Targeting lysine acetylation in inflammatory lung diseases

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Chapter 1

Introduction and scope of the thesis

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Lysine acetylation is a reversible post translational modification (PTM) of cellular proteins and represents an important regulatory switch in signal transduction cascades (1) (2). An increasing number of studies highlight the importance of lysine acetylation as a key PTM directing both the outcomes as well as the activation levels of important signal transduction pathways such as the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway. For example, acetylation of NF-κB transcription factors p65 and p50 plays an important role in their nuclear localization and transcriptional activity (3). Similar phenomena have been observed for other pathways (4). Next to this, acetylation of histones connected to specific genes plays an important role in gene specific transcription in the NF-κB pathway (3).

Lysine acetylations are generally regulated by writers and erasers, which are denoted as histone acetyltransferases (HATs) and histone deacetylases (HDACs), respectively, due to their original discovery as histone modifying enzymes. An important future challenge is to identify and quantify distinct HAT and HDAC activities in distinct signaling pathways such as the NF-κB pathway, as well as their aberrations in disease (models). Considering the importance of lysine acetylation in the NF-κB pathway (Fig. 1), small molecule modulators of HATs and HDACs have great potential to specifically regulate this signaling cascade, which is an important aim in drug discovery. Focusing on the NF-κB pathway, in this chapter we summarize the effects of lysine acetylation of the p65 transcription factor as well as histones. Furthermore, importantly, we discuss the effects of frequently used small molecule HAT and HDAC inhibitors on the NF-κB signal transduction pathway and inflammatory responses *in vitro* and *in vivo*. Finally, we then introduce NF-κB as an alternative therapeutic target

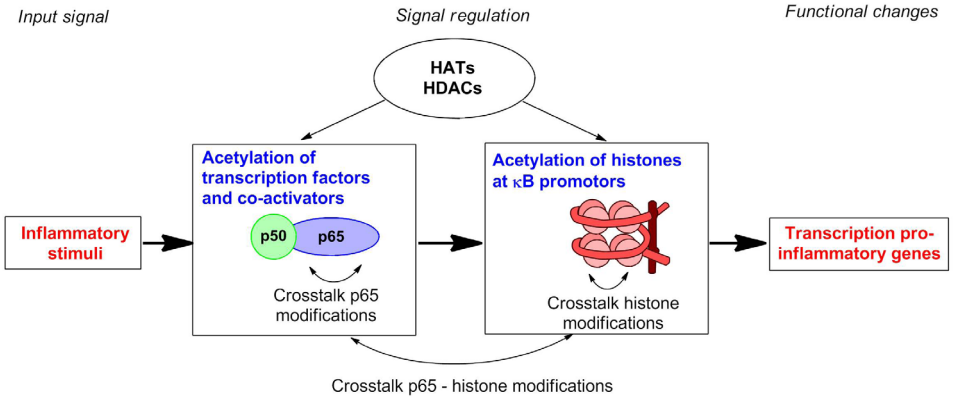

Figure 1. Schematic representation of the diverse roles of lysine acetylation in the activation of the NF-κB pathway. Lysine acetylations of the transcription factors as well as their co-activators play an important role in the duration of the response and the signaling output. Lysine acetylation status of the histones works in concert with acetylation status of the transcription factors to enable or disable transcription of specific genes.

for inflammatory airway diseases such as asthma and COPD, and outline how we have addressed this using HAT and HDAC inhibitors in this thesis.

The NF-κB pathway

The NF-κB transcription factors are a family of inducible transcription factors that play a central role in the expression of various cytokines, chemokines and adhesion molecules, which are involved in cell survival and inflammation (5). NF-κB transcription factors exist in homo- or hetero-dimeric complexes consisting of different members of the Rel family of proteins. The most prevalent and best-studied of these complexes is the p50-p65 heterodimer. In quiescent cells, the p50-p65 complex is present in the cytoplasm in an inactive form, bound to inhibitory proteins known as IκBs. The NF-κB pathway can be activated via two different routes; the canonical pathway or the alternative pathway. The canonical pathway is activated by inducers such as inflammatory cytokines, i.e., TNFa, interleukin (IL)-1; bacterial products (LPS) or oxidative stress (H_2O_2), resulting in phosphorylation, ubiquitylation and finally degradation of IκBα. Subsequently, the p50-p65 heterodimer is released and translocates into the nucleus, followed by specific upregulation of gene expression (6). The alternative pathway is activated by lymphotoxin (LT) β, CD40 ligand, B cell activating factor (BAFF), and receptor activator of NF-κB ligand (RANKL), resulting in activation of RelB/p52 complexes (7). Activation of the alternative pathway regulates genes required for lymph-organogenesis and B-cell activation. The canonical pathway plays an important role in chronic inflammatory diseases like inflammatory bowel disease, rheumatoid arthritis, asthma and COPD (8) (9).

Lysine acetylation as a regulator of the NF-κB pathway

In 2001, it was discovered that acetylation of p65 inhibits binding to the inhibitory complex IκBα, and thus stimulates gene transcription; whereas deacetylation promotes IκBα binding and nuclear export (10). This study triggered intense interest in lysine acetylations of the seven lysine residues (122, 123, 218, 221, 310, 314, 315) of p65 that are subject to this PTM. These acetylations have specific roles in activation of the NF-κB pathway and have been previously reviewed (11) (12). Importantly, acetylation of lysines 122 and 123 decreases DNA binding (13); acetylation at lysines 218 and 221 increases binding to κB enhancers; and acetylation at lysine 310 is essential for full transcriptional activity (14). In addition, acetylations of specific lysine residues in histone H3 and H4 play an important role in NF-κB mediated gene transcription as reviewed (3).

HATs in the NF-κB pathway

Based on their primary structure homology, HATs have been divided into five families. Three families that have been studied extensively are the GNAT (GCN5-related N-acetyltransferase) family, represented by PCAF (p300/CBP associated factor) and GCN5

(general control nonderepressible 5); the p300/CBP family, including CBP (cAMP-response-element-binding-protein-binding-protein) and p300; and the MYST family including MOF and Tip60 (TAT-interacting protein 60) (15). The HATs p300 and PCAF acetylate lysine 122 and 123 of p65 (13) and p300 has been described to acetylate lysine 310, 314 and 315 (16). The role of HATs in acetylation of the NF-κB transcription factor as well as acetylation of the histones connected to κB promoters has been reviewed (3). Interestingly, a recent study demonstrates that Tip60 is a co-activator of several NF-κB targets genes and exerts its action via protein-protein interactions with p65. It appears that Tip60 binds earlier to the κB promotors than p65 and simultaneously promotes histone acetylation, which indicates that Tip60 could serve as a platform to promote NF-κB mediated gene transcription (17).

