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XMEN: welcome to the glycosphere

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Commentary

XMEN (X-linked immunodeficiency with magnesium defect, EBV infection, and neoplasia) is a complex primary immunological deficiency caused by mutations in *MAGT1*, a putative magnesium transporter. In this issue of the*JCI*, Ravell et al. greatly expand the clinical picture. The authors investigated patients' mutations and symptoms and reported distinguishing immunophenotypes. They also showed that MAGT1 is required for N-glycosylation of key T cell and NK cell receptors that can account for some of the clinical features. Notably, transfection of the affected lymphocytes with *MAGT1* mRNA restored both N-glycosylation and receptor function. Now we can add XMEN to the ever-growing family of congenital disorders of glycosylation (CDG).

XMEN: welcome to the glycosphere

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A comprehensive clinical description of patients with XMEN

XMEN is the attention-grabbing name for a rare primary immunodeficiency (X-linked immunodeficiency with magnesium defect, EBV infection, and neoplasia) first described for 3 male patients in 2011 (1). Mutations in the X-linked gene *MAGT1* were thought to disrupt magnesium (Mg^{2+}) transport and homeostasis; a few case reports and reviews restated that perspective (2–4). In the current issue of the *JCI*, Ravell et al. (5) thoroughly analyzed 23 patients and broadened the clinical picture. Importantly, they revised their mechanistic perspective by showing that *MAGT1* mutations reduce N-glycosylation of selected critical T cell receptors that can explain the immunological impact. On the basis of a glycoproteomic analysis, they offer a N-glycosylation–dependent diagnostic approach and demonstrate that XMEN is actually a glycoprotein-selective congenital disorder of glycosylation (CDG) (5).

In July 2011, the Lenardo laboratory described 3 patients with XMEN with CD4 lymphopenia, severe chronic viral infections, and defective T lymphocyte activation (1). They had mutations in *MAGT1*, considered to be a Mg^{2+} transporter (6, 7). Antigen receptor stimulation of T cells causes a transient rapid Mg^{2+} influx (8). MAGT1 deficiency abrogates that influx and impairs antigen receptor engagement, suggesting that Mg^{2+} is an intracellular second messenger. However, both total and serum Mg^{2+} concentrations were normal and not diagnostic of XMEN. A separate commentary (9) about this work proposed that MAGT1 could have other, as-yet unidentified functions and that it might be interesting to determine the effects of MAGT1 deficiency in other cell types. Ravell et al. phenotyped 23 patients, 8 of whom were EBV naive. They confirmed original symptoms of an inverted CD4/CD8 ratio, CD4$^+$ T cell lymphocytopenia, dysgammaglobulinemia, increased B cells, and decreased expression of the NK cell group 2, member D (NKG2D) receptor. NKG2D loss predisposes individuals to EBV-driven lymphoproliferative disease (LPD) and lymphoma; Hodgkin's lymphoma was frequent in EBV-infected patients, but not in EBV-naive patients. The immune phenotype of EBV-infected and EBV-naive XMEN patients was very similar, showing decreased IgG and IgA and recurrent ear and sinus infections (5).

Notably, the clinical features in XMEN resembled autoimmune lymphoproliferative syndrome (ALPS). Consistently, the authors found increased CD4$^-$CD8$^-$B220$^-$ TCRab$^+$ T (αβDNT) cells, lymphadenopathy, various cytopenias, and liver disease. Further, B cell malignancies were frequent in the EBV-infected patients. However, deep immunophenotyping (via time-of-flight mass cytometry [CyTOF]) enabled Ravell and colleagues to distinguish patients with XMEN from patients with ALPS and from healthy individuals (5).

Eight patients underwent brain imaging, some of whom showed atrophy of the cerebrum, cerebellum, brainstem, and spinal cord, but no intellectual disability or facial dysmorphism. All mutations tested abolished MAGT1 protein expression. Decreased NKG2D surface expression on CD8$^+$ T cells and NK cells was the best immune-related diagnostic indicator (5).

These results (5) provide the most comprehensive clinical description of patients with XMEN, but they also show that *MAGT1* mutations affect other systems, as the commentators suggested in 2011 (9).

