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Detection of phosphoglucomutase-3 (PGM3) deficiency by lectin-based flow cytometry

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To the Editor

Phosphoglucomutase 3 (PGM3) deficiency is an autosomal recessive syndrome with immunologic phenotypes ranging from a hyper-IgE syndrome to severe combined

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Conflicts of Interest

The authors declare no conflict of interest.

immunodeficiency (SCID). To date, PGM3 deficiency has been described in twenty-nine individuals from thirteen families, with the majority (23 individuals from 9 families) presenting with a hyper-IgE phenotype (1–5). Hypomorphic *PGM3* mutations result in altered glycan expression, making this deficiency a congenital disorder of glycosylation (CDG). Among individuals with partially preserved immune function and associated serum IgE elevations, the phenotype is complex and includes severe atopic disease, Th17-associated autoimmunity, and infectious susceptibility, in addition to connective tissue and neurologic abnormalities. PGM3 converts *N*-acetylglucosamine-6-phosphate (GlcNAc-6-P) to GlcNAc-1-P. The fundamental defect in PGM3 deficiency appears to arise from inadequate production of GlcNAc-1-P. The resulting reduction in substrate for the subsequent enzymatic step in the hexosamine biosynthetic pathway causes a secondary deficiency of uracil diphosphate (UDP)-GlcNAc in primary dermal fibroblasts (1) and in peripheral blood mononuclear cells (PBMCs) (Fig 1A).

UDP-GlcNAc is fundamental to normal *N*-linked glycosylation of proteins. It is incorporated into the core structure of all *N*-linked glycoproteins and is a key component of branching structures of complex *N*-glycans (Fig 1B). *N*-glycan complexity has been shown to have dramatic effects on T cell function including altering the activation threshold of the TCR and promoting CTLA-4-mediated growth arrest (6, 7). Diminished intracellular concentrations of UDP-GlcNAc seen in PGM3 deficiency have been associated with altered *N*-glycosylation of serum proteins but alterations in cellular glycan expression have not yet been characterized (1).

Complete loss of function in PGM3 is embryonically lethal in mice (8). To date, all individuals identified with PGM3 deficiency express mutant protein, thus limiting the ability to screen for this disorder using conventional protein detection assays. Furthermore, functional enzymatic activity assays are complicated and require specialized expertise. To get around these obstacles, we exploited predicted glycan differences to develop a lectin-based flow cytometric assay that can quantify expression of complex branched *N*-glycans and successfully identify PGM3 deficient individuals (see the Online Repository Methods for a complete description of assays).

N-glycan branching was measured using a fluorescein-conjugated plant lectin, L-phytohemagglutinin (L-PHA), which has specificity for 2,6-branched complex *N*-glycans with bisecting *N*-acetylglucosamine (GlcNAc) (Fig 1B) (9). The dependence of L-PHA staining on *N*-glycan expression was confirmed by treatment of peripheral blood mononuclear cells (PBMCs) with PNGaseF – an enzyme that hydrolyzes nearly all *N*-glycans – resulting in a significant reduction in staining intensity (Fig 1C).

We examined PBMCs isolated from PGM3 deficient individuals and controls, as well as from other disorders with significant elevations in IgE (atopic dermatitis, DOCK8 deficiency, and dominant negative *STAT3* mutations resulting in loss of function) and from patients with congenital disorders of glycosylation (CDGs) (caused by autosomal recessive loss of function mutations in *ALG13*, *PMM2*, *ALG12*, or *MOGS*) and deglycosylation (*NGLY1*). Patient ages, allergic disease prevalence, serum IgE, and absolute eosinophil counts are listed in Table I. Phosphomannomutase-2 (PMM2) facilitates conversion of

phosphorylated mannose in a manner similar to PGM3, potentially limiting mannose substrate for all *N*-glycans, but not specifically limiting *N*-glycan branching or complexity (E1). Asparagine-linked glycosylation homologs 13 and 12 (ALG13/ALG12) assist in the addition of sugars to the *N*-glycan core structure, and therefore are predicted to affect total *N*-glycosylation expression (E2). Mannosyl-oligosaccharide glucosidase (MOGS) and *N*-glycanase 1 (NGLY1) are glycosidases involved in glycoprotein quality control, and defects may result in accumulation of high-mannose type glycans for the former (E3, E4).