HAT inhibitors mainly lead to inhibition of the NF-κB pathway

Several inhibitors of histone acetyltransferases (HATi) are known and have been previously reviewed (18) (3). The natural product anacardic acid (AA) (Fig. 2, Table 1) is a small molecule inhibitor of HATs such as p300 and PCAF (19) and inhibits NF-κB-mediated gene transcription (20). This inhibitor has been used as a starting point for development of novel inhibitors such as the alkylidene malonates (21). The novel anacardic acid derivative MG149 (Fig. 2, Table 1) demonstrates selectivity towards the MYST type of HATs Tip60 and MOF (22) and this molecule effectively suppresses SAHA induced hyperacetylation (23). In addition, DNA microarrays demonstrated that MG149 inhibits the p53 and the NF-κB pathways and a very limited number of other pathways (23).

Next to this, high throughput screening identified the isothiazolones as HATi (24). However, attempts to optimize this class failed to give inhibitors with higher potency and selectivity (25) (26) (27), which could mainly be attributed to the exceptionally high reactivity of isothiazolones for thiolates (28). Fortuitously, virtual screening enabled the identification of C646 (Fig. 2, Table 1) as the first potent, selective and cell-permeable p300 HATi (K_i 0,4 μM) (29). In addition, another recent virtual screening study describes the identification of a novel cell-permeable inhibitor (**1a** Zeng; Fig. 2, Table 1) of p300, which is active at the same concentrations as C646 (30). This demonstrates the strength of virtual screening as a strategy for identification of novel inhibitors for challenging targets such as the HATs.

Regarding the use of C646 in cellular models, one study on prostate cancer cell lines demonstrates that both siRNA-mediated and C646-mediated inhibition of p300 increase apoptosis, which is, among others, caused by inhibition of the androgen receptor and the NF-κB pathway (31). Another group demonstrated that p300 binds to the COX-2 promotor and that inhibition of p300 activity by C646 diminished both the p300 promotor binding and the expression of COX-2. Interestingly, this study was done in animal models in which C646 was administered via a lumbar intrathecal catheter, which demonstrates that HATi can be administered locally in in animal models (32). Thus, these data demonstrate

that C646 performs very well in both cell-based studies and upon local administration in animal models. Inhibition of p300 by both siRNA and the chemical inhibitor C646 leads to inhibition of, among others, the NF-κB pathway.

HDAC classes

Mammalian HDACs are classified into four main groups based on their homology with yeast orthologues (33). Class I HDACs, including HDAC1, 2, 3, and 8 are predominantly found within the nucleus, due to the presence of a nuclear localization sequence and the absence of a nuclear export signal sequence within HDAC1, 2, and 8. However, HDAC3 has both a nuclear import and export signal, allowing for localization in both the cytoplasm and the nucleus (33) (34). Class I HDACs have a ubiquitous tissue distribution (33). Class II HDACs are subdivided into two groups, IIA (HDAC4, 5, 7, 9) and IIB (HDAC6 and 10), and are predominantly found in the cytoplasm (35). Class II HDACs are able to shuttle between the cytoplasm and the nucleus, and have a more tissue-specific distribution than class I HDACs (33). Class IV consists only of HDAC11, which shares similarities with both class I and II HDACs. Class I, II and IV HDACs are zinc dependent. Class III HDACs are also called sirtuins (SIRT1-SIRT7) and are found in the cytoplasm. Sirtuins act via different mechanisms and require the co-factor NAD^+ for their activity (36).

Inhibitors of zinc dependent HDACs have ambiguous effects on the NF-κB pathway

Several HDAC inhibitors (HDACi) are known. HDACi can be grouped in four main classes based on their chemical structure i.e. hydroxamic acids, 2-amino-benzamides, cyclic peptides and short-chain fatty acids. From these, the first two classes have been studied most intensively (37). Three HDACi of the hydroxamic acid type which are non-selective among the Zn^{2+} dependent HDACs, suberanilohydroxamic acid (SAHA) (Fig. 2, Table 1), Belinostat (Fig. 2, Table 1) and Panobinostat (Fig. 2, Table 1), obtained FDA approval for treatment of hematological cancers (38).

Although HDACi were initially discovered as anti-cancer agents, many studies indicate their ability to suppress inflammatory responses. In this respect, early evidence stems from a study demonstrating that phenylbutyrate, as well as trichostatin A (TSA) (Fig. 2, Table 1), inhibit TNFα expression in inflamed tissues in a rheumatoid arthritis animal model (39). Another early study demonstrates that HDACi SAHA inhibits LPS induced cytokine release *in vitro* and *in vivo* (40). This anti-inflammatory effect was observed at much lower concentrations than tumor suppressive effects *in vitro*. The anti-inflammatory potency of HDACi has also been described in several reviews (41) (42) (43). It was observed that HDACi such as SAHA and TSA delay and reduce NF-κB nuclear translocation and gene expression upon TNFα stimulation (44). Most interestingly, a recent study applied the HDACi Givinostat (Fig. 2, Table 1) in a relatively small patient group suffering from

systemic-onset juvenile idiopathic arthritis, and demonstrated a clear therapeutic benefit and excellent safety profile (45).

In contrast to inhibiting inflammation, other studies have also demonstrated that certain HDACi can also lead to the stimulation of pro-inflammatory gene transcription. Initial studies on NF-κB acetylation reported that HDACs 1-3 (class I) can deacetylate p65 and negatively regulate gene transcription (10) (46). In addition, HDAC activity leads to histone deacetylation, which is generally associated with inhibition of gene transcription. Both factors imply that HDACi stimulate pro-inflammatory gene transcription, which is supported by several studies. Indeed, it was demonstrated that SAHA (47), TSA (48) (49), and LBH-589 (Fig. 2, Table 1) (50) increase NF-κB activation. Such contradictory findings can be explained by applications of different cell types and the lack of selectivity of the employed HDACi. Most frequently, applied HDACi target all zinc dependent HDACs, as most rely on the strongly zinc coordinating hydroxamic acid functionality (carried by Pan HDACi in Fig. 2 and Table 1) (51). This hampers elucidation of the relevance of HDAC activity in specific disease models. However, over the past few years, more selective inhibitors have become available. An example includes MS-275 (or Entinostat) (Fig. 2, Table 1), which is HDAC1-3 selective (51).

