ASIAN PACIFIC JOURNAL OF ALLERGY AND IMMUNOLOGY (2009) 27: 161-165

CASE REPORT

A Novel Mutation of the *IL12RB1* Gene in a Child with Nocardiosis, Recurrent Salmonellosis and Neurofibromatosis Type I: First Case Report from Thailand

Voravich Luangwedchakarn[1], Orathai Jirapongsaranuruk[2], Julie E. Niemela[3], Charin Thepthai[1], Kulkanya Chokephaibulkit[2], Sanya Sukpanichnant[4], Punchama Pacharn[2], Nualanong Visitsunthorn[2], Pakit Vichyanond[2], Surapon Piboonpocanun[5] and Thomas A. Fleisher[3]

SUMMARY Genetic defects of interleukin (IL)-12/23-and interferon (IFN)-γ-mediated immunity can cause increased susceptibility to intracellular microbes. Among these defects, a mutation of the gene encoding the IL-12 receptor β1 (IL-12Rβ1) is the most common worldwide. A 12-year old Thai boy with pre-existing neurofibromatosis type 1 (NF1) was evaluated for primary immunodeficiency after a history of tuberculous lymphadenitis, recurrent *Salmonella* infections and nocardiosis. Flow cytometry of phytohemagglutinin (PHA)-stimulated peripheral blood mononuclear cells (PBMCs) revealed a defect in the IL-12Rβ1 surface expression. A genetic study showed a novel nonsense homozygous mutation of the *IL12RB1* gene in exon 4 (402C>A), confirming the diagnosis of IL-12Rβ1 deficiency. This is the first case report of a primary IL-12Rβ1 deficiency in Thailand with the interesting finding of a coexisting NF1.

Interleukin (IL)-12 is an important cytokine for the protective immunity against intracellular pathogens such as *Salmonell*a spp. and *Mycobacterium*.[1,2] IL-12 exerts its activities particularly on T lymphocytes and NK cells through high affinity receptors which are composed of IL-12Rβ1 and IL-12Rβ2.[3] Signal transduction through these receptors drive $CD4^+$T-cells to differentiate to T-helper (T_H) 1 cells and promote interferon (IFN)-γ secretion from both T_H1 and NK cells. IFN-γ-activated macrophages become more capable of eliminating intracellular microorganisms. IL-12Rβ1 is also a necessary component of the IL-23 receptor complex, mediating the effect of IL-23 in a special T-cell subset to produce IL-17 and IFN-γ.[3,4] Mutation of the genes involved in this interactive pathway of IL-12/23 and IFN-γ can cause primary immunodeficiency diseases in humans characterized by a markedly increased

From the [1]Department of Immunology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand, [2]Department of Pediatrics, Faculty of Medicine Siriraj Hospital, Mahidol University, [3]Department of Laboratory Medicine, Warren G. Magnuson Clinical Center, National Institutes of Health, USA, [4]Department of Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University, [5]Institute of Molecular Biology and Genetics, Mahidol University, Nakorn Pathom, Thailand.
Correspondence: Voravich Luangwedchakarn
E-mail: sivlw@mahidol.ac.th

susceptibility to intracellular microbes.[5,6] More than 290 cases from 43 countries across all continents have been identified with genetic defects of the IL-12/23 and IFN-γ-mediated immunity.[7] Thirteen different genetic disorders are caused by mutations involving six genes in this interactive pathway. The most common etiology being an IL-12Rβ1 deficiency represents approximately 40% of all cases worldwide.[7]

We report the first case of an IL-12Rβ1 deficiency in a boy with nocardial infection and neurofibromatosis type 1 (NF1) confirmed by a defective receptor expression upon T cell stimulation and mutation analysis. Neither infection with *Nocardia* spp. nor concurrent NF1 has been reported in patients with IL-12Rβ1 deficiency.

CASE REPORT

A 12-year old Thai boy with underlying neurofibromatosis, presented with left lower quadrant abdominal pain and watery diarrhea for 2 weeks. Three months earlier, he had low grade fever along with productive cough and yellowish sputum which partially improved after a course of antibiotics. The past medical history was significant for tuberculous lymphadenitis at 9 months of age, and five separate episodes of *Salmonella* septicemia at 8 years of age. During an eighteen month interval, his blood cultures grew *Salmonella typhi* twice and *Salmonella* group D three times and his bone marrow culture also grew *Salmonella* group D once. There was no history of consanguinity, or recurrent infection in other family members but his mother also had neurofibromatosis. Physical examination revealed that his weight and height were below the third percentile for his age and he had the following findings: multiple café-au-lait spots on his trunk, a non-tender enlarged right axillary lymph node (1 cm. in diameter), and hepatosplenomegaly.

