Projet financé par la Fondation SCOR

Identifying inborn errors of immunity in tuberculosis (TB) to propose new immune system-restoring treatments of TB

Principal Investigator: Laurent Abel

Dr. Laurent ABEL, DRCE Inserm

Directeur du Laboratoire de Génétique Humaine des Maladies Infectieuses

Institut Imagine, Paris

Juin 2021

General context and objectives of the project

Tuberculosis (TB) is caused by *Mycobacterium tuberculosis* (Mtb). More than nine million individuals worldwide develop TB each year, and an estimated one third of the world population is infected with Mtb[1,2]. Most infected subjects develop latent TB infection, with only ~5% going on to develop clinical TB within two years of infection[3,4]. This primary TB is particularly common in children, some of whom develop a hematogenous disseminated form[5-7]. In ~5% of patients with latent infection, TB develops later in life, principally as a pulmonary disease in adults, typically due to reactivation of the original infection[3]. While TB remains a leading cause of mortality and morbidity worldwide, the key events in the pathogenesis of TB are unknown, and the considerable variability of infection outcomes, in particular the fact that ~90% of infected individuals do not develop the disease, remains largely unexplained. Faced with this fundamental paradox, understanding the pathogenesis of TB is a top priority in the field to find new ways of combating this disease. BCG vaccination and the development of anti-mycobacterial antibiotics have greatly decreased mortality due to childhood TB. However, BCG vaccination is clearly incompletely effective (~ 50%) and more than 80,000 children per year are still dying from TB[1,2]. In addition, BCG vaccination has almost no protective effect in the development of pulmonary TB in adults. Moreover, the use of old and novel antibiotics to treat this disease entails a long-term risk of the selection of drug-resistant mycobacteria. Multidrug-resistant (MDR) TB, defined as resistance to the two key drugs isoniazid and rifampicin, has now emerged as a global threat with an estimated 4% of new cases and 21% of re-treated cases who were MDR-TB in 2015[1,2]. Among these MDR forms, extensively drug-resistant TB forms (XDR-TB) were reported in 117 countries with an overall proportion of 10% of XDR-TB among MDR-TB[1,2].

In light of this, there is an urgent need to develop novel approaches to the treatment of TB. This process should begin with the long-awaited dissection of TB pathogenesis. What accounts for the natural resistance of most infected individuals, and the predisposition to TB of only a few? This is the most fundamental question in the field of TB, and despite 50 years of research into the mechanisms of protective immunity to Mtb, it remains unanswered. A century of surveys in genetic epidemiology (e.g. twin studies)[8,9], half a century of genetic studies in animal models of TB[3,10], and a decade of investigations in human molecular genetics[3,11,12], have provided firm foundations for the investigation of TB from the standpoint of human genetics. In particular, the investigation of a rare condition

characterized by selective susceptibility to weakly virulent mycobacteria (BCG vaccine and environmental mycobacteria), and designated as Mendelian susceptibility to mycobacterial disease (MSMD) provided the first genetic clues. Mutations of 17 genes related to the IFN-γ pathway have been found to underlie MSMD, and paved the way for the demonstration that TB can be monogenic[13,14]. TB patients carrying rare mutations in MSMD genes (in particular *IL12RB1* and *TYK2*) provided proof-of-concept [15-21]. The combined results of these studies have led to the view that TB is not only an infectious disease but also a *bona fide* genetic disorder[11,12].

The hypothesis of this project was that TB results to a large extent from a collection of rare, single-gene inborn errors of immunity, ie diverse variants of genes with a strong impact on immunity during Mtb infection. This hypothesis has become experimentally testable with the advent of next-generation sequencing (NGS) technologies, whole-exome sequencing (WES) in particular. Therefore, the objectives of the project were to search for and characterize the underlying genetic defects using

1) cutting-edge genome-wide strategies, including WES analyses,
2) in-depth functional studies to validate the causal role of the genetic variants identified.

