Tocilizumab for the treatment of pigmentary hypertrichosis and non-autoimmune insulin-dependent diabetes mellitus (PHID)

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Abbreviations

IL	Interleukin
JIA	Juvenile Idiopathic Arthritis
PHID	Pigmentary hypertrichosis and non-autoimmune insulin-dependent diabetes mellitus Syndrome
TRAPS	Tumour Necrosis Factor Receptor Associated Periodic Syndrome

HIDS	Hyperimmunoglobulinemia D syndrome
SAA	Serum Amyloid A
CRP	C-Reactive Protein
ESR	Erythrocyte Sedimentation Rate
PGA	Physician global assessment

Contributors' Statements:

Nadia Kausar Rafiq drafted the initial manuscript, and approved the final manuscript as submitted.

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Abstract

Pigmentary hypertrichosis and non-autoimmune insulin-dependent diabetes mellitus (PHID) is associated with recessive mutations in *SLC29A3*, encoding the equilibrative nucleoside transporter hENT3 expressed in mitochondria, causing PHID and H syndromes, familial Rosai-Dorfman disease, and histiocytosis-lymphadenopathy-plus syndrome. Autoinflammation is increasingly recognized in these syndromes. We previously reported a 16-year-old girl with PHID associated with severe autoinflammation that was recalcitrant to interleukin-1 or tumor necrosis factor-α blockade. Tocilizumab is a humanized, monoclonal, anti-human IL-6 receptor (IL-6R) antibody routinely used to treat arthritis in children and adults. Herein we report the first case of successful treatment of PHID syndrome using tocilizumab. Prior to commencing tocilizumab, there was evidence of significant systemic inflammation, and progressive sclerodermatous changes (physician global assessment [PGA] 7/10). Twelve weeks after starting tocilizumab (8 mg/kg every 2 weeks, intravenously) systemic inflammatory symptoms improved, and acute phase response markers normalized: serum amyloid A reduced from 178 to 8.4mg/L. Following a dose increase to 12 mg/kg every 2 weeks energy levels, appetite, fevers, and night sweats further improved. Less skin tightness (PGA 5/10) was documented 12 months later. This excellent clinical and serological response was sustained over 48 months, and cutaneous sclerosis had improved further (PGA 3/10). Height remained well below the 0.4th centile, and tocilizumab also had no impact on her diabetes or exocrine pancreatic insufficiency. Whilst the mechanism of autoinflammation of PHID remains uncertain, we suggest that tocilizumab should be the first choice when considering treatment for the autoinflammatory and/or cutaneous manifestations of this genetic disease.

(245/250 words)

Introduction

PHID is an autosomal recessive genetic disease characterized by the childhood onset of pigmented hypertrichotic skin lesions associated with autoantibody-negative insulin-dependent diabetes mellitus. It is associated with recessive mutations in *SLC29A3* which encodes for the equilibrative nucleoside transporter hENT3 expressed in mitochondria, leading to PHID and H syndromes, familial Rosai-Dorfman disease, and histiocytosis-lymphadenopathy plus syndrome. We previously reported a case of a 16-year-old girl of Pakistani origin with pigmentary hypertrichosis and non-autoimmune insulin-dependent diabetes mellitus (PHID) syndrome, with severe systemic inflammation and cardiomyopathy which was recalcitrant to treatment with blockade of IL-1 and TNF-α [1]. IL-6 is a physiologically important cytokine involved in physiological immune responses and inflammation [2]. Abnormal continuous production of IL-6 is associated with pathogenesis of various autoimmune disorders [3-4]. Tocilizumab is a humanised, monoclonal, antihuman IL-6 receptor (IL-6R) antibody that binds to soluble IL-6R present in serum and joint fluid, as well as membrane-bound IL-6R expressed on the surface of cells, leading to inhibition of receptor-mediated IL-6 signaling and suppression of several physiological roles of IL-6 [2,4]. Tocilizumab has been used to successfully treat rheumatoid arthritis (RA), systemic JIA (sJIA) [5-6], and more recently polyarticular JIA [7]. Experience of tocilizumab for the treatment of monogenic autoinflammatory diseases is limited. We herein describe our experience of using tocilizumab for the treatment of autoinflammation associated with PHID syndrome, which to the best of our knowledge is the first report of successful response to IL-6 blockade in this rare genetic disease.

