Complementation of a pathogenic IFNγR2 misfolding mutation with modifiers of N-glycosylation

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Germline mutations may cause human disease by various mechanisms. Missense and other in-frame mutations may be deleterious because the mutant proteins are not correctly targeted, do not function correctly, or both. We studied a child with mycobacterial disease caused by homozygosity for a novel in-frame microinsertion in IFNγR2. In cells transfected with the mutant allele, most of the interferon γ receptor 2 (IFNγR2) protein was retained within the cell, and that expressed on the cell surface had an abnormally high molecular weight (MW). The misfolding mutation was not gain–of–glycosylation, as it created no new N-glycosylation site. The mutant IFNγR2 allele was null, as the patient’s cells did not respond to IFN–γ. Based on the well-established relationship between protein N-glycosylation and protein quality control processes, we tested 29 compounds affecting maturation by N-glycosylation in the secretory pathway. Remarkably, up to 13 of these compounds reduced the MW of surface-expressed mutant IFN–γR2 molecules and restored cellular responsiveness to IFN–γ. Modifiers of N-glycosylation may therefore complement human cells carrying in-frame and misfolding, but not necessarily gain–of–glycosylation, mutations in genes encoding proteins subject to trafficking via the secretory pathway. Some of these compounds are available for clinical use, paving the way for clinical trials of chemical complementation for various human genetic traits.

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These genes display high levels of allelic heterogeneity, resulting in the definition of at least 13 different disorders (3–5). Four forms of IFN-γR2 deficiency have been described at the cellular level, although all known patients with IFN-γR2 deficiency present only one of the three types of recessive MSMD. The first type was first identified in 1998 in a patient with complete IFN-γR2 deficiency, homozygous for a loss-of-expression mutation (6). Patients with complete IFN-γR2 deficiency caused by surface-expressed, nonfunctional IFN-γR2 molecules were later reported (7, 8). Finally, a child homozygous for a hypomorphic IFNγR2 mutation and residual IFN-γ responsiveness was reported, defining partial IFN-γR2 deficiency (9). Dominant IFN-γR2 deficiency has not yet been reported in any symptomatic patient, but it was recently shown that cells from a healthy heterozygous carrier display weak IFN-γ responses, suggesting that there may be patients with novel, disease-causing, dominant mutations in IFNγR2 (10). In any event, patients with complete IFN-γR2 deficiency have a much more severe prognosis than patients with partial deficiency (5).

We studied a new patient with MSMD in whom we diagnosed complete IFN-γR2 deficiency. She was homozygous for an in-frame IFNγR2 mutation (382–387dup), potentially associated with a lack of production, a lack of surface expression, or the surface expression of nonfunctional receptors. Most of the mutant 382–387dup IFN-γR2 protein was of low molecular weight (MW) and failed to mature normally. However, mature, Endoglycosidase H (Endo-H)–resistant proteins of high MW, reflecting excessive N-glycosylation, were also found. Unlike the T168N mutation previously reported in IFNγR2 (7), it was not found in 50 and 77 unrelated healthy individuals of European and Arabian descent, respectively. The parents and one sibling of this patient are heterozygous for the mutation, which was not found in the other sibling. No IFN-γ–activated sequence (GAS)–binding proteins were detected in EMSAs with EBV-transformed B cells from P1 stimulated with IFN-γ (Fig. 1 D). Similarly, no such proteins were detected in cells from two previously described children homozygous for other mutations: an in-frame 27-bp microdeletion of nucleotides 663–689 (designated 663del27 in P2, another in-frame IFNγR2 mutation) (7) and the 278delAG–null frameshift deletion (negative control, C–, lacking IFN-γR2 expression, used as a negative control; Fig. 1, C and D) (6). IFN-γR2–deficient EBV-transformed B cells homozygous for the 278delAG allele (6) were complemented by the WT allele but not by any of the three mutant IFNγR2 alleles (Fig. 1 E). Thus, P1, like P2, was homozygous for an in-frame IFNγR2 mutation causing complete functional IFN-γR2 deficiency (7), an allele conferring a complete lack of cellular responses to IFN-γ.

