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CHAPTER 3
Transcription Factors and Inflammatory Lung Disease

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1. Introduction

Inflammation is a central feature of many lung diseases, including asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, fibrosing alveolitis and adult or acute respiratory distress syndrome (ARDS). The specific characteristics of the inflammatory response and the site of inflammation differ between these diseases, but all involve the recruitment and activation of inflammatory cells and chances in the structural cells of the lung. These diseases are characterized by an increased expression of many proteins involved in the complex inflammatory cascade. These

inflammatory proteins include cytokines, enzymes that produce inflammatory mediators, receptors and adhesion molecules. The increased expression of most of these proteins is the result of increased gene transcription; many of the genes are not expressed in normal cells but are induced in certain cell types in these inflammatory diseases. Changes in gene transcription are regulated by transcription factors, which are proteins that bind to DNA. This suggests that transcription factors may play a key role in the pathophysiology of inflammatory diseases, because they regulate the increased gene expression that may underlie the acute and chronic inflammatory mechanisms that characterize these diseases. Corticosteroids are the most effective therapy in the long-term control of asthma and appear to reduce inflammation in asthmatic airways largely by inhibiting the transcription factors that regulate abnormal gene expression. Corticosteroids may also have a beneficial effect in other inflammatory lung diseases, although this is less marked than in asthma, indicating that different genes and transcription factors are involved.

2. Transcription Factors

Transcription factors are proteins that bind to regulatory sequences, usually in the 5′-upstream promoter region of target genes, to increase (or sometimes decrease) the rate of gene transcription. This may result in increased or decreased protein synthesis and altered cellular function. Transcription factors may be activated by many extracellular influences acting via surface receptors which lead to phosphorylation by several types of kinase [1, 2], or may be directly activated by ligands (such as corticosteroids, thyroid hormone and vitamins). Transcription factors may therefore convert transient environmental signals at the cell surface into long-term changes in gene transcription, thus acting as "nuclear messengers" [3]. Transcription factors may be activated within the nucleus, often with the transcription factor bound to DNA, or within the cytoplasm, resulting in exposure of nuclear localization signals and targeting to the nucleus. Thus transcription factors convert environmental signals into altered gene expression. In the context of inflammatory diseases, transcription factors activated by inflammatory stimuli (such as cytokines or viruses) switch on inflammatory genes, leading to increased synthesis of inflammatory proteins (Figure 1). In this way transcription factors may amplify and perpetuate the inflammatory process and this makes them an important potential target in the development of new anti-inflammatory drugs. It is possible that abnormal functioning of transcription factors may determine disease severity and responsiveness to treatment [4]. Of particular importance is the demonstration that transcription factors may physically interact with each other, resulting in inhibition or enhancement of transcriptional activity.

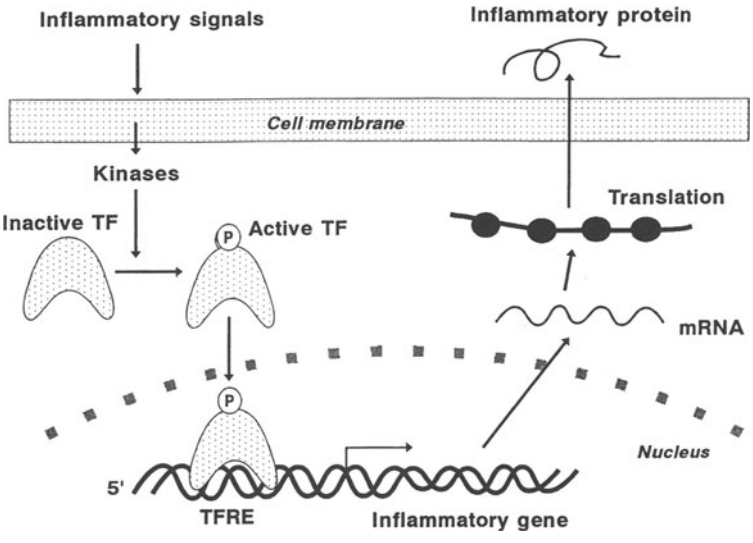

Figure 1. Transcription factors may play a key role in the amplification and perpetuation of inflammatory diseases. Inflammatory signals activate transcription factors (TF) by phosphorylation; these then bind to recognition elements (TFRE) in the promoter regions of inflammatory genes to increase the synthesis of inflammatory proteins.

2.1. Transcription Factor Families

Several families of transcription factors exist and members of each family may share structural characteristics. These families include helix–turn–helix (e.g. POU), zinc finger (e.g. glucocorticoid receptors), basic protein-leucine zipper (cyclic AMP response element-binding factor or CREB, nuclear factor-κB (NF-κB], activator protein-1 [AP-1]) and β-sheet motifs (e.g. HU) [5]. Many transcription factors are common to several cell types (ubiquitous), whereas others are cell specific and determine the phenotypic characteristics of a cell.

2.2. Methods for Studying Transcription Factors

There is relatively little information about the regulation of transcription factors in the airways, particularly in diseases such as asthma. However, molecular methods have been developed for investigation of transcription factor expression and activity. These methods include immunoblotting and immunocytochemistry to detect the transcription factor protein, electrophoretic mobility shift assays to measure transcription factor binding to DNA and DNA footprinting to determine binding to recognition sequences in particular genes. Many transcription factors have now been discovered,

but we will concentrate on some of the transcription factors that may be relevant in inflammatory lung diseases. In the future, the genetic control of transcription factor expression may be an increasingly important aspect of research, because this may be one of the critical mechanisms regulating expression of disease phenotypes and their responsiveness to therapy.

2.3. Transcription Factor Interactions

One of the most important concepts to have emerged is that transcription factors may interact with other transcription factors. This then allows cross-talk between different signal transduction pathways at the level of gene expression [6]. Indeed, it is the interaction of transcription factors that may give them different properties in different cell types, because the presence of other transcription factors will profoundly influence the effect exerted by a particular factor on gene expression. Interaction between transcription factors is particularly relevant to the action of drugs, such as corticosteroids and cyclosporin A, which activate or block transcription factors that subsequently modulate other transcription factors.

2.4. Basal Transcription Machinery

Binding of transcription factors to their specific recognition sequences, or activation of the already bound transcription factors in the control regions (promoters) of target genes, is communicated to the basal transcription machinery bound to the TATA box near the start site of transcription. This then leads to activation of the critical enzyme RNA polymerase II, via a chain of basal factors, resulting in increased transcription of the gene and formation of messenger RNA (mRNA), which in turn is then translated into a protein. Binding of transcription factors to their specific binding motifs in the promoter region may alter transcription by interacting directly with components of the basal transcription apparatus or via co-factors that link the transcription factor to the basal transcription apparatus [7] (Figure 2). As DNA loops around histone residues, binding of a transcription factor even far from the TATA boy may interact.

Large proteins that bind to the basal transcription machinery may interact with many transcription factors and thus act as integrators of gene transcription. These co-activator molecules include CREB-binding protein (CBP), which was first recognized as a protein that the transcription factor CREB had to bind to in order to exert its effects [8]. Other co-activator molecules include p300, and these co-activators may bind multiple transcription factors, thus allowing complex interactions between different signalling pathways.

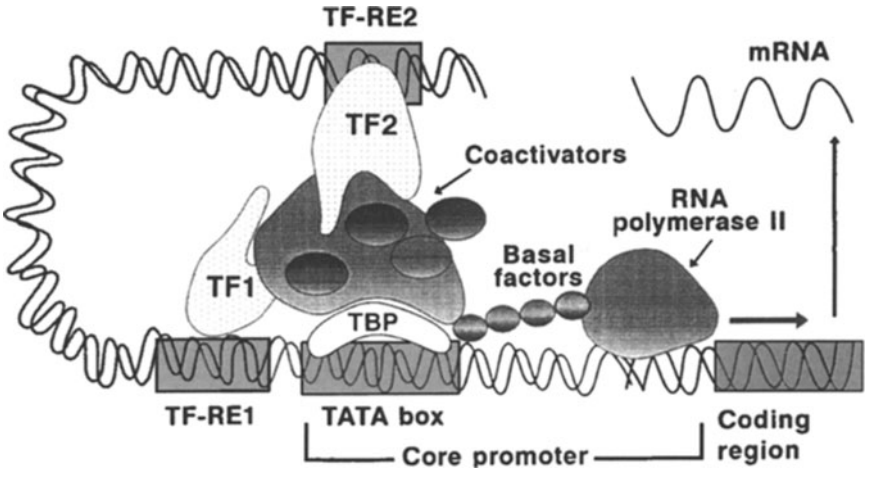

Figure 2. The basal transcription apparatus consists of various proteins that bind to the TATA box at the start site of transcription. These factors include co-activators that bind to transcription factors, some of which may be situated far from the start site as DNA loops around. Transcription factors (TF) bind to specific recognition sequences in the 5′-promoter region (TFRE), resulting in increased transcription by activating RNA polymerase II which in turn, results in transcription of messenger RNA (mRNA).

2.5. Specific and Ubiquitous Transcription Factors

Many transcription factors have now been identified and a large proportion of the genome appears to code for these proteins. Many transcription factors are cell specific and are responsible for the selective expression of genes that characterize a particular cell in terms of its structural characteristics, differentiation or function. One example of a specific transcription factor is nuclear factor of activated T cells (NF-AT) which regulates the expression of the lymphocyte proliferative factor interleukin-2 (IL-2) in T lymphocytes. Many transcription factors, such as AP-1 and nuclear factor κB (NF-κB), are ubiquitous and regulate large sets of genes, so that a coordinated cellular response is produced. There may also be important interactions between transcription factors, so that it is necessary to have coincident activation of several transcription factors in order to have maximal gene expression. For example, IL-8 is regulated by NF-κB and C/EBPβ (or nuclear factor of IL-6). C/EBPβ binding alone has little effect on IL-6 transcription but markedly enhances the effect of NF-κB binding, resulting in maximal gene expression [9]. This means that IL-8 will be maximally transcribed only when both transcription factors are activated simultaneously by coincident activating signals. These sorts of interaction explain how transcription factors that are ubiquitous may regulate particular genes in certain types of cell.

3. Nuclear Factor-κB (NF-κB)

NF-κB is a ubiquitous transcription factor that appears to be of particular importance in inflammatory and immune responses. There is increasing evidence that NF-κB plays a pivotal role in orchestrating the inflammatory response and acts as an amplifying and perpetuating mechanism [10]. NF-κB was first identified as a regulator of immunoglobulin κ light chain gene expression in murine B lymphocytes [11], but has subsequently been identified in most cell types. NF-κB binds to the κB DNA sequence 5′-GGGACTTTCC-3′. Several different NF-κB proteins have been characterized and belong to the Rel family of proteins, which share a region of about 300 amino acids known as the Rel homology domain and containing the DNA-binding elements [12, 13]. The activated form of NF-κB is a heterodimer, which usually consists of two subunits, p65 (RelA) and p50, although other forms such as Rel, RelB, v-Rel, p52, p105 (NF-κB1) and p100 (NF-κB2) may also occur. The p50 may be constitutively bound to DNA but requires p65 for transactivation activity. Rel proteins were identified even in invertebrates, such as *Drosophila* sp., where they play an important role in both development and primitive inflammatory responses. Targeted disruption of the genes coding for p65 or p50 in mice ("knock-outs") results in severe immune deficiency which is lethal in the case of p65 [14, 15].

