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Follicular bronchiolitis as phenotype associated with CD25 deficiency

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Summary

Regulatory T cells [T_{regs}; $CD4^+CD25^+$ forkhead box protein 3 ($FoxP3^+$)] are subsets of T cells involved in the maintenance of peripheral self-tolerance by actively suppressing the activation and expansion of autoreactive T cells. Signalling through the interleukin-2 receptor (IL-2R) contributes to T cell tolerance by controlling three important aspects of regulatory T cell (T_{reg}) biology. CD25 is the α-chain of the IL-2R that, in concert with the β-chain and γ-chain, constitutes the complete IL-2R. CD25 contributes only to IL-2 binding affinity but not to the recruitment of signalling molecules. However, its importance in the development of a normal immune response is emphasized by the finding that a truncation mutant of CD25 results in an immunodeficiency in humans characterized by an increased susceptibility to viral, bacterial and fungal infections. In 1997, Sharfe *et al.* described an infant with severe bacterial, viral and fungal infections. Counts of autologous T lymphocytes were moderately low, T cells displayed a weak proliferative response to mitogens *in vitro* and the patient displayed no rejection of an allogeneic skin graft. However, unlike children with severe combined immunodeficiency (SCID), besides not having circulating T cells, the patient also developed peripheral lymphocytic proliferation and autoimmune primary biliary cirrhosis. We present the first female Argentine patient with mutation in CD25 associated with chronic and severe inflammatory lung disease (follicular bronchiolitis with lymphocyte hyperplasia), eczema and infections. She has no expression of CD25 on $CD4^+$ T cells and an extremely low amount of T_{regs}. The molecular study confirmed homozygous missense mutation in the alpha subunit of the IL-2 receptor (CD25αR) (c. 122 a > c; p. Y41S).

Keywords: CD25 deficiency, follicular bronchiolitis, $FoxP3^+$, IPEX-like, regulatory T cells

Introduction

Regulatory T (T_{reg}) cells are subsets of T cells involved in the maintenance of peripheral self-tolerance by actively suppressing the activation and expansion of autoreactive T cells. Several types of T_{reg} cells have been characterized, most prominently natural and inducible $CD4^+CD25^+$ T_{reg} cells [1].

Adaptive T_{reg} cells secrete the immunosuppressive cytokines interleukin (IL)-10 and transforming growth factor (TGF)-β, both endowed with immunosuppressive functions, which play a critical role in T_{reg} cell biology. In recent years there have been improvements in the knowledge of various molecular and cellular mechanisms involved in the generation of T_{reg} cells, the molecules involved and their role in immune homeostasis, autoimmunity and inflammation [2].

T_{reg} cell deficiency, lymphoproliferation and autoimmunity were described by defects along the IL-2/IL-2R pathway. Approximately 40% of patients with an immune dysregulation, polyendocrinopathy, X-linked-like syndrome (IPEX-like) phenotype lack detectable mutations in the forkhead box protein 3 (FoxP3) gene. T_{reg} cells express all three components of the IL-2R, including the α, β and γ chains (CD25, CD122 and CD132, respectively)[3]. T_{reg} cells do not secrete IL-2, but are dependent upon the provision

of this cytokine by paracrine sources to expand in the periphery and activate their immunosuppressive function [4].

Whereas deficiency of the IL-2Rγ chain is associated with X-linked severe combined immunodeficiency, genetic defects in the other components of the IL-2/IL-2R axis, all of which are autosomal, are predictive of abnormalities in the development and function of $CD4^+CD25^+$ T_{reg} cells [2,4–7].

IL-2Rα only contributes to IL-2 binding affinity but not to the recruitment of signalling molecules. However, its importance in the development of a normal immune response is emphasized by the finding that a truncation mutant of IL-2Rα results in an immunodeficiency in humans characterized by an increased susceptibility to viral, bacterial and fungal infections [5].

In 1997, Sharfe *et al*. described an infant with severe bacterial, viral and fungal infections [8]. Counts of autologous T lymphocytes were moderately low, T cells displayed a weak proliferative response to mitogens *in vitro* and the patient displayed no rejection of an allogeneic skin graft. However, unlike children with severe combined immunodeficiency (SCID), the patient not only had circulating T cells but also developed peripheral lymphocytic proliferation and autoimmune primary biliary cirrhosis [6]. In 2003, Caudy *et al*. described an 8-year-old boy with clinical manifestations of IPEX that had a normal FoxP3 gene, but with a CD25 deficiency due to autosomal recessive mutations in this gene. This patient exhibited defective IL-10 expression from CD4 lymphocytes, whereas a FoxP3-deficient patient expressed normal levels of IL-10 [2].