The 382–387dup and 663del27 IFNγR2 alleles encode misfolded proteins

We investigated the subcellular distribution of the mutant IFN-γR2 molecules by confocal microscopy in IFN-γR2–deficient SV40-transformed fibroblasts transformed with WT, 382–387dup, and 663del27 IFN-γR2–eGFP constructs. WT IFN-γR2–deficient EBV-transformed B cells homozygous for the 278delAG allele (6) were complemented by the WT allele but not by any of the three mutant IFNγR2 alleles (Fig. 1 E). Thus, P1, like P2, was homozygous for an in-frame IFNγR2 mutation causing complete functional IFN-γR2 deficiency (7), an allele conferring a complete lack of cellular responses to IFN-γ.

RESULTS AND DISCUSSION

A novel loss-of-function IFNγR2 allele (382–387dup)

Patient 1 (P1) with MSMD was homozygous for a previously unidentified in-frame microduplication of six nucleotides (382–387) in IFNγR2 (designated 382–387dup; Fig. 1 A). This mutation is predicted to result in the duplication of amino acids T128 and M129 in the protein (WVTMPW→WVTMTMPW; Fig. 1, B and C). This duplication neither creates nor deletes known consensus sites for posttranslational modifications; in particular, it is not a gain-of-glycosylation mutation, as it creates no consensus site for N-glycosylation, unlike the previously reported T168N mutation in IFNγR2 (7). It was not found in 50 and 77 unrelated healthy individuals of European and Arabian descent, respectively. The parents and one sibling of this patient are heterozygous for the mutation, which was not found in the other sibling. No IFN-γ–activated sequence (GAS)–binding proteins were detected in EMSAs with EBV-transformed B cells from P1 stimulated with IFN-γ (Fig. 1 D). Similarly, no such proteins were detected in cells from two previously described children homozygous for other mutations: an in-frame 27-bp microdeletion of nucleotides 663–689 (designated 663del27 in P2, another in-frame IFNγR2 mutation) (7) and the 278delAG–null frameshift deletion (negative control, C–, lacking IFN-γR2 expression, used as a negative control; Fig. 1, C and D) (6). IFN-γR2–deficient EBV-transformed B cells homozygous for the 278delAG allele (6) were complemented by the WT allele but not by any of the three mutant IFNγR2 alleles (Fig. 1 E). Thus, P1, like P2, was homozygous for an in-frame IFNγR2 mutation causing complete functional IFN-γR2 deficiency (7), an allele conferring a complete lack of cellular responses to IFN-γ.

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resulting in an abnormally high MW). In both cases, most proteins were of low MW and failed to mature normally, as shown by their sensitivity to Endo-H (Fig. 2 A) (20). The high MW of mature, Endo–H-resistant proteins reflected insufficient maturation of the glycosylated proteins, as shown by their digestion with PNGase-F. Polyubiquitinylation, which has been reported to be responsible for the high MW of other misfolded proteins (21), was not involved in this case, because digestion with PNGase-F resulted in the complete normalization of MW (Fig. S1 B). Thus, the two in-frame loss-of-function IFNγR2 alleles (382-387dup in P1 and 663del27 in P2) encoded misfolded proteins that were abnormally N-glycosylated.

Maturation of the 382-387dup IFN–γR2 protein with NB-DNJ, castanospermine, or kifunensine Glucosidases I and II are involved in the folding of glycoproteins in the ER, their concomitant quality control by calnexin–calreticulin, and their subsequent trafficking (12–18). ER–mannosidase I is also involved in glycoprotein folding.

![Figure 1](jem.rupress.org)
quality control, and trafficking (Fig. 2B) (12–18). We therefore hypothesized that NB-DNJ (Zavesca) and castanospermine (22), which inhibit glucosidases I and II, and kifunensine (22), an inhibitor of ER–mannosidase I, might improve the glycosylation or trafficking of the nonnative IFN-γR2 molecules (Fig. 2B; and Table S1, available at http://www.jem.org/cgi/content/full/jem.20071987/DC1). NB-DNJ and castanospermine had no detectable impact on WT IFN-γR2 glycoproteins (MW of ~60), but most of the mutant molecules, including Endo–H–resistant proteins of high MW, displayed a shift in MW to ~60 (Fig. 2A). In contrast, kifunensine impaired the maturation of both WT and mutant molecules, which became sensitive to Endo–H (Fig. 2A). We assessed the surface expression of IFN-γR2 by biotinylating

