Supplementary Methods

Sequencing

Genomic DNA was PCR-amplified and analyzed by cycle sequencing using the ABI PRISM BigDye Terminator v1.1 cycle sequencing kit (Applied Biosystems) as earlier described {Moens, 2014 #1}. DNA extraction was performed using the FlexiGene or the Qiamp DNA FFPE kits (Qiagen) according to the supplied protocols. For extraction from paraffin, the tissue slides were first incubated for 5 minutes in xylene and vigorously vortexed. After centrifugation and removal of the xylene the sample was washed with 99.5% ethanol, centrifuged at 14,000x g and all ethanol carefully removed by a pipette. PCR was performed using the HotStarTaq plus DNA polymerase kit (Qiagen) according to manufacturer's protocol. Primers used were, forward: 5'-TTT GGT AGC CTT TAG AAC TCT C-3', reverse: 5'-TGA GGG CAG TAT CTT TCT CAC-3', and forward 2: 5'- TGT AGA CCA TAC TTT TGA TGG CA-3', reverse 2: 5'-GAA ACT ACC AGA AAA CAA AAA TGG-3'. For both PCR-sets an annealing temperature of 53 °C was used.

Cell culturing

EBV-transformation. Whole EDTA-blood was diluted 1:2 in RPMI 1640 (Life Technologies), with 20 % DMSO, and aliquots of 1.5 ml frozen at -80 °C. To ascertain enough amounts of cells for further analysis, an aliquot was subsequently transformed with Epstein Barr Virus (EBV). Briefly, cells were rapidly thawed and washed in RPMI 1640 containing 20 % heat-inactivated fetal bovine serum (RPMI-20) and re-suspended in filtered EBV B95-8 supernatant, at 10^7 cells/ml. After 90 minutes at 37 °C the cells were diluted with RPMI-20, pelleted and resuspended at 2 x 10^6 cells/ml in RPMI-20 supplemented with 10 mM HEPES, 1x PEST (Life Technologies) and 1 μg Cyclosporin A (CsA)(Sigma)/ml, and placed in a 96-well round bottom plate. Half of the culture medium in the wells was exchanged every 4-5 days and CsA was withdrawn after 2-3 weeks. EBV-transformed cells were expanded and cultured in RPMI-20 containing 50 mg/ml Gentamicin (Life Technologies), at 37 °C in a humidified incubator with 5 % CO_2 atmosphere.

Mitogen stimulation (FASCIA). Whole blood with Heparin was diluted 1:9 in RPMI 1640 medium supplemented with 100 IU/mL penicillin, 100 IU/mL streptomycin (all from Gibco,), 2 mM L-glutamine (Invitrogen) and stimuli as indicated below, in polypropylen round bottom tubes with caps (BD Biosciences). 450 μL of diluted blood was incubated with 50 μL of the agents listed below, using concentrations as indicated: PHA (Phytohemagglutinin, Sigma-Aldrich) 10 μg/ml; PWM (Pokeweed mitogen, Sigma-Aldrich) 5 μg/mL; ConA (Concanavalin A, Sigma-Aldrich) 10 μg/mL Pneumococci (Prevenar 13 vaccine, Pfizer) diluted 1:100; Tetanus toxin 40 IU/ml (Statens serum institute, Copenhagen) undiluted; Influenza (Influenza A vaccine, Fluarix, GlaxoSmithKline AB) diluted 1:100; PPD (Tuberculin purified protein derivative, Statens serum institute, Copenhagen) 10 μg/mL; Candida (Candida albicans, Greer) 20 μg/mL; Staphylococcal enterotoxin A (Staphylococcal enterotoxin, Sigma-Aldrich) 100 ng/mL; Varicella zoster (Varizella zoster virus vaccine, Varilrix , GlaxoSmithKline AB) diluted 1:100.