Recently, Goudy *et al*. published the case of a girl with severe autoimmune enteritis and viral infections due to a novel IL-2RA mutation. T_{regs} were present and she also had pronounced lymphoproliferation and impared antigen-specific responses *in vivo* and *in vitro* [9].

We present the first female Argentine patient with mutation in CD25 associated with chronic and severe inflammatory lung disease, eczema and infections.

Methods

Laboratory evaluation

Serum immunoglobulins (IgG, IgA and IgM) were measured by kinetic nephelometry (IMMAGE® 800 System; Beckman Coulter, Buenos Aires, Argentina), and IgE was measured by chemiluminescence (AxSYM Plus Abbott; Abbott Laboratories, Maidenhead, UK).

Humoral functionality tests were conducted to assess the ability of the immune system to respond against a specific challenge. We determined: (i) protective levels of anti-toxoid antibodies, by measuring this using a home-made indirect enzyme-linked immunosorbent assay (ELISA) (≥ 0·1 U/ml protective device) and (ii) the ability of specific antibody production against polysaccharide antigens by challenging with a 23-valent pneumococcal vaccine. Assessing response to the vaccine was performed by ELISA using a commercial kit (The Binding Site, Birmingham, UK) (response criteria: consecutive titre after stimulus ≥ 113 mg /l as the IDP Working Group consensus of the Argentina Society of Pediatrics). As a confirmatory method we used the measurement of 10 specific serotype antibodies by an international standardized third-generation ELISA protocol, according to the World Health Organization (WHO). A titre ≥ 1·3 μg/ml in 50% of the serotypes evaluated for children under 5 years of age and 70% for children over 5 years of age was considered an appropriate response.

Anti-nuclear antibodies (ANA) were determined by indirect immunofluorescence: anti-smooth muscle (SMA), anti-mitochondrial (AMA), anti-liver/kidney microsome (LKM), anti-native double-strand DNA (dsDNA) and anti-neutrophil antibodies [cytoplasmic anti-neutrophil cytoplasmic antibodies (c-ANCA) and perinuclear ANCA (p-ANCA)]; using ELISA: anti-(Sm, RNP, SS-A/Ro, SS-B/La, scl-70, Jo-1 and histones) and anti-transglutaminase-IgA (aTg-A) antibodies.

Flow cytometry

Cells were stained and analysed on a FACSCalibur flow cytometer using BD CellQuest™ Pro Software (BD Biosciences, San Jose, CA, USA) and FACSCanto flow cytometer using Infinicyt™ 1·6·0v (Flow Cytometry Software, Cytognos, Salamanca, Spain); peripheral blood lymphocyte subsets (BD Multitest™ 6-colour TBNK reagent; Becton Dickinson Co., BD Biosciences, San Jose, CA, USA); naive and memory B cells [IgD-FITC, CD24-phycoerythrin (PE), CD4-peridinin chlorophyll-cyanin (PerCP-Cy)™ 5·5, CD27 PerCP-Cy™ 5·5, CD24 PE-Cy™ 7, CD38-allophycocyanin (APC) and CD19 APC-H7]; naive and memory T cells (CD45RA-FITC, CD45RO-PE, CD4-PerCP-Cy™ 5·5, human leucocyte antigen D-related (HLA-DR)-PE-Cy™ 7, CD31 Alexa Fluor® 647 and CD8 APC-H7; BD Biosciences); T_{regs} CD25-FITC and CD4-PerCP-Cy™ 5·5 (BD Biosciences). Antibodies to human FoxP3 (clone 2A3) and isotype control IgG1k with phycoerythrin (BD Pharmingen™). We confirmed the expression of IL-2 beta chain receptors with CD122-PE and gamma common chain receptor with CD132-APC (BD Biosciences).

Activation of CD4 lymphocytes

PBMCs were obtained using Ficoll-Hypaque gradient (Histopaque® 1077; Sigma, St Louis, MO, USA). CD4 lymphocytes were cultured with phytohaemagglutinin (PHA) (Gibco™, Invitrogen Corporation, Carlsbad, CA, USA) in the presence of IL-2 (7 UI/ml) (BD Pharmingen™), in order to evaluate the up-regulation of CD25. DR expression was used as a positive control of stimulated cells.