Achievements and results

Collection of patients and whole exome sequencing (WES) analysis

Through an important and large network of national and international collaborations we have established over the last 20 years, we could extend our recruitment of TB patients during the project. For childhood TB, the patients included in this project are children with a life-threatening, disseminated form of TB requiring hospitalization. These forms include, in particular, miliary TB, meningitis, peritonitis, and osteomyelitis (such as Pott's disease, in particular). These patients are mainly recruited from North Africa, Turkey, and Middle-Eastern countries. We have now more than 400 children with TB and available WES data. For adult TB, only patients > 18 years with bacteriologically confirmed pulmonary TB are included. Our main sample was collected in Morocco, and we have now in total more than 1200 pulmonary TB patients as well as 1000 healthy controls TB infected as assessed by positive Tuberculin skin test and Quantiferon test. The WES performed during the project led to a total of 500 pulmonary TB patients and 250 TB infected healthy controls with available sequencing data. In addition, we have also collected MSMD patients, as the investigation of those patients could provide critical information for the genetic basis of TB as previously shown[13,14]. The analysis of the WES data of these TB and MSMD patients provided several major findings in the three last years that are detailed in the next sections.

Identification of homozygosity for the TYK2 P1104A as the first identified common monogenic cause of TB (articles 1 and 2)

TYK2 is a Janus kinase involved in four cytokine signaling pathways (those mediated by IL-12, IL-23, IFN-α and IL-10) [21-27]. Patients with complete AR TYK2 deficiency homozygous for very rare loss of function (LOF) mutations mainly suffer from MSMD and/or TB[21]. By analysing the WES data of TB and MSMD patients, we found that homozygosity for a common *TYK2* missense variant (P1104A) was strongly predisposing to TB (odds ratio (OR) 89.3 (95% CI, 14.7–1,725)) with high penetrance (at least 50%) in areas of endemic disease[28]. Homozygous carriers also displayed a predisposition to MSMD, albeit to a lesser extent[28]. The minor allele frequency (MAF) of P1104A is 4.2% in the European

population (from gnomAD), leading to a frequency of homozygous individuals of about 1/600 in Europe. Homozygosity for P1104A selectively disrupts the induction of IFN-γ by IL-23, as seen in patients with complete TYK2 deficiency, whereas the other three cytokines responsive pathways are unaffected[28]. In addition, we found that the frequency of P1104A was higher in ancient human DNAs (~4,000 years ago) than presently, consistent with a purging operated by endemic infections such as TB. We could further confirm the role of P1104A homozygosity in TB in the United Kingdom (UK) Biobank cohort, a large cohort of European ancestry [29]. Overall, homozygosity for the *TYK2* P1104A variant is the first identified common monogenic cause of TB, and may underlie a sizable proportion of TB cases, accounting for ~1% of TB cases in Europeans and ~0.33% of cases in most other regions of the world (i.e. outside sub-Saharan Africa, and Eastern Asia).

- **Article 1:** Boisson-Dupuis S, Ramirez-Alejo N, Li Z, Patin E, Rao G, Kerner G, Lim CK, Krementsov DN, Hernandez N, Ma CS, Zhang Q, Markle J, Martinez-Barricarte R, Payne K, Fisch R, Deswarte C, Halpern J, Bouaziz M, Mulwa J, Sivanesan D, Lazarov T, Naves R, Garcia P, Itan Y, Boisson B, Checchi A, Jabot-Hanin F, Cobat A, Guennoun A, Jackson CC, Pekcan S, Caliskaner Z, Inostroza J, Costa-Carvalho BT, de Albuquerque JAT, Garcia-Ortiz H, Orozco L, Ozcelik T, Abid A, Rhorfi IA, Souhi H, Amrani HN, Zegmout A, Geissmann F, Michnick SW, Muller-Fleckenstein I, Fleckenstein B, Puel A, Ciancanelli MJ, Marr N, Abolhassani H, Balcells ME, Condino-Neto A, Strickler A, Abarca K, Teuscher C, Ochs HD, Reisli I, Sayar EH, El-Baghdadi J, Bustamante J, Hammarström L, Tangye SG, Pellegrini S, Quintana-Murci L, Abel L, Casanova JL. Tuberculosis and impaired IL-23-dependent IFN-γ immunity in humans homozygous for a common *TYK2* missense variant. ***Sci Immunol. 2018*** Dec 21;3(30):eaau8714. doi: 10.1126/sciimmunol.aau8714. PMID: 30578352; PMCID: PMC6341984.
- **Article 2:** Kerner G, Ramirez-Alejo N, Seeleuthner Y, Yang R, Ogishi M, Cobat A, Patin E, Quintana-Murci L, Boisson-Dupuis S, Casanova JL, Abel L. Homozygosity for *TYK2* P1104A underlies tuberculosis in about 1% of patients in a cohort of European ancestry. ***Proc Natl Acad Sci U S A. 2019*** May 21;116(21):10430-10434. doi: 10.1073/pnas.1903561116. Epub 2019 May 8. PMID: 31068474; PMCID: PMC6534977.

