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New asthma drugs acting on gene expression

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Abstract

New asthma drugs acting on transcription are transcription factor agonists (dissociated steroids, peroxisome proliferator-activated receptor gamma agonists), transcription factor inhibitors (NF-κB / AP-1 inhibitors, STAT6 inhibitors), inhibitors of protein kinases acting on transcription factors (p38 MAP kinase inhibitors), and chromatin modifying agents. Pharmacological approach of translation in asthma includes therapeutic ribozymes and antisense oligonucleotides targeting receptors (adenosine A1 receptor, alpha chain of IL-5 receptor, common beta chain of IL-3/IL-5/GM-CSF receptor), cytokines (IL-4, IL-5, SCF), signal transduction molecules (Syk, Lyn), transcription factors (STAT-6, GATA-3). Some of these drugs acting on gene expression have the potential to improve therapeutic benefits compared with traditional drugs.

Keywords: gene expression - transcription - transcription factors - translation

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Introduction

A goal in modern molecular pharmacology is to gain control over the gene expression, using new molecules. Asthma is an airway inflammatory disease characterized at molecular level by chronically increased expression of multiple inflammatory proteins, including cytokines, chemokines, adhesion molecules, enzymes and receptors. Proteomics research is very important in evaluating molecular targets of new drugs and gene expression analysis provides valuable information about the effects of potential drugs for asthma.

The targets for therapeutic intervention of new asthma drugs acting on gene expression are transcription, post-transcriptional events, translation and post-translational events.

Pharmacological approach of transcription

Pharmacological approach of transcription in asthma includes transcription factor agonists and inhibitors, inhibitors of protein kinases acting on transcription factors that regulate genes involved in inflammation and chromatin modifying agents [1].

Transcription factor agonists and inhibitors

Transcription factor agonists and inhibitors are important tools in asthma treatment, because of their mechanism of action and molecular targets.

Transcription factors are proteins that bind to DNA-regulatory sequences of target genes to modify the rate of gene transcription.

Important transcription factors that modulate airway inflammation in asthma belong to the NF-*k*B and NF-AT families (class: Rel homology region, superclass: Beta-scaffold factors with minor groove contacts), and AP-1 family and CREB (class: leucine zipper factors, superclass: basic domains).

Two important transcription factors that modulate Th_2 differentiation are STAT6 (class: Signal transducer and activator of transcription, superclass: Beta-scaffold factors with minor groove contacts), and GATA3 (class: diverse Cys4 zinc fingers, superclass: zinc-coordinating DNA-binding domains).

Cys4 zinc finger of nuclear receptor type transcription factors, glucocorticoid receptor (GR) and peroxisome proliferator activated receptor (PPAR), are involved in modulation of drug responses. These two factors belong also to the superclass of zinc-coordinating DNA-binding domains, GR to the family of steroid hormone receptors and PPAR to the family of thyroid hormone receptor-like factors.

Cys4 zinc finger transcription factors can insert into a helix into the major groove of DNA, each having two fingers and binding to DNA as dimers.

Cys4 zinc finger of nuclear receptor type transcription factor agonists

Cys4 zinc finger of nuclear receptor type transcription factor agonists are of great importance in asthma treatment, because corticosteroids (classic and dissociated steroids) are in fact GR ligands, and PPAR gamma agonists may be a novel approach for asthma treatment.

Corticosteroids (CS) have a privileged position in the pharmacological approach of transcription. Inhaled CS are the most effective therapy in the long-term control of asthma and they produce their effects on responsive cells by activating the glucocorticoid receptor (GR) to directly or indirectly regulate the transcription of the target genes. GR is a Cys4 zinc finger transcription factor (Fig. 1), widely distributed within human lung. After passive penetration of the cell membrane, the corticosteroid binds to a hormone binding domain at the C-terminal end of the cytoplasmatic wild-type isoform alpha GR molecule. The GR activation necessitates dissociation of multi-protein chaperone heterocomplex, containing two molecules of heat shock protein of 90 kDa (hsp90). The hsp90 chaperone cycle involves also immunophilin p59, p23 and other hsp90-partner proteins (hsp40, hsp70, HiP, HOP).

Molecular mechanisms by which CS modify gene expression include mainly transactivation (positive regulation of transcription) and transrepression (negative regulation of transcription) [1,2].

Transactivation (TA) is mediated by binding of the hormone-activated GR to a DNA sequence called glucocorticoid response element (GRE), after nuclear translocation of the conformational changed GR *via* a nuclear pore complex by virtue

