

## **TNF-alpha in asthma**

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## Abstract

Although only 5-10% of patients with asthma are relatively unresponsive to treatment with inhaled corticosteroids, refractory asthma represents an important condition as these patients suffer considerable morbidity and mortality and consume a disproportionately large amount of health resource. Treatment options are limited and there is a large unmet clinical need for additional therapies. TNF- $\alpha$  is a proinflammatory cytokine, which has been implicated in many aspects of the airway pathology in asthma and has recently been highlighted as potentially important in refractory asthma. The development of neutralising biological agents against TNF- $\alpha$  has allowed the testing of its role in vivo. Preliminary studies have demonstrated an improvement in lung function, airway hyperresponsiveness, asthma quality of life and a reduction in exacerbation frequency in patients treated following anti-TNF- $\alpha$  therapy. We have reviewed the evidence associating TNF- $\alpha$  with asthma airway biology and summarise the findings of currently published clinical trials of anti-TNF- $\alpha$  therapy in asthma.

## 1.0 Biological Activities of TNF $\alpha$ in relation to asthma

TNF- $\alpha$  is an important cytokine in the innate immune response which provides immediate host defence against invading organisms prior to activation of the adaptive immune system<sup>1</sup>. It is principally produced by macrophages in response to activation of membrane bound pattern recognition molecules, which detect common bacterial cell surface products such as polysaccharide, carbohydrates and lipopolysaccharides. It is initially produced as a biologically active 26kD membrane anchored precursor protein<sup>2</sup> which is subsequently cleaved, principally by TNF- $\alpha$  converting enzyme (TACE)<sup>3</sup>, to release the 17kD free protein. These proteins form into biologically active homotrimers<sup>4</sup> which act on the ubiquitously expressed TNF- $\alpha$  receptors 1 and 2 (p55 and p75 or TNFRi and TNFRii)<sup>5</sup>. The receptor-ligand interaction causes intracellular signalling without internalisation of the complex, which leads to phosphorylation of NF- $\kappa$ B to activate the p50-p65 subunit, which interacts with the DNA chromatin structure to increase transcription of pro-inflammatory genes, such as IL-8, IL-6 and TNF- $\alpha$  itself. Response to TNF- $\alpha$  activation is balanced by shedding of the extracellular domain of the TNF- $\alpha$  receptors.

Dysregulated TNF- $\alpha$  response has been implicated in a number of inflammatory conditions. In rheumatoid arthritis, a common destructive arthropathy in which TNF- $\alpha$  is produced by macrophages and monocytes in response to activation by CD4+ T cells, TNF- $\alpha$  is measurable in increased concentration in the synovial fluid and in the serum<sup>6</sup>. Antagonism of TNF- $\alpha$  either by treatment with recombinant soluble receptors or neutralising antibodies in patients with rheumatoid disease leads to improvement in disease activity scores<sup>7</sup>. Similarly positive results are seen in

treatment of other conditions that are thought to be mediated by TNF-  $\alpha$ , including Crohn's disease and Beçhet's disease.

The possibility that TNF-  $\alpha$  contributes to the dysregulated inflammatory response seen in the asthmatic airway is raised by the findings of increased TNF-  $\alpha$  mRNA<sup>8</sup> and protein<sup>9</sup> in the airway of patients with asthma. Moreover, the administration of inhaled recombinant TNF-  $\alpha$  to normal subjects led to the development of airway hyperresponsiveness and an airway neutrophilia<sup>10</sup>. The administration of TNF-  $\alpha$  to patients with asthma leads to an increase in airway hyperresponsiveness as measured by a reduction in methacholine PC<sub>20</sub><sup>11</sup>. The mechanism behind these observations has not been fully elucidated: it could represent a direct effect of TNF-  $\alpha$  on airway smooth muscle<sup>12</sup> or be mediated by the release of the cysteinyl-leukotrienes LT<sub>C4</sub> and LT<sub>D4</sub><sup>13</sup>. The release of mediators from mast cells localised to the airway smooth muscle has recently been suggested to be important in the pathogenesis of airway hyperresponsiveness and bronchoconstriction in asthma<sup>14</sup>. TNF-  $\alpha$  induces histamine release from human mast cells directly<sup>15</sup> and participates in a positive autocrine loop that potentiates human mast cell cytokine secretion<sup>16</sup>. It is possible therefore that TNF-  $\alpha$  is involved in mast cell/smooth muscle interaction and that this is particularly important in the development of airway hyper-responsiveness.

