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Clinical Immunology 235 (2022) 108932

Contents lists available at ScienceDirect

Clinical Immunology

journal homepage: www.elsevier.com/locate/yclim

Lessons learned from the diagnostic work-up of a patient with the bare lymphocyte syndrome type II

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ARTICLE INFO

Keywords:
Primary immunodeficiency
MHC class II deficiency
Diagnosis

ABSTRACT

A patient presented severe combined immunodeficiency (SCID)-like symptoms. The presence of a substantial number of $CD4^+$ T-cells in the peripheral blood was not explained by maternal engraftment. Genetic analysis revealed a novel *RFXANK* mutation, c.232C > T, resulting in a stop codon, with consequently defective transcription of MHC class II resulting in bare lymphocyte syndrome (BLS) type II. The initial unawareness of complete absence of MHC class II expression and normal T-cell receptor excision circles (TREC)-levels delayed the final diagnosis. After identification of the genetic defect the patient was scheduled for hematopoietic stem cell transplantation (HSCT). Here, we present and discuss the diagnostic and therapeutic approach of a novel case of BLS type II in relation to T-cell development.

1. Introduction

The bare lymphocyte syndrome (BLS) has first been described in 1978 by Touraine et al. [1]. This involved a patient lacking major histocompatibility complex (MHC) class I expression, being referred to as BLS type I. Next, also cases deficient in MHC class II expression, *i.e.*, BLS type II, were described [2]. While these patients lack MHC class II expression, genetic analyses have revealed that the underlying cause of the disease involves defects in either one of the four transcription factors that regulate MHC class II expression. These factors include class II transcription activator (CIITA), regulatory factor X (RFX)-5, RFX associated protein (RFX-AP), and RFX associated protein containing ankyrin repeats (RFX-ANK) [3]. Mutations in the genes are located on autosomal chromosomes and have a recessive mode of inheritance.

MHC class II expression is essential for activation of $CD4^+$ T-cells, consequently, BLS type II is characterized by repeated and often severe infections that may also be of opportunistic nature due to inadequate cellular and subsequent humoral immune responses. Since the clinical manifestations are generally less profound than in patients with severe combined immunodeficiency (SCID), BLS type II is defined by the International Union of Immunological Societies (IUIS) as combined immunodeficiency (CID) [4]. Next to activation of $CD4^+$ T-cells, MHC class II molecules are important in the positive selection of these cells in the thymus. Therefore, it may be anticipated that patients with BLS type II lack circulating $CD4^+$ T-cells. Although $CD4^+$ T-cell counts have been reported to be reduced in these patients, reflecting abnormal selection and maturation, the phenotype and function of residual $CD4^+$ T-cells have been reported to be largely normal, although obviously lacking MHC class II expression [2].

In this paper we describe a patient, clinically suspected of a severe immunodeficiency, that was eventually identified as having BLS type II. Genetic analysis revealed a homozygous mutation in the *RFXANK* gene, resulting in a stop codon and defective MHC class II expression. Reconstitution of immunity after hematopoietic stem cell transplantation (HSCT) is discussed.

2. Case report

A young boy, 5 months of age, was admitted to the pediatric intensive care unit with respiratory insufficiency and diagnosed with

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https://doi.org/10.1016/j.clim.2022.108932
Received 19 September 2021; Accepted 15 January 2022
Available online 19 January 2022

Pneumocystis jirovecii pneumonia (PCP), requiring supplemental oxygen with high-flow nasal canula. He was treated with a combination of high dose co-trimoxazole (20/100 mg/kg/day) and additional prednisolone (2 mg/kg/day). His past medical history showed diarrhea, failure to thrive, and rhinovirus infection. The boy was born term, but with low weight for gestational age. His parents were from Afghanistan and consanguineous. He received the first vaccinations according to the Dutch national vaccination program, not including any life-attenuated vaccines, without any complications.