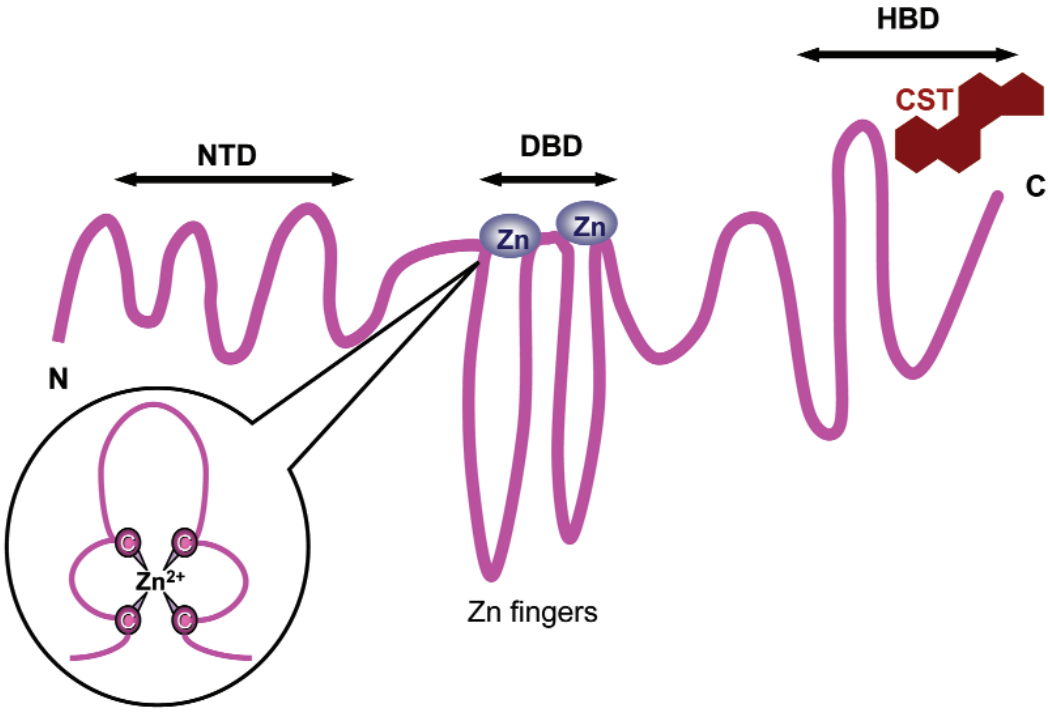

Fig. 1 Functional domains of the human glucocorticoid receptor (GR). The three major protein domains are the N-terminal domain (NTD), the DNA binding domain (DBD), and the hormone binding domain (HBD). The cytoplasmatic GR is a Cys4 zinc finger of nuclear receptor type transcription factor and the corticosteroid (CST) molecule binds its HBD [1,2].

of the nuclear localization sequence within the DNA-binding domain.

Activated GR binds classical GRE sites as a homodimer. TA may result in some pharmacological benefits (upregulation of beta$_2$-adrenoreceptor, lipocortin I and secretory leukocyte protease inhibitor genes) and many CS adverse effects (diabetes, arterial hypertension, edema, hypokalemia, glaucoma).

DNA binding dependent transrepression (TR) with binding of the GR to a negative GRE is involved in some adverse effects, because of inhibition of the expression of osteocalcin and adrenocorticotropin precursor (pro-opiomelanocortin) genes. That is the reason why TR mechanisms rather than TA may underlie CS-induced osteoporosis and inhibition of the hypothalamic-pituitary-adrenal axis.

Multiple transrepressive (TR) mechanisms without DNA binding, acting in concert, are involved in the inhibition of expression of various proinflammatory proteins (cytokines, chemokines, adhesion molecules, proinflammatory receptors and enzymes). These include the I*k*Balpha upregulatory model, the protein-protein interaction model and the cofactor competition model.

Protein-protein interaction model of TR without DNA-binding with mutual masking of TA domains between GR as monomer and other transcription factors, such as AP-1, NF-*k*B, STAT5 etc, appears to be important because negative GRE are not generally found in the promoters of inflammatory genes (Fig. 2A).

Cofactor competition model of TR without DNA-binding (Fig. 2B) is of special interest because various transcription factors (GR, NF-*k*B, AP-1, STATs) converge to CREB-binding protein (CBP) / p300, with limited availability. GR dimers bind to steroid-receptor co-activators (SRCs) constitutively bound to CBP, which regulates transcription *via* its association with RNA polymerase II and through its intrinsic histone acetyltransferase (HAT) activity.

GR binding to SRC results in histone deacetylation, tightening up DNA coiling (condensed inactive chromatin), restricting access of transcription factors to promoter regions of inflammatory genes.

