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IFN-α cannot substitute lack of IFN-γ responsiveness in cells of an IFN-γR1 deficient patient

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Abstract Patients with complete IFN-γR deficiency are unable to respond to IFN-γ and have impaired Th1-immunity and recurrent, severe infections with weakly virulent *Mycobacteria*. Since IFN-α and IFN-γ share signalling pathways, treatment with IFN-α has been proposed in complete IFN-γR deficiency. We stimulated cells from healthy controls and from a patient lacking IFN-γR1 with IFN-α and IFN-γ, to establish whether IFN-α would substitute for IFN-γ effects. IFN-α induced STAT1 phosphorylation in monocytes of the IFN-γR1$^{-/-}$ patient, but did not prime for LPS-induced IL-12p70, IL-12p40, IL-23 or TNF production. In control cells, IFN-α inhibited the priming effect of IFN-γ on LPS-induced pro-inflammatory cytokine release. Finally, IFN-γ but not IFN-α induced killing of *M. smegmatis* in cultured macrophages. In conclusion, no evidence was found to support the use of IFN-α in IFN-γR-deficient patients as intervention against mycobacterial infection; on the contrary, treatment of individuals with IFN-α may even adversely affect host defence against *Mycobacteria*.

1. Introduction

Patients with unusual, persistent and severe infections caused by otherwise poorly pathogenic *Mycobacteria* have a condition known as Mendelian Susceptibility to Mycobacterial Disease (MSMD). MSMD is a heterogeneous disorder that can be caused by mutations in the *IL12B*, *IL12RB1*, *IFNGR1*, *IFNGR2* and *STAT1* genes that are involved in the IL-12/-23/IFN-γ cytokine signalling cascade [1]. Due to these defects, no effective immune response is generated in response to mycobacterial infection. Patients with IL-12p40 or IL-12Rβ1 deficiency are unable to produce or respond to IL-12 and IL-23. Since IL-12 and IL-23 signalling is imperative for IFN-γ production, these patients do not produce sufficient IFN-γ to control infections. These patients benefit from treatment with recombinant IFN-γ in addition to antibiotics [2]. Patients with partial IFN-γR deficiency also benefit from treatment with high dose recombinant IFN-γ [3]. A more severe clinical course is seen in complete IFN-γR1 and IFN-γR2 deficient patients whereby these individuals often succumb to mycobacterial infections very early in life [1]. Patients with complete IFN-γR1 or IFN-γR2 deficiencies are unable to respond to IFN-γ and thus will not benefit from treatment with recombinant IFN-γ. The only currently

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available curative treatment of complete IFN-γR deficiency is hematopoietic stem cell transplantation; however, the overall success rate of stem cell transplantation in this setting is low.

Treatment with IFN-α as additional therapy has been described in two patients, with complete IFN-γR1 and complete IFN-γR2 deficiency, respectively, suffering from disseminated infection with *Mycobacterium avium* complex [4,5]. The rationale behind this therapy being that IFN-α and IFN-γ activate common signalling pathways and the induced genes and biological effects partly overlap. Through the activation of overlapping effects of IFN-α and IFN-γ, treatment with exogenous IFN-α is thought to (partly) compensate for the absence of IFN-γ signalling in patients deficient in IFN-γR1 or IFN-γR2. The IFN-α receptor is composed of IFN-αR1 and IFN-αR2 subunits, associated with Janus kinases (JAKs) TYK2 and JAK1. In response to IFN-α, these JAKs are activated and subsequently phosphorylate signal transducer and activator of transcription (STAT) 1 and STAT2 [6]. STAT1 and STAT2 form heterodimers, which associate with interferon regulatory factor-9 (IRF-9) to form a STAT1/STAT2/IRF-9 complex (also known as IFN-stimulated gene factor 3 (ISGF3)) [7]. This complex migrates to the nucleus and regulates primary response genes by binding specific DNA response elements. IFN-γ signals via the IFN-γR1 and IFN-γR2. Upon binding of IFN-γ, receptor-associated JAK1 and JAK2 are phosphorylated, which in mature monocytes and macrophages leads to phosphorylation of STAT1 [8]. STAT1 dimerizes and translocates to the nucleus to regulate primary response gene transcription [8]. STAT1 can, in theory, also form homodimers in response to IFN-α [9]. IFN-α signalling is not affected by deficiencies of the IFN-γR1 or the IFN-γR2.

The potential effect of IFN-α treatment in patients with complete IFN-γR deficiency has not been investigated at the cellular level. We therefore determined whether IFN-α can (partly) compensate for absence of IFN-γ signalling in cells obtained from an IFN-γR1 deficient patient.