The clinical presentation was highly suspicious for a severe immunodeficiency. Therefore, immunological screening for immunoglobulins and lymphocytes was performed. In addition, infectious workup revealed negative results for human immunodeficiency virus (HIV), herpes simplex virus (HSV), varicella zoster virus (VZV), and Epstein-Barr virus (EBV). IgG antibodies (probably of maternal origin) to cytomegalovirus (CMV) were found in the absence of IgM antibodies or viral DNA.

After intensive care recovery intravenous immunoglobulin (IVIG) supplementation was given (500 mg/kg) as well as co-trimoxazole in a prophylactic dose (4/20 mg/kg/day). One month after admission, the patient presented with rhinovirus and norovirus infection. At the same time, throat and perineum cultures were tested positive for *Candida albicans*. The patient seemed unable to efficiently clear these infections.

3. Diagnostics

The diagnostic work-up for SCID included analysis of serum immunoglobulins, immune phenotyping of lymphocyte subsets, and genetic analyses for mutations in relevant target genes.

Immunoglobulin levels were slightly elevated as compared to age-dependent upper reference values (IgG 12.8 g/L [<11.3]; IgA 1.73 g/L [<0.90]; IgM 1.97 g/L [<1.20]). The hypergammaglobulinemia involved mostly IgG1 (11.4 g/L [<7.0]) and had a polyclonal appearance on serum protein electrophoresis. Responses to *Streptococcus pneumonia* conjugate (10 serotypes) vaccine were insufficient.

Immunophenotyping showed NK-cells (134 cells/μL; normal 100–1000) and B-cells (1234 cells/μL; normal 700–2500) to be within normal ranges. However, the percentage of isotype switched memory B-cells was rather low (1.5%). Interestingly, absolute numbers of both $CD4^+$ T-cells (506 cells/μL; normal 1400–5100) and $CD8^+$ T-cells (174 cells/μL; normal 600–2200) were low, but not absent. They exhibited a predominantly naïve phenotype, *i.e.*, CD45RA. Maternal engraftment was suspected and made unlikely with a low HLA-DR expression on T-cells and subsequently excluded by short tandem repeat (STR) analysis.

