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Characterization of a "high" TNF-α phenotype in moderate-to-severe asthmatic children

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Summary

Systemic TNF-α expression is increased in a subset of children with moderate-to-severe asthma despite aggressive corticosteroid treatment and is associated with poor asthma control. Phenotypic-directed TNF-α inhibition may be of benefit in some asthmatic children.

Keywords

Severe asthma; Children; Phenotype; Tumor necrosis factor alpha (TNF-α); Nuclear factor kappa B (NF-κB); Inflammation

To the Editor

Severe asthma is a heterogeneous disorder associated with multiple clinical phenotypes. Recognizing that the treatment of severe asthma may require targeted biologic approaches, we have read with interest reports of tumor necrosis factor alpha **(TNF-α)** antagonism in asthmatic adults.[1, 2] TNF-α is a pleiotropic cytokine that binds to the type 1 tumor necrosis factor receptor and upon activation, induces canonical activation of nuclear factor kappa B **(NF-κB)**. While previous studies have demonstrated increased expression of both TNF-α and NF-κB in patients with severe asthma,[1, 3, 4] non phenotypic-directed antagonism of TNF-α in general populations of moderate-to-severe asthmatic adults has failed to improve asthma outcomes.[2] However, smaller, non-placebo-controlled studies of adults with corticosteroid-refractory asthma and high TNF-α expression have demonstrated clinical improvements with TNF-α antagonism,[1, 4] suggesting that treatment efficacy may be limited to selected phenotypes.

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Given that children are not "small adults" and that children with asthma may require unique pharmacologic interventions,[5] the purpose of this study was to quantify expression of TNF-α in children with moderate-to-severe persistent asthma and to further assess the associations between increased TNF-α expression and clinical features. While children with moderate-to-severe asthma as a whole were characterized by increased TNF-α and NF-κB expression, we report that a subpopulation with "high" TNF-α expression (despite intensive corticosteroid therapy) is distinguished by the highest degree of symptoms and healthcare utilization. These findings highlight the multiple endotypes that likely exist within the severe asthma population and suggest that phenotypic-directed therapy may be warranted for these children.

Healthy non-asthmatic children age 6-17 years and children with asthma confirmed by either ≥ 12% reversibility in the forced expiratory volume in one second **(FEV_1)** after albuterol administration or airway hyperresponsiveness to methacholine were enrolled from a severe asthma clinic in Atlanta, Georgia. Criteria for mild asthma included no treatment with asthma controller medications for at least one month and well-controlled asthma evidenced by an Asthma Control Questionnaire Score **(ACQ)** < 0.75.[6] Children were classified as having moderate-to-severe asthma if they were receiving Step 5 or Step 6 therapy according to the National Asthma Education and Prevention Program asthma treatment guidelines (Online Repository Table E1). [7] Children treated with Step 4 therapy were also eligible provided they had poor asthma control evidenced by either an ACQ score ≥ 1.25,[6] daytime asthma symptoms more than twice weekly, or nighttime asthma symptoms at least once per week. All children underwent phenotypic characterization as described in the **Online Repository.** A subset of children with moderate-to-severe asthma further underwent formal corticosteroid responsiveness testing with systemic triamcinolone acetonide.

Peripheral blood mononuclear cells **(PBMCs)** were isolated from whole blood through a density gradient. Plasma TNF-α concentrations were quantified using a commercially available, high-sensitivity kit with a detection limit of 0.106 pg/mL (R&D Systems, Minneapolis, Minnesota). PBMC mRNA gene expression was determined with a real-time polymerase chain reaction array (Human Antibacterial Response RT^2 *Profiler*,™ SABiosciences, Frederick, MD) after isolation of RNA using a commercial kit (RNeasy® Mini, Qiagen, Valencia, California). 8 μl of the first-strand cDNA synthesis reaction was pre-amplified (RT^{2}PreAMP PCR Master Mix and RT^2 PreAMP Human Antibacterial Response Primer Mix, SA Biosciences) and the equivalent of 0.8 μl cDNA was added to each well of the array plate. Genes of interest included *CHUK*, *IKBKB*, *NFKB1*, *NFKBIA* and *TNF* (Online Repository Table E2). The cycle threshold **(CT)** values of the target cDNAs for each subject were normalized to the average CT of 5 housekeeping genes (*GAPDH, ACTB, RPL13A, B2M* and *HPRT1*).

Seventy-two children, including 15 healthy non-asthmatic children, 8 children with mild, well-controlled asthma and 49 children with moderate-to-severe asthma were included in the final analyses (Online repository Figure E1). Features of the groups are shown in Table 1. Children with moderate-to-severe asthma were more likely to be obese males and had increased serum IgE and exhaled nitric oxide concentrations, greater airflow obstruction, poorer asthma control and decreased asthma-related quality of life despite a median dose of

1000 mcg of inhaled fluticasone per day (mean ± SD, 818 ± 287 mcg). Children with moderate-to-severe asthma also had higher plasma TNF-α concentrations and increased mRNA expression of TNF-α and other genes downstream of TNF-α activation and binding, including *NFKB1*. (Figure 1).