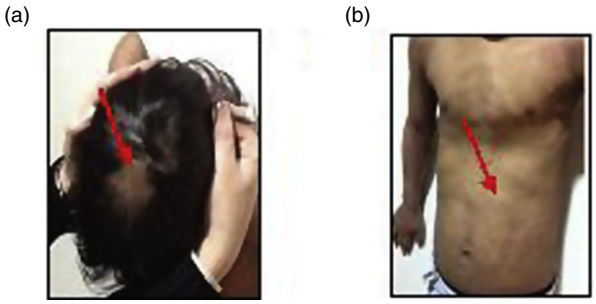

Fig. 1. (a) Patient's alopecia, (b) Severe eczema (see arrows).

Lymphoproliferation assays

We performed lymphoproliferation assays using PHA to stimulate T cells. Cells were cultured in the presence or absence of IL-2 during 5 days at 37°C with 5% of CO_2 and the proliferation assay was measured as the frequency of dividing cells (defined as carboxyfluorescein succinimidyl ester; CFSE) (CellTrace™ CFSE Proliferation Kit; Invitrogen, Molecular Probes®, Eugene, OR, USA.

Molecular biology

Molecular sequencing was done at Hôpital Universitaire Necker-Enfants Malades, Paris, France.

To predict the possible impact of amino acid substitutions on the structure and function of the protein, we used the PolyPhen-2 software tool.

Case presentation

Patient demographics and history. The patient is an adopted 5-year-old girl who developed severe atopic dermatitis, chronic diarrhoea and several respiratory infections at 6 days of age, needing extended hospitalization until the age of 6 months. Initially it was assumed to be an allergy to cow's milk protein, but the disease did not respond to a restriction diet. In this period she also presented with severe varicella infection. After she was discharged she began to suffer from alopecia, continued with bronchospasms, several lower and upper respiratory infections and exacerbations of her dermatitis (Fig. 1).

She had a torpid pneumonia needing permanent oxygen therapy. As a consequence, she was admitted for the first time to our hospital at 4 years of age. At this point we noticed characteristic features such as a prominent forehead and a saddle nose in a non-dwarfism girl. Due to severely compromised lung parenchyma on tomography, lung biopsy was performed, showing follicular bronchiolitis with lymphocyte hyperplasia (Fig. 2) (Table 1).

Taking her personal history and these results into account, we assumed it to be a dysregulation syndrome and began immunosuppressive treatment with corticosteroids, antibiotic prophylaxis, rapamycin and intravenous gammaglobulin. Under this regimen her condition improved, and oxygen therapy was no longer necessary.

Considering the immunosuppressive treatment, routine laboratory tests were performed, and no abnormalities in haematological cell counts, liver enzymes, cholesterol levels or renal function were noted. At the moment she is clinically stable, waiting for stem cell transplantation.

Patient's laboratory and diagnostic data. The patient presented with hypergammaglobulinaemia, absent IgG_4 and impaired specific polysaccharide response. IgA, IgM and IgE were within the normal range by age, with normal counts of lymphocyte subsets, slightly low naive and normal memory T cells and the lymphocyte B compartment presented normal naive B cells and low memory B cells. Regulatory T cells were extremely low (Fig. 3). She has high titres of antinuclear antibodies in a homogeneous and speckle pattern in different episodes, with positive c-ANCA. All other studied autoantibodies were negative (Table 2). She presented a normal phytohaemagglutinin (PHA)-stimulated T cell proliferation assay with no difference between PHA and PHA + IL-2 tubes (Fig. 4). Activation of CD4 lymphocytes with anti-CD3 in the presence of IL-2 showed DR expression, but no CD25 up-regulation (Fig. 5). We confirmed the expression of IL-2 beta receptor (CD122) and common gamma (CD132) chains (Fig. 6).

The molecular analysis of the IL-2 receptor (CD25αR) gene of the patient's DNA revealed the presence of a homozygous missense mutation: c. 122 a > c; p. Y41S, leading to an amino acid substitution in the extracellular domain, at position 41 of the protein.

In order to evaluate the functional compromise of the protein with this specific mutation we used the software tool polymorphism phenotyping (PolyPhen2), and found that this mutation is predicted to be probably damaging with a score of 1·000.

Discussion

CD25 deficiency is a disease with immune dysregulation that should be suspected on the basis of clinical and laboratory features. Early diagnosis is important to improve the clinical evolution of these patients [7].

IL-2 was identified originally based on its potent T cell growth factor activity and is considered widely to be a key cytokine in T cell-dependent immune responses. Regulatory T cells are subsets of T cells involved in the maintenance of peripheral self-tolerance and in regulating the immune response to non-self-antigens.

