

Published in final edited form as:

Magnes Res. 2011 September ; 24(3): S109–S114. doi:10.1684/mrh.2011.0286.

Loss of MAGT1 abrogates a Mg^{2+} flux required for T cell signaling and leads to a novel human primary immunodeficiency

Feng-Yen Li^{1,2}, Michael J. Lenardo¹, and Benjamin Chaigne-Delalande¹

¹Molecular Development Section, Lymphocyte Molecular Genetics Unit, Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD

²Biomedical Sciences Graduate Program, University of California-San Francisco, San Francisco, CA

Abstract

Although Mg^{2+} has a well-recognized role as an essential cofactor for all ATP-binding enzymes, its role as a signaling ion like Ca^{2+} has been controversial. A requirement for Mg^{2+} for optimal T lymphocyte stimulation was demonstrated more than 30 years ago, but the mechanism of its synergistic effect with Ca^{2+} in T cell activation remains elusive. Here, we summarize our recent discovery of a signaling role for Mg^{2+} in the T cell antigen receptor (TCR) signaling pathway from the study of a novel primary immunodeficiency now named X-linked immunodeficiency with Mg^{2+} defect, EBV infection and neoplasia (XMEN). XMEN patients were found to have a deficiency in Magnesium Transporter 1 (MAGT1), a Mg^{2+} -specific transporter, which leads to the absence of a TCR-stimulated Mg^{2+} flux and attenuation of T cell activation. We further showed that this Mg^{2+} flux is required proximally for the temporal orchestration of phospholipase C- γ 1 (PLC γ 1) activation. Thus, our study not only provides a second messenger role for Mg^{2+} to explain its synergism with calcium in T cell signaling, it also identifies a potential extracellular therapeutic target for T cell-specific immunomodulation.

Introduction

Mg^{2+} is the most abundant divalent cation in mammalian cells. Although, cells exhibit high concentrations of intracellular Mg^{2+} (10-30 mM), most of it is bound to proteins, phospholipids, nucleic acids, and particularly ATP [1-3]. However, the free cytosolic Mg^{2+} (0.2-1 mM) represents only 1-5 % of the total Mg^{2+} , and is actively maintained at 50 to 100-fold lower than its electrochemical equilibrium potential [4]. Unlike Ca^{2+} , the role of Mg^{2+} as a second messenger is still controversial [5]. Nevertheless, within the last thirty years, an increasing number of observations have been reported that implicated Mg^{2+} in various cell functions in response to extracellular stimuli [6, 7]. For example, the role of intracellular free Mg^{2+} in growth factor-induced cell proliferation, originally thought to be strictly a cofactor effect, is now reevaluated as a possible second messenger role [8]. In the immune system, several studies have shown that extracellular Mg^{2+} acts synergistically with Ca^{2+} in T cell activation but not in B cells stimulated with lipopolysaccharide (LPS) [9-11]. Moreover, in suboptimal extracellular concentrations of Ca^{2+} , extracellular Mg^{2+} is able to restore interleukin-2 (IL-2) receptor expression and cell proliferation in T cells stimulated by phytohemagglutinin (PHA) but not by ionomycin [12]. As ionomycin is a Ca^{2+} ionophore that bypasses proximal T cell receptor (TCR) signaling events to induce Ca^{2+} signaling, this result suggests that optimal T cell activation requires a Mg^{2+} -dependent process upstream of

the Ca^{2+} flux induction. In all these cases, the mechanism of action of the Mg^{2+} as a signaling ion remained elusive.

Recently, we reported a novel primary immunodeficiency named X-linked immunodeficiency with magnesium defect, EBV infection and neoplasia (XMEN) [13]. Our work unraveled a signaling role for Mg^{2+} in TCR signaling by identifying the loss of the Mg^{2+} transporter MAGT1 as the cause of this disease. This study demonstrated that TCR stimulation induced a Mg^{2+} flux through MAGT1 that was important for the activation of PLC γ 1 and its downstream effects, including the production of inositol triphosphate (IP3) and the consequent release of Ca^{2+} .

Description of the patients and TCR defect

Our study began with two young boys (patient A.1 and A.2) with a history of recurrent viral infections including chronic active Epstein-Barr virus (EBV) infections. These patients exhibited low CD4^+ T cells counts, leading to an inverted CD4:CD8 ratio. Other lymphocytes populations did not show major disturbances. We first ruled out well-established causes of low CD4^+ T cells counts, like infections with human immunodeficiency virus (HIV) or cytomegalovirus (CMV), Bare-Lymphocyte syndrome, Wiskott-Aldrich syndrome, and Di-George syndrome. Then we assessed the CD31^+ cell population amongst the CD4^+ CD27^+ CD45RO^- naïve T cells and found this population reduced in the patients compared to normal controls, indicating a diminished thymic output of CD4^+ T cells.