Given the heterogeneity in TNF-α expression levels observed in children with moderate-to-severe asthma and the overlap in TNF-α values between groups, a TNF-α cut-point of 1.25 pg/mL was selected for further stratification. This cut-point had sensitivity and specificity of 59% and 91%, respectively, for detection of moderate-to-severe asthma as compared to the other groups (Online Repository, Figure E2). The demographic features of the resulting TNF-α "low" and TNF-α "high" groups were not statistically different (Online Repository, Table E3). However, moderate-to-severe asthmatic children with "high" TNF-α concentrations were characterized by increased healthcare utilization in the previous year, poorer symptom control, and greater airflow obstruction than children with "low" TNF-α concentrations (Online Repository, Table E4). In the subset of children who underwent treatment with systemic triamcinolone (Online Repository, Table E5), TNF-α concentrations remained stable in most children, although a few outliers were noted (Online Repository, Figure E3). Similar results were also noted when a TNF-α cut-point of 1.05 pg/mL was used, although some significance was lost (Online Repository, Table E6).

These preliminary observations suggest that, similar to studies in adults,[1, 4] there is a subpopulation of moderate-to-severe asthmatic children with persistently high TNF-α expression and poor asthma control despite aggressive corticosteroid treatment who might benefit from anti-TNF-α therapy. Although our focus on systemic TNF-α expression may be criticized, we[8] and others[9] have previously described systemic inflammatory alterations in severe asthma that are similar to those in the airways. The use of PMBCs also permitted the inclusion of a healthy children and children with mild asthma. Nonetheless, these preliminary findings highlight a potential "endotype" or mechanism within the larger, heterogeneous severe asthma population that may contribute to poor asthma control. However, additional studies on TNF-α polymorphisms, TNF-α expression in other resident cells, and the longitudinal stability of TNF-α expression in relation to key asthma clinical outcomes such as exacerbations may be useful.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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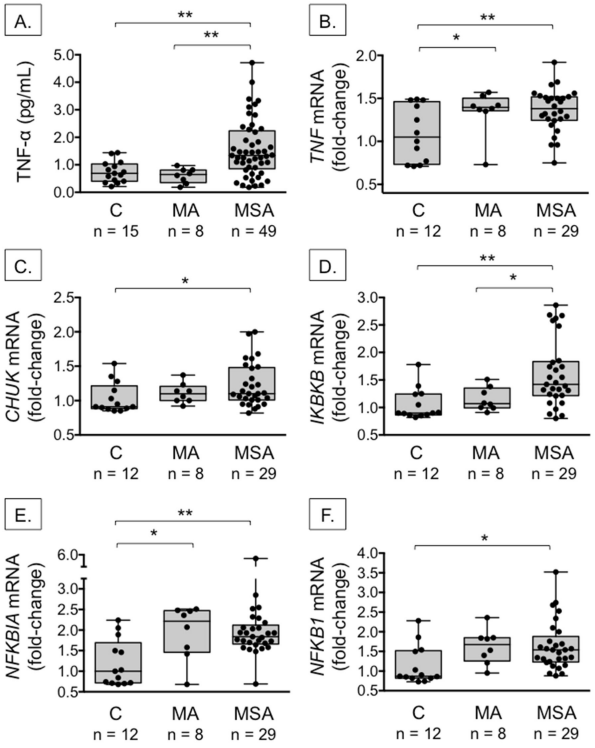

Figure 1.

(A) Plasma concentrations of TNF-α and mRNA gene expression of (B) *TNF*, (C) *CHUK*, (D) *IKBKB*, (E) *NFKBIA*, and (F) *NFKB1* in healthy controls ("C"), children with mild asthma ("MA"), and children with moderate-to-severe asthma ("MSA"). Horizontal lines represent the median. *$p < 0.05$, **$p < 0.01$

Table 1

Features of the participants. Data are shown as the median (interquartile range) or the number (percent).

	Healthy controls N = 15	Mild, well-controlled asthma N = 8	Moderate-to-severe asthma N = 49
Age (years)	10 (8 – 11)	12 (9 – 17)	12 (10 – 15)
Males	3 (20)	4 (50)	35 (71) §
Race			
White	1 (7)	0	4 (8)
Black	11 (73)	8 (100)	38 (78)
More than one race	3 (20)	0	7 (14)
Body mass index percentile >95%	0	1 (13)	17 (35) §
Asthma duration (years)	--	6 (3 – 17)	11 (7 – 14)
Serum IgE (kU/L)[1]	46 (26 – 68)	34 (22 – 200)	204 (70 – 567) §¶
Exhaled nitric oxide (ppb)[1]	11 (8 – 12)	22 (13 – 56) §	28 (16 – 54) §
Pulmonary function			
FVC (% predicted)	100 (89 – 115)	106 (93 – 121)	103 (91 – 114)
FEV_1 (% predicted)	99 (93 – 110)	96 (84 – 114)	89 (76 – 102) §
FEV_1/FVC	0.88 (0.84 – 0.94)	0.84 (0.74 – 0.90)	0.76 (0.70 – 0.83) §
FEF_{25-75} (% predicted)	98 (82 – 122)	89 (55 – 109)	67 (44 – 79) §
ACQ score[2]	--	0.14 (0 – 0.29)	1.29 (0.57 – 1.86) ¶
AQLQ score[2]	--	6.86 (6.58 – 6.98)	5.52 (4.76 – 6.32) ¶

[1] Data were logarithmically transformed prior to analyses.

[2] ACQ = Asthma Control Questionnaire. Scores on the ACQ range from 0 to 6, with lower scores indicating better asthma control and 0.5 as the minimal important difference

[3] AQLQ = Asthma Quality of Life Questionnaire. Scores on the AQLQ range from 1 to 7, with higher scores indicating better quality of life and 0.5 as the minimal important difference.

§ $p < 0.05$ versus controls,

¶ $p < 0.05$ versus mild asthma