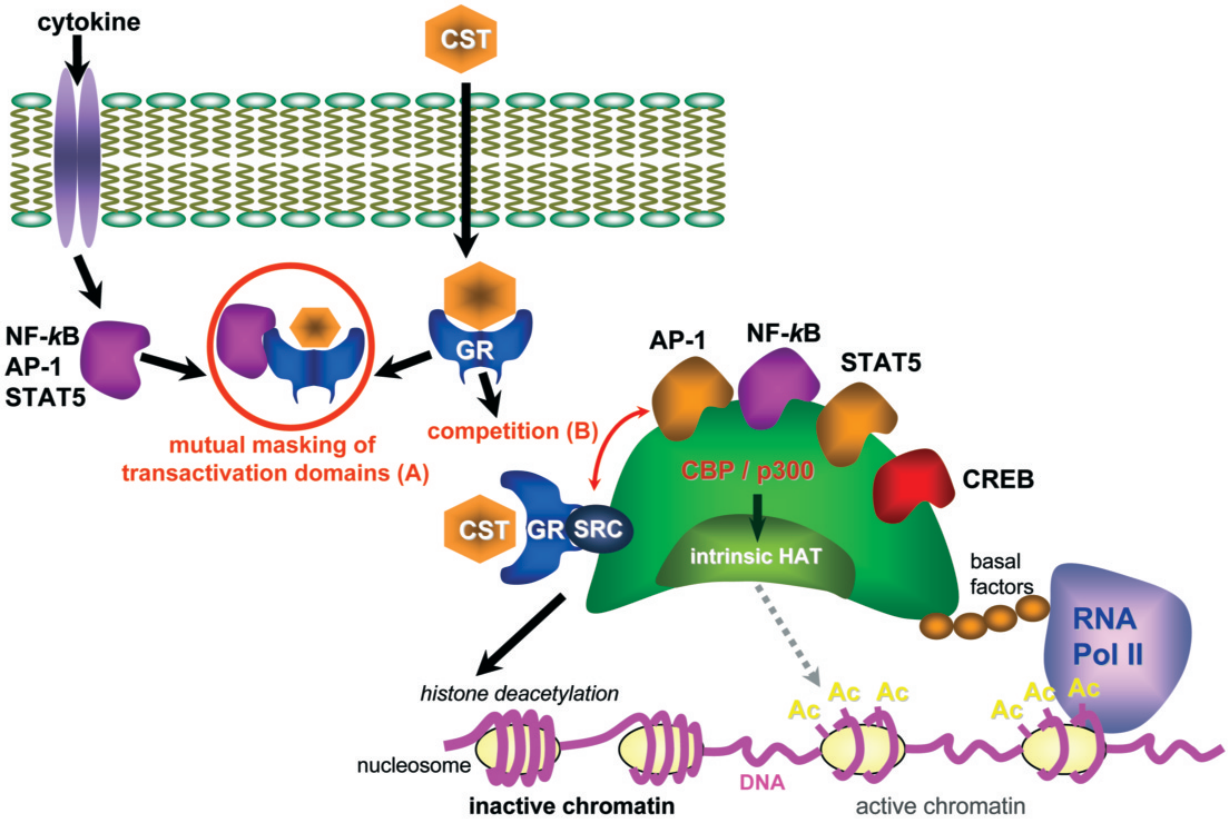

Fig. 2 Protein-protein interaction and cofactor competition models of corticosteroid (CST) transrepression without DNA-binding important in inhibition of expression of various proinflammatory proteins. Mutual masking of transactivation domains between glucocorticoid receptor (GR) monomers and other transcription factors represents the protein-protein interaction model (A). Cofactor competition model (B) is also critical because various transcription factors converge to CREB-binding protein (CBP)/p300. GR dimers bind to steroid-receptor co-activators (SRCs) and regulates transcription via CBP/p300 intrinsic histone acetyltransferase (HAT) activity. Histone deacetylation induces restricted acces of transcription factors (TF) to promoter regions of inflammatory genes [1,2,16].

Classic corticosteroids are "symmetrical" compounds that induce strong TA and TR. The rank order of TR potencies of currently available inhaled CS, in A549 lung cells transiently transfected with an AP-1 or NF-*k*B-dependent luciferase gene, is fluticasone propionate > budesonide > beclomethasone dipropionate, triamcinolone acetonide, and flunisolide. In this context, fluticasone propionate seems to partially dissociate TR from TA. It is interesting to mention that long-acting beta$_2$-agonists may have cellular mechanisms of interaction with inhaled corticosteroids, not only by promoting nuclear translocation of the GR in some cells, but also possibly through GR priming *via* a mitogen activated protein kinase stimulation mechanism, through decreasing I*k*Balpha degradation and inhibition of NF-*k*B mediated gene transcription *via* elevated cAMP levels or through induction of a cAMP mediated transcriptional repressor (ICER) [3].

Dissociated steroids are a novel class of transactivating defective synthetic steroids. Screening more than 200 synthetic corticoids, some molecules were found with dissociated characteristics: RU 24782, RU 24858, RU 40066. Transrepressive defective steroids (ZK 57740, ZK 77945) have no anti-inflammatory effects.

Preliminary data suggest that RU 24858, a prototypical dissociated steroid, exerts strong TR on AP-1, but little or no TA, and exhibits comparable anti-inflammatory activity to budesonide (Sephadex-induced lung edema model in rats).

However, the effects observed on bone metabolism (femur model of CS-induced osteopenia, femoral head histology and serum bone markers) suggest that *in vitro* separation of TR from TA activity does not necessarily translate to an increased therapeutic ratio *in vivo* and/or that some adverse effects are a consequence of TR [4]. More studies are required to establish whether dissociated steroids can be used *in vivo* to produce anti-inflammatory effects with reduced side effects.

Non-glucocorticoid steroids are novel anti-asthma drugs recently evaluated. EPI-12323 (Naturasone) is an inhaled small non-glucocorticoid steroid molecule, with positive preclinical data, and recently entered Phase I clinical trials. It has a long duration anti-inflammatory activity (inhibits Th_2 type inflammatory reaction, reduces the adenosine hypersecretion in the asthmatic lung and have pronounced effect on neutrophils) and a known safety profile (causing no bone demineralization).

