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Immune tolerance strategies in siblings with infantile Pompe disease — Advantages for a preemptive approach to high-sustained antibody titers

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ABSTRACT

Enzyme replacement therapy (ERT) has led to a significant improvement in the clinical course of patients with infantile Pompe disease (IPD), an autosomal recessive glycogen storage disorder characterized by the deficiency in lysosomal α-glucosidase. A subset of IPD patients mounts a substantial immune response to ERT developing high sustained anti-rhGAA IgG antibody titers (HSAT) leading to the ineffectiveness of this treatment. HSAT have been challenging to treat, although preemptive approaches have shown success in high-risk patients (those who are cross-reactive immunological material [CRIM]-negative). More recently, the addition of bortezomib, a proteasome inhibitor known to target plasma cells, to immunotherapy with rituximab, methotrexate, and intravenous immunoglobulin has shown success at significantly reducing the anti-rhGAA antibody titers in three patients with HSAT. In this report, we present the successful use of a bortezomib-based approach in a CRIM-positive IPD patient with HSAT and the use of a preemptive approach to prevent immunologic response in an affected younger sibling. We highlight the significant difference in clinical course between the two patients, particularly that a pre-emptive approach was simple and effective in preventing the development of high antibody titers in the younger sibling, thus supporting the role of immune tolerance induction (ITI) in the ERT-naïve high-risk setting.

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1. Introduction

Enzyme replacement therapies (ERT) have significantly improved the lifespan and clinical outcomes of patients with lysosomal storage disorders and other inherited disorders, such as Pompe disease. Pompe disease is an autosomal recessive glycogen storage disorder characterized by a deficiency in lysosomal α-glucosidase (GAA) [1]. The classic infantile form of Pompe disease (IPD) results from complete or near complete deficiency of GAA leading to a severe clinical phenotype [2,3]. Untreated IPD leads to death by 2 years of age due to cardiorespiratory failure [4,5]. Recombinant human GAA (rhGAA, alglucosidase alfa, Genzyme, Cambridge, MA) was FDA approved in 2006 for the treatment of Pompe disease, and its use in IPD has significantly improved the course of disease [2,3,6–9]. The effectiveness of rhGAA treatment depends on several factors including age, stage of disease at start of treatment, and the development of anti-rhGAA IgG antibodies [10,11].

Anti-rhGAA IgG antibodies develop in 89% of IPD patients treated with rhGAA [12], with a large majority developing immunological tolerance with continued treatment [10,11]. A subset of IPD patients forms high sustained anti-rhGAA IgG antibody titers (HSAT); defined as ≥1:51,200 on two occasions ≥6 months from the start of ERT) which have been shown to negatively impact clinical outcomes [11,13]. The majority of patients with HSAT produce no native GAA enzyme and are designated cross-reactive immunological material (CRIM)-negative [14]. CRIM-positive patients produce low levels of GAA enzyme (either functioning or non-functioning), and while they are expected to form tolerance to rhGAA, a subset of CRIM-positive patients (both infantile and late onset cases) also develops HSAT [14,15].

The elimination of anti-rhGAA IgG antibodies, particularly HSAT, has been challenging. Initial immune suppression strategies (with combinations of rituximab, intravenous immunoglobulin [IVIG], plasmapheresis, methotrexate, and cyclophosphamide) have required prolonged use of these agents and have been met with limited to no success in patients with HSAT, suggesting that it is extremely hard to eliminate long-lived plasma cells. Timing of immune tolerance induction (ITI) is thus critical in order to prevent an immune response [13,16]. Prophylactic ITI has therefore been instituted in high-risk CRIM-negative patients, and these regimens have been shown to prevent the development of anti-rhGAA IgG antibodies and achieve immune tolerance [17]. The failure of ITI with previous regimens, particularly in the setting of HSAT, has been hypothesized to be due to failure to target antibody-secreting plasma cells [13]. Bortezomib, a proteasome inhibitor that is FDA approved for the treatment of multiple myeloma, depletes plasma cells

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and may mitigate damage to skeletal muscle [18–21]. Based upon these effects, bortezomib was added to an ITI regimen of rituximab, IVIG, and methotrexate and was shown to successfully eliminate HSAT in three IPD patients (two CRIM-positive, one CRIM-negative) who had concomitant improvements in clinical measures [22].