Despite having reduced UDP-GlcNAc pools, L-PHA staining in PGM3-deficient PBMCs *en masse* displayed no discernable difference from controls or other genetic disorders (Fig 1D). However, examining distinct leukocyte populations (for gating strategy see Fig E2), significant but relatively modest reductions were observed in multiple cell populations, most significantly within the $CD8^+$ $CD45RO^-$ and $CD45RO^+$ pools, with the notable exception of $CD19^+$ B cells. While statistically significant, L-PHA staining intensity of these PGM3 deficient cell populations overlapped with one or more control population, therefore none of these differences was large enough to discern every individual with PGM3 deficiency clearly from the other disorders (Fig 1E, F; E1). However, closer inspection of $CD4^+$ $CD45RO^-$ cells, revealed a marked reduction in *N*-glycan complexity in PGM3 deficiency compared to all other samples, clearly distinguishing PGM3 deficiency from all other disorders of hyper-IgE as well as all other CDGs tested (Fig 1E, G). These differences appeared specific to detection of 2,6-branching complex *N*-glycans since staining with concanavalin A (Con A), a lectin which binds alpha-linked mannose residues present on all *N*-glycans, failed to demonstrate such a defect (Fig E3). Despite the marked reductions seen in $CD4^+$ $CD45RO^-$ cells, the complex *N*-glycan expression in the $CD4^+$ $CD45RO^+$ population appeared relatively unperturbed. We hypothesize the differences seen between these populations is related to metabolic changes that are known to occur with lymphocyte activation.

We have developed a lectin-based flow cytometric assay that can clearly identify PGM3 deficiency by examining extracellular L-PHA staining of $CD4^+$ $CD45RO^-$ T cells. It is possible that other glycosylation disorders could result in a similar glycan pattern, and follow-up sequencing remains necessary. However, we predict that similar glycosylation defects in lymphocytes will likely be associated with clinical phenotypes resembling PGM3 deficiency. Thus, this and other lectin-based flow cytometry may also aid in screening for and identifying CDGs associated with immune dysregulation. This assay is easy to perform with low cost, using a lectin conjugate and conjugated antibodies standard to flow cytometry labs, and can help stratify individuals for subsequent targeted or genomic sequencing.

Hematopoietic stem cell transplantation (HSCT) has demonstrated efficacy in PGM3 deficiency-associated SCID. Because of the high morbidity associated with the hyper-IgE phenotype, HSCT may also be considered for this presentation in the future. Lectin-based flow cytometry-screening could aid in early diagnosis without delaying HSCT in this clinical context.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used

PGM3	Phosphoglucomutase-3
CDG	congenital disorder of glycosylation
SCID	severe combined immunodeficiency
GlcNAc	*N*-acetylglucosamine
UDP	uracil diphosphate
HSCT	hematopoietic stem cell transplant
DOCK8	dedicator of cytokinesis 8
STAT3	signal transducer and activator of transcription 3
PMM2	phosphomannomutase-2
ALG13	asparagine-linked glycosylation homolog 13
ALG12	asparagine-linked glycosylation homologs 12
MOGS	mannosyl-oligosaccharide glucosidase
NGLY1	*N*-glycanase 1
L-PHA	L-phytohemagglutinin
PBMC	peripheral blood mononuclear cell
MFI	mean fluorescence intensity
Con A	concanavalin A

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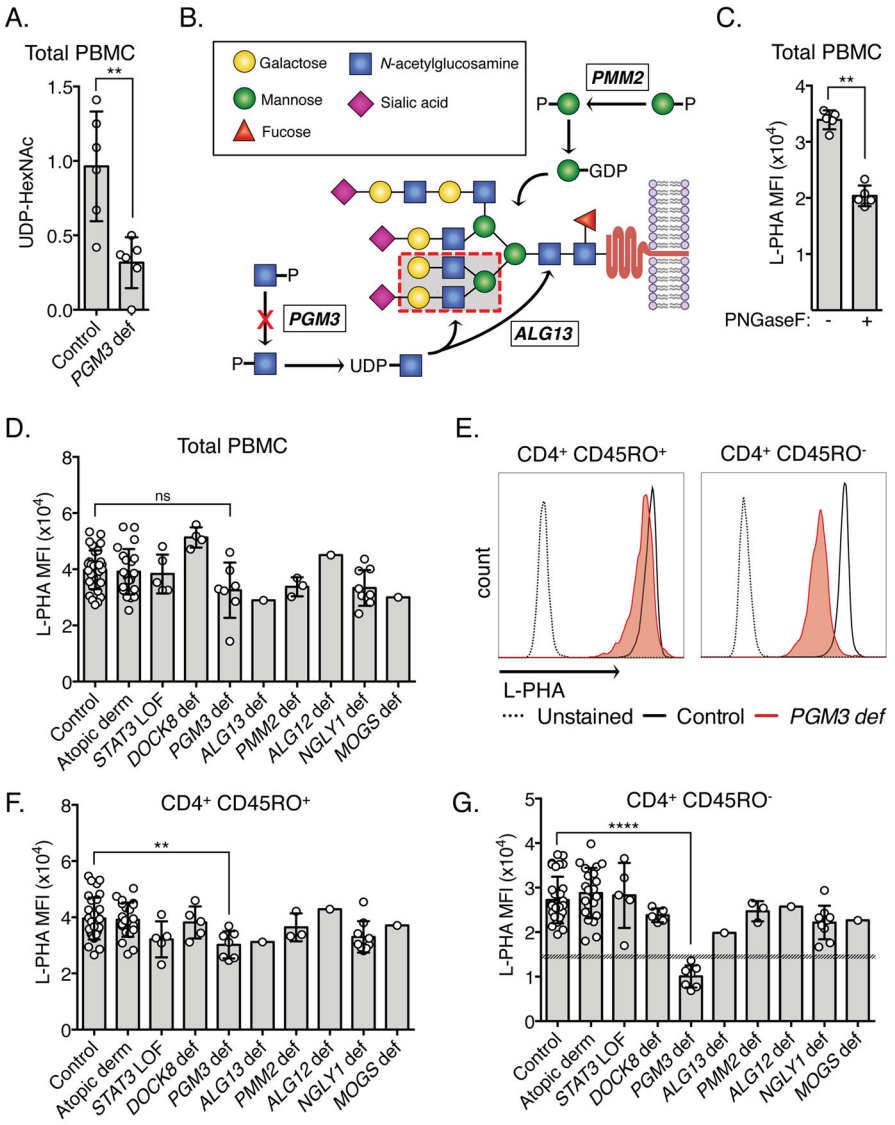

