MAGT1 gene mutation causes myositis and CD127 expression downregulation

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Abstract

X-linked immunodeficiency with Magnesium defect, Epstein-Barr virus infection, and Neoplasia (XMEN) is a primary immune deficiency caused by mutations in MAGT1 and is characterized by chronic infection with Epstein-Barr virus (EBV), CD4 lymphopenia, severe chronic viral infections, and defective T-lymphocyte activation. Herein, we described an XMEN patient carrying a MAGT1 p.Arg331* mutation that presented with prominent muscle involvement. The PET-CT and lower limbs MRI showed diffuse muscle inflammation. Histopathology staining revealed the infiltrated lymphocytes were stained with CD3, CD20, and EBER. We monitored the patient's in-depth immunophenotype at the first visit and after two months of oral Mg 2+ supplementation. We noticed obviously decreased CD127 expression in CD4 + T cells, which could be reversed by Mg 2+ supplementation. Our findings suggested that the decreased CD127 expression may be an additional biomarker of XMEN.

Introduction

X-linked immunodeficiency with Magnesium defect, Epstein-Barr virus infection, and Neoplasia (XMEN) is a primary immune deficiency caused by mutations in *MAGT1* and is characterized by chronic infection with Epstein-Barr virus (EBV), CD4 lymphopenia, severe chronic viral infections, and defective T-lymphocyte activation [1]. The clinical spectrum of XMEN was described as recurrent infections by viruses, lymphoproliferative disease, thrombocytopenia, and hemolytic anemia.

MAGT1 encodes a magnesium transporter protein 1 (MAGT1) which was reported to be involved in Mg^{2+} transport in vertebrate cells [2]. A previous study indicated that the stimulation of the TCR in normal T cells triggers a transient increase of intracellular Mg^{2+} through MAGT1. This transient Mg^{2+} flux is essential for the activation of phospholipase C g1 (PLCg1) required for the downstream generation of Ca^{2+} flux by promoting the release of Ca^{2+} from the endoplasmic reticulum (ER) via inositol 1,4,5-triphosphate (IP3) and its receptor [3]. Early studies on XMEN patients suggested that the loss-of-function (LOF) mutations in *MAGT1* result in dysfunction of this process [1], decrease the NKG2D expression, and consequently NK and $CD8^+$ T-cell cytotoxicity [4].

Recently, MAGT1 had been reported to share up to 66% amino acid sequence similarity to the human Tumor Suppressor Candidate 3 protein (TUSC3), and act as a subunit of the STT3B isoform of the N-oligosaccharyltransferase (OST) complex [2, 5]. Thus, either MAGT1 or TUSC3 can facilitate the asparagine (N)-linked glycosylation (NLG) of a subset of STT3B-dependent glycoproteins in a mutually exclusive way [6]. The increased expression of TUSC3 in cells devoid of MAGT1, except for lymphocytes, was able to rescue the glycosylation defect [7, 8]. The key pathogenesis of XMEN may revolve around defective glycosylation in addition to or even instead of the alterations of Mg^{2+} transport.

Herein, we described a patient presenting with recurrent muscle weakness, symptomatic epilepsy, and chronic infection with Epstein-Barr virus (EBV) while carrying the same mutation (*MAGT1* p.Arg331*) previously reported in XMEN [9] and *MAGT1* associated congenital disorders of glycosylation (CDG) [7].

In brief, we demonstrated that the function of the NK and $CD8^+$T cell cytotoxicity in the patient was impaired and the lesions in his muscles were EBV infiltrated. Furthermore, the CD127 expression in his $CD4^+$T cells was decreased and oral Mg^{2+} supplementation did increase the CD127 expression but not ameliorated his symptoms.

Materials And Methods

Ethical approval

This study was approved by the Ethics Committee of Huashan Hospital of Fudan University. Written informed consent was signed by each participant before inclusion in the study.