Herein we present two brothers with IPD treated with ITI—one with this four drug bortezomib-based ITI regimen in the setting of HSAT and the second as prophylaxis with the three drug ITI regimen (rituximab, methotrexate and IVIG) during initiation of treatment with rhGAA. Differences are highlighted showing the advantages of earlier treatment as well as institution of an ITI regimen in the prophylactic setting in a high-risk infant.

2. Methods

Both patients met criteria for classic IPD per standard criteria [22] and were CRIM-positive by mutation analysis and Western blot (Patient 1: Integrated Genetics, Westborough, MA; Patient 2: Duke University, Durham, NC). Anti-rhGAA IgG antibody titers were evaluated serially by Genzyme. Analysis of left ventricular mass index (LVMI, in g/m²) was performed as previously described [2]. To monitor B-cell recovery, flow cytometry was used to assess CD19% using standard methods. Both patients were enrolled in this study (# 00001562) for CRIM determination and longitudinal follow-up which was Institutional Review Board (IRB)-approved through Duke University (LDN6709 Site 206; NCT01665326). Written informed consent for ITI was obtained from the patients’ parents for this study in November 2013. Patient 1 (index case) developed HSAT and received a bortezomib-based regimen as outlined in Fig. 1 and based on published case series [22]. Patient 2 received prophylactic ITI at the time of initiation of treatment with rhGAA as outlined in Fig. 2 and as previously published [17]. Followup is reported on both patients through December 2014.

3. Results

Patient 1 was a 10-month-old African American male born full term and diagnosed as a neonate with classic IPD. Shortly after birth, he developed supraventricular tachycardia with Wolff–Parkinson–White syndrome and hypertrophic cardiomyopathy. These findings, in addition to mild macroglossia and hypotonia, prompted evaluation for IPD. Genetic testing demonstrated a missense change in allele 1 (c.2105G>T) and a nonsense mutation in allele 2 (c.2512C>T) in the GAA gene. Parental testing confirmed that each parent carried one mutation. The missense mutation in allele 1 results in leucine replacing arginine 702 (p.Arg702Leu), a nucleotide change not previously reported but predicted to be disease causing. CRIM status was determined as CRIM-positive by Western blot analysis (Integrated Genetics). Treatment with alglucosidase alfa (20 mg/kg every 2 weeks) was initiated at age 23 days with improvement in clinical status, particularly LVMI (Fig. 1). Swallow study as a neonate demonstrated aspiration, therefore he received feeds via a nasogastric tube.

Seronegative status was confirmed prior to initiation of ERT. After approximately 5 months of ERT, the patient developed detectable anti-rhGAA IgG antibodies, initially at a titer of 1:25,600 (Fig. 1). He then developed HSAT with titer of 1:102,400 on two occasions with concomitant worsening of clinical status. Treatment with a bortezomib-based ITI was initiated based on published data [22] around 11 months of age with subsequent decline in antibody titers. Three and a half months into ITI, bortezomib was repeated (due to antibody titer remaining at 1:51,200) resulting in a subsequent decline to 1:1200. CD19+ B-cells decreased to <1% of total while blood cell (WBC) count approximately 2 months from the start of ITI and remained <1% (Table 1). Rituximab was weaned to every 8 weeks beginning approximately 9 months into ITI.

Prior to initiation of ITI, the patient was not requiring respiratory support. He developed respiratory failure requiring invasive ventilation on three occasions, all due to viral respiratory tract infections (with respiratory syncytial virus, rhinovirus, and rhinovirus/adenovirus respectively) and/or aspiration. Infecation and/or aspiration in the setting of advanced disease were clinically felt to be the primary trigger for these events. Given that the patient was receiving IVIG and had normal WBC count and absolute neutrophil count throughout his admissions,
免疫抑制从ITI被临床认为具有次要的病因学作用。在第二次入院后，他被带回家中在晚上使用BiPAP。在第三次入院（约20个月大）时，由于反复的拔管失败，放置了气管造口术。胃造口术在19个月大时被放置，最终由于担心反流而转换为胃空肠管。在前几次入院时，气管和胃造口术都被推荐，但家庭不愿意进行直到第三次入院。这些程序的延迟可能加剧了反复和持续的住院。