Identification of PD-1 deficiency, as a novel rare TB-causing gene (article 3)

Our WES analyses also identified a patient homozygous for a frameshift insertion in *PDCD1,* encoding PD-1[30]. The patient suffered from disseminated TB and died of pulmonary autoimmunity after a course of type I diabetes and thyroiditis. The older brother of the patient suffered from autoimmunity and died at 3 years old from pulmonary disease. Even though no biological material could be recovered, we assume that he too had PD-1 deficiency. PD-1 is an immune checkpoint inhibitor that decreases signals from the TCR when it binds to its ligand PD-L1 and PD-L2 [31]. PD-1 knockout mice spontaneously develop autoimmune diseases that vary depending on their genetic background and are extremely susceptible to *Mtb* [32-37]. Moreover, cancer patients treated with PD-1 antibodies develop auto-immune manifestations and reactivate TB [38-41], highlighting an essential role of PD-1 in the maintenance of tolerance to self and immunity to infection. In HEK293T cells as well as in PBMC (CD4 and CD8 T cells, B cells and monocyte) of the patient, no PD-1 could be detected by flow cytometry, implying that the mutant allele is loss-of-expression. The patient's lymphocytes produced only small amounts of IFN-γ upon mycobacterial stimuli, similarly to patients with inborn errors of IFN-γ production who are vulnerable to TB. Our work highlights the indispensable role of human PD-1 in

governing both antimycobacterial immunity and self-tolerance while identifying potentially actionable molecular targets for the diagnostic and therapeutic management of TB and autoimmunity in patients on PD-1 blockade.

- **Article 3:** Ogishi M, Yang R, Aytekin C, Langlais D, Bourgey M, Khan T, Ali FA, Rahman M, Delmonte OM, Chrabieh M, Zhang P, Gruber C, Pelham SJ, Spaan AN, Rosain J, Lei WT, Drutman S, Hellmann MD, Callahan MK, Adamow M, Wong P, Wolchok JD, Rao G, Ma CS, Nakajima Y, Yaguchi T, Chamoto K, Williams SC, Emile JF, Rozenberg F, Glickman MS, Rapaport F, Kerner G, Allington G, Tezcan I, Cagdas D, Hosnut FO, Dogu F, Ikinciogullari A, Rao VK, Kainulainen L, Béziat V, Bustamante J, Vilarinho S, Lifton RP, Boisson B, Abel L, Bogunovic D, Marr N, Notarangelo LD, Tangye SG, Honjo T, Gros P, Boisson-Dupuis S, Casanova JL. Inherited PD-1 deficiency underlies tuberculosis and autoimmunity in a child. ***Nat Med. 2021*** Jun 28. doi: 10.1038/s41591-021-01388-5. Epub ahead of print. PMID: 34183838.

Identification of ZNFX1 deficiency, as a genetic etiology of MSMD or TB (article 4)

Analysis of WES data also identified four patients from two unrelated kindreds with intermittent monocytosis and mycobacterial disease, including BCG-osis and disseminated TB, and without any known inborn error of IFN-γ. The patients are homozygous for *ZNFX1* mutations predicted to be LOF. ZNFX1 is a conserved and broadly expressed helicase, but its biology remains largely unknown. Mutations in the ZNFX1 gene resulted in a defect in the expression of the ZNFX1 protein, which is expressed in myeloid cells called monocytes. We also found a link between the ZNFX1 protein and ribonucleoprotein granules, called stress granules, which play a role in protecting the body from stress, in transcribing mRNA and carrying out specific biological and immune processes. Overall, we discovered that Inherited deficiency of stress granule-associated ZNFX1 is a novel genetic etiology of MSMD or TB.

- **Article 4:** Le Voyer T, Neehus AL, Yang R, Ogishi M, Rosain J, Alroqi F, Alshalan M, Blumental S, Al Ali F, Khan T, Ata M, Rozen L, Demulder A, Bastard P, Gruber C, Roynard M, Seeleuthener Y, Rapaport F, Bigio B, Chrabieh M, Sng D, Berteloot L, Boddaert N, Rozenberg F, Al-Muhsen S, Bertoli-Avella A, Abel L, Bogunovic D, Marr N, Mansouri D, Al Mutairi F, Béziat V, Weil D, Mahdaviani SA, Ferster A, Zhang SY, Reversade B, Boisson-Dupuis S, Casanova JL, Bustamante J. Inherited deficiency of stress granule ZNFX1 in patients with monocytosis and mycobacterial disease. ***Proc Natl Acad Sci U S A. 2021*** Apr 13;118(15):e2102804118. doi: 10.1073/pnas.2102804118. PMID: 33876776; PMCID: PMC8053974.

