Heterozygous mutations in RelB can be associated with immune dysregulation and lymphoma

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ABSTRACT

Background: The nuclear factor kappa-B (NFκB) family of transcription factors is essential for numerous processes, including the development and function of the innate and adaptive immune response, inflammation and cell growth, differentiation, and survival. Recently, patients with homozygous mutations in the gene for the NFκB transcription factor *RelB* have been described as presenting with features of combined immunodeficiency such as recurrent infection and failure to thrive as well as reduced response to mitogens and an inability to maintain an adequate antibody response to immunizations.

Methods: The immune status and genetics of the parents of patients with homozygous *RelB* mutations were assessed. In vitro mitogen stimulation, flow cytometry, and cytokine ELISA were used to assess immunological status and signal transduction pathways.

Results: Four patients were confirmed to have heterozygous *RelB* mutations. The majority of patients had evidence of immune dysfunction with impaired in vitro responses to PHA and antigens. One patient developed lymphoma.

Conclusion: Heterozygous *RelB* mutations can be associated with immune dysregulation with impaired mitogen and antigen responses and lymphoma. It is likely that the immune defects apparent in *RelB* deficient humans are due to a wider effect of RelB on the classical NFκB pathway (involving RelA and c-Rel) through cross-regulation of activation and expression in addition to RelB's function within the alternate pathway.

Statement of novelty: We describe for the first time the immune abnormalities in patients with heterozygous *RelB* mutations.

Introduction

The nuclear factor kappa-B (NFκB) family of transcription factors is essential for numerous processes, including the development and function of the innate and adaptive immune response, inflammation and cell growth, differentiation, and survival (Sun 2011). The NFκB signaling pathway consists of 5 major proteins including NFκB 1 p50/p105, NFκB 2 p52/p100, RelA/p65, RelB, and c-Rel (Sun 2011; Millet et al. 2013). These proteins function as dimeric transcription factors that regulate the expression of genes influencing a range of processes including innate and adaptive immunity. The NFκB pathway is divided into 2 main signaling pathways, the classical or canonical pathway mediated by RelA and the alternative or noncanonical pathway mediated by RelB (Sun 2011). The canonical signaling pathway is initiated by a surface receptor, such as TLR4, TNFR, and TCR, causing activation of the IKK complex (IKKβ, IKKα, NEMO), which in turn phosphorylates IκB proteins leading to their proteasomal degradation and release of RelA and p50 heterodimers

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(Millet et al. 2013). The RelA and p50 heterodimers then translocate into the nucleus where they bind NFkB sites and induce target gene expression (Millet et al. 2013).

The noncanonical pathway is activated through extracellular signals, such as LTβR, CD40L, BAFF, and TWEAK, which cause TRAF3 degradation in the proteosome and stabilization of NIK (Millet et al. 2013). IKKα is then activated, resulting in the proteosomal processing of p100, converting it to p52 (Millet et al. 2013). This creates transcriptionally competent NFκB p52/RelB complexes that translocate to the nucleus and induce target gene expression (Millet et al. 2013). In addition to its receptor activated functions, the presence of RelB is proposed to be necessary for the regulation of other family members (Oeckinghaus et al. 2011; Sun 2011; Millet et al. 2013). *RelB* knockout mice show that the protein plays an important role in the development of lymphoid tissue as knockout mice often lack splenic and thymic structures, including the germinal centers, marginal zones, and thymic medulla (Burkly et al. 1995; Weih et al. 1995). Furthermore, *RelB* knockout mice often die early of severe multisystem inflammatory syndrome with T-cell and monocytic infiltrates in multiple organs (Burkly et al. 1995).

Primary immunodeficiency can result from mutations at various stages in the NFκB pathway. Specifically, mutations in NFκB Essential Modulator (NEMO, also known as inhibitor factor of nuclear factor kappa-B kinase subunit gamma (IKKγ)), IκBα, and inhibitor of NFκ-B kinase subunit beta (IKKβ, also known as IKK2) have been described (Doffinger et al. 2001; Courtois et al. 2003; Janssen et al. 2004; Orange et al. 2004; Lopez-Granados et al. 2008; Picard et al. 2011; Pannicke et al. 2013; Schimke et al. 2013). Recently, Merico et al. (2015) described homozygous mutations in the gene encoding *RelB* (c.1191 C > A; Y397 stop) resulting in the premature stop and the ablation of RelB expression. The 3 patients described had features of combined immunodeficiency with repeated infections and failure to thrive. Immune assessment revealed normal to increased numbers of circulating lymphocytes that were unresponsive to mitogens or antigens in vitro and poor specific antibody response. Further studies reported that these patients highlighted that absence of RelB expression lead to intrinsic defects in both T and B lymphocyte maturation and function resulting in combined immunodeficiency and autoimmunity (Sharfe et al. 2015).

Here we present the parents of the 3 patients previously described with homozygous *RelB* mutations and highlight that heterozygous mutations in *RelB* can be associated with immune dysregulation and lymphoma.

