ESID Long-term fellowship 2010

Function and phenotype of a novel IκBα mutation in anhidrotic ectodermal dysplasia with immunodeficiency

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Anhidrotic ectodermal dysplasia with immunodeficiency (EDA-ID) is a rare syndrome characterized by partial or complete absence of eccrine sweat glands, sparse hair growth and conical teeth, combined with severe bacterial and viral infections, and defective immunoglobulin production (Abinun 1995; Carrol et al. 2003). EDA-ID is caused by mutations in genes encoding proteins involved in the activation of Nuclear Factor κB (NF-κB), a transcription factor pivotal in innate and adaptive immune responses, cell adhesion, cell growth, apoptosis, and ectodermal development (Courtois 2005). A schematic overview of NF-κB activation by a variety of stimuli including pro-inflammatory cytokines, tumor necrosis factor (TNF)-α, bacterial lipopolysaccharide (LPS), viral proteins or stress is shown in Figure 1.

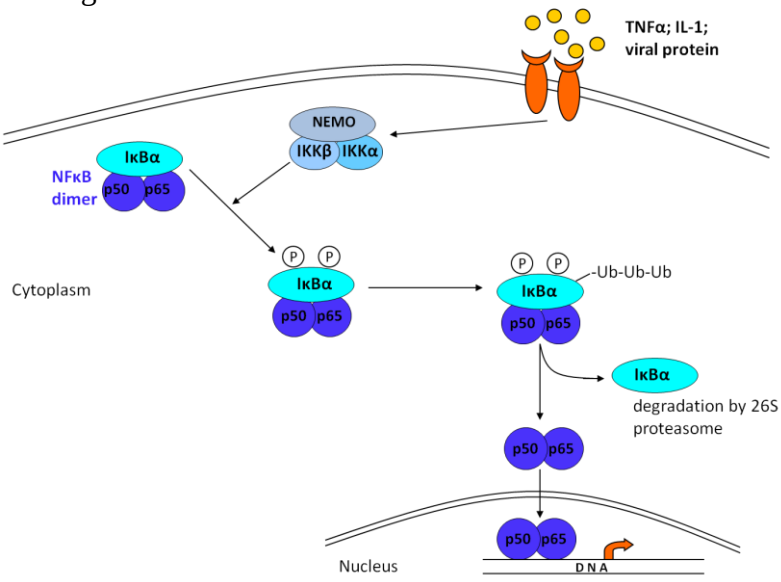

Figure 1: Schematic of events involved in NF-κB activation. Briefly, cytokine or toll-like receptor (TLR) ligand stimulation causes the IκB kinase complex (containing NEMO, IKKα and IKKß) to phosphorylate IκBα on serines 32 and 36 leading to its polyubiquitination and degradation by the 26S proteasome. This unmasks the nuclear localization signals on the NF-κB subunits allowing them to translocate to the nucleus where they regulate transcription of target genes. (Puel et al. 2004)

The majority of patients with this disorder have the X-linked form (XL-EDA-ID) caused by hypomorphic mutations in the gene encoding the Nuclear Factor κB Essential Modulator (NEMO; also known as IKKγ). In addition, to date five patients with EDA-ID and additionally marked lymphocytosis and significant, albeit variable T cell immunodeficiency (Picard et al. 2011) and autosomal dominant inheritance (AD-EDA-ID) caused by hypermorphic mutations in the gene encoding the NF-κB inhibitor IκBα (*NFKBIA;* also known as *IKBA*) have been described (Puel et al. 2004). Three of these patients show point mutations affecting the critical phosphoserine residue at position 32 (p.S32I) (Courtois et al. 2003; Janssen et al. 2004) and 2 show nonsense mutations causing premature termination codons at positions 11 (p.W11X) and 14 (p.E14X) (McDonald et al. 2007; Lopez-Granados et al. 2008). All of these mutations result in an IκBα protein that is resistant to degradation and therefore impair NF-κB activation (Lopez-Granados et al. 2008).

We identified a novel IκBα mutation (p.M37K) in a boy with many features of AD-EDA-ID with additional clinical manifestations not in general associated with this disorder. We aimed to characterize the clinical course of the IκBα defect and to analyze the functional consequences of the p.M37K mutation on NF-κB activation and to determine whether this mutation causes disease by preventing degradation of the IκBα protein thus exerting a dominant-negative effect on NF-κB signaling.