Identification of T-bet deficiency, as a genetic etiology of mycobacterial infections (article 5)

Analysis of WES data further identified a patient with disseminated BCG disease who was homozygous for a LOF mutation of the gene encoding the transcription factor T-bet. This mutation reduced the production of IFN-γ by natural killer (NK) cells, lymphocytes of the innate immune system capable of killing infected cells, and innate-like adaptive lymphocytes.

Overall, we discovered the first human T-bet deficiency that underlies mycobacterial disease by preventing the development of innate (NK) and innate-like adaptive lymphocytes.

- **Article 5:** Yang R, Mele F, Worley L, Langlais D, Rosain J, Benhsaien I, Elarabi H, Croft CA, Doisne JM, Zhang P, Weisshaar M, Jarrossay D, Latorre D, Shen Y, Han J, Ogishi M, Gruber C, Markle J,

Al Ali F, Rahman M, Khan T, Seeleuthner Y, Kerner G, Husquin LT, Maclsaac JL, Jeljeli M, Errami A, Ailal F, Kobor MS, Oleaga-Quintas C, Roynard M, Bourgey M, El Baghdadi J, Boisson-Dupuis S, Puel A, Batteux F, Rozenberg F, Marr N, Pan-Hammarström Q, Bogunovic D, Quintana-Murci L, Carroll T, Ma CS, Abel L, Bousfiha A, Di Santo JP, Glimcher LH, Gros P, Tangye SG, Sallusto F, Bustamante J, Casanova JL. Human T-bet Governs Innate and Innate-like Adaptive IFN-γ Immunity against Mycobacteria. ***Cell. 2020*** Dec 23;183(7):1826-1847.e31. doi: 10.1016/j.cell.2020.10.046. Epub 2020 Dec 8. PMID: 33296702; PMCID: PMC7770098.

Identification of homozygous mutations in the gene encoding IFN-γ, as a genetic etiology of mycobacterial infections (article 6)

Since the first MSMD-causing mutations were reported in 1996, biallelic mutations in the genes encoding the two chains of the IFN-γ receptor 1 (*IFNGR1* and *IFNGR2*) have been reported in many patients of diverse ancestries. Surprisingly, mutations of the gene encoding the IFN-γ cytokine itself have not been reported, raising the remote possibility that there might be other agonists of the IFN-γ receptor. The analysis of our WES data identified 2 Lebanese cousins with MSMD who are both homozygous for a small deletion within the *IFNG* gene. The mutant allele is loss of expression and loss of function. The blood T and NK lymphocytes from these patients also failed to produce and secrete detectable amounts of IFN-γ. Finally, we show that human *IFNG* has evolved under stronger negative selection than *IFNGR1* or *IFNGR2*, suggesting that it is less tolerant to heterozygous deleterious mutations than *IFNGR1* or *IFNGR2*. Overall we reported the first complete IFN-γ deficiency which is causing severe mycobacterial infections.

- **Article 6:** Kerner G, Rosain J, Guérin A, Al-Khabaz A, Oleaga-Quintas C, Rapaport F, Massaad MJ, Ding JY, Khan T, Ali FA, Rahman M, Deswarte C, Martinez-Barricarte R, Geha RS, Jeanne-Julien V, Garcia D, Chi CY, Yang R, Roynard M, Fleckenstein B, Rozenberg F, Boisson-Dupuis S, Ku CL, Seeleuthner Y, Béziat V, Marr N, Abel L, Al-Herz W, Casanova JL, Bustamante J. Inherited human IFN-γ deficiency underlies mycobacterial disease. ***J Clin Invest. 2020*** Jun 1;130(6):3158-3171. doi: 10.1172/JCI135460. PMID: 32163377; PMCID: PMC7260033.

