

RLTPR deficiency : a new genetic etiology of combined immunodeficiency

Yi Wang

▶ **To cite this version:**

Yi Wang. RLTPR deficiency : a new genetic etiology of combined immunodeficiency. Immunology. Université Sorbonne Paris Cité, 2016. English. NNT : 2016USPCB111 . tel-02145632

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THESE DE DOCTORAT EN SCIENCES

UNIVERSITE PARIS DESCARTES – DIDEROT

Pour l'obtention du diplôme de

DOCTEUR DE L'UNIVERSITE PARIS V - PARIS VII
SPECIALITE: IMMUNOLOGIE

Présentée et soutenue publiquement par

Yi WANG

Le 25 Novembre 2016

Sujet de thèse :

RLTPR DEFICIENCY:

A NEW GENETIC ETIOLOGY OF COMBINED IMMUNODEFICIENCY

JURY

Monsieur le Docteur Andrew GENNERY, Rapporteur

Monsieur le Professeur Stephan EHL, Rapporteur

Monsieur le Professeur Alain HOVNANIAN, Examinateur

Madame le Docteur Vanessa SANCHO-SHIMIZU, Examinateur

Madame le Docteur Emmanuelle JOUANGUY, Directrice de Thèse

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"I am among those who think that science has great beauty."

-Marie Curie

"The most beautiful experience we can have is the mysterious. It is the fundamental emotion that stands at the cradle of true art and true science."

-Albert Einstein

"The scientist is not a person who gives the right answers, he's one who asks the right questions."

-Claude Lévi-Strauss

Acknowledgements

First, thanks to Dr. Andrew Gennery and Pr. Stephan Ehl for accepting to be the reporters of my thesis, and to Pr. Alain Hovnanian and Dr. Vanessa Sancho Shimizu for accepting to be examiners for my thesis.

I will forever be thankful to the director of the lab, Jean-Laurent Casanova, thank you for giving me the chance to know science, how beautiful it is, how true it is and also how cruel it is. I was a kid for science when I came to the lab 5 years ago, I felt like I was in a huge ball pool, fun but also confused, the ball pool called human genetics of infectious diseases though. Thanks to your patience, encouragement and lectures during my thesis, I am on my way to be someone who is not only trying to give right answers but also ask the right questions. I hope that I could be as lively, enthusiastic, and energetic as you, never stop making efforts to science.

Thanks to Laurent Abel, the co-director of the lab, you've been always very kind, supportive and dependable. Thanks for the reception of these five years first, and the helpful discussion in the lab meetings, I will benefit all your scientific suggestions for my future research.

A very very big thank to Emmanuelle Jouanguy, director of my thesis and also the mentor of my master's internship. Words can not describe the feeling of me for you. You were a strict teacher for pushing me to have great achievements, discussions in the evenings, weekends or holidays. You made me grow up in light speed to a big girl who can be independent, serious and strong for research. Thanks to you, before I design any experiment, I must ask myself, do you have negative control, positive control? Do you set different conditions? See, such a nice impact on me for doing research. And now, you are not only my teacher, with ups and downs, we bond together. I will never forget your help and support in my future life. Thanks for giving me your knowledge. I will take three more things from you to my future, three sketches, first, you were smoking and holding a coffee in your left hand in Necker; second, my face was hit by ball, you were carrying an ice bag and ran to me in Jura; last, you were working in front of computer.

A huge thank to Vivien Béziat, who is my defined "Chihuahua" and "Sheldon", such smart, serious and dependable. When a window closed to me, you opened a door for me. Working with you has always been adorable and memorable, I learnt so much about immunology and flow cytometry from you. The nights we spent in front of flow cytometry machines, the scientific questions we discussed, the anxiety you passed to me, the force you gave to me, the joy when we had great results, the beers we had for celebrating our first paper. Thank you so much for everything you did for me during my thesis, I am sure you will do better and happier science in future and please give my best wishes to little Baptiste! More and more grateful words keep in my heart, Bazinga!

My dear Monsieur Lazaro Lorenzo, “papa” of my thesis life. Thank you very much for leading me to bench work, from how to extract RNA till I become as good as you do on bench, or even better! We like old friends, not only because you are from Cuba I am from China, but also for being faithful and helpful. Thank you for teaching me a lot of experiments with your patience and encouragement, thanks for discussing with me when I had difficulties, thanks for being a bin when I complained, thanks for making all kinds of jokes to make me forget stress and pain. Our virus zones, Necker or Broussais or Imagine, are full of your songs, dances and jokes which gave me so much force to work hard and keep moving on! Dear papa, thank you for everything, I’ve never thought can have a friend like you, a teacher like you, the lab not only gave me a beautiful paper and a memorable PhD life, but also a such precious gift, you! I wish your dream comes true as soon as possible!

I also want to thank virus group, Laure and Julien in Paris branch. Thank you very much, Laure, you taught me so many tips while doing experiments, with you, my techniques of experiments were progressing day by day. Thanks for helping me with techniques issues those Saturdays, thanks to you, I do better and beautiful experiments! Julien, “brother” of virus group, thanks for discussing with me, helping me, making jokes and playing music. You are such rock n’roll that without you, our office is so quite. Wish you a big success in your career. Ofhlyn, my apple, thanks for always being with me whether I am sad or happy, your support is really important to me, your tender voice always makes me calm and feel better, with our special Asian connection, we will enjoy a lot of great time together. For NYC branch, I’d like to thank Jill, Fabien, Serkan for all helpful discussions and advices of my projects and future career, working with you guys, makes me always feel that I have so many things to learn, thanks for giving me so many motivations to do better. Shen-Ying, thank you for all kind and helpful scientific discussions, being like a sister to support me and encourage me. Moreover, we have long time to share all our scientific thoughts, crazy ideas, happiness and precious moments of our life in future, we will get there!

A Big thank to CMC group, not only because I worked with you, but also I love all people of CMC group. Anne Puel, hottest senior for me, thank you for all helpful discussions and suggestions for projects, thank you for encouraging me to do whatever I want for the future, seems like you give me wings, each time after talking with you, I can fly anywhere with your support, hope and trust. Thank you, Anne. Yun Ling, my Chinese brother, who helped me so much when I just arrived in the lab. Thank you, my brother, our discussions, your tips of techniques, your humors, the stories you told in Chinese, nice dinners and picnics with your family, especially with Lila, my cutie bunny, I want to thank all your help and support for these years. Erika Della Mina, such a beautiful Italian girl, how can I forget all the days and nights we spent in the lab and my studio, thanks for always supporting me and helping me. Share all happiness and tough time with me, you are such a wonderful friend, whenever I need a shoulder, you’ve been always there. I also want to thank Laura,

Melanie, Yoann, Romain L, Romain G, Juan, Luyan, your humors, your help, your support and the time with you for working or having fun. Yoann, thanks for relaxing me by your baby's pics, I wish you and your little mickey all the best!

Maya, my dear Maya, thank you very much for being a nice sister, always makes me calm down and relax, whatever happened, with you, I can chillax immediately, you see your magic? Thanks for taking care of me as your own sister during these years, many thanks! Carmen and Joelle, two young girls, like my little sisters, thanks for having you during lunch time, tea time and candy time, thanks to Carmen's cat face, you smooth my troubles; Joelle, thanks to your famous quote: let it go, I become stronger and stronger! Silvia, my sunshine, my giggle girl, thanks for your hugs, your laughter and your love, I know there is always a room for me in Florence, and Dante! Alejandro, thanks for being my fans, you've always been supportive and nice! Maria, although we know each other for a short time, but your smile is a good painkiller! Caroline, my TripAdvisor, my Allocine, thanks for making my life outside the lab so amazing and colorful. Antoine, my dear brother, thanks for the discussion of experiments, thanks for your unique hugs with warm and love, thanks for all jokes which like sunshine of my lab life, thanks for being as crazy as me about spicy food, I wish you have a great paper and postdoc in Australia. I also want to thank Jacinta, thanks for the discussion of patients, giving me precious suggestions of my project, thanks for your care and all help.

I'd like to thank Aziz, Vimel, Gaspar, Aurelie, Fredegonde, Jean, Quentin and all help and valuable discussions from dry lab, and everyone of U980 of Paris and NYC who I didn't write the name one by one, thanks!

Thanks to the help from Celine, Martine and Lahouari for my contracts and documents, without you I can't focus on my work, thank you very much.

A big thank to ANRS, for supplying fellowship to my thesis, with the strong support I can continue my study and work. Thanks to the fellowship of the fourth year of Imagine Institute, without your support, I can't finish my thesis.

It's time to thank my friends outside the lab, Qianwen, my dear, thanks for being with me since 2010, you have no idea that how important you are in my life, thank you for always supporting me, encouraging me and loving me! Di, my dear sister, thanks for all good times we had in Paris, please wait for me in Chengdu with my dear godson! Shu, thanks for meeting you in Imagine, days, nights and weekends we spent in the lab, it's memorable and so warm. We always make jokes on it, who knows Imagine after 23h, the answer is US! Thanks for the discussions, encouragements, flowers, and everything you did for me and with me, I can't wait to see little monkey, pearl and you in China! Thank you, Liang Ning, Ma Kuiying, Fang Bin, Chuande, Xiao bai, Haidai, Hua Lin and all Chinese friends I met in Paris, thanks for all great Chinese meals and good times, your help and support. I wish all of you great successes in the

future; let's make an effort to Chinese academia together! I also want to thank other besties, Chang Hongying, Zhou Ye, Yang Fan, Qian Yu…you are faithful friends, as long as I need you, you are all there! How lucky I am!

I appreciate a lot of people who were so supportive and friendly to me, Kevin, Louis, Sina, Caroline Alby, Anne, Lucas, Yi Hao, Marc… our neighbors of Necker and Imagine, thank you.

I'd like to thank all patients and their families from Morocco, France and Turkey, without you, I won't have this great paper, thanks for your contribution to my work and research. A big thank to all physicians, Dr. Aziz Bousfiha, Dr. Fatima Ailal, Dr. Yildiz Camcioglu, Dr. Serge Jacquot (a very nice meeting with you at ESID), and Dr. Alain Hovnanian, thanks for all help and comments for this project! And all our collaborators, Stuart Tangye, Cindy Ma, Bernard Malissen…Thanks for your great work and collaborations!

Finally, I want to thank my parents, the greatest parents in the world, always be generous, caring and loving, you tolerant all my bad tempers, and never give up your crazy daughter, whatever I do, you are always there with me. You always say that you are proud of me; the truth is that I am so proud of you, I love you

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1. **Yi Wang**, Cindy S. Ma, Yun Ling, Aziz Bousfiha, Yildiz Camcioglu, Serge Jacquot, Kathryn Payne, Elena Crestani, Romain Roncagalli, Aziz Belkadi, Gaspard Kerner, Lazaro Lorenzo, Caroline Deswarte, Maya Chrabieh, Etienne Patin, Quentin B. Vincent, Ingrid Muller-Fleckenstein, Bernhard Fleckenstein, Fatima Ailal, Lluis Quintana-Murci, Sylvie Fraitag, Marie‐Alexandra Alyanakian, Marianne Leruez‐Ville, Capucine Picard, Anne Puel, Jacinta Bustamante, Stéphanie Boisson‐Dupuis, Marie Malissen, Bernard Malissen, Laurent Abel, Alain Hovnanian, Luigi D. Notarangelo, Emmanuelle Jouanguy, Stuart G. Tangye, Vivien Béziat, and Jean-Laurent Casanova

Dual T cell– and B cell– intrinsic deficiency in humans with biallelic RLTPR mutations (2016) ***J. Exp Med*** 2016 2016 Oct 17;213(11):2413-2435.

2. Julien Cottineau, Molly C. Kottemann[#], Francis P. Lach[#], Young-Hoon Kang[#], Frédéric Vély[#], Elissa K. Deenick*, Tomi Lazarov*, Laure Gineau*, **Yi Wang***, Andrea Farina, Marie Chansel, Lazaro Lorenzo, Christelle Piperoglou, Cindy S. Ma, Patrick Nitschke, Aziz Belkadi,Yuval Itan, Bertrand Boisson, Fabienne Jabot-Hanin, Capucine Picard, Jacinta Bustamante, Céline Eidenschenk, Soraya Boucherit, Nathalie Aladjidi, Didier Lacombe,Pascal Barat, Waseem Qasim, Jane A. Hurst, Andrew J. Pollard, Holm H. Uhlig, Claire Fieschi, Jean Michon, Vladimir P. Bermudez, Laurent Abel, Jean-Pierre de Villartay, Frédéric Geissmann, Stuart G. Tangye, Jerard Hurwitz, Eric Vivier, Emmanuelle Jouanguy, Agata Smogorzewska, and Jean-Laurent Casanova

Human inherited GINS1 deficiency: impaired DNA replication, growth retardation, and an atypical immunodeficiency (2016) Submitted

3. Vivien Béziat, Cindy S Ma[#], Aziz Bousfiha[#], Isabelle Pellier[#], Yoann Zerbib[#], Juan Li[#], **Yi Wang**[#], Romain Lévy, Frédégonde About, Ai Ing Lim, Geetha Rao, Kathryn

Payne, Simon J Pelham, Danielle T Avery, Elissa K Deenick, Chou Janet, Romain Guéry, Aziz Belkadi, Antoine Guérin, Mélanie Migaud, Emmanuelle Jouanguy, Ingrid Müller-Fleckenstein, Bernhard Fleckenstein, Baris Safa, Ozen Ahmet, Somer Ayper, Fatima Ailal, Ibtissame Naim, Ibtihal Benhsaien, Isabelle Meyts, Olivia Chandesris, Mathias Titeux, Sylvie Fraitag, Marie-Alexandra Alyanakian, Capucine Picard, James Di Santo, Alain Hovnanian, Keles Sevgi, Talal Chatila, Laurent Abel, Stuart Tangye, Anne Puel, and Jean-Laurent Casanova

Inherited Toubkal deficiency in patients with mucocutaneous candidiasis and allergy

(2016) Submitted

List of Abbreviation:

AR: Autosomal recessive

Ab: Antibody

BCR: B cell receptor

BCG: Bacillus Calmette-Guérin

CID: Combined immunodeficiency

CMC: Chronic mucocutaneous candidiasis

DPT: Diphtheria, pertussis (whooping cough), and tetanus.

ENU: N-ethyl-N-nitrosourea

EBV: Epstein-Barr virus

GDI: Gene damage index

GWL: Genome-wide linkage

IVIG: Intravenous IgG

LRR: Leucine rich repeat

LOD: Logarithm (base 10) of odds

MFI: Fluorescence intensity

NF-κB: Nuclear factor-kappa B

PID: Primary immunodeficiency

PBMC: Peripheral blood mononuclear cell

PMA: Phorbol 12-myristate 13-acetate

PHA: Phytohaemagglutinin

SCID: Severe combined immunodeficiency

STAT: Signal transducer and activator of transcription

TCR: T cell receptor

TB: Tuberculosis

WES: Whole-exome sequencing

WT: Wild- type

Abstract

Combined immunodeficiency (CID) refers to inborn errors of human T cells that also affect B cells, due to the T cell deficit or an additional B cell-intrinsic deficit. The identification of new genetic etiologies of CID is important to better understand the immune responses to infectious agents in humans, and to better manage patients with infectious diseases. We herein report 6 patients from 3 unrelated families (Moroccan, Tunisian and Turkish) with bi-allelic mutations in RLTPR, the mouse orthologue of which is essential for CD28 signaling. The patients developed a variety of infections, including invasive tuberculosis and mucocutaneous candidiasis but also presented cutaneous and pulmonary allergic manifestations. The identification of autosomal recessive RLTPR deficiency elucidates a new genetic etiology for CID patients. This work focused on the role of human RLTPR in T cell and B cell immunity. In RLTPR-deficient patients, proportions of circulating regulatory T cells, memory $CD4^+$ T cells and central memory $CD8^+$ T cells are reduced. In vitro, proliferation of patients' T cells is diminished in response to various stimuli, including mitogens and antigens. The RLTPR-deficient $CD4^+$ T cells did not respond to CD28 stimulation in terms of the production of IFN-γ, TNF and IL-2, as well as the phosphorylation of P65. The $CD4^+$ T cells exhibit a Th2 bias ex vivo and when cultured in vitro, contrasting with the paucity of Th1, Th17, and Tfh cells. The patients also displayed a deficiency of memory B cells and poor Ab responses. This B cell phenotype does not result solely from the T cell deficiency, as the patients' B cells fail to activate NF-κB upon BCR stimulation. Our study strongly suggests that human RLTPR deficiency is a CID affecting at least the CD28 responsive pathway in T cells and the BCR responsive pathway in B cells.