The complete blood count showed hemoglobin 11 g/dL, hematocrit 32.2%, white blood cell count 6,900/mm^3, neutrophils 52.4%, lymphocytes 19.4%, monocytes 6.9%, eosinophils 21%, and platelets 94,000/mm^3. The erythrocyte sedimentation rate was 116 mm/hr. Blood culture and stool culture yielded no organisms and a gastric wash for acid-fast bacilli was negative on 3 consecutive days. The investigations for parasitic infections including stool for ova and parasites, anti-cysticercosis antibody (Ab), anti-Angiostrongylus Ab, and anti-*Gnathostoma spinigerum* Ab were negative. Thoraco-abdominal computerized tomography (CT) demonstrated generalized pre-aortic, paratracheal, and intra-abdominal lymphadenopathy. Sampling of an axillary lymp node revealed chronic non-granulomatous inflammation. *Nocardia* spp. was found in a modified acid-fast stained section of the lymphoid tissue but the tissue culture was negative. His serum immunoglobulin levels were elevated, especially IgE at 5,621 IU/ml (normal range < 100 IU/ml). A tuberculin skin test was non-reactive while a response to other two recall antigens was present. A lymphocyte subpopulation enumeration was normal. A defect of the phagocyte oxidative burst was ruled out by a normal dihydrorhodamine (DHR) assay.

Because of the pattern of susceptibility to intracellular pathogens, a defect in IL-12/23-and IFN-γ mediated immunity was suspected. The cell surface expression of IFN-γR1 and IL-12Rβ1, the two most commonly affected proteins in this group of genetic disorders, were studied by flow cytometry. The IFN-γR1 expression of the patient's lymphocytes was normal. However, the IL-12Rβ1 expression of PHA-stimulated mononuclear cells was absent, supporting the diagnosis of IL-12Rβ1 deficiency.[5,6,8] (Fig. 1)

To investigate the underlying genetic defect causing the absence of surface protein expression, we sequenced the *IL12RB1* locus as described below. A nonsense homozygous mutation was detected in exon 4 (c.402C->A, p.Y133X) (Fig. 2). This nonsense mutation in the extracellular region of IL12RB1 would be expected to prevent cell surface expression of the protein, presumably due to nonsense-mediated decay. Neither of his parents has been studied for the presence of this mutation.

The patient was treated with antimicrobial agents then discharged home on cotrimoxazole prophylaxis. Recombinant human IFN-γ was not given due to the unavailability in Thailand. For the past three years up to the time of this report in 2008, the patient had been well without further infection from intracellular organisms.

Analysis of *IL12RB1*

The coding regions of the IL12RB1 locus were PCR-amplified using exon-specific primers (primer sequences available upon request) and the PCR products were directly sequenced in both the forward and reverse direction using BigDye (version 1.1) terminators (Applied Biosystems, Foster City, CA). All identified mutations were confirmed by sequencing a second PCR product. The coding sequence was compared with RefSeq NM 005535. Nucleotide and amino acid numbering was done according to the HGVS nomenclature guidelines: the 'A' of the ATG-translation initiation codon and the initiator Methionine are numbered as '1'.

DISCUSSION

The patient in this report presented not only the typical manifestations of IL-12Rβ1 deficiency including mycobacterial infections and recurrent *Salmonella* septicemia but also nocardial infection. Nocardiosis was previously reported in a patient with an IL-12p40 defect.[7,9,10] Other evidence to directly support IL-12 as a key player in the immune response to nocardiosis is unavailable, although IFN-γ prophylaxis was shown to reduce the rate of infection by this pathogen in chronic granulomatous disease.[11] These observations highlight the important role of IL-12/23-and IFN-γ mediated immunity against *Nocardia* spp. The clinical course of this case followed a pattern similar to those described in previous reports.[7,12] Most patients with IL-12Rβ1 defect suffered from recurrent salmonellosis but not from repeated mycobacterial infection. Those who did not develop disease from the Calmette-Guérin bacillus vaccine later had mycobacterial diseases.