Identification of a novel locus controlling resistance to infection by Mtb (article 7)

We also interested ourselves in the resistance to *Mtb* infection itself. Indeed, some highly exposed individuals remain resistant to *Mtb* infection, as inferred by tuberculin skin test (TST) or interferon-gamma release assays (IGRAs). We performed a genome-wide association study in an endemic region of Southern Vietnam. We enrolled household contacts (HHC) of pulmonary TB cases and compared subjects who were negative for both TST and IGRA with infected individuals who were either positive for both TST and IGRA or had a diagnosis of TB. We found a genome-wide significant locus on chromosome 10 with a cluster of variants associated with strong protection against *Mtb* infection. The locus was replicated in a French multi-ethnic HHC cohort and a familial admixed cohort from a hyper-endemic area of South Africa. The variants are located in intronic regions and upstream of C10orf90, a tumor suppressor gene that encodes an ubiquitin ligase activating the transcription factor p53. In silico analysis showed that the protective alleles were associated with a decreased expression in monocytes of the nearby gene ADAM12 which could lead to an enhanced response of Th17 lymphocytes. Our results reveal a novel locus controlling resistance to Mtb infection across

different populations.

- **Article 7:** Quistrebert J, Orlova M, Kerner G, Ton LT, Luong NT, Danh NT, Vincent QB, Jabot-Hanin F, Seeleuthner Y, Bustamante J, Boisson-Dupuis S, Huong NT, Ba NN, Casanova JL, Delacourt C, Hoal EG, Alcaïs A, Thai VH, Thành LT, Abel L, Schurr E, Cobat A. Genome-wide association study of resistance to Mycobacterium tuberculosis infection identifies a locus at 10q26.2 in three distinct populations***. PLoS Genet. 2021*** Mar 4;17(3):e1009392. doi: 10.1371/journal.pgen.1009392. PMID: 33661925; PMCID: PMC7963100.

Conclusion

Overall, during these 3 years, we have identified several novel genetic etiologies of TB and/or MSMD. These findings led to 7 articles in journals of the highest level including Cell, PNAS, Science Immunology, Nature Medicine, or Journal of Clinical Investigation in which the SCOR Foundation is acknowledged. All the identified defects, except ZNFX1, are located in the IFN-γ pathway confirming the major role of this circuit in the development of TB. The ongoing investigations of ZNFX1, a gene with a largely unknown biology, may provide clues in novel pathways involved in the response to mycobacteria. Of importance, we have also found the first complete deficiency of the IFN-γ gene itself.

Our most important result is probably the identification of homozygosity for the *TYK2* P1104A as the first identified common monogenic cause of TB. Homozygosity for the *TYK2* P1104A variant underlies a sizeable proportion of TB cases, perhaps accounting for ~1% of TB cases in Europeans and ~0.5% of cases in most other regions of the world (i.e. outside Europe and Eastern Asia). A large number of P1104A-related TB cases would have major implications for the prevention and treatment of TB. It should make it easier to target individuals at high risk of TB when defining optimal cohorts for trials of TB candidate vaccines. Indeed, vaccination strategies should aim at protecting the 5% of individuals who are not naturally, genetically resistant to *M. tuberculosis*. Genetic testing for this variant may also be warranted before travel to countries highly endemic for TB. The vast majority of P1104A homozygotes living in the most developed countries are asymptomatic, as they have not been exposed to *M. tuberculosis*. The diagnosis of P1104A homozygosity in patients with TB could also pave the way for genetic counseling in their families.

The notion that 0.5 to 1% of TB is autosomal recessive and accounted for by homozygosity for a common *TYK2* variant has far-reaching implications for the genetic study of TB and other common, severe infectious diseases. This discovery further blurs the dichotomy between rare monogenic etiologies (rare variants with a large effect) and common risk factors (common variants with a modest effect). We are now searching for other monogenic but common causes of TB taking advantage of our unique cohort of TB patients we have collected.

All our findings pave the way for a novel and paradigm-shifting approach to the rational design of novel immune system-restoring treatments of TB, fundamentally different from current vaccines and antibiotics. The basic concept is to restore a partially deficient immunity in some “predisposed’ subjects at the level of most infected ‘resistant” individuals who are naturally protected against the development of clinical TB. These novel immune system-restoring treatments of TB are of major interest to complement classical antibiotic treatments in the present context of an increasing number

of MDR and XDR Mtb strains with some of them being totally drug-resistant (TDR). The best example of these treatments is provided by injections of recombinant IFN-γ that be should beneficial in patients with impaired production of IFN-γ[42,43] such as patients identified in this project who are homozygous for TYK2 P1104A, or deficient inPD-1, T-bet, or IFN-γ.