Peroxisome proliferator-activated receptor gamma ligands have potential therapeutic role in asthma. PPAR has three isoforms designated PPAR alpha, PPAR beta/delta and PPAR gamma. The last isoform of ligand-activated nuclear receptors, belonging to the thyroid hormone receptor-like transcription factors, is positioned at the crossroads between lipid metabolism and inflammation. The mechanisms of gene expression regulation by PPAR are *via* DNA binding (PPAR/RXR heterodimeric complex binds to a peroxisome proliferator-response element, PPRE), co-activator recruitment and negative interfering with the NF-*k*B, STAT and AP-1 signaling pathways, and even PPAR-gamma independent mechanisms. The consequences are inhibition of proinflammatory genes expression (IL-2, IL-6, IL-8, TNFalpha, GM-CSF), inhibition of cell proliferation and induction of apoptosis [5].

Natural PPAR gamma agonists are cyclopentenone prostaglandins, such as 15-deoxy-delta^{12,14}-PGJ_2 (15d-PGJ_2), obtained *via* albumin-catalyzed nonenzymatic hydrolysis of PGD_2 released from stimulated mast cells. They are "clavulone"-type marine eicosanoids from the marine polyp *Clavularia viridis*. Synthetic PPAR gamma agonists are the thiazolidinediones (glitazones), such as ciglitazone and troglitazone (older representatives with hepatic toxicity), rosiglitazone and pioglitazone (approved as oral antidiabetic drugs).

PPAR gamma agonists, 15d-PGJ_2 and ciglitazone, inhibit the cytokine-induced iNOS expression in airway epithelial cells in a dose-dependent manner. The thiazolidinedione dramatically inhibits the cytokine-induced secretion of IL-8 in IL-4-pretreated human epithelial A549 cells. PPAR gamma ligands and LXA_4, an endogenous anti-inflammatory eicosanoid, decrease transcription of IL-8 gene by interfering with NF-*k*B pathway, inhibiting chemotaxis and activation of neutrophils and eosinophils [6].

Regarding the effect of PPAR agonists on gene expression in airway smooth muscle cells, actively involved in the inflammatory process and remodeling in asthma, the synthetic PPAR-gamma ligand, ciglitazone, inhibits the IL-1 beta-induced release of GM-CSF and G-CSF from cultured human airway smooth muscle (HASM) cells, while WY-14643 (a synthetic PPAR-alpha ligand) has no significant effect on the release of colony-stimulating factors [7].

PPAR gamma ligands, 15d-PGJ_2 and rosiglitazone, have similar antimitogenic effects, but different effects on cytokine release in HASM.

Rosiglitazone inhibits IL-1 induced GM-CSF release and IL-8 release (measured in supernatants of cultured HASM cells from lung resection specimens, preincubated with the retinoid X receptor ligand, 9-*cis* retinoic acid), while the PGD_2 metabolite induce increasing effects. The bFGF (basic fibroblast growth factor)-induced HASM cell proliferation is reduced by rosiglitazone. The elevation in cyclin D1 levels with bFGF is inhibited by 15d-PGJ_2, but not by rosiglitazone. The mitogen-independent antiproliferative effects of PPAR gamma agonists differ from corticosteroids, which prevent passage through the restriction point in G1 phase of the cell cycle [8].

Considered together, these data support the idea that agonists of the transcription factor PPAR gamma represent a potential anti-inflammatory and antiproliferative treatment in asthma, with additional effects to those of corticosteroids. PPAR gamma ligands inhibit the cytokine-induced expression of important inflammatory mediators in airway epithelial cells, inhibit the release of colony-stimulating factors from airway smooth muscle cells, limiting the recruitment and activation of infiltrating leukocytes, and decrease the proliferation in human airway smooth muscle, indicating their potential to

