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ORIGINAL INVESTIGATION

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A new mutation in exon 7 of *NEMO* gene: late skewed X-chromosome inactivation in an incontinentia pigmenti female patient with immunodeficiency

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Abstract Incontinentia pigmenti is an X-linked genodermatosis, lethal in males. Affected females survive because of X-chromosome dizygosity and negative selection of cells carrying the mutant X-chromosome, and for this reason the skewed X inactivation pattern is often used to confirm the diagnosis. The most frequent mutation is a deletion of part of the *NEMO* gene (*NEMO*Δ*4–10)*, although other mutations have been reported. Mutations of *NEMO* which do not abolish NF-κB activity totally permit male survival, causing an allelic variant of IP called hypohidrotic ectodermal dysplasia and immunodeficiency (HED-ID). We present a non-classical IP female patient who also suffered transient immunodeficiency because of a late and progressive selection against peripheral blood cells carrying an active mutated X-chromosome. This finding suggests that in the absence of known mutation the X-inactivation studies used in genetic counselling can induce mistakes with some female patients. At the age of 3 years and 6 months, all immunodeficiency signs disappeared, and the X-chromosome inactivation pattern was completely skewed. The low T cell proliferation and CD40L expression corroborate the important role of NEMO/NF-κB pathway in T cell homeostasis. The decreased NEMO protein amount and the impaired IkBα degradation suggest that this new mutation, NM_003639: c.1049dupA, causes RNA or protein instability. To our knowledge, this is the first time that selection against the mutated X-chromosome in X-linked disease has been documented in vivo.

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Introduction

Familial incontinentia pigmenti (IP; [MIM 308310]) is an X-linked dominant genodermatosis that is usually lethal prenatally in males. In affected females, it causes highly variable abnormalities of the skin, hair, nails, teeth, eyes, and central nervous system. The prominent skin signs occur in four classic cutaneous stages: perinatal inflammatory vesicles, verrucous patches, a distinctive pattern of hyperpigmentation and dermal scarring (Sulzberger 1993; Landy and Donnai 1993; Hadj-Rabia et al. 2003). IP males typically fail to survive, whereas affected females survive due to X chromosome dizygosity and negative selection of cells expressing the mutant X chromosome. Such negative selection usually results in all IP females showing an extremely skewed X-inactivation pattern compared to approximately 10% in a healthy population (Sharp et al. 2000; Parrish et al. 1996). For this reason, skewing of the X-chromosome is one of the constant diagnostic criteria of IP.

The International IP Consortium demonstrated that mutations in the *NEMO* (for NF-κB Essential Modulator) gene cause IP (International IP Consortium 2000). The *NEMO* (or IKBKG) gene, which has been mapped to chromosome location Xq28, spans 23 kb organized in 10 exons (GenBank accession no. AJ271718), and encodes a modulator protein. NEMO functions as a scaffolding protein that binds to two kinase proteins (IKKα and IKKβ) to form the IKK complex, indispensable for the activation of NF-κB

transcription factor. The latter is sequestered in the cytoplasm by IκB inhibitory proteins. A different stimulus activates the IKK complex leading to the phosphorylation of IkB and its degradation by the ubiquitin 26S proteasome pathway. Removal of IkBs proteins allows the translocation of NF-κB to the nucleus and the transcription of multiple gene targets (Rothwarf et al. 1998; Israel 2000). The NF-κB pathway is fundamental for cell development, survival and function.

The most frequent mutation found in IP is a genomic rearrangement resulting in the deletion of part of the *NEMO* gene (*NEMOΔ4–10)*, but microdeletions, duplications and nucleotide substitutions have also been reported (Aradhya et al. 2001a, b), (Fusco et al. 2004). The phenotypic expression is highly variable, even among related patients with the same mutation. In female patients with classic deletion, the phenotypic variation could be related to skewed X-chromosome inactivation (Fusco et al. 2004) which may vary between different tissues in the same individual (Sharp et al. 2000).