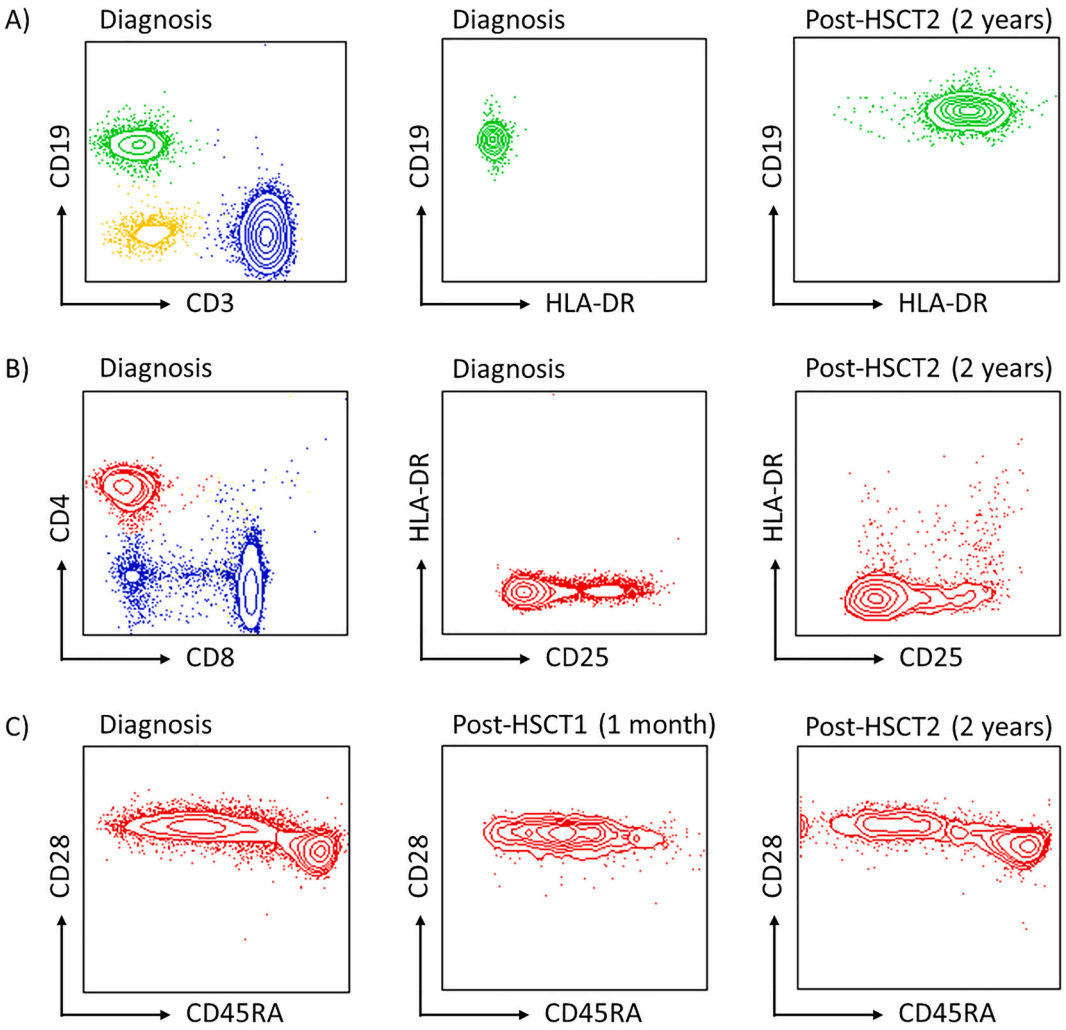

Fig. 1. FACS analysis of lymphocytes at the timepoint of diagnosis and follow-up. A) Lymphocyte populations; B-cells (green), T-cells (blue), and NK-cells (yellow) at the time of diagnosis (left). HLA-Dr expression on B-cells was absent at the time of diagnosis (middle) and present 2 years after the second HSCT (right). B) T-cells are gated into $CD4^+$ (red) and $CD4^-$ (blue) (left). HLA-Dr expression on $CD4^+$ T-cells was absent at the time of diagnosis (middle) and present 2 years after the second HSCT (right). C) Naïve and memory $CD4^+$ T-cell populations at the time of diagnosis (left). Naïve $CD4^+$ T-cells were almost absent 1 month after the first HSCT (middle), but clearly present 2 years after the second HSCT (right). HSCT; hematopoietic stem cell transplantation.

T-cells were polyclonal using *TCRB* gene scan analysis, and analysis of T-cell receptor excision circles (TREC) on peripheral blood was positive.

Revision of the immunophenotyping results revealed that the MHC class II (HLA-DR) expression on T-cells turned out to be not just 'low' but completely absent. In addition, MHC class II expression on B-cells (Fig. 1) and monocytes was lacking completely. This finding prompted the search for genetic mutations in the transcription factors related to BLS type II. Genetic analysis (PCR and Sanger sequencing) revealed a homozygous mutation in exon 4 of the *RFXANK* gene, substituting cytosine with thymine on position 232 (c.232C > T), resulting in a direct stop codon (p.Arg78X). The same (heterozygous) mutation was present in both parents.

4. Treatment

Treatment options for BLS type II are limited to either HSCT or continuous prophylactic therapy. HSCT outcomes, specifically for BLS type II cases with *RFXANK* mutations, have been described before [5,6]. HSCT in BLS type II is particularly complicated by infections, graft failure, and graft *versus* host disease (GVHD), possibly due to aberrant intra-thymic T-cell selection. Intensive conditioning is needed to eliminate the residual T-cells that may prevent successful engraftment. Additionally, HSCT will not restore MHC class II expression on thymic epithelial cells precluding appropriate $CD4^+$ T-cell development post-transplant. On the other hand, life expectancy on prophylactic therapy only is also limited. The decision was made to prepare the patient for HSCT.