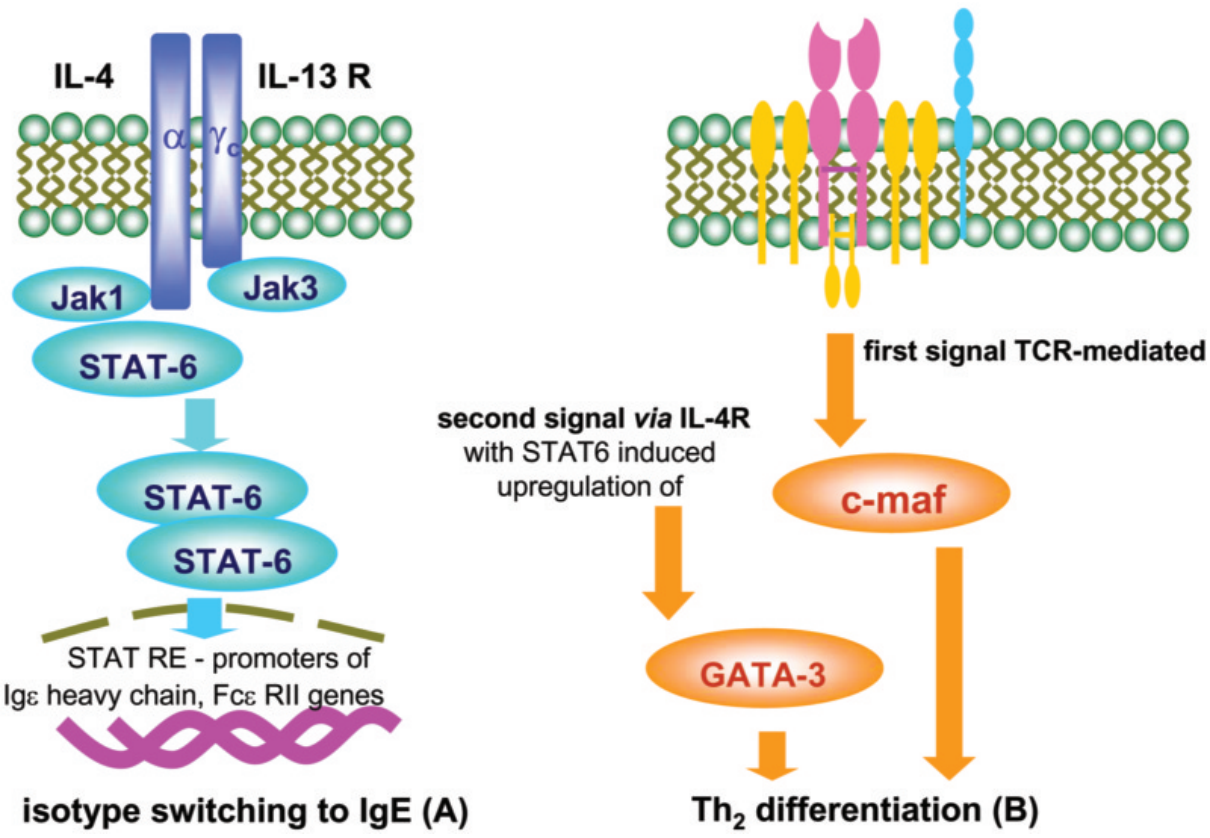

Fig. 3 Important transcription factors that modulate class switching to IgE (A) and Th2 cell differentiation (B). The signal transducer and activator of transcription 6 (STAT6) is an obligatory transcription factor in IL-4 and IL-13 signaling. Receptor ligation leads to activation of kinases Jak-1 and Jak-3. Activated STAT6 dimer is translocated to the nucleus where it binds specific STAT response elements in the promotor of IL-4/IL-13-responsive genes. Transcriptional activation of the germline epsilon promoter is required for class switching to the IgE isotype (A). Th2 differentiation requires two signals, the first is TCR-mediated and induces c-maf, the second one is via IL-4 receptor with activation of STAT6 and upregulation of GATA-3. GATA-3 and c-maf are Th2 specific transcription factors critical for Th2 differentiation [14,30].

treat the hyperplastic response in asthma. Fibroblast, macrophage, endothelial and T-cell apoptotic death in response to thiazolidinediones has also been documented.

Inhibitors of transcription factors

Inhibitors of transcription factors involved in airway inflammation or Th_2 differentiation and isotype switching to IgE (Fig. 3) are a new class of potential asthma drugs acting on transcription.

Nuclear factor-kB (NF-*k*B) inhibition represents a key strategy in asthma treatment. The activity of NF-*k*B is regulated by interaction with inhibitory I*k*B proteins. Almost all of the signals that lead to activation of NF-*k*B converge on a high molecular weight signalsome complex that contains two serine/threonine kinases (IKK-alpha and IKK-beta) and a regulatory/docking protein named NEMO (NF-*k*B essential modulator) or IKK-gamma.

Cell-permeable peptide inhibitors of NF-kB, small peptides that mimic NBD (NEMO-binding domain) that can specifically block the activation of NF-*k*B, administered intranasal in mouse models are likely to represent a novel strategy for the treatment of asthma, because of potent anti-inflammatory effects [9].

Synthetic NF-*k*B inhibitors SP650003 and SP100030 are capable of significantly attenuating NF-*k*B/AP-1-dependent gene transcription. The NF-*k*B / AP-1 inhibitor SP100030, studied in sensi-

tized Brown-Norway rats, intraperitoneally in daily dose of 20 mg/kg, for three days prior to allergen challenge, inhibits mRNA expression of both Th_1 and Th_2 cytokines, but not allergen-induced airway eosinophilia and bronchial hyperresponsiveness [10].

A triazolo-pyridazinyl-2-butynoate derivate, MOL 294, is a small molecule inhibitor of NF-*k*B and AP-1 based upon a beta-strand template that binds to and inhibits the cellular redox protein thioredoxin. This potent nonpeptide inhibitor of redox-regulated NF-*k*B and AP-1 transcription, administered intranasal in a mouse model of asthma, reduces IL-13 and eotaxin levels in bronchoalveolar lavage fluid, decreases airway eosinophilia and hiperresponsiveness to methacholine [11].

A small molecule NF-*k*B inhibitor, IPL 576092, based on molecule originally isolated from a sea sponge, was studied in phase II asthma trials.

Furthermore, naturally occurring NF-*k*B inhibitors have also been identified, such as the *Aspergillus* derivative, gliotoxin [12]. Inhibition of NF-*k*B activation by gliotoxin may un-mask the ability of TNF-alpha to induce eosinophil apoptosis [13].

The signal transducer and activator of transcription 6 (STAT6) is an obligatory transcription factor in IL-4 and IL-13 signaling, it is essential for IgE switch recombination and contributes to the Th_2 cell differentiation, therefore it is a key transcription factor in the pathophysiology of allergic asthma. STAT6 inhibitors have demonstrated evidence of efficacy in *in vitro* models involving expression of IL-4 induced genes associated with allergic hypersensitivity and asthma. STAT-induced STAT inhibitor-1 (SSI-1) / suppressor of cytokine signaling 1 (SOCS1) / JAB (JAK-binding protein) is a SH2-domain-containing protein that can be an useful tool to shut down the atopic response. A peptide STAT6 inhibitor (Stat6BP), derived from the STAT6-binding region of IL-4 receptor alpha, inhibits IL-4 dependent phosphorylation of STAT6, in different human and murine cell lines [14]. Heterocyclic inhibitors of STAT6 are represented by several isoxazoles, thyazoles, thyadiazoles, that inhibit STAT6 activation in micromolar concentrations. A thyadiazole STAT6 activation inhibitor has efficacy *in vitro* involving expression of IL-4 induced genes, and it is a potential oral drug for asthma treatment at a very early step in the disease process.