Altogether, lysine acetylation is a key regulator of the NF-κB pathway. Importantly, small molecule modulators of its writers (HATs) or erasers (HDACs), have been demonstrated to regulate NF-κB signaling, suggesting that these are potential drugs for inflammatory diseases. An ongoing challenge towards regulation of the NF-κB pathway is the development of highly potent molecules that selectively target specific HATs or HDACs. Fortuitously, this field has seen remarkable progress over the past few years. Several inhibitors now demonstrate specific effects in distinct disease models in both cellular systems and animal studies, which is very promising for drug discovery. A noteworthy example is HATi C646. Due to its high selectivity and potency for p300, this inhibitor mainly leads to inhibition of pathways that are connected to NF-κB, the androgen receptor, and the glucocorticoid receptor, in cellular and animal models. Another promising example is HATi MG149, which mainly inhibits expression related to the NF-κB and p53 pathways. Additionally, some currently described HDACi show encouraging effects in animal models and even patients; pan HDACi Givinostat displayed promising therapeutic benefits in patients suffering from juvenile idiopathic arthritis. An important consideration in the development of such agents is the capacity for HDACi to show either activation or inhibition of inflammatory responses. We presume that a main cause of these ambiguities is that different reports employed different cell types. In addition, we speculate that they may also be explained by HDAC (a)selectivity of the inhibitors used.

Model diseases in this thesis: inflammatory airway diseases asthma and COPD

NF-κB plays an important role in the inflammation in asthma and chronic obstructive pulmonary disease (COPD) (55). Asthma and COPD are common inflammatory airway

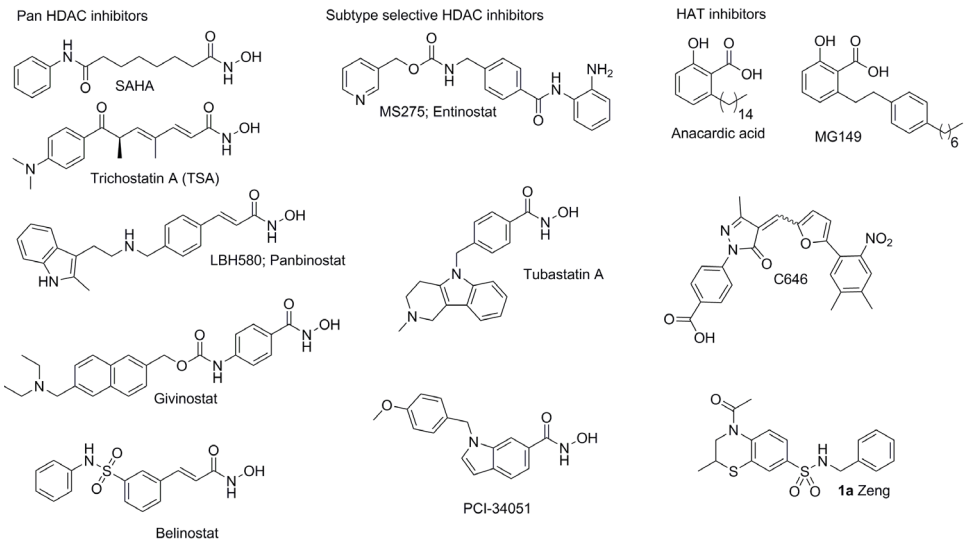

Figure 2. Structures of small molecules that interact with writers (HATs) or erasers (HDACs) of lysine acetylations.

diseases. These diseases affect millions of people world-wide, and globally, the incidence is increasing. For asthma, the clinical symptoms include among others wheezing and shortness of breath. COPD is characterized by shortness of breath upon exercise and a largely irreversible and progressive airflow limitation (55) (56) (57) (58). In asthma the larger airways are mainly affected, whereas in COPD, the lung parenchyma and peripheral airways are mainly affected (57). The immunopathology of asthma and COPD is characterized by different types of inflammatory cells which are at play. For example, asthma is driven by T helper 2 cells, dendritic cells, and is characterized by eosinophilic inflammation. In asthma there is mast-cell sensitization by IgE, and multiple bronchoconstrictors are released (55) (56). On the other hand, COPD is characterized by T helper 1 cells, cytotoxic T cells and neutrophilic inflammation (59) (57). The inflammation in COPD is also characterized by increased numbers of macrophages, neutrophils, and innate lymphoid cells recruited from the circulation. A variety of pro-inflammatory mediators, including cytokines, chemokines, growth factors, and lipid mediators are secreted by these cell types (59). Currently, glucocorticoids are the cornerstone in the treatment of asthma and COPD, however, this is not effective in all patients. Severe asthmatics display glucocorticoid resistance (60), and the effectivity of glucocorticoids in COPD patients is subject to debate (61) (62). Alternative therapeutic strategies are needed for these patients, which currently form a major health burden for society due to high socioeconomic costs (55) (56) (57) (58) (63).

The NF-κB pathway may be a new therapeutic target for these diseases. The NF-

Table 1. Small molecules that interfere with writers (HATs) or erasers (HDACs) of lysine acetylations, and their selectivity and effects on disease models for NF-κB mediated inflammation.

Compound	Selectivity	Effects in disease models	Ref.
HAT inhibitors			
Anacardic acid	p300 and PCAF inhibitor	Inhibits the NF-κB pathway.	(20)
MG149	Tip60 and MOF selective inhibitor	Inhibits SAHA induced hyperacetylation. Inhibits the NF-κB and p53 signaling pathways.	(22) (23)
C646	p300 selective inhibitor (K_i 0,4 μM)	Increases apoptosis by inhibition of the androgen receptor and NF-κB pathway. Repression of gene expression. Inhibition of COX-2 expression.	(31,52) (32) (53)
1a Zeng	p300 HAT inhibitor (IC_{50} 3.4 μM)	-	(30)
HDAC inhibitors			
SAHA	Pan-HDAC inhibitor	Suppression of LPS-induced cytokine release *in vitro* and *in vivo*.	(40) (51)
Trichostatin A (TSA)	Pan-HDAC inhibitor	Inhibition of TNFα in vitro.	(39) (51)
LBH580 (Panibinostat)	Pan-HDAC inhibitor	Enhances NF-κB activation.	(50) (51)
ITF2357 (Givinostat)	Pan-HDAC inhibitor	Therapeutic benefit in patients suffering from juvenile idiopathic arthritis.	(45) (51)
Belinostat	Pan-HDAC inhibitor	-	(38)
MS275 (Entinostat);	HDAC1-3 selective inhibitor	Enhances NF-κB activation.	(47) (51)
Tubastatin A	HDAC6 selective inhibitor	-	(54)
PCI-34051	HDAC8 selective inhibitor	-	(54)

κB transcription factor plays an important role in macrophages in the expression of pro-inflammatory cytokines and chemokines, which then attract other inflammatory cell types. This plays a central role in the orchestration of asthma and COPD (64). Next to this, upregulated NF-κB activity has been reported in asthma (65) and COPD (66) (67), and increased nuclear localization of p65 was observed in sputum macrophages during exacerbations of COPD (68) as well as in bronchial biopsies of stable COPD patients (69). Several lines of research have focused on modulating NF-κB activity as a novel therapeutic strategy for the treatment of asthma and COPD (66). This gives rise to an interesting research line approaching this through investigating the capability of HATi and HDACi in regulating NF-κB-mediated inflammatory responses in models systems for asthma and COPD.