3.1. Activation of NF-κB

In unstimulated cells. NF-κB is localized to the cytoplasm because of binding to inhibitory proteins (IκB), of which several isoforms exist (IκB-α, IκB-β, IκB-γ, IκB-δ, IκB-ε), the most abundant being IκB-α [16, 17]. When the cell is appropriately stimulated, specific IκB kinases phosphorylate IκB, leading to the rapid addition of ubiquitin residues (ubiquitination) which make it a substrate for the proteasome, a multifunctional cellular protease [18, 19] (Figure 3). A specific IκB-α kinase complex (IKK) has now been identified and contains at least two interacting subunits [20]. Several signal transduction pathways are involved in the activation of NF-κB and enzymes from the mitogen-activated protein (MAP) kinase pathways may interact at various points in the activation of NF-κB [21]. A newly described kinase, NF-κB-inducing kinase (NIK), is a MAP3K-related enzyme involved in the activation of IKK by tumour nectoris factor-α (TNF-α) and IL-1β [22]. A key enzyme in the stress-activated MAP kinase pathway, which leads to the activation of c-Jun N-terminal kinase, is MEKK1, which also activates the IκB-α kinase complex, indicating that mechanisms that activate JNK and AP-1 may also activate NF-κB [23].

Degradation of IκB uncovers nuclear localization signals on p65 and p50, so it is rapidly transported into the nucleus where it binds to specific

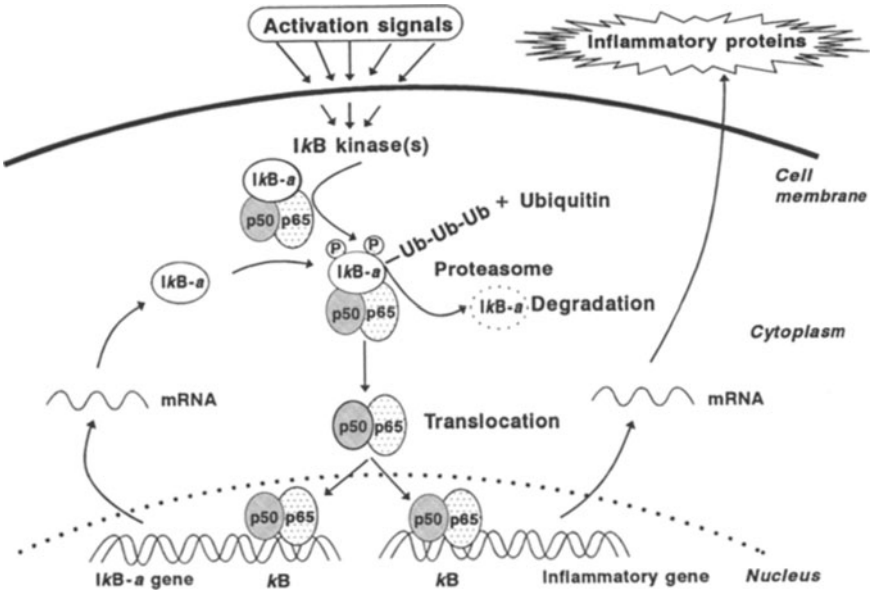

Figure 3. Activation of nuclear factor-κB (NF-κB) involves phosphorylation of the inhibitory protein IκB by specific kinase(s), with subsequent ubiquitination and proteolytic degradation by the proteasome. The free NF-κB then translocates to the nucleus, where it binds to κB sites in the promoter regions of inflammatory genes. Activation of the IκB-α gene results in increased synthesis of IκB-α to terminate the activation of NF-κB.

κB recognition elements in the promoter regions of target genes. The IκB-α gene (MAD-3) itself has several κB sequences in its promoter region, so that NF-κB induces the synthesis of IκB-α, which enters the nucleus to bind NF-κB and induce its export to the cytoplasm, thus terminating activation [24]. Newly synthesized IκB-α interacts with and binds to NF-κB heterodimers within the cytoplasm and to NF-κB bound to κB sites within the nucleus [25]. Targeted disruption of the IκB-α gene results in prolonged activation of NF-κB and animals die of inflammation [26]. By contrast, IκB-β is not induced by NF-κB, so NF-κB is likely to be activated for a more prolonged period in cell types in which IκB-β predominates [27].

Many stimuli activate NF-κB, including cytokines IL-1β, TNF-α, IL-2 and granulocyte-macrophage colony-stimulating factor (GM-CSF) [12], oxidative stress (particularly hydrogen peroxide) [28], viruses (such as rhinovirus and adenovirus) [29], phorbol esters, lipopolysaccharide, and B- and T-lymphocyte activation. Several signal transduction pathways may be involved in this activation, but all of these stimuli appear to act via rapidly activated protein kinases that lead to IκB phosphorylation. The activation of these protein kinases may be blocked by antioxidants, such as pyrrolidine dithiocarbamate (PDTC) and *N*-acetylcysteine, suggesting that reac-

tive oxygen species may act as intermediary molecules in NF-κB activation in response to a wide range of stimuli [30].

3.2. *Inflammatory and Immune Genes*

NF-κB is now known to regulate the expression of many inflammatory and immune genes. Many of these genes are induced in inflammatory and structural cells and play an important role in the inflammatory process. Although NF-κB is not the only transcription factor involved in regulation of the expression of these genes, it often appears to have a decisive regulatory role. NF-κB often functions in cooperation with other transcription factors, such as AP-1 and C/EBP, which are also involved in regulation of inflammatory and immune genes [9, 31]. Genes induced by NF-κB include those for the inflammatory cytokines IL-1β, TNF-α and GM-CSF, and the chemokines IL-8, macrophage inflammatory protein-1α (MIP-1α), macrophage chemotactic protein-1 (MCP-1), RANTES and eotaxin, which are largely responsible for attracting inflammatory cells into sites of inflammation [12, 32–35]. NF-κB also regulates the expression of inflammatory enzymes, including the inducible form of nitric oxide synthase (iNOS), which produces large amounts of NO [36], and inducible cyclo-oxygenase (COX-2), which produces prostanoids [37, 38]. NF-κB also plays an important role in regulating expression of adhesion molecules, such as E-selectin, vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molcule-1 (ICAM-1), which are expressed on endothelial and epithelial cells at inflammatory sites and play a key role in the initial recruitment of inflammatory cells [39, 40]. This suggests that activation of NF-κB leads to the coordinated induction of multiple genes that are expressed in inflammatory and immune responses.

Products of genes that are regulated by NF-κB also cause its activation. Thus the proinflammatory cytokines IL-1β and TNF-α both activate and are activated by NF-κB; this may result in a positive regulatory loop which may be important in amplifying and perpetuating the inflammatory response at the local site (Figure 4).

NF-κB also plays a complex role in apoptosis. Inhibition of NF-κB increases apoptosis in response to TNF-α in several cell types, including lymphocytes, suggesting that NF-κB counteracts apoptosis [41, 42].

3.3. *Role in Inflammatory Lung Diseases*

NF-κB may be activated by many of the stimuli that exacerbate asthmatic inflammation. In experimental animals allergen exposure in sensitized animals activates NF-κB in the lung [43] with concomitant expression of iNOS and chemokines [44]. In animal studies *in vivo* activation of T lym-

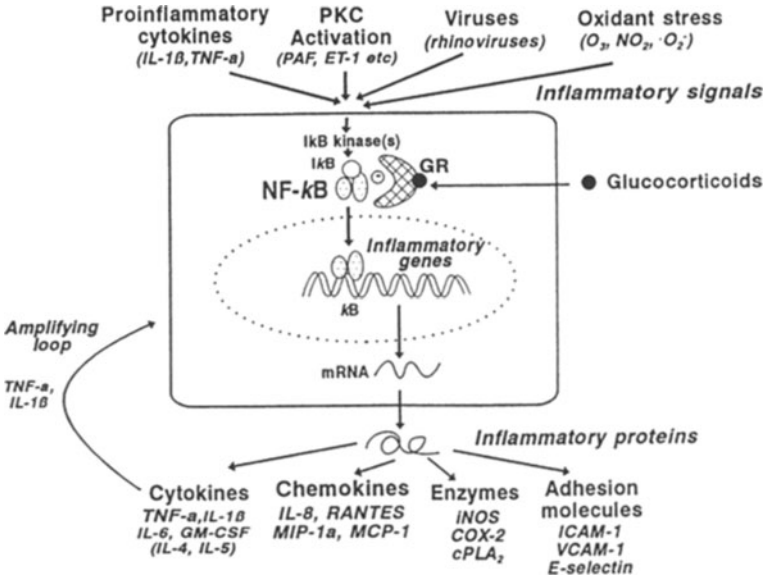

Figure 4. NF-κB may be activated by a variety of inflammatory signals, resulting in the coordinated expression of multiple inflammatory genes, including cytokines, chemokines, enzymes and adhesion molecules. The cytokines IL-1β and TNF-α both activate and are regulated by NF-κB and may act as an amplifying feed-forward loop. The actions of NF-κB are inhibited by glucocorticoids by binding to activated glucocorticoid receptors (GR).

phocytes with CD3 antibodies results in marked activation of NF-κB [45]. Oxidants activate NF-κB in a human epithelial line, resulting in increased expression of iNOS [46], and exposure of animals to the oxidant ozone results in NF-κB expression in lung [47]. Exposure of human peripheral blood mononuclear cells, epithelial cells and lung tissue to proinflammatory cytokines results in marked activation of NF-κB which may be prolonged [48–50]. Virus infections are common triggers of acute severe exacerbations of asthma and are thought to initiate a prolonged inflammatory response. Thus experimental rhinovirus infection results in activation of NF-κB and IL-6 secretion in nasal epithelial cells [29]. Viruses may activate NF-κB through mechanisms that involve generation of reactive oxygen intermediates [28]. There is also evidence for activation of NF-κB in biopsies of patients with asthma and in inflammatory cells in the sputum [51].

Many of the inflammatory and immunoregulatory genes (cytokines, enzymes, adhesion molecules) expressed in asthma are regulated predominantly by NF-κB. One such gene that has been studied extensively is iNOS which is expressed in airway epithelial cells and macrophages in asthma [52]. This increased iNOS expression is reflected by increased amounts of NO in exhaled air of asthmatic patients [53].

Although there are many similarities between the inflammatory responses in arthritis, asthma, inflammatory bowel disease and other inflammatory diseases, there are also important differences and clearly factors other than NF-κB are involved [10]. These differences may relate to the secretion of specific cytokines, such as IL-5 in asthmatic inflammation which promotes an eosinophilic inflammation. The role of NF-κB should be seen as an amplifying and perpetuating mechanism that will exaggerate the disease-specific inflammatory process through the coordinated activation of multiple inflammatory genes. Thus, IL-5 alone results in relatively little accumulation of eosinophils within tissues, but this is enormously amplified by the local injection of the eosinophil-specific chemokine, eotaxin, which is regulated via NF-κB [54].

NF-κB is also likely to be involved in the alveolar inflammation of fibrosing lung disease. Although no direct measurements of NF-κB have been made, there is evidence for increased expression of iNOS, which is largely regulated by NF-κB [55], and there is an increase in exhaled NO in patients with active alveolitis [56]. Asbestos exposure also activates NF-κB in lungs of experimental animals [57], and thus may be linked to the fibrotic process.

NF-κB is also likely to be involved in ARDS [58]. Endotoxin is a potent activator of NF-κB in lungs and alveolar macrophages of experimental animals and this may underlie the neutrophil response in the lungs [59]. The antioxidant *N*-acetylcysteine inhibits this endotoxin-mediated NF-κB activation and the neutrophilic response. NF-κB activation is likely to underlie the increased expression of iNOS in the lungs of rats exposed to endotoxin [60]. Alveolar macrophages from patients with ARDS have increased activation of NF-κB compared with patients with other severe diseases [61]. This would be consistent with reports of increased IL-8 in bronchoalveolar lavage fluid of patients with ARDS [62].