Inhibitors of protein kinases acting on transcription factors

Inhibitors of protein kinases acting on transcription factors that regulate genes involved in inflammation were recently studied:

- selective p38 mitogen-activated protein (MAP) kinase inhibitors (SB 239063), because p38 MAP kinase is involved in regulation of various transcription factors: ELK-1, STAT1, ATF2, CREB (*via* MSK1);
- selective extracellular signal regulated kinase 1 (ERK1) inhibitors (PD98059), because ERK is involved in regulation of SRF (serum response factor) accessory factor (ELK1) and MSK1;
- potent mitogen and stress activated kinase 1 (MSK1) inhibitors (H89), because MSK regulates CREB and nucleosomal proteins: histone H3 and HMG-14 (high mobility group proteins) [15,16].

Transcription factors activation in chronic airway inflammation is complex and involves intracellular signal transduction pathways, including protein kinases.

The role of **p38 MAP kinase inhibitors** in asthma treatment has recently been established, because it was shown that activation of the p38 kinase pathway by various stimuli results in the translationally regulated production of many inflammatory mediators.

First generation p38 MAP kinase inhibitors (SB 202190, SB 203580) have partially selectivity on p38 MAPK (acting also on c-Raf), but those of second generation (SB 239063) with comparable potency, have improved selectivity.

SB 239063, with potent inhibitory activity on p38 MAP kinase (alpha / beta isoforms), without direct inhibition of other kinases involved in inflammation (c-Raf, JNK1, ERK1), inhibits *in vitro* the proinflammatory cytokine production (IL-1 and TNF-alpha production of human monocytes, eotaxin-induced IL-8, GM-CSF production of epithelial cells) [17]. SB 239063, orally dose of 10 and 30 mg/kg 1 hour before and 4 hours post-antigen inhalation, inhibits the chemotaxis of

eosinophils into the airways. The demonstration of a dose related increase in apoptosis of cultured airway eosinophils isolated from guinea pig lung, in the presence of IL-5 provides an additional beneficent mechanism of action [15].

In addition, p38 MAP kinase inhibitors may have a potential in reversing glucocorticoid receptor insensitivity and reestablishing the beneficial effects of glucocorticoids in patients with severe asthma [18].

Chromatin modifying agents

Chromatin modifying agents may be used in the future for gene control in asthma. Chromatin is the protein-DNA complex, and histones are the major protein component. Core histone tail modification is of major importance in transcriptional control. Histone acetylation by histone acetyltransferases (HATs), such as p300 / CBP (CREB binding protein) and PCAF (p300/CBP-associated factor) is associated with transcriptional activation, whereas histone deacetylation induced by histone deacetylases 1-8 (HDACs) is associated with transcriptional repression.

Chromatin modification has a critical role in inflammatory gene transcription in asthma. Subjects with asthma had reduced HDAC enzymatic activity, reduced HDAC1 and HDAC2 protein expression, and increased HAT activity in bronchial biopsies. Regarding HDAC / HAT modulators, steroids recruit HDACs to the site of inflammatory gene transcription, increase HDAC activity and suppress HAT activity, greater than they induce effects on HDAC. Combination of steroids with theophylline (direct activator of HDAC activity) induces IL-1β-beta-stimulated histone H4 acetylation at GM-CSF promoter. HDAC inhibitors (trichostatin A) can potentiate some glucocorticoid receptor actions.

Modulation of HAT and HDAC activity may be important new targets for drug development. Specific HAT inhibitors are able to modulate NF-*k*B - mediated inflammatory gene expression. HDAC inhibitors need to be used with caution, because they alter the transcription of many genes and exert pan-cellular effects [19].

Pharmacological approach of translation

Pharmacological approach of translation is a great new wave in molecular pharmacology. Antisense therapy downregulating gene expression with therapeutic potential in asthma includes ribozymes and antisense oligonucleotides.

Ribozymes

Ribozymes are synthetically engineered catalytic RNA that act as “molecular scissors”, cleaving mRNA. Several classes of therapeutic ribozymes have been described, the most important are hairpin ribozymes and hammerhead ribozymes. The mechanism of action involves binding to specific mRNA through Watson Crick base pair hybridization, cleaving mRNA in a highly sequence-specific manner (well defined target of approximately 15 nucleotides), thus preventing translation of the protein causing the disease. Modified hammerhead ribozymes, the most advanced in therapeutic applications, present a catalytic core that cleaves a site immediately 3’ to a GUX sequence (X = A, C, U), two helices that bind the target mRNA and provide quick dissociation of the cleaved products (potential of turnover). There are several chemical modification for stabilization (2’-O-methyl nucleotides) and for nuclease resistance (3-4 phosphorothioate modifications at the 5’ end).