HAT and HDAC expression and activity in asthma and COPD

It is worthwhile to mention that asthma is characterized by increased HAT activity (70). For instance, it was demonstrated that there is increased HAT activity, and decreased HDAC

activity and HDAC1 and HDAC2 expression, in bronchial biopsies and bronchoalveolar lavage (BAL) macrophages obtained from asthmatics compared to healthy adults (71) (72), thereby shifting the balance towards HAT activity. Another study showed that HAT activity is increased in peripheral blood mononuclear cells (PBMCs) obtained from mild and severe asthmatic children compared to healthy children, whereas HDAC activity is decreased. (73). However, another study did not find decreased expression of HDAC1 and HDAC2 mRNA or protein in endobronchial biopsies in a large set of severe asthmatics compared to healthy controls (74). Yet another study found that in neutrophilic asthma, there was increased HAT and decreased HDAC activity in isolated PBMCs. However, there were no differences in the expression levels of the HATs p300, KAT2B, cAMP-response-element-binding-protein-binding-protein (CBP) or the HDACs HDAC1, HDAC2 or HDAC3, which could indicate differences in activity due to post-translational effects (75). More recently, it has been identified that acetylation of histone H3 lysine 18 (H3K18) is elevated in asthma epithelium compared to healthy subjects (76), which is in line with a shift towards increased HAT activity.

COPD is characterized by a loss of SIRT1 (77), and HDAC2 expression and activity (78) (70). In more recent studies, decreased HDAC2 expression was also found in PBMCs and lymphocytes in COPD (79) (67). In COPD, the decreased HDAC2 expression and activity can be linked to glucocorticoid resistance (80). This is because glucocorticoids are dependent on HDAC2 for their immunosuppressive activity. Glucocorticoids diffuse through the cellular membrane and bind the glucocorticoid receptor (GR) which is present in the cytoplasm. The GR is normally bound by heat shock proteins (HSP), but upon binding of glucocorticoids, is released and translocated into the nucleus where it binds to target genes with glucocorticoid response elements. This includes multiple inflammatory genes. GR then recruits HDAC2, which leads to transcriptional repression (80). It is interesting to note that also in severe asthmatics, there are indications that decreased HDAC2 expression and activity can be linked to steroid resistance (81), and that passive smoking reduced HDAC2 expression in severe asthmatic children (82). In summary, both asthma and COPD are characterized by increased HAT and decreased HDAC activity. Importantly, however, the expression and activity have not been well-characterized for most HAT and HDAC isoforms.

HATi and HDACi in asthma and COPD

Not much is known about the effects of HATi in models systems for inflammatory lung diseases such as asthma and COPD. It was, however, previously shown that anacardic acid decreased the expression of IL-4, IL-5 and IL-13 in T cells isolated from mice challenged with ovalbumin to model allergic asthma. Upon re-administering these T cells to mice, the balance between HDAC and HAT activities were changed in lung tissue towards more HDAC activity (83). Furthermore, in a mouse model, anacardic acid was found to ameliorate lung damage which was induced by exposure of the mice to diesel exhaust particles. This effect

was attributed to reduced levels of neutrophils in the lung parenchyma and reduced TNF-α levels in the BALF supernatant (84). Taken together, this indicates that the effects of HATi such as anacardic acid in asthma and COPD models are promising but need to be further explored, including their effects on the NF-κB pathway in these model systems.

Interestingly, anti-inflammatory effects of HDACi have been reported in a number of studies using mouse models of asthma, mostly using the non-selective HDACi TSA, which inhibits class I and II HDACs (51). TSA reduced T cell infiltration and expression of the T helper 2 cytokines IL-4 and IL-5, and IgE in an ovalbumin sensitization and challenge mouse asthma model (85). In another study, the effects of TSA were also evaluated in an ovalbumin model. TSA-treated mice had a reduced number of total inflammatory cells and eosinophils in the BAL fluid compared to vehicle-treated mice. Furthermore, airway remodeling changes were significantly reduced with TSA compared to vehicle-treated mice, with fewer goblet cells, less subepithelial collagen deposition and attenuated airway hyperresponsivenes induced by methacholine (86). Another study also showed that TSA inhibited methacholine-induced airway hyperresponsiveness in mice sensitized and challenged with *Aspergillus fumigatus* antigen. However, this was not related to anti-inflammatory effects of TSA, since no effects were observed on leukocyte trafficking or concentrations of cytokines in BAL fluid in antigen-challenged mice. Instead, using human precision-cut lung slices and airway smooth muscle cells, an inhibiting effect on smooth muscle contraction was observed upon TSA treatment (87). In a recent study using an *Alternaria* mouse allergic model, TSA downregulated the number of innate lymphoid group 2 cells (ILC2; an important source of the cytokines IL-5 and IL-13 which are critical to allergic airway inflammation) expressing IL-5, IL-13 and IL-33 upon *Alternaria* extract challenge, and reduced lung eosinophilia and mucus hypersecretion. TSA treatment also decreased *Alternaria* extract-induced pro-inflammatory cytokines and chemokines including KC (murine IL-8), TNF-α and GM-CSF (88). Another recent study using an ovalbumin mouse model demonstrated that TSA decreased IL-17 level in the BAL fluid (89). On the other hand, it was shown that TSA increased pro-inflammatory cytokine levels in the culture supernatant of memory T cells which were isolated from ovalbumin asthmatic mice; and when these memory T cells (treated with TSA) were adoptively transferred into naive mice, increased pro-inflammatory cytokine levels were also found in the BAL fluid (90). Another study did not use TSA, but other HDACi including tubastatin A (an HDAC6i) (Fig. 2, Table 1), PCI-34051 (an HDAC8i) (Fig. 2, Table 1), and the non-selective HDACi givinostat (Fig. 2, Table 1). These were tested in a mouse ovalbumin asthma model and all reduced inflammatory cell counts in the BAL fluid of these mice (54). Taken together, HDACi generally reduce inflammation in mouse asthma models. However, this has mostly been studied using the non-selective HDACi TSA, and the underlying biological mechanisms which could explain these anti-inflammatory effects, including the effects on the NF-κB pathway in these model systems, remain unclear. Next to this, the potential of HDACi in COPD mouse models has remained unexplored.