4. Activator Protein-1 (AP-1)

AP-1 is a heterodimer of Fos and Jun oncoproteins, which is a member of the basic leucine zipper (bZIP) transcription family, characterized by a basic leucine-rich area that is involved with dimerization with other transcription factors (Figure 5). AP-1 was originally described by binding to the TPA (tetradecanoylphorbol-13-acetate) response element (TRE: 5′-TGAC/GTCA-3′) and is responsible for the transcriptional activation of various genes that were activated by phorbol esters (such as TPA, also known as phorbol mysistate acetate or PMA) via activation of protein kinase C (PKC) [63]. It is now apparent that AP-1 is a collection of related transcription factors belonging to the Fos (c-Fos, FosB, Fra1, Fra2) and Jun (c-Jun, JunB, JunD) families which dimerize in various combinations through their leucine zipper region. Fos/Jun heterodimers bind with the

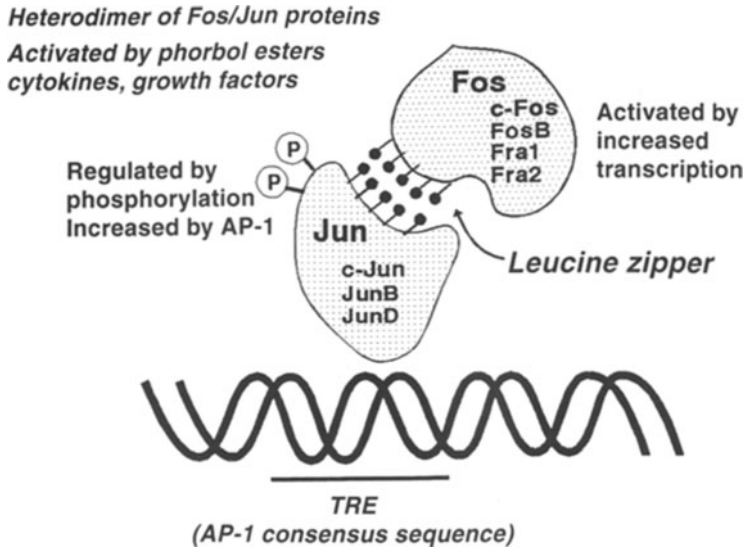

Figure 5. AP-1 is a heterodimer of Fos and Jun proteins.

greatest affinity and are the predominant form of AP-1 in most cells, whereas Jun/Jun homodimers bind with low affinity. AP-1 proteins may also form functionally distinct dimeric complexes with members of the related bZIP family of ATF/CREB transcription factors.

AP-1 may be activated via PKC and by various cytokines, including TNF-α and IL-1β via several types of protein tyrosine kinase (PTK) and mitogen-activated protein (MAP) kinase, which themselves activate a cascade of intracellular kinases [64, 65]. Both TNF-α and IL-1β activate TNF-associated factors (TRAF), which subsequently activate MAP kinases [21]. Recent studies suggest that there may be interactions between the AP-1 activating pathways and NF-κB pathways, in that TRAF2 (activated by TNF-α) and TRAF-6 (activated by IL-1β) may both activate NIK and then IKK [21] (Figure 6).

We have demonstrated the activation of AP-1 in human lung after stimulation with PMA, TNF-α and IL-1β [50, 66], and in peripheral blood mononuclear cells after activation with PMA [48]. Certain signals rapidly increase the transcription of the *fos* gene, resulting in increased synthesis of Fos protein. Other signals lead to activation of kinases that phosphorylate c-Jun, resulting in increased activation. Several specific c-Jun N-terminal kinases (JNK) are now recognized, and may play an important role in the regulation of cellular responsiveness to cytokine signals [65, 67, 68]. Conversely a Jun phosphatase counteracts the activation of AP-1, and a deficiency of this enzyme might lead to amplification of chronic inflammation.

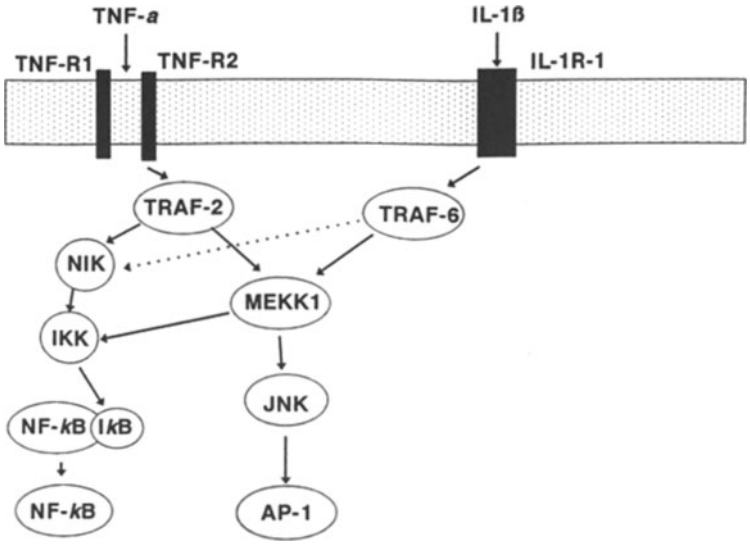

Figure 6. Interaction between AP-1 and NF-κB activating pathways: TNF-α binding to TNF-receptor-2 (TNF-R2) activates TNF-associated factor-2 (TRAF-2) which then activates NF-κB-inducing kinase (NIK); this in turn, leads to activation of NF-κB via activation of the IκB kinase complex (IKK). TRAF-2 also activates a MAP kinase enzyme, MEKK1, which leads to activation of Jun N-terminal kinase (JNK) and activation of AP-1. Similarly, IL-1β binds to the IL-1 receptor (IL-1R-1), leading to activation of TRAF-6, which also activates NIK and MEKK1, resulting in parallel activation of AP-1 and NF-κB.

The role of AP-1 in other inflammatory lung diseases has not been investigated. Endotoxin activates AP-1, so that it is likely to be involved with NF-κB in the regulation of inflammatory genes in this condition. For example, endotoxin induces haem oxygenase-1 via AP-1 activation [69].

There is evidence for increased expression of c-Fos in epithelial cells in asthmatic airways [70], and many of the stimuli relevant to asthma that activate NF-κB will also activate AP-1. AP-1, like NF-κB, regulates many of the inflammatory and immune genes that are over-expressed in asthma. Indeed many of these genes require the simultaneous activation of both transcription factors that work together cooperatively.

The recognition that AP-1 may interact with other transcription factors indicates that cross-talk between different signal transduction pathways is possible [71]. The activated glucocorticoid receptor (GR) directly interacts with activated AP-1, and this may be an important action of steroids to inhibit cytokine-mediated inflammatory responses (see below). AP-1 also interacts with cell-specific transcription factors, such as nuclear factor of activated T cells (NF-AT) (see below).

5. CAAT/Enhancer-Binding Proteins

C/EBP are transcription factors important in IL-1, IL-6 and lipopolysaccharide (LPS)-dependent signal transduction and bind to a consensus sequence ATTGCGCAAT, which includes the CAAT box. These transcription factors are members of the bZIP class of transcription factors and include C/EBPα, C/EBPβ (formerly called nuclear factor for IL-6), C/EBPγ and C/EBPδ [72]. These transcription factors are activated by pathways that involve PKC and regulate the expression of several inflammatory and immune genes. They often cooperate positivelly with other transcription factors, particularly other bZIP proteins, such as AP-1, ATF and CREB, but also with NF-κB. Thus, in the regulation of IL-8 gene expression there is a marked enhancement of transcription when C/EBPβ is activated together with NF-κB, whereas C/EBPβ activation alone has little effect [9]. Splice isoforms of these transcription factors, which appear to have blocking effects on transcription, have been identified.

The role of C/EBP in asthma has not yet been defined, but it is likely that activation of this transcription factor by inflammatory signals is an important amplifying mechanism for the expression of inflammatory genes, such as iNOS, COX-2 and certain chemokines, which have C/EBPβ recognition sequences in their promoter regions. Many of the effects of IL-6 are mediated through activation of C/EBPβ and this cytokine is produced in increased amounts from macrophages of asthmatic patients [73] and is further enhanced by allergen exposure via low-affinity immunoglobulin E (IgE) receptors (FcεRII) [74]. Rhinovirus infection markedly increases the concentrations of IL-6 in induced sputum and levels remain elevated for several days [75].

6. JAK/STAT Family

Several cytokines, including interferons, activate specific cytosolic tyrosine kinases known as Janus kinases (JAK) [76, 77] (Figure 7). Members of the JAK family include JAK1, JAK2, JAK3, TyK1 and TyK2 and may be differentially activated by different cytokines. Thus IL-2 activates JAK1 and JAK3, IL-6 activates JAK1, JAK2 and JAK3, whereas IL-5 activates only JAK2. JAKs are constitutively associated with the cytoplasmic domains of cytokine receptors and become activated upon ligand-induced receptor homo- or heterodimerization. JAKs then phosphorylate tyrosine residues on the cytoplasmic domains of cytokine receptors, which create docking domains for a family of transcription factors known as signal transducers and activators of transcription (STATs) [72]. Phosphorylation of the SH2 domain of STATs results in the formation of homo- or heterodimers which migrate to the nucleus, where they bind to response elements on promoter sequences to regulate the transcription of specific genes. An increasing number of STAT proteins have now been identified and, again,

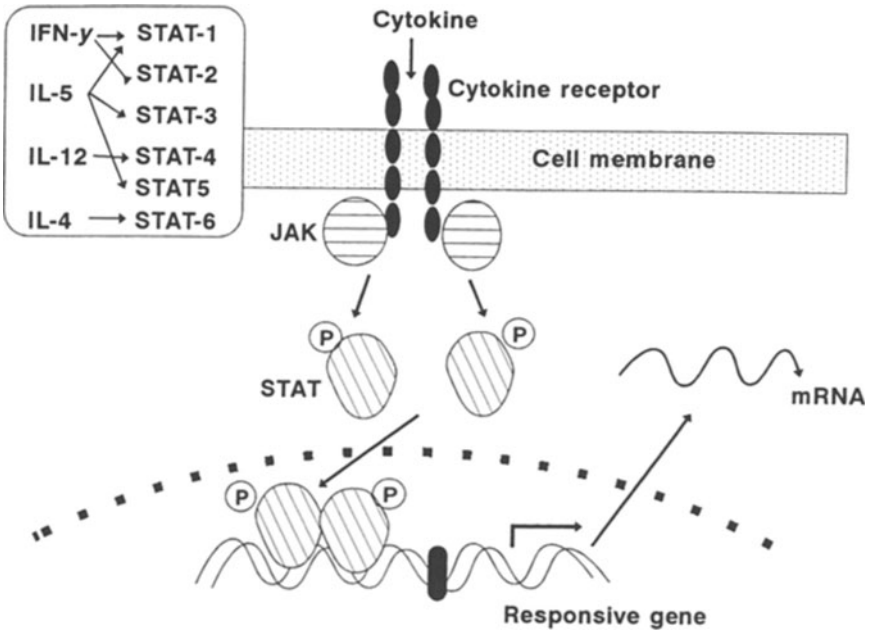

Figure 7. JAK-STAT pathways: cytokine binding to its receptor results in activation of Janus kinases (JAK) which phosphorylate intracellular domains of the receptor, resulting in phosphorylation of signal transduction-activated transcription factors (STATs). Activated STATs dimerize and translocate to the nucleus where they bind to recognition elements on certain genes.

there is specificity with particular cytokines. Thus INF-γ activates STAT1 only, whereas IFN-α activates STAT1 and STAT2 to form a STAT1/STAT2 heterodimer; these, in turn bind to IFN-γ activation sequences (GAS). STATs affect transcription by interacting with the co-activator molecules CBP and p300 [78, 79].

There is specificity in JAK-STAT pathways. Thus IL-6 activates STAT3, whereas IL-4 activates STAT6 [80, 81]. STAT6 therefore provides a novel target for blocking the effects of IL-4 as a potential treatment for allergic asthma. STAT6 knock-out mice have no response to IL-4, fail to produce IgE on allergen sensitization and do not develop Th2 cells in response to IL-4, indicating the critical role of STAT6 in allergic responses [82]. By contrast IL-12 signals via activation of STAT4 [83]. STAT4 knock-out mice have no response to IL-12 and have a propensity to develop Th2 lymphocytes [84]. IL-5 appears to activate several STATs, including STAT1, STAT3 and STAT5α [85–87]. STAT5, originally identified as a mediator of the growth effects of prolactin, also mediates the effects of GM-CSF [87].