Ribozymes are a potential powerful treatment tool in asthma, hammerhead and hairpin ribozymes targeting conserved sequences within IL-5, ICAM-1 and NF-*k*B mRNA have been already designed. Pharmacokinetics in mice revealed that intratracheal administration increase exposure in lungs [20].

Ribozymes in clinical trials for other disease than asthma are to date well tolerated. There are studies with Angiozyme targeting *Flt*-1 VEGFR (Vascular Endothelial Growth Factor Receptor) mRNA for breast and colon cancer; Heptazyme which targets HCV RNA; Herzyme targeting HER-2 (human epidermal growth factor-2) mRNA for breast and ovarian cancers; and anti-HIV ribozyme which targets the Tat and Tat/rev regions of HIV viral genome.

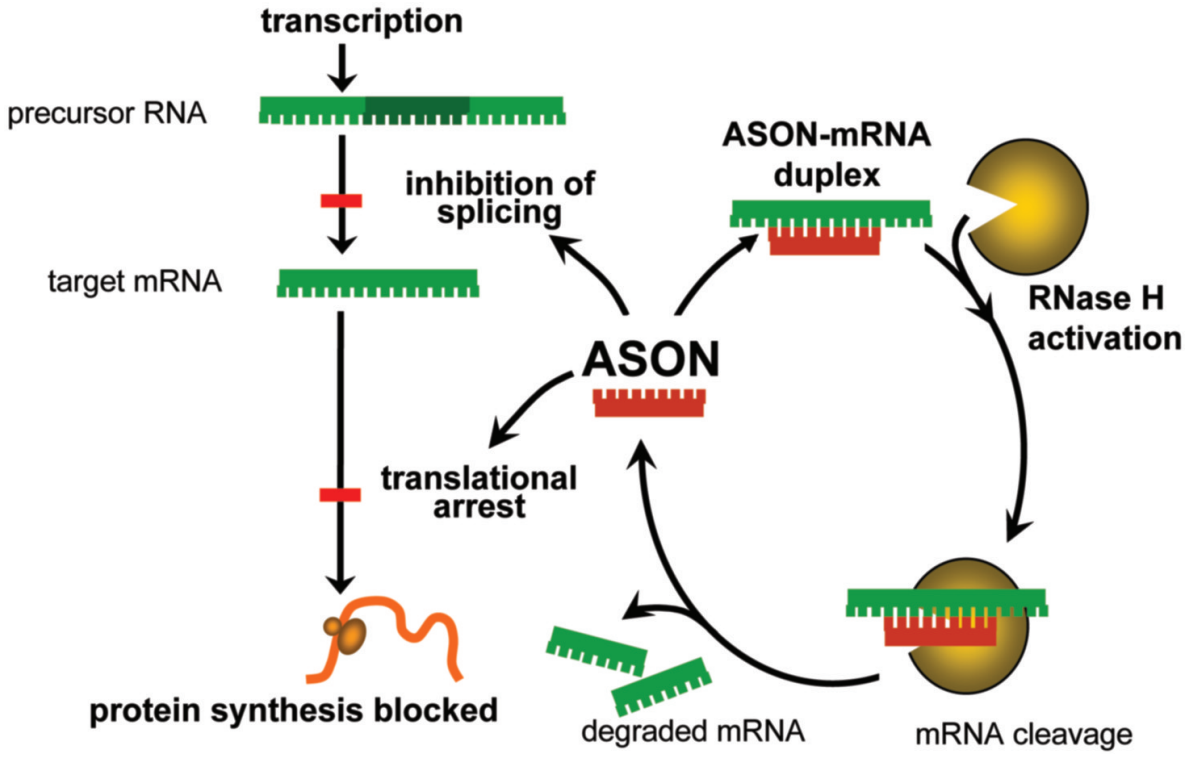

Fig. 4 Antisense oligonucleotide (ASON) mechanisms of action. Duplex ASON-mRNA forming, with subsequent RNase H activation, inhibition of splicing and translational arrest are ASON complex mechanisms of action by which the synthesis of the protein encoded is blocked [21].

Antisense oligonucleotides

Antisense oligonucleotides (ASONs) are short, single-stranded synthetic nucleic acid polymers (usually 18-25 nucleotides) interacting *via* Watson-Crick base pairing with target mRNA. Advantages are represented by high specificity (complementary binding to very specific sequence of the target mRNA), high avidity for targets (many hydrogen bondings between ON-mRNA) and optimal resistance to nucleases (phosphorothioates ONs have an oxygen atom in the internucleotide bridge substituted with sulphur).

ASON mechanism of action involves forming a duplex with mRNA, inhibition of splicing and translational arrest, disrupting ribosome assembly. ASON-mRNA duplex formation induces RNase H activation, which degrades mRNA. Thus, the synthesis of the protein that it encodes is blocked. ASONs prevent the production of a disease causing protein, while traditional drugs do not act until the protein has already been produced (Fig. 4).

The targets for ASONs are receptors (adenosine A1 receptor, alpha chain of IL-5 receptor, common beta chain of IL-3/IL-5/GM-CSF receptor), cytokines (IL-4, IL-5, SCF), signal transduction molecules (Syk, Lyn), transcription factors (STAT-6, GATA-3), etc.