Most of *NEMO* mutations eliminate NF-κB activity and cause potentially widespread disruption of downstream cellular responses. On rare occasions, hypomorphic mutations of *NEMO* do not completely abolish NF-κB activity and permit male survival. However, these males show hypohidrotic ectodermal dysplasia and immunodeficiency (HED-ID), an allelic variant of IP characterized by the absence or hypoplasia of ectodermal derived tissues (hair, teeth and sweat glands). Immunodeficiency is variable: hyperimmunoglobulinemia M, disgammaglobulinemia, defective antipolysaccharide antibody synthesis, accompanied by elevated susceptibility to infections (Zonana et al. 2000; Aradhya et al. 2001a, b; Jain et al. 2001; Kosaki et al. 2001). Occasionally, males with hypomorphic *NEMO* mutations do not present any sign of ectodermal dysplasia (Niehues et al. 2004; Orange et al. 2004). Furthermore, Nishikomori reported a patient with a novel type of EDA-ID whose NEMO expression varied among cell lineages due to reversion mosaicism of a portion of NEMO gene (Nishikomori et al. 2004). On the other hand, a hypermorphic IkBα mutation resulted in impaired NF-κB activation, and is associated with autosomal dominant anhydrotic ectodermal dysplasia and T cell immunodeficiency (Courtois et al. 2003; Janssen et al. 2004).

Here, for the first time we describe the association between alterations in T cell function and IP in an affected female patient who went through progressive inactivation of the mutated X-chromosome, starting at the age of 30 months. By the age of 3 years and 6 months, the immunodeficiency had disappeared, and a completely skewed X- chromosome inactivation pattern was observed.

Subjects and methods

Case report

Our female patient was born from healthy non-consanguineous parents; at birth, she presented the four classical dermatological stages of IP, together with extracutaneous manifestations, such as dental, ocular and central nervous system disorders that include motor retardation. At 2 weeks, she had respiratory sincitial virus (RSV) positive bronchopneumonitis, persistent diarrhea and oral thrush. At 3 months, she was admitted to hospital because of parainfluenza III virus and *Staphylococcus aureus* positive bronchopneumonitis; 1 week later, a Candida sepsis was diagnosed. At this time, laboratory studies revealed eosinophilia, CD8 lymphopenia, discretely low IgG levels and high IgM serum levels. Immunodeficiency was suspected and she underwent substitutive intravenous gammaglobulin (IVIG) therapy, 400 mg/body weight every 21 days; 3 months later, she was diagnosed as having *Aspergillus fumigatus* osteomyelitis of the right knee, together with a new outbreak of verrucous lesions. In the next months the girl remained in a good condition; persistent low levels CD8 lymphocytes, normal IgA and IgM serum levels were presented. No clear diagnosis of immunodeficiency was established and IVIG therapy was discontinued when she was 18 months of age. At 20 months, the values of natural pneumococcal and hemophilus specific IgG antibody were elevated: 450 UA/ml and 8.7 mg/ml, respectively.

In 2001, at the age of 30 months, the girl was diagnosed as having infectious mononucleosis (laboratory diagnosis showed Epstein–Barr virus specific IgM antibody), and 15 days later she was admitted to a hospital with a parainfluenza III virus and *Streptococcus pneumoniae* bronchopneumonia complicated with significant pleural effusion (Table 1). At this moment, laboratory findings showed high IgM serum levels. After this last admission to hospital, the immunodeficiency symptoms completely disappeared, and at present, the girl remains in a healthy immune condition.

Molecular analysis

Screening for NEMOΔ4–10 deletion and Genomic sequencing of the NEMO gene

Genomic DNA from parents and proband were extracted from leukocytes with the proteinase K digestion and salting out method, and stored at −70°C. The proband was screened for the common deletion with a PCR test with previously reported primers, and system 3 of the EXPAND-LONG kit (Roche, Mannhein, Germany).