Key words: Combined immunodeficiency (CID); RLTPR; CD28 signaling; T cells; B cells

Section I. Introduction

I.1 Primary Immunodeficiencies (PIDs)

The field of primary immunodeficiency diseases (PIDs) was born in the middle of 20th century, with the description of Bruton agammaglobulinemia (XLA)[1] and Kostmann's congenital neutropenia[2]. These patients had an absence of gamma globulins and severe neutropenia, respectively, and developed various infections, pneumococcal sepsis and bacterial infections, respectively. PIDs have since been typically associated with multiple life-threatening recurrent infections and abnormal immunological features. Gradually, it appeared that PIDs can underlie five major categories of phenotypes, including infection, allergy, malignancy, autoimmunity and autoinflammation[3] (Fig.1). To date, PIDs comprise more than 260 genetic disorders. For decades, PIDs were however confined to rare, fully penetrant, familial diseases and recessive traits[4,5,6].

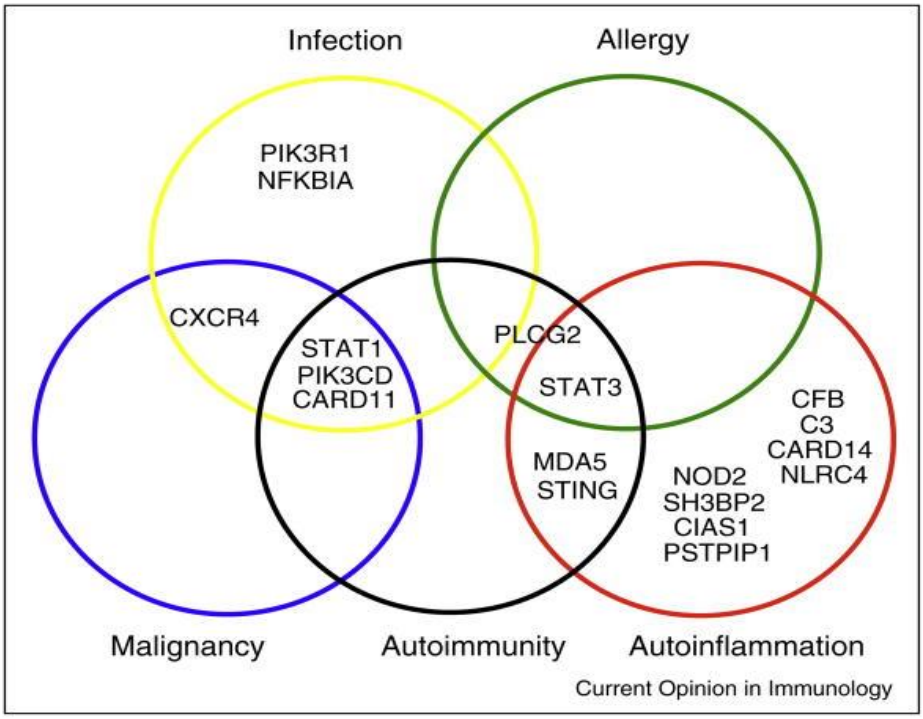


Fig.1. Manifestations of PIDs include five major categories, infection, allergy, malignancy, autoimmunity and autoinflammation. (Adapted from Boisson et al, 2015[3])

Since the last 15 years, their definition has been modified with the emergence of PIDs characterized by susceptibility to a single pathogen, the identification of dominant diseases, and the description of non-hematopoietic, tissue-intrinsic defective immunity[4]. Among classical PIDs, the IUIS classification of 2015 reported 9 major different groups: (i) severe combined immunodeficiency (SCID), characterized by the absence of autologous T cells; (ii) combined immunodeficiencies with associated or syndromic features, these disorders show a wide range of abnormalities of T and/or B cells; (iii) antibodies deficiencies in which patients are susceptible to bacterial infections; (iv) immune dysregulation diseases, with significant autoimmune manifestations and impaired cell-mediated cytotoxicity; (v) phagocytes' numbers and/or function; (vi) defects in intrinsic and innate immunity, patients display the clinical phenotypes which resulting from various infections; (vii) autoinflammatory disorders, the hallmark of these disorders are abnormally increased inflammation, mediated mainly by the cells and molecules of innate immune system; (viii) complement defects and (ix) phenocopies of PID[7].

Among these different categories, some might share common clinical manifestations, as higher susceptibility to bacterial infections, reported in hyper IgE syndromes (CID) and in TIR signaling pathway defect (Innate immunity) or autoimmunity, reported in some CIDs and in some immune dysregulation diseases. For example, severe bacterial infections are observed in patients with STAT3- and IRAK4-deficient patients, whereas only STAT3 deficiency is associated with hyper IgE[8–11]. Although, mutations in STIM1 and CTLA4 are associated with autoimmunity phenotype, STIM1 deficiency belongs to CID subgroup whereas CTLA4 in immune dysregulation diseases subgroup [12-13,14]. Both medical and scientific contributions,

with a strong clinical description (initial and follow-up) and a deep biological phenotype (immunological and biochemical), lead to a better understanding of the function of a protein, a pathway, a cell type in the immune response and to improve the diagnosis and treatments to PIDs. Nowadays, diverse treatments are available to improve the conditions of patients with PIDs. Immunoglobulin replacement therapy (IVIG) is one of the efficient therapies[15], for humoral deficiencies and antibody deficiency, but not only, as also in reducing the occurrence of some infections. Immunosuppressive treatments are helping people who suffer from inflammatory diseases. Furthermore, hematopoietic cell transplantation (HCT)[16] is the only potentially curative therapy available for many primary immunodeficiencies by now. Some other treatments, like gene therapy, have shown to be efficient for a few hematopoietic lineage defects, such as SCID. The clinical experience gathered throughout the years endorses disease awareness and should guide research into disease progression, newborn screening, and future therapeutic strategies[17].

I.2 Combined immunodeficiencies (CIDs) and T cell immunity to infections

The phenotype of SCID patients is characterized by a wide variety of life-threatening infections of infancy and the absence of autologous T cells[7,18]. By contrast, the term combined immunodeficiency (CID) is used to define related conditions in which T cells are present (with normal or decreased numbers) but defective, and an impaired antibody response is observed[19]. The B cell phenotype can be either a strict consequence of the T cell deficit, or the combination of the T cell defect and an additional B cell-intrinsic defect. Patients with CIDs suffer from various infections and often also from autoimmunity, allergy, or both[17]. Distinct groups of CIDs have been reported with or without syndromic features. The molecular and cellular understanding of each new CID gives new insights into T and B cell immunity. Many T cell functional defect cases elucidated contribute to the study of T

cell and B cell interaction. The non- or poor- functional T cells may also explain why patients are susceptible to infections.

It is well known that TCR and co-stimulation signaling pathways both contribute to T-B cell interactions[20]. By the help of T cells, B cells produce specific antibodies[21]. Full T cell activation and differentiation require 3 signals. “Signal No.1” supplies specific antigen on T cell responses that is triggered by the interaction of the T cell antigen receptor (TCR) and class I and class II molecules of the major histocompatibility complex (MHC). The second signal, or costimulatory signal, is delivered by the co-receptor CD28 expressed on antigen-presenting cells (APCs) after binding of its ligands CD80 and CD86. The best characterized T cell co-stimulation pathway is B7-1/B7-2-CD28/CTLA4 which is essential for T cell activation and T cell tolerance[22,23]. CD28, in synergy with TCR, activates the NF-κB signaling pathway[24]. Moreover, classical NF-κB signaling pathway is activated by T cells and B cells antigens through specific receptors[25] (Fig.2).

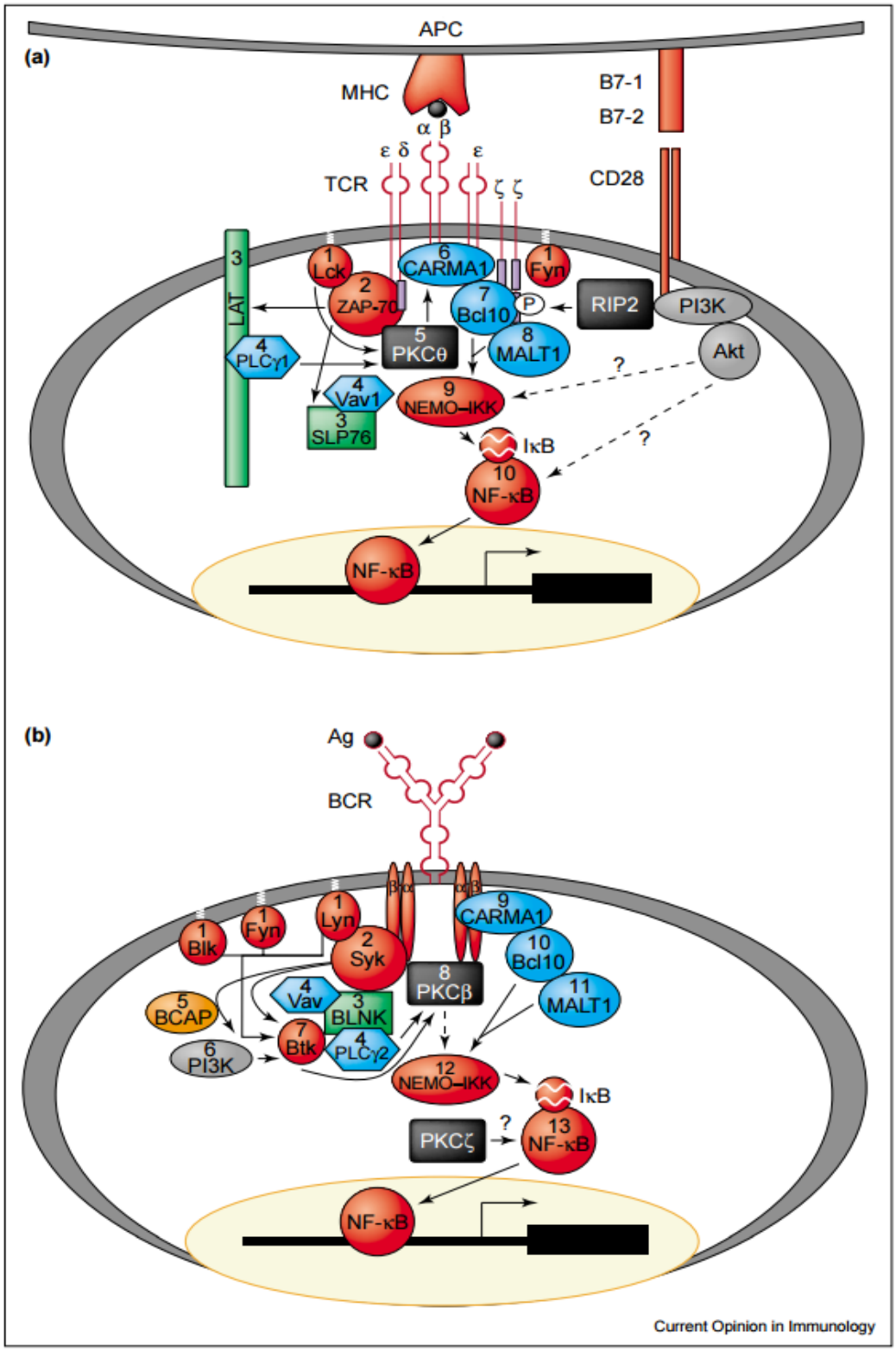


Fig.2. Antigen-mediated NF-κB activation in T and B lymphocytes. (Adapted from Weil et al 2004[25]).

The Th subsets are defined by the expression of transcription factors and/or by the cytokines they produced[26]. The "Signal No. 3"[27,28] is important for naïve T cells to Th subset polarization. Th1 subset is the major producer of IFNγ and its differentiation is induced by IL-12 and STAT4 signaling pathway. IL-4 plays a major role in the Th2 generation, via STAT6 signaling, leading to the production of IL-5 and IL-13. Th17 differentiation requires a cytokines cocktail, including TGF-beta, IL-23, IL-1 and IL-6. Some cytokines are either a promoter or an inhibitor for the polarization process. As an example, IL-12 promotes Th1 differentiation but inhibits Th2 polarization. All regulatory mechanisms involved in Th differentiation are still not totally understood and the identification of PIDs affecting one or other Th subsets will also help for this fundamental question.

There are several molecules identified for PIDs which affect TCR and/or BCR signaling pathways[20] (Fig.3). Common variable immunodeficiency (CVID) patients with impaired production of antibody, e.g. patients with mutations in ICOS[29-32], who displayed reduced Ag-specific Ab response and decreased serum IgG/A/E level. These patients also had impaired IgM^+ memory B cells, absent IgG^+ and IgA^+ memory B cells, and an impaired development and/or function of T follicular helper (T_{FH}) cells. There are some other PIDs affecting genes that contribute to the development of B cells, like AR HIGM (autosomal recessive hyper IgM syndrome) due to mutations in CD40[32,33]. Their immunological phenotype is characterized by the presence of IgM^+ and IgD^+ circulating B cells only, normal or increased serum IgM level whereas all other Ig subclasses are absent. These patients also displayed diminished T_{FH} cells that results from affected ICOS-L expression downstream of CD40/CD40L signaling[29–31]. Recent studies of how T cell and B cell interaction contribute to primary immunodeficiencies have expanded our knowledge of PIDs.

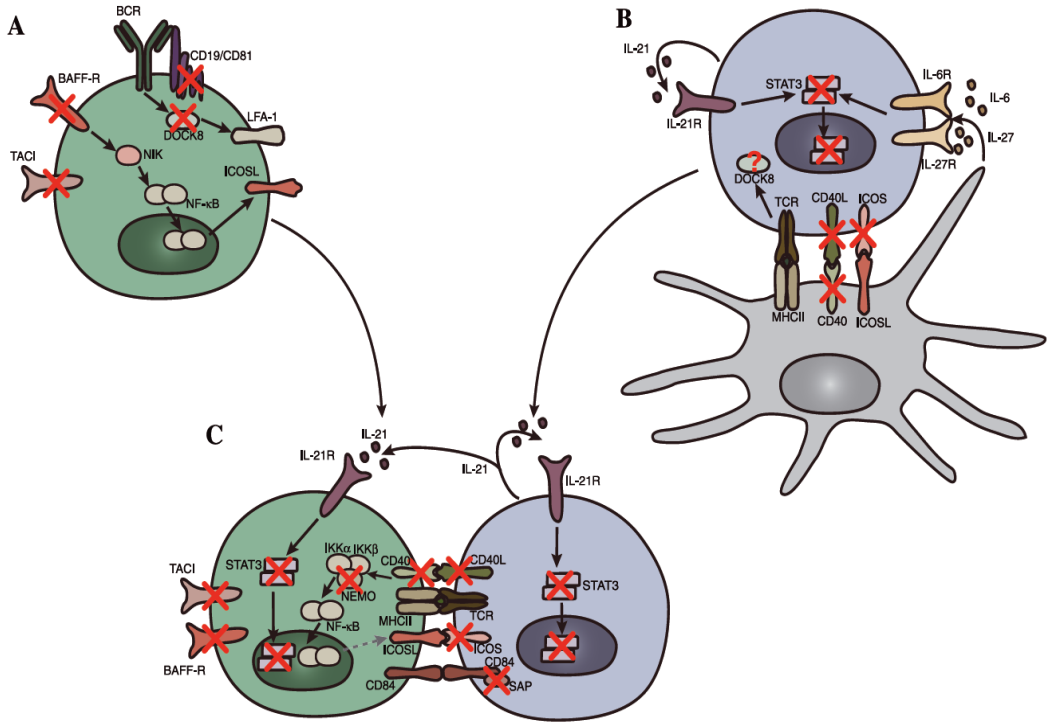


Fig.3. Identified molecules in PIDs which affect TCR and BCR signaling pathway marked with red crosses. (Adapted from Tangye et al 2012[20]).