Figure 2. Biochemical properties of IFN-γR2 under various chemical treatments. (A) Hek-293 cells were transfected with 278delAG, WT, 382-387dup, and 663del27 IFN-γR2–tagged V5 constructs. They were then incubated alone or with 1 μM kifunensine, 1.5 mM NB-DNJ, or 2 mM castanospermine for 48 h. Whole-cell extracts were generated, and left untreated or digested with Endo-H overnight. They were then subjected to SDS-PAGE and immunoblotting with an anti-V5 antibody. (B) Maturation of N-linked oligosaccharides: the triglucosylated polymannose oligosaccharides (GlcNac, yellow squares; mannose, blue circles; glucose, red circles; galactose, orange circles) transferred to the asparagine residues of proteins (green) from dolichol phosphate during the translation of mRNA on ribosomes. This oligosaccharide was then matured by incubation with a series of enzymes, including glucosidase I or II (G-I or G-II), glucosyl transferase (UGTI), ERP57, calnexin (CNX), calreticulin (Crt), ER–mannosidase I or II (ERM-I or ERM-II), M9-mannase (M9M), Golgi–mannosidase I or II (GM-I or GM-II), and glucosyl transferase I (GnT-I). Alternative pathways involving mannosidases (M*) or Golgi-endomannosidase (G-EM) are able to bypass the classical pathway. Some pathways for unfolded proteins lead to proteasomal or nonproteasomal proteolysis. (C) IFN-γR2–deficient SV40-transformed fibroblasts were transformed with the 278delAG, WT, 382-387dup, and 663del27 IFN-γR2–tagged V5 constructs, either without prior treatment or 48 h after treatment with 1.5 mM NB-DNJ, 2 mM castanospermine, or 1 μM kifunensine. Cell surfaces were biotinylated, and precipitates (IP-Strept) were analyzed by Western blotting with HRP-conjugated anti-V5 antibody. (D) IFN-γR2–deficient SV40-transformed fibroblasts were transformed with the 278delAG, WT, 382-387dup, and 663del27 IFN-γR2–tagged V5 constructs, either without prior treatment or 48 h after treatment with 1.5 mM NB-DNJ, 2 mM castanospermine, or 1 μM kifunensine. Cell surfaces were biotinylated, and total extract (−) or flow through (+) from the biotinylated cells was analyzed by Western blotting with HRP-conjugated anti-V5 antibody. White lines indicate that intervening lanes have been spliced out.
surface-expressed proteins in IFN-γR2–deficient fibroblasts (6) transfected with various V5-tagged IFN-γR2 constructs, and assessing IFN-γR2 production in total extracts, the streptavidin–bound fraction, and flow through by Western blotting with a V5–specific antibody (Fig. 2, C and D). Most WT molecules with a MW >60 were expressed at the cell surface, except in the presence of kifunensine. In contrast, only a small fraction of mutant molecules was expressed at the surface, even after treatment with NB-DNJ, castanospermine, or kifunensine. However, after treatment with any of these three drugs, mutant IFN-γR2 molecules with a MW of ~60, corresponding to the MW of WT molecules, appeared on the cell surface (Fig. 2 C). The maturation of the 382-387dup and 663del27 IFN-γR2 molecules in the presence of NB-DNJ, castanospermine, or kifunensine thus resulted in the expression of a detectable fraction of receptors at the cell surface with a MW similar to that of WT proteins under the same conditions, with 663del27 molecules being somewhat lighter owing to the deletion of nine residues. It is unclear whether the surface-expressed mutant IFN-γR2 molecules of normal MW detected in the presence of these compounds were generated by the maturation of IFN-γR2 molecules of low MW that would otherwise have been retained in the secretory pathway, or by the maturation of high MW molecules that had reached the cell surface. The time-course experiments revealed that NB-DNJ exerted its effects on MW after 9 h, whereas castanospermine and kifunensine exerted their effects only after 24 h (Fig. S2, A and B). The exact MW of the IFN-γR2 WT molecules responding to IFN-γ is unknown. We can only predict that the mutant IFN-γR2 molecules with a MW closer to that of the WT in the presence of glycosylation inhibitors are the most likely to be active. Finally, the stability and natural degradation of WT and mutant IFN-γR2 molecules were independent of the proteasome, as shown by experiments with the inhibitor MG132 (Fig. S3). Moreover, mutant and WT proteins behaved similarly in the presence of cycloheximide, a protein synthesis inhibitor (Fig. S3).