Signalling through the IL-2 receptor (IL-2R) contributes to T cell tolerance by controlling important aspects of T_{reg} biology. IL-2 is essential for thymic T_{reg} development and regulates T_{reg} homeostasis and suppressive function. Analogous to activated conventional T lymphocytes, IL-2R

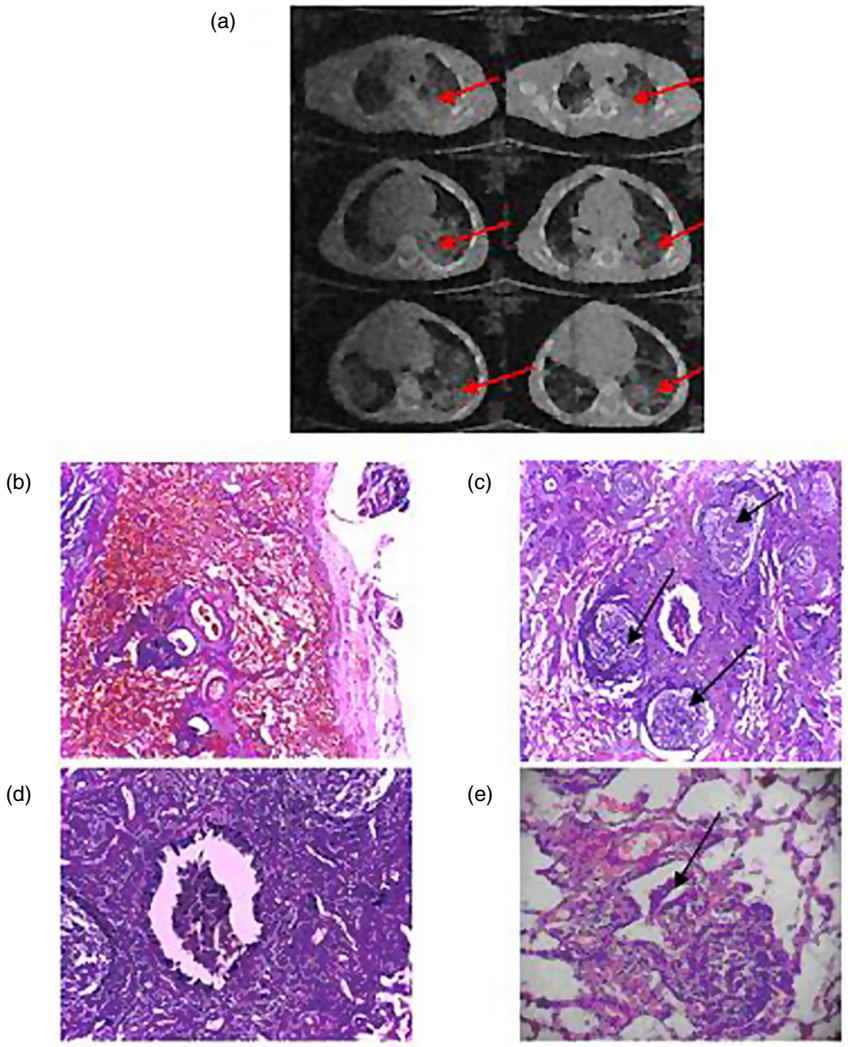

Fig. 2. (a) Computerized tomography (CT) scan of the lungs of the patient, showing areas of consolidation (indicated by arrows). (b) Lymphocytic infiltrate that is arranged in follicles surrounding the bronchioles. (c,d) Peribronchial lynphoid follicle [haematoxylin and eosin (H&E) ×4 and ×10, respectively]. (e) Peribronchiolar lymphoid accumulations (H&E ×10) (see arrows).

signalling also plays an important part in T_{reg} cell growth, survival and effector differentiation. In particular, T_{reg} cells require essentially only IL-2-dependent receptor proximal signal transducer and activator of transcription 5 (STAT-5) activation, as they contain inhibitory pathways to minimize IL-2R-dependent activation of the phosphatidyinositol 3-kinase/Akt pathway [10]. Moreover, many IL-2R-dependent activities, including full induction of FoxP3 expression, in T_{reg} cells require minimal and transient STAT-5 activation [11].