As mentioned, TCR activation contributes to cytokine production, cell proliferation, differentiation and survival[34]. The analysis of T cell immunity to infection has benefited from genetic studies. SCID or CID patients are susceptible to a broad spectrum of infections[17]. One example is the infection by *Candida albicans*, a major fungus that can cause recurrent or persistent infection of the skin, nails, and mucosae. This disease has been described as chronic mucocutaneous candidiasis (CMC)[35–39]. CMC occurs commonly in infancy, and also in patients with T cells immunodeficiencies[40]. Besides infectious diseases, some CMC patients also develop autoimmune diseases[36,41,42, 43]. The first cases were reported in 1960s[44–47]. Since then, more than 15 genes were identified as CMC disease-causing genes[42].

For some reported CMC cases, there is a primary T-cell immunodeficiency and a susceptibility to viral and/or bacterial infections. Autosomal recessive CD25-deficient patients had T lymphopenia, impaired T cell proliferation and

developed oral thrush, esophageal candidiasis and autoimmunity[48,49]. Patients with autosomal recessive DOCK8 deficiency associated with hyper IgE syndrome, had T cell development and function defect, decreased B cell count and memory B cells[50,51]. Patients, who carry bi-allelic mutations in TCR-α, display normal T and B cell count, but TCR αβ T-cell and T cell proliferation defect, those patients also develop recurrent viral, bacterial and fungal infections[52.] Patients with X-linked recessive NF-κB essential modulator (NEMO) deficiency or autosomal dominant IκBα deficiency, display low frequency of T cells and memory B cells and are severely infected by mycobacteria and pyogens[53]. CMC is also reported in CIDs patients with autosomal recessive TYK2, ORAI1, MST1 or IRF8 deficiency, these patients all display T cell disorders and severe infections diseases[13,54–56]. In a word, the role of human T cells in mucocutaneous immunity against *Candida* species is essential.

Besides primary T-cell immunodeficiencies with CMC, important observations have been reported on isolated CMC as well[42]. Inborn errors of IL-17A and IL-17F, the Th17 signature cytokines, underlie CMC for both conditions. T cell development and function mediated by IL-17 dependent T-cell immunity were shown to underlie Mendelian susceptibility to CMC[42,57,58] (Fig. 4).

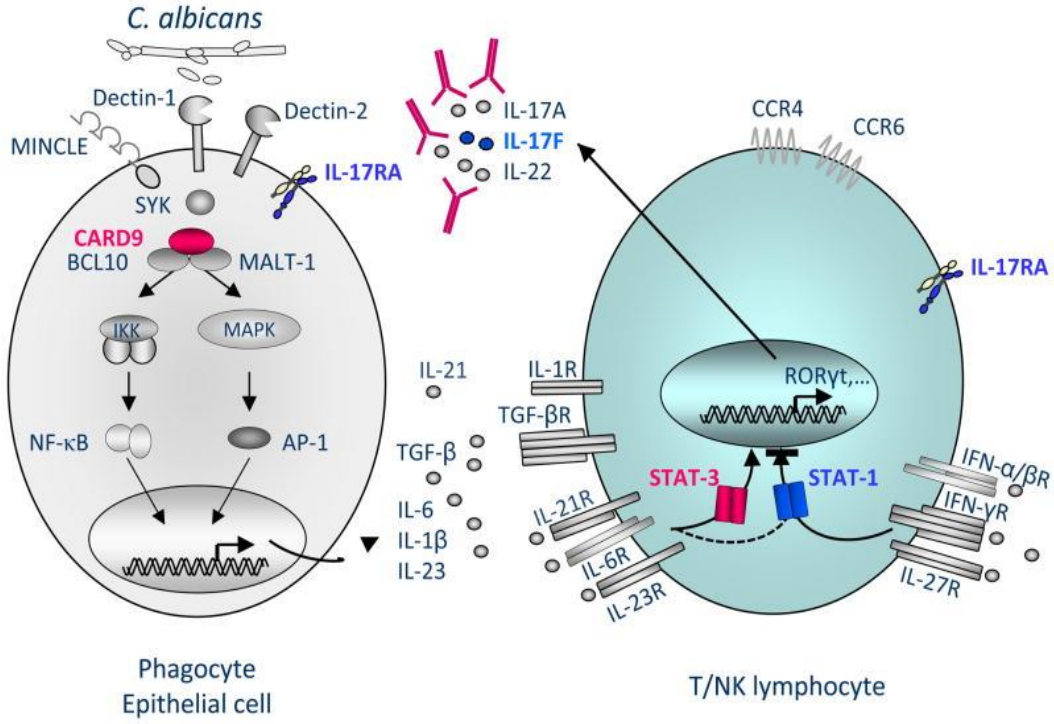


Fig.4. IL-17 immunity with primary defects underlies isolated mucocutaneous candidiasis. (Adapted from Puel et al, 2012[42])

Staphylococcus aureus and other species of staphylococci are microorganisms also related to mucocutaneous immunity. Nevertheless, unlike the findings of genetic studies of mucocutaneous immunity to *Candida*, the understanding of the mechanism of host defense in mucocutaneous staphylococcosis is still incomplete. Recent studies show that STAT1- and STAT3-mediated signaling pathways[59], IL-17 signaling and also IL-6 receptor-mediated signaling[60,61] are responsible for clinical manifestations of PIDs patients with *Staphylococcus aureus* infection.

Conversely, inborn errors of T cells can be implicated in the pathogenesis of infections caused by environmental mycobacteria, including BCG vaccine, because they occur in patients with pure forms of SCID, in which only T cells are intrinsically affected. In general, onset of mycobacterial disease is in infancy, and some PIDs cases are associated with mycobacterial disease which is narrowly susceptible than most PIDs which are broad susceptible to infections. These patients are also prone to

the more devastating *Mycobacterium tuberculosis* (*(M. tuberculosis)* which causes tuberculosis (TB)[62]. Robert Koch discovered the specific causative agent of tuberculosis in 1882[63]. Nowadays TB is still a main infectious disease killer all over the world. Even in 2014, there are about 1 million children developed TB and 1% of these children are killed by TB. Typical TB is pulmonary TB which affects the lungs, yet *Mycobacterium tuberculosis* can also cause extrapulmonary TB which affects the other sites[64]. BCG is used to vaccinate against TB in neonates. However, the protection of BCG vaccination is not complete[65]. With epidemiological survey, clinical investigation, familial studies and a series of animal model experiments which began in the 1930s[66–69], the findings demonstrated clearly that, first, infection phenotypes of TB are highly heritable; second, $CD4^+$ T cells were required for immunity to *M. tuberculosis* in knockout mice model[68]; third, in humans, CD4-mediated immunity and the IL-12/IFNγ signaling pathway are important to *M. tuberculosis*[69]; fourth, primary TB may result from inborn errors of immunity, as exemplified by severe TB in children with classical PIDs[70].

Recent genetic studies of mendelian susceptibility to mycobacterial disease (MSMD) have revealed new inborn errors of immunity underlying mycobacterial infections. Some patients with IFN-γR1 deficiency display susceptibility to TB in addition to other mycobacterial infection[62]. Complete or partial IFN-γR1-deficiency is associated with disseminated BCG or environmental mycobacteria infections. Some patients, only had TB infected by *M.bovis*[71] or *M. avium*[72]*;* one single case with partial recessive IFN-γR1 deficiency developed TB[73]. Other genetic disorders were found in TB patients, cells of these patients do no respond to or produce IL-12 and IL-23, hence, IFN-γ production is affected in T and NK cells suggesting that among all T cells, Th1 subset is important in the control of mycobacterial infections because of the IFN-γ production. Bi-allelic loss-of-function mutations in IL-12p40 and IL-12Rβ1 were identified in these patients[74,75, 76]. Interestingly, patients with complete IL-12p40 deficiency suffered from MSMD but also from *Salmonella* infection[77,78],

which are not reported in IFN-γR deficiencies, suggesting that IL-12 is a key player of anti-salmonella immune response in an IFN-γ-independent mechanism. Furthermore, the Mendelian findings increase the possibilities that patients infected by mycobacteria including *M. tuberculosis* in their childhood may carry a monogenic PID which may be already known or not[62,66].

CID patients are also susceptible to viral infections. Inborn errors of $CD8^+$ T cells can disrupt immunity to specific viruses, as exemplified by X-linked lymphoproliferative disease (XLP) due to inactivating mutations in SH2D1A encoding SAP[79], for example. XLP is an X-linked primary immunodeficiency; patients with fulminant infectious mononucleosis (FIM), hemophagocytic lymphohistiocytosis (HLH), hypogammaglobulinemia and B-cell lymphoma, resulting from EBV infection, which is the single pathogen of XLP. XLP kills patients in 2 months if the patients present with EBV-induced FIM/HLH[80–91]. In 1998, the first genetic defect underlying XLP was identified, with loss-of-function mutations in SH2D1A encoding SAP. XLP patients with SAP deficiency developed impaired effector functions of NK cell cytotoxicity, $CD8^+$ T cells of patients selectively impaired the response to B cells. That explains why XLP patients are susceptible to EBV infection and disease, also put insight to the frequent development of B-lymphoma in these patients[92–94]. XLP is another good example of PID that expanded our knowledge of T cell immunity to infection.

The combined studies of CIDs and other immune deficiencies, related to specific infectious susceptibility, are important to understand the role of the cytokines, signaling pathways and/or cell subsets in a deeper way. We studied six patients from three unrelated kindreds, with unusual histories of mycobacterial diseases, mucocutaneous candidiasis, silent but detectable EBV viremia, and/or staphylococcal diseases, in the context of pulmonary and cutaneous allergy. We tested the hypothesis that they suffered from a novel T cell deficit.

Section II. Human RLTPR deficiency

II.1 Clinical phenotype

We investigated 6 patients from 3 unrelated families. P1, P2 and P3 (kindred A) were born from a Moroccan consanguineous family. P1 developed various infections during his childhood, as fungal infections (almost of all nails of fingers and toes with perionyxis) (Fig.5), multifocal tuberculosis (TB) but no cutaneous abscesses. He died at age 17 due to worsening respiratory distress. His 2-year-old dizygotic twin sisters, P2 and P3, developed CMC (almost of all nails of fingers and toes with perionyxis as their brother) (Fig.5) and recurrent pulmonary bacterial infections.

P4 and P5 of kindred B were born to second-degree cousins' parents originating from Tunisia. They suffered from sub-cutaneous staphylococcal abscesses (Fig.5), P4 presented with "cold" sub-cutaneous abscesses, without fever, unlike P5 who developed painful abscesses with fever at 39°C. They had *Molluscum contagiosium* eruption, eczema, asthma and recurrent infections of the upper and lower respiratory tracts.

P6 (kindred C) was born to Turkish non-consanguineous parents, he presented with miliary TB at 9 years of age. He also developed seborrheic dermatitis (Fig.5) which was on his scalp and eyebrow but unresponsive to therapy. He is now aged 18 and doing well.

All patients were born with normal skin yet gradually developed clinical manifestations, including severe allergic lesions. Patients did not present with any severe illnesses caused by common viruses, as concluded from viral serology. At last follow-up, P4 was treated with intravenous IgG (IVIG), not for other patients yet. In conclusion, these patients suffered from a broad and partly overlapping phenotype of recurrent infectious diseases caused by multiple pathogens, including *Candida*,

Staphylococcus, and *Mycobacterium*, suggesting a combined immunodeficiency with cutaneous and pulmonary infections (Tables 1, 2, 3, and 4).

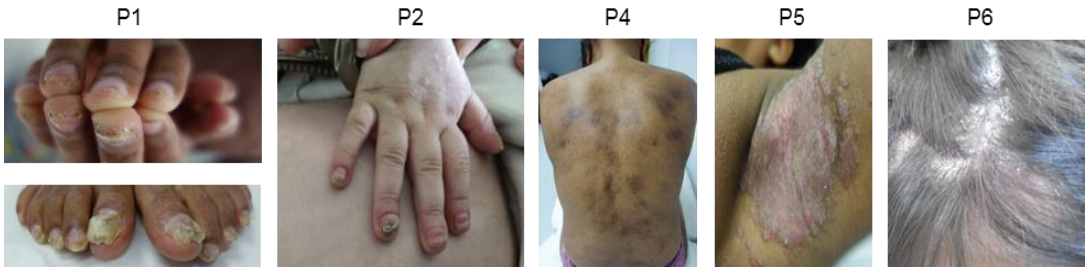


Fig.5. Skin phenotypes of patients.

Patient Origin Gender Present age (yo)	Infectious diseases	Cutaneous features	Others	Level of IgE (KIU/ml)
P1 Morocco Male Died at 17yo	Purulent otitis	Scaly erythroderma	Bronchitis	<5
	Multifocal tuberculosis	Focal alopecia		
	Onycomycosis with perionyxis	Pustular like and scaly lesions on the soles	Peptic stenosis of the esophagus	
	CMC (perionyxis)			
P2 Morocco Female 2yo	CMC (perionyxis and oral thrush)	Scaly erythroderma	Bilateral broncho alveolar syndrome	nt
	Recurrent bacterial lung infections			
P3 Morocco Female 2yo	CMC (perionyxis)	Scaly erythroderma	Recurrent bacterial lung infections	nt
	Bilateral broncho alveolar syndrome			
P4 Tunisia Female 27yo	Recurrent purulent otitis	Eczema	Bronchial dilatation	1381
	Sub-cutaneous abscessess	Cold urticaria	Asthma	
	Molluscum contagiosum	Lupus-like facial erythema		
	Recurrent bacterial lung infections	hyperpigmented lesions	Allergy to Yolk egg and peanut	
	Gastritis by Helicobacter pylori			
P5 Tunisia Female 26yo	Pneunomia	Eczema	Recurrent bronchopneumonia Bronchial dilatation	nt
		Psoriasis guttata “like” lesions		
	Sub-cutaneous abscessess	Mild ichthyosis		
		Sun intolerance		
	Molluscum contagiosum	Dry skin	Asthma	
		Large inflammatory and erosive inverted psoriasis “like” plaques in the folds		
P6 Turkey Male 19yo	Miliary tuberculosis	Seborrheic dermatitis	Asthma	34,7

Table 1. Clinical phenotype of patients

		P1	P6		P4	P5	
		17yo	**18yo**		**24yo**	**24yo**	
	Units			Normal range			Normal range
Lymphocytes	x10*9/l	6,7		1-4	2,0	2,6	1-4
T lymphocyte							
CD3+	/µl	5494	4351	1000-2200	1740	2080	807-1844
CD4+	/µl	2412	1665	530-1300	740	754	460-1232
CD8+	/µl	2077	2256	330-920	680	962	187-844
B lymphocyte							
CD19+%	%	5	15	6-23	4	8	6-17
CD19+	/µl	335	806	110-570	80	208	92-420
NK lymphocyte							
CD16+CD56+%	%	2	3	3-22	8	3	5-20
CD16+CD56+	/µ	134	161	70-480	160	78	89-362
Neutrophils	x10*9/l	6,6	nt	1,5-7	2,6	1,8	1,5-7
Eosinophils	x10*9/l	0,5	nt	0-0,5	1,1	0,4	0-0,5
Basophils	x10*9/l	0,0	nt	0-0,2	0,0	0,0	0-0,2
Monocytes	x10*9/l	1,2	nt	0,2-1	0,6	0,7	0,2-1

