Role and Pharmacogenomics of TNF-α in Asthma

Dimosthenis Lykouras, Fotis Sampsonas, Alexandros Kaparianos, Kiriakos Karkoulias and Kostas Spiropoulos*

University of Patras, University Hospital of Patras, Department of Internal Medicine, Division of Pneumonology, Rio, Patras 26500, Greece

Abstract: Asthma is a chronic heterogeneous inflammatory disease of the respiratory system in which numerous cytokines play a significant role. Among them TNF-α (tumour necrosis factor α), a proinflammatory cytokine, has a predominant role in orchestrating airway inflammation and affecting treatment outcome.

In this review we attempt to summarize the involvement of TNF-α in the pathogenesis of asthma, illustrate variations of TNF-α gene that potentially influence asthma phenotype and highlight promising therapies by blocking the production of TNF-α or inhibiting its action.

A cytokine specific target therapy seems to be very promising since agents that block TNF-α slow disease progression, suppress inflammation and in some cases induce remission of chronic inflammation.

INTRODUCTION

Asthma is an inflammatory disease of the airways that has increasing morbidity and mortality rates. In this paper we want to summarize the major pathways through which TNF-α is involved in asthma and discuss a possible role of anti-TNF-α therapies for special populations of patients suffering from asthma.

ASTHMA: AN INFLAMMATORY CONDITION OF THE AIRWAYS

Asthma is an inflammatory disease, characterized by airway hyperreactivity, chronic eosinophilic inflammation, episodes of reversible bronchoconstriction, and mucus hypersecretion [1]. The eosinophilic inflammation associated with asthma is typically coupled with increased numbers of CD4+ T lymphocytes that produce increased levels of TH2type cytokines and decreased levels of γ-interferon [2]. Of note, different allergic phenotypes cannot be distinguished strictly on the basis of TH1 and TH2 cytokines [3]. Generally, asthma and allergic disorders are casually characterized by elevated Th2 cytokines (IL-4, IL-5, IL-13) and the chronic inflammatory response in asthmatic airways is maintained by Th1 cytokines.

TNF-α: A DISTINCTIVE PROINFLAMMATORY CYTOKINE

What is TNF-α

TNFα is the most widely studied cytokine member of Tumour Necrosis Factor (TNF) super family. It is secreted by lipopolysaccharide stimulated macrophages and causes necrosis of tumor *in vivo* when injected into tumor bearing mice [4]. Experimentally, TNFα causes cytolysis or cytostasis of certain transformed cells [5] being synergistic with gamma interferon in its cytotoxicity [6].

TNF-*α*, a cytokine that plays a role in many inflammatory diseases, is produced by several pro-inflammatory cells (mainly macrophages, but also monocytes, dendritic cells, B-cells, CD4+ cells, neutrophils, mast cells and eosinophils) and structural cells (fibroblasts, epithelial cells and smooth muscle cells) known to be crucial in the pathogenesis of asthma.Large amounts of TNF-*α* are generated in response to bacteria or parasitic proteins, but all potentially noxious stimuli ranging from physical, chemical to immunological can rapidly induce production and release of TNF-*α*. Moreover, TNF-*α* can also be generated as a consequence of stimulation of a wide range of pro-inflammatory cytokines including TNF-*α* itself. For example, mast cells are known to release and respond to TNF-*α*, indicating a positive autocrine loop leading to augmentation of mast cell activation [7].

The biological function of TNF-*α* includes the modulation of growth differentiation and proliferation of a variety of cell types, but it is also important in the causation of apoptosis. Besides these effects, TNF-*α* is also a well-known inducer of the inflammatory response and a regulator of immunity. Its inflammatory properties are classically mediated by means of a wide variety of pro-inflammatory cytokines, including IL (interleukin)- 1, IL-2, IL-4, IL-6, IL-10, IL-12, IFN-*γ* (interferon-*γ*) and TGF-*β* (transforming growth factor-*β*), generated mainly through NF-*κ*B (nuclear factor *κ*B) activation [8].

Stimulation of TNF-α

Physiological stimuli for the synthesis of TNFα are IL-1, bacterial endotoxins, TNF, platelet derived growth factor (PDGF), and Oncostatin M. In fibroblasts the synthesis of TNFα is stimulated by IFNβ, TNFα, PDGF, and viral infections. In thymic stromal cells the synthesis of TNFα can be induced by neuronal growth factor (NGF). TNFα can also stimulate or inhibits its own synthesis, depending upon the

*Address correspondence to this author at the Professor of Pneumology, University Hospital of Patras, Rio, Patras 26500, Greece; Tel: +302610999523; Fax: +302610999523;
E-mail: k-spiropoulos@hotmail.com

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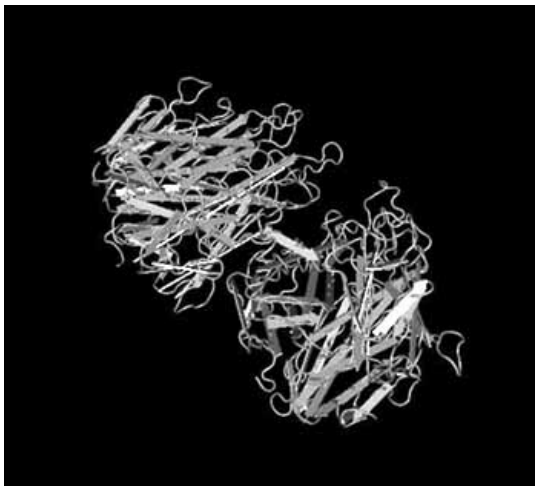
cell type. In epithelial, endothelial, and fibroblastic cells secretion of TNFα is induced by IL-17.



