

Letter to the Editor

Phagocyte nicotinamide adenine dinucleotide phosphate oxidase activity in patients with inherited IFN- γ R1 or IFN- γ R2 deficiency

To the Editor:

An understanding of protective immunity to mycobacterial infection is critical for the development of effective strategies to control tuberculosis (TB), a major public health problem worldwide. Mendelian susceptibility to mycobacterial disease (MSMD) is a rare condition characterized by clinical disease caused by weakly virulent mycobacteria, such as *Mycobacterium bovis* Bacille Calmette-Guérin (BCG) vaccines and nontuberculous, environmental mycobacteria (OMIM209950).¹ Patients are also susceptible to *M. tuberculosis*. Nine genes have been found to be mutated in patients with MSMD (*IFNGR1*, *IFNGR2*, *STAT1*, *IL12B*, *IL12RB1*, *IRF8*, *ISG15*, *NEMO*, and *CYBB*). All these genes are involved in IFN- γ immunity, which is therefore essential for defense against mycobacterial infections in humans.² X-linked recessive (XR2) MSMD caused by mutations in *CYBB* results in impaired nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity in monocyte-derived macrophages (MDMs) and EBV-transformed B (EBV-B) cell lines but not in monocytes or granulocytes.³ In contrast, germline mutations in *CYBB* that impair NADPH oxidase activity in all cell types result in X-linked chronic granulomatous disease (CGD).⁴ An increasing number of case reports from various countries have shown that BCG disease and TB are important features of CGD, particularly in countries in which BCG vaccine is routinely administered, TB is endemic, or both.⁴

Macrophages are known to be the first line of defense against mycobacteria, generating the reactive oxygen species and probably responsible for microbicidal activity.⁵ Phagocyte NADPH oxidase activity can be enhanced by treatment with IFN- γ , and the corresponding genes can also be induced by IFN- γ .⁶ A contribution of NADPH oxidase deficiency to mycobacterial disease in patients with inborn errors of IFN- γ is however uncertain. The occurrence of BCG disease and TB in patients with CGD and in patients with macrophage-tropic mutations of the NADPH oxidase complex suggests that impaired macrophage NADPH oxidase activity may contribute to both diseases in patients with IFN- γ R deficiency.⁴ We therefore tested the hypothesis that the function of the NADPH oxidase complex might be partly dependent on IFN- γ , at least in human MDMs *in vitro*.

We first evaluated NADPH oxidase activity in EBV-B cells from patients with MSMD without cellular responses to IFN- γ (IFN- γ R1/IFN- γ R2 [c] complete deficiencies), patients without NADPH oxidase activity (CGD), and healthy controls. We selected EBV-B cells from patients with cIFN- γ R1 (n = 9) or cIFN- γ R2 (n = 6) deficiencies, XR2-MSMD (n = 6), or CGD (n = 18), and healthy controls. We assessed the ability of EBV-B cells to produce superoxide (O₂⁻) by the cytochrome-c reduction assay and to release hydrogen peroxide (H₂O₂) by reducing 10-acetyl-3,7 dihydroxyphenoxazine (Resurin or Amplex Red). We also investigated the NADPH oxidase activity by using luminal and isoluminol assays (see Fig E1, A and B, in this article's [Online Repository](#) at www.jacionline.org). As previously reported,³ the EBV-B cells of healthy controls produced and

released reactive oxygen species after stimulation with a phorbol ester, such as phorbol 12-myristate 13-acetate, for 2 hours. All EBV-B cells from patients with cIFN- γ R1/cIFN- γ R2 had levels of O₂⁻ production similar to those of healthy controls (Fig 1, A, and Fig E1, A), unlike the EBV-B cells of patients with CGD and XR2-MSMD. However, H₂O₂ release from the EBV-B cells of all patients with cIFN- γ R1/cIFN- γ R2 deficiencies was severely impaired (Fig 1, B, and Fig E1, B).

We further explored MDMs from patients with cIFN- γ R1 (n = 1) or cIFN- γ R2 (n = 2) deficiency. MDMs from these patients produced very small amounts of H₂O₂ after stimulation with IFN- γ or PPD (purified protein derivative from *M. tuberculosis*), suggesting an impairment in NADPH oxidase activity (Fig 1, C and D). This is consistent with the findings for EBV-B cells (Fig 1, B). We also assessed NADPH oxidase activity in monocyte-derived dendritic cells (MDDCs) from all patients. MDDCs from patients with XR2-MSMD have not been tested previously.³ We derived MDDCs *in vitro* by treatment with GM-CSF plus IL-13,⁷ and the respiratory burst was evaluated with Amplex Red. MDDCs from healthy controls released H₂O₂, and, as expected, MDDCs from patients with CGD did not (Fig 1, E). Notably, MDDCs from patients with XR2-MSMD (n = 4) and cIFN- γ R1 (n = 1) or cIFN- γ R2 (n = 1) deficiency produced amounts of H₂O₂ similar to those obtained from healthy controls (Fig 1, E). The impairment of NADPH oxidase activity was, therefore, cell-specific and restricted to MDMs in these patients. Conversely, the normal respiratory burst activity of granulocytes and monocytes from patients with MSMD with cIFN- γ R1 or cIFN- γ R2 deficiency (data not shown), like that herein documented in MDDCs, may account for their protection against fungi and bacteria other than mycobacteria, whereas patients with CGD are typically susceptible to these microbes.