Muscle Histopathology

Muscle biopsy was obtained from vastus lateralis by using standard technique. Frozen sections were stained by hematoxylin-eosin, Modified Gomori's trichrome, NADH-tetrazolium reductase reaction, Cytochrome C oxidase, Periodic acid Schiff. The following primary antibodies were used for immunohistochemistry with standard procedures: CD3, CD4, CD8, CD20, CD68, MAC, MHC-I. A normal control was labeled at the same magnification and exposure to allow direct comparison between the patient and control sections.

Exome sequencing and analysis.

Genomic DNA was isolated from the patient's and healthy controls' peripheral blood mononuclear cells (PBMCs) using a QIAamp DNA blood mini kit (Qiagen). Targeted exome sequencing was executed on the Illumina HiSeq 2000 platform according to previous description [10, 11]. The resulting reads were mapped to the reference genome hg19 and variants were further evaluated according to the American College of Medical Genetics and Genomics (ACMG) guidelines [12].

RNA extraction and PCR analysis

Total RNAs were extracted from peripheral blood mononuclear cells (PBMCs) and muscle cells using RNAiso Plus (Takara) according to the manufacturer's instructions. The cDNAs were synthesized using the PrimeScript™ II 1st Strand cDNA Synthesis Kit (Takara). Our in-house primers were designed for reverse-transcription Polymerase Chain Reaction (RT-PCR) amplifying, Sanger sequencing, and quantification real-time PCR (qRT-PCR) (*MAGT1*-term-F: TGCTTGCTATGACATCTGGTC, *MAGT1*-term-R: CTCATCAGAAAGCTGTATGGG). The PCR products for *MAGT1* spanned the mutation site. RT-PCR products were further examined by 1.5% agarose gel electrophoresis. The relative expression in qRT-PCR was determined with the $2^{-\Delta\Delta CT}$ method.

Flow cytometry

For flow cytometry, 100μL of the subject's EDTA anticoagulated blood was stained with the appropriate mixture of fluorescein-conjugated antibodies (detailed multi-color panels were listed in the **Supplementary Table 1**), and incubated at routine temperature for 20 minutes. Appropriate fluorescein-conjugated, isotype-matched, irrelevant mAbs were used as negative controls. Cells were then treated with Red Blood Cell lysis buffer (TIANGEN) for 5 minutes and washed twice in phosphate buffered saline (PBS). Cells were subsequently mounted on the Attune NxT Flow Cytometer (Thermo Fisher Scientific) and analyzed by the FlowJo V10.5.3 Software (FlowJo, LLC).

TCR-induced Mg^{2+} influx detection

The TCR-induced Mg^{2+} influx detection was performed using MagFluo4-AM probe (Invitrogen) according to a previous report [1]. Briefly, the PBMCs isolated using the Ficoll method were loaded with 1μM MagFluo4-AM and stained with the appropriate volume of anti-CD4/CD8a. The mixture was incubated at 37℃ for 20 minutes. Then, cells were washed in incubation buffer (120 mM N-methyl-D-glucamine, 20 mM HEPES, 4.7 mM KCl, 1.2 mM, KH2PO4, 10 mM glucose, pH 7.4). The Mg^{2+} influx was detected using a CytoFLEX flow cytometer (Beckman Coulter). For TCR stimulation, 5μg/mL anti-CD3 was added in the reaction. The loading efficiency was assessed later by adding 1 mM calcimycin. Kinetic analyses were performed using the FlowJo V10.5.3 Software (FlowJo, LLC), with the percentage of responding cells defined as 95th percentile of unstimulated baseline.

Statistics

Statistical analyses were performed in GraphPad Prism 8.0.1. The Mann-Whitney test was used to compare control versus patient samples. $P < 0.05$ was considered significant.

