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Tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) and receptors in type 1, type 2 and type 17 inflammation in cross-sectional asthma study

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ABSTRACT

Tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) reportedly promotes, or conversely, resolves inflammation in asthma. In this study of TRAIL and cell receptors in sputum, bronchoalveolar lavage and biopsy from subjects in the Severe Asthma Research Program at Wake Forest, the high TRAIL group had significant increases in all leucocytes, and was associated with increased type 1, type 2 and type 17 cytokines, but not type 9 interleukin 9. Two variants at loci in the TRAIL gene were associated with higher sputum levels of TRAIL. Increased TRAIL decoy receptor R3/DcR1 was observed on sputum leucocytes compared with death receptor R1/DR4, suggesting reduced apoptosis and prolonged cellular inflammation.

BACKGROUND

Tumour necrosis factor-related apoptosis-inducing ligand (TRAIL/TNFSF10) is a cytokine of the tumour necrosis factor (TNF) superfamily with a potential role in allergic asthma.[1 2] TRAIL induces apoptosis in a variety of cells thereby resolving inflammation.[1 3] Conversely, TRAIL has been shown to promote eosinophil survival in patients with asthma following segmental antigen challenge.[2] These counteracting effects suggest divergent roles for TRAIL in lung diseases.[4] Response to TRAIL depends on ligand interaction with five TRAIL receptors. TRAIL R1/DR4 and R2/DR5 contain a 'death domain' and lead to apoptosis. TRAIL R3/DcR1, R4/DcR2 and soluble decoy osteoprotegerin are truncated without a 'death domain', prevent cell apoptosis,[2] but may result in non-canonical signalling through the receptor-interacting serine/threonine-protein kinase 1, TNF receptor-associated factor 2 and inhibitor of NF-κ subunit gamma.[4] Genetic variation in the TNFSF10 gene has been associated with asthma, but whether variation altered protein levels was unexplored.[5]

We previously examined TRAIL levels in bronchoalveolar lavage (BAL) fluid, and TRAIL receptors on BAL leucocytes, in eosinophilic inflammation following segmental allergen challenge.[2] Weckmann *et al* reported increased TRAIL in sputum from asthmatics compared with controls, and activation of type 2 inflammation via CCL20/MIP3α.[6] A TRAIL−/− mouse model of allergic asthma had reduced airway remodelling, including peribronchial fibrosis, smooth muscle hypertrophy and mucus hypersecretion,[7] but without confirmation in humans.

We recently reported TRAIL/TNFSF10 associated with increased bronchial epithelial cells in sputum from asthmatics, and found TRAIL more strongly associated with Th1 cytokines, such as interleukin 6 (IL6), CXCL9, CXCL10, and CXCL11.[8] Our objectives here were to examine whether TRAIL in sputum or BAL was characteristic of more severe asthma, including airway remodelling; whether differential expression of TRAIL receptors indicated imbalance between apoptotic and non-canonical signalling in immune cells; and whether genetic variants in TRAIL related to increased TRAIL.

MATERIALS AND METHODS

Non-smoking (<5 pack years) Wake Forest subjects with asthma (American Thoracic Society,ATS criteria) underwent comprehensive phenotypic characterisation as approved by Institutional Review Board (IRB00021507); samples from sputum induction (n=116),[8 9] and in subjects consenting to bronchoscopy, BAL and biopsies (n=59)[10] were obtained. Observed sputum TRAIL values spanned more than three log values (minimum 2 pg/mL to maximum 3473 pg/mL); both median (422 pg/mL) and mean (653 pg/mL) divided the cohort into nearly equal numbers for low and high TRAIL groups (median: n=58 each low and high; mean: n=59 and n=57, low and high groups, respectively). Subjects were therefore stratified into low and high TRAIL groups based on the higher mean concentration. Standard parametric or non-parametric statistical tests were performed ($p<0.05$ accepted as significant). Details are provided in the online supplementary.

RESULTS

Subjects with high sputum TRAIL levels had lower maximal FEV_1 per cent predicted ($p=0.043$) and a higher maximum bronchodilator reversal ($p=0.046$) compared with patients with low TRAIL levels (table 1).

In addition, there was a trend towards lower prebronchodilator FEV1/FVC in the high TRAIL group ($p=0.071$). Spearman correlation analyses of lung function variables with TRAIL found weak but significant negative associations with FVC%predicted ($\rho=-0.191$; $p=0.062$) and with FEV1/FVC ratio ($\rho=-0.457$, $p<0.0001$). Age, gender, race, age of asthma onset or duration of disease did not differ.