Recently, inhibitors of STATs have been identified which are themselves regulated through JAK-STAT pathways and therefore provide a mechanism for switching of cytokine-triggered cellular signalling [88, 89].

7. Cyclic AMP Response Element-Binding Protein

Increased concentrations of cAMP also result in the activation or inhibition of gene transcription; cAMP activates protein kinase A, which phosphorylates the transcription factor CREB, which in turn binds to a CRE in the promoter region of certain genes [90]. CREB is a member of large family of CRE-binding proteins, including members of the activating transcription factor (ATF) family. CREB itself binds to CBP which acts as a co-activator molecule that binds to the TATA box and initiates transcription [91]. CREB may be counteracted by another transcription factor called CRE modulator (CREM) which may block the effects of CREB on CRE (although some splice variants appear to increase CRE binding). CREB appears to be important in the regulation of β_2-adrenoceptor expression [92]. It is activated by relatively high concentrations of β_2-agonists in lung [93] and may play a role in the down-regulation of β_2-receptors after chronic exposure to β_2-agonists [94, 95]. CREB also regulates the expression of several immune and inflammatory genes, including GM-CSF and IL-5.

CREB interacts directly with other transcription factors, allowing cross-talk between different signalling pathways. Thus CREB has a negative effect on AP-1 [96] and GR [97]. High concentrations of β-agonists inhibit the binding of GR to DNA [93, 98]. This may interfere with the anti-inflammatory effects of steroids and may account for the deleterious effects of high-dose inhaled β-agonists in some patients with asthma [99].

8. CREB-Binding Protein

Recent evidence suggests that several transcription factors interact with large co-activator molecules, such as CBP and the related p300, which bind to the basal transcription factor apparatus [100]. Several transcription factors have now been shown to bind directly to CBP, including AP-1, NF-κB and STATs [79, 101–103]. As binding sites on this molecule may be limited, this could result in competition between transcription factors for the limited binding sites available, so that there is an indirect rather than a direct protein–protein interaction (Figure 8). CBP also interacts with nuclear hormone receptors, such as glucocorticoid receptor (GR) and retinoic acid. These nuclear hormone receptors may interact with CBP and the basal transcriptional apparatus through binding to other nuclear co-activator proteins, including steroid receptor co-activator-1 (SRC-1) [104, 105], transcription factor intermediary factor-2 (TIF2) or glucocorticoid receptor-interacting protein-1 (GRIP-1 for the GR) [106]. A newly described nuclear protein called p300/CBP co-integrator-associated protein (p/CIP) appears to be particularly important in the binding of several nuclear receptors to CBP/p300 [107]. These nuclear activator proteins associate with nuclear receptors via a common sequence LXXLL (where L is lysine and X is any amino acid) [108].

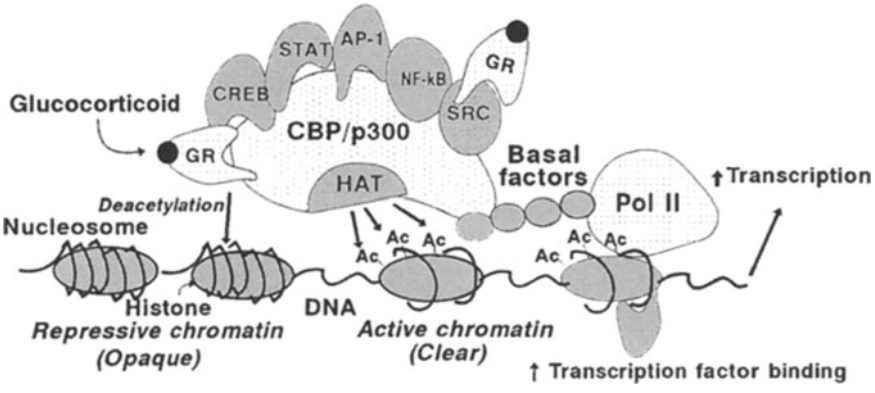

Figure 8. Co-activator molecules: transcription factors, such as STATs, AP-1 and NF-κB, bind to co-activator molecules, such as CREB-binding protein (CBP) or p300, which have intrinsic histone acetyltransferase (HAT) activity, resulting in acetylation (Ac) of histone proteins around which DNA is wound in the chromosome. This leads to unwinding of DNA and so allows increased binding of transcription factors, resulting in increased gene transcription. Glucocorticoid receptors (GR), after activation by corticosteroids, bind to a steroid receptor co-activator which is bound to CBP. This results in deacetylation of histone, with increased coiling of DNA around histone, thus preventing transcription factor binding leading to gene repression.

DNA is wound around histone proteins to form nucleosomes and the chromatin fibre in chromosomes. It has long been recognized at a microscopic level that chromatin may become dense or opaque as a result of the winding or unwinding of DNA around the histone core. CBP and p300 have histone acetylation activity which is activated by the binding of transcription factors, such as AP-1 and NF-κB [109]. Acetylation of histone residues results in unwinding of DNA coiled around the histone core, thus opening up the chromatin structure; this allows transcription factors to bind more readily, thereby increasing transcription (Figure 8). Repression of genes reverses this process by histone deacetylation [110]. The process of deacetylation involves the binding of hormone or vitamin receptors to co-repressor molecules, such as nuclear receptor co-repressor (N-CoR) which forms a complex with another repressor molecule Sin3 and a histone deacetylase [111, 112]. Deacetylation of histone increases the winding of DNA round histone residues, resulting in dense chromatin structure and reduced access of transcription factors to their binding sites, thereby leading to repressed transcription of inflammatory genes.

9. Glucocorticoid Receptors (GRs)

Glucocorticoid receptors are members of the nuclear receptor superfamily which includes other steroids (oestrogen, progesterone), and receptors for vitamins (vitamins A and D) and thyroid hormone. GRs are transcription

factors that regulate the transcription of several steroid-responsive target genes [113]. They are expressed in most types of cell, and in human lung there is a high level of expression in airway epithelium and the endothelium of bronchial vessels [114]. The inactive GR is bound to a protein complex which includes two molecules of 90-kDa heat shock protein (hsp90) and an immunophilin; these act as molecular chaperones, protecting the nuclear localization site. Glucocorticoids bind to GRs in the cytoplasm, resulting in dissociation of these molecules and rapid nuclear localization and DNA binding. GRs form homodimers to interact with glucocorticoid response elements (GRE: GGTACAnnnTGTTCT), resulting in increased gene transcription [115]. Recently, it has become apparent that steroid receptors bound to DNA may interact with CBP to enhance transcription and the adenovirus protein E1A, which inactivates CBP, interfere with the action of steroids [104]. Steroids interact with CBP through binding of their ligand-activated GRs to p/CIP and other nuclear proteins [107].

Relatively few genes have GREs. One well-studied example is the human β_2-adrenoceptor gene which has at least three GREs [92]. Corticosteroids increase transcription of β_2-receptors in animal and human lung and this may prevent tolerance to the effects of β_2-agonists by compensating for their down-regulation [95, 116]. Corticosteroids also increase the transcription of several anti-inflammatory proteins, including lipocortin-1, secretory leukoprotease inhibitor, CC-10 and IL-1 receptor antagonist, and these effects are presumably also mediated via GREs in the promoter regions of these genes [117]. Corticosteroids have also been reported to increase the expression of IκB-α in lymphocytes and thus to inhibit NF-κB [45, 118], but this has not been seen in other cell types [119–121]. The IκB-α gene does not appear to have any GRE consensus sequence so any efect of corticosteroids is probably mediated via other transcription factors.

9.1. Interaction With Other Transcription factors

The major anti-inflammatory effects of corticosteroids are through repression of inflammatory and immune genes, and it was believed that this was likely to be mediated through negative GREs resulting in gene repression. However, none of the inflammatory and immune genes that are switched off by steroids in asthma appears to have negative GREs in their promoter sequences, suggesting that there must be some less direct inhibitory mechanism. The inhibitory effect of corticosteroids appears to result largely from a protein–protein interaction between activated GRs and transcription factors, such as AP-1, NF-κB and C/EBP, which mediate the expression of these inflammatory genes [122] (Figure 9). Direct protein–protein interactions have been demonstrated between GRs and AP-1 [6, 71], and between the p65 component of NF-κB [48, 123, 124] and some STAT proteins, such as STAT5 [125], suggesting that corticosteroids block the

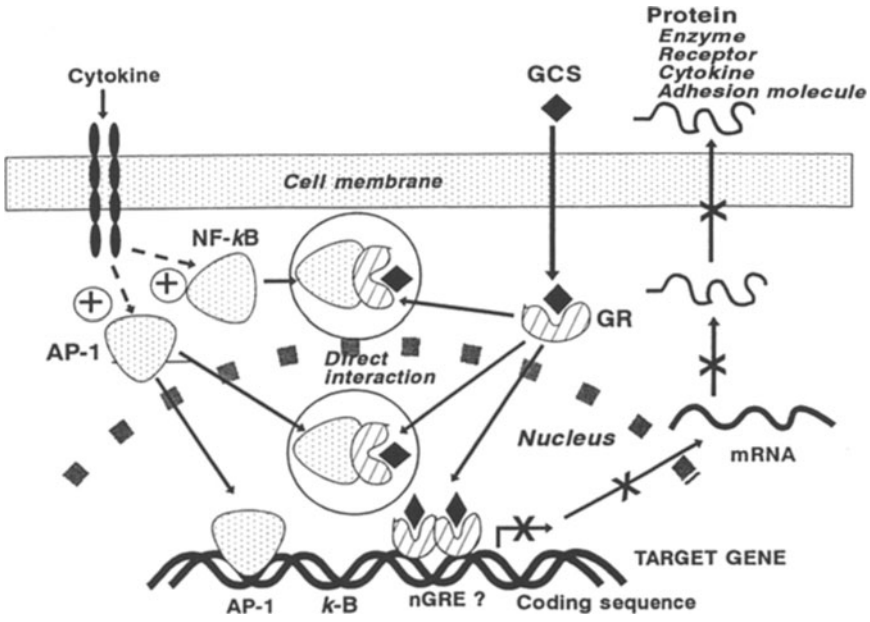

Figure 9. Mechanism of gene repression by corticosteroids. There is little evidence that glucocorticoid receptors (GR) interact with negative glucocorticoid response elements (nGRE). Direct interaction between the transcription factors activator protein-1 (AP-1) and nuclear factor-κB (NF-κB) and the GR may result in mutual repression. In this way steroids may counteract the chronic inflammatory effects of cytokines and other stimuli that activate these transcription factors.

binding or activation of these transcription factors and thus suppress activated inflammatory genes.

There has recently been increasing evidence that corticosteroids may have effects on the chromatin structure of DNA. The repressive action of steroids may be a result of competition between GRs and the binding sites on CBP for other transcription factors, including AP-1, NF-κB and STATs [79, 101–103]. Activated GRs may bind to several transcription co-repressor molecules which associate with proteins that have histone deacetylase activity, resulting in deacetylation of histone, increased winding of DNA round histone residues, reduced access of transcription factors to their binding sites, and therefore repression of inflammatory genes [110] (see Figure 8).

A good example is the inhibitory effect of steroids on iNOS expression; iNOS is largely regulated via NF-κB [36] and the inhibitory effect of glucocorticoids on induction of iNOS appears to result from a direct interaction of GRs and the p65 component of NF-κB [126]. This results in reduced iNOS expression in asthmatic airways [127] and a reduction in exhaled nitric oxide [128]. Similarly, the eosinophil chemotactic cytokine RANTES, which is up-regulated in asthmatic airways, is inhibited by cor-

ticosteroids [129–131]. This is probably the result of an interaction of GRs with NF-κB and AP-1, which are important determinants of RANTES expression [33].