References

1. Dheda K, Barry CE, 3rd, Maartens G. Tuberculosis. *Lancet* 2016; **387**(10024): 1211-26.
2. WHO. Global tuberculosis report. *Geneva* 2016; **World Health Organization**.
3. O'Garra A, Redford PS, McNab FW, Bloom CI, Wilkinson RJ, Berry MP. The immune response in tuberculosis. *Annu Rev Immunol* 2013; **31**: 475-527.
4. Zumla A, Raviglione M, Hafner R, von Reyn CF. Tuberculosis. *N Engl J Med* 2013; **368**(8): 745-55.
5. Alcais A, Fieschi C, Abel L, Casanova JL. Tuberculosis in children and adults: two distinct genetic diseases. *J Exp Med* 2005; **202**(12): 1617-21.
6. Cruz AT, Starke JR. Clinical manifestations of tuberculosis in children. *Paediatr Respir Rev* 2007; **8**(2): 107-17.
7. Marais BJ, Gie RP, Schaaf HS, Beyers N, Donald PR, Starke JR. Childhood pulmonary tuberculosis: old wisdom and new challenges. *Am J Respir Crit Care Med* 2006; **173**(10): 1078-90.
8. Casanova JL, Abel L. The genetic theory of infectious diseases: a brief history and selected illustrations. *Annu Rev Genomics Hum Genet* 2013; **14**: 215-43.
9. Puffer R. Familial susceptibility to tuberculosis; Its importance as a public health problem. Cambridge, MA: Harvard University Press; 1944.
10. Fortin A, Abel L, Casanova JL, Gros P. Host genetics of mycobacterial diseases in mice and men: forward genetic studies of BCG-osis and tuberculosis. *Annu Rev Genomics Hum Genet* 2007; **8**: 163-92.
11. Abel L, El-Baghdadi J, Bousfiha AA, Casanova JL, Schurr E. Human genetics of tuberculosis: a long and winding road. *Philos Trans R Soc Lond B Biol Sci* 2014; **369**(1645): 20130428.
12. Boisson-Dupuis S, Bustamante J, El-Baghdadi J, et al. Inherited and acquired immunodeficiencies underlying tuberculosis in childhood. *Immunol Rev* 2015; **264**(1): 103-20.
13. Boisson-Dupuis S. The monogenic basis of human tuberculosis. *Hum Genet* 2020.
14. Bustamante J. Mendelian susceptibility to mycobacterial disease: recent discoveries. *Hum Genet* 2020.
15. Boisson-Dupuis S, El Baghdadi J, Parvaneh N, et al. IL-12Rb1 deficiency in two of fifty children with severe tuberculosis from Iran, Morocco, and Turkey. *PLoS One* 2011; **6**(4): e18524.
16. Caragol I, Raspall M, Fieschi C, et al. Clinical tuberculosis in 2 of 3 siblings with interleukin-12 receptor beta1 deficiency. *Clin Infect Dis* 2003; **37**(2): 302-6.
17. Özbek N, Fieschi C, Yilmaz BT, et al. Interleukin-12 receptor beta 1 chain deficiency in a child with disseminated tuberculosis. *Clin Infect Dis* 2005; **40**(6): e55-8.
18. Altare F, Ensser A, Breiman A, et al. Interleukin-12 receptor beta1 deficiency in a patient with abdominal tuberculosis. *J Infect Dis* 2001; **184**(2): 231-6.
19. Tabarsi P, Marjani M, Mansouri N, et al. Lethal tuberculosis in a previously healthy adult with IL-12 receptor deficiency. *J Clin Immunol* 2011; **31**(4): 537-9.
20. Boisson-Dupuis S, Bustamante J, El-Baghdadi J, et al. Inherited and acquired immunodeficiencies underlying tuberculosis in childhood. *Immunol Rev* 2015; **264**(1): 103-20.
21. Kreins AY, Ciancanelli MJ, Okada S, et al. Human TYK2 deficiency: Mycobacterial and viral infections without hyper-IgE syndrome. *J Exp Med* 2015; **212**(10): 1641-62.
22. Casanova JL, Holland SM, Notarangelo LD. Inborn Errors of Human JAKs & STATs. *Immunity* 2012; **36**(4): 518-28.
23. Ihle JN, Kerr IM. Jaks and Stats in signaling by the cytokine receptor superfamily. *Trends in genetics : TIG* 1995; **11**(2): 69-74.
24. Leonard WJ, O'Shea JJ. Jaks and STATs: biological implications. *Annual review of immunology* 1998; **16**: 293-322.
25. Minegishi Y, Saito M, Morio T, et al. Human tyrosine kinase 2 deficiency reveals its requisite roles in multiple cytokine signals involved in innate and acquired immunity. *Immunity* 2006; **25**(5): 745-55.