The tubes were then incubated for 7 days in a cell culture incubator at 37 °C with 5% CO_2 and with loose caps. At day 7 medium was removed and frozen for cytokine analysis and the cells were stained with CD3-FITC/CD4-PE Simultest mix

(BD Biosciences). In addition, the tubes with medium control and PWM were stained with CD19-PC7 (Beckman Coulter) alternatively CD19-PerCP-Cy (BD) or CD-APC (BD). Erythrocytes were lysed with 1× IOTest lysing solution (Beckman Coulter) and the cell pellets were washed and resuspended in 450 µL PBS. Cells were counted using a FC500 or Navios flow cytometer (Beckman Coulter). To obtain an absolute number of proliferative responses per µL whole blood, Trucount tubes (BD Biosciences) were used and cells/µL blood calculated as described in Marits P. et al. Clin Immunol. 2014 Aug;153(2):332-42.

Immunoblotting

EBV-transformed cells were used for Western Blot and RNA extraction. Cells were counted and pelleted at 400x g and immediately lysed with RIPA buffer (50 mM Tris-HCl, pH 7.4 with 50 mM sodium fluoride, 150 mM sodium chloride, 2 mM EDTA, 1.0 % NP-40, 0.5 % sodium deoxycholate, and 0.1 % sodium dodecyl sulfate (SDS)), containing an EDTA-free complete inhibitor cocktail (Roche). Samples were analyzed by SDS-polyacrylamide electrophoresis on 4 to 12 % Bis-Tris-glycine gels (Life Technologies), and transferred to nitrocellulose membranes using the iBlot Dry-Blotting system (Life Technologies). The membranes were treated with Odyssey blocking buffer (LI-COR Biosciences), and probed with rabbit polyclonal anti-PGM3 antibodies (PA5-22353, Thermo Scientific) at dilution 1:2000 and mouse monoclonal anti-Actin (A-5441, Sigma) diluted 1:100,000. Secondary antibodies goat anti-mouse-800CW and goat anti-rabbit-680 (LI-COR Biosciences) were used in a dilution of 1:20,000. The membranes were scanned with the Odyssey infrared imaging system (LI-COR Biosciences). Different amounts of cell lysates corresponding to between 0.5 and 2 $x10^6$ cells were tested to verify that the signals for both proteins were within a linear range.

Quantitative RT-PCR

At the same time as cells were harvested for immunoblotting, an aliquot was pelleted separately and immediately resuspended in Trizol reagent (Life Technologies). RNA was extracted according to the manufacturer's protocol and the concentration determined by spectroscopy. For the first strand synthesis an amount of 500 ng total RNA was used per reaction in the high capacity cDNA reverse transcriptase kit (Applied Biosystems) with random hexamer primers. The specific mRNA levels were subsequently analyzed using the Quantitect SYBR green PCR kit (Qiagen) according to protocol. Primers used were for *PGM3* forward: 5'-GAG TTA TTA GCA CTA CCG ATG-3', reverse: 5'-GAT CTG CAC TTT CTT GTG AG-3' at 0.3 mM and for *HPRT* forward: 5'-GAC TTT GCT TTC CTT GGT CAG-3' reverse: 5'-GGC TTA TAT CCA ACA CTT CGT GGG-3' at 0.1 µM final concentration. A dilution curve was obtained for one of the samples and the relative quantity in the different samples calculated.

Expression and purification of recombinant PGM3 protein

cDNAs encoding wild type (wt) and mutant (Ile322Thr) PGM3 were synthesized (GenScript) with codons optimized for expression in *E. coli.* The cDNAs were subcloned into the NcoI and BamHI sites of the pETM-11 vector (EMBL collection), which includes an N-terminal His_6-tag and a TEV (tobacco etch virus) protease cleavage site in front of the inserted gene. *PGM3* constructs were transformed into

E. coli BL21 (DE3) RIL Codon Plus cells (Stratagene) for overexpression. Cultures were grown in LB medium. Protein expression was induced when the cell density reached an OD600 of ~ 0.80 by addition of isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 0.25 mM. Induced cells were grown for 18 h at 18 °C prior to harvesting by centrifugation. Cell pellets were resuspended in 50 mM Tris-HCl pH 8.0 and 300 mM NaCl; the cells were lysed by sonication. Cellular debris was removed by centrifugation, and the supernatant was applied to an Ni-NTA resin equilibrated in lysis buffer and eluted using 300 mM imidazole. The purified protein was dialyzed against 20 mM Tris pH 8.0, 100 mM NaCl and 10 mM β-ME. Glycerol was added to a final concentration 50 % (v/v) and the samples were frozen before further use.