该患者的运动发育有显著下降。在第一次入院后，患者被送入住院康复院17天，在此期间他能挺直站立和独立坐立。这些发育进步与抗体滴度的下降相吻合。在第二次入院后，他被送入住院康复院14天，在此期间他不再能挺直站立，但能直立坐着。他能用手抓物，拍手，和将物体放入口中，他能保持良好的头部控制。在最后一次由物理医学和康复医学评估（2014年12月），他无法保持头部直立或将手伸到嘴中。他能将被放进他手里的物体握在手中，但除此之外，他在四肢运动上没有明显变化。

患者2是一个3周大的非洲裔美国男性，足月出生，由于其兄弟已知的疾病状态，预产期被诊断为常规IPD。在18周的羊水穿刺中进行了基因检测，结果显示在1号（c.2105G>T）和在2号（c.2512C>T）等位基因中存在错义突变（与兄弟相同的检测）。该诊断由生后2天的血清GAA酶活性水平得到证实。基于他兄弟的疾病表型（尽管在启动ERT之前早期即开始使用INF），在第一次启动alglucosidase alfa（20 mg/kg每2周；图2）时，就启动了预防性免疫耐受诱导（ITI）法，包括利妥昔单抗，IVIG和甲氨蝶呤。在启动ERT之前，患者已经接受了五周的预防性ITI。图中显示了在启动ERT前，左心室质量指数（LVMI；g/m²，按已发表报告中定义的上正常LVMI限值为64 g/m²）达到峰值，随后下降。在ITI期间和之后的ERT中，抗体滴度一直保持较低水平。

表1
- ITI对CD19+ B淋巴细胞百分比和绝对数的影响。

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</table>

图2
- 预防性免疫耐受诱导（ITI）在患者2中的应用。患者2在启动ERT前5周接受了预防性ITI（利妥昔单抗，IVIG和甲氨蝶呤），启动了启动alglucosidase alfa（20 mg/kg每2周）。左心室质量指数（LVMI；g/m²）在启动ERT前达到峰值，随后下降。在ITI期间和之后的ERT中，抗体滴度保持较低水平。

患者2是一个3周大的非洲裔美国男性，足月出生，由于其兄弟已知的疾病状态，预产期被诊断为常规IPD。基因检测显示在1号（c.2105G>T）和在2号（c.2512C>T）等位基因中存在错义突变（与兄弟相同的检测）。该诊断由生后2天的血清GAA酶活性水平得到证实。基于他兄弟的疾病表型（尽管在启动ERT之前早期即开始使用INF），他就启动了预防性免疫耐受诱导（ITI）法，包括利妥昔单抗，甲氨蝶呤，和IVIG。该法启动时，为了防止启动ERT时首次alglucosidase alfa（20 mg/kg每2周）的抗体滴度。在开始ITI时，他已对rhGAA IgG抗体滴度的确认。随着ITI的进行，抗体滴度保持低水平，范围从1:200到1:3200。他的免疫抑制在持续治疗期间得到恢复，他的免疫抑制在持续治疗期间得到恢复，他的免疫抑制在持续治疗期间得到恢复。他正在接受物理和职业治疗，以治疗轻度发育迟缓。在12个月大时，他能独立坐立，而在18个月大时，他开始走路。他能通过口中的正常饮食。他的体重从第三百分位数增加到第15百分位数，他的身高保持在15–50百分位数。他的疫苗接种在启动ITI后暂停，但随着B细胞计数的恢复，他们已经接种完成。

图2
- 预防性免疫耐受诱导（ITI）在患者2中的应用。患者2在启动ERT前5周接受了预防性ITI（利妥昔单抗，IVIG和甲氨蝶呤），启动了启动alglucosidase alfa（20 mg/kg每2周）。左心室质量指数（LVMI；g/m²）在启动ERT前达到峰值，随后下降。在ITI期间和之后的ERT中，抗体滴度保持较低水平。
4. Discussion