Table 1 Laboratory and clinical findings

Age (months)	Serum Igs levels			Lymphocyte phenotype						Absolute subpopulations	
	IgG	IgA	IgM	CD3%	CD4%	CD8%	CD19%	CD45RA /CD4%	CD45RO /CD4%	CD4	CD8
0.5[a]	**550 L** (758–1,800)	7 (0–10)	**160 H** (10–20)	**42 L** (60–87)	**32 L** (41–64)	**10 L** (16–35)	7 (4–25)	81 (41–88)	6 (17–73)	3,200 (1,460–5,110)	1,000 (650–2,450)
2	**130 L** (200–750)	14 (10–50)	51 (10–70)	62 (60–87)	43 (41–64)	19 (16–35)	21 (14–37)	78 (57–81)	22 (10–84)	2,580 (1,460–5,110)	1,140 (650–2,450)
3[a]	**227** (200–750)	43 (10–50)	88 (10–70)	60 (60–87)	**40 L** (41–64)	17 (16–35)	25 (14–37)	80 (64–96)	20 (14–62)	**920 L** (1,460–5,110)	**391 L** (650–2,450)
6[a]	901[b]	35 (10–70)	146 **H** (20–140)	**50 L** (57–84)	**35 L** (36–61)	**13 L** (16–34)	**36 H** (13–35)			1,925 (1,950–4,600)	**714 L** (720–2,490)
7	1,150[b]	59 (10–70)	135 (20–140)	**55 L** (57–84)	40 (36–61)	**15 L** (16–34)	25 (13–35)			1,720 (1,950–4,600)	**645 L** (720–2,490)
12	833[b]	57 (30–90)	143 (50–200)	**52 L** (53–81)	37 (31–54)	**13 L** (16–34)	28 (17–36)	78 (65–97)	22 (11–65)	1,739 (1,020–3,600)	**611 L** (720–2,490)
18	1,090[b]	80 (30–90)	143 (50–200)	**52 L** (53–81)	38 (31–54)	**14 L** (16–38)	27 (17–36)	84 (65–97)	16 (11–65)	1634 (1,020–3,600)	**602 L** (720–2,490)
24	503 (300–1,000)	51 (30–90)	88 (50–200)	**50 L** (53–81)	37 (31–54)	**12 L** (16–38)	25 (19–40)			1517 (1020–3,600)	**492 L** (570–2,230)
30[a]	711 (300–1,000)	114 (40–150)	**469 H** (50–200)	**60 L** (62–80)	**34 L** (35–51)	26 (22–38)	14 (19–40)			1156 (900–2,860)	884 (630–1,919)
48	1010 (500–1,400)	80 (50–180)	181 (50–200)	62 (62–80)	39 (35–51)	18 (22–38)	27 (19–40)	67 (35–70)	**33 L** (47–81)	1191 (900–2,860)	882 (630–1,919)

At the age of 3 months, immunodeficiency was suspected. The neutrophil oxidation test using nitroblue tetrazolium (NBT) and complement function (CH50) were normal, and the patient was included on therapy with continuous monthly IVIG infusions. Normal values were taken from a study by our laboratory in the population of Balearic Islands. In the case of the normal proportions of CD45RA or CD45RO positive CD4 cells, the normal values were extracted from de Vries (de Vries et al. 2000). The low or high values are indicated in bold letters: *H* High, *L* low. Clinical findings at 0.5 months: bronchopneumonitis (Respiratory sincityal virus); at 3 months bronchopneumonitis *Staphylococcus aureus* and parainfluenza III virus and Candida sepsis; at 6 months *Aspergillus fumigatus* osteomyelitis; at 30 months; mononucleosis (EBV) and bronchopneumonitis (*Streptococcus pneumoniae* and parainfluenza III virus)

[a]The patient was infected

[b]The patient was under IVIG treatment

Mutation analysis of individual exons of the NEMO gene was performed by PCR. Amplification of genomic DNA was carried out by use of AmpliTaq Gold polymerase and previously reported primers (International IP Consortium, 2000). DNA sequencing was conducted with the Big Dye Terminator Cycle sequencing kit v.3.1 (Applied Biosystems, Foster City, CA, USA). The products were evaluated on an ABI 3100 DNA sequencer (Applied Biosystems).

X-inactivation analysis

DNA was obtained from the proband's PBMC, T cells, fibroblasts, and granulocytes (purified from peripheral blood cells by a dextran method in the last ones). X-inactivation analysis was performed by the method previously described (Allen et al. 1992). Briefly, this method consists of the analysis of methylation at the Androgen receptor (AR) locus. PCR is performed on genomic DNA digested with the methylation-sensitive restriction enzyme *Hpa*II, and only the AR gene residing in the inactivated X-chromosome is amplified. Conditions were the same as those described in our previous article (Heine-Suner et al. 2003).