TRAIL high and low groups did not differ for proportion of subjects identified as severe

Table 1 Demographics and clinical characteristics of subject cohort and stratified by tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) high and low levels in sputum

	Whole cohort	TRAIL low group	TRAIL high group	P value high versus low
N	116	59	57	
Age (year)	36.89±12.0	37.4±12.0	36.4±12.1	0.327
Asthma duration (year)	22.6±12.2	23.4±12.8	21.7±11.6	0.237
BMI	30.0 (25.1–36.4)	30.3 (24.8–36.5)	29.8 (25.3–36.3)	0.804
Age onset (year)	11 (4–22)	10 (3–22)	12 (4.5–22)	0.576
Baseline FEV_1 %prd	76.6±17.4	79.1±16.9	74.1±17.6	0.118
Baseline FVC %prd	88.9±15.9	91.9±17.4	86.7±14.6	0.113
preBD FEV_1/FVC	0.74 (0.65–0.79)	0.75 (0.70–0.81)	0.73 (0.62–0.77)	*0.071*
Max FEV_1 %prd	89.6±15.8	93.4±15.9	86.9±15.2	**0.043**
Max FVC %prd	97.0±15.4	98.9±18.2	95.6±12.9	0.302
Max reversal	11.3 (7.6–20.2)	9.7 (6.8–18.1)	12.9 (8.5–24.4)	**0.046**
PC20	1.0 (0.25–3.12)	1.1 (0.3–3.8)	0.8 (0.2–2.4)	0.409
IgE	169 (62–388)	129 (49–285)	188 (96–413)	0.126
FeNO	27±2.3	26±2.4	29±2.2	0.503
Gender (% female)	68	75	61	0.186
Race (%C/%AA/%other)	55/40/5	59/36/5	51/44/5	0.423
Number + Skin Prick Tests to 14 allergens	4 (2–7)	4 (2–6)	4 (2–7)	0.544
Sputum WCC count (× 10^6/mL)	0.95 (0.56–2.10)	0.70 (0.32–1.20)	1.90 (0.86–3.31)	**<0.001**
Macro/mono count (×10^4/mL)	41.5 (18.03–87.24)	28.6 (13.5–61.7)	49.1 (31.5–124.6)	**<0.001**
Lymphocyte count (×10^4/mL)	1.13 (0.30–3.47)	0.68 (0.24–1.91)	2.42 (0.67–5.34)	**0.002**
Neutrophil count (×10^4/mL)	38.6 (10.6–94.5)	21.4 (7.9–52.0)	70.8 (32.2–160.1)	**<0.001**
Eosinophil count (×10^4/mL)	0.71 (0.11–4.54)	0.60 (0.001–1.91)	1.08 (0.17–8.35)	**0.030**
IL-4 (pg/mL)	4.55±8.51	1.16±4.70	2.34±4.39	**0.045**
IL-5 (pg/mL)	1.76 (1.18–3.32)	1.29 (1.02–2.14)	2.43 (1.38–5.00)	**0.001**
IL-13 (pg/mL)	1.97 (1.20–2.76)	1.61 (0.94–2.28)	2.51 (1.53–3.76)	**0.001**
IL-33 (pg/mL)	3.93 (2.34–5.84)	3.05 (1.52–4.80)	4.63 (3.05–7.23)	**0.001**
CCL5/RANTES (pg/mL)	14.11±23.25	2.46±4.47	8.51±4.09	**0.001**
CCL11/Eotaxin (pg/mL)	16.5±9.98	11.91±1.59	16.60±1.70	**0.002**
IL-9 (pg/mL)	0.56 (0.34–0.88)	0.54 (0.29–0.90)	0.60 (0.39–0.87)	0.594
IL-10 (pg/mL)	2.92 (1.71–4.36)	2.35 (1.50–3.46)	3.49 (2.13–6.11)	**0.001**
IL-17A (pg/mL)	1.09 (0.43–2.08)	0.84 (0.43–1.37)	1.47 (0.73–3.33)	**0.002**
IL-23 (pg/mL)	34.11 (15.96–59.60)	27.86 (15.60–53.21)	40.46 (26.03–70.96)	**0.021**
IFNg (pg/mL)	2.54 (1.55–4.87)	2.22 (1.24–3.93)	3.66 (1.90–5.74)	**0.013**
TNFa (pg/mL)	4.62 (1.98–9.45)	2.96 (1.56–5.53)	7.00 (3.73–13.00)	**0.001**

Bold font for p values indicates statistical significance. Italicized font for p value indicates trend toward significance.
BMI, body mass index; FeNO, fractional concentation of exhaled nitric oxide; IFN, interferon; IL, interleukin; TNF, tumour necrosis factor; WCC, white cell count.

