kany S, Vollrath JT, Relja B (2019) Cytokines in inflammatory disease. Int J Mol Sci 20(23)
16. Domin R et al (2021) Effect of various exercise regimens on selected exercise-induced cytokines in healthy people. Int J Environ Res Public Health 18(3)
17. Hamer M, Steptoe A (2007) Association between physical fitness, parasympathetic control, and proinflammatory responses to mental stress. Psychosom Med 69(7):660–666
18. Marsland AL et al (2017) The effects of acute psychological stress on circulating and stimulated inflammatory markers: a systematic review and meta-analysis. Brain Behav Immun 64:208–219
19. Steptoe A, Hamer M, Chida Y (2007) The effects of acute psychological stress on circulating inflammatory factors in humans: a review and meta-analysis. Brain Behav Immun 21(7):901–912
20. Bhattacharyya S et al (2007) Macrophage glucocorticoid receptors regulate Toll-like receptor 4-mediated inflammatory responses by selective inhibition of p38 MAP kinase. Blood 109(10):4313–4319
21. Horton DL, Remick DG (2010) Delayed addition of glucocorticoids selectively suppresses cytokine production in stimulated human whole blood. Clin Vaccine Immunol 17(6):979–985
22. Quax RA et al (2013) Glucocorticoid sensitivity in health and disease. Nat Rev Endocrinol 9(11):670–686
23. Schaefer A et al (2006) Mitochondrial disease in adults: a scale to monitor progression and treatment. Neurology 66(12):1932–1934
24. Chow JC et al (1999) Toll-like receptor-4 mediates lipopolysaccharide-induced signal transduction. J Biol Chem 274(16):10689–10692

25. Pugin J, Schürer-Maly CC, Leturcq D, Moriarty A, Ulevitch RJ, Tobias PS (1993) Lipopolysaccharide activation of human endothelial and epithelial cells is mediated by lipopolysaccharide-binding protein and soluble CD14. Proc Natl Acad Sci USA 90:p 2744–2748
26. Strahler J, Rohleder N, Wolf JM (2015) Acute psychosocial stress induces differential short-term changes in catecholamine sensitivity of stimulated inflammatory cytokine production. Brain Behav Immun 43:139–148
27. Alm JJ et al (2012) Transient 100 nM dexamethasone treatment reduces inter- and intraindividual variations in osteoblastic differentiation of bone marrow-derived human mesenchymal stem cells. Tissue Eng Part C Methods 18(9):658–666
28. Hedges LV, Olkin I (1985) Statistical methods in meta-analysis. 1st ed: Acad Press Inc
29. Barnes PJ, Adcock IM (2009) Glucocorticoid resistance in inflammatory diseases. The Lancet 373
30. Pickett SJ et al (2018) Phenotypic heterogeneity in m.3243A>G mitochondrial disease: The role of nuclear factors. Ann Clin Transl Neurol 5(3):333–345
31. Vattemi G et al (2013) Overexpression of TNF-alpha in mitochondrial diseases caused by mutations in mtDNA: evidence for signaling through its receptors on mitochondria. Free Radic Biol Med 63:108–114
32. Maresca A et al (2020) Expanding and validating the biomarkers for mitochondrial diseases. J Mol Med (Berl) 98(10):1467–1478
33. Lei Y et al (2021) Elevated type I interferon responses potentiate metabolic dysfunction, inflammation, and accelerated aging in mtDNA mutator mice. Sci Adv 7(22):eabe7548
34. Stokes JC et al (2022) Leukocytes mediate disease pathogenesis in the Ndufs4(KO) mouse model of Leigh syndrome. JCI Insight
35. Yu AK et al (2015) Mitochondrial complex I deficiency leads to inflammation and retinal ganglion cell death in the Ndufs4 mouse. Hum Mol Genet 24(10):2848–2860
36. Jin Z et al (2014) Mitochondrial complex i activity suppresses inflammation and enhances bone resorption by shifting macrophage-osteoclast polarization. Cell Metab 20(3):483–498
37. Tarasenko TN et al (2017) Cytochrome c oxidase activity is a metabolic checkpoint that regulates cell fate decisions during t cell activation and differentiation. Cell Metab 25(6):p 1254–1268 e7
38. Seok J et al (2013) Genomic responses in mouse models poorly mimic human inflammatory diseases. Proc Natl Acad Sci 110(9):3507–3512
39. Pace TW, Hu F, Miller AH (2007) Cytokine-effects on glucocorticoid receptor function: relevance to glucocorticoid resistance and the pathophysiology and treatment of major depression. Brain Behav Immun 21(1):9–19
40. King EM et al (2013) Glucocorticoid repression of inflammatory gene expression shows differential responsiveness by transactivation- and transrepression-dependent mechanisms. PLoS ONE 8(1):e53936
41. Yang N, Ray DW, Matthews LC (2012) Current concepts in glucocorticoid resistance. Steroids 77(11):1041–1049
42. Neigh GN et al (2004) Pyruvate prevents restraint-induced immunosuppression via alterations in glucocorticoid responses. Endocrinology 145(9):4309–4319
43. Russell G, Lightman S (2019) The human stress response. Nat Rev Endocrinol 15(9):525–534
44. Meimaridou E et al (2012) Mutations in NNT encoding nicotinamide nucleotide transhydrogenase cause familial glucocorticoid deficiency. Nat Genet 44(7):740–742
45. Picard M et al (2015) Mitochondrial functions modulate neuroendocrine, metabolic, inflammatory, and transcriptional responses to acute psychological stress. Proc Natl Acad Sci U S A 112(48):E6614–E6623
46. Jeppesen TD, Duno M, Vissing J (2020) Mutation load of single, large-scale deletions of mtDNA in mitotic and postmitotic tissues. Front Genet 11:547638
47. Lee HF et al (2007) The neurological evolution of Pearson syndrome: case report and literature review. Eur J Paediatr Neurol 11(4):208–214
48. Trifunov S et al (2018) Clonal expansion of mtDNA deletions: different disease models assessed by digital droplet PCR in single muscle cells. Sci Rep 8(1):11682
49. Palozzi JM, Jeedigunta SP, Hurd TR (2018) Mitochondrial DNA purifying selection in mammals and invertebrates. J Mol Biol 430(24):4834–4848
50. Walker MA et al (2020) Purifying selection against pathogenic mitochondrial DNA in human T cells. N Engl J Med 383(16):1556–1563
51. Forsström SJC, Carroll CJ, Kuronen M, Pirinen E, Pradhan S, Marmyleva A, Auranen M, Kleine IM, Khan NA, Roivainen A et al (2019) Fibroblast growth factor 21 drives dynamics of local and systemic stress responses in mitochondrial myopathy with mtDNA deletions. Cell Metab 30:p 1040–1054
52. Lehtonen JM, Bottani E, Viscomi C, Baris OR, Isoniemi H, Höckerstedt K, Österlund P, Hurme M, Jylhävä J, Leppä S et al (2016) FGF21 is a biomarker for mitochondrial translation and mtDNA maintenance disorders. Neurology 29(87):p 2290–2299
53. Sharma R et al (2021) Circulating markers of NADH-reductive stress correlate with mitochondrial disease severity. J Clin Invest 131(2)