The inactivation ratio for each of the homologous X-chromosome was ascertained by densitometry using the Genetools Image analysis software (SynGene). This ratio was calculated by dividing the total density of the band corresponding to one of the homologous X-chromosomes by the sum of the densities corresponding to the two bands of both homologous X-chromosomes.

Western blot analysis

Fibroblast cells were washed with PBS and resuspended in hypotonic solution (10 mM HEPES, pH 7.8, 10 mM KCl, 2 mM $MgCl_2$, 1 mM DTT, 0.1 mM EDTA) supplemented with protease inhibitor. After 10 min at 4°C, NP-40 was added to 1% and the cells centrifuged in a microfuge for 20 s. The supernatant containing the cytoplasmic fraction was recovered. One volume of 2× Laemmli buffer was added, and the samples were boiled for 5 min and fractionated on 10% SDS-polyacrylamid gels and transferred onto Immobilion membranes (Millipore). Blots were revealed with an enhanced chemiluminiscence detection system (ECL, Amersham). Two antibodies raised against NEMO were used. Mouse anti-IKKγ directed against the whole NEMO was purchased from Santa Cruz Biotechnology. Rabbit anti-NEMO (33.28) was a kind gift from G. Courtois. Goat anti-actin and rabbit anti-IkBα antibodies were also purchased from Santa Cruz Biotechnology. Secondary antibodies raised against mouse, rabbit and goat Immunoglobulin conjugated to peroxidase were purchased from Amersham Biosciences.

IkBα degradation

Fibroblast cells were treated for the indicated time with recombinant TNF-α (10 ng/ml) purchased from R&D Systems for the indicated time and cytoplasmic extracts were prepared and analyzed after Western blotting with anti- IkBα antibody.

Cell preparation and culture conditions for T cell function evaluation

To evaluate the impact of the *NEMO* mutation in T cell function, we obtained T lymphocytes by negative selection using a mixture of mouse IgG monoclonal antibodies against CD14, CD16, CD56 and HLA class II and polyestyrene beads coated with human IgG4 antibody against mouse IgG (Dynabeads; Dynal, Oslo, Norway) cells. Purity of T cells defined as CD3+ cells was greater than 95%. For T cell stimulation, 20 mg/ml of goat anti-mouse IgG (Fc specific) (Sigma-Aldrich, St. Louis, MO, USA) was added to the plates and kept at 4°C overnight. Then, 10^5 T cells per well in 96-well plates, were stimulated with anti-CD3 (Becton Dickinson, San Jose, CA, USA) at 1 mg/ml or with anti-CD3 at 0.01 mg/ml plus 5 mg/ml of anti-CD28 (BD) and maintained at 37°C in a 5% CO_2 atmosphere for 48 h to measure CD40L membrane expression capacity with PE-conjugated anti-CD154 (Pharmingen, San Diego, CA, USA) or for 72 h to measure proliferation, with incorporation of 3H-thymidine by T cells.

Results

Screening for *NEMOΔ4–10* deletion and Genomic sequencing of the *NEMO* gene

The typical rearrangement present in the majority of IP patients was not detected in our patient (data not shown). Direct sequencing of individual exons of *NEMO* revealed duplication of an adenosine in exon 7 (NM_003639: c.1049dupA) within a run of three adenosines of the wild-type gene (Fig. 1). This insertion resulted in a novel amino acid sequence from codon 264 to a premature stop at codon position 284 (CAB93146:-pLys264 fs) (Fig. 2). The 1049dupA occurs on *NEMO* gene and not on its pseudogene located 30 kb distal, because we found the same mutation when selective PCR amplification of *NEMO* gene, primed with E2F primer, specific to *NEMO* gene is performed (*NEMO* pseudogene lack exon 2). No sequence mutation was detected in the *NEMO* gene of probands parents.