Results

Clinical Manifestation

Our patient was originally referred to the Department of Rheumatology at the age of 24 year of age, with 6-year intermittent weakness of lower limbs and 4-year epilepsy period of attacks. In his childhood, he had mild developmental delay on language and sports, and had several fevers stemming from upper respiratory tract infections, requiring no hospital admission. At 18 years old, he suddenly felt the weakness of lower limbs during a run without any aura and loss of consciousness for the first time, with recovery after 10 minutes. The symptom relapsed 1-3 times every year and there was no movement disorder in the inter-ictal phase. At 20 years old, he began to suffer from epileptic seizures once each year. After his fourth seizure at 24-years of age, the symptoms became more noticeable. His weakness of lower limbs began to aggravate with longer-lasting (15-30 minutes) and more frequent attacks. Before being admitted to the hospital, he was suffering from uncontrolled seizures every day for nearly two weeks.

He was found to have mild neutropenia and thrombocytopenia for more than three years. Clinical evaluation of the patient was initially performed by a physician, and no specific noticeable body

examinations were found, including normal myodynamia and muscular tension. Initial investigations revealed markedly elevated creatine kinase (1540U/L). Serology testing for antibodies to viruses were positive for an EBV IgG, and later viral DNA detection confirmed a high EBV-DNA load (7.93×10^6 copies/ml). An antinuclear antibodies profile and myositis-specific autoantibodies were negative. Electromyography (EMG) revealed myopathic changes that were most pronounced in both arms and legs. The PET-CT showed widespread adenopathy and skeletal muscle with avid fluorodeoxyglucose (FDG) uptake (**Figure 1A a**). Lower limbs MRI confirmed diffuse muscle inflammation (**Figure 1A b&c**). IVIG and a low dose of steroids were attempted to ameliorate the inflammation in muscles for less than a week, and creatine kinase descended to 385U/L (normal range). However, three months later, the patient began to suffer sustained muscle weakness and had a difficulty walking independently. After admission, ultrasound examination suggested generalized superficial lymphadenopathy. A muscle biopsy was performed to investigate histological changes. The pathology of muscle suggested that plenty of lymphocytes infiltrated into the endomysium. Histopathology staining revealed the infiltrated lymphocytes were stained with CD3, CD20, and EBER (**Figure 1B**). Bone marrow examination showed normal trilineage hematopoiesis. Considering the patient's clinical and pathological manifestations, a targeted exome sequencing for immune deficiency was conducted.

Mutational Analysis

Targeted exome sequencing using PBMC revealed that the patient was carrying a hemizygous loss-of-function mutation in the exon 8 of *MAGT1*: c.991C>T, p.Arg331*, while his mother was normal (**Figure 1C**). RT-PCR amplifying was performed in the PBMCs and muscles. Unsurprisingly, the *MAGT1* expression was significantly decreased in the patient's PBMCs, as well as in his muscles (**Figure 1D**). Sanger sequencing using the patient's muscle revealed the same single base substitution (**Figure 1E)**. Given the predominant susceptibility of the patient to EBV infection, the patient was then diagnosed as XMEN.

In-depth immunophenotype analysis of the patient

We determined the immunophenotype of the patient. Not surprisingly, the CD4/CD8 ratio decreased to 0.9. However, we found an increased intermediate expression of CD4 in T cells (CD4-int cells) (**Figure 2A**), while the T helper (Th) proportions, including Th1, Th2, Th9, Th17, Th17.1, Th22, follicle T helper (Tfh), and regulatory T (Treg) cells, were in the normal range (**Table 1 and Supplementary Figure 1**). Meanwhile, we noticed a decreased expression of CD127 in $CD4^+$T cells (**Figure 2B**). Moreover, the NKG2D expression was much lower in our patient compared with healthy control both in $CD8^+$CTLs and NK cells (**Figure 2C**).

TCR-induced Mg^{2+} influx in PBMCs was then measured as previously described[1]. The patient's Mg^{2+} influx induced by anti-CD3 in $CD8^+$ cells was not detectable comparing to his mother. Similar results were shown in his $CD4^+$T cells (**Supplementary Figure 2**).