Respirable antisense oligonucleotides (RASONs) are a new class of respiratory drugs delivered by inhalation directly to the lungs, which are an exceptional target for ASON delivery because their extremely large surface area. Surfactant, containing zwitterionic lipids with cationic properties, increase the uptake and distribution, reducing the effective doses and minimizing the risk of non-antisense systemic side effects, such as hypotension with phosphorothioate backbones. Nuclease sensitivity is titrated to be sufficiently nuclease resistant to be effective, but not so nuclease resistant to enter in the circulation undegraded [21].

Adenosine A_1 receptor RASON (Durason, EPI-2010) is a 21-mer phosphorothioate RASON (21

nucleotides) targeting a region of the A_1 receptor mRNA overlapping the initiation codon. There are no specific sequence motifs (GGGG) associated with sequence-dependent non-antisense effects (gene activation). EPI-2010 was extensively studied because adenosine is an important mediator of asthma, the adenosine A_1 receptor (a G-protein-coupled-receptor) is involved in inflammation, bronchoconstriction, and surfactant depletion observed in asthma and A1 receptor levels are upregulated both in animal models of asthma and asthmatic patients. Nebulization of EPI-2010 in house-dust mite mix-sensitized, New Zealand White rabbits reduces adenosine A_1 receptor (and not adenosine A_2 or bradykinin B_2 receptor) number in bronchial smooth muscle and significantly improves allergen-induced airway obstruction and bronchial hyperresponsiveness [22]. In phase I clinical trials EPI-2010 was effective at single inhaled doses on the order of 50 mcg/kg and the effect duration (the ability to block hyperresponsiveness to inhaled adenosine) was 6.8 days (range 4-11 days), giving it the potential to be the first once-per-week treatment for asthma. Durason decreases bronchodilator p.r.n. use and reduces the symptom scores. EPI-2010 is targeted for moderate to severe asthma and is applicable as a controller medication intended for chronic treatment. It is currently in early Phase II clinical trials.

Systemic IL-5 receptor alpha ASON (10-20 mg/kg/day i.v.) reduces eosinophilia in an ragweed-sensitized peritonitis murine model, because IL-5 is a critical cytokine that regulates maturation, chemotaxis, activation and survival of eosinophils [23]. IL-4 and IL-5 ASONs, in an *ex vivo* model, decreased IL-4 and IL-5 expression consequent to the transfer of antisense-treated T cells, but adoptively transferred late allergic response is inhibited by IL-4, but not IL-5, ASON [24].

Intratracheal administration (200 μg) of the common beta chain of IL-3/IL-5/GM-CSF receptor ASON (AS143), reduces lung beta chain mRNA and protein expression, allergen-induced lung eosinophilia and airway hyperresponsiveness to LTD_4, in a Brown Norway rat model of asthma [25].

Intranasal stem cell factor (SCF) ASON in murine model of asthma, in a high dose (a total of 1.11 mg administered over three consecutive days, equivalent to 56 mg/kg), results in uptake in interstitial lung cells, reduction in intracellular SCF expression and eosinophilic infiltration, SCF being an important activating and chemotactic factor not only for mast cells, but also for eosinophils [26].

Nebulized Syk ASON (60-mers) complexed with liposomes, in rats sensitized with either *Nippostrongylus brasiliensis* or ovalbumin, reduces Syk mRNA expression in alveolar macrophages and antigen-induced pulmonary inflammation, because Syk protein kinase mediates the inflammatory response after cross-linking of FcεR / FcγR in many cell types as an early event in the inflammatory process [27].

Lyn ASON blocks the expression of Lyn in eosinophils [28]. This may be an interesting therapeutic approach, Lyn tyrosine kinase being critical for antiapoptotic effect of IL-5 in eosinophils.

STAT-6 ASON administration using cationic lipid-mediated transfection in human lung epithelial A549 cells induces a dose-dependent STAT6 mRNA and protein down-regulation and inhibition of germline C*epsilon* transcription required for IgE isotype switching in B cells [29].

Intranasal GATA-3 ASON (18-mer, fully phosphorothioated, lacking CpG dinucleotides, targeting the 5′ end of the GATA-3 translation initiation site) in a mice model of asthma, 200 μg per day for four consecutive days (equivalent to 40 mg/kg), significant reduces Th_2 cytokine production, eosinophilic infiltration and airway hyperresponsiveness to methacholine, because GATA3 is a Cys4 zinc finger "master switch" transcription factor of Th_2 cell differentiation. It is an advantage that GATA-3 ASON reduces the expression of various Th_2 cytokines simultaneously, rather than suppressing a single cytokine [30].

Finally, it is important to mention the RASONs already used in preclinical trials for asthma, such as EPI-4067, a multi-target RASON, and EPI-30051, a RASON-targets transcription factor, both with anti-inflammatory and Th_2 lineage inhibitor effects.

Conclusions

There are several potential advantages of new molecular pharmacological approach of modulating transcription and translation over traditional respiratory drugs: attenuation of the disease process obtained at a point more proximal to the cause, the

abnormal expressed genes, and a more rational drug design.

Although not every gene relevant to asthma is likely to represent a pharmacological target for modulation of expression, some new drugs acting on transcription or translation have the potential to improve therapeutic benefits [31]. A major revolution in pharmacology has begun, science is clarifying the roles of gene expressions involved in asthma, but only thinking about the big picture will result in realistic ideas for new drugs.

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