No matched family donor was available, and a mismatched (9/10) unrelated umbilical cord blood donor was found. HSCT was performed at the age of 13 months. The pre-HSCT treatment included intestinal decontamination and a conditioning regimen comprising of anti-thymocyte globulin (2 mg/kg/day, total 8 mg/kg) from day −9 to −6, treosulfan (14 g/m^2, total 52 g/m^2) from day −7 to −5, and fludarabine (30 mg/m^2, total 150 mg/m^2) from day −7 to −3. Following HSCT, a combination of cyclosporine A and prednisolone (1 mg/kg/day for 4 weeks) was given as GVHD prophylaxis. Post-HSCT leukocytes were above 0.5×10^9 cells/L on day +21. The number increased to 3.6×10^9 cells/L (day +31) and predominantly consisted of neutrophils (64%) and monocytes (33%). Lymphocyte numbers remained very low (182 cells/μL). Chimerism analyses showed that all mononuclear cells and granulocytes were of recipient origin.

A second HSCT was performed at the age of 16 months with another mismatched (8/10) unrelated umbilical cord blood graft. Pre-HSCT treatment was similar to the first HSCT with the exception of anti-thymocyte globulin being replaced with alemtuzumab therapy (0.1 mg/kg/day and 0.2 mg/kg/day, total 0.3 mg/kg) on day −10 and − 9. Two months later the patient presented with skin lesions and diarrhea, and he was still Norovirus positive. GVHD of the skin and gut was pathologically confirmed. The acute and chronic GVHD was treated with a combination of rapamycin, steroids, and eventually also with imatinib.

Follow-up immunophenotyping revealed a total T-cell count of 222 cells/μL 4 months post-HSCT, increasing to 1300 cells/μL one month later. T-cells consisted primarily out of $CD4^+$ T-cells (176 cells/μL and 1170 cells/μL) and to a far lesser extent out of $CD8^+$ T-cells (22 cells/μL and 132 cells/μL) at 4 and 5 months post-HSCT, respectively. T-cell chimerism after the second transplantation was 100% donor type. Both $CD4^+$ and $CD8^+$ T-cells had a memory phenotype suggestive of low thymic output. In line with the chimerism, the MHC class II defect was restored in the leukocytes since B-cells and monocytes expressed a normal level of HLA-DR. Two years post-HSCT the $CD4^+$ T-cell compartment expanded to 2359 cells/μL and contained a substantial population of naïve $CD4^+$ T-cells (54%). The T-cell reconstitution was in the first period after HSCT slow resulting in clear immune dysregulation with severe GVHD and a mild Coombs positive hemolytic anemia. As time progressed the lymphocyte phenotype normalized and the GVHD resolved.

5. Discussion

In the current report, we have described a patient who presented with SCID-like clinical manifestations. Further immunophenotyping and genetic analyses, however, revealed a novel mutation in *RFXANK* and a diagnosis of BLS type II. For these patients, HSCT is the only curative treatment to date. However, HSCT success rates are relatively low and immune recovery is usually slow [5–9]. Since early detection and subsequent treatment is associated with improved survival outcome [7,10]. We discuss the respective diagnostic and therapeutic process in this case.

Our patient was clinically suspected of a form of severe immunodeficiency with reduced T-cell numbers. The combination of high CD45RA expression and absent MHC class II expression on T-cells was interpreted as absence of maternal activated T-cells, as also confirmed by STR analysis. However, initially the complete lack of MHC class II expression remained unnoticed. This would not have been prevented by novel flow cytometry protocols for diagnostic screening of PID of the lymphoid system, *i.e.*, the EuroFlow PID orientation tube, since this protocol does not include HLA-DR detection [11]. However, using the EuroFlow SCID-RTE tube, which is used in diagnostics for children with a suspicion for a severe PID, it would have been identified [12]. In combination with substantial numbers of circulating $CD4^+$ T-cells, this resulted in a delayed diagnosis of BLS type II and infectious complications. The diagnostic delay could have been reduced by a "genetics-first" approach [13], currently becoming common practice in the Netherlands. Indeed, a broad genetic analysis by next generation sequencing would have accelerated the finding of the *RFXANK* mutation.