In conclusion, these results support a role for the IFN- γ pathway in the upregulation of NADPH oxidase activity in MDMs and EBV-B cells and suggest that impairment of the phagocyte respiratory burst contributes to BCG disease and TB in patients with inborn errors of IFN- γ immunity. IFN- γ can regulate NADPH oxidase activity in different cell types.⁸ O₂⁻ is rapidly converted to H₂O₂ by spontaneous and enzymatic dismutation. Differences between the amounts of H₂O₂ and O₂⁻ produced in response to phorbol 12-myristate 13-acetate were detected in the same cells. There are several possible reasons for this, including the role of endogenous superoxide dismutase in catalyzing the dismutation of O₂⁻ to H₂O₂, differences in kinetics and the techniques used to measure O₂⁻ and H₂O₂ levels, and variability due to differences in the pH of assay buffers. It was striking that some mycobacteria, for example, *M. tuberculosis*, produced the enzyme such as KatG, a catalase-peroxidase, that protect from killing by H₂O₂ but not O₂⁻ in mice.⁹ Probably NADPH oxidase activity contributes to cytokine production, granuloma genesis, or autophagy more than does the killing of mycobacteria.⁵ The mechanisms affecting this activity in MDMs and EBV-B cells from patients with MSMD remain unclear and require further investigation.

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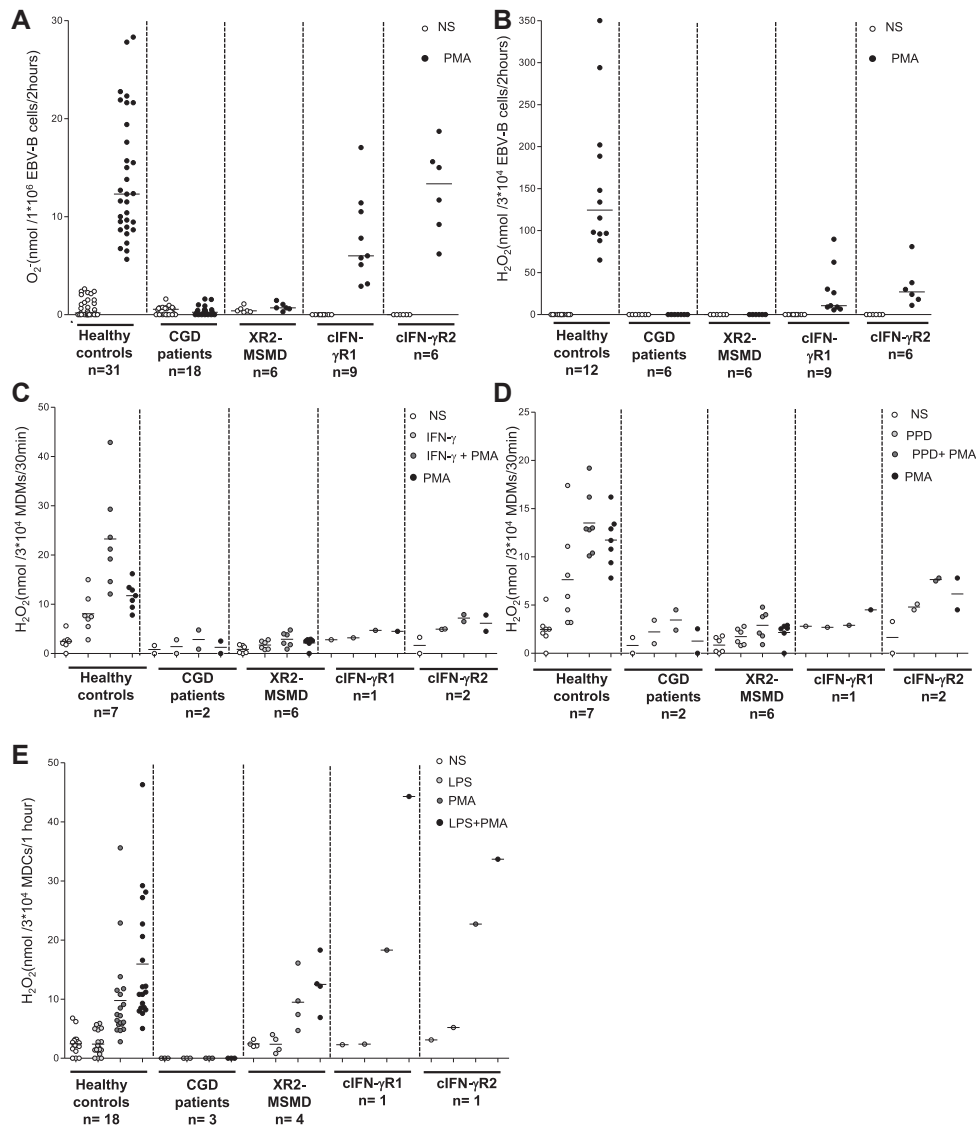