Compound 1. TNF-α 3D structure retrieved from NCBI structure database.

Structure of TNF-α

TNF-*α* is a non-glycosylated protein of 17 kDa with 157 amino acids and belongs to a family of peptide ligands that activate a corresponding set of structurally related receptors [9,10], [Compound (1)]. The soluble 17 kDa form ofTNF-*α* is generated by cleaving the 26 kDa trans-membrane precursor by TACE (TNF-*α*-converting enzyme). The tumour necrosis factor-α converting enzyme is also known as ADAM 17 as it is part of a larger ADAM family (ADAM: proteins containing a disintegrin and metalloproteinase domain), and is inhibited by tissue inhibitor of metalloproteinase-3 (TIMP-3) [11]. This enzyme is also responsible for the liberation of other membrane-bound proteins, including TNF receptors, transforming growth factor-α, and the adhesion molecule, L-selectin [12,13]. Various studies have indicated that is possible to inhibit TACE, and hence TNF-α release, by agents such as, hydroxamic acid derivatives [14,15]. These inhibitors may have less specificity upon TNF-α than was first thought, perhaps because TACE has other functions than just cleaving TNF-α. As yet, there are few reports relating to MMP in asthma, and none on TACE or TIMP3 in this condition.

Both soluble and membrane-bound forms of TNF-*α* are biologically active, although they have different affinities for the two receptors. After separating from the cell membrane, soluble TNF-*α* aggregates into trimolecular complexes (51 kDa homotrimers) that subsequently bind to the receptors. TACE also cleaves the extracellular domain of its complementary receptor forming sTNFRs (soluble TNF-*α* receptors) that are free to bind to trimolecular TNF-*α* rendering it biologically inactive, resulting in diminished cellular signalling of TNF and thus acting as a soluble natural inhibitor of TNF-*α* bioactivity *in vivo*.

Receptors of TNF-α

Biological responses of TNF-*α* are mediated by specific binding either *via* a Type I (TNFR1; p55 or CD120a) or a Type II (TNFR2; p75 or CD120b) receptor. These two receptors are expressed on the surface of many cell types, and a recent model of receptor-mediated signalling proposes that TNFR1 is expressed on cells susceptible to the cytotoxic action of TNF-*α*, whereas TNFR2is expressed strongly on stimulated B- andT-cells [16]. Binding of TNF-*α* to its receptors results in activation of intracellular signalling processes that lead to a remarkably diverse set of cellular responses, including differentiation, activation, release of pro-inflammatory mediators and apoptosis, through the recruitment and activation of adaptor proteins [17]. It is clear that the ratio of TNFR1/TNFR2 dictates the final outcome of the cellular response upon TNF-*α* stimulation (Fig. (**1**)).

ROLE OF TNF-A IN THE PROGRESSION OF AIRWAY INFLAMMATION

Inflammation is believed to be the key event of TNFα-dependent pathophysiological events. Deregulated recruitment of leukocytes and lymphocytes at the inflamed foci leads to injury. TNF-α also depletes cellular glutathione (GSH), a cellular antioxidant [18].

The exact mechanism through which TNF-α decreases the levels of glutathione in pulmonary tissues has not yet been fully determined. Both *in vitro* and *in vivo* studies show that TNF-α stimulates the reactive oxygen species (ROS) generation from pulmonary and non-pulmonary tissues.

It is generally assumed that in physiological homeostatic conditions most of the ROS generation takes place at the phagocytic cells such as macrophages or neutrophils and the ROS generation by nonphagocytic cells is a minor fraction to that of the phagocytic cells.

At the subcellular level NADPH oxidase and mitochondria are the potential sites of TNF-α-dependent ROS generation and subsequent oxidative stress in endothelial cells. Recent studies indicate that NADPH oxidase and mitochondria are linked through a feedback mechanism [19]. Interestingly, a recent study has reported that the two sources ROS (mitochondria and NADPH oxidase) may have divergent pathways in endothelial cells: The mitochondrial pathway is suppressed by rotenone and appears to be directly involved in TNF-α induced apoptosis by activating caspase 3. Another pathway is a membranedependent pathway that is associated with the NADPH oxidase and protects against TNFα-induced cell death by activation of small GTPase Rac1 (a component of NADPH oxidase) [20]. Thus, depending upon the type of stimulus and the levels of generation of ROS, cells may undergo either pro-survival or pro-apoptotic pathway in response to TNF-α (Fig. (**2**)).

