

Asthma and the CCAAT-enhancer binding proteins: A holistic view on airway inflammation and remodeling

Peter Borger, PhD,^{a,b} Judith L. Black, MD, PhD,^{a,b} and Michael Roth, PhD^{a,b} Sydney, Australia

Asthma is an airway disease with increasing prevalence characterized by intermittent reversible airway obstruction, airway inflammation, and airway wall remodeling. The disease is generally triggered by inhalation of allergens, but nonallergic asthma triggers are quite common. The pathogenesis of asthma is well documented, and a great deal of research has been carried out to elucidate the underlying mechanisms. A multitude of articles have focused on cells alleged to be involved in the pathogenesis, including circulating cells from the immunologic compartment (ie, eosinophils and T lymphocytes) and resident cells, such as fibroblasts, airway smooth muscle cells, and, more recently, the airway epithelium. Despite the enormous amount of research, it is still unclear what exactly causes asthma. A general feature of most studies is an enhanced activation status of asthmatic cells, suggesting a general defect with respect to regulation of cellular responses. Here we discuss the ubiquitous transcription factor family of CCAAT-enhancer binding proteins (C/EBPs) and its involvement in inflammation and proliferation. We propose that an imbalance of C/EBP isoform expression might lead to an enhanced activity of asthmatic cells and provide an overall hypothesis that both airway inflammation and remodeling can be conceived as the result of an imbalance of C/EBP isoform expression. (*J Allergy Clin Immunol* 2002;110:841-6.)

Key words: Asthma, inflammation, remodeling, CCAAT-enhancer binding proteins

Asthma is an airway disease with increasing prevalence characterized by intermittent reversible airway obstruction, airway inflammation, and airway wall remodeling. The disease is generally triggered by inhalation of allergens, but nonallergic asthma triggers are quite common. Many cell types are alleged to be involved in the pathogenesis of asthma, including circulating cells from the immunologic compartment (ie, eosinophils and T lymphocytes) and resident cells, such as fibroblasts, airway smooth muscle cells, and, more recently, the airway epithelium. A general finding of most studies is an enhanced activation status of asthmatic cells, suggesting a general defect with respect to regu-

Abbreviations used

AMP: Adenosine monophosphate
cdk: Cyclin-dependent kinase
C/EBP: CCAAT-enhancer binding protein
CREB: Cyclic AMP response element binding protein
GR: Glucocorticoid receptor
HASM: Human airway smooth muscle
NF: Nuclear factor
PKA: Protein kinase A
TGF: Transforming growth factor

lation of cellular responses. In the present review we discuss the ubiquitous transcription factor family of CCAAT-enhancer binding proteins (C/EBPs) because they have recently been shown to be crucial in the regulation of both inflammation and proliferation. We propose that an imbalance of C/EBP-isoform expression might underlie an enhanced activity of asthmatic cells.

A COMPLEX DISORDER INVOLVING MANY CELL TYPES

The inflammation of the lungs in asthma has been extensively studied, and the predominant cells infiltrating the airways are eosinophils.¹ Release of eosinophil-derived cytotoxic proteins, such as major basic protein and cationic proteins, in the lung is a major pathogenic effect in asthma. In addition, the T_H2-like T lymphocytes that produce IL-4 and IL-5 have been found in higher numbers in the lungs of asthmatic patients compared with in those of healthy control subjects.^{2,3} The infiltrating T lymphocytes exhibit an activated phenotype demonstrated by enhanced IL-2 receptor (CD25) expression⁴ and are characterized by a primed phenotype with higher levels of IL-4 and IL-5 mRNA and protein on stimulation.^{5,6} These T_H2-like cytokines are considered to be responsible, at least in part, for the eosinophilia and IgE-mediated mast cell degranulation observed in asthma. However, not only T_H2-type cytokines seem to be enhanced, but also increased levels of other cytokines have been documented in the asthmatic bronchoalveolar lavage fluid, peripheral blood, or both, including IL-2,² IL-3, GM-CSF,⁷ and IL-6.⁸ Furthermore, mast cells from asthmatic patients,⁹ as well as neutrophils¹⁰ and macrophages,¹¹ exhibit increased cellular activation, function, or both. It is thought that continued exposure to allergen might prime the asthmatic cells and contribute to enhanced production of these messengers.