TNF-  $\alpha$  has a number of other actions which may be relevant to asthma: it is chemoattractant for neutrophils and eosinophils<sup>17</sup> it increases the cytotoxic effect of eosinophils on endothelial cells<sup>18</sup>, it is involved in activation and cytokine release by T-Cells<sup>19</sup> and it increases epithelial expression of adhesion molecules such as ICAM-

1 and V-CAM-1<sup>20</sup> which play an important role is the conduction of T-Cells to the lung and in the subsequent development of airway hyper-responsiveness<sup>21</sup>.

In addition to its relevance to asthma in general, TNF-  $\alpha$  has a number of properties that might be relevant to refractory asthma, including: recruitment of neutrophils<sup>10</sup>, induction of glucocorticoid resistance<sup>22</sup>, myocyte proliferation<sup>23</sup> and stimulation of fibroblast growth and maturation to myofibroblasts by promoting TGF-  $\alpha$  expression<sup>24,25</sup>.

## 2.0 Clinical Trials of Anti-TNF- $\alpha$ therapy. (Summarised in Table 1)

The current commercially available TNF- $\alpha$  blockers are infliximab which is a chimeric mouse/human monoclonal antibody, etanercept which is a soluble fusion protein combining two p75 TNF receptors with a Fc fragment of human IgG<sub>1</sub> and adalimumab, a fully human monoclonal antibody.

In an uncontrolled study of etanercept in severe (GINA stage V) asthma Howarth and colleagues demonstrated a significant 2.5 doubling concentration improvement in methacholine airway hyperresponsiveness, a 240ml improvement in FEV<sub>1</sub> and an improvement in asthma quality of life<sup>26</sup>. These findings were repeated in a randomised, placebo controlled study in which 12 weeks treatment with etanercept lead to a similar improvement in PC<sub>20</sub> and FEV<sub>1</sub> as well as an improvement in asthma related quality of life<sup>27</sup>. One of the striking aspects of this study was that the clinical response correlated closely with the expression of TNF $\alpha$  and TNF $\alpha$ R1 on monocytes. Increased expression of TNF $\alpha$  was only noted in patients with severe disease. This suggests that TNF $\alpha$  may only be involved in a subset of severe

asthmatics and that measurement of monocyte expression of TNF $\alpha$  may be a useful biomarker of responsiveness. Another interesting aspect of the study was that there was no effect on the number of sputum eosinophils or neutrophils suggesting that TNF $\alpha$  antagonism was working through the airway smooth muscle axis rather than an inflammatory pathway. Erin and colleagues performed a randomised, placebo controlled study with infliximab in patients with moderately severe asthma in which they did not find any significant improvement in lung function but noted a 50% reduction in the number of exacerbations encountered<sup>28</sup>. It is possible that the lack of effect on lung function was due to the selection of patients with less severe disease or that there is a therapeutic difference between etanercept and infliximab.

However we are aware of two unpublished studies of etanercept (the preliminary data from one of these has been published in abstract form (<sup>29</sup>) which were larger than those quoted above which demonstrated no beneficial effect. This could be due to different degrees of severity but it does suggest that if anti-TNF $\alpha$  is effective in asthma it will only be on a relatively small sub-group of patients, possibly defined by an increased TNF $\alpha$  axis.

Another concern is the safety of TNF- $\alpha$ <sup>30</sup>. A recent report of the use of Infliximab in COPD for six months apart from showing no benefit, recorded 9 malignancies out of 157 treated patients as oppose to one in 77 placebo treated subjects together with an increased risk of pneumonia <sup>31</sup>. There has also been an excess of malignancy and infection reported in patients treated with anti-TNF- $\alpha$  for rheumatoid arthritis.

In summary therefore TNF- $\alpha$  is a potentially important cytokine in refractory asthma and preliminary studies on small numbers of patients have demonstrated an improvement in lung function, airway hyperresponsiveness, asthma quality of life and exacerbation rate with anti-TNF therapy. These findings have, however, not been consistently repeatable and any potential role for TNF- $\alpha$  antagonism in refractory asthma needs to be established in a sufficiently powered large scale clinical trial. There also needs to be a focus on drug safety, particularly in respect of susceptibility to severe infection and the development of solid organ malignancy. Heterogeneity in response to TNF- $\alpha$  antagonism could lie in the genetics of TNF- $\alpha$  as polymorphisms in the cytokine gene, particularly in the G to A transition at position 308 and have been associated with increased risk of asthma in some studies <sup>32</sup> This aspect also needs to be addressed in future studies of anti-TNF $\alpha$  therapy. Clearly any further studies of anti-TNF $\alpha$  need to focus on identifying a treatment responsive subset of patients.

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