Tumour necrosis factor-α is known to up-regulate adhesion molecules, such as E-selectin, vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1 or CD54); [21,22]. Tumour necrosis factor-α induction of adhesion molecules, such as VCAM-1, on pulmonary endothelium is important for eosinophil recruitment [23,24] and in addition, biopsies of asthmatic bronchial wall have shown VCAM-1 to be up-regulated [25].

TNF induces the expression of multiple airway epithelial cell genes, including cytokines (IL-5, IL-6, IL-8, G-CSF,

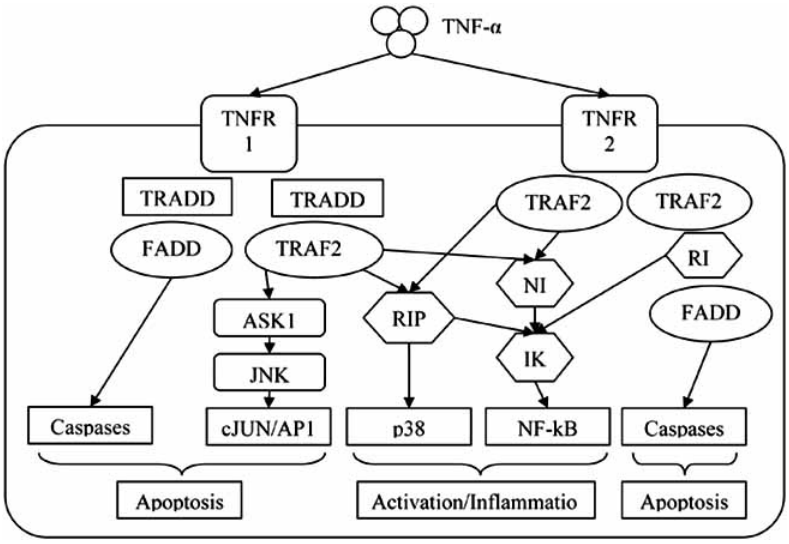


Fig. (1). TNF-α signalling pathways Binding of TNF-α to TNFR1 results in the configuration of TRADD (TNFR-associated death domain) and FADD (Fas-associated death domain). TRADD complex recruits the adapter protein TRAF-2 (TNFR-associated factor 2), whereas FADD stimulates the caspase cascade. Known downstream signalling molecules that interact with TRAF-2 are NIK (NF-κB-inducing kinase), RIP (receptor-interacting protein) and ASK1 (apoptosis signal-regulating kinase 1) and these are capable of channelling signals towards cell death and inflammation. Binding of TNF-α to TNFR2 recruits the adapter protein TRAF-2, which directly activates the inflammatory cascade via the generation of NF-κB or p38 MAPK (mitogen-activated protein kinase) and activates caspase-mediated cell death through recruitment of FADD and RIP.

GM-CSF), chemokines (eotaxin, MCP-1, RANTES), adhesion molecules (ICAM-1), extracellular matrix glycoproteins (tenascin), neuropeptides (endothelin-1), mucins (MUC-1, MUC-2, MUC-5AC), and cytosolic phospholipase A2 [26-28]. TNF increases the adhesion of activated eosinophils to respiratory epithelial cell cultures and promotes neutrophil chemotaxis, adherence, and transendothelial and transepithelial migration [29]. IgE receptor activation induces TNF release from human lung tissue and upregulates eosinophil TNF mRNA levels [30]. An association between asthma and polymorphisms in the TNF locus that correlate with increased TNF secretion has been proposed [31,32].

The adhesion described above is performed through the enhanced release of pro-inflammatory/chemotactic mediators and up-regulation of adhesion molecules, such as E-selectin, VCAM-1 (vascular cell adhesion molecule- 1) and ICAM-1 (intercellular cell-adhesion molecule-1), thus facilitating the migration of eosinophils and neutrophils [33-35]. Moreover the proteolytic enzyme MMP-9 (matrix metalloproteinase-9)

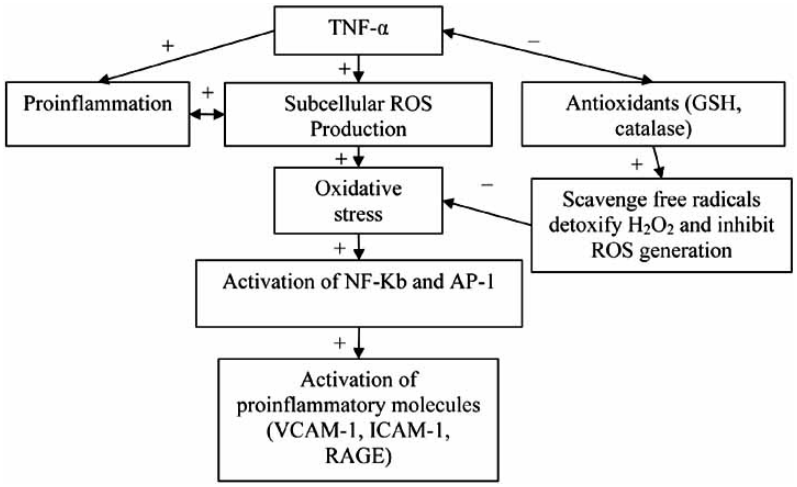


Fig. (2). Schematic diagram of the mechanism of action of TNFα. While TNFα-dependent activation of reactive oxygen species (ROS) generation enhances oxidative stress of cells and subsequent activation of pro-inflammatory and pro-oxidative transcription factors nuclear factor kappa-B (NF-kB) and the activator protein one (AP-1), antioxidants namely GSH attenuates oxidative stress and subsequent activation of NF-κB and AP-1. NF-κB and AP-1 are involved in the activation of pro-inflammatory molecules like, vascular cell adhesion molecule one (VCAM-1), intercellular adhesion molecule one (ICAM-1) and receptor for advanced glycation end products (RAGE). + indicates activation, - indicates inhibition → and ↔ indicate one way and two way flow of signals respectively.