Functional complementation of the 382–387dup IFNGR2 allele in EBV-transformed B cells, SV40-transformed fibroblasts, and blood cells

We therefore investigated whether cells from P1 or P2 could be functionally complemented by culturing EBV-transformed B cells with NB-DNJ, castanospermine, or kifunensine for 16 h, and then stimulating them with IFN-γ for 30 min (Fig. 3 A). WT cells responded to IFN-γ in the presence of each of these compounds, as shown by EMSA. Cells from a negative control (C–) (6) and cells from P2 (663del27) did not respond to IFN-γ, even in the presence of these drugs. However, cells from P1 (382–387dup) regained responsiveness to IFN-γ upon treatment with NB-DNJ or castanospermine. Surprisingly, they responded to an even greater extent when treated with kifunensine, with responses reaching normal levels (Fig. 3 A). This functional complementation did not result from the trimming of extended IFN-γR2 glycans impairing IFN-γ binding to IFN-γR1, as the patient’s cells had normal numbers of IFN-γ binding sites of normal affinity (unpublished data). When EBV-transformed B cells from P1 were treated with NB-DNJ, castanospermine, or kifunensine...
gene with various tagged IFN-γ/H9253 constructs (24–26). The 382-387dup IFN-γ/H9253 molecules were not of normal MW for 16 h and were stimulated with various doses of IFN-γ for 30 min; they responded to as little as 100 IU/ml IFN-γ. Interestingly, these cells were more sensitive to IFN-γ than cells from other patients, responding normally even to 0.01 IU/ml IFN-γ (Fig. S4 B, available at http://www.jem.org/cgi/content/full/jem.20071987/DC1). We then assayed a more distal event, using flow cytometry to test the induction of HLA-DR on the surface of SV40-transformed fibroblasts. Cells from P1, unlike those from all other patients, responded normally to IFN-γ, after treatment with any of the three molecules tested (Fig. 3 B). Cells from P1, whether immortalized lymphoid and fibroblastic cell lines or freshly prepared blood cells, were complemented by modifiers of glycosylation.

We assessed the specificity of enzyme inhibition by transfecting human cells defective for the glucosidase I or glucosidase II enzymes, as they cannot produce the only commercially available compound, NB-DNJ (Zavesca). NB-DNJ restored the response of control cells to the induction of the glucosidase II substrates, including the expression of HLA-DR molecules (Fig. 3 B). Finally, we stimulated freshly prepared PBMCs with live BCG, BCG plus IFN-γ, or IFN-γ alone, and determined IL-12p40 and p70 levels by ELISA (23), in the presence or absence of the only commercially available compound, NB-DNJ (Zavesca). NB-DNJ restored the responsiveness of PBMCs from P1 (Fig. S5 and Fig. S6). Unfortunately, our patient died before we could test kifunensine. Thus, IFN-γ–deficient cells from P1, whether immortalized lymphoid and fibroblastic cell lines or freshly prepared blood cells, were complemented by modifiers of glycosylation.

Table 1. Complementation of the 382-387dup IFN-γR2 allele with multiple modifiers of N-glycosylation.

We used flow cytometry to test the induction of HLA-DR on the surface of SV40-transformed fibroblasts. Cells from P1, unlike those from all other patients, responded normally to IFN-γ, after treatment with any of the three molecules tested (Fig. 3 B). Finally, we stimulated freshly prepared PBMCs with live BCG, BCG plus IFN-γ, or IFN-γ alone, and determined IL-12p40 and p70 levels by ELISA (23), in the presence or absence of the only commercially available compound, NB-DNJ (Zavesca). NB-DNJ restored the responsiveness of PBMCs from P1 (Fig. S5 and Fig. S6). Unfortunately, our patient died before we could test kifunensine. Thus, IFN-γ–deficient cells from P1, whether immortalized lymphoid and fibroblastic cell lines or freshly prepared blood cells, were complemented by modifiers of glycosylation.