Case report

A 16.5 year old girl with PHID caused by homozygous x-x *SLC29A3* mutation [8], was born at term to consanguineous parents of Pakistani origin. She has three siblings, two unaffected; and a twenty-two-year-old female sibling who also has PHID syndrome caused by the same homozygous mutation. The full clinical course and detailed investigations have previously been reported by our group [1]. In summary, she first presented with features of PHID at the age of nine months with hyperpigmentation and hypertrichosis affecting her back and legs. Lesional skin biopsy revealed non-specific inflammatory changes. Throughout her childhood and adolescence, she had poor weight gain, stunted growth, recurrent severe pyrexias and night sweats, fatigue and chronic diarrhea. Acute phase markers were constantly elevated: erythrocyte sedimentation rate (ESR) was consistently 160 mm/h or higher; C reactive protein (CRP) was usually greater than 60 mg/L; and there was significant anaemia (Hb 60-90 g/L). At the age of four years she developed diabetes mellitus necessitating insulin treatment. Islet cell and glutamic acid decarboxylase antibodies were negative; exocrine pancreatic insufficiency was also detected around this time, and required pancreatic enzyme replacement therapy. The skin on her arms and legs showed increased tightening the typical sclerodermatous-like changes over the years. Biopsies from lymph nodes and subcutaneous tissue showed fibro-fatty connective tissue with fibrosis and mononuclear cell infiltrates around the larger vessels. Inflammatory markers such as the ESR, CRP and SAA were constantly raised. She also had persistent hepatomegaly and splenomegaly. Despite evidence of systemic involvement her cognition remained normal.

Daily prednisolone (varying from 0.2 to 1 mg/kg daily) was required for most of her early life to control systemic inflammatory features. This temporarily improved her inflammatory

symptoms and ESR; upon weaning, however, her inflammatory symptoms and acute phase markers deteriorated. She was therefore treated with high dose pulsed intravenous methylprednisolone (30 mg/kg daily for 3 days), plus subcutaneous methotrexate (15mg/m^2) in an attempt to spare the daily prednisolone dose. Nine months after commencing methotrexate there was partial improvement in skin tightness but still significant systemic inflammation. Her blood results at this point revealed elevated serum amyloid A (SAA) 178mg/L; CRP 54mg/L; and ESR 86mm/hr.

At the age of ten years, peripheral blood cytokine analysis, measured by enzyme-linked immunosorbent assay, was performed in an attempt to identify potential therapeutic targets. These revealed a normal IL-6 level of 4.9 pg/mL (reference range 0.43–8.9 pg/mL), and a raised tumor necrosis factor-α (TNF-α) of 68 pg/mL (reference <15.6 pg/mL). IL-1β has a very short half-life, and usually functions in an autocrine and/or paracrine manner; hence measurement of this cytokine in peripheral blood is usually uninformative and was thus not assessed in this case. Due to persistently raised SAA measurements ranging between 99mg/L and 127mg/L we gave her a trial of daily anakinra (IL-1 blockade), 50 mg (2.3mg/kg/day subcutaneously) for a month. This had no clinical impact on her systemic inflammatory features or serological markers of inflammation: SAA remained elevated (104-164 mg/L) 4 weeks after starting, despite a dose increase to 100 mg daily. Anakinra was subsequently stopped. Due to persistent systemic inflammation, one year later she started subcutaneous adalimumab (a humanised monoclonal antibody against TNF-α) 40 mg every 2 weeks. Despite this she continued to have severe ongoing inflammatory symptoms, and raised inflammatory markers. Adalimumab was therefore discontinued four months later.

At the age of twelve, she reported exertional dyspnoea; cardiac magnetic resonance imaging revealed hypertrophic cardiomyopathy, not typical of cardiac amyloidosis, as previously reported [1].