We assessed the function of the T cells by testing the ability of the patients' T cells to normally activate upon TCR engagement using an agonist anti-CD3 antibody (OKT3). We found that patients' T cells exhibited defective induction of several activation markers, such as CD69, CD25, Fas (CD95) and CTLA-4, in response to TCR stimulation (figure 1A). Looking at earlier signaling events, we observed that TCR-induced translocation of essential transcription factors, such as NF- κ B and NFAT, were impaired as well in the patients' cells (figure 1B). However, these defects were rescued by using the second messenger inducers phorbol 12-myristate 13-acetate (PMA) and ionomycin, showing that the patients had a proximal TCR signaling defect prior to the induction of the Ca^{2+} flux. Unlike T cells, the patients' B cells showed normal activation upon B cell receptor (BCR) stimulation.

Characterization of the genetic defect

As the two patients were boys, we hypothesized that the defect could be X-linked. To assess this possibility we tested for random lyonization or inactivation of each of the mother's X chromosomes [14]. When one of the X chromosomes carries a genetic mutation conferring a selective disadvantage to the cell (e.g. impacting proliferation or survival), then skewed lyonization reflects the loss of cells with an active X chromosome carrying the mutation. Based on that hypothesis, we performed a lyonization assay and showed that the mother's T cells exhibited inactivation of only one of her X chromosomes instead of the expected random inactivation of both X chromosomes. This skewed lyonization led us to search for a genetic defect on the X chromosome. We performed X-chromosome exon-capture and single-end Solexa sequencing on genomic DNA from the mother and the two boys. According to our X-linked genetic model with skewed lyonization in the mother, we hypothesized that the defect should be present in both brothers, heterozygous in the mother and not present in the mother's cDNA. Using these criteria, we initiated the analysis by looking for single nucleotide polymorphisms (SNPs) or 1-2 bp deletion-insertion polymorphisms (DIPs). We did not find any nonsense mutations or 1-2 bp DIPs. DIPs larger than 2 bp are not revealed by single-end Illumina sequencing, so we ruled out large DIPs (>1000 bp) using a comparative genomic hybridization array. For the intermediate

DIPs (3-1000 bp), we searched for missing coverage in the patients but not in the mother by developing a new computational tool. This approach allowed us to identify a 10 bp deletion present in both patients in *MAGT1*, a gene coding for a Mg^{2+} transporter. Consistent with our genetic model, the deletion was not found in the mother's cDNA nor in 100 normal controls. Finally, we confirmed the presence of the deletion by Sanger sequencing in both patients and showed it was heterozygous in the mother's genomic DNA. Moreover, this mutation was also carried by the grandmother and the great-grandmother of the patients. The deletion was located in the 3' exon-intron junction of the exon 7 of *MAGT1* and induced the splicing of exon 6 with exon 9, creating a premature stop codon. This early termination led to the decrease of *MAGT1* mRNA of $\approx 80\%$ by nonsense-mediated decay leading to the loss of MAGT1 protein expression in the patients.

MAGT1 deficiency impairs TCR-induced Mg^{2+} and Ca^{2+} influxes

Little was known about the function of MAGT1 in lymphocytes before our study. However, previous studies in transfected *Xenopus laevis* oocytes and the human embryonic kidney 293T cell line (HEK 293T) showed that MAGT1 induces a highly selective Mg^{2+} uptake with almost no permeability to other cations including Ca^{2+} [15, 16]. Using fluorescent probes for Mg^{2+} (Mag Fluo 4-AM) and Ca^{2+} (Fluo 4-AM), we assessed Mg^{2+} and Ca^{2+} uptake in T cells from the patients and normal controls after addition of 1 mM Ca^{2+} or Mg^{2+} . We observed that the basal level of free Mg^{2+} was decreased in patients' T cells and there was impaired Mg^{2+} uptake, which was consistent with MAGT1 as a Mg^{2+} transporter in lymphocytes. Nevertheless, although the patients' T cells had defective Mg^{2+} uptake, the total amount of Ca^{2+} and Mg^{2+} in purified $CD4^+$ and $CD8^+$ T cells, measured by inductively coupled plasma mass spectrometry, was not different from normal control cells. As the free Mg^{2+} represents only a small fraction of the total cellular Mg^{2+} and the fluorescent probes only detect the free cytosolic Mg^{2+} , the defect we observed was limited to the free Mg^{2+} .