The effect of steroid receptor activation is to interfere with the activation of CBP, which regulates acetylation of the histone around which DNA is coiled. The net effect results in deacetylation of histone residues and thus tighter coiling of DNA, excluding transcription factors such as NF-κB and AP-1 from binding to DNA [102].

9.2. Steroid-Resistant Inflammation

A small proportion of asthmatic patients are steroid resistant and fail to respond to even high doses of oral steroids [132–134]. Similar resistance is seen in other chronic inflammatory diseases, such as inflammatory bowel disease and rheumatoid arthritis, but it has been studied most carefully in patients with asthma. This defect is also seen in mononuclear cells and T lymphocytes isolated from these patients. A reduction in the number or affinity of GRs cannot account for the profound loss of steroid responsiveness, but we have found a marked impairment of GRE binding after exposure of mononuclear cells to steroids *in vitro* [135]. This is associated with a marked reduction in the number of activated GRs available for binding. In the same patients there is a reduced inhibitory effect of corticosteroids on AP-1 activation, but not on NF-κB or CREB activation [136]. Furthermore, there is an increase in the baseline activity of AP-1 and activation of AP-1 with phorbol esters shows a greatly exaggerated expression of c-Fos as a result of increased gene transcription [137]. This appears to be caused by excessive activation of JNK at baseline and in response to TNF-α [138]. The increased activation of AP-1 may result in sequestration of GRs so that no receptors are available for inhibiting NF-κB, C/EBP, etc., resulting in steroid resistance. This resistance will be seen at the site of inflammation where cytokines are produced, i.e. in the airways of asthmatic patients, but not at non-inflamed sites. This may explain why patients with steroid-resistant asthma are not resistant to the endocrine and metabolic effects of steroids, and why they develop steroid side effects [139]. Whether this abnormality is inherited is not yet certain, although there is often a positive family history of asthma in patients with steroid-resistant asthma, indicating that genetic factors may be important.

10. Nuclear Factor of Activated T Cells

The nuclear factor of activated T cells (NF-AT) is a good example of a cell-specific transcription factor, because it is predominantly found in T lymphocytes, although it is also found in other cells such as mast cells. NF-

AT is of key importance in the regulation of the expression of IL-2 and probably other T cell-derived cytokines, such as IL-4 and IL-5. Activation of T cells results in activation of the phosphatase calcineurin, which in turn, activates a preformed cytoplasmic NF-AT (NF-ATp) (Figure 10). Calcineurin binds tightly to NF-ATp and is transported into the nucleus where it continues to dephosphorylate NF-AT, counteracting an NF-AT kinase [140]. At least three other forms of NF-AT have now been identified (NF-ATc, NF-AT3 and NF-AT4) and these are differentially expressed in different tissues, although all have a Rel-like domain [141]. AP-1 forms a transcriptional complex with NF-AT (and is the nuclear NF-AT previously identified) by interacting with the Rel domain to increase IL-2 gene expression [142–144]. This may be inhibited by cyclosporin A and tacrolimus (FK 506) which both inhibit calcineurin, or by steroids which inhibit AP-1 directly. This predicts that there is a synergistic interaction between cyclosporin A and steroid in inhibiting cytokine gene expression in T cells, which has recently been confirmed in studies of lymphocyte proliferation and transcription factor suppression [145].

NF-ATp is important for the regulation of IL-4 and IL-5 genes [146]. NF-AT cooperates with AP-1 in the expression of IL-4 in mice [147] and

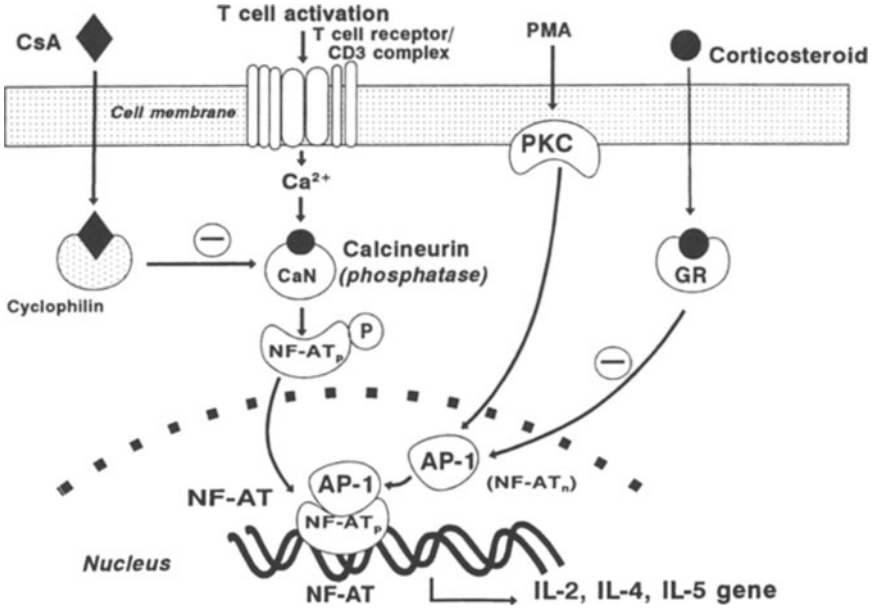

Figure 10. Nuclear factor of activated T cells regulates expression of IL-2, IL-4 and IL-5. It is made up of a cytoplasmic component (NF-AT) and AP-1. Cyclosporin inhibits NF-AT by inhibiting the activity of calcineurin (CaN) which is needed for activation of NF-AT, whereas steroids inhibit by blocking the AP-1 component. This predicts a synergy between these two drugs.

knockout of the NF-ATp gene results in defective IL-4 production [148]. Recently, an additional transcription factor called NF-AT-interacting protein (NIP-45) has been described which appears to be involved in the activation of NF-AT and the proto-oncogene c-*Maf* to activate IL-4 transcription [149]. NF-AT is also implicated in the transcription of IL-5 in T lymphocytes and mast cells, acting in concert with another transcription factor GATA [150, 151]. Corticosteroids potently inhibit IL-5 gene transcription and this may be via inhibitory effects on AP-1 which interacts with NF-ATp [152].

11. Therapeutic Implications

The increased understanding of transcription factors has given new insights into the pathophysiology of inflammatory diseases, such as asthma, but has also opened up an opportunity for the development of new anti-inflammatory treatments. Several new therapies, based on interaction with specific transcription factors or their activation pathways, are now being developed for the treatment of chronic inflammatory diseases and several drugs already in clinical use (corticosteroids, retinoic acid, cyclosporin A) work via transcription factors [153]. One concern about this approach is the specificity of such drugs, but it is clear that transcription factors have selective effects on the expression of certain genes and this may make it possible to be more selective. In addition, there are cell-specific transcription factors that may be targeted for inhibition, which could provide selectivity of drug action. One such example is NF-AT, blocked by cyclosporin A and tacrolimus, which has a restricted cellular distribution. In asthma it may be possible to target drugs to the airways by inhalation, e.g. with inhaled corticosteroids to avoid any systemic effects.

11.1. New Steroids

The recognition that most of the anti-inflammatory effects of steroids are mediated by repression of transcription factors (transrepression), whereas the endocrine and metabolic effects of steroids are likely to be mediated via GRE binding (transactivation), has led to a search for novel corticosteroids that selectively transrepress, thus reducing the risk of systemic side effects. As corticosteroids bind to the same GR, this seems at first to be an unlikely possibility, but although GRE binding involved a GR homodimer, interaction with transcription factors AP-1 and NF-κB involves only a single GR. A separation of transactivation and transrepression has been demonstrated using reporter gene constructs in transfected cells and selective mutations of GRs [154]. Furthermore, some steroids, such as the antagonist RU486, have a greater transrepression than transactivation effect.

Indeed, the topical steroids used in asthma therapy today, such as fluticasone propionate and budesonide, appear to have more potent transrepression than transactivation effects, which may account for their selection as potent anti-inflammatory agents [155]. Recently, a novel class of steroids has been described in which there is potent transrepression with relatively little transactivation. These "dissociated" steroids, including RU24858 and RU40066, have anti-inflammatory effects *in vivo* [156]. This suggests that the development of steroids with a greater margin of safety is possible and may predict the development of oral steroids which could be safe to use in asthma and other inflammatory diseases.

11.2. NF-κB Inhibitors

As NF-κB may play a pivotal role in inflammatory diseases, this has suggested that specific NF-κB inhibitors might be beneficial and could avoid some of the metabolic effects seen with inhaled steroids [10]. There has therefore been interest in NF-κB inhibitors in asthma therapy [157]. Antioxidants have the ability to block activation of NF-κB in response to a wide variety of stimuli, and drugs, such as pyrrolidine dithiocarbamate, have proved useful for *in vitro* studies, but are too toxic for *in vivo* development [28]. Spin-trap antioxidants may be more effective because they work at an intracellular level [158]. However, antioxidants do not block all of the effects of NF-κB and this may require the development of novel drugs.

Some naturally occurring NF-κB inhibitors have already been identified. Thus gliotoxin, derived from *Aspergillus* sp., is a potent NF-κB inhibitor which appears to be relatively specific [159]. The anti-inflammatory cytokine IL-10 also has an inhibitory effect on NF-κB, via an effect on IκB-α [160], and is another therapeutic possibility, particularly as there appears to be a deficit in IL-10 secretion in airway macrophages from asthmatic patients which correlates with increased secretion of proinflammatory cytokines and chemokines [161].

Novel approaches to inhibition of NF-κB would be to develop specific inhibitors of IκB kinases involved in the initial activation of NF-κB or to block the signal transduction pathways leading to activation of IκB kinases. Now that IκB kinases have been identified, it may be possible to screen and design specific inhibitors. It may also be possible to inhibit the activity of the enzymes responsible for its degradation of the IκB complex, although the proteasome has many other important functions and its inhibition is likely to produce severe side effects. Recently, it has been possible to block NF-κB function by targeting of a specific enzyme (ubiquitin ligase) involved in conjugation of ubiquitin [162]. It may be more difficult to develop drugs to inhibit the components of NF-κB itself directly, but antisense oligonucleotides have been shown to be effective inhibitors *in vitro* and stable

cell permeable phosphorothioate oligonucleotides are a therapeutic possibility in the future. Recently, adenovirus-mediated gene transfer of IκB-α has been reported to inhibit endothelial cell activation [163].

However, it may be unwise to block NF-κB for prolonged periods, because it plays such a critical role in immune and host defence responses. Targeted disruption ("knock-out") of p65 is lethal because of developmental abnormalities [14], whereas lack of p50 results in immune deficiencies and increased susceptibility to infection [15]. Topical application of NF-κB inhibitors of inhalation may prove to be safe, however.

11.3. Drug Interactions

One of the most important implications of research on transcription factors is that multiple and complex interactions between these proteins are possible and that this leads to cross-talk between different signal transduction pathways. This might be exploited therapeutically by the combination of drugs that act on different transcription factors or pathways which may work together cooperatively. For example, NF-AT has a cytoplasmic component (NF-ATp) which is blocked by cyclosporin and tacrolimus, and a nuclear component AP-1, which is blocked by corticosteroids (see Figure 10). Combining steroids and cyclosporin may therefore have a synergistic inhibitory effect on the expression of genes such as those for IL-2, IL-4 and IL-5. This has indeed been demonstrated for IL-2 in human T cells, where a combination of both drugs has a much greater suppressive effect than either drug alone [145]. This suggests that a dose of cyclosporin A that is too low to give nephrotoxic side effects may be combined with an inhaled steroids, so that this synergistic interaction is confined to the airways.