The patient was then prescribed with oral Mg^{2+} supplementation and discharged. In the subsequent 2 months, the patient's muscle weakness did not change but had fewer seizure attacks. The flow cytometry

analysis revealed a decreased number of CD4-int (**Figure 2A**) and $CD4^+CD127^-$cells (**Figure 2B**). Nevertheless, the NKG2D expression remained unchanged both in $CD8^+$CTLs and NK cells (**Figure 2C**).

Discussion

XMEN is a primary immune deficiency caused by mutations in *MAGT1* and characterized by chronic infection with Epstein-Barr virus (EBV), CD4 lymphopenia, severe chronic viral infections, and defective T-lymphocyte activation [1]. In this study, we reported a patient carrying de novo *MAGT1* mutation c.991C>T, which presented with recurrent muscle weakness, symptomatic epilepsy, and chronic infection with Epstein-Barr virus (EBV). This mutation was a known mutation originally associated with CDG [7] and led to premature termination of MAGT1 protein translation. More recently, this mutation was reported in an XMEN patient [9]. A segregation study revealed that the mutation was de novo. RT-PCR and qRT-PCR experiments further indicated that the mutation resulted in a significant decrease in *MAGT1* mRNA expression both in PBMC and muscle. Thus, we classified this mutation as pathogenic for XMEN according to the ACMG guidelines [12].

It is well known that XMEN can present with multi-system abnormalities. A previous study including 23 patients from 17 unrelated families demonstrated that patients may present with recurrent infections, chronic lymphadenopathy (LAD) and EBV-driven lymphoproliferative disease (LPD), splenomegaly, severe autoimmune cytopenias, and even malignancy [9]. Transient and asymptomatic elevations of serum muscle creatine phosphokinase (CPK) were also noted [9]. However, this is the first report with muscle involvement during disease progression. We further demonstrated that the lesions in his muscle was EBER positive. The EBV infection resulted from a severe immune deficiency, which facilitated inflammatory lymphocytes infiltrated into the endomysium. These findings broadened the clinical spectrum of XMEN.

A significant expression CD127 decrease was observed in $CD4^+$T cells. It is known that CD127, which is critical in the maintenance of T cell homeostasis, is expressed on T cells at almost all stages of development. While T cells downregulate CD127 whenever they undergo prominent expansion [13]. Thus, one plausible explanation for our observation was that the downregulation of CD127 may reflect an increased level of immune activation present in those individuals facing viremia [14]. Another explanation may be that the downregulation of CD127 may be due to the feedback regulation of the NLG deficiency in CD127. It was reported that the downregulating N-glycan branching in CD127 may facilitate the enhancement of TCR signaling and activation of peripheral T cells [15]. However, since partially-impaired TCR signaling was observed XMEN, the CD127 expression continuously decreased to maintain the T cell activation. Interestingly, the transcriptome analysis in $CD8^+$T cells from XMEN also indicated the downregulation of CD127, together with other genes associated with immune function, suggesting a common basal transcriptional and functional defect [8] (**Figure 3**). The latter explanation may be supported by the following two aspects: 1) the increasing intermediate expressing of CD4 probably due to the apoptosis of the over-activated T cells; 2) our follow-up CD127 monitoring, which might be reversible because the functional study had revealed that the glycosylation defect was caused by decreasing the

free basal Mg^{2+} levels [8]. After 2-month of oral Mg^{2+} supplementation, we observed that the patient's CD127 expression increased. In contrast, we observed elevated NKG2D expression did not change, suggesting a different pathogenesis.

In conclusion, we described an XMEN patient carrying a *MAGT1* p.Arg331* mutation that presented with prominent muscle involvement for the first time. Histopathology staining revealed the infiltrated lymphocytes were stained with CD3, CD20, and EBER. We monitored the patient's in-depth immunophenotype at the first visit and after two months of oral Mg^{2+} supplementation. We noticed obviously decreased CD127 expression in $CD4^{+}$T cells, which could be reversed by Mg^{2+} supplementation. Our findings suggested that the decreased CD127 expression may be an additional biomarker of XMEN.