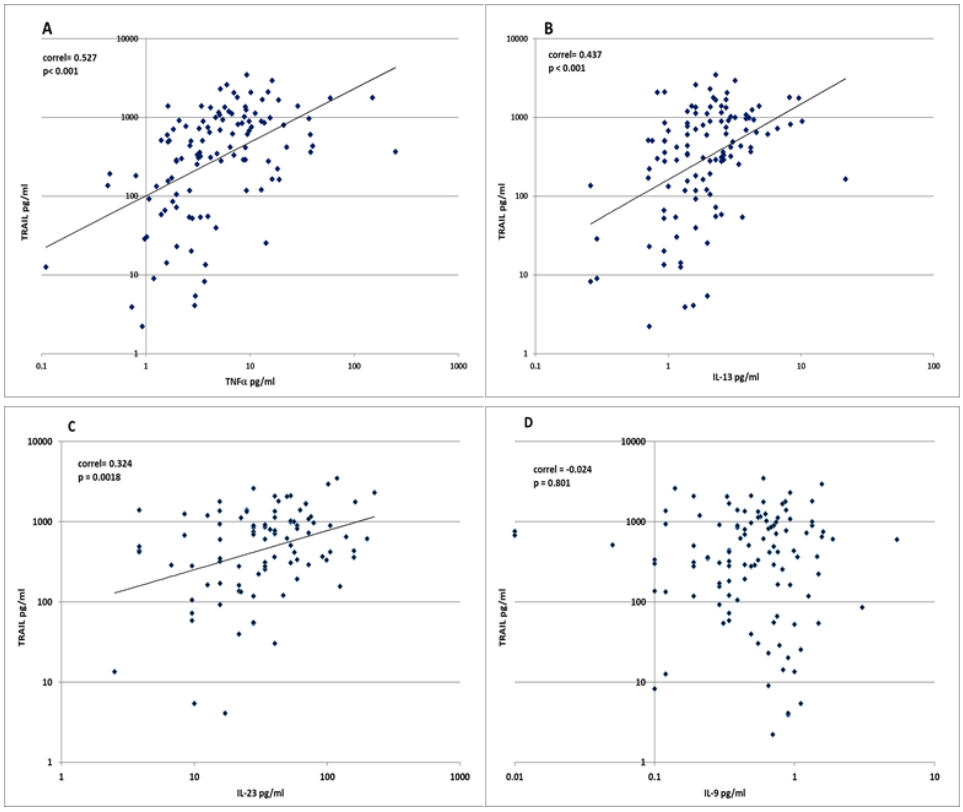

Figure 1 Association of tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) levels with representative mediators of type 1 (A, tumour necrosis factor α (TNFα)), type 2 (B, interleukin (IL)-13), and type 17 (C, IL-23) inflammatory mediators which were positively associated with TRAIL, and type 9 (D, IL-9) which was not significantly associated with TRAIL.

(p=0.293), nor in use of inhaled corticosteroids, systemic steroid bursts in the past year, or long-acting β-agonist use (online supplementary table S1, online supplementary). However, the high TRAIL group had greater proportion of subjects with exacerbations provoked by physical activity (p=0.050; online supplementary table S1).

Specific leucocyte percentages in sputum differentials did not differ between low and high TRAIL groups. However, all leucocyte counts/mL were significantly increased in the high TRAIL group due to increased white cell count (WCC) in sputum (table 1, p<0.001).

Increased sputum supernatant levels for other inflammatory proteins were observed in the high TRAIL group, including type 2 cytokines, IL-4, IL-5, IL-13, IL-33, CCL5/RANTES, and CCL11/Eotaxin 1; type 1 and type 17 cytokines, respectively, IL-10, IFNγ, TNFα, and IL-17 and IL-23; but not IL-9/Th9 inflammation (table 1 and figure 1).