and the matrix glycoprotein tenascin are abundant in thickened asthmatic subepithelial basement membrane. The production of extracellular matrix glycoproteins is mainly derived by the activation of myofibroblasts and fibroblasts in the proximity of the subepithelial basement membrane [36] TNF-*α* is known to be implicated in the proliferation and activation of subepithelial myofibroblasts/fibroblasts thus contributing to development of fibrosis below the bronchial basement membrane of the epithelial layer and to tissue remodelling in general [37,38]. Moreover, airway epithelial cells also secrete mucus when stimulated with TNF-*α* [39]. TNF-*α* is also likely to have a more integrated role in airway remodelling, since it appears to modulate the EGFR (epidermal growth factor receptor)- dependent stress and repair response that occurs as a result of the inflammatory response that is associated with airway epithelial injury [40].

TNF-α AFFECTS BOTH INTRACELLULAR CA AND AIRWAY SMOOTH MUSCLE (ASM) HYPERRESPONSIVENESS

Intracellular Ca and TNF-α

Growing evidence suggests that intracellular calcium plays an important role in mediating the biologic effects of cytokines. TNF-α-induced calcium signals may be stimulated by several distinct pathways, including an indirect emptying of intracellular calcium stores through the generation of second messengers. TNF-α stimulates the expression of a calcium-binding protein, calbindin-D28K, in neuronal cells [41], and increases activity of proteins with calcium pumping properties in fetal pancreatic islets [42]. A number of studies [43-45], suggest that changes in calcium homeostasis represent a new mechanism by which TNF-α may regulate cellular responses.

Airway Smooth Muscle (ASM) Hyperresponsiveness

ASM exposed to TNF-α either *in vitro* or *in vivo* become hyper-responsive to many contractile agonists. TNF-α significantly enhances phosphoinositide turnover in response to bradykinin. The use of agonistic antibodies or recombinant proteins of TNF-α that specifically activate either TNFR1 or TNFR2 receptor led to the conclusion that TNF-α mediates most of its cellular effects by activating the TNFR1 receptor [46].

In parallel studies, TNF-a increased cytosolic calcium levels induced by NaF [47], an agent that directly activates G-protein in ASM cells, which strongly suggests that TNF- α effects occur downstream from the receptor, possibly at the level of the G-proteins (Fig. (**3**)).

Together, these studies suggest that brief treatment with TNF-α may modulate ASM contractile response by directly inducing calcium sensitization of intracellular contractile elements, rather than by modulating agonist-evoked calcium mobilization induced by longer pretreatment with TNF-α. Fig. (**3**) summarizes potential mechanisms by which TNF-a modulates ASM responsiveness.

TNF-α activates at least two cell-surface receptors – TNFR1 (55 kDa) and TNFR2 (75 kDa) – that are expressed in most cell types [48]. Both receptors are expressed on cultivated human ASM cells and on native ASM.

CURRENT THERAPEUTICAL PRACTICE AGAINST ASTHMA

Inhaled Corticosteroids

Inhaled glucocorticosteroids are currently the most effective anti-inflammatory medications for the treatment of persistent asthma. Studies have demonstrated their efficacy in reducing asthma symptoms, improving quality of life, improving lung function [49], decreasing airway hyperresponsiveness [50], controlling airway inflammation [51], reducing frequency and severity of exacerbations, and reducing asthma mortality. However, they do not cure asthma, and when they are discontinued deterioration of clinical control

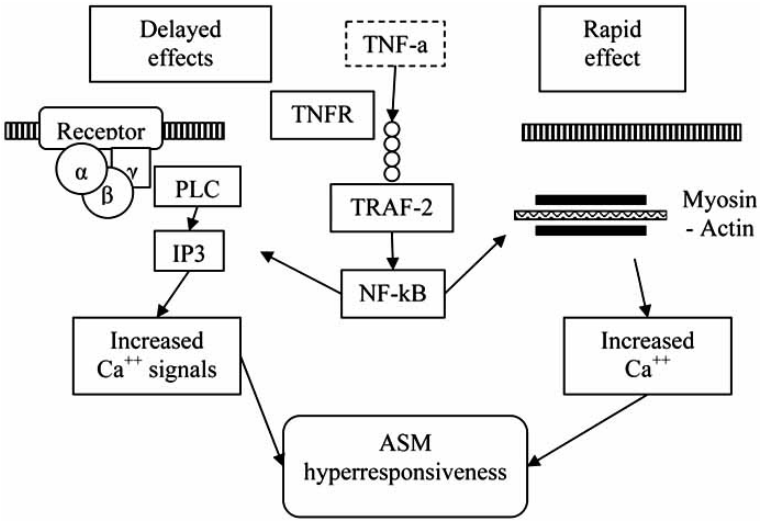
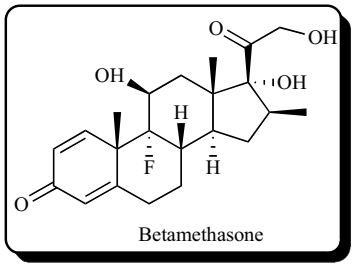