Table 2. Patients' immunophenotyping performed on whole-blood samples

Patient	Age (y)	IgG (g/l)	IgA (g/l)	IgM (g/l)	IgE (kU/l)	IgG1 (g/l)	IgG2 (g/l)	IgG3 (g/l)	IgG4 (g/l)
Normal range	>10	6,65-12,78	0,7-3,44	0,5-2,09	<114	>4	>0,6	>0,17	
	6-11	5,5-10,8	0,45-1,5	0,55-1,55	<148				
	2	4,82-8,96	0,33-1,22	0,5-1,53	<40.3	>4	<0,3	>0,13	
P1	17	8,27	2,48	3,62	<5	6,9	3,15	0,83	0,003
P2	2	7,01	2,32	3,06	<2	4,5	3,57	0,61	0,003
P3	2	6,38	1,32	1,87	<2	4,8	1,22	0,49	0,004
P4	17	14	2,94	3,1	1493	nt	nt	nt	nt
	17	nt	nt	nt	996	nt	nt	nt	nt
	17	nt	nt	nt	2496	nt	nt	nt	nt
	19	nt	nt	nt	2679	nt	nt	nt	nt
	21	15,2	3,97	4,1	>5000	nt	nt	nt	nt
	23	15,74	3,84	4,11	1714	8,9	4,47	1,06	0,064
	25	nt	nt	nt	383	nt	nt	nt	nt
	25	nt	nt	nt	305	nt	nt	nt	nt
	25	17,9	3,79	3,7	nt	nt	nt	nt	nt
	25	14	3,8	3,43	nt	nt	nt	nt	nt
	25	16,78	3,32	3,72	377	10,6	3,59	0,81	0,05
	26	nt	nt	nt	1381	nt	nt	nt	nt
	26	16,89	3,87	3,82	2158	9,2	3,5	0,91	0,06
P5	6	15	0,59	1,92	nt	nt	nt	nt	nt
	24	12,61	2,56	3,49	nt	nt	nt	nt	nt
	24	13,74	2,52	3,71	<2	7,8	2,87	0,65	0,011
	24	12,56	2,4	3,44	2,3	7	2,24	0,93	0,013
P6	9	14,4	2,19	1,14	<17	nt	nt	nt	nt
	13	nt	nt	nt	77,6	nt	nt	nt	nt
	17	9,66	1,83	1,03	17,4	nt	nt	nt	nt
	17	9,9	1,92	0,98	28,2	5,7	3,78	0,63	0,014
	17	13,52	1,32	1,67	35,2	8,1	2,45	1,14	0,22
	18	9,23	1,97	0,99	34,7	nt	nt	nt	nt

Table 3. Immunoglobulins of patients

Serology (detection threshold)		P1	P2	P3	P4	P5	P6
Anti-VZV IgG (<165)		287	Neg	Neg	894	167	865
Anti-CMV IgG (<14)		92	80	82,3	104	118	130
Anti-HSV IgG		HSV1: 4,06 HSV2: Neg	Neg	Neg	HSV1: 9,2 HSV2: 0,76	3	>62
Anti-Rubella virus IgG (<5)		nt	Neg	11.1	225	>350	>350
Anti-Parvovirus B19 IgG (<1.1)		nt			7,7	nt	nt
Anti-Measles virus IgG		Neg	Neg	Neg	130	Neg	Neg
Anti-Mumps virus IgG		Neg	Neg	Neg	24	50	<5
Anti-Influenza A virus IgG (<4)		4			32	nt	nt
Anti-Influenza B virus IgG (<4)		<4			8	nt	nt
Anti-Parainfluenza virus IgG (<4)		nt			64	nt	nt
Anti-VRS IgG (<4)		nt			64	nt	nt
Anti-Adenovirus IgG (<4)		nt			16	nt	nt
Anti-HBV IgG (<10)		nt			161	nt	nt
Anti-HCV IgG (<1)		nt			0,09	nt	nt
Anti-HAV IgG (<1)		Neg			10,81	nt	nt
EBV	Anti-EBNA IgG(<20.0U/ml)	112,0	Neg	Neg	263	<3,0	Neg
	Anti-VCA IgG(<20.0U/ml)	53,8	Neg	66,4	Pos	>750,0	322

EBV PCR								
	Age (yo)	PCR	Age (yo)	PCR	Age (yo)	PCR	Age (yo)	PCR
P1	15	2,6*						
P2	2	Neg**						
P3	2	2						
P4	23	Neg**	25	3,1*	25	Neg**	26	Neg**
P5	24	3,4*	25	Neg**	25	Neg**		
P6	17	Neg**	16	Neg*				

* performed on whole blood, normal range: <2.3 Log cp/ml

** performed on plasma, normal range: <2.7 Log cp/ml

nt: not tested

Table 4. Viral serologies and loads

II.2 Human *RLTPR* mutations identification

We then analyzed the patients by whole-exome sequencing (WES) and we performed genome-wide linkage (GWL) on the two consanguineous families (Kindred A and B). The maximum LOD scores (2.53 for kindred A and 2.0556 for kindred B) were obtained for an overlapping region of 3.2 Mb on chromosome 16. *RLTPR* is the only one gene of the 141 protein-coding genes in the linked region, which carried homozygous rare variants in all 5 patients of kindred A and B. For P6, the homozygosity rate determined with WES data, was at 3-4%, strongly suggestive of parental consanguinity; he also carried a homozygous rare variant in *RLTPR*. Interestingly, the mouse ortholog of Rltpr is essential for CD28 co-stimulation of T cells[23]. By Sanger sequencing, we confirmed all variants. P1, P2 and P3 carried a homozygous nucleotide substitution (T>G) at position 1,115 in exon 14 of *RLTPR*, resulting in the replacement of a highly conserved leucine residue by an arginine (L372R) in the leucine-rich repeat (LRR) domain. P4 and P5 carried a nucleotide substitution (C>T) at position 2,557 in exon 25, resulting in the replacement of a glutamine residue by a stop codon (Q853X). P6 carried a nucleotide substitution (T>A) at position 1,574 in exon 17, resulting in the replacement of a highly conserved leucine residue with glutamine (L525Q) (Fig.6a and b), which also is located in the leucine-rich repeat (LRR) domain (Fig.6c). All parents and unaffected siblings were heterozygous or homozygous wild-type (WT). This suggests that RLTPR deficiency is an autosomal recessive (AR) trait with complete penetrance. None of the three variants were found in 1000 Genomes, Single Nucleotide Polymorphism (dbSNP), HapMap, EVS, and ExAC databases, nor in the Greater Middle-Eastern variome[95] or in our own WES database. No other homozygous, nonsense, essential splicing, or frameshift deletion/insertion variations were found in the above databases. Moreover, the gene damage index (GDI) score of *RLTPR* is 4.938, a moderate neutrality index score and purifying *f* parameter of 0.32 and 0.488, respectively, suggesting that

RLTPR is under purifying selection[96]. Overall, both family and population genetic studies strongly suggested that these six patients had AR RLTPR deficiency.

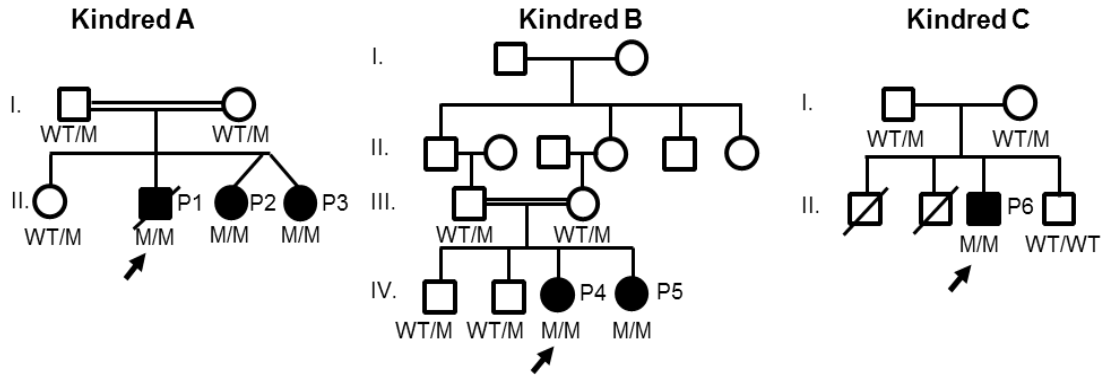


Fig.6a. Familial segregation of patients. (obtained from Yi WANG)

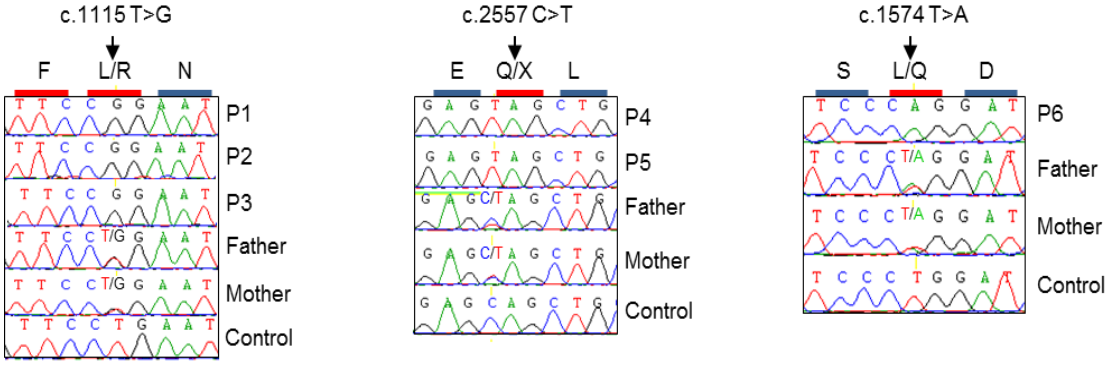


Fig.6b. Sequencing profiles showing the homozygous RLTPR of patients. (obtained from Yi WANG)

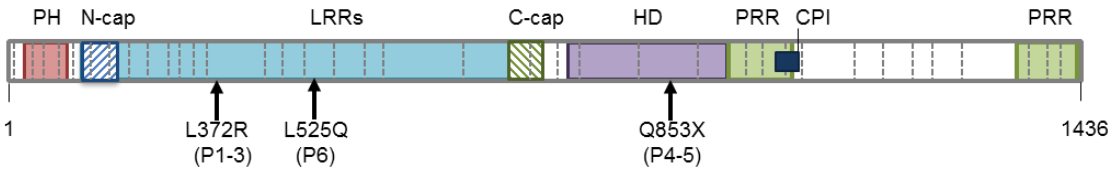


Fig.6c. Schematic representation of the RLTPR protein. The different domains are depicted as follows: the pleckstrin homology (PH) domain in pink, the leucine-rich region (LRRs) in light blue, the homodimerization domain (HD) in purple, the proline

rich regions (PRRs) in light green and the CP-interacting (CPI) domain in black. (obtained from Yi WANG)

II.3 Molecular characterization of mutant human *RLTPR* alleles

By western blot in overexpression experiment, we first showed that the expression of missense proteins (L372R and L525Q) was normal with two different antibodies (anti-N terminal and C-terminal anti-RLTPR Abs), and the nonsense mutation (Q853X) led to the expression of a truncated protein, detectable only with the anti-N-terminal RLTPR Ab (Fig.7a). Then, we quantified *RLTPR* mRNA by quantitative real-time PCR (qPCR) from EBV-B cells of P1, P5 and P6, and we observed that all alleles (WT, L372R, Q853X, and L525Q) were equally abundant. Similarly, higher or normal mRNA levels were observed in T-saimiri cells of P1 and P3, as compared with healthy controls, suggesting that the nonsense mutation is not related to nonsense-mediated mRNA decay. Meanwhile, we evaluated *RLTPR* protein expression in EBV-B cells, T-saimiri cells, and PHA-blasts from controls and patients by Western Blot. The L372R (P1) and L525Q (P6) variants of RLTPR show lower levels than WT proteins. The truncated Q853X protein (P4) was not detectable in patient-derived EBV-B cells, T-saimiri cells, and PHA-blasts by anti-C-term RLTPR. Unfortunately, none of the commercially available antibodies recognizing the N-term domain of RLTPR detected endogenous RLTPR in control cells. Therefore, we cannot conclude that a truncated Q853X protein is loss-of-expression in the patients' cells (Fig.7b). RLTPR (CARMIL2) belongs to the CARMIL family, which is characterized by a central helical domain (HD) and several leucine-rich repeat (LRR) domains. It has been shown that CARMIL1 is capable of dimerization through its HD domain. To go further, we co-transfected HEK-293T cells with DDK/Myc- and V5-C-terminal tagged version of WT or mutant RLTPR. By co-immunoprecipitation, we found that WT RLTPR was capable to homodimerize, as CARMIL1. In this

over-expression system, all mutant proteins (L372R, L525Q and Q853X) were capable of dimerization (Fig.7c).

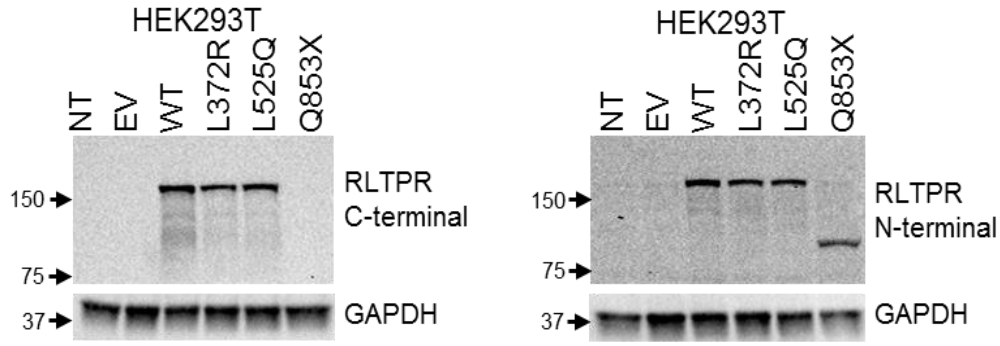


Fig.7a. Whole cell lysates were subjected to immunoblots against indicated RLTPR domains by mutations of patients. (obtained from Yi WANG, N=3)

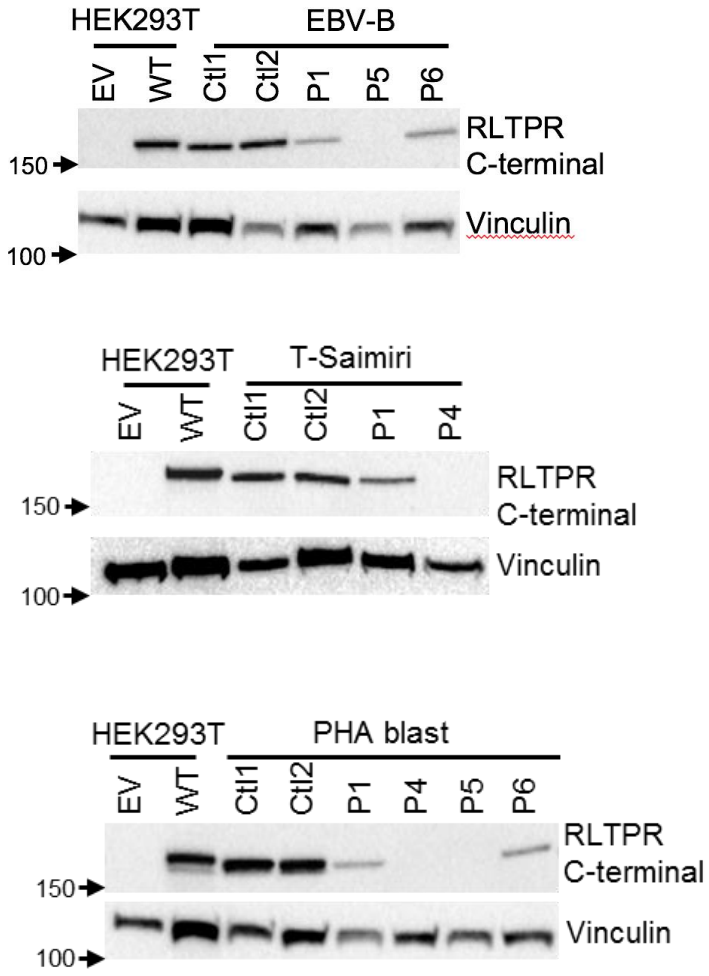


Fig.7b. Immunoblot analysis of RLTPR expression in whole protein extracts of HEK293T cells transfected with an empty pCMV6 plasmid (EV) or pCMV6 plasmids encoding WT RLTPR, or in indicated patients' derived EBV-B, T-Saimiri cells or PHA-blasts. (obtained from Yi WANG, on EBVB cell line: N=3; on T-saimiri cell line: N=4; on PHA blast: N=4 (controls, P5 and P6), N=3 (P1), N=2 (P4))