2. Materials and methods

2.1. Cell culture conditions and infections

Human CD14$^+$ cells and PBMCs were isolated from healthy donor buffy coats (Sanquin) or from blood from healthy consenting volunteers, or an IFN-γR1 deficient patient (P2 in [10]), by Ficoll-Amidotrizoate density gradient centrifugation and subsequent selection with anti-CD14 MACS beads (Miltenyi Biotech). The purity of monocytes selected with anti-CD14 MACS beads is typically 95–98% in our hands. CD14$^+$ cells were cultured in RPMI-1630 medium, supplemented with 20 mM GlutaMAX (Gibco/ Invitrogen), 10% FCS, 100 U/ml penicillin, and 100 μg/ml streptomycin (Gibco/ Invitrogen).

For assessment of in vitro mycobactericidal activity as described [12], CD14$^+$ cells were cultured for 6 days in the presence of 5 ng/ml GM-CSF (Biosource) to generate pro-inflammatory macrophages (Mφ1) as previously described [11]. These cells were seeded at 3×10^5 in 24-well plates, left to adhere then treated with IFN-α (IFN-α2b (Intron®A)/IFN-α2a (Roferon®A)) and/or IFN-γ (Immukine®, Boehringer) or H-89 (Sigma Aldrich) overnight prior to infecting with log phase growth *M. smegmatis*::GFP at an MOI of 1. Extracellular mycobacteria were eliminated by low dose gentamycin treatment (5 μg/ml) for the duration of the experiment. All cells were subsequently collected, lysed, plated out on 7H9 agar plates and levels of fluorescence were assayed in a Mithras LB940 plate reader after 24 h.

All research involving patient material was approved by the Leiden University Medical Center-Medical Ethical Committee. This research was conducted in accordance with the Declaration of Helsinki.

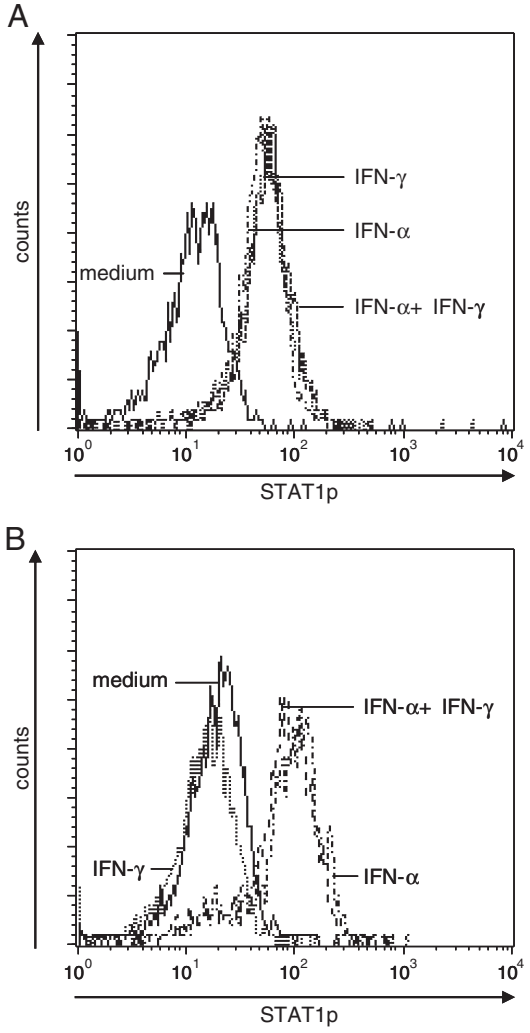

Figure 1 IFN-α induces STAT1 phosphorylation in monocytes from an IFNγR1 deficient patient. Healthy control (A) or patient (B) derived PBMC were stimulated with 1000 U/ml IFN-α, 2.5 ng/ml IFN-γ, 1000 U/ml IFN-α plus 2.5 ng/ml IFN-γ, or left unstimulated for 15 min. Cells were labeled with anti-human pSTAT1-Alexa 647 and CD64-FITC antibodies and analyzed by FACS. Histograms shown are gated on CD64 positive monocytes. In monocytes from a healthy control, IFN-α, IFN-γ and IFN-α plus IFN-γ induced STAT1 phosphorylation. In monocytes from the patient IFN-α and IFN-α plus IFN-γ induced STAT1 phosphorylation, whereas stimulation with IFN-γ alone did not.