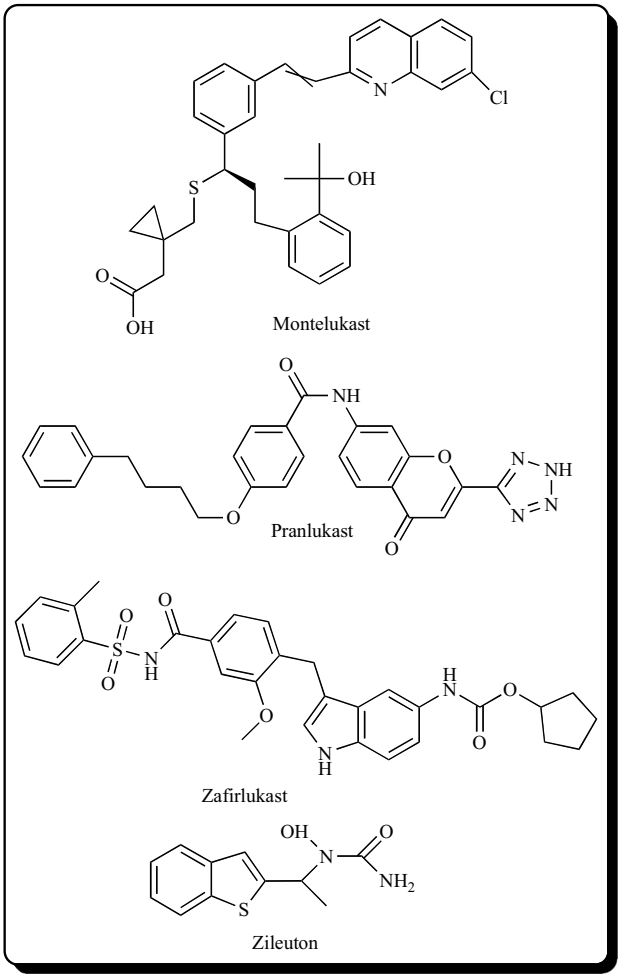
Fig. (3). Potential intracellular mechanisms involved in the modulation of ASM hyper-responsiveness induced by TNF-a via TNFR1. Activation of TNFR1 coupled to the TRAF2–NF-kB pathway induces a delayed effect, in which long-term pretreatment with TNF-a enhances G-protein-coupled signal transduction, leading to increased calcium signals to contractile agonists. Activation of TNFR1 may also be involved in a rapid effect, in which short-term pretreatment with TNF-a enhances the calcium sensitization of intracellular contractile elements. IP3, inositol-1,4,5-trisphosphate; PLC, phospholipase C.

follows within weeks to months in a proportion of patients [52].



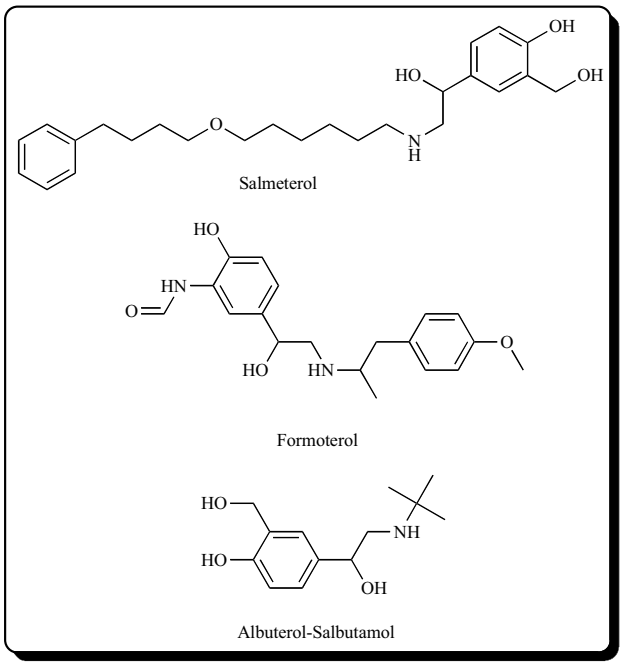
Leukotriene Modifiers

Leukotriene modifiers include cysteinylleukotriene 1 (CysLT1) receptor antagonists (montelukast, pranlukast, and zafirlukast) and a 5-lipoxygenase inhibitor (zileuton). Clinical studies have demonstrated that leukotriene modifiers have a small and variable bronchodilator effect, reduce symptoms including cough [53], improve lung function, and reduce airway inflammation and asthma exacerbations [54].