Another interaction that may be exploited therapeutically is that between retinoic acid and steroids. Retinoic acid (vitamin A) binds to retinoic acid receptors which, like GRs, bind to CBP. There appears to be a synergistic interaction between steroids and retinoic acid in repression of transcription factors, such as NF-κB and AP-1, presumably because of competition for binding sites on CBP. A synergistic interaction between retinoic acid and steroids has been demonstrated in suppression of GM-CSF release from cultured epithelial cells, suggesting that retinoic acid may potentiate the anti-inflammatory effects of steroids [164]. Novel retinoic acid derivatives activate a subtype of retinoic acid receptor (RXR) which interacts with these transcription factors, so that it may be possible to develop more selective retinoids for this purpose [165].

References

1 Hunter T, Karin M (1992) The regulation of transcription by phosphorylation. *Cell* 70: 375–387
2 Karin M, Smeal T (1993) Control of transcription factors by signal transduction pathways: the beginning of the end. *Trends Biochem Sci* 17: 418–422
3 Adcock IM, Barnes PJ (1996) Transcription factors. In: RG Crystal, JB West, WR Weibel, PJ Barnes (eds): *The Lung: Scientific foundations*. Lippincott-Raven, Philadelphia, 255–276
4 Latchman DS (1996) Transcription-factor mutations and disease. *N Engl J Med* 334: 28–33
5 Papavassilou AG (1995) Transcription factors. *N Engl J Med* 332: 45–47
6 Pfahl M (1993) Nuclear receptor/AP-1 interaction. *Endocr Rev* 14: 651–658
7 Tjian R, Maniatis T (1994) Transcriptional activation: a complex puzzle with few easy pieces. *Cell* 77: 5–8
8 Lalli E, Sassone-Corsi P (1994) Signal transduction and gene regulation: the nuclear response to cAMP. *J Biol Chem* 269: 17359–17362
9 Stein B, Baldwin AS (1993) Distinct mechanisms for the regulation of the interleukin-8 gene involve synergism and cooperativity between C/EBP and NF-κB. *Mol Cell Biol* 13: 7191–7198
10 Barnes PJ, Karin M (1997) Nuclear factor-κB: a pivotal transcription factor in chronic inflammatory diseases. *N Engl J Med* 336: 1066–1071
11 Sen R, Baltimore D (1986) Multiple nuclear factors interact with the immunoglobulin enhancer sequences. *Cell* 46: 705–716
12 Siebenlist U, Franzuso G, Brown R (1994) Structure, regulation and function of NF-κB. *Annu Rev Cell Biol* 10: 405–455
13 Baeuerle PA, Henkel T (1994) Function and activation of NF-κB in the immune system. *Annu Rev Immunol* 12: 141–179
14 Beg AA, Sha WC, Bronson RT, Ghosh S, Baltimore D (1995) Embroyonic lethality and liver degeneration in mice lacking the RelA component of NF-κB. *Nature* 376: 167–170
15 Sha WC, Liou HC, Tuomanen EI, Baltimore D (1995) Targeted disruption of the p50 subunit of NF-κB leads to multifocal defects in immune responses. *Cell* 80: 321–330
16 Baldwin AS (1996) The NF-κB and IκB proteins: new discoveries and insights. *Annu Rev Immunol* 14: 649–681
17 Baeuerle PA, Baltimore D (1996) NF-κB: ten years on. *Cell* 87: 13–20
18 DiDonato J, Mercurio F, Rosette C, Wu-Li J, Suyang H, Ghosh S, Karin M (1996) Mapping of the inducible IκB phosphorylation sites that signal its ubiquitination and degradation. *Mol Cell Biol* 16: 1295–1304
19 Chen ZJ, Parent L, Maniatis T (1996) Site-specific phosphorylation of IκBα by a novel ubiquitination-dependent protein kinase activity. *Cell* 84: 853–862
20 Zandi E, Rothwarf DM, Delhase M, Hayakawa M, Karin M (1997) The IκB kinase complex (IKK) contains two kinase subunits, IKKα and IKKβ, necessary for IκB phosphorylation and NF-κB activation. *Cell* 91: 243–252
21 Eder J (1997) Tumour necrosis factor α and interleukin 1 signalling: do MAPKK kinases connect it all? *Trends Pharmacol Sci* 18: 319–322
22 Malinin NL, Boldin MP, Kovalenko AV, Wallach D (1997) MAP3K-related kinase is involved in NF-κB induction by TNF, CD95 and IL-1. *Nature* 385: 540–544
23 Lee FS, Hagler J, Chen ZJ, Maniatis T (1997) Activation of the IκB-α kinase complex by MEKK1, a kinase of the JNK pathway. *Cell* 88: 213–222
24 Arenzana-Seisdedos F, Thomson J, Rodriguez MS, Bachelerie F, Thomas D, Hay RT (1995) Inducible nuclear expression of newly synthesized IκB-α negatively regulates DNA binding and transcriptional activity of NF-κB. *Mol Cell Biol* 15: 2689–2696
25 Adcock IM, Barnes PJ (1996) Tumour necrosis factor alpha causes retention of activated glucocorticoid receptor within the cytoplasm of A549 cells. *Biochem Biophys Res Commun* 225: 1127–1132
26 Klement JF, Rice NR, Car BD et al. (1996) IκB-α deficiency results in a sustained NF-κB response and severe widespread dermatitis in mice. *Mol Cell Biol* 16: 2341–2349
27 Thomspon JE, Phillips RJ, Erdjument-Bromage H, Tempst P, Ghosh S (1995) IκB-β regulates the persistent response in a biphasic activation of NF-κB. *Cell* 80: 573–582

28 Schreck R, Rieber P, Baeuerle PA (1991) Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-κB transcription factor and HIV-1. *EMBO J* 10: 2247–2258
29 Zhu Z, Tang W, Ray A et al. (1996) Rhinovirus stimulation of interleukin-6 *in vivo* and *in vitro*. Evidence for nuclear factor κB-dependent transcription activation. *J Clin Invest* 97: 421–430
30 Schreck R, Meier B, Männel DN, Dröge W, Baeuerle PA (1991) Dithiocarbamates as potent inhibitors of nuclear factor κB activation in intact cells. *J Exp Med* 175: 1181
31 Stein B, Baldwin AS, Ballard DW, Greene WC, Angel P, Herrlich P (1993) Cross-coupling of the NF-κB p65 and Fos/Jun transcription factors produces potential biological function. *EMBO J* 12: 3879–3891
32 Mukaido N, Morita M, Ishikawa Y, Rice N, Okamoto S, Kasahara T, Matsushima K (1994) Novel mechanisms of glucocorticoid-mediated gene repression: NF-κB is target for glucocorticoid-mediated IL-8 gene expression. *J Biol Chem* 269: 13289–13295
33 Nelson PJ, Kim HT, Manning WC, Goralski TJ, Krensky AM (1993) Genomic organisation and transcriptional regulation of the RANTES chemokine gene. *J Immunol* 151: 2601–2612
34 Ueda A, Okuda K, Shira A et al. (1994) NF-κB and Sp1 regulate transcription of the human monocyte chemoattractant protein-1 gene. *J Immunol* 153: 2052–2063
35 Lilly CM, Nakamura H, Kesselman H et al. (1997) Expression of eotaxin by human lung epithelial cells: induction by cytokines and inhibition by glucocorticoids. *J Clin Invest* 99: 1767–1773
36 Xie Q, Kashiwarbara Y, Nathan C (1994) Role of transcription factor NF-κB/Rel in induction of nitric oxide synthase. *J Biol Chem* 269: 4705–4708
37 Yamamoto K, Arakawa T, Ueda N, Yamamoto S (1995) Transcriptional roles of nuclear factor κB and nuclear factor-interleukin 6 in the tumor necrosis-α-dependent induction of cyclooxygenase-2 in MC3T3-E1 cells. *J Biol Chem* 270: 31315–31320
38 Newton R, Kuitert LM, Bergmann M, Adcock IM, Barnes PJ (1997) Evidence for involvement of NF-κB in the transcriptional control of COX-2 gene expression by IL-1β. *Biochem Biophys Res Commun* 237: 28–32
39 Iademarco MF, McQuillan JJ, Rosen GD, Dean DC (1995) Characterization of the promoter for vascular adhesion molecule-1 (VCAM-1). *J Biol Chem* 267: 16323–16329
40 Van De Stolpe A, Caldenhoven E, Stade BG, Koenderman L, Raaijmakers JA, Johnson JP, van Der Saag PT (1994) 12-O-Tetradecanoyl phorbol-13-acetate and tumor necrosis factor alpha-mediated induction of intercellular adhesion molecule-1 is inhibited by dexamethasone. Functional analysis of the human intercellular adhesion molecule-1 promoter. *J Biol Chem* 269: 6185–6192
41 Liu ZG, Hsu H, Goeddel DV, Karin M (1996) Dissection of TNF receptor 1 effector functions: JNK activation is not linked to apoptosis while NF-κB activation prevents cell death. *Cell* 87: 565–576
42 Beg AA, Baltimore D (1996) An essential role for NF-κB in preventing TNF-α-induced cell death. *Science* 274: 782–784
43 Liu SF, Haddad E, Adcock IM et al. (1997) Inducible nitric oxide synthase after sensitization and allergen challenge of Brown Norway rat lung. *Br J Pharmacol* 121: 1241–1246
44 Haddad E-B, Liu SF, Salmon M, Robichaud A, Barnes PJ, Chung KF (1995) Expression of inducible nitric oxide synthase mRNA in Brown-Norway rats exposed to ozone: effect of dexamethasone. *Eur J Pharmacol (Environ Toxicol Section)* 293: 287–290
45 Auphan N, DiDonato JA, Rosette C, Helmberg A, Karin M (1995) Immunosuppression by glucocorticoids: inhibition of NF-κB activity through induction of IκB synthesis. *Science* 270: 286–290
46 Adcock IM, Brown CR, Kwon OJ, Barnes PJ (1994) Oxidative stress induces NF-κB DNA binding and inducible NOS mRNA in human epithelial cells. *Biochem Biophys Res Commun* 199: 1518–1524
47 Haddad E-B, Salmon M, Koto H, Barnes PJ, Adcock I, Chung KF (1996) Ozone induction of cytokine-induced neutrophil chemoattractant and nuclear factor-κB in rat lung: inhibition by corticosteroids. *FEBS Lett* 379: 265–268
48 Adcock IM, Brown CR, Gelder CM, Shirasaki H, Peters MJ, Barnes PJ (1995) The effects of glucocorticoids on transcription factor activation in human peripheral blood mononuclear cells. *Am J Physiol* 37: C331–C338