26. Kilic SS, Hacimustafaoglu M, Boisson-Dupuis S, et al. A patient with tyrosine kinase 2 deficiency without hyper-IgE syndrome. *J Pediatr* 2012; **160**(6): 1055-7.
27. Wu P, Chen S, Wu B, Chen J, Lv G. A TYK2 Gene Mutation c.2395G>A Leads to TYK2 Deficiency: A Case Report and Literature Review. *Front Pediatr* 2020; **8**: 253.
28. Boisson-Dupuis S, Ramirez-Alejo N, Li Z, et al. Tuberculosis and impaired IL-23-dependent IFN-gamma immunity in humans homozygous for a common TYK2 missense variant. *Sci Immunol* 2018; **3**(30).
29. Kerner G, Ramirez-Alejo N, Seeleuthner Y, et al. Homozygosity for TYK2 P1104A underlies tuberculosis in about 1% of patients in a cohort of European ancestry. *Proc Natl Acad Sci U S A* 2019; **116**(21): 10430-4.
30. Chamoto K, Al-Habsi M, Honjo T. Role of PD-1 in Immunity and Diseases. *Curr Top Microbiol Immunol* 2017; **410**: 75-97.
31. Sharpe AH, Pauken KE. The diverse functions of the PD1 inhibitory pathway. *Nat Rev Immunol* 2018; **18**(3): 153-67.
32. Nishimura H, Nose M, Hiai H, Minato N, Honjo T. Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. *Immunity* 1999; **11**(2): 141-51.
33. Nishimura H, Okazaki T, Tanaka Y, et al. Autoimmune dilated cardiomyopathy in PD-1 receptor-deficient mice. *Science* 2001; **291**(5502): 319-22.
34. Wang J, Okazaki IM, Yoshida T, et al. PD-1 deficiency results in the development of fatal myocarditis in MRL mice. *Int Immunol* 2010; **22**(6): 443-52.
35. Lazar-Molnar E, Chen B, Sweeney KA, et al. Programmed death-1 (PD-1)-deficient mice are extraordinarily sensitive to tuberculosis. *Proc Natl Acad Sci U S A* 2010; **107**(30): 13402-7.
36. Tousif S, Singh Y, Prasad DV, Sharma P, Van Kaer L, Das G. T cells from Programmed Death-1 deficient mice respond poorly to Mycobacterium tuberculosis infection. *PLoS One* 2011; **6**(5): e19864.
37. Barber DL, Mayer-Barber KD, Feng CG, Sharpe AH, Sher A. CD4 T cells promote rather than control tuberculosis in the absence of PD-1-mediated inhibition. *J Immunol* 2011; **186**(3): 1598-607.
38. Jensen KH, Persson G, Bondgaard AL, Pohl M. Development of pulmonary tuberculosis following treatment with anti-PD-1 for non-small cell lung cancer. *Acta Oncol* 2018; **57**(8): 1127-8.
39. Picchi H, Mateus C, Chouaid C, et al. Infectious complications associated with the use of immune checkpoint inhibitors in oncology: reactivation of tuberculosis after anti PD-1 treatment. *Clin Microbiol Infect* 2018; **24**(3): 216-8.
40. Barber DL, Sakai S, Kudchadkar RR, et al. Tuberculosis following PD-1 blockade for cancer immunotherapy. *Sci Transl Med* 2019; **11**(475).
41. Iwai Y, Hamanishi J, Chamoto K, Honjo T. Cancer immunotherapies targeting the PD-1 signaling pathway. *J Biomed Sci* 2017; **24**(1): 26.
42. Alangari AA, Al-Zamil F, Al-Mazrou A, et al. Treatment of disseminated mycobacterial infection with high-dose IFN-gamma in a patient with IL-12Rbeta1 deficiency. *Clin Dev Immunol* 2011; **2011**: 691956.
43. Holland SM. Immunotherapy of mycobacterial infections. *Semin Respir Infect* 2001; **16**(1): 47-59.