Collectively, these results suggest that IL-2 is essential for the development, maintenance and function of $CD4^+CD25^+$ T_{regs}. Thus, *Foxp3*/*FOXP3* appears to be a master control gene for the development and function of natural $CD4^+CD25^+$ T_{regs}. Given that humans bear natural $CD4^+CD25^+$ T_{regs} with a phenotype and function comparable to those found in rodents, it is most likely that in IPEX, disruption of the *FOXP3* gene abrogates the development of thymic T_{regs}, leading to hyperactivation of T cells reactive with self-antigens, commensal bacteria in the intestine or innocuous environmental substances, thus causing autoimmune polyendocrinopathy, immune bowel disease or allergy, respectively. This has several implications for self-tolerance and autoimmune/inflammatory disease in humans: first, this is an example showing that an abnormality in naturally arising T_{regs} could cause human autoimmune disease, immune bowel disease and allergy; and secondly, the development of natural T_{regs} is, at least in part, genetically and developmentally programmed [12].

Our patient's clinical manifestations (eczema, chronic diarrhoea and infection susceptibility) are the result of the mutation in the CD25 molecule, due to the importance of an intact receptor function to control the immune system homeostasis, as cited in the previous paragraph.

The obliterant bronchiolitis was the main sign that led us to the diagnostic suspicion of this type of disease, as we have reported on other girls (data not shown) with STAT-5B deficiency that presented the same chronic lung compromise. We emphasize that pulmonary tissues are a typical

Table 1. Comparative table between the four patients with CD25 deficiency

Clinical features	1st patient Sharfe *et al*. 1997 [8]	2nd patient Caudy *et al*. 2007 [2]	3rd patient Goudy *et al*. 2013 [9]	Our patient
Age of onset	6 months	6 weeks	1 month	6 days
Consanguinity	Yes (first cousins)	No	Yes (first cousins)	Unknown
Chronic diarrhoea/ enteropathy	Severe (8 months)	Severe, with villous atrophy (6 weeks)	Severe, with villous atrophy (1 month)	Severe (6 days)
Severe eczema	–	Diffuse (2 years)	Diffuse (1 month)	Diffuse (6 days)
Alopecia universalis	–	–	+ (5 years)	+ (5 years)
Insulin-dependent diabetes	–	+ (6 weeks)	–	–
Autoimmune haemolytic anaemia	–	+ (3 years)	–	–
Hypothyroidism	–	+ (3 years)	–	–
Autoimmune neutropenia	–	+ (5 years)	–	–
Autoimmune thyroiditis	–	–	+ (4 years)	–
Hepatosplenomegaly	+ (8 months)	+ (2 years)	–	–
Lymphadenopathy	Yes (8 months)	Yes (2 years)	Yes (5 years)	
Viral infections	Recurrent CMV pneumonitis (6 months) + adenovirus gastroenteritis (6 months)	Recurrent CMV pneumonitis, urine tract infection and chronic instestinal inflammation (6 weeks) + EBV lymph nodes hyperplasia (2 years)	CMV intestinal infiltration (1 months)	Severe varicella (6 months)
Upper respiratory tract infections		Persistent sinusitis and otitis media (5 years)		Recurrent (6 months)
Lower respiratory tract infections	Recurrent (8 months)	Recurrent (5–8 years)		Recurrent (6 months)
Chronic lung disease	Right upper lobe (3 years)	–	–	Follicular bronchiolitis with lymphocyte hyperplasia (4 years)
Other	Persistent thrush + candida oesophagitis + failed to thrive (6 months)/ gingivitis + iron deficiency anaemia + chronic mandible inflammation (3 years)	Asthma (5–8 years)	Bullous pemphigoid (1 year)/psoriasiform dermatitis (5 years)/ bacterial cellulitis (8 years)	Asthma (6 months)
Mutation at DNA level	60–64 del-4 bp	301 C > T; 693 ins A	497 G > A	122 A > C
Mutation at protein level	21 FS and 46 Stop	101 Stop; 232 FS	166 S > N	41 Y > S

Age of onset of the first symptom in days (d), weeks (w) and months (m). Clinical features negative (–), positive (+) and between parentheses the age of the patient in days (d), months (m) and/or years (y). CMV: cytomegalovirus; bp: base pairs; EBV: Epstein–Barr virus; FS: frameshift.

