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Elevated TNF-α is associated with pain and physical disability in mucopolysaccharidosis types I, II, and VI

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ABSTRACT

Background: Children and adults with the lysosomal storage diseases mucopolysaccharidosis (MPS) types I, II and VI live shortened lives permeated by chronic pain and physical disability. Current treatments do not alleviate these problems. Thus there is a critical need to understand the mechanism of chronic pain and disability in MPS in order to improve the way we treat patients. A potential target is inflammation.
Hypothesis: We hypothesized that excessive inflammation mediated by the tumor necrosis factor-α (TNF-α) inflammatory pathway is the fundamental cause of much of the chronic pain and physical disability in MPS.
Methods: 55 patients with MPS I, II, or VI were enrolled over the course of a 5-year prospective longitudinal natural history study and evaluated annually for 2–5 years. 51 healthy controls were enrolled in a separate cross-sectional study of bone and energy metabolism. TNF-α was measured by ELISA. Pain and physical disability were measured by the Children's Health Questionnaire – Parent Form 50 (CHQ-PF50). Differences in log-transformed TNF-α levels and associations with CHQ domains were evaluated using a linear mixed effects model with random intercept.
Results: TNF-α levels were measured in 48 MPS (age: 5–17 years; 35% female) and 51 controls (age: 8–17 years; 53% female). Among MPS, 22 (46%) were treated with hematopoietic cell transplantation (HCT) alone, 24 (50%) with enzyme replacement therapy (ERT) alone, and 2 (4%) with both HCT and ERT. TNF-α levels are higher in MPS compared to healthy controls ($p < 0.001$). Higher TNF-α levels are associated with increased pain and decreased physical function, social limitations due to physical health, and physical summary score (all $p < 0.05$). TNF-α levels were not significantly associated with the general health score. TNF-α levels did not change significantly over time in MPS.
Conclusions: Higher TNF-α levels are implicated in the pain and decreased physical function present in individuals with MPS despite treatment with ERT and/or HCT, suggesting that TNF-a inhibition could potentially be a useful adjunctive therapy. Further investigation into the role of TNF-α inhibition in MPS to decrease pain and improve physical function is indicated.

1. Introduction

Children with the rare genetic disease mucopolysaccharidosis (MPS) continue to suffer and prematurely die from their disease. Enzyme replacement therapy (ERT) and/or hematopoietic cell transplantation (HCT) can help kids with MPS live longer, but these treatments are not cures, and only partially alleviate suffering. Neither ERT nor HCT adequately treats musculoskeletal disease, cardiac valvular disease, or central nervous system disease [1–17]. For example, individuals with MPS I, II and VI treated with ERT and/or HCT have progressive skeletal disease including hip dysplasia, kyphoscoliosis, bone density deficits, and short stature that is generally resistant to treatment with growth hormone [13–15, 18, 19], as well as progressive joint contractures, stiffness, and genu valgum [6, 8, 9, 11, 20, 21] despite these treatments. In fact, the majority of these individuals report chronic pain and have significant limitations in their activities of daily living due to their musculoskeletal disease. Thus there is a critical need to understand the causes of pain and physical disability in order to improve the way we treat MPS.

TNF-α is a potential therapeutic target. TNF-α is involved in a variety of inflammatory pathways that have destructive results such as

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increased endothelial permeability, inflammatory cell migration, matrix metalloproteinases (MMPs), and prostaglandins [22]. Elevated levels of TNF-α and other inflammatory markers in this pathway have been reported in animal models of MPS [23–26]. Importantly, decreasing inflammation through treatment of MPS animals with anti-TNF-α medications or another anti-inflammatory medication, has demonstrated improvements in mobility and exercise tolerance, resolution of joint inflammatory changes, and increased bone length [25–28]. In addition, the cardiovascular disease, which is common in MPS despite treatment with ERT and/or HCT, is likely inflammatory in nature as well [2, 29, 30].

For this first study of the role of TNF-α in humans with MPS, based on these preclinical data, we hypothesized that 1) TNF-α levels would be higher in individuals with MPS compared to healthy controls and 2) that elevated levels of TNF-α would be associated with more severe pain and physical disability due to more severe skeletal disease and activation of inflammatory pathways. We used data from a 5-year longitudinal observational natural history study of MPS I, II and VI and from a separate cross-sectional study of bone and energy metabolism in healthy children and adolescents to test these hypotheses.

2. Methods

Children and adolescents with MPS aged 5–17 years were enrolled in two 5-year longitudinal observational studies and evaluated on an annual basis. Inclusion criteria included diagnosis of MPS I, II, or VI, ability to travel to the study center, and English speaking. Exclusion criteria included pregnancy and inability to comply with study procedures. A healthy cohort age 8–17 was also enrolled from the local community; exclusion criteria for this cohort were diabetes, medications that altered insulin sensitivity, secretion or beta cell mass, concurrent participation in an intervention trial, and pregnancy. The University of Minnesota Institutional Review Board approved both studies. All clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki. Written informed consent was obtained from the participants.

TNF-α levels were measured annually for 2–5 years in MPS and at one time-point in the healthy cohort. Pain and physical function were measured with the Children's Health Questionnaire – Parent Form 50 (CHQ-PF50) annually for 2–5 years in the MPS group. In MPS participants, data are included from the following number visits: 1 visit for 12 (25%) participants, 2 visits for 9 (18%) participants, 3 visits for 14 (29%) participants, 4 visits for 12 (24%) participants, and 5 visits for 2 (4%) participants.

