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/TNFSF10) is a cytokine of the tumour necrosis factor (TNF) superfamily with a potential role in allergic asthma.12 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 countertacting effects suggest divergent roles for TRAIL in lung diseases.4 Response to TRAIL depends on ligand interaction with five receptors on BAL leucocytes, in eosinophilic alveolar lavage (BAL) fluid, and TRAIL 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/D4R, 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.12 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, 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),10 11 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 FEV1, 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 (p=−0.191; p=0.062) and with FEV1/FVC ratio (p=−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 FEV₁ %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 FEV₁/FVC           | 0.74 (0.65–0.79) | 0.75 (0.70–0.81) | 0.73 (0.62–0.77) | 0.071                  |
| Max FEV₁ %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 (× 10⁶/mL) | 0.95 (0.56–2.10) | 0.70 (0.32–1.20) | 1.90 (0.86–3.31) | <0.001                 |
| Macro/mono count (×10⁹/mL) | 41.5 (18.03–87.24) | 28.6 (13.5–61.7) | 49.1 (31.5–124.6) | <0.001                 |
| Lymphocyte count (×10⁹/mL) | 1.13 (0.30–3.47) | 0.68 (0.24–1.91) | 2.42 (0.67–5.34) | 0.002                  |
| Neutrophil count (×10⁹/mL) | 38.6 (10.6–94.5) | 21.4 (7.9–52.0) | 70.8 (32.2–160.1) | <0.001                 |
| Eosinophil count (×10⁹/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)      | 14.1±23.25   | 2.46±4.47       | 8.5±4.09         | 0.001                  |
| CCL11/Eotaxin (pg/mL)    | 16.5±9.98    | 11.9±1.59       | 16.6±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)            | 34.1 (15.96–59.60) | 27.86 (15.60–53.21) | 40.46 (26.03–70.96) | 0.021                  |
| IFNγ (pg/mL)             | 2.54 (1.55–4.87) | 2.22 (1.24–3.93) | 3.66 (1.90–5.74) | 0.013                  |
| TNFα (pg/mL)             | 4.62 (1.90–9.45) | 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 concentration of exhaled nitric oxide; IFN, interferon; IL, interleukin; TNF, tumour necrosis factor; WCC, white cell count.
Brief communication

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.

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.

(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...
We were not able to confirm in our smaller biopsy numbers DR4, suggesting reduced apoptosis in these leucocytes and R3/DcR1 on leucocytes than the death domain receptor R1/cells showed significantly greater density of the decoy receptor.

Airway smooth muscle area, basement membrane thickness in 4

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