The nonsense mutation found at the nucleotide position 402 of *IL12RB1* in this patient has not been previously reported. Forty-one identified mutations were located in exons 2, 3, 5-13, and 15.[7,13] The possibility of this finding being a polymorphism is unlikely. If the mutant mRNA was translated into protein and not degraded by nonsense-mediated decay, the protein would be severely truncated, lacking most of the fibronectin type-III domains, the transmembrane domain, and the intracellular domain.[13] It is more likely that this nonsense mutation leads to nonsense-mediated decay. Either of these scenarios is consistent with the lack of cell-surface expression, as shown by flow cytometry, similar to most cases of IL-12Rβ1 deficiency associated with mutations of

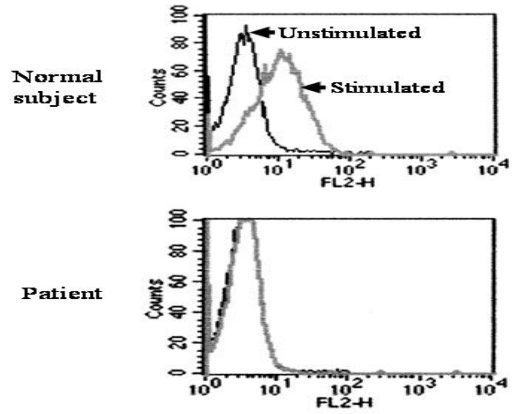

Fig. 1 Detection of surface IL-12Rβ1 expression by flow cytometry on PHA-stimulated PBMCs of a normal subject and the patient. In the normal subject, the stimulated cell shows increased fluorescence intensity. In the patient, there is no change in the fluorescence intensity after stimulation, indicating the absence of IL-12Rβ1 surface expression.

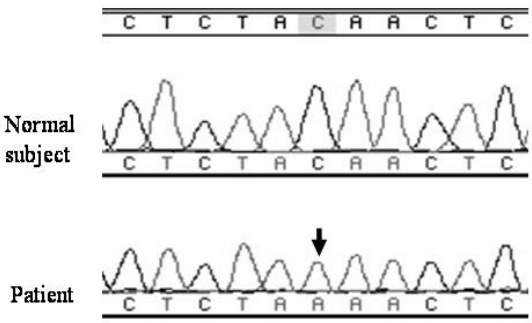

Fig. 2 Analysis of the *IL12RB1* exon 4 of a normal subject and the patient. A chromatogram of the *IL12RB1* exon 4 of the patient reveals a homozygous C to A mutation found at the nucleotide position 402 as indicated by an arrow. This nucleotide substitution results in a stop codon at position Tyr133 in the extracellular domain of IL12RB1, probably resulting in nonsense-mediated decay.

the extracellular portion.[7] The inheritance of this mutation is most compatible with an autosomal recessive pattern based on the unremarkable infection history of both parents and the homozygosity at this loci.

Although both T and B lymphocytes from neurofibromin deficient mice demonstrate an impaired proliferative response, NF1 patients are not known to have an increased susceptibility to infection suggesting a normal host defense against microbes in this disorder.[14-16] The connection between NF1 and malignancy of immune cells is much more widely recognized and is supportd by in vitro experiments of NF1-enhanced sensitivity to growth factors by myeloid cells.[14] The first report of a co-existing neurofibromin deficiency and a primary immune defect was in a Turkish NF1 boy with common variable immunodeficiency diagnosed following a history of recurrent upper respiratory tract infections, panhypogammaglobulinemia, and a low number of B lymphocytes (1.1% of lymphocytes; absolute count of 56/mm^3).[17] The underlying cause of the humoral immunodeficiency in that case was unexplained. Our case provides the first example of a co-existence of NF1 and a primary immunodeficiency involving a cytokine receptor. How these two genetic defects arose in one patient is uncertain. The chance of having a double mutation on both *NF1* and *IL12RB1* in the same individual is quite exceptional. The two genes are located on different chromosomes, preventing them from being a product of a large contiguous mutation. The gene encoding *IL12RB1* is on 19p13.1 while the gene for neurofibromin is on 17q11.2. Still the most plausible explanation for the concomitant conditions in this case is by chance because of the rather common incidence of NF1, approximately 1 in 3,000-4,000 worldwide.[14] How this unique situation affects the immune cell functions in this patient is an interesting subject for future study.

Eosinophilia is far more common in tissue-invading parasitic diseases than in intracellular bacterial infections. In this patient, the negative investigation results had ruled out parasitic diseases. Speculation could be made that the defect in T_H1-mediated immunity might convert the immune reaction more toward the T_H2-predominated response. But this particular group of patients has not been reported to have an increased risk of atopic diseases. Another explanation might come from the fact that human eosinophils express functional IL-12R, rendering them prone to apoptosis through the effect of IL-12.[18] As a consequence of the IL-12Rβ1 deficiency, it is possible that the eosinophilia found in this patient could be the result of prolonged cell survival although this has not been reported in other patients with a defect in this receptor. The increased serum IgE in this case might be a consequence of the skewed T_H2 response that could also explain the eosinophilia. However, neither of these findings has been demonstrated in IL-12Rβ1 deficient patients. Alternatively, the high serum IgE in this case may be related to the unexplained influence from his underlying NF1, as shown by the report of a small Brazilian NF1 cohort.[19] Forty percent of these patients (n = 75) had serum IgE levels above 502 IU/ml. This report noted that the size of the cutaneous neurofibromas and the elevation in serum IgE levels were related in male patients with plexiform neurofibromas although this correlation did not reach statistical significance.