Declarations

Acknowledgements

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Conflict of Interest

The authors declare no conflict of interest.

Availability of data and material

Data are available upon reasonable request to the corresponding author.

Authorship Contributions

S.C. and X.W. performed experiments, analyzed results, and drafted manuscript; C.S., B.Y.L., K.Q., J.Y., Y.C., J.Y.X. S.S.L., W.H.Z., C.B.Z. and J.H.L. were responsible for data collection and interpretation; J.L. designed the study and revised manuscript.

Ethics approval

This study was approved by the Ethics Committee of Huashan Hospital Fudan University.

Consent to participate

Written informed consent was signed by each participant before inclusion in the study.

Consent for publication

Informed consent was obtained from the study subjects prior to publication.

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Tables

Table 1. In-depth T cells immunophenotype analysis of the patient.

	Patient	Patient's mother (Healthy Control)
CD4 (% in PBMC)	17.7	30.0
CD8 (% in PBMC)	19.1	16.8
CD4 int (% in PBMC)	31.5	7.8
CD4/CD8 ratio	0.9	1.8
$CD4^+$T Subsets		
Th1	7.6	17.6
Th2	13.1	6.86
Th9	4.7	12.7
Th17	5.5	11.9
Th17.1	1.3	10.0
Th22	0.6	1.6
Tfh	10.7	28.3
$ICOS^+$ (% in Tfh)	3.7	4.0
$ICOS^-$ (% in Tfh)	96.3	96.0
Treg	5.7	5.2
TEMRA	0.7	3.7
TEM	3.9	5.0
TCM	72.1	51.7
TNAIVE	23.3	39.5
$CD8^+$T Subsets		
TEMRA	7.3	17.2
TEM	4.3	1.1
TCM	51.6	37.9
TNAIVE	36.8	43.8

PBMC: peripheral blood mononuclear cell; Th: T helper cell; Tfh: folliclar helper T cell; Treg: regulatory T cell; TEMRA: CD45RA-positive effector memory T cell; TEM: effector memory T cell; TCM: central memory T cell; TNAIVE: naïve T cell.