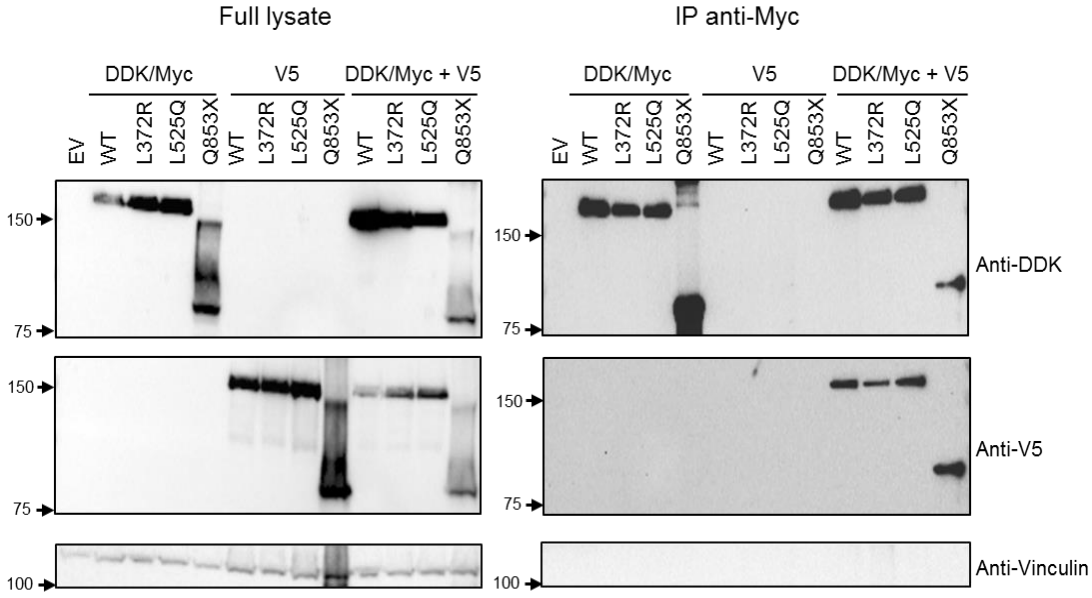


Fig.7c. Effect of mutations on RLTPR homodimerization. HEK293T cells were transfected with RLTPR wild type or L372R, L525Q or Q853X RLTPR mutant alleles tagged in C-terminal with either V5 or Myc/DDK. Full cell lysates (left panel) or anti-Myc immunoprecipitates are depicted (right panel). (obtained from Yi WANG, N=3)

II.4 RLTPR expression in leukocytes and patients' cells

We tested expression of WT RLTPR in leukocytes *ex vivo*. RLTPR is strongly expressed in $CD4^+$ and $CD8^+$ T cell subsets, B cells, NK cells, whereas poorly expressed in $CD14^+$ monocytes from healthy controls by western blot with anti-C-terminal RLTPR mAb. By flow cytometry, RLTPR expression was detected in naïve and memory B cells, in both $CD56^{bright}$ and $CD56^{dim}$ NK cells, naive, central

and effector memory $CD4^+$ and $CD8^+$ αβ T cells, Tregs, MAIT cells, γδ T cells, iNKT, mDC2, and pDCs, but only weakly detectable in monocytes and mDC1 (Fig.8). However, all tested patients did not display detectable RLTPR in any leukocyte population by flow cytometry. Stable transduction of wild-type *RLTPR* in T-saimiri cells of P4 restored normal expression of RLTPR, suggesting that the lack or poor expression of RLTPR was due to *RLTPR* mutations (Fig.9). Collectively, these data indicate that both missense RLTPR alleles are poorly expressed at the protein level in patients' cells, whereas the nonsense allele encodes a truncated protein, the levels of expression of which in the patients cells are unknown.

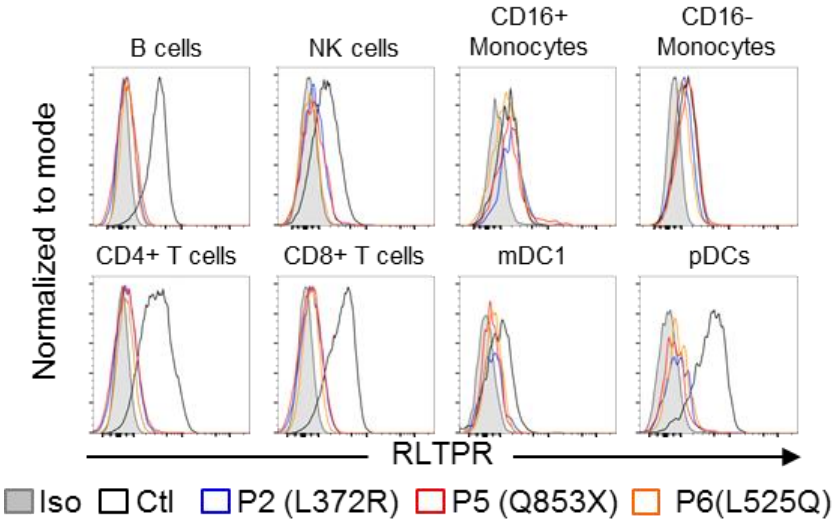


Fig.8. RLTPR expression determined by FACS in indicated leukocyte subsets from healthy control and patients. (obtained from Yi WANG, N=3)

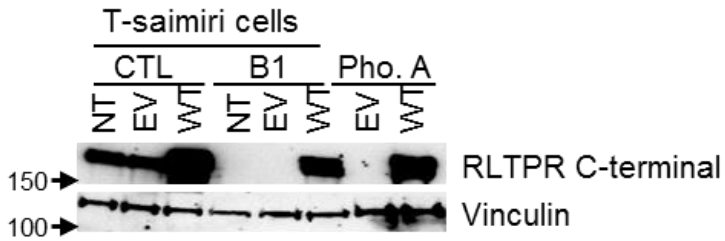


Fig.9. Immunoblot analysis of RLTPR expression in whole protein extracts of T-Saimiri cells from control or P4 were transduced with retrovirus encoding either a tag only (empty vector) or tagged WT RLTPR, or Phoenix A cells transfected with an empty pLZRS plasmid (EV) or pLZRS plasmid encoding WT RLTPR. (obtained from Yi WANG, N=3)

II.5 Leukocyte subsets development of RLTPR-deficient patients

All patients had normal counts of polymorphonuclear neutrophils, monocytes, B cells and NK cells. All patients except P4 had elevated counts of $CD4^+$ and $CD8^+$ T cells. Increased counts of eosinophils were documented twice in P4. In addition, we observed increased frequencies of naive $CD4^+$ T cells and reduced frequencies of $CD45RA^-CCR7^+$ central memory $CD4^+$ T and $CD8^+$ T cells (Fig.10a). Other subsets of memory T cells were not affected. Flow cytometry analysis of T cell subsets *ex vivo* revealed normal proportions of γδ T cells, NKT cells, and Th2 ($CCR6^-CXCR3^-$) but significantly decreased proportions of Tregs ($CD4^+FOXP3^+CD25^+CD127^{lo}$), Tfh ($CD4^+CXCR5^+$), Th17 ($CD4^+CCR6^+CXCR3^-$), and MAIT ($CD3^+CD161^+Vα7.2$) cells, and a slight but non-significant decrease of Th1 cells ($CD4^+CXCR3^+CCR6^-$) (Fig.10b). We further enumerated Th2-type cells by determining the proportions of memory $CD4^+$ T cells that expressed the surface receptor CRTh2, which is expressed on human $CD4^+$ T cells enriched for producing IL-4, IL-5 and IL-13[97,98]. This analysis revealed normal proportions of $CRTh2^+$ cells within the memory $CD4^+$ T cell subsets in RLTPR-deficient patients compared with controls.

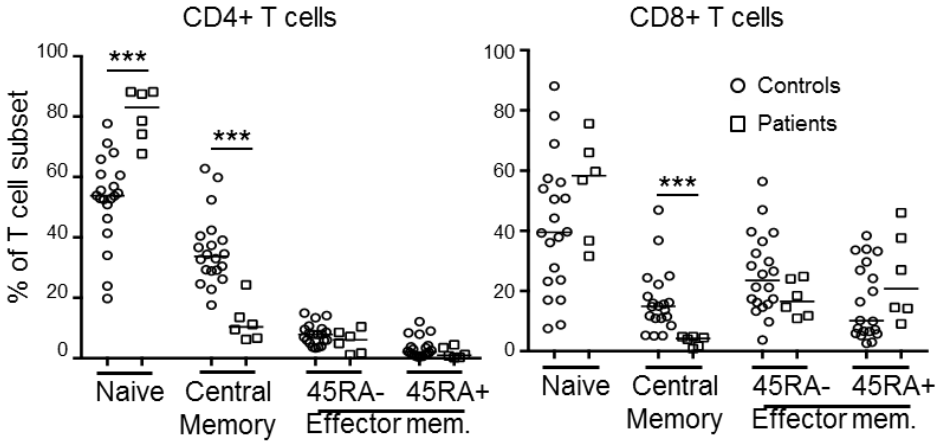


Fig.10a. Frequency of naïve ($CD45RA^{+}CCR7^{+}$), central memory ($CD45RA^{-}CCR7^{+}$) and effector memory ($CD45RA^{+/-}CCR7^{-}$) compartments in $CD4^{+}$ and $CD8^{+}$ T cells of controls and patients. (obtained from Yi WANG, N=3)

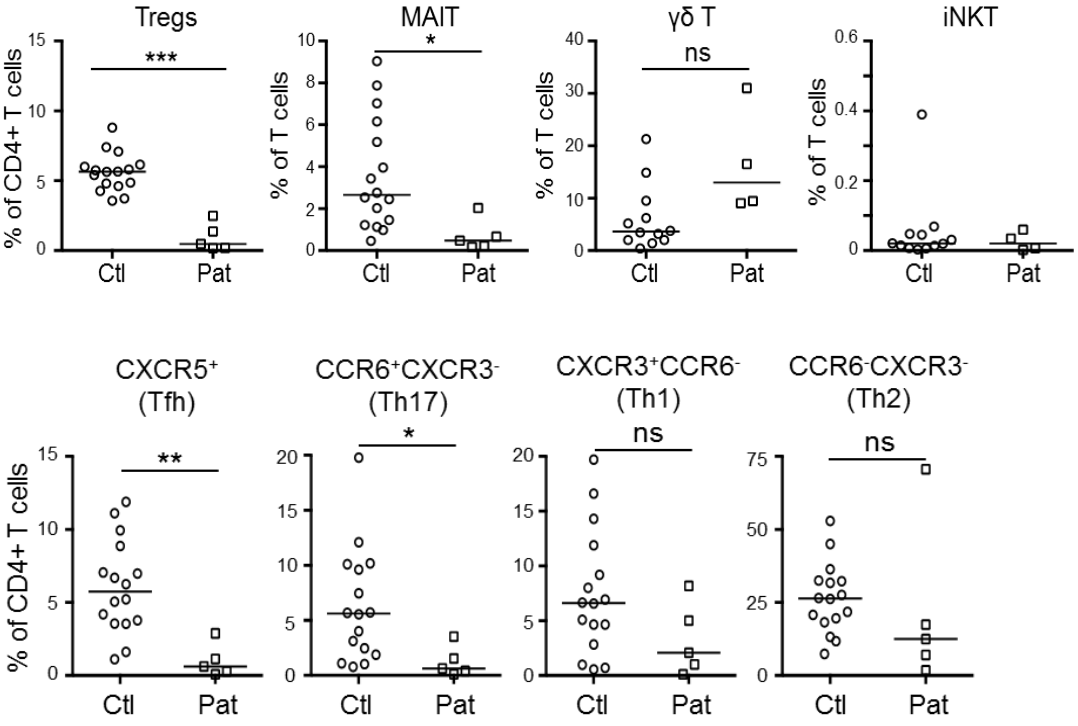


Fig.10b. Frequency of Tregs among $CD4^{+}$ T cells, frequencies of MAIT, γδ T cells and iNKT cells among $CD3^{+}$ T cells, frequency of T helper (Th) subsets in $CD4^{+}$ T-cells. (obtained from Cindy MA, N=3)

II.6 T cell defect in RLTPR-deficient patients

In mice, it has been reported that RLTPR is involved in the CD28 signaling pathway[23]. To go further, we tested proliferation of patients T cells *in vitro* in response to various stimuli. Upon mitogens, such as PMA/ionomycin, PHA and anti-CD3, and antigens stimulations, the proliferation of T cells was diminished in patients, as compared to controls. To determine if the decreased proliferation upon anti-CD3 and PHA might be due to a defect in CD28 signaling pathway, as observed in mouse model, we then analyzed the CD28 co-stimulation pathway in all patients. We first determined the CD28 expression in both $CD4^+$ and $CD8^+$ T cells in RLTPR-deficient patients, as defined by FACS mean fluorescence intensity (MFI). Interestingly, CD28 expression level was decreased around 40% compared to healthy controls on the surface of both naive $CD4^+$ and $CD8^+$ T cells. However, all naive and memory $CD4^+$ and all naive $CD8^+$ T cells express CD28 whereas the proportion of $CD28^+$ cells was significantly decreased in memory $CD8^+$ T cells in patients. Then, we assessed the impact of CD3 and CD28 co-stimulation on cytokine production using a redirected triggering assay against the P815 cell line. With specific Abs, we cross-linked CD3 and/or CD28 on T cells for 6 hours and assessed TNF, IFN-γ and IL-2 production by $CD4^+$ T cells, as well as TNF and IFN-γ production and CD107a surface expression by $CD8^+$ T cells. A poor response to CD3 and CD28 co-stimulation was observed in control naive $CD4^+$ and $CD8^+$ T cells in these experimental conditions, so we compared memory cells of control and patients. CD3 stimulation alone induced little or no TNF, IFN-γ and IL-2 in both control and patients $CD4^+$ memory T cells but equally in $CD8^+$ memory T cells. CD3 and CD28 co-stimulation of control $CD4^+$ memory T cells strongly synergized for TNF and IL-2, but not in patients' memory $CD4^+$ T cells. Interestingly, no synergic effect was observed for IFN-γ production in control and patients memory $CD4^+$ T cells (Fig.11a).