Scope of the thesis

In summary, lysine acetylation is a reversible PTM of cellular proteins and represents an important regulatory switch in signal transduction. Lysine acetylation directs both the outcomes as well as the activation levels of important signal transduction pathways such as the NF-κB pathway. Small molecule modulators of its writers (HATs) and erasers (HDACs) have been used to specifically regulate the NF-κB pathway. This thesis focuses on the effects of HATi and HDACi on the NF-κB signal transduction pathway and inflammatory responses, and their potential as novel drugs. We have a particular focus on inflammatory airway diseases such as asthma and COPD. Asthma and COPD are common inflammatory airway diseases that affect millions of people world-wide. The current therapy for these diseases constitutes glucocorticoids, however, not all patients respond to this therapy. Hence, alternative therapeutic targets and strategies are required. The NF-κB pathway plays a crucial role in the inflammation in asthma and COPD, for example in pro-inflammatory gene expression in macrophages. Based on the fact that HATi and HDACi can modulate NF-κB activity, these small molecules have potential in the treatment of asthma and COPD. In this thesis we evaluate and explore the potential of small molecule HATi and HDACi in attenuating NF-κB-mediated inflammatory responses in model systems for inflammatory airway diseases such as asthma and COPD.

In **chapter 2** we focus on C646, an inhibitor for the HAT p300 (KAT3B). With a K_i value of 0,4 μM, C646 is one of the most potent HATi described to date. Based on the crucial role of p300 in regulation of NF-κB acetylation, we investigate the effects of this compound on NF-κB activity in RAW264.7 murine macrophages. We report inhibition of NF-κB activity in the macrophages. We also explore the potential of this compound in attenuation of lipopolysaccharide and interferon gamma (LPS and IFNγ)-induced pro-inflammatory gene expression in RAW264.7 murine macrophages and murine lung tissue slices, and find inhibition of pro-inflammatory gene expression. Then, we proceed to explore the potential of this compound in inhibition of histone acetylation in the macrophages. Unexpectedly, we report increased histone acetylation upon treatment with this compound. We find this to correlate with inhibition of recombinant HDACs. Therefore, the results of this chapter have important implications for biochemical assays employing C646 as a p300 inhibitor.

In **chapter 3** we study MG149, an inhibitor for MYST type HATs Tip60 (KAT5) and MOF (KAT8) previously discovered at our lab. MG149 is a 6-alkylsalicylate. Previous literature has reported that the 6-alkylsalicylate anacardic acid (from which MG149 was derived) can attenuate inflammation in models for lung inflammation. In this chapter, we report that the 6-alkylsalicylate MG149 inhibits histone acetylation and LPS and IFNγ-induced pro-inflammatory gene expression in murine lung tissue slices. The chapter demonstrates the potential of 6-alkylsalicylates which inhibit HATs in attenuation of lung inflammation.

In **chapter 4** we study 4-amino-1-naphtol (compound **13**), a non-selective inhibitor for the HATs p300 (KAT3B), MOF (KAT8), and PCAF (KAT2B), which was recently discovered at our lab. In this chapter we investigate the effects of this compound in model systems for inflammatory airway diseases. We start with an investigation of the effects on histone acetylation in RAW264.7 macrophages. We then proceed to study the effects of the compound on histone acetylation in lung tissue slices. In addition, in both the macrophages and lung tissue slices, we study the effects of **13** on LPS and IFNγ-induced pro-inflammatory gene expression. The results described in this chapter demonstrate that **13** is a potent inhibitor of histone acetylation, and that **13** inhibits pro-inflammatory gene expression. Altogether, this suggests that the concept of HAT inhibition as an alternative therapeutic strategy for inflammatory airway diseases such as asthma and COPD, is promising.

In **chapter 5** we investigate the HDAC1-3 inhibitor MS-275. Based on the role of HDAC1-3 in NF-κB acetylation, we explore its effects on LPS and IFNγ-induced pro-inflammatory gene expression in RAW264.7 macrophages. We demonstrate that the HDAC1-3 inhibitor MS-275 gives rise to mixed effects on pro- and anti-inflammatory gene expression in RAW264.7 macrophages with upregulation of both pro- and anti-inflammatory genes. Importantly, MS-275 increases *IL10* expression. We then move on to study the underlying molecular pathways involved, and identify NF-κB as a regulator. MS-275 increases NF-κB promoter activity, acetylation, nuclear translocation and NF-κB p65 binding to the *IL10* promoter in RAW264.7 macrophages. Finally, we study the effects of this compound in a mouse COPD (cigarette smoke expose) model. We find anti-inflammatory effects on several parameters including increased anti-inflammatory *IL10* expression in lung macrophages, reduced KC (murine IL-8) expression and reduced neutrophilic influx upon MS-275 treatment, which is the first time that anti-inflammatory effects of a HDACi are being reported in a COPD mouse model.

In **chapter 6** the results of the thesis are summarized and discussed.