49 Jany B, Betz R, Schreck R (1995) Activation of the transcription factor NF-κB in human tracheobronchial epithelial cells by inflammatory stimuli. *Eur Respir J* 8: 387–391
50 Adock IM, Brown CR, Shirasaki H, Barnes PJ (1994) Effects of dexamethasone on cytokine and phorbol ester stimulated c-Fos and c-Jun DNA binding and gene expression in human lung. *Eur Respir J* 7: 2117–2123
51 Hart L, Krishnan VJ, Adcock IM, Barnes PJ, Chung KF (1998) Activation and localization of transcription factor nuclear factor-κB in asthma. *Am J Respir Crit Care Med, in press*
52 Hamid Q, Springall DR, Riveros-Moreno V et al. (1993) Induction of nitric oxide synthase in asthma. *Lancet* 342: 1510–1513
53 Kharitonov SA, Yates D, Robbins RA, Logan-Sinclair R, Shinebourne E, Barnes PJ (1994) Increased nitric oxide in exhaled air of asthmatic patients. *Lancet* 343: 133–135
54 Collins PD, Marleau S, Griffiths-Johnson DA, Jose PJ, Williams TJ (1995) Cooperation between interleukin-5 and the chemokine eotaxin to induce eosinophil accumulation *in vivo*. *J Exp Med* 182: 1169–1174
55 Saleh D, Barnes PJ, Giaid A (1997) Increased production of the potent oxidant peroxynitrite in the lungs of patients with pulmonary fibrosis. *Am J Respir Crit Care Med* 155: 1763–1769
56 Paredi P, Kharitonov SA, Maziak W, du Bois RM, Barnes PJ (1997) Exhaled nitric oxide (NO) a possible marker for disease activity in patients with interstitial lung disease. *Eur Respir J* 10 (suppl 25): 158S
57 Simeonova PP, Luster MI (1996) Asbestos induction of nuclear transcription factors and interleukin 8 gene regulation. *Am J Respir Cell Mol Biol* 15: 787–795
58 Blackwell TS, Christman JW (1997) The role of nuclear factor-κB in cytokine gene regulation. *Am J Respir Cell Mol Biol* 17: 3–9
59 Blackwell TS, Blackwell TR, Holden EP, Christman BW, Christman JW (1996) *In vivo* antioxidant treatment suppresses nuclear factor-κB activation and neutrophilic lung inflammation. *J Immunol* 157: 1630–1637
60 Liu S, Adcock IM, Old RW, Barnes PJ, Evans TW (1993) Lipopolysaccharide treatment *in vivo* induces widespread expression of inducible nitric oxide synthase mRNA. *Biochem Biophys Res Commun* 196: 1208–1213
61 Schwartz MD, Moore EE, Moore FA, Shenkar R, Moine P, Haenel JB, Abraham E (1996) Nuclear factor-κB is activated in alveolar macrophages from patients with acute respiratory distress syndrome. *Crit Care Med* 24: 1285–1292
62 Donnelly SC, Strieter RM, Kunkel SL et al. (1993) Interleukin-8 and development of adult respiratory distress syndrome in at-risk patient groups. *Lancet* 341: 643–647
63 Hai T, Curran T (1991) Cross-family dimerization of transcription factors Fos/Jun and ATF/CREB alters DNA binding specificity. *Proc Natl Acad Sci USA* 88: 1–5
64 Karin M (1995) The regulation of AP-1 activity by mitogen-activated protein kinases. *J Biol Chem* 270: 16483–16486
65 Karin M, Liu Zg, Zandi E (1997) AP-1 function and regulation. *Curr Opin Cell Biol* 9: 240–246
66 Adcock IM, Shirasaki H, Gelder CM, Peters MJ, Brown CR, Barnes PJ (1994) The effects of glucocorticoids on phorbol ester and cytokine stimulated transcription factor activation in human lung. *Life Sci* 55: 1147–1153
67 Kyriakis JM, Bangrjee P, Nikolakaki E et al. (1994) The stress-activated protein kinase subfamily of c-Jun kinases. *Nature* 369: 156–160
68 Westwick JK, Weitzel C, Minden A, Karin M, Brenner DA (1994) Tumor necrosis factor alpha stimulates AP-1 activity through prolonged activation of the c-Jun kinase. *J Biol Chem* 269: 26396–26401
69 Camhi SL, Alam J, Otterbein L, Sylvester SL, Choi AM (1995) Induction of heme oxygenase-1 gene expression by lipopolysaccharide is mediated by AP-1 activation. *Am J Respir Cell Mol Biol* 13: 387–398
70 Demoly P, Basset-Seguin N, Chanez P et al. (1992) c-*Fos* proto-oncogene expression in bronchial biopsies of asthmatics. *Am J Respir Cell Mol Biol* 7: 128–133
71 Ponta H, Cato ACB, Herrlick P (1992) Interference of specific transcription factors. *Biochim Biophys Acta* 1129: 255–261
72 Kishimoto T, Taga T, Akira S (1994) Cytokine signal transduction. *Cell* 76: 253–262
73 Gosset P, Tsicopoulos A, Wallaert B, Vannimenus C, Joseph M, Tonnel AB, Capron A (1991) Increased secretion by tumor necrosis factor α and interleukin 6 by alveolar macro-

phages consecutive to the development of the late asthmatic reaction. *J Allergy Clin Immunol* 88: 561–571
74 Gosset P, Tsicopoulos A, Wallaert B, Vannimenus C, Joseph M, Tonnel AB, Capron A (1992) Tumor necrosis factor α and interleukin-6 production by human mononuclear phagocytes from allergic asthmatics after IgE-dependent stimulation. *Am Rev Respir Dis* 146: 768–774
75 Grunberg K, Smits HH, Timers MC et al. (1997) Experimental rhinovirus 16 infection. Effects on cell differentials and soluble markers in sputum in asthmatic subjects. *Am J Respir Crit Care Med* 156: 609–616
76 Darnell JE, Kerr IM, Stark GR (1994) Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signalling proteins. *Science* 264: 1415–1421
77 Ihle JN, Witthuhn BA, Quelle FW, Yamamoto K, Thienfeloer WC, Kreider B, Silvennoinev O (1994) Signalling by the cytokine receptor superfamily: JAKs and STATs. *Trends Biochem Sci* 19: 222–225
78 Battacharya S, Eckner R, Grossman S, Oldread E, Arany Z, D'Andrea A, Livingston DM (1996) Cooperation of Stat 2 and p300/CBP in signaling induced by interferon-alpha. *Nature* 383: 344–347
79 Zhang JJ, Vinkemeier U, Gu W, Chakravarti D, Horvath CM, Darnell JE (1996) Two contact regions between STAT1 and CBP/p300 in interferon-γ signalling. *Proc Natl Acad Sci USA* 93: 15092–15096
80 Hou J, Schindler U, Henzel WJ, Ho TC, Brasseur M, McKnight SL (1994) An interleukin-4-induced transcription factor: IL-4 stat. *Science* 265: 1701–1706
81 Takeda K, Tanaka T, Shi W et al. (1996) Essential role for Stat6 in IL-4 signaling. *Nature* 380: 627–630
82 Kaplan MH, Schindler U, Smiley ST, Grusby MJ (1996) Stat6 is required for mediating responses to IL-4 and for the development of Th2 cells. *Immunity* 4: 313–319
83 Bacon CM, Petricoin EF, Ortaldo JR, Rees SC, Larner AC, Johnston JA, O'Shea JJ (1995) IL-12 induces tyrosine phosphorylation and activation of STAT-4 in human lymphocytes. *Proc Natl Acad Sci USA* 92: 7303–7311
84 Kaplan MH, Sun Y, Hoey T, Grusby MJ (1996) Impaired IL-12 responses and enhanced development of Th2 cells in Stat4-deficient mice. *Nature* 382: 174–177
85 Pazdrak K, Stafford S, Alam R (1995) The activation of the Jak-STAT 1 signalling pathway by IL-5 in eosinophils. *J Immunol* 155: 397–402
86 van der Bruggen T, Caldenhoven E, Kanters D, Coffer P, Raaijmakers JA, Lammers JW, Koenderman L (1995) Interleukin-5 signaling in human eosinophils involves JAK2 tyrosine kinase and Stat1α. *Blood* 85: 1442–1448
87 Mui ALF, Wakao H, O'Farrell AM, Miyajima A (1995) Interleukin-3, granulocyte-macrophage colony stimulating factor and interleukin-5 transduce signals through two STAT5 homologs. *EMBO J* 14: 1166–1175
88 Naka T, Narazaki M, Hirata M et al. (1997) Structure and function of a new STAT-induced STAT inhibitor. *Nature* 387: 924–929
89 Starr R, Willson TA, Viney EM et al. (1997) A family of cytokine-inducible inhibitors of signalling. *Nature* 387: 917–921
90 Yamamoto KK, Gonzalez GA, Biggs WH, Montminy MR (1988) Phosphorylation-induced binding and transcriptional efficacy of nuclear factor CREB. *Nature* 334: 494–498
91 Chrivia JC, Kwok RPS, Lamb N, Hagiwara M, Montminy MR, Goodman RH (1993) Phosphorylated CREB specifically binds to the nuclear factor CBP. *Nature* 365: 855–859
92 Collins S, Altschmied J, Herbsman O, Caron MG, Mellon PL, Lefkowitz RJ (1990) A cAMP element in the β_2-adrenerigc receptor gene confers autoregulation by cAMP. *J Biol Chem* 265: 19930–19935
93 Peters MJ, Adcock IM, Brown CR, Barnes PJ (1995) β-Adrenoceptor agonists interfere with glucocorticoid receptor DNA binding in rat lung. *Eur J Pharmacol (Mol Pharmacol Section)* 289: 275–281
94 Nishikawa M, Mak JCW, Shirasaki H, Barnes PJ (1993) Differential down-regulation of pulmonary β_1- and β_2-adrenoceptor messenger RNA with prolonged *in vivo* infusion of isoprenaline. *Eur J Pharmacol (Mol Section)* 247: 131–138
95 Mak JCW, Nishikawa M, Shirasaki H, Miyayasu K, Barnes PJ (1995) Protective effects of a glucocorticoid on down-regulation of pulmonary β_2-adrenergic receptors *in vivo*. *J Clin Invest* 96: 99–106

96 Masquilier D, Sassone-Corsi P (1992) Transcriptional cross talk: nuclear factors CREM and CREB bind to AP-1 sites and inhibit activation by Jun. *J Biol Chem* 267: 22460–22466
97 Imai F, Minger JN, Mitchell JA, Yamamoto KR, Granner DK (1993) Glucocorticoid receptor – cAMP response element-binding protein interaction and the response of the phosphoenolpyruvate carboxykinase gene to glucocorticoids. *J Biol Chem* 268: 5353–5356
98 Stevens DA, Barnes PJ, Adcock IM (1995) β-Agonists inhibit DNA binding of glucocorticoid receptors in human pulmonary and bronchial epithelial cells. *Am J Resp Crit Care Med* 151: A195
99 Adcock IM, Stevens DA, Barnes PJ (1996) Interactions between steroids and β_2-agonists. *Eur Respir J* 9: 160–168
100 Janknecht R, Hunter T (1996) A growing coactivator network *Nature* 383: 22–23
101 Arias J, Alberts AS, Brindle P et al. (1994) Activation of cAMP and mitogen responsive genes relies on a common nuclear factor. *Nature* 370: 226–229
102 Kamei Y, Xu L, Heinzel T et al. (1996) A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. *Cell* 85: 403–414
103 Perkins ND, Felzien LK, Betts JC, Leung K, Beach DH, Nabel GJ (1997) Regulation of NF-κB by cyclin-dependent kinases associated with the p300 coactivator. *Science* 275: 523–526
104 Smith CL, Onate SA, Tsai MJ, O'Malley BW (1996) CREB binding protein acts synergistically with steroid receptor coactivator-1 to enhance steroid receptor-dependent transcription. *Proc Natl Acad Sci USA* 93: 8884–8888
105 Yao PM, Buhler JM, D'Ortho MP, Lebargy F, Delclaux C, Harf A, Lafuma C (1996) Expression of matrix metalloproteinase gelatinases A and B by cultured epithelial cells from human bronchial explants. *J Biol Chem* 271: 15580–15589
106 Hong H, Kohli K, Garabedian MJ, Stallcup MR (1997) GRIP1, a transcriptional coactivator for the AF-2 transactivation domain of steroid, thyroid, retinoid, and vitamin D receptors. *Mol Cell Biol* 17: 2735–2744
107 Torchia J, Rose DW, Inostroza J, Kamei Y, Westin S, Glass CK, Rosenfeld MG (1997) The transcriptional co-activator p/CIP binds CBP and mediates nuclear receptor function. *Nature* 387: 677–684
108 Heery DM, Kalkhoven E, Hoare S, Parker MG (1997) A signature motif in transcriptional co-activators mediates binding to nuclear receptors. *Nature* 387: 733–736
109 Ogryzko VV, Schlitz RL, Russanova V, Howard BH, Nakatani Y (1996) The transcriptional coactivators p300 and CBP are histone acetyltransferases. *Cell* 87: 953–959
110 Wolffe AP (1997) Sinful repression. *Nature* 387: 16–17
111 Nagy L, Kao HY, Chakravarti D et al. (1997) Nuclear receptor repression mediated by a complex containing SMRT, mSin3A, and histone deacetylase. *Cell* 89: 373–380
112 Heinzel T, Lavinsky RM, Mullen TM et al. (1997) A complex containing N-CoR, mSin3 and histone deacetylase mediates transcriptional repression. *Nature* 387: 43–48
113 Beato M, Herrlich P, Schutz G (1995) Steroid hormone receptors: many actors in search of a plot. *Cell* 83: 851–857
114 Adcock IM, Gilbey T, Gelder CM, Chung KF, Barnes PJ (1996) Glucocorticoid receptor localization in normal human lung and asthmatic lung. *Am J Respir Crit Care Med* 154: 771–782
115 Truss M, Beato M (1993) Steroid hormone receptors: interaction with deoxyribonucleic acid and transcription factors. *Endocr Rev* 14: 459–479
116 Mak JCW, Grandordy B, Barnes PJ (1994) High affinity [^{3}H]formoterol binding sites in lung: characterization and autoradiographic mapping. *Eur J Pharmacol (Mol Section)* 269: 35–41
117 Barnes PJ (1996) Mechanism of action of glucocorticoids in asthma. *Am J Respir Crit Care Med* 154: S21–S27
118 Scheinman RI, Cogswell PC, Lofquist AK, Baldwin AS (1995) Role of transcriptional activation of IκB-α in mediating immunosuppression by glucocorticoids. *Science* 270: 283–286
119 Brostjan C, Anrather J, Csizmadia V, Stoka D, Soares M, Bach FH, Winkler H (1996) Glucocorticoid-mediated repression of NF-κB activity in endothelial cells does not involve induction of IκB α synthesis. *J Biol Chem* 271: 19612–19616