For this we evaluated the patient's immunologic phenotype and lymphocyte proliferation in response to mitogens and antigens using peripheral blood mononuclear cells (PBMCs) according to standard protocols. NF-κB signaling was evaluated by measuring IκBα

degradation in patient's fibroblasts by flow cytometry. In addition, in vitro experiments were performed to prove that the identified alteration is pathogenic and causes a dominant-negative effect using transient transfected HeLa cells expressing the IκBα-M37K mutation, the previously characterized IκBα-S36A-mutant, or IκBα wild type. IκBα degradation after TNF-α and TLR agonists' stimulation was measured by immunoblotting. NF-κB nuclear translocation after TNF-α stimulation was evaluated by electrophoretic mobility shift assay (EMSA) and NF-κB dependent gene transcription was analyzed using the Dual-Luciferase Reporter Assay kit (Promega) according to the manufacturer's instructions. NF-κB function was determined by measuring IL-6 and IL-10 production by patient's whole blood cell which was performed in collaboration with the laboratory of Dr. Jean Laurent Casanova.

All data obtained in this study are in preparation for publication (a copy of which I will submit following acceptance of the manuscript) and summarized here briefly. The clinical and immunologic findings comprised a classical ectodermal dysplasia phenotype and additional findings such as recurrent mucocutaneous candidiasis, autoimmune thyroiditis and panhypopituitarism, and low Th17 cell counts. IκBα degradation was abolished in patient's fibroblasts as well as the cell constructs expressing the IκBα-M37K mutant. Furthermore, we could show that the IκBα-M37K mutant results in impaired nuclear translocation of NF-κB and reduced NF-κB dependent gene transcription similar to the IκBα-S36A mutant indicating a dominant-negative effect of the novel mutation. These data is schematically summarized in Figure 2. The impaired NF-κB function was confirmed by no or reduced IL-6 and IL-10 production by the patient's whole blood leukocytes in response to different NF-κB activators.

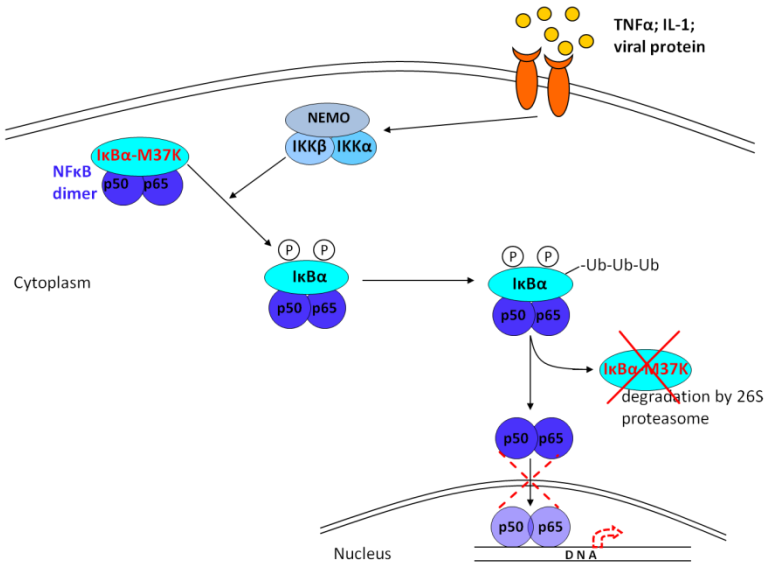

Figure 2: Schematic of functional effects of the novel mutation p.M37K
The IκBα-M37K protein is resistant to degradation (indicated by a red cross) and exerts a dominant-negative effect on NF-κB activation. Reduced NF-κB nuclear translocation and reduced NF-κB dependent gene transcription are indicated by red dashed lines.

Taken together, due to the results obtained during my research period it was possible to show the functional significance of the novel hypermorphic IκBα p.M37K mutation. This expands the spectrum of mutations capable of causing EDA-ID and provides insights in molecular biology mechanisms controlling NF-κB function and adequate immune responses.

Thank you very much for giving me the opportunity to perform this project and spent 5 month in the laboratory of Prof.s Troy R. Torgerson and Hans D. Ochs in Seattle. It was a great experience and important step in my professional career which expanded my knowledge in methodological and clinical aspects in the field of primary immunodeficiencies.

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