From ^athe Department of Pharmacology and ^bthe Woolcock Institute of Medical Research, Royal Prince Alfred Hospital, University of Sydney, Australia.

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Reprint requests: Peter Borger, PhD, Department of Pharmacology, University of Sydney, NSW 2006, Australia.

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TABLE I. C/EBP isoforms (α - ζ) and their alternative names, tissue distributions, and putative roles in asthma

Name	Alternative name	Expression pattern	Involvement in asthma
C/EBP α	C/EBP	Liver, adipose, intestine, lung, adrenal gland, granulocytes, T cells	Eosinophilia, activation of neutrophils, NF- κ B-mediated inflammation, steroid receptor signaling, β -receptor signaling, respiratory defects in knockout mice, excessive lung epithelial cell growth in knockout mice
C/EBP β	NF-IL6, IL-6DBP, LAP, CRP2, AGP/EBP, NF-M, ApC/EBP	Liver, intestine, lung, adipose	T _H 2 response, <i>IL4</i> gene expression, <i>IL5</i> gene expression, HASM cell proliferation inflammation
C/EBP γ	Ig/EBP	Ubiquitous	Not known
C/EBP δ	CELF, CRP3, NF-IL6b, R α C/EBP2	Liver, lung, adipose, intestine	<i>IL5</i> gene expression, HASM proliferation, epithelial growth
C/EBP ϵ	CRP1	T cells	Not known
C/EBP ζ	CHOP, Gadd 153	Ubiquitous	Not known

In addition, asthmatic patients demonstrate increased airway smooth muscle mass, most likely because of enhanced proliferation of human airway smooth muscle (HASM) cells.¹² HASM cells are presently regarded as prominent mediators of the chronic features of asthma-associated airway remodeling. This view is supported by observations that several agents that are increased in the asthmatic airway are mitogenic for HASM cells in vitro.¹³ Moreover, HASM cells isolated from asthmatic patients produce higher levels of growth factors, such as GM-CSF and connective tissue growth factor.¹⁴ Aberrant proliferation and repair in asthma-derived epithelial cells has also been reported. The expression of transforming growth factor (TGF) β and epidermal growth factor receptors seems to be enhanced in epithelial cells,^{15,16} yet this does not lead to an appropriate restitution of the airway epithelium.¹⁶

Overall, these data indicate that asthma is not a result of dysfunction of one particular cell type but might rather be the consequence of a defective regulatory mechanism involved in both proliferation and cytokine gene expression that is operable (and probably dysfunctional after exposure to allergen) in all asthma-implicated cells. The importance of transcriptional regulation in allergic inflammation has recently been reviewed by Escoubet-Lozach et al.¹⁷ Therefore a common regulatory factor involved in both inflammation and proliferation might well be responsible for the aberrant phenotype of asthma cells. As will be discussed in detail, the C/EBP transcription factors might include such a factor.

THE C/EBP FAMILY

The C/EBPs are a family of transcription factors involved in the regulation of cellular differentiation, cell-cycle regulation, and cytokine gene expression.¹⁸ The C/EBPs are modular proteins, consisting of an activation domain, a basic DNA-binding region, and a leucine-rich dimerization domain. Six members of the family have been characterized. They are designated C/EBP α to C/EBP ζ (Table I) and share a strong sequence homology.¹⁸

In most cell types C/EBP β and C/EBP δ induce cell-cycle progression, whereas C/EBP α and C/EBP ϵ pre-

dominantly inhibit proliferation.¹⁹⁻³³ C/EBP γ is a truncated form and serves to counteract the C/EBP α and C/EBP β isoforms. For fine tuning of cell-cycle progression and gene regulation, several C/EBP isoforms are transiently and differentially expressed in multiple tissues. The importance of C/EBP α and C/EBP β as modulators of cellular responses is most evident from truncated proteins produced by differential initiation of translation that demonstrate contrasting functions in gene activation and cell proliferation.¹⁹⁻²⁷ This has clearly been demonstrated in adipocytes, in which deregulated expression of truncated C/EBP α and C/EBP β isoforms interferes with terminal differentiation and induces cell transformation.²⁷

Furthermore, C/EBP isoforms are autoregulatory and cross-regulate each other's expression. For example, C/EBP β is able to induce its own transcription³⁴ and upregulates the expression of C/EBP α .³⁵ Changes in isoform ratio have been observed in the acute-phase response,³⁶ in liver development and regeneration,³⁶⁻³⁸ and in tumorigenic conversion.³⁹ These observations show C/EBPs to be strictly regulated and show that the ratio of C/EBP isoforms determines whether a cell proliferates or differentiates.