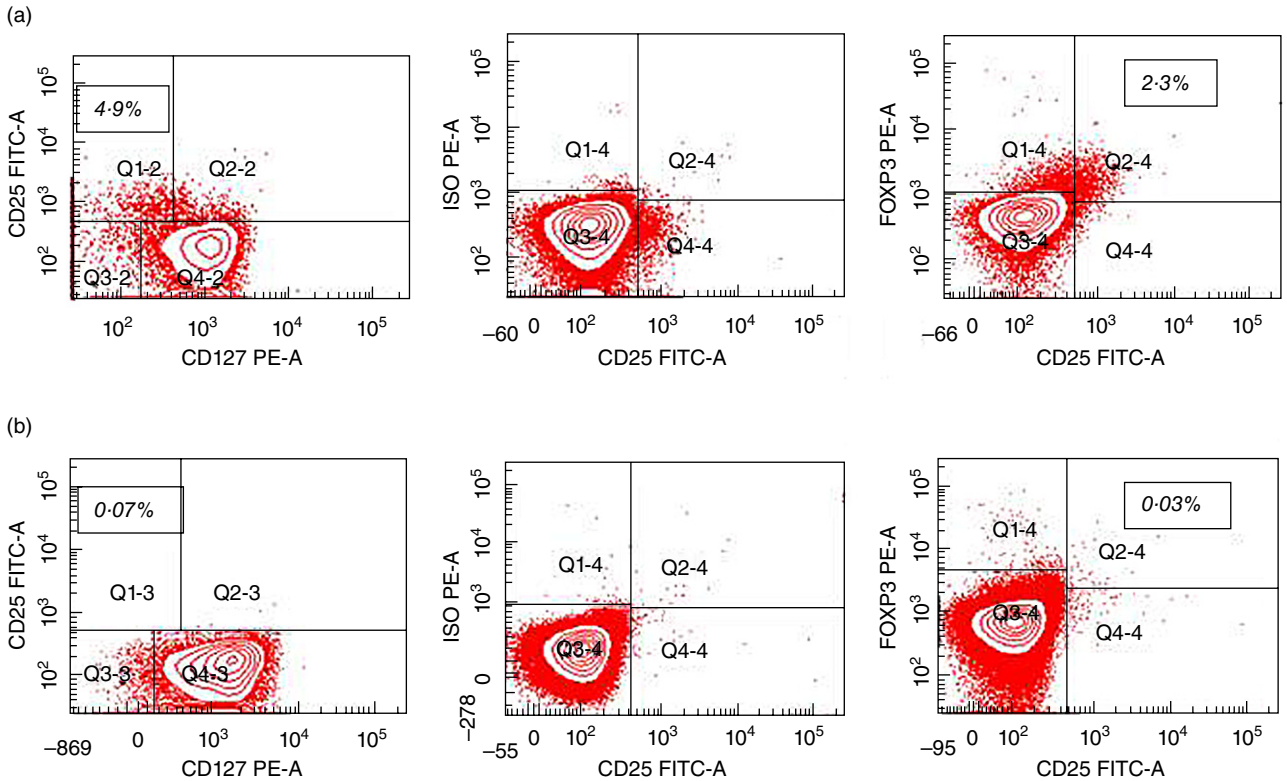

Fig. 3. Regulatory T cells dot-plot. Graphic of a control sample (a) with normal count of regulatory T cells (T_{regs}) and (b) patient sample with extremely low T_{regs} measured as $CD25^{+}CD127^{low}$ and $CD4^{+}$ (gate not shown) $CD25^{+}$forkhead box protein 3 ($FOXp3^{+}$).

target of lymphocytes immune dysregulation, where they infiltrate and can find no control of their cytotoxic activity inducing severe tissue damage [13].

The stained lung sections of our patient are reminiscent of those described in mice by Curotto de Lafaille *et al.*, in which chronic antigen stimulation through the airways leads to an abundance of organized lymphoid tissue [14], with defined T and B cell areas and germinal centres [15] (Fig. 2c).

Although our patient has hypergammaglobulinaemia, she has an impaired specific polysaccharide response which makes the immune system more susceptible to bacterial

Table 2. Laboratory at 4 years old

Serum Igs	Total IgG (g/%)	IgG1 (g/%)	IgG (g/%)	IgG3 (g/%)	IgG4 (g/%)	IgM (g/%)	IgA (g/%)	IgE (UI/ml)
Patient	1·9	1·1	0·29	0·06	n.d.	0·15	0·27	60
Normal values	(0·9–1·4)	(0·3–8·2)	(0·08–0·5)	(0·08–0·1)	(0·01–0·12)	(0·06–0·12)	(0·05–0·1)	(8–32)