120 Newton R, Hart LA, Adcock IM, Barnes PJ (1997) Effect of glucocorticoids on IL-1β-induced NF-κB binding and expression in type II alveolar cells – no evidence for down-regulation by IκB. *Am J Respir Crit Care Med* 155: A699
121 Heck S, Bender K, Kullmann M, Gottlicher M, Herrlich P, Cato AC (1997) IκBα-independent downregulation of NF-κB activity by glucocorticoid receptor. *EMBO J* 16: 4698–4707
122 Barnes PJ, Adcock IM (1993) Anti-inflammatory actions of steroids: molecular mechanisms. *Trends Pharmacol Sci* 14: 436–441
123 Ray A, Prefontaine KE (1994) Physical association and functional antagonism between the p65 subunit of transcription factor NF-κB and the glucocorticoid receptor. *Proc Natl Acad Sci USA* 91: 752–756
124 Caldenhoven E, Liden J, Wissink S et al. (1995) Negative cross-talk between RelA and the glucocorticoid receptor: a possible mechanism for the antiinflammatory action of glucocorticoids. *Mol Endocrinol* 9: 401–412
125 Stocklin E, Wisler M, Gouilleux F, Groner B (1996) Functional interactions between Stat5 and the glucocorticoid receptor. *Nature* 383: 726–728
126 Kleinert H, Euchenhofer C, Ihrig Biedert I, Forstermann U (1996) Glucocorticoids inhibit the induction of nitric oxide synthase II by down-regulating cytokine-induced activity of transcription factor nuclear factor-kappa B. *Mol Pharmacol* 49: 15–21
127 Giaid A, Saleh D, Lim S, Barnes PJ, Ernst P (1998) Formation of peroxynitrite in asthmatic airways. *Am J Respir Crit Care Med* 157: A870
128 Kharitonov SA, Yates D, Barnes PJ (1995) Increased nitric oxide in exhaled air of normal human subjects with upper respiratory tract infections. *Eur Respir J* 8: 295–297
129 Kwon OJ, Jose PJ, Robbins RA, Schall TJ, Williams TJ, Barnes PJ (1995) Glucocorticoids inhibition of RANTES expression in human lung epithelial cells. *Am J Respir Cell Mol Med* 12: 488–496
130 Berkman N, Robichaud A, Krishnan VL, Barnes PJ, Chung KF (1996) Expression of RANTES in human airway epithelial cells: effect of corticosteroids and interleukins-4, 10 and 13. *Immunology* 87: 599–603
131 Wang H, Devalia JL, Xia C, Sapsford RJ, Davies RJ (1996) Expression of RANTES by human bronchial epithelial cells *in vitro* and *in vivo* and the effect of corticosteroids. *Am J Respir Cell Mol Biol* 14: 27–35
132 Barnes PJ, Adcock IM (1995) Steroid-resistant asthma. *Q J Med* 88: 455–468
133 Barnes PJ, Greening AP, Crompton GK (1995) Glucocorticoid resistance in asthma. *Am J Respir Crit Care Med* 152: 125S–140S
134 Szefler SJ, Leung DY (1997) Glucocorticoid-resistant asthma: pathogenesis and clinical implications for management. *Eur Respir J* 10: 1640–1647
135 Adcock IM, Lane SJ, Brown CA, Peters MJ, Lee TH, Barnes PJ (1995) Differences in binding of glucocorticoid receptor to DNA in steroid-resistant asthma. *J Immunol* 154: 3000–3005
136 Adcock IM, Lane SJ, Brown CA, Lee TH, Barnes PJ (1995) Abnormal glucocorticoid receptor/AP-1 interaction in steroid resistant asthma. *J Exp Med* 182: 1951–1958
137 Adcock IM, Lane SJ, Barnes PJ, Lee TH (1996) Enhanced phorbol ester-induced c-Fos transcription and translation in steroid-resistant asthma. *Am J Respir Crit Care Med* 153: A682
138 Adcock IM, Brady H, Lim S, Karin M, Barnes PJ (1997) Increased JUN kinase activity in peripheral blood monocytes from steroid-resistant asthmatic subjects. *Am J Respir Crit Care Med* 155: A288
139 Lane SJ, Atkinson BA, Swimanathan R, Lee TH (1996) Hypothalamic–pituitary axis in corticosteroid-resistant asthma. *Am J Respir Crit Care Med* 153: 1510–1514
140 Shibasaki F, Price ER, Milan D, McKeon F (1996) Role of kinases and the phosphatase calcineurin in the nuclear shuttling of transcription factor NF-AT4. *Nature* 382: 370–373
141 Hoey T, Sun YL, Williamson K, Yu X (1995) Isolation of two new members of the NF-AT gene family and functional characterization of the NF-AT proteins. *Immunity* 2: 461–472
142 Jain J, McCaffrey PG, Valge Archer VE, Rao A (1992) Nuclear factor of activated T cells contains Fos and Jun. *Nature* 356: 801–804
143 Northrop JP, Ullman KS, Crabtree GR (1993) Characterization of the nuclear and cytoplasmic components of the lymphoid-specific nuclear factor of activated T cells (NF-AT). *J Biol Chem* 268: 2917–2923

144 Palmer JBD, Cuss FMC, Mulderry PK et al. (1987) Calcitonin gene-related peptide is localized to human airway nerves and potently constricts human airway smooth muscle. *Br J Pharmacol* 91: 95–101
145 Wright LC, Cammisuli S, Baboulene L, Fozzard J, Adcock IM, Barnes PJ (1995) Cyclosporin A and glucocorticoids interact synergistically in T lymphocytes: implications for asthma therapy. *Am J Resp Crit Care Med* 151: A675
146 Lee HJ, Matsuda I, Naito Y, Yokota T, Arai N, Arai K (1994) Signals and nuclear factors that regulate the expression of interleukin-4 and interleukin-5 genes in helper T cells. *J Allergy Clin Immunol* 94: 594–604
147 Hodge MR, Rooney JW, Glimcher LH (1995) The proximal promoter of the IL-4 gene is composed of multiple essential regulatory sites that bind at least two distinct factors. *J Immunol* 154: 6397–6405
148 Hodge MR, Ranger AM, Charles de la Brousse F, Hoey T, Grusby MJ, Glimcher LH (1996) Hyperproliferation and dysregulation of IL-4 expression in NF-ATp-deficient mice. *Immunity* 4: 397–405
149 Hodge MR, Chun HJ, Rengarajan J, Alt A, Lieberson R, Glimcher LH (1996) NF-AT-driven interleukin-4 transcription potentiated by NIP45. *Science* 274: 1903–1905
150 Lee HJ, Masuda ES, Arai N, Arai K, Yokota T (1995) Definition of *cis*-regulatory elements of the mouse interleukin-5 gene promoter. Involvement of nuclear factor of activated T cell-related factors in interleukin-5 expression. *J Biol Chem* 270: 17541–17550
151 Prieschl EE, Gouilleux Gruart V, Walker C, Harrer NE, Baumruker T (1995) A nuclear factor of activated T cell-like transcription factor in mast cells is involved in IL-5 gene regulation after IgE plus antigen stimulation. *J Immunol* 154: 6112–6119
152 Mori A, Kaminuma O, Suko M et al. (1997) Two distinct pathways of interleukin-5 synthesis in allergen-specific human T cell clones are suppressed by glucocorticoids. *Blood* 89: 2891–2900
153 Manning AM (1996) Transcription factors: a new frontier in drug discovery. *Drug Devel Ther* 1: 151–160
154 Heck S, Kullmann M, Grast A, Ponta H, Rahmsdorf HJ, Herrlich P, Cato ACB (1994) A distinct modulating domain in glucocorticoid receptor monomers in the repression of activity of the transcription factor AP-1. *EMBO J* 13: 4087–4095
155 Adcock IM, Barnes PJ (1996) Ligand-induced differentiation of glucocorticoid receptor (GR) transrepression and transactivation. *Am J Respir Crit Care Med* 153: A243
156 Vayssière BM, Dupont S, Choquart A et al. (1997) Synthetic glucocorticoids that dissociate transactivation and AP-1 transrepresson exhibit antiinflammatory activity *in vivo*. *Mol Endocrinol* 11: 1245–1255
157 Barnes PJ, Adcock IM (1997) NF-κB: a pivotal role in asthma and a new target for therapy. *Trends Pharmacol Sci* 18: 46–50
158 Miyajima T, Kotake Y (1995) Spin trapping agent, phenyl *N*-tert-butyl nitrone, inhibits induction of nitric oxide synthase in endotoxin-induced shock in mice. *Biochem Biophys Res Commun* 215: 114–121
159 Pahl HL, Krauss B, Schultze-Osthoff K et al. (1996) The immunosuppressive fungal metabolite gliotoxin specifically inhibits transcription factor NF-κB. *J Exp Med* 183: 1829–1840
160 Wang P, Wu P, Siegel MI, Egan RW, Billah MM (1995) Interleukin(IL)-10 inhibits nuclear factor kappa B activation in human monocytes. IL-10 and IL-4 suppress cytokine synthesis by different mechanisms. *J Biol Chem* 270: 9558–9563
161 John M, Lim S, Seybold J, Robichaud A, O'Connor B, Barnes PJ, Chung KF (1997) Inhaled corticosteroids increase IL-10 but reduce MIP-1α, GM-CSF and IFN-γ release from alveolar macrophages in asthma. *Am J Respir Crit Care Med*
162 Yaron A, Gonen H, Alkalay I et al. (1997) Inhibition of NF-κB cellular function via specific targeting of the IκB-ubiquitin ligase. *EMBO J* 16: 6486–6494
163 Wrighton CJ, Hofer-Warbinek R, Moll T, Eytner R, Bach FH, de Martin R (1996) Inhibition of endothelial cell activation by adenovirus-mediated expression of IκB-α, an inhibitor of transcription factor NF-κB. *J Exp Med* 183: 1013–1022
164 Wallace J, Adcock IM, Barnes PJ (1996) Retinoic acid potentiates the inhibitory effects of dexamethasone on AP-1 DNA binding in epithelial cells. *Am J Respir Crit Care Med* 153: A209
165 Rowe A (1997) Retinoid X receptors. *Int J Biochem Cell Biol* 29: 275–278