References

1. Kim SC, Sprung R, Chen Y, Xu Y, Ball H, Pei J, et al. Substrate and functional diversity of lysine acetylation revealed by a proteomics survey. Mol Cell 2006 Aug;23(4):607-618.
2. Choudhary C, Kumar C, Gnad F, Nielsen ML, Rehman M, Walther TC, et al. Lysine acetylation targets protein complexes and co-regulates major cellular functions. Science 2009 Aug 14;325(5942):834-840.
3. Ghizzoni M, Haisma HJ, Maarsingh H, Dekker FJ. Histone acetyltransferases are crucial regulators in NF-κB mediated inflammation. Drug Discov Today 2011;16(11-12):504-511.
4. Kusio-Kobialka M, Wolanin K, Podszywalow-Bartnicka P, Sikora E, Skowronek K, McKenna SL, et al. Increased acetylation of lysine 317/320 of p53 caused by BCR-ABL protects from cytoplasmic translocation of p53 and mitochondria-dependent apoptosis in response to DNA damage. Apoptosis 2012 Sep;17(9):950-963.
5. Chen LF, Greene WC. Shaping the nuclear action of NF-kappaB. Nat Rev Mol Cell Biol 2004 May;5(5):392-401.
6. Hoesel B, Schmid JA. The complexity of NF-kappaB signaling in inflammation and cancer. Mol Cancer 2013 Aug 2;12:86-4598-12-86.
7. Bonizzi G, Karin M. The two NF-kappaB activation pathways and their role in innate and adaptive immunity. Trends Immunol 2004 Jun;25(6):280-288.
8. Lawrence T. The nuclear factor NF-kappaB pathway in inflammation. Cold Spring Harb Perspect Biol 2009 Dec;1(6):a001651.
9. Wisastra R, Dekker FJ. Inflammation, Cancer and Oxidative Lipoxygenase Activity are Intimately Linked. Cancers (Basel) 2014 Jul 17;6(3):1500-1521.
10. Chen L, Fischle W, Verdin E, Greene WC. Duration of nuclear NF-kappaB action regulated by reversible acetylation. Science 2001 Aug 31;293(5535):1653-1657.
11. Perkins ND. Post-translational modifications regulating the activity and function of the nuclear factor kappa B pathway. Oncogene 2006 Oct 30;25(51):6717-6730.
12. Huang B, Yang XD, Lamb A, Chen LF. Posttranslational modifications of NF-kappaB: another layer of regulation for NF-kappaB signaling pathway. Cell Signal 2010 Sep;22(9):1282-1290.
13. Kiernan R, Bres V, Ng RW, Coudart MP, El Messaoudi S, Sardet C, et al. Post-activation turn-off of NF-kappa B-dependent transcription is regulated by acetylation of p65. J Biol Chem 2003 Jan 24;278(4):2758-2766.
14. Chen L-, Mu Y, Greene WC. Acetylation of RelA at discrete sites regulates distinct nuclear functions of NF-κB. EMBO J 2002;21(23):6539-6548.
15. Marmorstein R. Structure and function of histone acetyltransferases. Cell Mol Life Sci 2001 May;58(5-6):693-703.
16. Buerki C, Rothgiesser KM, Valovka T, Owen HR, Rehrauer H, Fey M, et al. Functional relevance of novel p300-mediated lysine 314 and 315 acetylation of RelA/p65. Nucleic Acids Res 2008 Mar;36(5):1665-1680.
17. Kim JW, Jang SM, Kim CH, An JH, Kang EJ, Choi KH. New molecular bridge between RelA/p65 and NF-kappaB target genes via histone acetyltransferase TIP60 cofactor. J Biol Chem 2012 Mar 2;287(10):7780-7791.
18. Dekker FJ, Haisma HJ. Histone acetyl transferases as emerging drug targets. Drug Discov Today 2009 Oct;14(19-20):942-948.
19. Balasubramanyam K, Swaminathan V, Ranganathan A, Kundu TK. Small molecule modulators of histone acetyltransferase p300. J Biol Chem 2003 May 23;278(21):19134-19140.

20. Sung B, Pandey MK, Ahn KS, Yi T, Chaturvedi MM, Liu M, et al. Anacardic acid (6-nonadecyl salicylic acid), an inhibitor of histone acetyltransferase, suppresses expression of nuclear factor-kappaB-regulated gene products involved in cell survival, proliferation, invasion, and inflammation through inhibition of the inhibitory subunit of nuclear factor-kappaBalpha kinase, leading to potentiation of apoptosis. Blood 2008 May 15;111(10):4880-4891.
21. Sbardella G, Castellano S, Vicidomini C, Rotili D, Nebbioso A, Miceli M, et al. Identification of long chain alkylidenemalonates as novel small molecule modulators of histone acetyltransferases. Bioorg Med Chem Lett 2008 May 1;18(9):2788-2792.
22. Ghizzoni M, Wu J, Gao T, Haisma HJ, Dekker FJ, George Zheng Y. 6-alkylsalicylates are selective Tip60 inhibitors and target the acetyl-CoA binding site. Eur J Med Chem 2012 Jan;47(1):337-344.
23. Legartova S, Stixova L, Strnad H, Kozubek S, Martinet N, Dekker FJ, et al. Basic nuclear processes affected by histone acetyltransferases and histone deacetylase inhibitors. Epigenomics 2013 Aug;5(4):379-396.
24. Stimson L, Rowlands MG, Newbatt YM, Smith NF, Raynaud FI, Rogers P, et al. Isothiazolones as inhibitors of PCAF and p300 histone acetyltransferase activity. Mol Cancer Ther 2005 Oct;4(10):1521-1532.
25. Dekker FJ, Ghizzoni M, van der Meer N, Wisastra R, Haisma HJ. Inhibition of the PCAF histone acetyl transferase and cell proliferation by isothiazolones. Bioorg Med Chem 2009 Jan 15;17(2):460-466.
26. Ghizzoni M, Haisma HJ, Dekker FJ. Reactivity of isothiazolones and isothiazolone-1-oxides in the inhibition of the PCAF histone acetyltransferase. Eur J Med Chem 2009 Dec;44(12):4855-4861.
27. Gorsuch S, Bavetsias V, Rowlands MG, Aherne GW, Workman P, Jarman M, et al. Synthesis of isothiazol-3-one derivatives as inhibitors of histone acetyltransferases (HATs). Bioorg Med Chem 2009 Jan 15;17(2):467-474.
28. Wisastra R, Ghizzoni M, Maarsingh H, Minnaard AJ, Haisma HJ, Dekker FJ. Isothiazolones; thiol-reactive inhibitors of cysteine protease cathepsin B and histone acetyltransferase PCAF. Org Biomol Chem 2011 Mar 21;9(6):1817-1822.
29. Bowers EM, Yan G, Mukherjee C, Orry A, Wang L, Holbert MA, et al. Virtual ligand screening of the p300/CBP histone acetyltransferase: identification of a selective small molecule inhibitor. Chem Biol 2010 May 28;17(5):471-482.
30. Zeng FQ, Peng SM, Li L, Mu LB, Zhang ZH, Zhang ZY, et al. Structure-based identification of drug-like inhibitors of p300 histone acetyltransferase. Yao Xue Xue Bao 2013 May;48(5):700-708.
31. Santer FR, Hoschele PP, Oh SJ, Erb HH, Bouchal J, Cavarretta IT, et al. Inhibition of the acetyltransferases p300 and CBP reveals a targetable function for p300 in the survival and invasion pathways of prostate cancer cell lines. Mol Cancer Ther 2011 Sep;10(9):1644-1655.
32. Zhu XY, Huang CS, Li Q, Chang RM, Song ZB, Zou WY, et al. p300 exerts an epigenetic role in chronic neuropathic pain through its acetyltransferase activity in rats following chronic constriction injury (CCI). Mol Pain 2012 Nov 23;8:84-8069-8-84.
33. Yang XJ, Seto E. The Rpd3/Hda1 family of lysine deacetylases: from bacteria and yeast to mice and men. Nat Rev Mol Cell Biol 2008 Mar;9(3):206-218.
34. de Ruijter AJ, van Gennip AH, Caron HN, Kemp S, van Kuilenburg AB. Histone deacetylases (HDACs): characterization of the classical HDAC family. Biochem J 2003 Mar 15;370(Pt 3):737-749.
35. Parra M, Verdin E. Regulatory signal transduction pathways for class IIa histone deacetylases. Curr Opin Pharmacol 2010 Aug;10(4):454-460.
36. Dali-Youcef N, Lagouge M, Froelich S, Koehl C, Schoonjans K, Auwerx J. Sirtuins: the 'magnificent seven', function, metabolism and longevity. Ann Med 2007;39(5):335-345.
37. Bolden JE, Peart MJ, Johnstone RW. Anticancer activities of histone deacetylase inhibitors. Nat Rev Drug Discov 2006 Sep;5(9):769-784.
38. Chun P. Histone deacetylase inhibitors in hematological malignancies and solid tumors. Arch Pharm Res 2015 Jun;38(6):933-949.
39. Chung YL, Lee MY, Wang AJ, Yao LF. A therapeutic strategy uses histone deacetylase inhibitors to modulate the expression of genes involved in the pathogenesis of rheumatoid arthritis. Mol Ther 2003 Nov;8(5):707-717.