ERTs have significantly improved clinical outcomes and survival in patients with many inherited diseases, but the development of immune responses to ERT can negate these effects. While CRIM-negative IPD patients are most likely to develop HSAT, they can occur in CRIM-positive patients [14,15,25]. Patients with IPD who develop HSAT have very poor treatment outcomes, and ITI regimens with rituximab, methotrexate, IVIG and bortezomib have shown success at reducing the antibody titers [22]. Without the addition of bortezomib, there has been limited to no success in the elimination of HSAT [11,13]. Given the success with the bortezomib-based regimen and the elimination of HSAT in patients with an established immune response, this regimen was initiated in Patient 1 and resulted in a 64-fold decrease in antibody titers [17,22]. This patient received only 2 cycles of bortezomib, showing rapid responsiveness to the high titers with addition of bortezomib, in contrast to prior publications without its use [13,26]. As there is significant concordance in clinical progression in classic IPD [27], the sibling was treated in the ERT-naïve setting and has continued to have low antibody titers. The two patients presented expand upon the use of a bortezomib-based regimen for HSAT and extend the use of preemptive ITI to a high-risk CRIM-positive patient, the latter of which is no different from the CRIM-negative high-risk setting. The proband developed high antibody titers despite starting ERT early (within the first month of life) further adding to the understanding, especially with newborn screening for Pompe disease on the horizon, that early ERT does not necessarily prevent the development of HSAT.

The three previously reported patients appeared to tolerate the bortezomib-based regimen well with steady clinical improvements correlating with decline in antibody titers [22]. Our Patient 1 also did not appear to have adverse events related to ITI, although he had a complicated clinical course felt to be secondary to advanced disease. At the beginning of each admission for respiratory failure, he had at least 1 virus isolated on nasopharyngeal culture, and 2 of 3 admissions were preceded by an event clinically consistent with aspiration. Aspiration was also felt to complicate his respiratory failure during each admission. His need for respiratory support appeared to escalate between admissions coinciding with continued disease progression in the setting of HSAT. Tracheostomy was discussed during each admission and gastrostomy tube during the latter two admissions, but the family preferred to continue to monitor him closely without these procedures. Delay in tracheostomy and gastrostomy tube placement may have contributed to recurrent aspiration events and admissions for respiratory failure. This highlights the importance of supportive care in these complicated patients.

While successful ITI using a bortezomib-based regimen was initiated after the development of HSAT in Patient 1, this case highlights several key observations: 1) the development of HSAT even when ERT is initiated early (in this case by age 26 days), 2) the challenges in abrogating antibody titers in the entrenched setting, and 3) the inability to completely recover from possibly irreversible muscle damage when titers are high, rendering ERT essentially ineffective. Regarding the latter point, as antibody titers take weeks to decline to clinically insignificant levels and continued accumulation of glycogen furthers muscle damage, enzyme is less likely to have an effect during this period of HSAT and disease continues to progress. These patients are also at high-risk for aspiration events and typically sensitive to minor viral respiratory infections, as evidenced by our patient’s clinical course. While the ITI regimen used is immune suppressive, this was felt to be a minor trigger for respiratory failure in our patient relative to his advanced IPD.

Comparable to that reported in high-risk CRIM negative patients, Patient 2 received a short course of three drug ITI beginning 1 day prior to the initiation of ERT at age 21 days (which is similar to the proband) [17]. There is little variability in the clinical course of IPD (in contrast to late onset disease) and significant concordance in clinical phenotype has been demonstrated between siblings (R = 0.6) [27]. Thus, it is very likely that this patient would have developed HSAT if not treated preemptively with ITI, and while the role of other minor factors in his favorable outcome cannot be excluded (such as favorable genetic polymorphisms), such factors are unlikely to have contributed significantly. The advantages of preemptive ITI are highlighted in this patient, including exposure to fewer ITI drugs, shorter duration of therapy, and improved clinical outcome by preventing prolonged exposure to HSAT. While both patients began ERT early in life, they have had significant differences in outcome related to the development (or prevention) of HSAT.

Precious time is wasted in a rapidly progressive disorder such as IPD. In patients at high-risk for developing HSAT, including CRIM-negative patients and affected siblings of CRIM-positive patients with HSAT, short course ITI with rituximab, IVIG and methotrexate can prevent the development of HSAT and improve clinical outcomes. In patients who have already developed HSAT, a bortezomib-based ITI can successfully and rapidly decrease antibody levels to a clinically insignificant level compared to regimens that do not target plasma cells. Similar to our previous report, this regimen was overall well tolerated with adverse events felt primarily to be due to advanced disease and not the ITI regimen. ITI should be initiated as early as possible, as clinical response to ERT is minimal with persistent HSAT. It is critical, therefore, that we continue to find ways to predict which patients are at high-risk for HSAT (aside from CRIM status) or treat all cases with a regimen that is safe and well tolerated so that preemptive ITI can be initiated in these patients to improve clinical outcomes.

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