Conclusion

In this report, the first case of IL-12Rβ1 deficiency from Thailand is demonstrated. The co-existence of an IL-12Rβ1 defect and neurofibromatosis type 1 in the patient also presents a unique opportunity to further explore the relationship between these two diseases and the downstream signaling cascades. The nocardial infection found in this case and another patient with IL-12 p40 deficiency support the role of IL-12 -mediated immunity against this organism.

REFERENCES

1. MacLennan C, Fieschi C, Lammas DA, Picard C, Dorman SE, Sanal O, *et al.* Interleukin (IL)-12 and IL-23 are key cytokines for immunity against *Salmonella* in humans. J Infect Dis 2004; 190: 1755-7.
2. Casanova JL, Abel L. Genetic dissection of immunity to mycobacteria: the human model. Ann Rev Immunol 2002; 20: 581-620.
3. Trinchieri G. Interleukin-12 and the regulation of innate resistance and adaptive immunity. Nature Immunol 2003; 3: 133-45.
4. Zhu J, Paul WE. CD4 T cells: fates, functions, and faults. Blood 2008; 112: 1557-69.

5. Altare F, Durandy A, Lammas D, *et al.* Impairment of mycobacterial immunity in human interleukin-12 receptor deficiency. Science 1998; 280: 1432-5.
6. de Jong R, Altare F, Haagen IA, *et al.* Severe mycobacterial and *Salmonella* infections in interleukin-12-deficient patients. Science 1998; 280: 1435-8.
7. Filipe-Santos O, Bustamanta J, Chapgier A, *et al.* Inborn errors of IL-12/23-and IFN-gamma-mediated immunity: molecular, cellular, and clinical features. Semin Immunol 2006; 18: 347-61.
8. Desai BB, Quinn PM, Wolitzky AG, Mongini PK, Chizzonite R, Gately MK. IL-12 receptor: II distribution and regulation of receptor expression. J Immunol 1992; 148: 3125-32.
9. Picard C, Fieschi C, Altare F, *et al.* Inherited interleukin-12 deficiency: *IL-12B* genotype and clinical phenotype of 13 patients from six kindreds. Am J Hum Genet 2002; 70: 336-48.
10. Fieschi C, Dupuis S, Catherinot E, *et al.* Low penetrance, broad resistance, and favorable outcome of interleukin 12 receptor β1 deficiency: medical and immunological implications. J Exp Med 2003; 197: 527-35.
11. Dorman SE, Guide SV, Conville PS, *et al.* Nocardial infection in chronic granulomatous disease. Clin Infect Dis 2002; 35: 390-4.
12. Fieschi C, Casanova JL. The role of interleukin-12 in human infectious diseases: only a faint signature. Eur J Immunol 2003; 33: 1461-64.
13. van de Vosse E, Lichtenauer-Kaligis E, van Dissel JT, Ottenhoff TH. Genetic variations in the interleukin-12/interleukin-23 receptor (β1) chain, and implications for IL-12 and IL-23 receptor structure and function. Immunogenetics 2003; 54: 817-29.
14. Trovó-Marqui AB, Tajara EH. Neurofibromin: a general outlook. Clin Genet 2006; 70: 1-13.
15. Kim TJ, Cariappa A, Iacomini J, *et al.* Defective proliferative responses in B lymphocytes and thymocytes that lack neurofibromin. Mol Immunol 2001; 38: 701-8.
16. Ingram DA, Zhang L, McCarthy J, *et al.* Lymphoproliferative defects in mice lacking the expression of neurofibromin: functional and biochemical consequences of *Nf1* deficiency in T-cell development and function. Blood 2002; 100: 3652-62.
17. Kiliç Ş, Tezcan I, Sanal Ö, Ersoy F. Common variable immunodeficiency in a patient with neurofibromatosis. Pediatr Intl 2001; 43: 691-3.
18. Nutku E, Zhuang Q, Soussi-Gounni A, Aris F, Mazer BD, Hamid Q. Functional expression of IL-12 receptor by human eosinophils: IL-12 promotes eosinophil apoptosis. J Immunol 2001; 167: 1039-46.
19. Geller M, Ribeiro MG, Araújo AP, de Oliveira LJ, Nunes FP. Serum IgE levels in neurofibromatosis type 1. Int J Immunogenet 2006; 33: 111-5.