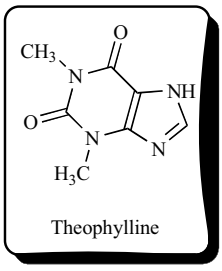
Long-Acting b-2 Agonists (LABA)

Long-acting inhaled b-2-agonists, including formoterol and salmeterol, are also used in asthma as these medications do not appear to influence the airway inflammation in asthma. They are most effective when combined with inhaled glucocorticosteroids [55], and this combination therapy is the preferred treatment when a medium dose of inhaled glucocorticosteroid alone fails to achieve control of asthma. Addition of long-acting inhaled b-2-agonists to a daily regimen of inhaled glucocorticosteroids improves symptom scores, decreases nocturnal asthma, improves lung function, decreases the use of rapid-acting inhaled b-2-agonists [56], reduces the number of exacerbations [57], and achieves clinical control of asthma in more patients, more rapidly, and at a lower dose of inhaled glucocorticosteroids than inhaled glucocorticosteroids given alone [58].



Theophylline

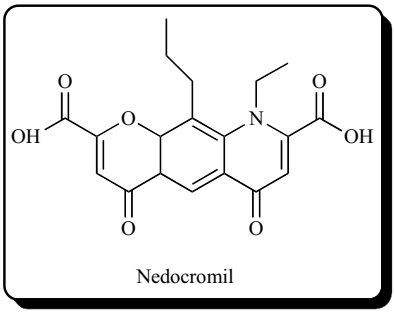
Theophylline is a bronchodilator and, when given in a lower dose, has modest anti-inflammatory properties [59]. Available evidence suggests that sustained-release theophylline has little effect as a first-line controller [60]. It may provide benefit as add-on therapy in patients who do not achieve control on inhaled glucocorticosteroids alone [61].



Cromones: Sodium Cromoglycate and Nedocromil Sodium

The role of sodium cromoglycate and nedocromil sodium in long-term treatment of asthma in adults is limited. Effi-

cacy has been reported in patients with mild persistent asthma and exercise-induced bronchospasm. Their anti-inflammatory effect is weak and they are less effective than a low dose of inhaled glucocorticosteroid [62]. They provoke stabilization of mast cells and prevent IgE-mediated release of mast cell mediators resulting to lower excretion of histamine and leukotrienes.



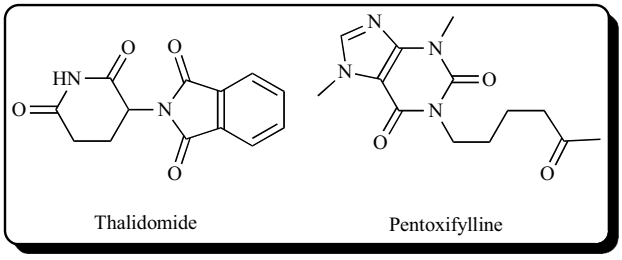
Nedocromil

Anti-IgE

Anti-IgE (omalizumab) is a treatment option limited to patients with elevated serum levels of IgE. Improved asthma control is reflected by fewer symptoms, less need for reliever medications, and fewer exacerbations [63,64]. Further investigations will likely provide additional clarification of the role of anti-IgE in other clinical settings.

ANTI-TNF THERAPIES IN MEDICINE

Involvement of TNF-α in various inflammatory disorders has led to the use of pharmacological agents that can either suppress the production of TNF-α or block its action. A variety of candidates are being studied including inhibitors of TNF-α mRNA transcription (e.g. pentoxifylline and phosphodiesterase inhibitors) [65,66], accelerators of TNF-α mRNA degradation (e.g. thalidomide) [67], inhibitors of TNF-α protein translation (e.g. tetravalent guanylhydrazones) [68] and the metalloproteinase inhibitors that prevent the cleavage of the 26 kDa membranebound protein to the active 17 kDa molecule [69]. Other approaches include TNF receptor fusion proteins [70] and monoclonal antibodies raised against TNF-α. The latter have been used in human subjects who have rheumatoid arthritis, usually as a humanized murine antibody [71]. Therefore, these pharmacological agents may have potential therapeutic value for a wide variety of TNF-α-mediated disorders.



Thalidomide Pentoxifylline

TNF-α is a major therapeutic target in a range of chronic inflammatory disorders in which neutrophils are involved. These include rheumatoid arthritis, juvenile arthritis, ankylosing spondylitis, Crohn's disease, psoriasis, glomerulonephritis, sarcoidosis, and Behcet's disease, all of which are characterized by TH1-type immune response associated with excess generation of TNF-α.

ANTI-TNF THERAPIES AGAINST ASTHMA

The ability of TNF to attenuate allergen-mediated airway inflammation may occur at two levels. First, chronic TNF stimulation has been reported to suppress T cell receptor signaling, proliferative responses, and cytokine production, thereby negatively regulating the function of both Th1- and Th2-type T cells [72,73]. Conversely, TNF inhibition enhances T cell proliferation and cytokine production. Second, TNF signaling has been proposed to repress IL-4 gene expression in Th2- type T cells *via* binding of the receptor-associated factor (TRAF2) to the nuclear factor of activated T cells (NFAT)-interacting protein (NIP45). The TRAF2-NIP45 interaction suppresses IL-4 production by inhibiting NIP45/NFATc2/c-maf transactivation of the IL-4 promoter [74].