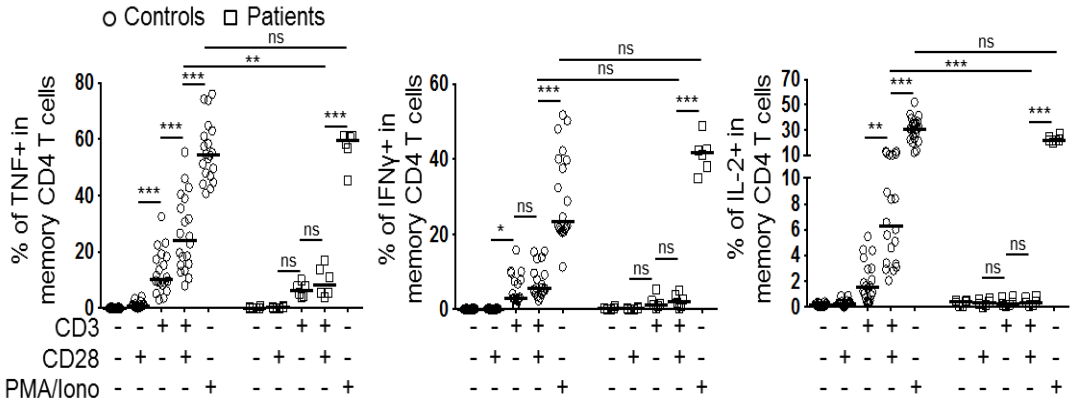


Fig.11a. Impaired frequency of TNF^{+}, IFN-γ^{+} and IL-2^{+} upon CD28 co-stimulation in patients memory CD4^{+} T cells. (obtained from Yi WANG, N=3)

In memory CD8^{+} T cells, the CD28 co-stimulation did not increase the production of TNF and IFN-γ and CD107a surface expression, suggesting that memory CD8^{+} T cell do not rely on CD28 co-stimulation for these three readouts (Fig.11b), regardless of CD28 expression. This is consistent with a lack of CD28 expression on many memory CD8^{+} T cells, and the potent effector function (cytotoxicity) of CD8^{+}CD28^{-}T cells that can be induced following TCR engagement[99]. Altogether, these results suggested that RLTPR plays an essential role in the CD28 co-stimulation pathway in human CD4^{+} T cell, at least in these experimental conditions.

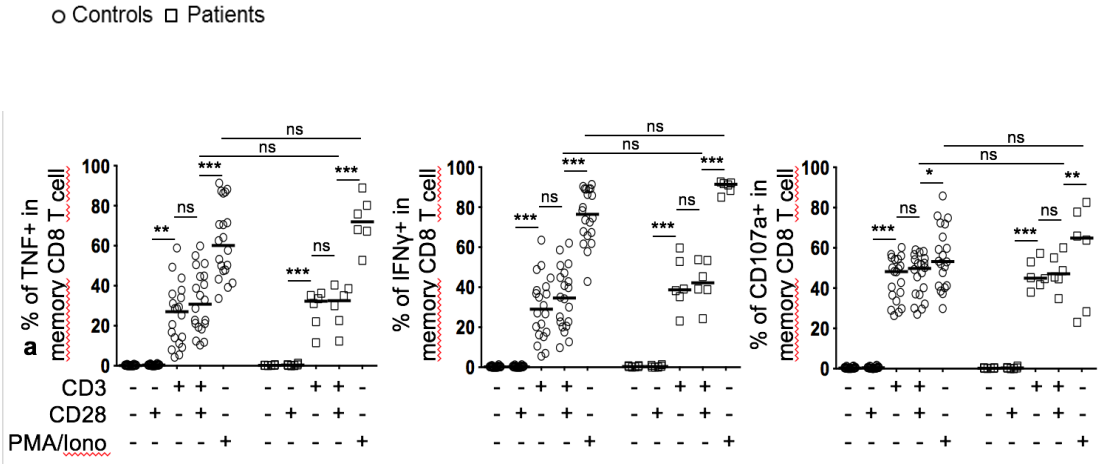


Fig.11b. Impaired frequency of TNF^+, IFN-γ^+ and CD107a upon CD28 co-stimulation in patients memory $CD8^+$ T cells. (obtained from Yi WANG, N=3)

We then sorted and labeled with CFSE naive and memory $CD4^+$ T cells, and analyzed their proliferation after culture under different conditions: anti-CD2/CD3/CD28 beads alone (Th0) or under Th1 (IL-12), Th2 (IL-4) or Th17 (ILs 1β, 6, 21, 23; TGF-β) polarizing conditions from healthy controls and patients. Cytokine production and cell division were determined at different times. Interestingly, after 4 days of culture, in Th0 condition, we observed reduced proliferation of naive $CD4^+$ T cells from RLTPR-deficient patients, when compared to healthy controls (Fig. 11c). The frequency of IL-2^+ cells of patients was reduced, as compared with controls, which might contribute to their proliferation defect (Fig. 11d). It demonstrates that CD2 signaling cannot completely overcome the CD28 signaling defect in naive $CD4^+$ T cells. Yet, when cultured under Th1 or Th2, but not Th17 conditions, proliferation of RLTPR-deficient naive $CD4^+$ T cells was restored to normal levels, suggesting that cytokines such as IL-12 or IL-4 can overcome the CD28-related defect in RLTPR-deficient $CD4^+$ T cells (Fig. 11e). In contrast with $CD4^+$ naive T cells, there was no reduction in proliferation and in frequency of IL-2 producing cells between patients and controls after four days culture of memory $CD4^+$ T cells cultured under Th0 conditions (Fig 11f and g).

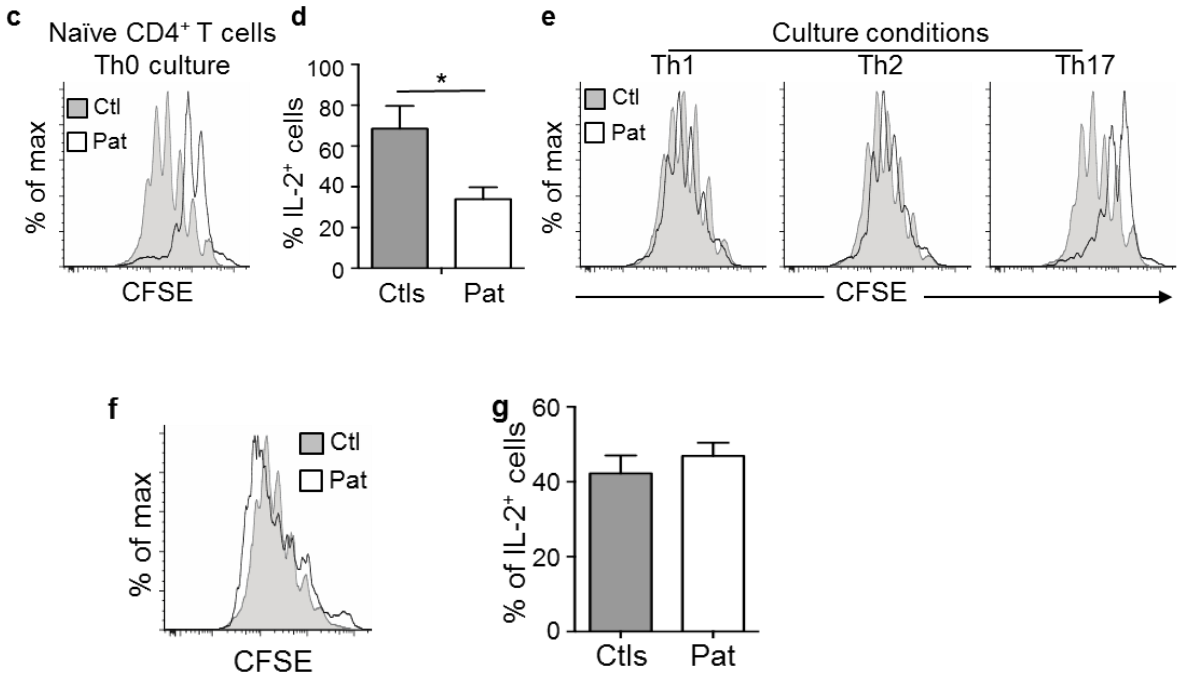


Fig.11(c-g) Proliferation of $CD4^+$ Th cells in vitro. (c) CFSE dilution of naive $CD4^+$ T cells sorted from a representative control (Ctl) and patient (Pat; P5) after 4 d of culture in the presence of CD2/CD3/CD28-coated beads (Th0). (d) Percent $IL\text{-}2^+$ naive $CD4^+$ T cells after 4 d of culture under Th0 conditions. Data are mean ± SEM. *, $P < 0.05$.N = 4–5. (e) CFSE dilution of naive $CD4^+$ T cells sorted from a representative control and patient (P5) after 4 d of culture under Th1 (IL-12), Th2 (IL-4), or Th17 (IL-1β, -6, -21, and -23; TGF-β) cell–polarizing conditions. Similar results were obtained when cells from three additional RLPTR-deficient cells were analyzed under the same conditions. (f) CFSE dilution of memory $CD4^+$ T cells sorted from a healthy control or RLTPR-deficient patient (P5) and then cultured for 4d in the presence of CD2/CD3/CD28-coated beads (Th0). (g) Percent $IL\text{-}2^+$ memory $CD4^+$ T cells after 4d of culture under Th0 conditions. CFSE profiles are representative of data derived from five independent experiments using cells from different donors and patients. Data are mean ± SEM. (obtained from Cindy MA)

NF-κB signaling pathway is involved in human T-cell activation following CD3/CD28 co-stimulation[100]. We then tested phosphorylation of P65 by flow cytometry in PHA-driven T cell blasts of P3, P4, P5 and P6 and 8 healthy controls with combinations of Abs against CD3, CD28 and/or CD2. In control $CD4^+$ T cells, isolated CD28 crosslinking induced strong P65 phosphorylation, which is not observed upon isolated CD3 and CD2 crosslinking, respectively. CD3 and CD28 co-stimulation did not markedly increase P65 phosphorylation, when compared with CD28 alone. In patients $CD4^+$ T cells, P65 phosphorylation was normal upon isolated CD3 or CD2 stimulation, whereas there was no P65 phosphorylation upon isolated CD28 stimulation. Moreover, P65 phosphorylation upon CD3 and CD28 co-stimulation was indistinguishable from that following CD28 stimulation alone. Whereas CD28 crosslinking alone induced weak/absent P65 phosphorylation and CD28 did not synergize with CD3 stimulation in controls $CD8^+$ PHA-T cells. As a result, no phenotype could be expected in patients $CD8^+$ T cells upon CD28 engagement. Altogether, these data indicate that RLTPR is required for CD28-mediated activation of NF-κB in human $CD4^+$ T cells (Fig.11h)

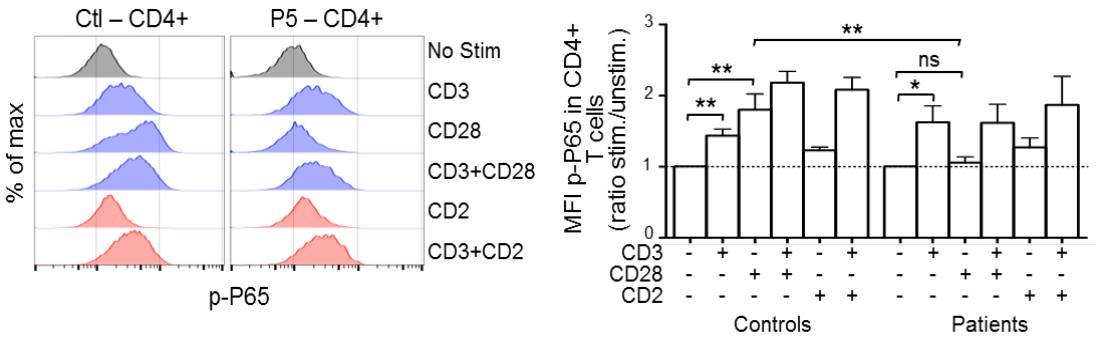


Fig.11h. Impaired CD28 co-stimulation in patients $CD4^+$ T cells. Phospho-P65 (p-P65) detection by flow cytometry in $CD4^+$ PHA blasts after crosslinking of indicated cell surface receptors. (obtained from Vivien BEZIAT, N=3)

To study the impact of RLTPR deficiency on the function of memory $CD4^+$ T cells and to determine whether defects in cytokine secretion by RLTPR-deficient memory $CD4^+$ T cells were intrinsic or extrinsic. We measured cytokine secretion following culture under Th0 conditions. RLTPR-deficient memory $CD4^+$ T cells exhibited dramatic reductions in production of IFN-γ, TNF, IL-17A/F and IL-22, as well as IL-6 and IL-10. In contrast, secretion of the Th2 cytokines IL-4, IL-5 and IL-13 was unaffected by RLTPR deficiency. RLTPR-deficient naive $CD4^+$ T cells showed impaired differentiation into IFN-γ/TNF and IL-17A/IL-17F-producing cells under Th1 and Th17 conditions. In contrast, production of IL-5 and IL-13 under Th2 conditions was only modestly affected (Fig.11i). We then studied by qPCR expression of the master regulators of Th1 (*TBX21*, encoding Tbet), Th2 (*GATA3*) and Th17 (*RORC*, encoding RORγt) differentiation in naive $CD4^+$ T cells following *in vitro* polarization. Surprisingly, induction of *TBX21* and *RORC* expression in RLTPR-deficient naive $CD4^+$ T cells was comparable to that observed for control naive $CD4^+$ T cells. Consistent with the cytokine data, induction of *GATA3* in RLTPR-deficient naive $CD4^+$ T cells was also intact. These data suggest that RLTPR is important for the initial activation and maintenance of naive $CD4^+$ T cells. We also assessed upregulation of CD40L and ICOS – two receptors critical for the function of Tfh cells on naive $CD4^+$ T cells following stimulation with anti-CD2/CD3/CD28 beads. Expression of CD40L and ICOS on RLTPR-deficient naive $CD4^+$ T cells was reduced, equating to ~65% and ~50% of the levels detected on cells from healthy controls. This underscores our finding of reduced proportions of circulating Tfh-type cells in the peripheral blood

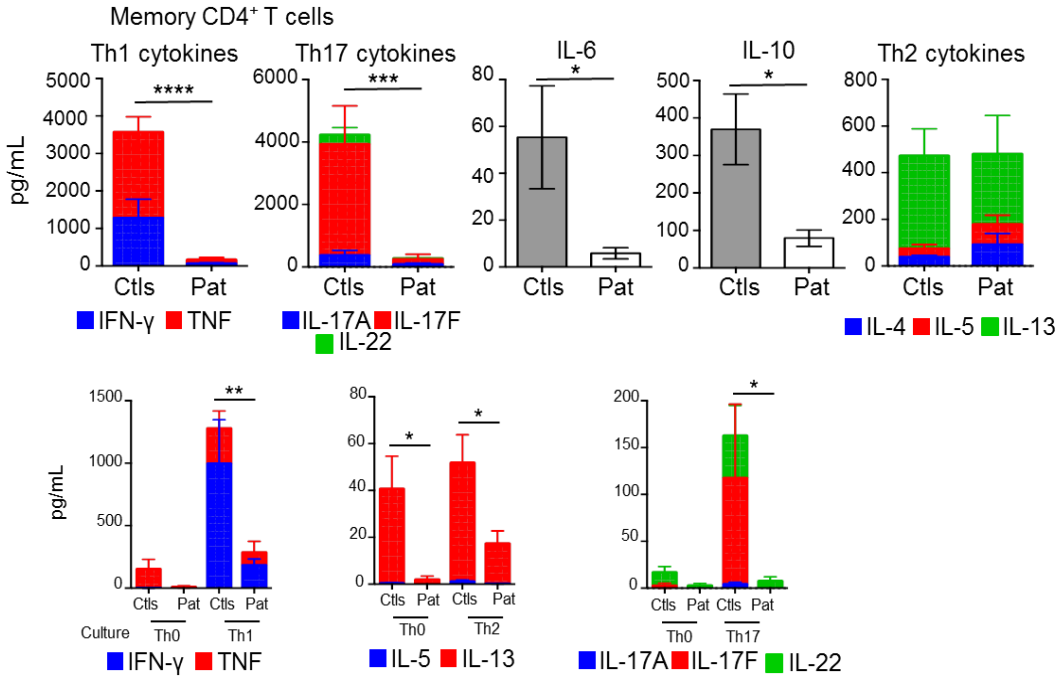


Fig.11i. Secretion of Th1 (TNF, IFN-γ) and Th17 (IL-17A, IL-17F, IL-22) cytokines, IL-6 and IL-10 and Th2 (IL-4, IL-5, IL-13) cytokines by memory $CD4^+$ T cells after four days of culture under Th0 conditions. And Secretion of the indicated Th1 (TNF, IFN-γ), Th2 (IL-5, IL-13) and Th17 (IL-17A, IL-17F, IL-22) cytokines by naive $CD4^+$ T cells after four days culture under Th1, Th2 or Th17 polarizing conditions. (obtained from Cindy Ma, N=3)

II.7 B cell deficiency in RLTPR-deficient patients

We also investigated B cell development and function in RLTPR-deficient patients. A normal count of B cells was observed in all patients except P4 who showed a slight decrease. Among all B cell subsets analyzed *ex vivo*, proportions of transitional B cells ($CD10^+$) were normal while there was a decrease in all memory ($CD27^+$) B cells and IgG^+ B cells (Fig.12a). Serum Ig isotypes levels were determined in all patients, including P4 who was then on IVIG replacement therapy. IgM levels were elevated in all patients except P4, whereas IgG and IgA levels were in the normal range. Serum IgE levels were at high level in P4, particularly during teenage

years. Antigen-specific antibodies to bacterial vaccines were undetectable in P4 and P5 including when tested after DPT vaccination recall for P4. P4 had antibodies against pneumococcal capsular glycans and all patients have detectable viral-specific antibodies. Despite the absence of clinical signs of autoimmunity, some autoantibodies were detectable in the patients (especially in P5), but none of them were directed against IL-17 or IFNs. Altogether, these data suggested that RLTPR-deficient patients displayed a deficiency of memory B cells, coupled to an Ab deficiency against some but not all antigens.