ANA	Anti-DNA Anti-histone	ANCA	ENA	aTg-A
Positive (640–2560) Homogeneous and speckle pattern	Negative	c-ANCA: positive ++ p-ANCA: negative	Sm(−) RNP(−) Ro(−) La(−) Scl70(−) Jo1(−)	Negative

T subsets (%)	$CD3^{+}$	$CD4^{+}$	$CD8^{+}$	$CD4^{+}CD45RA^{+}$	$CD4^{+}CD45RO^{+}$	$CD4^{+}CD25^{+}FoxP3^{+}$
Patient	61	47	11	46	50	0·03
Normal values	(60–81)	(26·8–42·3)	(17·5–35·0)	(57·8–81·0)	(17·9–39·0)	(1·0–3·1)

B subsets (%)	Naive	Memory $IgD^{+}CD27^{+}$	Memory $IgD^{-}CD27^{+}$
Patient	88	4	5
Normal values	(52·3–72·1)	(9·5–17·2)	(5·5–14·9)

Serum immunoglobulins (Igs), anti-nuclear antibodies (ANA) and phenotype (T and B cells subsets). Note that regulatory T cells are almost absent. Normal values from an age-matched control sample. ANCA: anti-neutrophil cytoplasmic antibody; ENA: extractable nuclear antigens; FoxP3: forkhead box protein 3; n.d.: not determined.

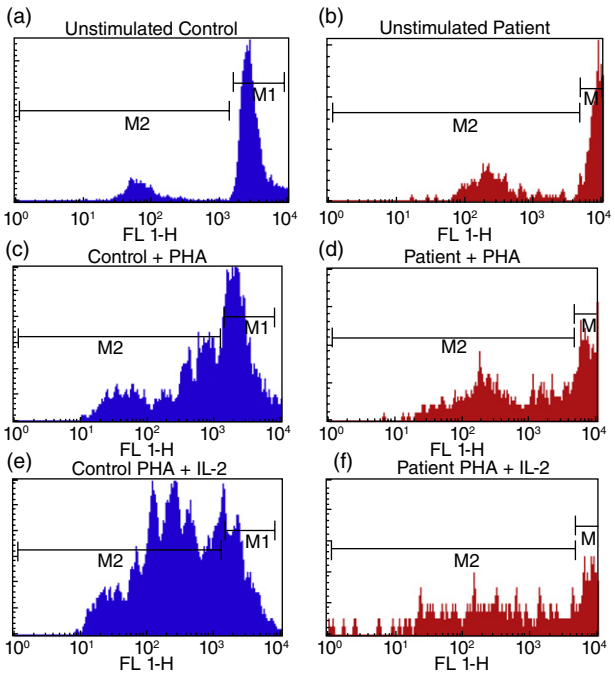

Fig. 4. Frequency of dividing cells [defined as carboxyfluorescein succinimidyl ester (CFSE) low fraction]. See the normal proliferation of cells in control (a) and patient (b) with phytohaemagglutinin (PHA) (c,d, respectively), but no difference between culture with phytohaemagglutinin (PHA) in the presence of interleukin (IL)-2 in the patient (f) as seen in the control sample (e). Index ratio (expressed as stimulated/unstimulated) (d) 1·65; (f) 1·92; (c) 3·80; (e) 5·71.