X-inactivation analysis

We assessed the X-inactivation status of peripheral blood cells of the patient at different periods of this study

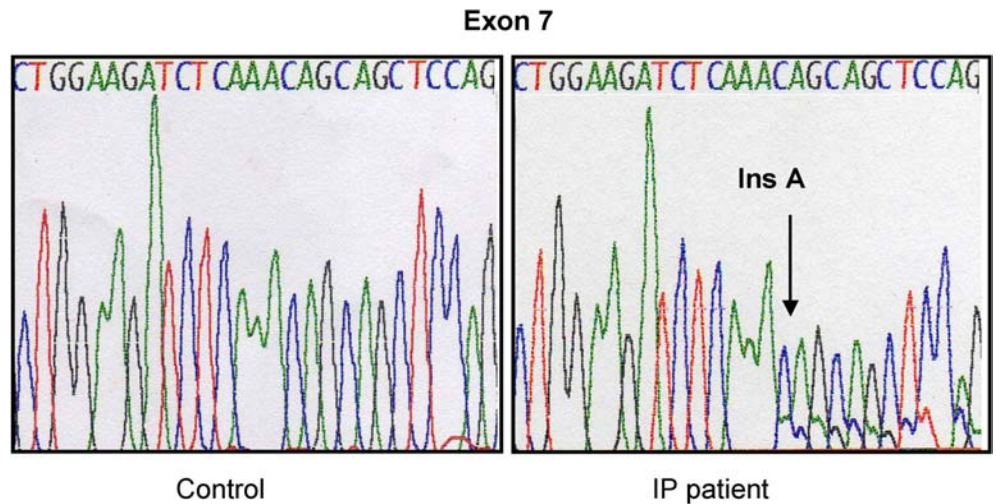

Fig. 1 The DNA sequence for the proband revealed a heterozygous insertion of adenine in *NEMO* exon 7 (NM_003639: c.1049dupA) (*right panel*). *Left panel* is a control sequence

(24, 30, 38, 48 months of age). At the age of 24 months, our patient showed random X-inactivation (55% of blood cells presented the paternal X-chromosome inactivated) in contrast to the skewed X-inactivation pattern usually presented in female IP patients, and also observed in our laboratory in 100% of IP female patients with known *NEMO* mutation and aged between 2 and 48 months (data not shown). However, the X-inactivation status changed over time, increasing from 58% of inactivation of the paternal X-chromosome at 30 months of age to 73% after 8 months. Finally, when the girl was 48 months old, a complete inactivation of the paternal X-chromosome was observed (Fig. 3a). The X-inactivation analysis of T cells and granulocytes at 24 months showed an identical random X-inactivation (Fig. 3b).

NEMO protein analysis

In order to test the relevance of the 1049dup on NEMO protein, protein studies were performed on fibroblast cells derived from skin biopsies of the patient carrying the c.1049dupA mutation, at 48 months of age. We checked the X-inactivation status of the patient's fibroblasts. The paternal and maternal alleles were present equally after *Hpa*II digestion (data not shown) which indicate that the cells expressing the *NEMO* mutation were still present and that the negative-cell selection did not occur during culture. The c.1049dupA is predicted to give a truncated protein. We used two different anti-*NEMO* antibodies to study *NEMO* protein in our patient. We failed to detect any abnormal band. Only a 48 kDa normal *NEMO* was observed in the IP patient as well as in a control (only results obtained with anti-IKK antibodies are shown, Fig. 4). Interestingly, we observed that the amount of *NEMO* decreased by at least twofold in the IP patient compared to control fibroblasts. Actin was used as control for sample quantification (Fig. 4). These results suggest that the 1049dupA mutation in *NEMO* gene causes either RNA or protein instability. The normal band corresponds to wild type *NEMO* encoded by the normal X chromosome in our IP patient.

IkBα degradation

We assessed the effect of the *NEMO* mutation detected in our patient on IkBα degradation. We stimulated patient and control fibroblasts with TNFα, a potent activator of NF-κB and determined IkBα cytoplasmic