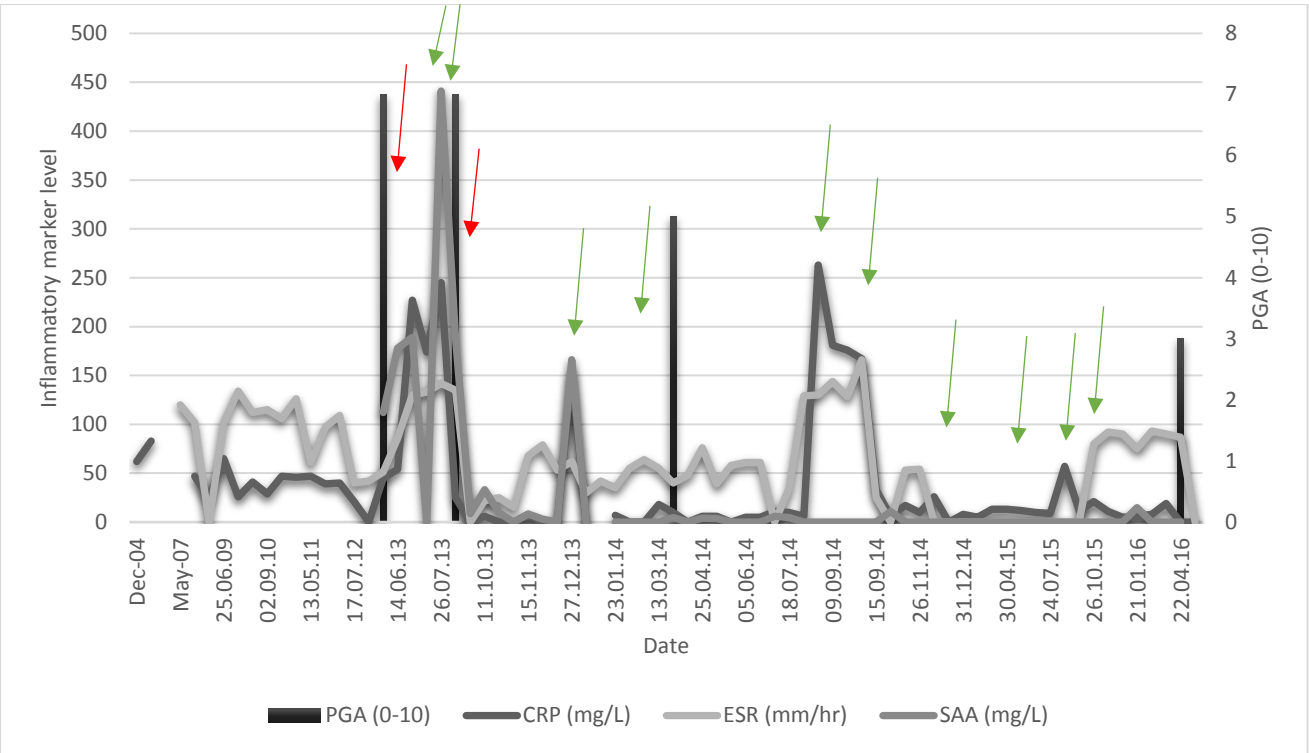
Clinical deterioration in the context of severe ongoing systemic inflammation which was recalcitrant to methotrexate, anakinra, and adalimumab necessitated consideration of empiric treatment with tocilizumab, despite normal circulating levels of IL-6. To assess her clinical response to this treatment we evaluated improvement based on patient reported improvement in fever episodes and night sweats; energy levels; growth; clinical response of the sclerotic skin changes using physician global assessment (PGA) of severity on a 10-point scale, 0 indicating no sclerotic skin disease, 10 signifying severe sclerotic skin disease; and change in acute phase markers (CRP, ESR, and SAA). Change in hypertrichosis was not possible to evaluate, since the patient regularly removed the hair from cutaneous lesions. Intravenous tocilizumab was started at 8mg/kg, every 2 weeks, initially combined with methotrexate (15 mg/m^2 per week) and prednisolone (0.4 mg/kg/day). At 12 weeks of treatment her inflammatory markers improved significantly: CRP <5mg/L, ESR 22mm/hr and SAA 8.4mg/L, although PGA of the skin remained 7/10. Her dose was therefore increased to 12mg/kg every two weeks. Almost immediately she reported marked clinical improvement in her energy levels, improved appetite, much improved fevers and night sweats (from greater than 5 episodes per week to approximately 1 per week) and subjectively less skin tightness. Prednisolone was gradually weaned and the methotrexate was discontinued nine months after commencing tocilizumab due to continued excellent clinical and serological response. Ten infections occurred following treatment with tocilizumab. These were mostly respiratory tract infections of presumed viral cause, four of them being defined as serious requiring hospital admission and empiric