Methods

Subjects

Subjects include the parents of patients recently described with homozygous *RelB* mutations (Merico et al. 2015). Patient data were compiled prospectively and retrospectively from medical records and were entered into the Canadian Centre for Primary Immunodeficiency Registry and tissue bank, which was approved by the SickKids Research Ethics Board (protocol # 1000005598). This included consent for patients and parents for genetic analysis, immune evaluation, collection of tissue and permission to publish.

T-cell proliferative responses

Lymphocyte proliferative responses to mitogens (including phytohemagglutinin (PHA) and anti-CD3 antibodies) and to a panel of recall antigens (including candida, tetanus, herpes zoster, and cytomegalovirus) were determined by thymidine incorporation at day 3 or day 6. All assays were performed in triplicate and were compared with simultaneously stimulated random normal controls.

Immunoglobulin and specific antibody determinations

Serum concentrations of immunoglobulins were measured by nephelometry. Serum IgE concentration was measured by radioimmunoassay with the IgE PRIST kit (Pharmacia Diagnostics, Quebec, Canada). Levels of serum antibodies to tetanus were measured by ELISA.

Cytokine secretion

Peripheral blood mononuclear cells (PBMCs) were cultured with PHA for 24 and 48 hours and culture supernatants collected for analysis of IL2 and IFNγ by ELISA. Kits were from R&D Systems Inc. (Minneapolis, MN).

Results

Case reports

Father 1 (F1) and Mother 1 (M1) are both of Irish descent and are the parents of patients 1 and 2 as

described in Merico et al. (2015). Medical history was remarkable for asthma in F1 and psoriasis and atopic dermatitis in M1.

Father 2 (F2) and Mother 2 (M2) are also of Irish descent and are the parents of patient 3 described in Merico et al. (2015) and Sharfe et al. (2015). M2 has an unremarkable past medical history. F2 had a diagnosis of asthma as a child. At the age of 27 years, F2 presented with soft tissue mass in the right supraclavicular area. CT scan of his neck, chest, abdomen, and pelvis reported multiple pathologically enlarged lymph nodes along the parotid chain. Excisional biopsy confirmed Hodgkin's lymphoma, nodular sclerosing subtype. Bone marrow biopsy had normal trilineage hematopoietic marrow with no morphologic evidence of Hodgkin's lymphoma. He was managed with chemotherapy and has been in remission for 2 years. Family history was remarkable for a first cousin who also had Hodgkin's lymphoma successfully treated with chemotherapy and a grandmother and grandfather with colon cancer.

Evaluation of the immune system

All patients displayed normal numbers of circulating white blood cells (Table 1). Lymphocyte immunophenotyping revealed that circulating lymphocyte numbers were normal to elevated in all 4 patients (Table 1). In vitro responses to PHA and antigens were low in all patients except M2. F2 and M2 had appropriate levels of immunoglobulins and response to vaccines (vaccine response was not measured in F1 and M1).

Cytokine secretion

PBMCs were cultures with PHA for 24 and 48 hours and culture supernatants collected for analysis of IL2 and IFNγ by ELISA. Patients heterozygous for the RelB mutation had markedly reduced PHA induced production of both IL2 and IFNγ (Figure 1).

Discussion

A novel type of combined immunodeficiency associated with a homozygous mutation in the *RelB* gene has recently been described (Merico et al. 2015; Sharfe

Table 1: **Immune evaluation**

	Family 1		Family 2		
	Father 1	Mother 1	Father 2	Mother 2	Normal
Clinical features	Asthma	Psoriasis, eczema	Lymphoma	—	—
White blood cell	11.0	6.0	12.4	6.2	$4.0–10 \times 10^9$/L
Lymphocyte count	3.0	2.65	3.91	1.62	$1.5–4.0 \times 10^9$/L
Markers (cells/μL)					
CD3	1932	2017	2564	1984	700–2100
CD4	1290	1396	1761	1460	300–1400
CD8	612	570	646	456	200–900
CD19	570	383	492	370	100–500
CD56	486	286	646	189	90–600
Response to mitogens					
Phytohemagglutinin	42	23	27	72	>50% of control
Response to antigens					
Candida	4.9	20.0	25	43	>25 SI
Tetanus	2.0	2.5	1.6	2.4	>25 SI
Zoster	7.0	9.8	6.9	64.6	>25 SI
Simplex	21.8	19.6	27	174.6	>25 SI
CMV	9.5	2.7	2.9	4.6	>25 SI
Immunoglobulins and antibodies					
IgG	11.9	11.2	8.7	13.8	7.2–15.8 g/L
IgA	1.5	1.1	0.5	2.1	0.5–3.5 g/L
IgM	1.4	1.9	0.3	1.9	0.2–3.1 g/L
IgE	ND	ND	103	51	<100 PU/mL
Anti-tetanus	ND	ND	0.35	0.51	>0.05 IU
TREC	319	487			

Note: ND, not performed.