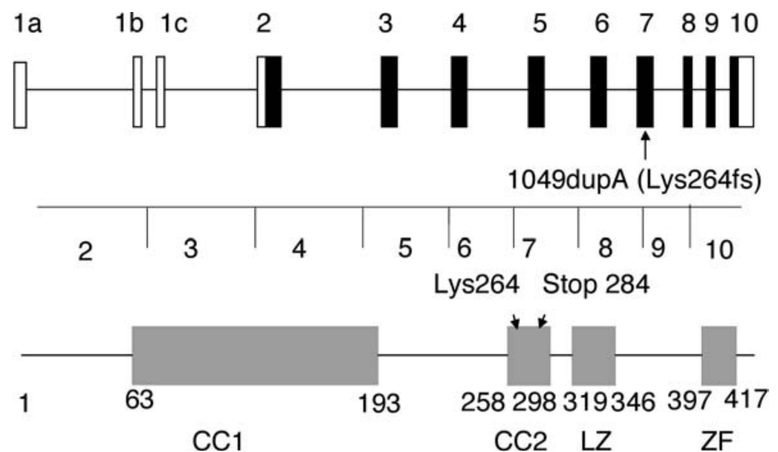

Fig. 2 The novel mutation in *NEMO* gene results in a truncated protein with a partial coiled-coil 2 (*CC2*) domain and lacks leuzine zipper (*LZ*) and ZF motifs causing a putative loss of *NEMO* function

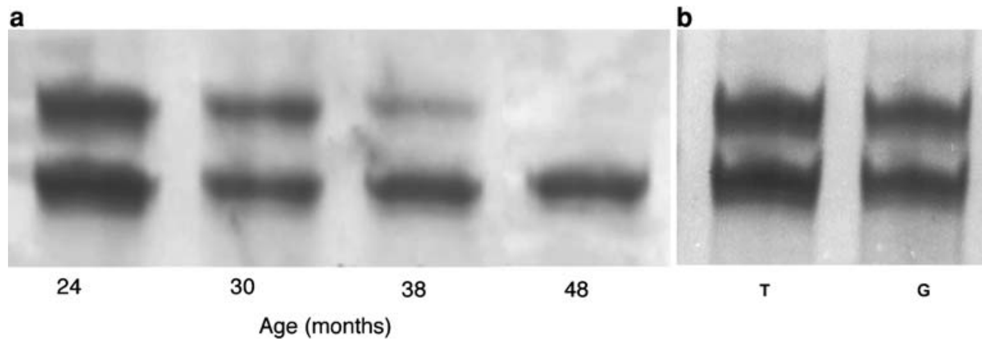

Fig. 3 The X chromosome inactivation pattern. **a** Peripheral blood cells of IP female patient showed a progressive skewed inactivation. The blood cells showed random X-inactivation (55%) when the patient was 24 months. When she was 30 months old, the skewed inactivation process started (58%) and finalized at 48 months of age (100%). **b** T and G lines showed the X-inactivation pattern of T cells and granulocytes, respectively, at 24 months

degradation. Whereas control fibroblasts exhibited complete disappearance of IkBα after 10 and 20 min of TNFα treatment (Fig. 5), our patient exhibited an impaired IkBα degradation (Fig. 5).

T cell function

In order to evaluate the importance of the new *NEMO* mutation on T cell functions, we measured the proliferation and induction of CD40L expression in purified T cells. T cell proliferation stimulated with anti-CD3 was diminished by at least 50% with respect to controls, but when stimulated with anti-CD3 plus anti-CD28 the level of proliferation was similar to controls. The expression of inducible surface CD40L was also decreased by 50% in anti-CD3 stimulated T cells, and it was not restored when anti-CD28 was added (Table 2). Eighteen months later, when a totally skewed X-inactivation pattern was observed in peripheral blood cells, we repeated these experiments. Then, T cell proliferation and CD40L expression were normal. All these observations prompted us to hypothesize that this mutation principally altered the T cell function.

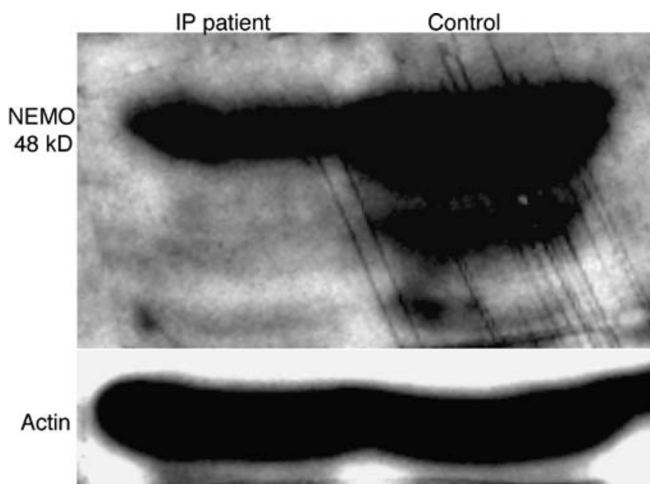

Fig. 4 Western blot analysis of *NEMO* protein in fibroblasts from an IP patient and a healthy control. Western blot revealed only a 48 kDa normal NEMO in the IP patient, but the amount of NEMO protein was decreased twofold in the IP patient compared to control fibroblasts