To link the TCR activation defect and the loss of MAGT1 in the patients, we examined TCR stimulation with an OKT3 antibody affects free Mg^{2+} level in T cells. Interestingly, we showed that TCR stimulation induced a Mg^{2+} flux in normal T cells, which was impaired in the patients' cells even with high doses of agonist (figure 1C). Moreover, the TCR-induced Ca^{2+} flux was drastically decreased in the patients' T cells, raising the possibility that the Ca^{2+} flux was dependent on Mg^{2+} (figure 1C). We explored this possibility by modulating extracellular $[Mg^{2+}]_e$ and $[Ca^{2+}]_e$. The TCR-induced Mg^{2+} and Ca^{2+} flux were both abrogated in absence of Mg^{2+} , whereas only the Ca^{2+} flux was impaired in absence of Ca^{2+} , showing that the Mg^{2+} flux was at least partially needed for the induction of the Ca^{2+} flux but not the converse. Moreover, we found that the BCR stimulation did not induce a Mg^{2+} flux, which is consistent with the fact that B cell activation was normal in the patients.

We were able to impair the TCR-induced Mg^{2+} flux as well as the NF- κ B translocation by knocking down MAGT1 in normal T cells, recapitulating the patients' phenotype. We also rescued the patients' T cells with ectopic expression of MAGT1, restoring the TCR-induced Mg^{2+} flux and the upregulation of activation markers upon TCR stimulation. These results established that MAGT1 mediated the Mg^{2+} influx required for full T cell activation. Thus, MAGT1 deficiency was the proximate cause of the cellular defect in XMEN disease.

Impairment of PLC γ 1 activation in MAGT1 deficient patients

Since we showed that the Mg^{2+} flux was necessary to induce a full Ca^{2+} response, we next wanted to dissect the TCR signaling event leading to the generation of the Ca^{2+} flux [17, 18]. Stimulation of the TCR induces the activation of the Lck kinase, which initiates a cascade of phosphorylation events leading to the activation of the PLC γ 1 which generates diacylglycerol (DAG) and IP3 (figure 2). IP3 then triggers the release of the Ca^{2+} from the

endoplasmic reticulum initiating the store operated calcium entry (SOCE), which is the Ca^{2+} flux induced by TCR. By assessing the phosphorylation status of the different components of the TCR signaling apparatus, we showed that the phosphorylation of PLC γ 1 was delayed in the patient cells after TCR stimulation. Indeed, the peak of phosphorylation of PLC γ 1 induced by OKT3 stimulation was shifted from 5 minutes in normal control T cells to 60 minutes in the patients' cells. As IP3 production was also delayed, we determined that PLC γ 1 activation was the proximal step regulated by Mg^{2+} . Thus, our findings suggested that Mg^{2+} acts as a second messenger in TCR signaling, leading to the activation of PLC γ 1.

Conclusion

Mg^{2+} plays a central role in many cellular functions by acting as a cofactor for all ATP consuming enzymes as well as a stabilizer for structural membrane phospholipids [19, 20]. However, our data showed that a transient, Mg^{2+} influx mediated by MAGT1 after TCR stimulation was able to modulate the activation of PLC γ 1, and the subsequent Ca^{2+} influx. These observations revealed an essential role for Mg^{2+} in the TCR signaling.

The study of the XMEN patients also revealed that their lymphocytes exhibited a defective Mg^{2+} uptake leading to lower basal level of free Mg^{2+} . However, this could not account for defective T cell activation, as the patient's B cells activated normally compared to control. Recently, the role of TRPM7 in Mg^{2+} homeostasis in B cell has been described, and a lack of TRPM7 can be compensated by overexpressing MAGT1 in B cell line [21]. These observations taken together with ours makes us think that TRPM7 may play a greater role in Mg^{2+} homeostasis in B cells than MAGT1. This selective function of MAGT1 in T cells could be used to develop new immune modulator drugs to target T cell-dependent autoimmunity or other immunological diseases.