Figures

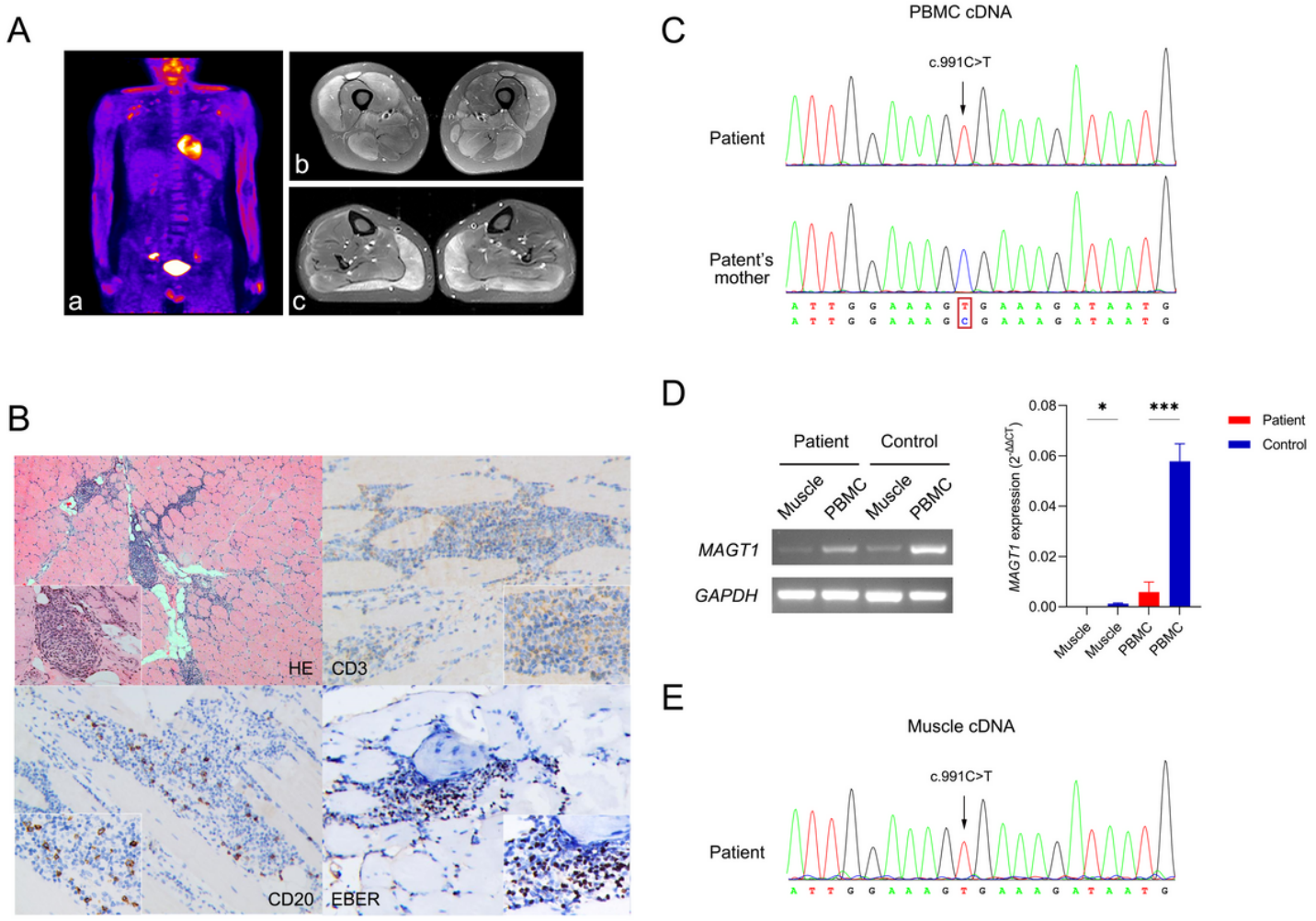

Figure 1

Clinical characteristics and mutational analysis. A. (a) The PET-CT showed widespread adenopathy and skeletal muscle with avid fluorodeoxyglucose (FDG) uptake. (b) T2-STIR (short time inversion recovery) MR of middle thigh revealed edema in the patient's vastus lateralis muscles. (c) T2-STIR MR of middle calf revealed edema in the patient's gastrocnemius muscles. B. Histopathology staining revealed the infiltrated lymphocytes were stained with CD3, CD20, and EBER. C. Sanger sequencing revealed that the patient was carrying a hemizygous loss-of-function mutation in the exon 8 of MAGT1: c.991C>T, p.Arg331*, while his mother was normal. D. RT-PCR and qRT-PCR revealed the MAGT1 expression was significantly decreased in the patient's PBMCs, as well as in his muscles. E. Sanger sequencing using the patient's muscles revealed the same MAGT1 c.991C>T single base substitution.