The role of glycosylation

In 2006, MAGT1 was called implantation-associated protein (IAP) and was identified as a component of the mammalian oligosaccharyl transferase (OST) complex (10). In 2008, a report (11) identified a putative mutation (p.V311G) causing "nonsyndromic mental retardation" (X-linked mental retardation [XLMR]), and some suggested it was involved in the recognition or utilization of N-glycosylation sites in specific glycoproteins (12). Later, that variant was found to be relatively common and unlikely to cause the phenotype. The few reported cases of XLMR document mutations in either IAP or its homolog tumor suppressor candidate 3 (TUSC3); however, the disease descriptions were

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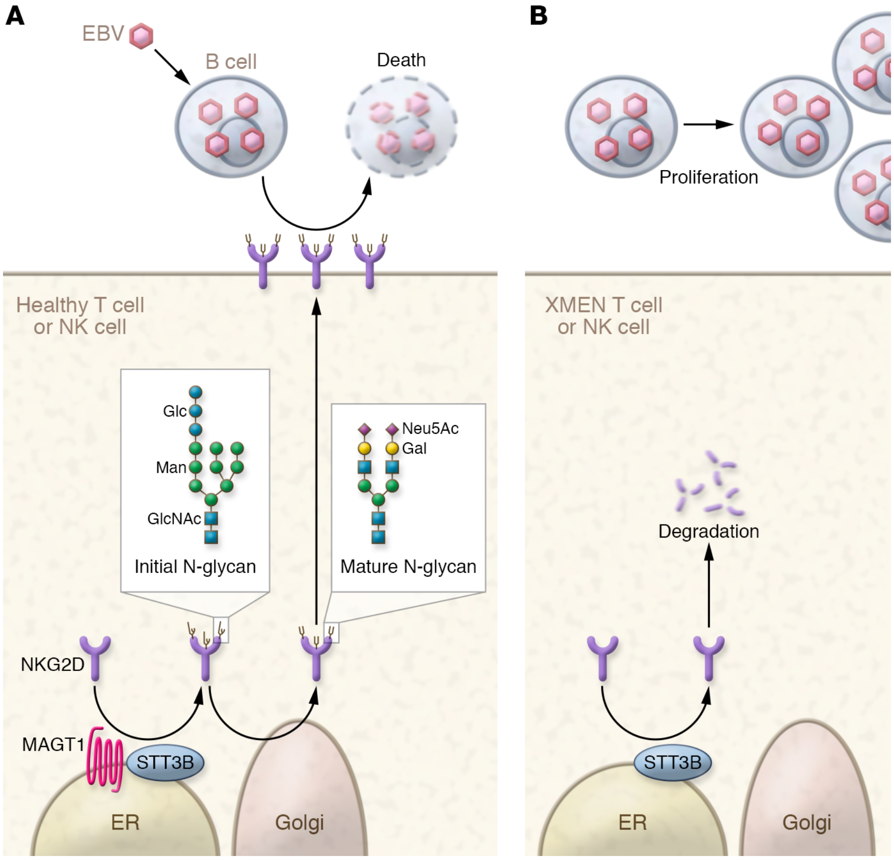

Figure 1. A model for XMEN pathology. In healthy T cells and NK cells, MAGT1 is required for N-glycosylation and the stability of key receptors. In the absence of MAGT1, underglycosylated receptors are degraded. This phenotype predisposes individuals to uncontrolled EBV infection.

incompatible with the clear immunological phenotype of XMEN.

The pioneering work of Gilmore focused on the structure and function of the OST complex, which is responsible for adding N-glycans to proteins in the ER (10, 13). He and his colleagues showed the existence of two separate complexes containing either catalytic subunit STT3A or STT3B. STT3A adds N-glycans cotranslationally and toward the N-terminal, whereas STT3B adds glycans to sites skipped by STT3A, to those near the C-terminus, or in short membrane loops. MAGT1 is exclusive to STT3B-containing complexes, and TUSC3 is found in STT3A complexes. Although many glycosylation sites are available for either complex, some glycosylation sites and proteins are specifically STT3B dependent (12). Extensive studies with these STT3A- versus STT3B-dependent proteins in cellular knockout models or in patients' fibroblasts deficient in either STT3A or STT3B confirmed a series of only STT3A- or STT3B-dependent proteins (14). Only a few months ago, this specificity strategy identified two patients with XLMR harboring *MAGT1* mutations, who resembled other patients with CDG. An additional patient had symptoms consistent with XMEN (15).