References

1. Cakmak I, Kirkby EA. Role of magnesium in carbon partitioning and alleviating photooxidative damage. *Physiol Plant*. 2008; 133(4):692–704. [PubMed: 18724409]
2. Cowan JA. Structural and catalytic chemistry of magnesium-dependent enzymes. *Biometals*. 2002; 15(3):225–35. [PubMed: 12206389]
3. Yang W, Lee JY, Nowotny M. Making and breaking nucleic acids: two-Mg²⁺-ion catalysis and substrate specificity. *Mol Cell*. 2006; 22(1):5–13. [PubMed: 16600865]
4. Romani AM. Cellular magnesium homeostasis. *Arch Biochem Biophys*. 2011; 512(1):1–23. [PubMed: 21640700]
5. Murphy E. Mysteries of magnesium homeostasis. *Circ Res*. 2000; 86(3):245–8. [PubMed: 10679471]
6. Vidair C, Rubin H. Mg²⁺ as activator of uridine phosphorylation in coordination with other cellular responses to growth factors. *Proc Natl Acad Sci U S A*. 2005; 102(3):662–6. [PubMed: 15647355]
7. Takaya J, Higashino H, Kobayashi Y. Can magnesium act as a second messenger? Current data on translocation induced by various biologically active substances. *Magnes Res*. 2000; 13(2):139–46. [PubMed: 10907231]
8. Rubin H. The logic of the Membrane, Magnesium, Mitosis (MMM) model for the regulation of animal cell proliferation. *Arch Biochem Biophys*. 2007; 458(1):16–23. [PubMed: 16750508]
9. Abboud CN, Scully SP, Lichtman AH, Brennan JK, Segel GB. The requirements for ionized calcium and magnesium in lymphocyte proliferation. *J Cell Physiol*. 1985; 122(1):64–72. [PubMed: 3965485]
10. Flynn A. Control of in vitro lymphocyte proliferation by copper, magnesium and zinc deficiency. *J Nutr*. 1984; 114(11):2034–42. [PubMed: 6333500]

11. Whitney RB, Sutherland RM. The influence of calcium, magnesium and cyclic adenosine 3',5'-monophosphate on the mixed lymphocyte reaction. *J Immunol.* 1972; 108(5):1179–83. [PubMed: 4337672]
12. Modiano JF, Kelepouris E, Kern JA, Nowell PC. Requirement for extracellular calcium or magnesium in mitogen-induced activation of human peripheral blood lymphocytes. *J Cell Physiol.* 1988; 135(3):451–8. [PubMed: 3135293]
13. Li FY, Chaigne-Delalande B, Kanellopoulou C, Davis JC, Matthews HF, Douek DC, Cohen JL, Uzel G, Su HC, Lenardo MJ. Second messenger role for Mg²⁺ revealed by human T-cell immunodeficiency. *Nature.* 2011; 475(7357):471–6. [PubMed: 21796205]
14. Wengler GS, Parolini O, Fiorini M, Mella P, Smith H, Ugazio AG, Notarangelo LD. A PCR-based non-radioactive X-chromosome inactivation assay for genetic counseling in X-linked primary immunodeficiencies. *Life Sci.* 1997; 61(14):1405–11. [PubMed: 9335230]
15. Goytain A, Quamme GA. Identification and characterization of a novel mammalian Mg²⁺ transporter with channel-like properties. *BMC Genomics.* 2005; 6(1):48. [PubMed: 15804357]
16. Zhou H, Clapham DE. Mammalian MagT1 and TUSC3 are required for cellular magnesium uptake and vertebrate embryonic development. *Proc Natl Acad Sci U S A.* 2009; 106(37):15750–5. [PubMed: 19717468]
17. Peterson EJ, Koretzky GA. Signal transduction in T lymphocytes. *Clin Exp Rheumatol.* 1999; 17(1):107–14. [PubMed: 10084044]
18. Weiss A, Littman DR. Signal transduction by lymphocyte antigen receptors. *Cell.* 1994; 76(2): 263–74. [PubMed: 8293463]
19. Grubbs RD, Maguire ME. Magnesium as a regulatory cation: criteria and evaluation. *Magnesium.* 1987; 6(3):113–27. [PubMed: 3306178]
20. Permyakov EA, Kretsinger RH. Cell signaling, beyond cytosolic calcium in eukaryotes. *J Inorg Biochem.* 2009; 103(1):77–86. [PubMed: 18954910]
21. Deason-Towne F, Perraud AL, Schmitz C. The Mg(2+) transporter MagT1 partially rescues cell growth and Mg(2+) uptake in cells lacking the channel-kinase TRPM7. *FEBS Lett.* 2011; 585(14): 2275–8. [PubMed: 21627970]