intravenous antibiotics. The only positive microbiology for these infections was a positive urine for *E.Coli* on one occasion. There were no other reported adverse events.

The serological and cutaneous response to tocilizumab, and episodes of infection are summarised in Figure 1. At final follow up aged 16.5 years, height (131.7 cm) and weight (35.9 kg) remained well below the 0.4^{th} centile despite the fact she has also been commenced on growth hormone treatment. She remains under cardiac review, and although she continues to have biventricular hypertrophy, her global systolic function is currently preserved.

Figure 1: Serological and cutaneous response to tocilizumab in PHID

Serological response (left Y axis) to tocilizumab in PHID. First dose of tocilizumab (8 mg/kg), and subsequent dose increase to 12 mg/kg are indicated by the red arrows. Infectious episodes are indicated by green arrows, and correspond to peaks in acute phase markers. Physician global assessment of sclerotic skin changes (right Y axis) is denoted by the black line.



Discussion

PHID syndrome has an important autoinflammatory component that is increasingly recognised in the context of the other cardinal features of the disease, namely pigmented hypertrichosis (usually with sclerodermatous changes), and both endocrine and exocrine pancreatic insufficiency. Virtually nothing is known about the mechanism of autoinflammation in PHID and related diseases caused by mutation in *SLC29A3*.One study showed that the inflammatory response involves activation of NF-κB (nuclear factor κ-light-chain-enhancer of activated B cells), a major transcription factor responsible for the regulation of cytokine production, suggesting that there could be increased production of TNF-α and other major pro-inflammatory cytokines [9]. In support of this, we documented significant elevation of serum

TNF-α, but not IL-6. Alternatively, nucleoside accumulation secondary to loss of function of hENT3 nucleoside transport activity in macrophages could contribute to excess cytokine production and immune activation [10-12]. In support of this, Hsu et al. [13] demonstrated altered macrophage function in a murine model of PHID, the ENT3 knockout mouse. ENT3 is an essential transmembrane transporter maintaining lysosomal integrity by regulating nucleoside trafficking. ENT3 deficiency, through defects in the lysosomal system, caused ineffective apoptotic cell clearance and increased macrophage colony-stimulating factor signaling that contributed to elevated numbers of macrophages and histiocytosis. These findings could provide an important cellular and molecular mechanism for the histiocytosis and autoinflammation seen in humans with PHID, and might predict that blockade of IL-1 and TNF-α would be ineffective, as in our case, and another case recently described [14].

We report the first case of PHID treated with IL-6 blockade, with successful outcome in relation to systemic inflammation, cutaneous skin lesions, and overall quality of life, which has so far been sustained over 48 months. Linear growth, diabetes and exocrine pancreatic insufficiency did not improve, however. Despite relatively mild infectious adverse events that we have documented (which are well known to be associated with tocilizumab in children and adults), the overall improvement in quality of life has been dramatic for this patient. Whilst the mechanism of autoinflammation of PHID remains uncertain, this case highlights an important role for IL-6 in the pathogenesis of autoinflammation associated with recessive mutations in *SLC29A3*. Our case also highlights yet again that peripheral blood cytokines are poor biomarkers on which to base predictions regarding efficacy for empiric treatments [15]. We suggest that tocilizumab should be the first choice when considering treatment of the autoinflammatory component of this extremely rare genetic disease. At the time of writing, her similarly affected sister is now also commencing tocilizumab.

Written informed consent was taken for the publication of this report.

(1580/1600 words)

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