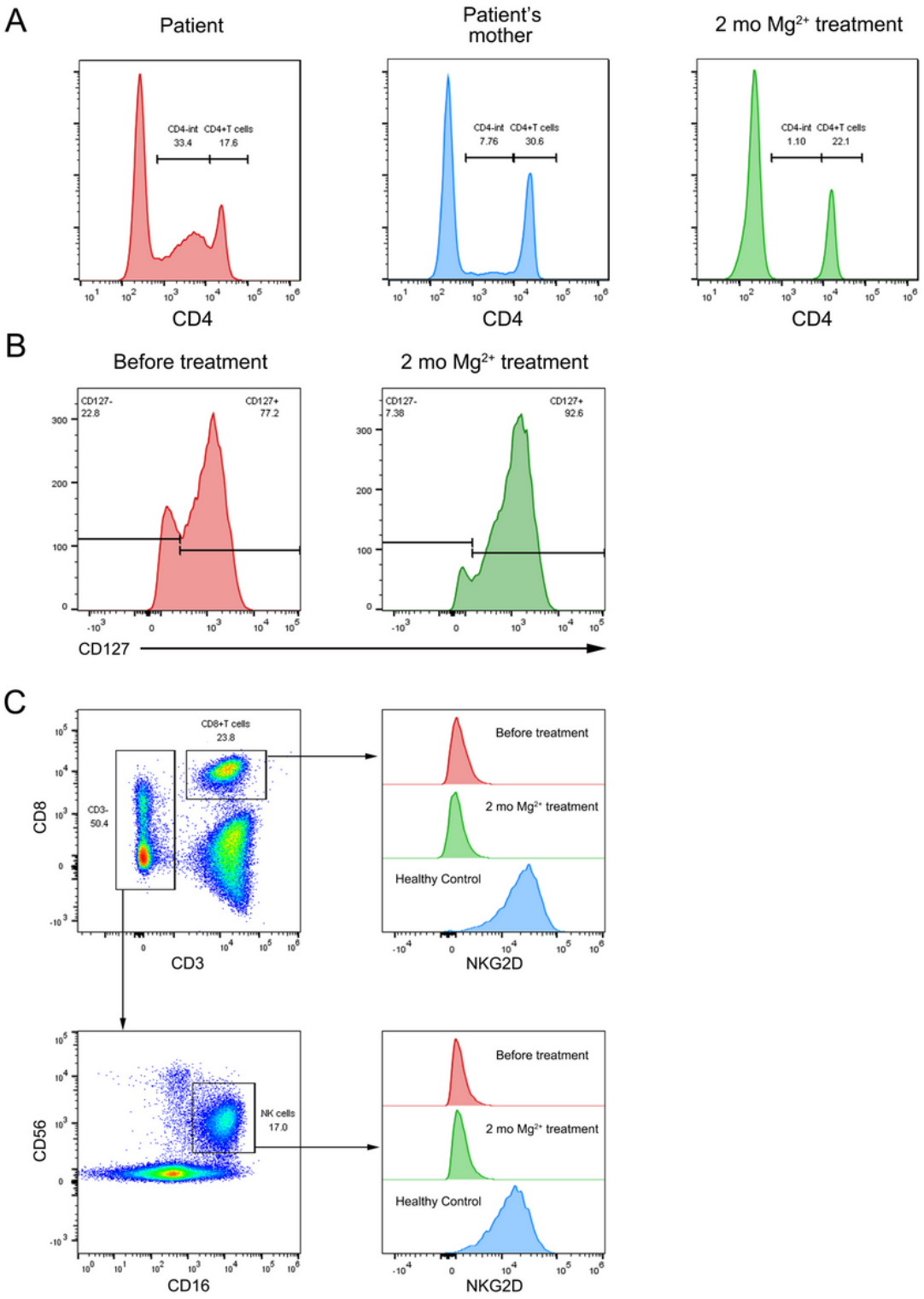

Figure 2

Flowcytometry analysis. A. Flowcytometry analysis revealed an increased intermediate expression of CD4 in T cells (CD4-int cells), which was decreased after 2 months oral Mg2+ supplementation. B. Flowcytometry analysis revealed a decreased expression of CD127 in CD4+T cells, which was increased after 2 months oral Mg2+ supplementation. C. The NKG2D expression was much lower in the patient

compared with healthy control both in CD8+CTLs and NK cells and remained unchanged after 2 months oral Mg2+ supplementation.