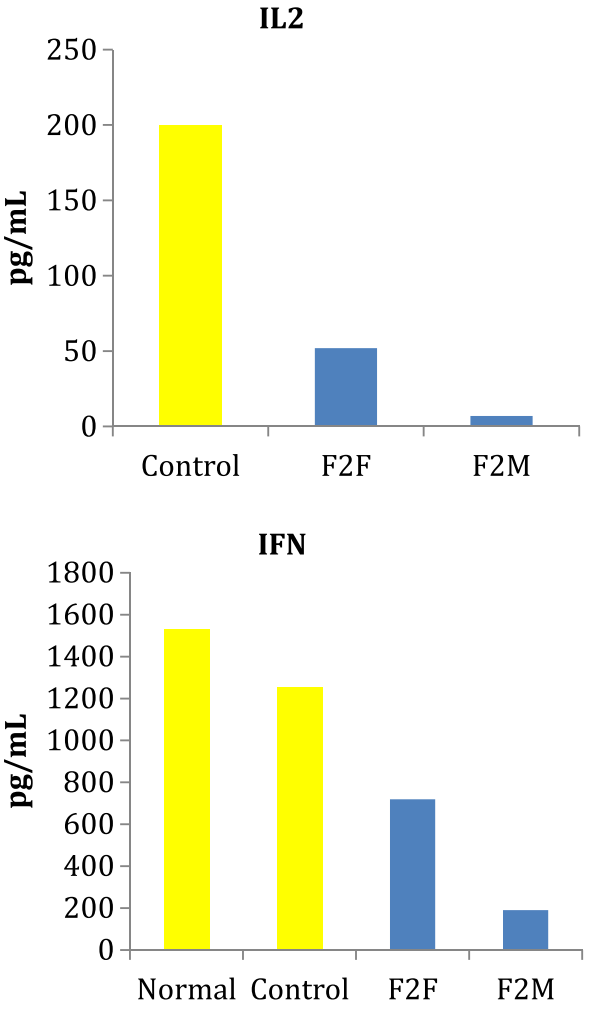

Figure 1: Peripheral blood mononuclear cells were cultures with phytohaemagglutinin for 24 and 48 hour and culture supernatants collected for analysis of IL2 and IFNγ by ELISA. Cultures at 48 hours post-stimulation yielded zero response (not shown). (F2F, XXXXX; F2M, XXXXX)

et al. 2015). Here we describe the clinical and immunologic features of 4 patients, the parents of the homozygous *RelB* patients, all whom were discovered to have heterozygous *RelB* mutations. The 3 patients described with homozygous *RelB* mutations were identified as having a premature stop codon mutation leading to markedly decreased levels of RelB mRNA, presumably due to message instability (Merico et al. 2015). Furthermore, RelB protein was undetectable in these patient's lymphocytes, suggesting that any potential fragment that may be translated is also unstable and subsequently degraded (Merico et al. 2015). Sharfe et al. (2015) detailed the effects of *RelB* deficiency on lymphocyte development and function (Sharfe et al. 2015). In particular, patients with homozygous *RelB* mutations have T-cell dysfunction as evident by markedly dysplastic thymus with reduced production of T cells, abnormal TCR repertoire with clonal expansion, depressed in vitro responses to T-cell mitogens and antigens, reduced production of IL2 and IFNγ, and exaggerated in vitro responses to anti-CD3 and CD28 (Sharfe et al. 2015). Furthermore, B-cell dysfunction was demonstrated with reduced BAFF receptor expression, absent memory/mature B cells and low to absent T-cell dependent antibody responses (Sharfe et al. 2015). The result of the intrinsic defects in both T- and B-lymphocyte maturation and function was a presentation consistent with combined immunodeficiency with features including repeated infections and failure to thrive. The 4 patients described here with heterozygous *RelB* mutations did not have recurrent infections as a clinical feature but did have evidence of immune dysregulation. Specifically, our patients also had impaired T-cell function with inadequate response to mitogens and antigens as well as reduced IL2 and IFNγ production following stimulation with PHA. These findings suggest that heterozygous *RelB* mutations also result in deficient T lymphocytes in vivo. The lack of recurrent infections in our patients may reflect residual RelB function and (or) the involvement of compensatory mechanisms protective against infection in the group with heterozygous *RelB* mutations.

Impaired immune functioning is also evident by the lymphoma that developed in F2. Aberrant NFκB activity has been observed in many cancers, including both solid and hematopoietic malignancies (Basseres and Baldwin 2006; Naugler and Karin 2008). Evidence suggests that NFκB pathways may mediate pro-apoptotic effect, growth arrest, and inhibition of cancer development (Perkins 2004; Chen and Castranova 2007). Specifically, a recent study demonstrated that RelB provides cell proliferation-inhibitory signals in murine fibroblasts and that RelB ectopic expression inhibits xenograft tumor growth in vivo, whereas RelB knockdown enhances it; Jacque et al. (2013) proposed these findings were secondary to direct transcriptional activation of the p53 tumor-suppressor gene by RelB. These findings highlight the importance of malignancy surveillance in this patient population.

Conclusion

Here we highlighted that heterozygous *RelB* mutations can be associated with immune dysregulation

and lymphoma. It is likely that the immune defects apparent in *RelB*-deficient humans are due to a wider effect of RelB on the classical NFκB pathway (involving RelA and c-Rel) through cross-regulation of activation and expression, in addition to RelB's function within the alternate pathway (Sharfe et al. 2015).

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