Sputum cell cytospins showed greater stain density for TRAIL decoy receptor R3/DcR1 than for death receptor R1/DR4 on leucocytes (figure 2 and online supplementary table S4; p=0.006). Squamous epithelial cells present in sputum cytospins did not differ for non-specific background stain between

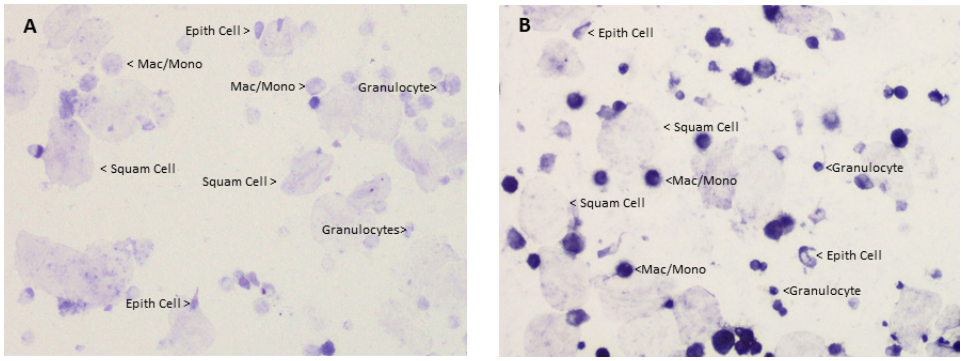

Figure 2 Representative sputum cell cytospins from the same subject immunostained for tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) receptor R1/DR4 (A) and R3/DcR1 (B). Sputum cells included epithelial cells, squamous cells, macrophages/monocytes and granulocytes, as indicated (arrows). Very few leucocytes stained strongly positive (dark purple blue) for death receptor TRAIL R1, whereas many leucocytes stained strongly positive for decoy TRAIL R3. The sum density for TRAIL receptor R3 leucocytes' stain was significantly increased over R1 (seven subject paired samples, p=0.006, online supplementary table S2) but background squamous cells' stain density did not differ (p=0.93). Thus, the decoy TRAIL receptor R3 predominated over death receptor R1 on sputum leucocytes from these asthmatics.

receptor types.

TRAIL BAL levels had fewer positive cytokine associations: type 2 cytokines, IL-5, MIP3a and Eotaxin 1 ($p<0.014$); type 1 cytokines, CXCL8, transforming growth factor alpha (TGFa $p<0.05$). Increased TRAIL BAL levels were not associated with any increased measures of airway remodelling: collagen III deposition in endobronchial biopsies ($p=0.995$), epithelial basement membrane thickness ($p=0.469$) or increased smooth muscle area proportion of biopsy ($p=0.186$).

All known TRAIL/TNFSF10 gene variations were examined for association with TRAIL sputum levels. Two specific alleles for known single nucleotide polymorphisms (SNPs) were associated with higher sputum TRAIL levels; at least one G allele at rs3136601 and one C allele at rs6763816 had higher sputum TRAIL levels (online supplementary figure 1S).

DISCUSSION

In summary, we report increased TRAIL/TNFSF10 in sputum supernatant associated with subjects having lower maximum FEV1%predicted, greater reversibility to bronchodilation and trends towards lower prebronchodilator FEV1/FVC, increased emergency visits and exacerbations. Thus, TRAIL appears associated with lower lung function and increased healthcare utilisation, characteristics typical for more severe asthma.[9]

Increased TRAIL associated with increased leucocyte counts in sputum corresponds to increased TRAIL associated with increased cytokines for types 1, 2 and 17 inflammation, although not type 9 inflammatory IL-9. Our data confirmed positive association between TRAIL and CCL20/MIP3α (not shown) as previously reported for activation of Th2 inflammation.[6] However, we were not able to confirm in our smaller biopsy numbers that TRAIL promotes airway remodelling[7]; collagen deposition, airway smooth muscle area, basement membrane thickness in bronchial biopsies did not differ with increased TRAIL.

Immunocytochemistry for two TRAIL receptors on sputum cells showed significantly greater density of the decoy receptor R3/DcR1 on leucocytes than the death domain receptor R1/DR4, suggesting reduced apoptosis in these leucocytes and possibly greater non-canonical signalling.[3]

Though this cohort was small for genetic analysis, variation in the TRAIL gene was associated with increased levels of gene product in airway secretions (online supplementary figure S1). This provides further support to haplotype analysis associating TRAIL/TNFSF10 with asthma.[5]

We conclude that increased immune regulator TRAIL positively associates with types 1, 2 and 17 inflammation in asthma. Increased decoy receptor on sputum leucocytes compared with death domain receptor for TRAIL may prolong airway inflammation, even in stable disease state.

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Contributors All authors contributed to the design, conception and analyses of the study. WCM, ATH, CS, ERB, DAM, MM and BR contributed to the acquisition of clinical samples and data. MM and ATH drafted the manuscript. All authors contributed to intellectual content and revision of the manuscript, and have approved submission of the manuscript for publication.

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