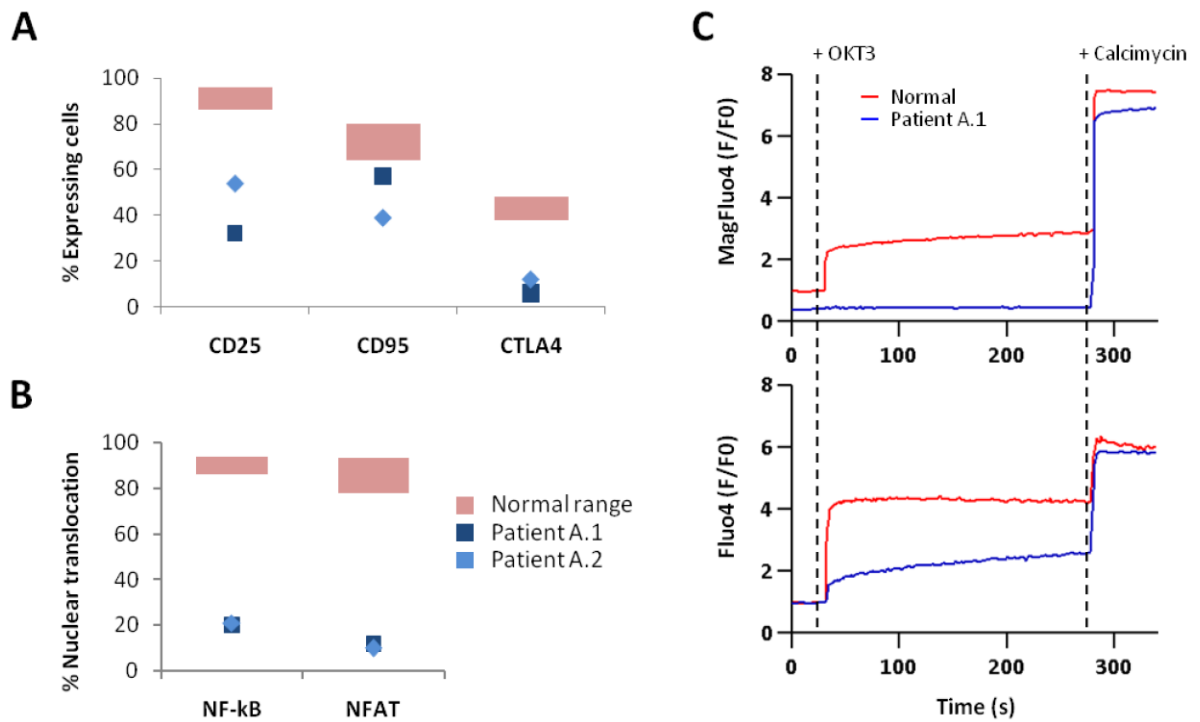


Figure 1. XMEN patients have a proximal TCR activation defect with loss of Mg^{2+} and Ca^{2+} fluxes

A) Graph representing the expression of the activation markers CD25, CD95 and CTLA4 in T cells after 3 days of stimulation with 1 ug/ml anti-CD3 (OKT3). The red box represents the normal range. **B)** Graph representing the nuclear translocation of NF- κ B and NFAT after 30 min stimulation with OKT3. **C)** Mg^{2+} (upper panel) and Ca^{2+} (lower panel) fluxes in T cells from normal control or Patient A.1 after 5 ug/ml OKT3 stimulation.

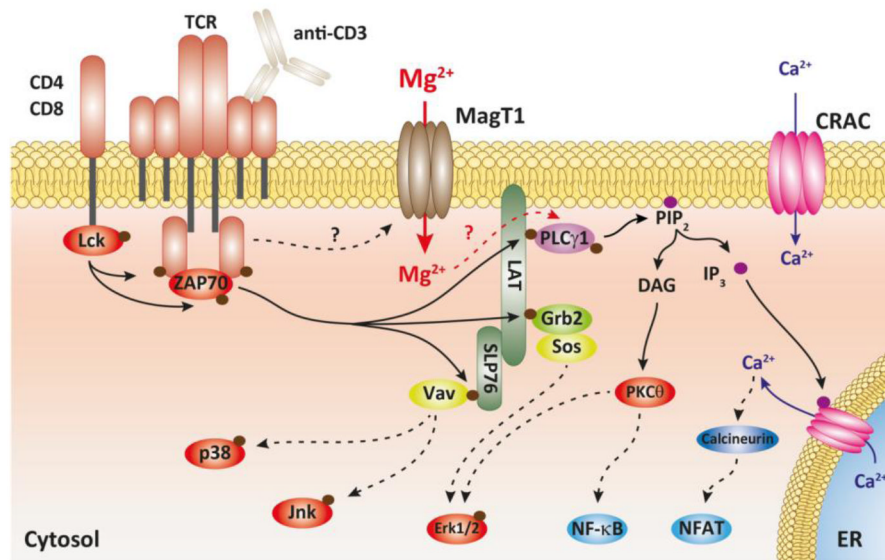


Figure 2. Hypothetical scheme depicting how the MagT1 mediated Mg^{2+} influx participates in TCR signaling
 Solid arrows indicate direct effects; dotted arrows indicate indirect effects.