To test whether there could be a B cell-intrinsic defect of RLTPR patients or a consequence of T cell defect, we assessed the NF-κB (degradation of IκBα; phosphorylation of P65) and MAP kinase (phosphorylation of ERK1/2) activation in primary $CD20^+$ B cells in response to BCR stimulation with either anti-IgM antibody or CD40 stimulation with pentameric CD40L by phospho-flow cytometry. All patients and control B cells responded strongly to PMA for the three readouts tested, indicating that RLTPR deficiency does not cause a general impairment in the ability of patient B cells to respond to exogenous stimuli. B cells from three RLTPR-deficient patients also showed a strong and normal NF-κB activation upon CD40 stimulation. Interestingly, while anti-IgM Abs induced NF-κB activation in controls, BCR engagement of RLTPR-deficient B cells failed to induce IκBα degradation or phosphorylation of P65. ERK1/2 phosphorylation upon IgM crosslinking was maintained in patients B cells, albeit slightly diminished in two of the three patients, indicating that the BCR responsive pathway was not fully abrogated in the absence of RLTPR (Fig.12b). These data show that RLTPR-deficient B cell have a partially defective signaling pathway, at least via NF-κB, but an intact CD40 signaling pathway, at least for the readouts tested. Beyond B cells and T cells, we also found decreased production of IFN-γ production by NK and T cells upon BCG plus IL-12 activation of whole blood from P1 and P4[9] and normal IL-10 and

IL-6 production by monocytes upon activation by TNF and microbial products, respectively[101]. Altogether, our results indicate that 6 patients from 3 kindreds with AR complete RLTPR deficiency display at least T cell- and B cell-intrinsic anomalies, accounting for the diversity and severity of their infectious phenotype.

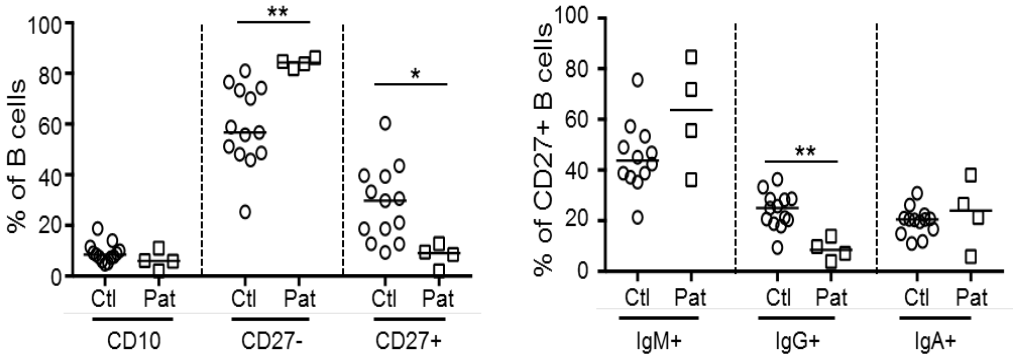


Fig.12a. Frequencies of transitional ($CD10^{+}CD27^{-}$), naive ($CD10^{+}CD27^{-}$) and memory ($CD10^{-}CD27^{+}$) B cells among $CD19^{+}$ B cells. (obtained from Cindy Ma, N=3)

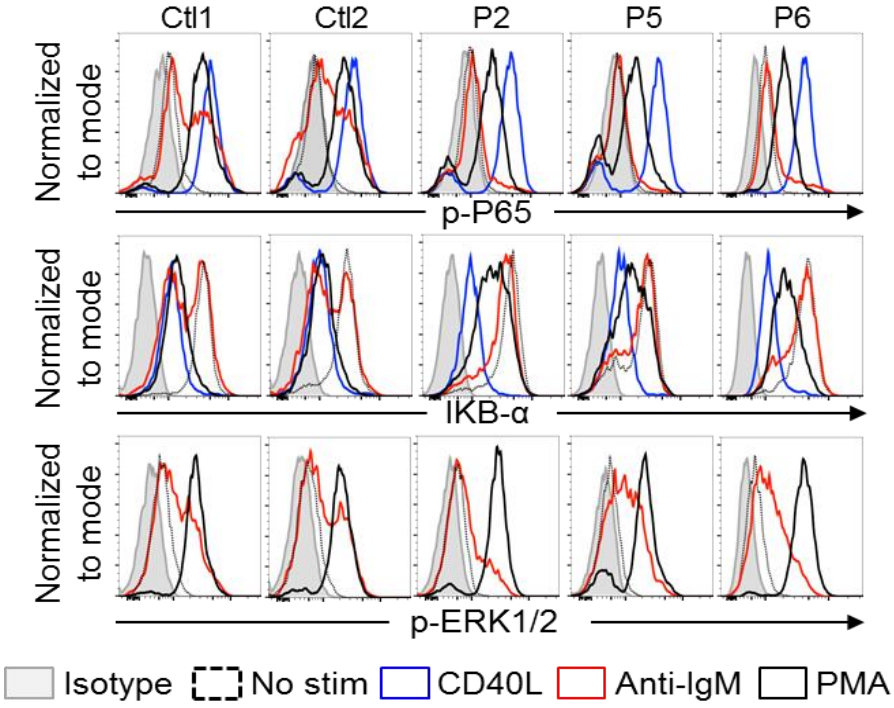


Fig.12b. Phosphorylation of P65, degradation of IκBα and Phosphorylation of ERK1/2 (pERK) after stimulation with CD40L (blue line) and/or anti-IgM (red line) or PMA (black line) of controls and patients. (obtained from Vivien BEZIAT, N=3)

Section III. Discussion

The identification of human RLTPR deficiency provides a new genetic etiology of CID and the first functional description of human RLTPR. In 2013, Liang et al showed that mouse Rltpr is essential for the CD28 co-stimulation signaling pathway[23] (Fig.13). In ENU-mutant mice, by AR trait, Basilic mutation of *Rltpr* was fixed in Lat (Y136F) background. Lat (Y136F)-mutated mice have accumulated $CD4^+$ T cells number, diminished $CD8^+$ T cells and abnormal activated B cell number and they also have lymphoproliferative disorder. Homozygous mutation L342P of $Rltpr^{Bas}$ is located at leucine rich repeat (LRR) region, as the two homozygous missense mutations identified in RLTPR-deficient patients which are located at the same region. These three missense mutations modified highly conserved leucines in LRRs. $Rltpr^{Bas}$ mice showed decreased Rltpr expression in thymocytes and peripheral T cells. Since Lat (Y136F) mice deprived of CD28 ligands, Liang et al investigated if Rltpr is related to CD28 co-stimulation signaling pathway. First of all, $Rltpr^{Bas}$ mice present normal CD28 expression in $CD4^+$ and $CD8^+$ T cells and normal development of T and B cells, but a strongly reduced counts/proportions of Treg cells and $CD4^+$ memory T cells. In our study, all patients develop normal counts of B and T cells, except P1 and P6 that had elevated $CD4^+$ T cells, and P4 that had elevated count of $CD8^+$ T cells. All patients had reduced Treg cells count. Second, in purified T cells of $Rltpr^{Bas}$ mice, defective proliferation after co-stimulation with anti-CD3 and anti-CD28 was detected in $CD4^+$ T cells, whereas only modest defect was reported in $CD8^+$ T cells. Meanwhile, impaired proliferation of T cells was observed in RLTPR-deficient patients upon various stimuli *in vitro*, such as stimulated by PMA/Ionomycin, PHA or OKT3 (anti-CD3 mAb), when compared to healthy controls. Further studies in mouse model of cytokine production upon CD-28 co-stimulation showed no synergy in term of IL-2 and IFN-γ production upon CD3/CD28 co-stimulation compared to CD3

stimulation alone in $CD4^+$ T cells and $CD8^+$ T cells, albeit to a lesser extent in $CD8^+$ T cells. *Rltpr* has been proved to be a key molecule in CD28 co-stimulation signaling pathway, Treg development and the development of Lat (Y136F) lymphoproliferative disorder. Interestingly, all patients had similar immunological features with $Rltpr^{Bas}$ mice. We investigated the cytokine production of IL-2, IFN-γ and TNF in patients $CD4^+$ T cells, and IFN-γ, TNF and CD107a in patients $CD8^+$ T cells. Compared to mouse model, CD28 co-stimulation had a strong impact on CD3/CD28-induced cytokine production in human $CD4^+$ T cells. In healthy controls, $CD8^+$ T cells did not respond to CD28 stimulation in our experimental settings. In contrast with mouse model, RLTPR deficiency had also an impact on BCR response, as shown with defective NF-κB response upon anti-IgM stimulation, but not upon CD40L stimulation. Overall, we showed not only that human RLTPR could affect CD28 co-stimulation signaling pathway, but also proved RLTPR deficiency could impact BCR response in B cells, reflecting cell-intrinsic phenotype and differences between both species[23, 102].

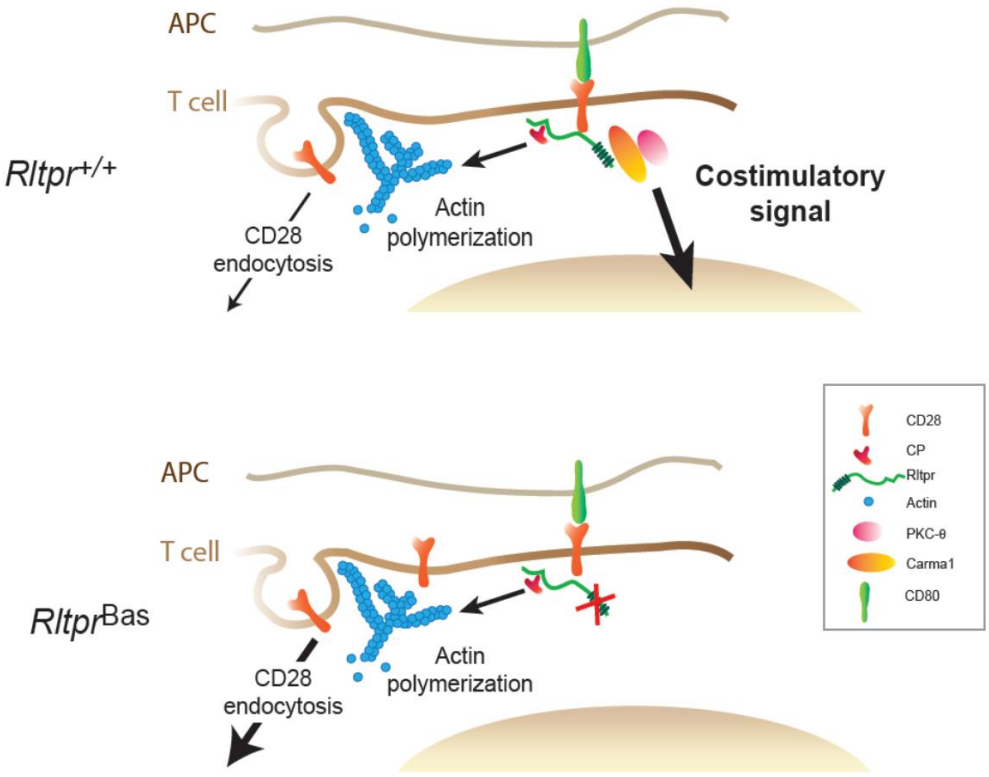


Fig.13. A model illustrating the multitasking role of Rltpr. Rltpr couples CD28 to PKC-θ and Carma1 via its LRRs. (Adapted from Liang et al, 2013[23])

In 2004, by using Affymetrix microarrays, a down-regulated gene was observed from affected psoriasis patients' tissues, which was the first report of RLTPR[103]. The *RLTPR* gene is at chromosome position 16q22 with 39 exons, also known as LRRC16C. "*In silico*" translation of the cDNA sequences indicates that the RLTPR protein is a member of the leucine-rich repeats (LRRs) family (residues 247–667) with a RGD motif, a homodimerization domain (HD; residues 728–905, as predicted by homology with CARMIL1, another member of CARMIL family[104]), a tropomodulin domain and a proline-rich domain (PRR). From 30 different tissues, RT-PCR detected *RLTPR* expression in thymus, spleen, colon, skin, skin keratinocytes, skin fibroblasts and fetal skin.

RLTPR is a large multidomain protein of the CARMIL family, called CARMIL2b. *In vitro* study has shown that CARMIL2 is co-localized with the vimentin filament network and controls cell polarity and motility[105].RLTPR is a large multidomain protein of the CARMIL family, called CARMIL2b, co-localized with the vimentin filament network. The loss of function of RLTPR affects cell polarity and motility[104]. Interestingly, dimerization of CARMIL1 occurs through both antiparallel HDs and contact between the two extremities of horseshoe-shaped LRRs. The canonical sequence of LRR motifs is LxxLxLxxN/CxL. L372 and L525 are both located at the second canonical position of their respective LRR motifs (Lxx**L**xLxxN/CxL) and highly conserved across species. The crystal structure of the LRR domain of CARMIL1 has been recently published[104]. CARMIL1 has 62% similarity with RLTPR, with a 43.8% sequence identity (73.7% similarity) between their LRR domains but only 24% sequence identity (64.7% similarity) between their HD domains, as defined by LALIGN software[106, 104,107]. HD domain of CARMIL1 was sufficient for dimerization. Additionally, not only HD domain participates in

dimerization interaction, but LRR domains may also be involved to protein-protein interactions (Fig.14). The results of co-immunoprecipitation with the Myc-Ab showed that WT RLTPR protein was able to dimerize. Overall, we show that the L372R, L525Q, and Q853X mutations do not impair dimerization, at least in this overexpression system. These data also suggest that the residues 853–905 of the predicted HD domain are not essential for dimerization, neither for LRR domain of CARMIL2b but, for now, we cannot exclude that RLTPR can form oligomers and complex with other protein partners.

