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Successful interferon-alpha 2b therapy for unremitting warts in a patient with DOCK8 deficiency

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Abstract The autosomal recessive form of the Hyper IgE syndrome (AR-HIES) with dedicator of cytokinesis 8 (DOCK8) deficiency is associated with difficult to treat persistent viral skin infections, including papilloma virus infection. Type I interferons play an important role in the defense against viruses. We examined the effect of therapy with IFN-α 2b in an 11-year old boy with DOCK8 deficiency due to a homozygous splice donor site mutation in *DOCK8* intron 40. His unremitting warts showed dramatic response to IFN-α 2b therapy. Immunological studies revealed decreased circulating plasmacytoid dendritic cells (pDCs) and profound deficiency of IFN-α production by his peripheral blood mononuclear cells in response to treatment with CpG oligonucleotides. These findings indicate that underlying pDC deficiency and impaired IFN-α production may predispose to chronic viral infections in DOCK8 deficiency. IFN-α 2b therapy maybe useful in controlling recalcitrant viral infections in these patients.

1. Introduction

Dedicator of cytokinesis 8 (DOCK8) deficiency is an autosomal recessive form of hyper-immunoglobulin E (HIES) syndrome that manifests recurrent sinopulmonary infections, viral skin infections, recurrent abscesses, mucocutaneous candidiasis, eczema, hepatic disorders, asthma, multiple food allergies and increased risk for malignancy and autoimmune diseases [1,2]. DOCK8-deficient patients are highly susceptible to viral skin infections including molluscum contagiosum and human papilloma virus that is usually progressive and unfortunately

Abbreviations: DOCK8, dedicator of cytokinesis 8 deficiency; DT, diphtheria and tetanus; EBV, Epstein–Barr virus; HIES, hyper-immunoglobulin E; IFN-α 2b, interferon-alpha 2b; MMR, measles, mumps and rubella; TLR, Toll-like receptors.

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may cause squamous cell carcinomas in some cases [1–3]. The laboratory evaluation usually reveals marked elevations in serum immunoglobulin E (IgE) level, eosinophilia and T-cell lymphopenia with impaired proliferative responses to mitogens [1–3]. We describe an 11 year-old boy with DOCK8 deficiency due to a splice junction mutation in DOCK8 who presented with severe generalized warts that responded to interferon-alpha 2b (IFN-α 2b) therapy.

2. Materials and methods

2.1. PCR and sequence analysis

Genomic DNA and total RNA were prepared from blood, and cDNA was synthesized as previously described [1]. Exons and flanking intron/exon boundaries from DOCK8 were amplified from genomic DNA by PCR according to standard protocols with Taq polymerase. PCR products were purified and the DNA was sequenced with the ABI PRISM BigDye Terminator kit V3.1 (Applied Biosystems, Foster City, Calif), the 3130xl Applied Biosystems Genetic Analyzer, DNA Sequencing Analysis software, version 5.2 (Applied Biosystems), and Sequencher, version 5.0 (Gene Codes Corp, Ann Arbor, Mich).

2.2. Immunoblotting

Epstein Barr virus (EBV)-transformed B cells were derived and lysed in lysis buffer, as described [4]. Whole cell lysates (50 μg/lane) were resolved by SDS-PAGE and transferred to nitrocellulose filters and immunoblotted with polyclonal rabbit anti-DOCK8 and monoclonal anti-human actin antibodies (Sigma-Aldrich). The blots were developed by using horseradish peroxidase-conjugated secondary antibodies and enzyme-linked chemiluminescence, and exposed to film.

2.3. Flow cytometry

For detection of pDCs, PBMCs were stained with anti-human CD123 (AC145) and BDCA-4 (AD5-17F6) mAbs (Miltenyi) with the appropriate isotype controls as described [4]. Standard flow cytometric methods were used for staining. Data collected with an LSRFortessa cell analyzer (BD Biosciences) were analyzed in the lymphocyte gate with FlowJo software (TreeStar).

2.4. IFN-α production

Human PBMCs were isolated from heparinized blood obtained from patients and normal donors using Ficoll-Hypaque (Pharmacia Biotech). Cells were suspended in RPMI-1640 containing 10% heat-inactivated FCS (Hyclone), 2 mM L-glutamine, 50 mg/ml streptomycin and 100 U/ml penicillin. PBMCs (2×10^6 cells/ml) were either unstimulated or stimulated with class A CpG ODN 2216 (0.5 mM Invivogen). IFN-α in culture supernatants was measured after 24 h by

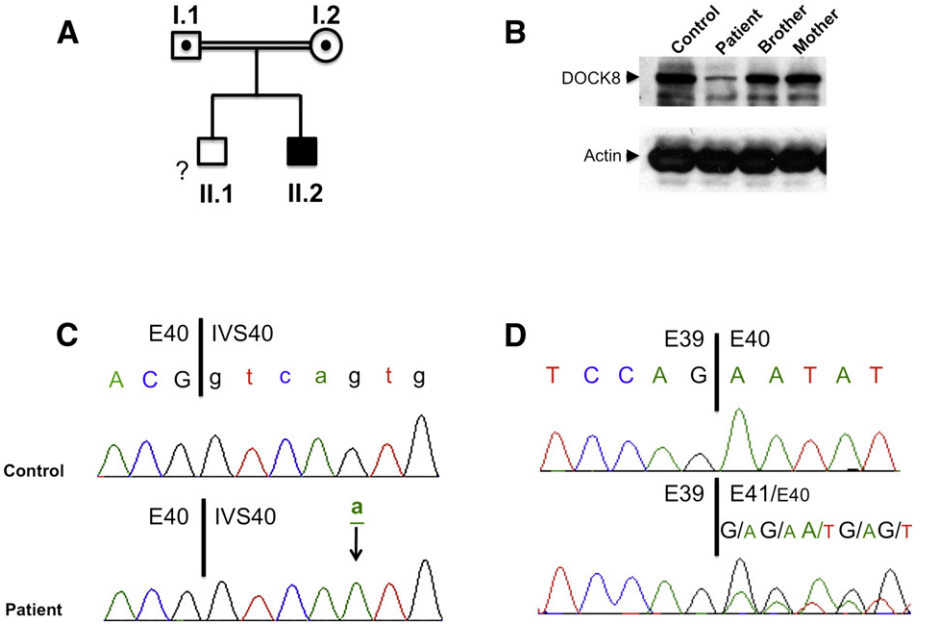

Figure 1 **A**. Pedigree of the patient's family. Each generation is designated by a Roman numeral (I–II), and each individual by an Arabic numeral. Squares, males; circle, female (mother). Filled symbol: patient. Dotted symbol: heterozygote carrier. The double lines connecting the parents denote consanguinity. The genetic status of the brother, who was otherwise healthy, was not determined and is indicated by '?'. **B**. Immunoblot analysis of DOCK8 protein expression in EBV cell lines derived from a control subject, the patient, his brother and mother. The blots were also probed for actin as a protein loading control. **C**. Sanger sequencing chromatograms of *DOCK8* exon (E)40/intervening sequence (IVS) 40 5′ splice junction. Exonic sequence is in upper case and the intronic sequence in lower case. The mutant base (g → a) is underlined. **D**. Sanger sequencing chromatograms of *DOCK8* cDNA at the junction of E39/E40 sequences. For patient sequence, the dominant E39:E41 trace is shown in upper caps while that of the minor E39:E40 trace in smaller caps.