40. Leoni F, Zaliani A, Bertolini G, Porro G, Pagani P, Pozzi P, et al. The antitumor histone deacetylase inhibitor suberoylanilide hydroxamic acid exhibits antiinflammatory properties via suppression of cytokines. Proc Natl Acad Sci U S A 2002 Mar 5;99(5):2995-3000.
41. Villagra A, Sotomayor EM, Seto E. Histone deacetylases and the immunological network: implications in cancer and inflammation. Oncogene 2010 Jan 14;29(2):157-173.
42. Grabiec AM, Tak PP, Reedquist KA. Function of histone deacetylase inhibitors in inflammation. Crit Rev Immunol 2011;31(3):233-263.
43. Dinarello CA, Fossati G, Mascagni P. Histone deacetylase inhibitors for treating a spectrum of diseases not related to cancer. Mol Med 2011 May-Jun;17(5-6):333-352.
44. Imre G, Gekeler V, Leja A, Beckers T, Boehm M. Histone deacetylase inhibitors suppress the inducibility of nuclear factor-kappaB by tumor necrosis factor-alpha receptor-1 down-regulation. Cancer Res 2006 May 15;66(10):5409-5418.
45. Vojinovic J, Damjanov N, D'Urzo C, Furlan A, Susic G, Pasic S, et al. Safety and efficacy of an oral histone deacetylase inhibitor in systemic-onset juvenile idiopathic arthritis. Arthritis Rheum 2011 May;63(5):1452-1458.
46. Ashburner BP, Westerheide SD, Baldwin AS,Jr. The p65 (RelA) subunit of NF-kappaB interacts with the histone deacetylase (HDAC) corepressors HDAC1 and HDAC2 to negatively regulate gene expression. Mol Cell Biol 2001 Oct;21(20):7065-7077.
47. Dai Y, Rahmani M, Dent P, Grant S. Blockade of histone deacetylase inhibitor-induced RelA/p65 acetylation and NF-kappaB activation potentiates apoptosis in leukemia cells through a process mediated by oxidative damage, XIAP downregulation, and c-Jun N-terminal kinase 1 activation. Mol Cell Biol 2005 Jul;25(13):5429-5444.
48. Kim YK, Lee EK, Kang JK, Kim JA, You JS, Park JH, et al. Activation of NF-kappaB by HDAC inhibitor apicidin through Sp1-dependent de novo protein synthesis: its implication for resistance to apoptosis. Cell Death Differ 2006 Dec;13(12):2033-2041.
49. Sato T, Kotake D, Hiratsuka M, Hirasawa N. Enhancement of inflammatory protein expression and nuclear factor Kappab (NF-Kappab) activity by trichostatin A (TSA) in OP9 preadipocytes. PLoS One 2013;8(3):e59702.
50. Rosato RR, Kolla SS, Hock SK, Almenara JA, Patel A, Amin S, et al. Histone deacetylase inhibitors activate NF-kappaB in human leukemia cells through an ATM/NEMO-related pathway. J Biol Chem 2010 Mar 26;285(13):10064-10077.
51. Khan N, Jeffers M, Kumar S, Hackett C, Boldog F, Khramtsov N, et al. Determination of the class and isoform selectivity of small-molecule histone deacetylase inhibitors. Biochem J 2008 Jan 15;409(2):581-589.
52. Bowers EM, Yan G, Mukherjee C, Orry A, Wang L, Holbert MA, et al. Virtual ligand screening of the p300/CBP histone acetyltransferase: identification of a selective small molecule inhibitor. Chem Biol 2010 May 28;17(5):471-482.
53. Yan G, Eller MS, Elm C, Larocca CA, Ryu B, Panova IP, et al. Selective inhibition of p300 HAT blocks cell cycle progression, induces cellular senescence, and inhibits the DNA damage response in melanoma cells. J Invest Dermatol 2013 Oct;133(10):2444-2452.
54. Ren Y, Su X, Kong L, Li M, Zhao X, Yu N, et al. Therapeutic effects of histone deacetylase inhibitors in a murine asthma model. Inflamm Res 2016 Dec;65(12):995-1008.
55. Barnes PJ. Immunology of asthma and chronic obstructive pulmonary disease. Nat Rev Immunol 2008 Mar;8(3):183-192.
56. Kim HY, DeKruyff RH, Umetsu DT. The many paths to asthma: phenotype shaped by innate and adaptive immunity. Nat Immunol 2010 Jul;11(7):577-584.
57. Barnes PJ. The cytokine network in asthma and chronic obstructive pulmonary disease. J Clin Invest 2008 Nov;118(11):3546-3556.
58. Caramori G, Casolari P, Barczyk A, Durham AL, Di Stefano A, Adcock I. COPD immunopathology. Semin Immunopathol 2016 Jul;38(4):497-515.

59. Barnes PJ. Inflammatory mechanisms in patients with chronic obstructive pulmonary disease. J Allergy Clin Immunol 2016 Jul;138(1):16-27.

60. Keenan CR, Salem S, Fietz ER, Gualano RC, Stewart AG. Glucocorticoid-resistant asthma and novel anti-inflammatory drugs. Drug Discov Today 2012 Sep;17(17-18):1031-1038.

61. Telenga ED, Kerstjens HA, Postma DS, Ten Hacken NH, van den Berge M. Inhaled corticosteroids in chronic obstructive pulmonary disease: a review. Expert Opin Pharmacother 2010 Feb;11(3):405-421.

62. Babu KS, Kastelik JA, Morjaria JB. Inhaled corticosteroids in chronic obstructive pulmonary disease: a pro-con perspective. Br J Clin Pharmacol 2014 Aug;78(2):282-300.

63. Rabe KF, Hurd S, Anzueto A, Barnes PJ, Buist SA, Calverley P, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. Am J Respir Crit Care Med 2007 Sep 15;176(6):532-555.