CDG defines approximately 140 rare disorders caused by mutations that affect one or more glycosylation pathways (16, 17). Approximately half of the disorders affect N-glycosylation of potentially hundreds of proteins carrying N-glycans, since essentially all secreted, cell-surface, and extracellular matrix proteins have N-glycans. Patients often have multisystemic symptoms, and the majority include intellectual disability, failure to thrive, and a host of neurological abnormalities. Many of these disorders also compromise the immune system (18). The N-glycosylation disorders fall into two groups: (a) those that limit the synthesis and OST-dependent transfer of a universal precursor to acceptor sites on the client proteins and (b) those that impair the complete maturation of those glycans. The glycosylation status of serum carbohydrate-deficient transferrin (CDT) is often used as a simple diagnostic surrogate to distinguish these groups. The absence of glycans can sometimes lead to protein instability and degradation. Clearly, if a specific mature glycan structure is needed for a physiological activity, the entire absence of that glycan will reduce or eliminate that function.

Ravell et al. hypothesized that selective N-glycosylation deficiency of multiple immune proteins was the basis of XMEN pathology (Figure 1). Indeed, they found that selective reduction of NKG2D was due to poor glycosylation that greatly reduced the presence of the receptor (5). Previous studies had shown that silencing the expression of human NKG2D or DAP10 decreases the cytotoxic effector function of $CD8^+$ T cells and NK cells, leading to impaired EBV antiviral immunity (19). But perhaps other proteins were also affected. To answer this, the authors performed extensive glycoproteomic site occupancy analysis of normal T cells and T cells from patients with XMEN (5).

Proteomic analysis using liquid chromatography with tandem mass spectrometry revealed no major differences in peptide abundance, however, the authors found that patients with XMEN had more peptides with lower glycosylation than did controls. The analysis of 2481 peptides from 1421 proteins showed that only a small set (73 proteins) were affected in XMEN. Decreased glycan site occupancy was observed for the proteins CD28, CD70, HLA-DRβ1, T cell receptor α chain (TCR-α), ceramide synthase 2 (CERS2), and solute carrier family 4 member 7 (SLC4A7). CD28, CD70, and HLA-DRB1 had lower surface expression in T cells. CD28, CD70, HLA-DRβ1, TCR-β, CERS2, and SLC4A7 had a reduction in fully or partly glycosylated species. All XMEN patients tested ($n = 10$) possessed a mild, but distinctly abnormal transferrin glycosylation–typical type I CDG. They also found mild changes in apolipoprotein CIII (Apo-CIII), which only contains O-linked glycans. Further, 36% and 17% of the differentially glycosylated peptides mapped to STT3B- and STT3A-predicted motifs, respectively. Importantly, transfection of MAGT1 into PBMCs restored defective glycosylation of NKG2D, CD70, CERS2, SLC4A7, and TCR-β. Diagnostically, surface staining for NKG2D and CDT together offers the best test for patients with XMEN (5).

In a separate study (20), researchers from this group showed that MAGT1 was present in T cells, B cells, NK cells, and monocytes, whereas TUSC3 was absent. These results suggest that these cells may rely on STT3B-dependent glycosylation more than do other types of cells.

Conclusions

Clearly, the analysis of T cell glycoproteins has provided reasonable explanations for some key pathological features of XMEN. Two questions remain unanswered: Why do severe mutations that seem to eliminate MAGT1 generate the XLMR versus the XMEN phenotype in patients? And what specific proteins in other cell types might account for the nonimmunological maladies seen in the expanded spectrum of patients with XMEN? Perhaps, once again, there are other currently unknown functions of MAGT1. For now, XMEN is definitely a CDG. Welcome!

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