In a recent study [75] it is shown that patients with severe corticosteroid-dependent asthma have higher concentrations of TNF-α in their bronchoalveolar lavage (BAL) fluid, whereas there was no difference in levels between patients with mild asthma and healthy control subjects. The increased TNF-α level is a feature of more persistent and corticosteroid-refractory asthma rather than of asthma per se and extends previous reports in which increased TNF-α-immunoreactive cells and a 30-fold greater TNF-α gene expression in bronchial biopsy specimens from patients with severe asthma compared with those with symptomatic nonsteroid-treated asthma [76].

Compared with healthy subjects or those with well-controlled asthma, their circulating mononuclear cells express increased cellsurface levels of membrane-associated TNF-a, TNF-α-converting enzyme (a disintegrin and metalloprotease 17 [ADAM17]), and the TNF receptors p55 and p75. Because anti-TNF-a strategies modify inflammatory diseases that persist despite corticosteroid therapy [77], there has been assessment of anti-TNF-α in severe asthma. In an openlabel study, p75 fusion protein etanercept was used. Etanercept interacts with both TNF-α and TNF-b to prevent cytokine binding to the p55 and p75 cell-surface receptors. Patients with severe asthma treated with high-dose inhaled and oral corticosteroids received etanercept, 25 mg subcutaneously twice weekly for 12 weeks. This treatment resulted in a marked improvement in asthma symptoms, lung function, and BHR.

Failure of inhaled corticosteroids to reduce TNF-*α* to a significant level in asthmatic airways may explain to a certain extent why these anti-inflammatory drugs appear to have limited effects in the more severe forms of asthma. Considering the critical role of TNF-*α* in the pathogenesis of asthma and the need for alternative treatments for those asthmatic patients with severe disease who are particularly resistant to conventional therapy, molecules targeted at blocking the effects of TNF-*α* are likely to constitute a considerable advance in the management of these difficult patients. The currently commercially available TNF-*α* blockers [infliximab (a chimaeric mouse/human monoclonal anti-TNF-*α* antibody), etanercept (a soluble fusion protein combining two p75 TNFRs with an Fc fragment of human IgG1), and adalimu-

mab (a fully human monoclonal anti-TNF-α antibody)] have proved to be remarkably effective and safe in well-conducted clinical trials of patients with RA refractory to conventional therapy [78]. The newer TNF-α targeting immunobiologicals that are being developed are a PEG [poly(ethylene glycol)]-bound p55 TNFR (PEG–TNFR1), PEGylated TNF-α antibody fragments (CDP-870) and TACE inhibitors [79].

PHARMACOGENOMICS: A PROMISING FIELD

What is Pharmacogenomics

It is well recognized that most drug therapies exhibit wide variability among individuals in their efficacy and toxicity. A study conducted in the US estimated that over 100,000 patients die and 2.2 million are injured annually by adverse drug reactions. The incidence of serious and fatal cases in hospitalized patients is reported at 6–7%, making adverse drug reactions the fourth leading cause of death in the US [80]. Currently, negative effects from medications are monitored only after prescribed to patients. On a case-by-case basis, clinicians adjust dosage and treatment type according to the reported reactions of individual patients. This 'trial and error' approach has been criticized for exposing patients to potentially harmful drug therapies as well as exacting inefficient use of costly clinical consultation time. Adverse drug reactions have come under greater scrutiny as an area ripe for intervention with the use of genetic sequencing information and technology. For many medications, differences in reactions are due, in part, to polymorphisms in genes coding drug-metabolizing enzymes, drug transporters, and/or drug targets. Through the study of human genetic variation, the hope is that adverse drug reactions will be minimized and safety and efficacy will be improved in the creation of genomic-based medications [81,82]. The promise of personalized medicine suggests that individuals will receive the "right" medication as dictated by their unique genetic signatures.

Making good on the promise of pharmacogenomics, however, is contingent on putting several important measures in place in the health-care system. In order to produce more efficient drug dosing based on genotyping, routine pharmacogenetic testing of the population is fundamental. The management of such data will be a significant challenge with the maintenance of ''personal pharmacogenetic profiles'' most likely falling on the shoulders of health-care organizations. Given these challenges and the unlikely event that genotyping becomes so ubiquitous that patients are able to identify themselves by the multitude of SNPs involved in drug metabolism, one could predict that pharmacogenomics will be directed at potential markets that are easily identifiable. Physicians routinely make clinical decisions that assume genetic differences based on individuals' perceived race [83,84].

Current Use of Pharmacogenomics

Genetic and genomic testing and analysis are already being incorporated into treatment decisions for patients with many diseases. For example, estrogen receptor and progesterone receptor status are used to select breast cancer patients likely to respond to hormone therapy [85]. Human epidermal growth factor receptor (HER-2 [c-erbB2/neu]) expression is also used to help guide treatment selection in women with breast cancer, with significant overall survival benefit following chemotherapy in targeted patients [86]. Results from studies of patients with other malignancies demonstrated clear genetic determination of responses to therapy [87,88].