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of glucosidase I, ER–mannosidase I, or Golgi–mannosidase II alone was insufficient to complement cells from P1 (Fig. 2B and Table S1). We tested combinations of two compounds, each used at its minimal effective concentration (Fig. 4 and Table S1). No inhibitory effect was detected, but additive effects were observed for several combinations and synergic effects were observed in several cases (Fig. S8). Interestingly, no complementation was observed if australone was used with bromoconduritol or miglitol (Fig. 4), suggesting that NB-DNJ and castanospermine also inhibited another, as yet unknown, enzyme in the N-glycosylation machinery. Further evidence to support this hypothesis is provided by the synergy conferred by NB-DGJ. Nonetheless, australone and castanospermine acted in synergy, suggesting that a potent and combined inhibition of glucosidase I and II is efficient. Paradoxically, kifunensine and swainsonine complemented the cells when used together, whereas DMJ alone did not (Fig. 4). The complementation of cells from P1 may thus reflect the combined inhibition of several enzymes, such as glucosidases I and II, ER–mannosidase I, and other, as yet unknown, enzymes.

Concluding remarks
Chemical chaperones, such as DMSO and glycerol, have been reported to complement some cell lines in vitro, albeit with low specificity and high toxicity, preventing their use in clinical trials (27–30). None of these compounds or the proteasome inhibitor tested (MG132) complemented the cell lines tested in our study (unpublished data). Curcumin was reported to complement delF508-CFTR in one study (28) but not in another (30), and it did not complement the cell lines studied in this report (unpublished data). Pharmacological chaperones, designed for the specific complementation of target proteins, are less toxic and therefore more promising, but their specificity is not compatible with broad application (29, 31, 32). Our report of modifiers of N-glycosylation complementing misfolding alleles is consistent with the well-established role of glycans in protein folding and quality control in the ER. (12–16, 18). We could not assess whether the unfolded protein responses were triggered by the mutant proteins, as X-box binding protein 1–specific antibodies gave negative results in Western blots of HEK epithelial cells transfected with the different constructs, whereas we detected X-box binding protein 1 in similar amounts in WT and mutant SV40–transformed fibroblastic cells (unpublished data). Unlike chemical and pharmacological chaperones, inhibitors of glycosylation do not interact directly. Instead, they have an indirect effect via the inhibition of selected glycosylation enzymes. Our data identify no simple, specific mechanism of complementation, but they do show convincingly that multiple modifiers of N-glycosylation, alone or in combination, can efficiently complement the IFNGR2 mutation, strongly suggesting that the key process involved is the modification of N-glycosylation. Deciphering the exact molecular mechanism of action of these compounds is, however, beyond the scope of this report.

Our successful complementation of misfolding mutations by this novel class of chemical chaperone–like molecules opens up new avenues of research. Several modifiers of N-glycosylation complemented a misfolding mutation in IFNGR2. They might complement mutations in other genes. Moreover, unlike most other previously reported chemical and pharmacological chaperones, which were active in transfected or tumor cells overproducing the mutant protein, these compounds complemented germline cells in vitro and ex vivo. Our findings raise hopes that chemical modifiers of N-glycosylation could be curative in some patients bearing mutations in genes encoding proteins subject to trafficking via the secretory pathway: not only patients with gain-of-glycosylation mutations (7, 8), but also patients bearing other types of misfolding mutation. Kifunensine is a good candidate, as it has a wide window of efficacy without toxicity in vitro; unlike chaperones, which work at millimolar concentrations, it worked at below micromolar concentrations. Derivatives of castanospermine are also potentially useful (33). Nevertheless, modifiers of glycosylation should be tested carefully, as they may result in an acquired form of congenital disorders of glycosylation (26). In this context, NB-DNJ (Zavesca, also known as Miglustat) is especially promising, as this molecule has displayed no overt toxicity in human clinical trials (34) and is currently used in patients with type I Gaucher’s disease because of its ability to inhibit glucosylceramide synthase at concentrations lower than those used in our study (35–37). It will be essential to define the concentrations of modifiers of N-glycosylation that rescue ad hoc mutant proteins without compromising cell viability in vitro and in vivo.

MATERIALS AND METHODS
Affected individuals. We studied three children from three unrelated families with severe mycobacterial disease. All three families were consanguineous. P1 developed disseminated Mycobacterium avium disease at the age of 2 yr. She tested negative for HIV, CMV, and EBV in serological tests. Despite antimycobacterial treatment with rifamycin, moxifloxacin, hydroxychloroquine, clofazimine, and ethambutol, P1 died at the age of 5 yr because of dissemination and uncontrolled M. avium infection. Neither of her two brothers has ever developed mycobacterial infection. P2 developed disseminated M. avium infection, which responded to antibiotics, at the age of 3 yr. He has received continuous antibiotic treatment for the last 7 yr. P3 developed disseminated BCG vaccine infection at the age of 1 mo; he is now 2.5 yr old and is still on antibiotic treatment. P2 and P3 have been previously described (7). This study was approved by the local institutional review committee (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale), and informed consent was obtained from all families.