Discussion

The International IP Consortium (2000) demonstrated that *NEMO* gene mutations cause IP in female patients whereas males die prenatally. Female survival is due to the negative selection of cells expressing the mutated X-chromosome. This results in a completely skewed pattern of X-inactivation, which reflects that all cells carry an inactivated mutated X-chromosome. This process occurs soon after birth in IP females which allows the survival of these patients, and the skewed X-inactivation pattern is often used to confirm the diagnosis. However, the present report suggests that X-inactivation studies for genetic counselling when the mutation is unknown can induce mistakes with some female patients. Here we describe a female with a severe immunodeficiency in her first 30 months of life caused by a novel mutation (c.1049dupA) in the *NEMO* gene.

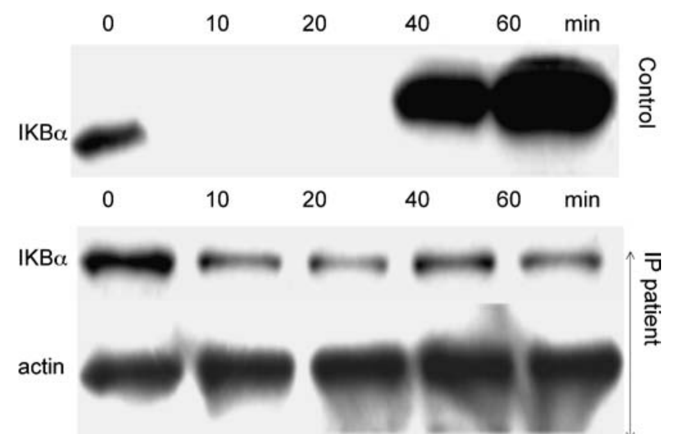

Fig. 5 Defect of IκBα degradation in IP patient fibroblasts treated with TNFα. Time course analysis of TNFα induced IκBα degradation as detected by Western blots showed that the IP patient exhibited an impaired IκBα degradation

Table 2 T cell function

	CD3	CD3 + CD28	CD3	CD3 + CD28
T cell proliferation	25 ± 13 (96 ± 14)	90 ± 8 (80 ± 15)	80 ± 10 (96 ± 14)	75 ± 8 (80 ± 15)
CD40L expression	22 (67)	27 (68)	60 (67)	62 (68)
X-inactivation	Azar	Azar	Skewed	Skewed
Age (months)	24	24	48	48

Proliferation was measured as c.p.m. The median of proliferation values are expressed in 10^3 cpm ± standard deviation. A diminished anti-CD3 T cell proliferation response versus control T cells was found. We observed diminished CD40L expression (measured as percentage of CD4 + T cells that expressed CD40L) after anti-CD3 or anti-CD3 plus anti-CD28 stimulation. Normal T cell proliferation and CD40L expression related to completely skewed X-chromosome inactivation pattern

X-inactivation studies showed that during the first 30 months, the X-inactivation pattern was random. At this point, cell selection started and finalized at 48 months of age, when a completely skewed X-inactivation pattern was detected, with the paternal X-chromosome completely inactivated in all of her peripheral blood cells. This result confirms that this is a de novo mutation in the parental X-chromosome. This process correlated with the disappearance of all clinical and laboratory manifestations of immunodeficiency. At this moment, clinical features and laboratory findings of immunodeficiency were totally normal. Interestingly the X-inactivation study performed in fibroblast cells and peripheral blood cells at the same time (48-month-old) showed different inactivation patterns: random in fibroblast cells and skewed in peripheral blood cells. These results corroborate the idea that the X-inactivation is variable between different tissues in the same individual (Sharp et al. 2000).