Genotype has also been demonstrated to influence responses to therapy in hepatitis C patients. Results from a recent study of patients with chronic hepatitis C indicated that increased hepatic expression of suppressor of cytokine signaling (SOCS)-3 mRNA was significantly associated with lack of response to IFN treatment [89].

Genetic polymorphisms that influence responsiveness to antidepressant therapy have also been identified. Although substantial further research is required, in the future, pharmacogenetic approaches may potentially affect the treatment of major depression [90]. Specific genetic variants have also been associated with responses to therapy in patients with schizophrenia [91].

An early yet growing body of evidence shows that incorporating our understanding of genomics into clinical practice can lead to clinical benefit. Genetic predictors of responses to specific therapies could be helpful in patients with asthma and clinicians should be educated regarding these determinants [92,93]. Therefore, there is a great need to investigate the existence and map certain polymorphisms and SNPs of TNF-α so in order to allow the design and production of individual drugs that would be more accurate and safer.

POLYMORPHISMS OF TNF-α PERMIT THE DEVELOPMENT OF MORE EFFECTIVE AND ACCURATE DRUGS

Genetic polymorphism of the TNF-α promoter and heterogeneity of the TNF-α receptor gene may play a significant role in the non-responsiveness of anti-TNF-α therapy.

Studies on the relevance of TNF promoter polymorphisms in asthma have produced mixed results.Whereas some studies have found evidence for an association between −308A and asthma with the A allele being more frequent in asthma cases [94,95], other investigations failed to find any association [96,97]. Interestingly, a study considering the extensive linkage disequilibrium present on chromosome 6 has found that extended haplotypes account for the association of TNF SNPs (single nucleotide polymorphisms) with asthma [98]. The extended haplotype LTαNco*1/ TNF–308*2/HLA-DRB1*02 was observed to have a remarkable association with asthma [OR (odds ratio) 6.68] and this resulted in an even stronger association with BHR (OR 21.9) in a study of over 1000 patients. Another study also found that BHR was associated with −308A, but again through an extended haplotype [99].

Another meta-analysis [100] clearly suggests that the TNF2 allele is a genetic contributor to overall asthma susceptibility. The TNF2/2 homozygote had a stronger association with asthma susceptibility than the TNF 2/1 heterozygote, indicating a dose response. These results suggest a possible beneficial role for the TNF1 allele versus the TNF2 allele in asthma, as well as synergy of the TNF2-TNF2 alleles. The TNF2/2 homozygote represents a greater risk fac-

tor for the development of asthma. These findings are consistent with TNF-α contributing to asthma inflammation both *in vitro* and *in vivo*. This reflects a direct functional effect of the TNF-α gene through its upregulation of TNF-α levels in asthma. Based on known or presumed mechanisms of disease pathophysiology, candidate gene strategies provide a useful approach for evaluating gene-disease associations. However, candidate gene case-control studies have been criticised because of a lack of replication. Some TNF-α studies have found positive associations between the TNF2 allele and asthma but others have not. Several factors may be influencing these differences. Firstly, if another variant in or near the TNF-α gene was the causal variant, the true association could easily be missed. Different linkage disequilibrium patterns with the functional variant may lead to variable results in different populations. It is feasible that the TNF-α gene variant is playing a role in asthma in cooperation with other gene variants exhibiting a more limited biology. Previous studies have shown that there is a strong linkage disequilibrium between TNF-α and LTa polymorphism [101]. A haplotype analysis by Bilolikar *et al.* showed that the LTa 252A/TNF2 combination was associated with a markedly increased risk for both asthma and infant wheezing. The study by Randolph *et al.* supported the suggestion that the haplotype LTa-1/LTa 4371T/TNF1/ TNF1078G is associated with both asthma overall and asthmatic phenotypes. Of note, the TNF-α gene is found within a 7 kb section of the informative major histocompatibility complex (MHC) class III region. Due to the close proximity of the TNF locus to HLA loci, linkage disequilibrium exists with several HLA alleles. For example, TNF2 is strongly associated with the HLA A1, B8 and DR3 alleles [102]. Moreover, Moffatt *et al.* found that the extended haplotype LTa-1/TNF2/HLA-DRB2 was associated with asthma (OR 6.68, p=0.002) and even more strongly associated with bronchial hyperresponsiveness (OR 21.9, p,0.0001) in a study of over 1000 patients.

CONCLUSION

In conclusion, summarising the variety of the available therapies against asthma we can assume a number of different approaches and potential therapeutic targets (present and potential). Moreover, each of these targets has representative therapeutic agents that have varying effectiveness.

However, despite all these available drugs, asthma still remains a condition concerning many patients worldwide and affecting their quality of life or may threaten their lives. Thus, a lot of work needs to be done towards the development of very specific drugs that have improved therapeutic result with minimum adverse reactions. This can only be achieved if "individual – personal" drugs are designed, special for the genetic profile of each patient.

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