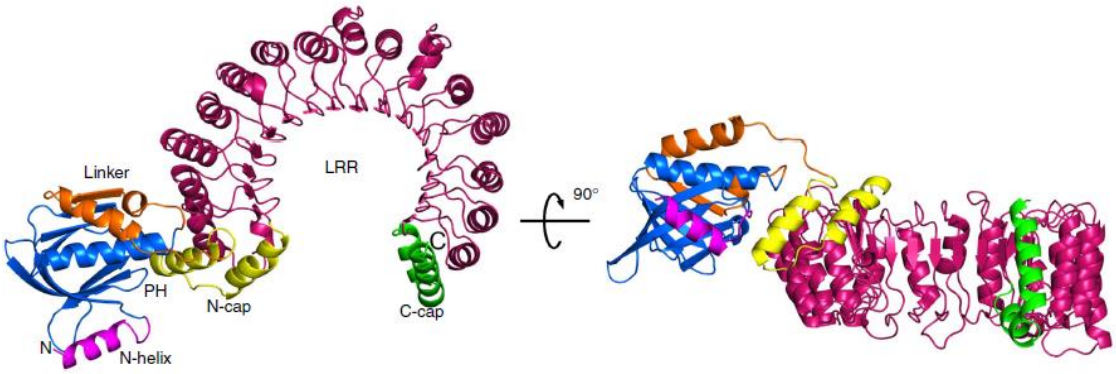


Fig.14 The crystal structure of $CARMIL_{1–668}$ of CARMIL1. (Adapted from Zwolak et al, 2013[104])

Functional studies of RLTPR in mice and our report showed that RLTPR is related to CD28 and NF-κB signaling pathways. TCR- and CD28-mediated signals are essential to activate T cells and to induce optimal levels of cytokines production and cell proliferation. Recently, Malissen group has studied RLTPR interactome in mouse thymocytes and in human Jurkat cell line[102]. First, they showed that RLTPR was interacting with CD28 and CARMA1, which is belonging to CARMA1-BCL10-MALT1 (CBM) complex. It has already been proven that CBM complex is involved in NF-κB activation upon TCR/CD28 co-stimulation[108]. They also showed that the LRR, PRR and pleckstrin domains of Rltpr are important for the CD28-signaling pathway. Finally, they have identified partners of Rltpr in non-activated and activated conditions[102]. Interestingly, DOCK8 is a partner of Rltpr but only after thymocytes activation in thymocytes. Further studies in human and

mice have to be conducted to dissect the role of these different partners in the activation of T cells.

RLTPR is also expressed in human and mouse B cells. Surprisingly, no B cell phenotype has been observed in Rltpr-deficient mouse, as proven by normal BCR-signaling and normal T-cell independent B cell response. In contrast, in human, it has been shown an impaired NF-κB activation upon BCR stimulation, but not upon CD40 ligand stimulation, suggesting that RLTPR is involved in BCR signaling. As CD28 is not expressed in B cells, it suggests that RLTPR has CD28-independent function. Further studies have to be done to determine the partners of RLTPR in B cells and to better understand its exact function in B cells.

The discovery of human RLTPR broadened the perspective of the knowledge of CID. RLTPR-deficient patients developed diverse infectious phenotypes, such as mycobacterial, fungal, staphylococcal and EBV infections. Especially, all patients display severe skin infections. *Candida albicans* and *Staphylococcus aureus* are the main microorganisms associated with skin infections. *Staphylococcus aureus* is responsible of abscesses and might also play a role in the induction and maintenance of psoriasis[109,110]. Herein, we reported that RLTPR is necessary for the development of Th1, Th17, and Tfh cells subsets. In addition, T cells of RLTPR-deficient patients are biased toward Th2 cells, as observed in patients with DOCK8, RORC or STAT3 deficiency[7]. Some of these immunological features are already associated with higher susceptibility to some microbes whereas others are apparently silent clinically, such as low count of Tregs and MAIT cells. For example, the occurrence of invasive tuberculosis and mucocutaneous candidiasis is frequently associated with impaired Th1 and Th17, respectively[42,57], or cutaneous staphylococcal and pulmonary bacterial diseases with low Th17 and Tfh cells, respectively[111,112]. The histology of skin lesions of patients with eczema and psoriasis showed that most of the lesions contained $CD8^+$ T cells, some macrophages and fewer mastocytes. These observations demonstrated the importance of immune system in controlling, forming and maintaining the skin

microbiota[113,117,118]. Despite some reports, such as Puel *et al* and Kreins *et al* describing patients with staphylococcal diseases associated with auto-Abs against IL-6 or impaired IL-6 response, respectively[14,60], the pathogenesis of staphylococcal disease is still not clear. Likewise, we noticed that RLTPR-deficient patients produced less IL-6 by memory $CD4^+$ T cells *in vitro*, and had an impaired development of Tfh cells and poor Ab response, which may also be related to staphylococcal disease. Finally, EBV viremia was detected in 2/3 of RLTPR-deficient patients, although none of these patients developed the illnesses caused by EBV infection now, they may be susceptible to EBV disease later in life[79].

In functional studies of RLTPR, we showed that RLTPR is related to CD28 co-stimulation signaling pathway and the underlying mechanism may involve NF-κB signaling pathway downstream of CD28. Co-stimulation signals, particularly, the very important CD28-mediated signal additionally provided by antigen-presenting cells (APCs) are required for activation of T cells, in view of the insignificancy of TCR-CD3 complex stimulating T cells alone to induce optimal levels of cytokine productions and cell proliferation. CD28 stimulation is also critical for NF-κB activation. We indicated that full T cell activation by OKT3 or recall antigens, in our experimental conditions, including the presence of APCs, requires an intact RLTPR, probably because of its requirement downstream of CD28 (which can be activated in this assay by antigen-presenting cells in the presence of OKT3 or PHA). Impaired responses to PMA/Ionomycin probably reflects the low proportion of memory T cells in the patients, as control naive T cells proliferate less well than memory T cells in response to this stimulus (unpublished data). Those responses also determined the potential role of RLTPR in CD28 signaling in human T cells by *in vitro* proliferation of naïve and memory $CD4^+$ T cells from healthy controls and patients. RLTPR deficiency compromises proliferation of naïve but not memory $CD4^+$ T cells. Impaired the synergy of cytokine production of IFN-γ, TNF and IL-2 after CD3 and CD28 co-stimulation and abolished P65 phosphorylation in patients' $CD4^+$ T cells

provided an important clue that RLTPR involves impaired CD28 co-stimulation pathway and NF-κB signaling downstream of CD28. Moreover, impaired NF-κB activation was found in patients B cells after BCR stimulation but not CD40 ligand, suggesting that RLTPR in antigen-receptor signaling in B cells.

The immunological phenotype of RLTPR-deficient patients shared some similarities, but also some differences, with other CIDs, such as DOCK8[51,114] or CBM complex[24,115-128]. DOCK8-deficient patients with loss-of-function bi-allelic DOCK8 mutations are associated with autosomal recessive hyper-IgE syndrome, have T cell lymphopenia[51], Treg defective function[116], and presented impaired proliferation upon CD3/CD28 stimulation in $CD8^+$ T cells[51,114,117]. Impaired proliferation upon CD3/CD28 stimulation in $CD8^+$ T cells was observed in these patients[51,114,117]. Most of mutations identified in *DOCK8* are large deletions, which also have been proven to be responsible not only for a T cell defect but also for a B cell-intrinsic defect[118]. However, DOCK8-deficient patients had shown a strong defect of $CD8^+$ T cells, both on development and function. Besides the reduction of central memory $CD8^+$ T cells, patients also had strong affected naïve and effector memory $CD8^+$ T cells. Moreover, the impaired function of $CD8^+$ T cells in DOCK8-deficient patients has been reported as they do not proliferate *in vitro* under TCR activation. Which is not the case of RLTPR-deficient patients[117]. Other immunological findings of patients with DOCK8 mutations, showed that be different from RLTPR-deficient patients, DOCK8-deficient patients developed affected NK cell function and NKT cell development[119,120]. Patients with inborn errors in *CARMA1*, *BCL10* or *MALT1* displayed normal counts of T and B cells, but impaired T cell proliferation upon TCR stimulation. Patients with bi-allelic CARD11 mutations present agammaglobulinemia, a predominance of transitional B lymphocytes was detected in the blood. Patients with CARMA1 or BCL10 deficiency have reduced Treg and Th17 cells but without autoimmunity manifestation, as observed in RLTPR-deficient patients[114,116,122]. Interestingly, some

of these PIDs have also B cell defect. DOCK8 deficiency have also intrinsic-B cell defect, with affected B cell numbers[51,118] and hyper-IgE syndrome[51,114]. CBM-deficient patients also presented B cell phenotype, with impaired development due to a blockade in naïve stage. CARMA1- and MALT1-deficient patients have also an impaired NF-κB activation upon BCR stimulation. These similarities might be explained by the fact that both DOCK8 and CARMA1 are partners of RLTPR, as described by Roncagalli et al[102]. However, the function of DOCK8 and RLTPR interaction is still unknown, as well as their function in B cells. Further studies have to be done to figure it out.

The clinical phenotype of RLTPR-, DOCK8- and CBM-deficient patients has some similarities and differences. All of them are sensitive to bacterial and fungal infections, which can be explained by T/B cell defects and reduced Th17 numbers, respectively. In addition, all developed skin phenotype, such as eczema. However, their clinical and immunological phenotype has also some differences. Patients with bi-allelic CARD11 mutations developed *P. jirovecii*, recurrent sino-pulmonary bacterial infections, and severe eczematous dermatitis[124–126]. In contrast, DOCK8-deficient patients presented with hyper IgE syndrome and were sensitive to cutaneous viral infections, due to *Molluscum contagiosum*, herpes simplex virus and human papillomavirus. The patients also had eosinophilia and severe food and environmental allergies. Up to 80-90% of DOCK8-deficient patients develop eczema[51, 114, 113,127], upper and lower respiratory tract infections[51,113,114,127] and viral infections[51]. Most of the patients present recurrent skin abscesses, caused by *Staphylococcus aureus*. Some patients also have post-inflammatory hyperpigmentation of skin[113,128]. Meanwhile, regarding to various infectious phenotypes, impaired CD28 co-stimulation alone can not contribute to all infections observed in patients, but with partial BCR signaling pathway defect, may give us a

clue that human RLTPR-dependent CD28 co-stimulation with CD3 in T cells and BCR activation of NF-κB in B cells which contribute to against infectious agents.

All RLTPR-deficient patients had detectable auto-abs. and displayed a significant reduced proportion of $Foxp3^+$ Treg cells, but none of them developed severe clinical autoimmune manifestation by now. This is not the first report of reduced Treg cells and absence of autoimmunity. Patients with CARD11 or BCL10 mutations had also low Treg cells and no autoimmune diseases[115,121]. Interestingly, Rltpr-deficient mice with impaired CD28 co-stimulation and decreased Treg cells had no autoimmune manifestations either. This is unlike previous reported PIDs, such as FOXP3, LRBA, CTLA-4 deficiencies, who displayed impaired development of Treg cells and autoimmunity[129]. Interestingly, CARD11[124,125], BCL10[121] and CD28 deficiencies are responsible of a activation defect of effector T cells response[130]. One plausible explanation of absence of autoimmunity in RLTPR deficient-patients is that the diminution of Treg cell is compensated by the lack of CD28 activation of effector $CD4^+$ T cells, poor generation, maintenance or expansion of memory $CD4^+$ T cells, and impaired survival of memory $CD4^+$ T cells because of compromised CD28-mediated induction of Bcl-xL.

Section IV. Conclusion and future directions

We identified RLTPR deficiency in 6 patients from 3 independent families with CID from Morocco, Tunisia and Turkey; the three identified mutations are different and the patients are aged from 2 to 27 years. These patients displayed a complex phenotype with cutaneous, pulmonary infections, including invasive tuberculosis, mucocutaneous candidiasis and staphylococcal infection, skin and allergic manifestations. We therefore showed that all patients had impaired development of lymphocytes, in particularly, reduced Th17 and Tfh cells, with slightly decreased Th1 cells, which respectively elucidated the occurrence of mucocutaneous candidiasis, cutaneous staphylococcal, pulmonary bacterial diseases, and tuberculosis. By further studies, we detected reduced production of IFN-γ, TNF, IL-17A/F and IL-22 in patients' memory $CD4^+$ T cells under Th0 condition, the same to IL-6 and Il-10 secretion. In addition, when we investigated the differentiation from naïve to Th subsets of patients' $CD4^+$ T cells, we observed a strongly impaired differentiation into IFN-γ, TNF, IL-17A/F producing cells under Th1 or Th17 condition. Despite these results, we cannot explain yet all clinical phenotypes of RLTPR-deficient patients. As the patients are still young, some manifestations may occur later in life, such as autoimmunity. Further studies are necessary to understand better how RLTPR contributes to CID.

Our study has first shed light on the function of human RLTPR, which is a new genetic etiology of CID and the first one affecting the CD28 co-stimulation signaling pathway. We first showed that homozygous RLTPR mutations were rare and with complete penetrance. Patients have an impaired cytokines production and proliferation upon CD3 and CD28 co-stimulation in $CD4^+$ T cells. They also have an impaired NK-κB signaling downstream of CD28, as inferred from the abolished P65 phosphorylation in $CD4^+$ T cells. Intriguingly, NF-κB activation was also impaired in

patients B cells upon BCR, but not CD40, activation, suggesting a role of RLTPR in antigen-receptor signaling in B cells. As we cannot detect any functional defect in $CD8^+$ T cells in our experimental setting, further studies about, at least, the RLTPR partners in $CD4^+$ and in $CD8^+$ T cells will be necessary in human and mouse.

CARMIL family member interacts with actin filament and it is also important for cell motility[104]. Moreover, actin cytoskeletal regulatory genes are involved in a number of immune cell functions and they are critical for normal immune system activity[131]. Patients with Wiskott-Aldrich syndrome (WAS) have a severe clinical phenotype, including allergy, infections, autoimmunity and malignancy. This phenotype overlaps with some symptoms observed in DOCK8- and RLTPR-deficient patients. The identified disease-causing gene is *WAS*, and WAS protein (WASp) has been shown to belong to a family of proteins that relay signals from the surface of the cell to the actin cytoskeleton[132]. A recent paper showed that DOCK8 and WASp are interacting together and link the TCR to the actin cytoskeleton[133]. As DOCK8 belongs to the RLTPR interactome in some activated thymocytes, further studies would have to determine whether RLTPR-deficient patients have an impaired actin filament network and defective cell motility. This study may complete knowledge of RLTPR.

As only very few studies have been reported on *RLTPR*, this gene is waiting for being explored in more depth. By now, the location of RLTPR in BCR signaling pathway is still a mystery. A shared mechanism in T and B cells may involve the disruption of the CARMA1–RLTPR interaction has been shown this year[102]. Further study would start with CD28-CARMA1-RLTPR signaling pathway, to define how CARMA1-RLTPR interaction works in TCR and BCR signaling pathway.

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ANNEX

Publication

Dual T cell– and B cell– intrinsic deficiency in humans with biallelic RLTPR mutations

Yi Wang, Cindy S. Ma, Yun Ling, Aziz Bousfiha, Yildiz Camcioglu, Serge Jacquot, Kathryn Payne, Elena Crestani, Romain Roncagalli, Aziz Belkadi, Gaspard Kerner, Lazaro Lorenzo, Caroline Deswarte, Maya Chrabieh, Etienne Patin, Quentin B. Vincent, Ingrid Muller-Fleckenstein, Bernhard Fleckenstein, Fatima Ailal, Lluis Quintana-Murci, Sylvie Fraitag, Marie‐Alexandra Alyanakian, Marianne Leruez‐Ville, Capucine Picard, Anne Puel, Jacinta Bustamante, Stéphanie Boisson‐Dupuis, Marie Malissen, Bernard Malissen, Laurent Abel, Alain Hovnanian, Luigi D. Notarangelo, Emmanuelle Jouanguy, Stuart G. Tangye, Vivien Béziat, and Jean-Laurent Casanova

J. Exp Med 2016 2016 Oct 17;213(11):2413-2435.