FIG 1. NADPH oxidase activity in human EBV-B cells, MDDCs, and MDMs. **A**, O_2^- generation of EBV-B cells from healthy controls ($n = 31$), patients with CGD ($n = 18$) and XR2-MSMD ($n = 6$), and patients with IFN- γ R1/IFN- γ R2 complete (c) deficiencies ($n = 9$ and 6 , respectively), measured by cytochrome-c reduction test after 2 hours of PMA (400 ng/mL) activation. Each symbol represents an individual subject. **B**, Fluorimetric quantification of H_2O_2 release from EBV-B cells of healthy controls ($n = 12$), patients with CGD ($n = 6$) and XR2-MSMD ($n = 6$), and patients with cIFN- γ R1/cIFN- γ R2 deficiency ($n = 9$ and 6 , respectively), measured by Amplex Red assay after 2 hours PMA (400 ng/mL) activation. **C** and **D**, Fluorimetric quantification of H_2O_2 release after 30 minutes from MDMs of healthy controls ($n = 7$), patients with CGD ($n = 2$), and patients with cIFN- γ R1/cIFN- γ R2 deficiency ($n = 1$ and 2 , respectively), measured by Amplex Red assay and then left untreated (NS) or treated for 18 hours with IFN- γ (1×10^5 IU/mL), PPD (1 mg/mL), followed by no trigger or by treatment with PMA (400 ng/mL) activation. **E**, Release of H_2O_2 from MDDCs obtained from healthy controls ($n = 18$), patients with CGD ($n = 3$), patients with cIFN- γ R1/cIFN- γ R2 deficiency ($n = 1$ and 1 , respectively), and patients with XR2-MSMD ($n = 4$), then left untreated (NS) or treated with LPS, followed by no trigger or by treatment with PMA (400 ng/mL). Each symbol represents an individual subject. Data are representative of 2 experiments (Fig 1, A and B; mean of duplicates) and mean of duplicates (Fig 1, C-E). PMA, Phorbol 12-myristate 13-acetate; PPD, purified protein derivative from *M tuberculosis*.

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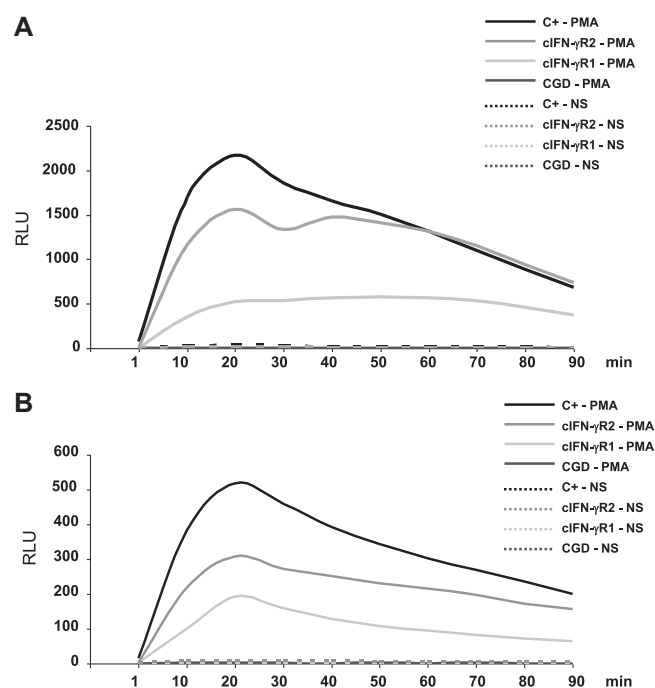


FIG E1. NADPH oxidase activity in human EBV-B cells. **A**, Monitoring of intracellular activity by luminol untreated or treated with PMA (400 ng/mL) using 350,000 EBV-B cells from healthy controls ($n = 7$), patients with CGD ($n = 3$), and patients with cIFN- γ R1 ($n = 5$) and cIFN- γ R2 ($n = 3$) deficiency. **B**, Monitoring of extracellular activity by isoluminol in response to PMA (400 ng/mL). We used 350,000 EBV-B cells from healthy controls ($n = 7$), patients with CGD ($n = 3$), and patients with cIFN- γ R1 ($n = 5$) and cIFN- γ R2 ($n = 3$) deficiency. PMA, Phorbol 12-myristate 13-acetate; RLU, relative light units. The kinetic data are mean of duplicates for 2 independent experiments.