Cell culture and transfection. EBV-transformed B cells and SV40-transformed fibroblasts were cultured in RPMI 1640 supplemented with 10% heat-inactivated bovine fetal serum (complete medium; Invitrogen). Transfection was performed as previously described (7).

Cytokines, enzymes, and antibodies. We used recombinant nonglycosylated human IFN-γ (Imukin), recombinant IFN-α2b (R&D Systems), anti-V5 antibody (1:5,000; Invitrogen), Endo-H (New England Biolabs, Inc.), PNGase-F (New England Biolabs, Inc.), NB-DNJ (Sigma-Aldrich), castanospermine (Sigma-Aldrich), MG132 (Sigma-Aldrich), cycloheximide
(Sigma-Aldrich), streptavidin-agarose (Invitrogen), PE-conjugated mouse anti-HLA-DR antibody (Becton Dickinson), and horseradish peroxidase (HRP)-labeled anti-mouse Ig antibody (1:10,000; GE Healthcare), NB-DNJ, and australine (Qbiogene). All other drugs were obtained from Toronto Research Chemicals Inc. All chemical compounds were dissolved or suspended in water.

**Expression vectors.** We used AmpliTag DNA polymerase (Applied Biosystems) to amplify cDNA fragments encoding human IFNγ-R2 and other genes. PCR products were digested with BglII and XmnI and inserted into pcGFP-N1 (Clontech Laboratories, Inc.) or the pcDNA3 (Invitrogen) vector, with or without a V5/His Tag, by a directional topoisomerase-based method. We used a Stratagene kit, according to the manufacturer’s instructions, for direct mutagenesis.

**Cell-surface biotinylation.** 2 d after transfection, SV40-transformed fibroblasts were labeled by incubation for 30 min at 4°C in PBS (pH 8), with or without sulfo-NHS-LC-Biotin (Thermo Fisher Scientific). They were then washed twice in PBS. The cross-linking reaction was stopped by adding 50 mM NH4Cl in PBS. Cells were harvested by cell lysis in an Eppendorf tube (1.5 ml) and were washed three times with PBS. Biotinylated SV40-transformed fibroblasts were lysed with the appropriate buffer for immunoprecipitation (as recommended by the manufacturer) and incubated overnight with streptavidin-agarose. Immunoprecipitates were analyzed by SDS-PAGE, using an anti-V5-antibody and the enhanced chemiluminescence system for detection.

**EMSA.** EBV-transformed B cells were analyzed by EMSA, as previously described (7).

**Endo-H digestions and SDS-PAGE.** Endo-H digestions were analyzed by SDS-PAGE, as previously described (7).

**Flow cytometry.** The HLA-DR expression profile of SV40-transformed fibroblasts was investigated by flow cytometry, as previously described (7).

**PBMC cultures and activation by BCG.** PBMCs were obtained from whole blood by centrifugal separation through a Ficoll gradient. We cultured 2 × 10^6 cells in triplicate in 200 μl RPMI 1640, supplemented with 10% FCS per well, in a 96-well plate. The plate was incubated for 48 h at 37°C under an atmosphere of 5% CO2/95% air, and was treated with NB-DNJ or castanospermine at 37°C, with medium alone, with live BCG (M. boris BCG, Pasteur substrate) at a multiplicity of infection of 20 (BCG/leu- kocytes) and with BCG plus 5,000 IU/ml IFN-γ, as previously described (7). Final results were standardized per million PBMCs, and are expressed as pg/ml/10^6 PBMCs.

**Cytokine ELISA.** Cytokine concentrations were analyzed by ELISA using the human Quantikine IL-12p40 and IL-12p70 kits from R&D Systems and the human Pelipair IFN-γ kit from Sangum, according to the manufacturers’ instructions. Optical density was determined as previously described (7).