TNF-α was measured in plasma by enzyme-linked immunosorbent assay (ELISA) at the University of Minnesota Cytokine Reference Lab. The CHQ-PF50 evaluates parent-report of both severity and frequency of their child's bodily pain and limitations in their child's physical function in activities such as play, getting around school, climbing stairs, and taking care of activities of daily living (e.g. eating, dressing, bathing, going to the toilet). BMI was calculated by weight (kg) divided by height squared (m^2).

2.1. Statistical analysis

Descriptive analyses of baseline characteristics and outcomes included means and standard deviations for continuous variables and frequencies for categorical variables. Differences in log-transformed TNF-α levels were evaluated using a linear mixed effects model with random intercept to account for the correlated nature of multiple measurements from the same individual. Similarly, associations of log transformed TNF-α with CHQ domains were also evaluated using linear mixed models with random intercept. The distribution of TNF-α levels among individuals treated with ERT versus those treated with HCT was very similar and therefore these groups were grouped together for all analyses. Statistical significance was considered as $p < 0.05$. All statistical analyses were conducted using R v.3.1.1 [31].

Table 1
Population characteristics at baseline. Mean ± SD or N (%) are presented.

Covariate	Control (N = 51)	MPS (N = 49)	MPSIH (N = 22)	ERT MPS (N = 27)
Female	27 (53%)	17 (35%)	13 (59%)	4 (15%)
Male	24 (47%)	32 (65%)	9 (41%)	23 (85%)
MPS IH[a]	0 (0%)	22 (45%)	22 (100%)	0 (0%)
MPS IA	0 (0%)	8 (16%)	0 (0%)	8 (30%)
MPS II	0 (0%)	12 (25%)	0 (0%)	12 (44%)
MPS VI[a]	0 (0%)	7 (14%)	0 (0%)	7 (26)
Race				
White	33 (64%)	45 (92%)	22 (100%)	23 (84%)
Black	5 (10%)	1 (2%)	0 (0%)	1 (4%)
American Indian	1 (2%)	1 (2%)	0 (0%)	1 (4%)
Asian or Pacific Islander	0 (0%)	1 (2%)	0 (0%)	1 (4%)
Other or mixed	7 (14%)	1 (2%)	0 (0%)	1 (4%)
Unknown/not reported	5 (10%)	0 (0%)	0 (0%)	0 (0%)
Tanner stage[b]				
1	3 (6%)	25 (51%)	13 (59%)	12 (44%)
2 or 3	9 (18%)	8 (16%)	4 (18%)	4 (15%)
4 or 5	39 (76%)	14 (28%)	5 (23%)	9 (33%)
Age	14.6 (2.0)	11.2 (4.3)	9.4 (3.3)	12.7 (4.5)
BMI[b]	27.1 (8.6)	19.8 (4.6)	18.6 (3.9)	20.8 (4.9)
Bone age[b]	15.1 (2.1)	10.0 (4.7)	8.9 (4.1)	10.9 (5.0)
TNF-alpha	0.9 (0.4)	2.4 (6.7)	1.4 (1.1)	3.1 (9.0)

MPS = mucopolysaccharidosis, ERT = enzyme replacement therapy, BMI = body mass index, TNF-alpha = tumor necrosis factor-alpha, IH = Hurler syndrome, IA = Hurler-Scheie or Scheie (attenuated), II = Hunter syndrome, VI = Maroteaux-Lamy syndrome.
[a] All MPS IH treated with HCT, 2 MPS VI treated with HCT, remainder of subjects treated with ERT for >1 year.
[b] Missing data on Tanner stage and bone age in two and BMI in one MPS subject(s).

3. Results

Characteristics of the MPS and healthy control cohorts are detailed in Table 1. In brief, there was a greater percent female in Controls, which is expected since MPS II is an X-linked disease. There was also a greater percent of non-white and higher body mass index (BMI) in Controls. Finally, Controls were more advanced in their pubertal stage.

TNF-α is significantly higher in children with MPS compared to healthy children (Fig. 1, $p < 0.001$). Higher TNF-α levels were associated with more pain, decreased physical function, increased social limitations due to physical disability, and overall decreased quality of life

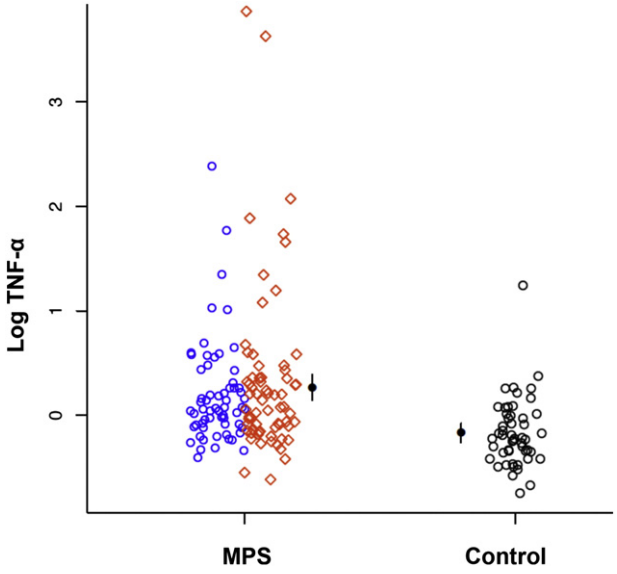

Fig. 1. Comparison of TNF-α levels (repeated measurements over 2–5 years) in MPS (blue circles = HCT treated; red diamonds = ERT treated) versus healthy controls. Mean and 95% confidence intervals are indicated by filled circle and vertical lines.