Figure 1. Reduced naïve CD4 *N*-glycan complexity identifies PGM3 deficiency from other congenital glycosylation and hyper-IgE disorders

(A) Intracellular UDP-HexNAc in PGM3 deficient (n = 6) and control PBMCs (n = 6). Unpaired t-test; **P<0.01. **(B)** Schematic of complex *N*-glycan formation with L-PHA binding site shown as a red dashed box. **(C)** L-PHA staining intensity of PBMCs before and after PNGAseF treatment (n = 5). Mann-Whitney test; **P<0.01. **(D)** L-PHA staining intensity of PBMCs from controls (n = 32), individuals with atopic dermatitis (AD, n = 22), DOCK8 deficiency (n = 4), STAT3 loss-of-function (STAT3 LOF, n = 5), PGM3 deficiency (n = 7), ALG13 deficiency (n = 1), PMM2 deficiency (n = 3), ALG12 deficiency (n = 1), NGLY1 deficiency (n = 9), and MOGS deficiency (n = 1). Representative **(E)** and combined **(F, G)** L-PHA staining of $CD45RO^+$ and $CD45RO^-$ $CD4^+$ cells. Shaded area in **(G)** delineates the upper 99% confidence interval (CI) of PGM3 deficient cells and the closest lower 99% CI (STAT3 LOF). Mann-Whitney test; **P<0.01, ****P<0.0001.

Table I

Clinical and laboratory data from screened individuals

Diagnosis (N)	Age (yr) Median, (range)	Serum IgE (IU/mL) Median, (range)	Eosinophils (cells/μL) Median, (range)	Allergy N (%)	Clinical Features (N)
PGM3 deficiency (N = 7)	**33 (4–38)**	**27,200 (1,927–35,445)**	**900 (200–3,600)**	**7 (100%)**	AD (7), food allergy (5), asthma (1), *S. aureus*[*] (5)
Atopic dermatitis (N = 22)	16 (6–52)	20,689 (5,129–185,033)	1,545 (500–4,550)	22 (100%)	AD (22), food allergy (20), asthma (18), *S. aureus* (17)
DOCK8 deficiency (N = 4)	27 (22–31)	7,408 (1,162–31,403)	2,735 (560–6,750)	4 (100%)	AD (4), food allergy (4), asthma (1),
STAT3 LOF (N = 5)	19 (12–57)	3,670 (3,219–29,611)	630 (530–2,220)	5 (100%)	AD (5), food allergy (2), asthma (1), *S. aureus* (5)
ALG13 deficiency (N = 1)	9	9	80	none	-
PMM2 deficiency (N = 3)	4 (3–5)	8 (0–27)	110 (0–170)	2 (100%)	AD (2) - mild
ALG12 deficiency (N = 1)	20	2	60	none	-
NGLY1 deficiency (N = 9)	8 (3–18)	26 (2–92)	230 (40–1,260)	1 (11%)	AD (1) - mild
MOGS deficiency (N = 1)	17	9	300	1 (100%)	food allergy (1) – egg, unconfirmed

[*] Susceptibility to skin and soft tissue infection with *Staphylococcus aureus* (*S. aureus*).