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NFKB1 regulates human NK cell maturation and effector functions

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ABSTRACT

NFKB1, a component of the canonical NF-κB pathway, was recently reported to be mutated in a limited number of CVID patients. CVID-associated mutations in *NFKB2* (non-canonical pathway) have previously been shown to impair NK cell cytotoxic activity. Although a biological function of NFKB1 in non-human NK cells has been reported, the role of *NFKB1* mutations for human NK cell biology and disease has not been investigated yet. We decided therefore to evaluate the role of monoallelic *NFKB1* mutations in human NK cell maturation and functions. We show that *NFKB1* mutated NK cells present impaired maturation, defective cytotoxicity and reduced IFN-γ production upon in vitro stimulation. Furthermore, human IL-2 activated NFKB1 mutated NK cells fail to upregulate the expression of the activating marker NKp44 and show reduced proliferative capacity. These data suggest that NFKB1 plays an essential novel role for human NK cell maturation and effector functions.

1. Introduction

The NF-κB (NF-kappaB; nuclear factor of kappa light polypeptide gene enhancer in B cells) signaling pathway plays an important role both in the innate and the adaptive immune system [1–3]. The NF-κB transcription factor family consists of five members: NF-κB1, NF-κB2, RelA, RelB and c-Rel. *NFKB1* encodes the precursor p105 which is processed to the mature p50. *NFKB2* encodes the precursor p100 and the mature p52. The canonical pathway, which includes NFKB1, mediates numerous immunological and inflammatory cellular responses, and may be activated upon stimulation with a broad range of stimuli, including proinflammatory cytokines, activation of innate immune receptors, T-cell receptor (TCR) and B-cell receptor (BCR) signaling, and others [1–3]. The non-canonical pathway, which involves NFKB2, has more restricted immunological functions mainly focusing on B cell homeostasis and is activated upon engagement of a limited set of members of the TNF receptor superfamily, including BAFF receptor, CD40 and the lymphotoxin receptor [2–4].

Data on the NF-κB involvement in NK cell function and maturation are limited. The first indirect description of the biological role of NF-κB in NK cells was derived from in vitro pharmacological NF-κB inhibition that led to impaired NK cell cytotoxicity [5]. Studies on Interleukin-2 (IL-2) induced NK cell functional stimulation have implicated the activation of NF-κB in both, cytotoxic activity and production of Interferon-gamma (IFN-γ) [6]. However, most of these studies did not investigate the role of individual components of the NF-κB signaling pathway. Observations in *Nfkb1*-deficient mice suggested that p50 is a negative regulator of NK cell proliferation and IFN-γ production [7].

Primary immunodeficiencies provide unique opportunities for a better understanding of the human immune system. For instance, congenital mutations in *NIK* [8] or *NEMO* [9], both of which encode upstream components of the NF-κB pathways, have demonstrated their importance for NK cell function. Monoallelic mutations in *NFKB2*, causing a functional haploinsufficiency due to expression of unprocessable p100 precursors have been reported in CVID patients [10–11] and were shown to impair NK cell cytotoxic activity [12]. Recently, monoallelic mutations in *NFKB1* leading to p50 haploinsufficiency have also been reported in three CVID families [13]. However, the impact of these *NFKB1* mutations in human NK cell biology has not been investigated. In the present study, we analyzed NK cell

Abbreviations: NFKB1, Nuclear Factor kappa-B, subunit 1; CVID, Common Variable Immunodeficiency; NFKB2, Nuclear Factor kappa-B, subunit 2; NK cells, natural killer cells; IFN-γ, Interferon-gamma; MFI, mean fluorescence intensity; IL-2, Interleukin-2; IL-12, Interleukin 12; IL-18, Interleukin 18; CMV, Cytomegalovirus; EBV, Epstein Barr Virus; JCV, Poliomavirus JC.

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Table 1
Clinical information of the 7 NFKB1 mutated CVID patients.

Patient ID	Nationality	Sex	Year of birth	Year of diagnosis	IgG[a]	IgA[a]	IgM[a]	Clinical features	Viral infections
Patient 1 [14]	Italian	M	1976	1989	160	10	10	Recurrent pulmonary infections, autoimmune thyroiditis, autoimmune enteropathy, gastric adenoma	No EBV, CMV, HBV, HCV (PCR neg.)
Patient 2[b,c] (Fam191_02)	German	F	1961	1994 (2008)	670 (251)	30 (<6)	30 (<16)	Pneumonias, necrotizing tonsillitis, autoimmune cytopenia (ITP, intermittent leukopenia, anemia), splenomegaly, lymphadenopathy, interstitial lung disease, periodontitis, multiple liver hemangiomas	Herpes zoster (2 episodes) CMV viremia (8500 IE/ml) with CMV colitis treated with valganciclovir; intermittent low grade EBV + CMV replication
Patient 3[b,c] (Fam191_01)	German	M	1963	2006	8	5	5	Recurrent sinusitis, recurrent otitis, pneumonia, salmonella enteritis, autoimmune cytopenia, vitiligo, arthritis, splenomegaly, lymphadenopathy, granulomatous lung disease, persistent CRP elevation	Herpes zoster (3 episodes) No EBV, CMV, HBV (PCR neg.); adenovirus + respiratory virus panel for respiratory infections neg.
Patient 4 [13] (FamNL1-36)	Dutch	F	1961	1991	181	6	48	Recurrent sinusitis, pneumonia, otitis media, severe salmonella enteritis	n.a.
Patient 5 [15] (Fam089II2)	German	F	1979	1995 (2006)	629 (441)	<30 (<7)	<30 (2)	Recurrent pulmonary infections, ITP, autoimmune hemolytic anemia, hepatomegaly, lymphadenopathy	Herpes zoster (2 episodes) No EBV, CMV, HBV, HCV, HIV, HHV8 (PCR neg.); enteritis virus panel for chronic diarrhoea neg.
Patient 6 [15] (Fam830_01)	German	M	1956	2003	270	<6	21	Chronic sinusitis, recurrent otitis, pneumonia, skin abscesses, atopic dermatitis with fungal superinfections, autoimmune enteropathy, nodular regenerative hyperplasia, splenomegaly, lymphadenopathy, thrombocytopenia	JC virus encephalitis Norovirus2 infection with intermittent bloody diarrhoea Herpes zoster (2 episodes) EBV reactivation with 1000 copies/ml, no specific therapy, on follow-up after 1 y neg.
Patient 7[c] (Fam695_01)	German	F	1957	1987 (2014)	n.a. (462)	n.a. (6)	n.a. (15)	Recurrent bronchitis and sinusitis, enteropathy, splenomegaly, basal cell carcinoma, osteoporosis	No EBV, CMV, HBV, HCV (PCR neg.)

Numbers in brackets indicate follow-up examinations.
n.a. not available.
[b] Patients 2 and 3 are siblings.
[a] Expressed in mg/dl.
[c] Not previously described patients.