Recently, HED-ID has been described in males associated with hypomorphic *NEMO* mutations. Mutations found in exon 10 in HED-ID patients either delete or alter a putative zinc-finger (ZF) domain at the C-terminal end of the *NEMO* protein. Mutations in this domain are reported to result in a loss of function of the NF-κB activation, but must preserve a partial IKKγ function (Aradhya et al. 2001a, b; Jain et al. 2001; Makris et al. 2002; Zonana et al. 2000). In 2001, Kosaki described a female patient with HED-ID who presented a mutation in exon 10, which resulted in a premature stop codon at the 394 aa position (Kosaki et al. 2001). Interestingly, as in our patient, this HED-ID female patient showed a random inactivation of the mutated X-chromosome.

The 1049dupA mutation present in our female patient is predicted to result in an inactive truncated protein with a partial coiled-coil 2 (CC-2) domain; lacking both leuzine zipper (LZ) and zinc finger (ZF) domains. We failed to detect the predicted truncated *NEMO*, probably because the mutation causes instability of either the transcript carrying the mutation or the encoded protein. This is not caused by a lack of expression of the mutated gene, because cells carrying an active mutated X-chromosome were still present during culture, and because both paternal and maternal alleles at HUMARA locus are present at the same level. This was confirmed by a decrease in the amount of *NEMO* protein in the patient's fibroblasts when compared to a normal control. Thus, the identified mutation causes an alteration of *NEMO* function, and a defective activation of NF-κB pathway as IκBα degradation was also found to be impaired in the fibroblasts carrying the mutation.

Interestingly, not all cell populations were found to be equally affected by this mutation. We observed low levels of CD8 T cells in the first 30 months of life accompanied by normal levels of the other peripheral cells populations. However, the X-inactivation pattern did not show differences between granulocytes, T cells, and total peripheral blood cells at 24 months of age. T cell proliferation and CD40L expression were both diminished in our patient after anti-CD3 stimulation. Furthermore, the analysis exhibited a low proliferation response which normalized when anti-CD28 was added. We hypothesize that low constitutive expression of CD28 in CD8 T cells could make them more sensitive to a defective NF-κB pathway. Our results confirm the recent report of Nishikomori, who presented a patient with a novel type of X-linked HED-ID, due to a reversion mosaicism of a 4.4 kb duplication of a portion of the *NEMO* gene (Nishikomori et al. 2004). This patient presented atypical features of a naïve phenotype on T cells and defective induced mitogen proliferation of mononuclear blood cells. Morever, Courtois et al. (2003) describe a patient with autosomal dominant form of HED-ID associated to a mutation of IkBα, who suffered a severe T cell immunodeficiency related to unresponsiveness of naïve T cells to CD3-TCR stimulus. Furthermore, recent reports showed that mutant mice with abnormally low NF-κB activity showed alterations in T cell proliferation, development and survival; reduced numbers of mature T cells in *NEMO* knock-out mice have also been described (Schmidt-Supprian et al. 2000, 2003). Interestingly, the HED-ID patients showed a defective response to a polysaccharide but our female patient showed a good response to pneumococcal and hemophilus polysaccharides.

To our knowledge, this is the first description of a late inactivation of the mutated X-chromosome in a female patient with IP and immunodeficiency. Furthermore, we speculate that our patient represents a clinical status ranging between classical IP patients, with no immunodeficiency and early skewed X inactivation of the mutated X-chromosome, and HED-ID male patients, or HED-ID female patient with random X-inactivation

pattern. However, the relationship between this mutation and the abnormal persistence of cells expressing the X-mutated chromosome is not well understood at present. Random X-inactivation could be observed indistinctly in peripheral blood cells, or in specific cell populations like fibroblasts, T cells, and granulocytes before 30 months of age. Although the IκBα degradation shows that this mutation causes an alteration of *NEMO* function, we can only hypothesize that the *NEMO* mutation present in our patient was less detrimental than the classical deletions seen in the majority of IP female patients because it only affects the CD8 T cell population. This may have favored a delay in the inactivation process of the X-mutated chromosome and allowed the survival of cells containing an active X-mutated chromosome causing an immunodeficiency.

In conclusion, we describe a case of a *NEMO* mutation that causes IP and transient immunodeficiency in a female patient with an atypical late inactivation of the mutated X-chromosome. To our knowledge, this is the first time that a progressive inactivation process of the mutated X-chromosome has been documented in vivo.

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