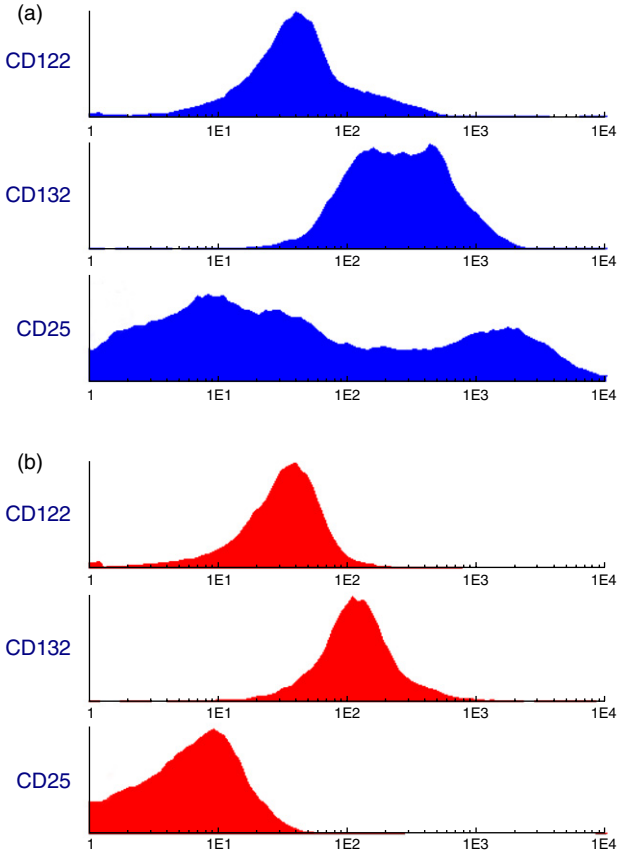

Fig. 6. Expression of CD122[interleukin (IL)-2Rβ], CD132 IL-2Rγc. Normal expression of beta and gamma common IL-2 receptor chains in control (a) and patient (b).

infections. As we observed low memory B cells, we hypothesized that there might be a Th2 cytokine dysregulation involved in this brake.

Published patients with CD25 deficiency had their first symptom before 6 months of age, and our patient presented her first clinical manifestation during her first week of life. Severe chronic diarrhoea was the most common and early symptom in all of them. The other three patients had cytomegalovirus (CMV) infections, while our patient had severe herpes virus infections. Only one had multiple endocrinopathies and the other two had autoimmunity.

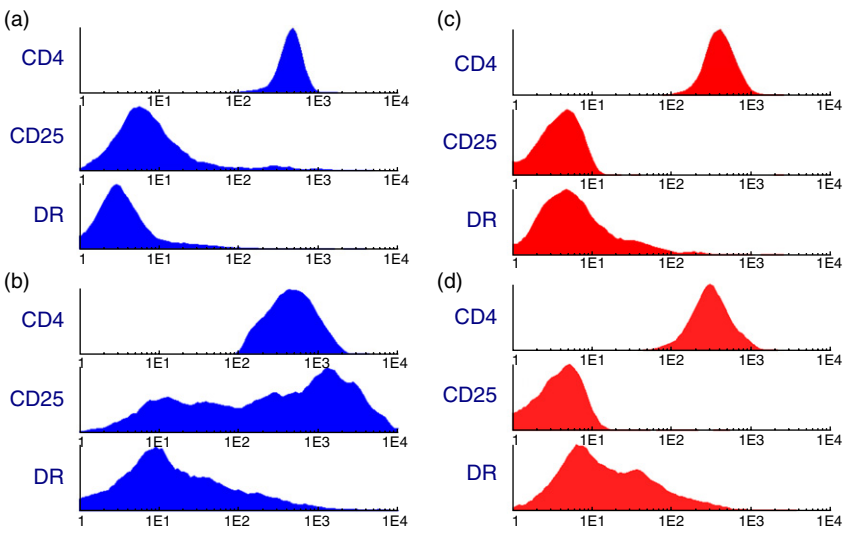

Fig. 5. Activation of CD4 lymphocytes: gated on $CD4^+$, cells were activated with phytohaemagglutinin (PHA). (a,c) Unstimulated control and patient, respectively; (b,d) stimulated control and patient, respectively, as seen on DR over-expression. There is no up-regulation of CD25 in the patient (d) after stimulus compared with the control sample.

These cases show the heterogeneity of the clinical manifestations of the dysregulatory syndromes due to CD25 deficiency, being early-onset severe enteropathy and common viral infections the common feature between all of them (Table 1).

The association of immunodeficiency and autoimmunity in patients with CD25 deficiency reflects the biological role of IL-2-mediated signalling. In fact, interaction of IL-2 with its high-affinity receptor, composed of IL-2Rα, IL-2Rβ and γc, is important both for the activation of effector $CD4^+$ and $CD8^+$ T cells as for the generation of $FoxP3^+$-induced T_{reg} (iT_{reg}) lymphocytes from naive peripheral T lymphocytes [16].

Rapamycin is an excellent option to control both clinical and pathological gastrointestinal manifestations of the disease. Sirolimus interacts with an FK binding protein and the mammalian target of rapamycin in T cells to inhibit cell cycle progression, in part by controlling the phosphorylation of several translational and cell cycle regulatory factors. Emerging evidence suggest that sirolimus may selectively inhibit effector T cells while allowing regulatory T cell expansion [17].

The identification of genetic defects in a variety of immune dysregulation syndromes is yielding important insights into basic mechanisms of autoimmunity in humans [2].

Unlike other patients who have been reported as severe combined immunodeficiencies, our case has a particular presentation, with chronic clinical evolution and the hallmarks of several infections and no autoimmunity as in other cases.

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Disclosure

There are no conflicts of interest.

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