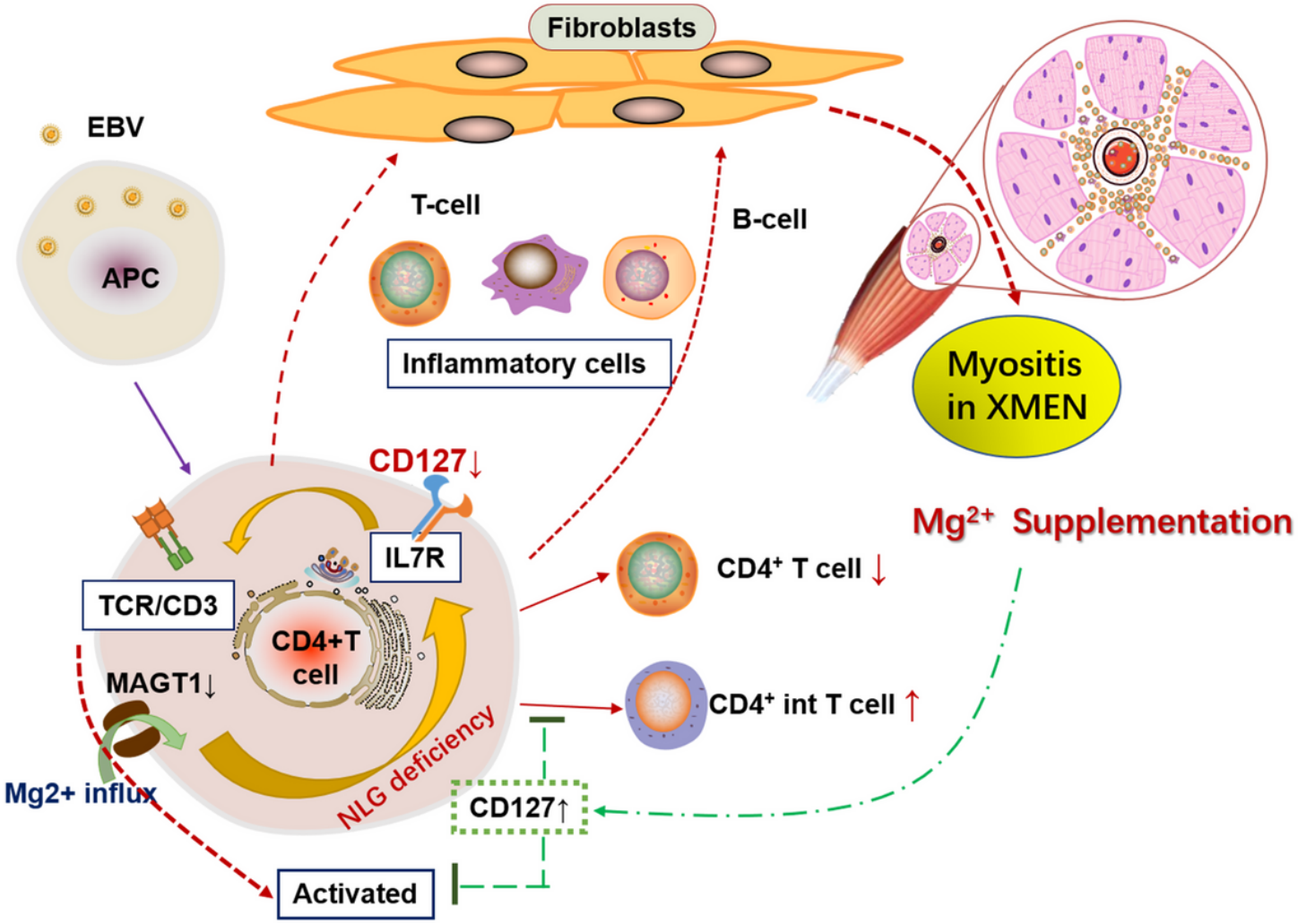

Figure 3

MAGT1 deficiency caused TCR deficiency and downregulation of CD127 in T cells, which facilitated EBV and inflammatory lymphocytes infiltrated into the endomysium.

Supplementary Files

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- SupplementaryFigure1.tif
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- Supplementarytable1.docx