64. Barnes PJ. Alveolar macrophages as orchestrators of COPD. COPD 2004 Apr;1(1):59-70.

65. Caramori G, Oates T, Nicholson AG, Casolari P, Ito K, Barnes PJ, et al. Activation of NF-kappaB transcription factor in asthma death. Histopathology 2009 Mar;54(4):507-509.

66. Edwards MR, Bartlett NW, Clarke D, Birrell M, Belvisi M, Johnston SL. Targeting the NF-kappaB pathway in asthma and chronic obstructive pulmonary disease. Pharmacol Ther 2009 Jan;121(1):1-13.

67. Tan C, Xuan L, Cao S, Yu G, Hou Q, Wang H. Decreased Histone Deacetylase 2 (HDAC2) in Peripheral Blood Monocytes (PBMCs) of COPD Patients. PLoS One 2016 Jan 25;11(1):e0147380.

68. Caramori G, Romagnoli M, Casolari P, Bellettato C, Casoni G, Boschetto P, et al. Nuclear localisation of p65 in sputum macrophages but not in sputum neutrophils during COPD exacerbations. Thorax 2003 Apr;58(4):348-351.

69. Di Stefano A, Caramori G, Oates T, Capelli A, Lusuardi M, Gnemmi I, et al. Increased expression of nuclear factor-kappaB in bronchial biopsies from smokers and patients with COPD. Eur Respir J 2002 Sep;20(3):556-563.

70. Barnes PJ, Adcock IM, Ito K. Histone acetylation and deacetylation: importance in inflammatory lung diseases. Eur Respir J 2005 Mar;25(3):552-563.

71. Ito K, Caramori G, Lim S, Oates T, Chung KF, Barnes PJ, et al. Expression and activity of histone deacetylases in human asthmatic airways. Am J Respir Crit Care Med 2002 Aug 1;166(3):392-396.

72. Cosio BG, Mann B, Ito K, Jazrawi E, Barnes PJ, Chung KF, et al. Histone acetylase and deacetylase activity in alveolar macrophages and blood mononocytes in asthma. Am J Respir Crit Care Med 2004 Jul 15;170(2):141-147.

73. Su RC, Becker AB, Kozyrskyj AL, Hayglass KT. Altered epigenetic regulation and increasing severity of bronchial hyperresponsiveness in atopic asthmatic children. J Allergy Clin Immunol 2009 Nov;124(5):1116-1118.

74. Butler CA, McQuaid S, Taggart CC, Weldon S, Carter R, Skibinski G, et al. Glucocorticoid receptor beta and histone deacetylase 1 and 2 expression in the airways of severe asthma. Thorax 2012 May;67(5):392-398.

75. Gunawardhana LP, Gibson PG, Simpson JL, Powell H, Baines KJ. Activity and expression of histone acetylases and deacetylases in inflammatory phenotypes of asthma. Clin Exp Allergy 2014 Jan;44(1):47-57.

76. Stefanowicz D, Lee JY, Lee K, Shaheen F, Koo HK, Booth S, et al. Elevated H3K18 acetylation in airway epithelial cells of asthmatic subjects. Respir Res 2015 Aug 5;16:95-015-0254-y.

77. Rajendrasozhan S, Yang SR, Kinnula VL, Rahman I. SIRT1, an antiinflammatory and antiaging protein, is decreased in lungs of patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2008 Apr 15;177(8):861-870.

78. Ito K, Ito M, Elliott WM, Cosio B, Caramori G, Kon OM, et al. Decreased histone deacetylase activity in chronic obstructive pulmonary disease. N Engl J Med 2005 May 12;352(19):1967-1976.

79. Hodge G, Jersmann H, Tran HB, Roscioli E, Holmes M, Reynolds PN, et al. Lymphocyte senescence in COPD is associated with decreased histone deacetylase 2 expression by pro-inflammatory lymphocytes. Respir Res 2015 Oct 24;16:130-015-0287-2.

80. Barnes PJ. Glucocorticosteroids: current and future directions. Br J Pharmacol 2011 May;163(1):29-43.
81. Hew M, Bhavsar P, Torrego A, Meah S, Khorasani N, Barnes PJ, et al. Relative corticosteroid insensitivity of peripheral blood mononuclear cells in severe asthma. Am J Respir Crit Care Med 2006 Jul 15;174(2):134-141.
82. Kobayashi Y, Bossley C, Gupta A, Akashi K, Tsartsali L, Mercado N, et al. Passive smoking impairs histone deacetylase-2 in children with severe asthma. Chest 2014 Feb;145(2):305-312.
83. Zhang HP, Wang L, Fu JJ, Fan T, Wang ZL, Wang G. Association between histone hyperacetylation status in memory T lymphocytes and allergen-induced eosinophilic airway inflammation. Respirology 2016 Mar 17.
84. Carvalho AL, Annoni R, Torres LH, Durao AC, Shimada AL, Almeida FM, et al. Anacardic acids from cashew nuts ameliorate lung damage induced by exposure to diesel exhaust particles in mice. Evid Based Complement Alternat Med 2013;2013:549879.
85. Choi JH, Oh SW, Kang MS, Kwon HJ, Oh GT, Kim DY. Trichostatin A attenuates airway inflammation in mouse asthma model. Clin Exp Allergy 2005 Jan;35(1):89-96.
86. Royce SG, Dang W, Yuan G, Tran J, El-Osta A, Karagiannis TC, et al. Effects of the histone deacetylase inhibitor, trichostatin A, in a chronic allergic airways disease model in mice. Arch Immunol Ther Exp (Warsz) 2012 Aug;60(4):295-306.
87. Banerjee A, Trivedi CM, Damera G, Jiang M, Jester W, Hoshi T, et al. Trichostatin A abrogates airway constriction, but not inflammation, in murine and human asthma models. Am J Respir Cell Mol Biol 2012 Feb;46(2):132-138.
88. Toki S, Goleniewska K, Reiss S, Zhou W, Newcomb DC, Bloodworth MH, et al. The histone deacetylase inhibitor trichostatin A suppresses murine innate allergic inflammation by blocking group 2 innate lymphoid cell (ILC2) activation. Thorax 2016 Jul;71(7):633-645.
89. Hou X, Wan H, Ai X, Shi Y, Ni Y, Tang W, et al. Histone deacetylase inhibitor regulates the balance of Th17/Treg in allergic asthma. Clin Respir J 2016 May;10(3):371-379.
90. Zhang HP, Wang L, Fu JJ, Fan T, Wang ZL, Wang G. Association between histone hyperacetylation status in memory T lymphocytes and allergen-induced eosinophilic airway inflammation. Respirology 2016 Jul;21(5):850-857.