Next to early genetic analysis, also newborn screening for (S)CID may result in an early diagnosis before infections manifest. Newborn screening for PID involving TREC analyses is clinically effective for the detection of SCID and severe T-cell lymphopenia and is now incorporated in national newborn screening programs of multiple countries [13]. In the Netherlands this has started just recently (January 2021) [14,15]. However, in our patient substantial amounts of TREC were measured during the diagnostic work-up. This is in line with previously reported cases [16]. However, there is large heterogeneity with respect to the amount of residual $CD4^+$ T-cells in patients with CID, and even in patients with BLS type II [5,17]. Therefore, normal TREC-levels in newborn screening does not exclude BLS type II.

The patient of this case report presented with reduced, but substantial amounts of $CD4^+$ T-cells and delayed reconstitution of $CD4^+$ T-cells after HSCT. These phenomena have been reported in some, but not all BLS type II patients [2,5,6,18]. MHC class II expression on thymic epithelial cells is considered essential for the generation of $CD4^+$ T-cells. Therefore, it is unexpected that BLS type II patients sometimes present with a substantial amount of autologous $CD4^+$ T-cells. In addition, the absence of MHC class II expression on thymic epithelial cells will not be restored by HSCT. Hence, reconstitution of the $CD4^+$ T-cell compartment would still be impaired after HSCT. These findings question the regulation of $CD4^+$ T-cell development, thymic output and MHC class II expression on thymic epithelial cells. It has been established for CIITA that distinct promoter regions are differentially used in different cell types [19]. Therefore, it might be anticipated that this has an effect on the obligatory role of other transcription factors, including RFX-ANK, in MHC class II expression. In particular after HSCT, migration of allogeneic MHC class II expressing cells into the thymus could drive positive selection, eventually resulting in the generation of $CD4^+$ T-cells. These mechanisms need to be further explored, for instance by using thymic organ cultures originating from cells of BLS type II patients.

The delay in T-cell reconstitution post-HSCT poses great challenges and greatly affects outcome. Due to the substantial amounts of residual T-cells in patients with BLS type II, pre-HSCT treatment requires conditioning regimens to sufficiently deplete T-cells in order to optimize engraftment of allogeneic stem cells. Serotherapy and treosulfan based conditioning regimes have been used successfully [20]. Although such

conditioning regimens deplete autologous T-cells, they may also affect T-cell reconstitution post-HSCT [21,22]. Particularly, the timing and dosing of the serotherapy remains important for successful T-cell reconstitution. Moreover, treatments interfering with the IL-2 – IL-2R pathway [23], *i.e.*, cyclosporine A and rapamycin, were given for GVHD prophylaxis and treatment, respectively. These drugs not only prevent T-cell proliferation, but also hamper thymocyte maturation and appropriate selection [24,25]. Due to defective negative selection in the thymus, there is a relative increase in autoreactive T-cells that may eventually cause autoimmune phenomena [26,27]. This may have led to the extensive chronic GVHD and the observed Coombs positive hemolytic anemia in our patient. Finally, also corticosteroids treatment will further reduce good thymus function and subsequent T-cell reconstitution [28]. Strategies that could accelerate T-cell reconstitution would benefit clinical outcome. In case of BLS type II, defective RFX-ANK expression in for instance thymic organoids could potentially be restored by transfection with the *RFXANK* gene. Such add on cell therapy may be further explored in a HSCT setting where immune restoration is hampered by poor thymic function.

6. Conclusion

We have described a patient with a novel *RFXANK* mutation resulting in BLS type II. The patient presented with repeated severe opportunistic infections despite having substantial amounts of residual peripheral blood $CD4^+$ T-cells. The flow cytometry protocol we used, did not adequately show the lack of MHC class II expression because only T-cells were analyzed for HLA-DR expression in light of activation due to maternal engraftment. Eventually, a second HSCT engrafted and despite severe chronic GVHD the patient fully recovered. BLS type II questions the role of RFX-ANK, and possibly the other related transcription factors, in the expression of MHC class II on thymic epithelial cells in combination with $CD4^+$ T-cell reconstitution after HSCT.

Ethics

Informed consent was obtained from the parents of the presented patient for the anonymous publication of this case report.

Conflict of interest statement

None of the authors has any potential financial conflict of interest related to this manuscript.

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