Supplementary tables

Since 1984 A.D. receives prophylactic immunoglobulin substitution and a summary of laboratory data from selected time points since then is provided in Supplementary Table I and Fig. S2. At three occasions the IgE level was measured and found to be in the high normal or just above the normal range

Supplementary Table I

Historical and recent laboratory data for patient A.D.							
Year Date	1984 25/5	1985 14/3	1994 13/12	1997 3/2	1998 9/9	2004 27/10	2014 6/2
Platelets x10^9 /L	**453** (150-350)	307 (150-350)	**405** (150-350)	257 (150-350)	**444** (150-350)	306 (165 – 387)	318 (165 – 387)
Leukocytes x10^9 /L	4.5 (3.5-9.0)	**2.9** (3.5-9.0)	**2.6** (3.5-9.0)	3.9 (3.5-9.0)	4 (3.5-9.0)	**2.5** (3.5 – 8.8)	4.4 (3.5 – 8.8)
Lymphocytes x10^9 /L	1.3	**1.74**[a]	**0.59** (1.4-4.0)	**0.58** (1.0-3.5)	**0.4** (1.0-3.5)	**0.8** (1.0-3.5)	**0.6** (1.0-4.0)
Basophils x10^9 /L	NT	NT	**0.04** (0-0.01)	**0.03** (0-0.01)	0.0 (0-0.01)	**0.03** (0 – 0.1)	<0.1 (0 – 0.1)
Neutrophils x10^9 /L	2.7	**0.67**	**1.25** (1.8-6.3)	**1.65** (1.8-6.3)	2.6 (1.8-6.3)	**0.7** (1.8-6.3)	2.2 (1.6-7.5)
Eosinophils x10^9 /L	**0.36**	**0.49**	**0.33** (0.10-0.30)	**1.11** (0.07-0.3)	**0.5** (0.07-0.3)	**0.6** (0.07-0.3)	**0.9** (0.0 – 0.5)
IgE 10^3 IU/L	NT	NT	**152** (<149)	85 (<94)	**109** (<91)	NT	19 (< 122)

Values outside normal intervals are indicated in bold. Reference intervals are given within parentheses. NT, not tested. IU, International unites. [a]) corresponding to 60 % of leukocytes (normal range 25-45%)

Activation of different lymphocyte subsets by indicated stimuli was analyzed for patient A.D. and the healthy control M.D. in blood samples taken in February 2014. The analysis was made by the standard FASCIA (Flow cytometric assay of specific cell-mediated immune response in activated whole blood) method used at the Karolinska Hospital Clinical Immunology laboratory, according to the description in Supplementary Methods

Supplementary Table II

Lymphocyte responses 2014			
Lymphocyte subpopulation and stimulus	Patient **A.D.**	Healthy sister **M.D.**	Reference values proliferating cells/μL
CD4 PHA	1256	**3891**	170-3499
CD8 PHA	1870	**4467**	76-3640
CD4 PWM	593	1477	233-2189
CD8 PWM	473	264	50-549
CD19 PWM	**35**	**824**	42-741
CD4 ConA	1880	**4565**	620-3800
CD8 ConA	1070	999	180-1757
CD4 Pneumococcal polysaccharide vaccine	0	12	0-269
CD8 Pneumococcal polysaccharide vaccine	0	1	0-13
CD4 Tetanus toxin	0	0	0-306
CD8 Tetanus toxin	0	1	0-14
CD4 Influenza A vaccine	52	133	19-1050
CD8 Influenza A vaccine	0	17	0-202
CD4 PPD	12	314	11-2022
CD8 PPD	**45**	14	0-29
CD4 *Candida albicans*	**0**	**2449**	51-1014
CD8 *Candida albicans*	0	11	0-49
CD4 *S. aureus* enterotoxin A	3461	6975	553-7743
CD8 *S. aureus* enterotoxin A	**2802**	780	123-2365
CD4 Varicella Zoster virus vaccine	7	118	0-154
CD8 Varicella Zoster virus vaccine	0	11	0-23

Values outside normal intervals are indicated in bold