**Online supplemental material.** Fig. S1 depicts subcellular distribution of WT, 382-387dup, and 663del27 IFN-γR2 in SV40-transformed fibroblasts, as shown by indirect immunofluorescence on confocal microscopy with IFN-γR2-eGFP constructs. This figure also shows that the high MW of the mutant IFN-γR2 proteins results from abnormal glycosylation. Fig. S2 shows biochemical properties of IFN-γR2 molecules, in the presence of NB-DNJ, castanospermine, or kifuneninse, in a time-course experiment. Fig. S3 depicts biochemical properties of IFN-γR2 molecules, in the presence of inhibitors of the proteasome or of protein synthesis, in a time-course experiment. Fig. S4 shows an IFN-γ or IFN-α response of EBV-transformed B cells and SV40-transformed fibroblasts from a positive control, the patient, and a negative control in the presence of NB-DNJ, castanospermine, or kifuneninse. This figure also shows that the IFN-α response of EBV-transformed B cells is not altered by NB-DNJ or castanospermine treatment. Fig. S5 shows IFN-γ responses of PBMCs from a positive control and the patient in the presence of NB-DNJ and castanospermine, as assessed by quantifying the production of IL-12p40 and IL-12p70. Fig. S6 depicts IFN-γ responses of PBMCs from positive controls in the presence of NB-DNJ and castanospermine and from negative controls, as assessed by quantifying the production of IL-12p40. Fig. S7 depicts IFN-γ responses of SV40-transformed fibroblasts from a positive control, the patient, and a negative control in the presence of 13 inhibitors of N-glycosylation, as assessed by measurements of HLA-DR induction. Fig. S8 shows examples of the additive and synergistic effects of the different drugs on the patient’s IFN-γ response, as assessed by measuring HLA-DR induction in SV40-transformed cells. Supplemental discussion contains information about N-glycosylation and quality control of proteins undergoing trafficking in the secretory pathway. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20071987/DC1.

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**REFERENCES**


SUPPLEMENTAL DISCUSSION

N-glycosylation and quality control of proteins undergoing trafficking in the secretory pathway

In the secretory pathway of mammalian cells, glycosidases play a key role in trimming and editing the peptide-linked oligosaccharide, the folding of newly formed glycoproteins, and quality control for nascent proteins in the ER (Fig. 2 B) (1–9). Glucosidases I and II in the ER remove glucose residues from the common oligosaccharide of the nascent polypeptides. This step occurs early in the N-glycosylation maturation process, before other enzymes further trim and edit the glycan moieties on glycoproteins. Moreover, by producing monoglucosylated oligosaccharides, these two enzymes also contribute to the proper folding and subsequent trafficking of glycoproteins in the ER, facilitating concomitant quality control by calnexin-calreticulin. The N-glycans are then processed by ER-mannosidases (such as ER-mannosidase I), which are also involved in glycoprotein folding, quality control, and trafficking. ER-mannosidases may be involved in the proteasomal or nonproteasomal proteolysis of misfolded glycoproteins. Finally, Golgi-endomannosidases provide an alternative pathway for glycan editing, facilitating quality control by calnexin-calreticulin for glycoproteins. During this step, the proteasomal proteolysis of misfolded glycoproteins is promoted. The editing of glycoproteins continues in the Golgi apparatus, with enzymes such as Golgi-mannosidases I and II. A more comprehensive description of the N-glycosylation and quality control processes along the secretory pathway is available from the references (1–9).

REFERENCES

Table S1. Chemical compounds used and their known targets, as reported in published studies

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Chemical compounds complementing the 382–387 mutation are indicated in black (column C). The full name and abbreviation of each chemical compound are indicated. Glu-I, Glu-II, ERM-I, ERM-II, M9M, GM-I, GnT-I, and GM-II are enzymes known to be associated with maturation by N-glycosylation. The effects on these enzymes of the chemical compounds tested are indicated in color (red, inhibition; gray, no effect; white, undescribed effects on N-glycosylation enzymes).

*An inhibitor of glucosidase I (Toronto Research Chemicals Inc.).
*A potent inhibitor of α-mannosidase (Toronto Research Chemicals Inc.).
*A selective and very strong inhibitor of beta-glucosidase (Toronto Research Chemicals Inc.).
*A glucosidase inhibitor (Toronto Research Chemicals Inc.).
*A glucosidase inhibitor (Toronto Research Chemicals Inc.).

REFERENCES