C/EBPs AND T_H2-LIKE INFLAMMATION

Both C/EBP β and C/EBP δ are readily induced by proinflammatory stimuli, such as LPS, glucagon, and cytokines, including IL-1 and IL-6.¹⁸ The IL-5 promoter contains 4 C/EBP consensus sequences, and at least one of them (at position -235) interacts with C/EBP β and C/EBP δ . Mutations within this particular C/EBP binding sequence reduced the promoter activity by more than 50%.⁴⁰ Moreover, it has recently been shown that *IL4* gene expression can be upregulated by IL-6, probably through the enhanced DNA-binding capacity of C/EBP β (personal communication with Dr H. F. Kauffman, Groningen, The Netherlands, 2002). Mutation analysis revealed that the *IL4* gene contains a CCAAT consensus site in its promoter and thus can be regulated by C/EBP proteins.⁴¹ Therefore C/EBPs fulfill important transactivating functions in both *IL4* and *IL5* gene expression and

might be abnormally expressed, activated, or both in T lymphocytes isolated from asthmatic patients.

C/EBP α is also pivotal in the generation and development of granulocytes. Knocking out the *C/EBP α* gene in a mouse model demonstrated that these mice completely lack eosinophils.⁴² Eosinophil-specific gene expression and the production of eosinophils in the bone marrow critically depend on the presence of functional C/EBP α .⁴³ The latter observation implies that increased activation, DNA binding, or both of C/EBP α might enhance the production of eosinophils. Thus an imbalance of C/EBP isoform expression might also be a key to explaining high levels of eosinophils in the peripheral blood of asthmatic patients. Interestingly, major basic protein, an eosinophil-derived protein suspected to contribute to airway damage in asthma, also contains a CCAAT consensus sequences in its promoter, indicating its regulation by C/EBPs.⁴⁴

C/EBPs IN CELL-CYCLE REGULATION AND REMODELING

It is becoming evident that remodeling of the airways is a major feature of the asthmatic lung. In particular, HASM cells have been implicated in the remodeling process because asthmatic patients demonstrate increased airway muscle mass, possibly as a result of increased proliferation. Indeed, cultured HASM cells isolated from asthmatic patients show an increased proliferation rate compared with those of nonasthmatic control subjects.⁴⁵ It is known that C/EBPs regulate the balance between cell proliferation and growth arrest during terminal differentiation.¹⁸ Cell-cycle progression requires cyclin-dependent protein kinases (cdks) that must be activated to move the cells through the different stages of mitosis. The activation of cdks is regulated by cyclin polypeptides, which are stringently controlled by transcriptional and posttranscriptional mechanisms. The predominant effect of C/EBP α is inhibition of cell-cycle progression. Recently, Wang et al⁴⁶ demonstrated that this inhibitory effect is mediated by C/EBP-cyclin interactions that are independent from the DNA-binding domain of C/EBP α . In contrast to C/EBP α , the isoforms C/EBP β and C/EBP δ are expressed in proliferating cells. The mechanism of how C/EBP β and C/EBP δ induce cell-cycle progression is still not completely understood, but one possibility is through interference with C/EBP α . Interestingly, the C/EBP α promoter contains a CCAAT consensus site for autoregulation but also for competition with C/EBP β .³⁵ Furthermore, C/EBP β and C/EBP δ can exert a direct effect on genes involved in proliferation and cell-cycle progression.^{47,48}

Cell-cycle progression of HASM cells has been shown to be mediated by cyclins and cdks.⁴⁹ Growth-arrested HASM cells constitutively express considerable levels of C/EBP α , C/EBP β and C/EBP δ (personal communication with Dr A. J. Ammit, University of Sydney, Sydney, Australia, 2002). Because both cyclins and C/EBPs seem to be involved, we propose that similar interactions, as observed by Wang et al,⁴⁶ regulate cell-cycle progression of HASM

cells. Data on other cell types clearly indicate the importance of a balanced expression of isoforms of the C/EBP transcription factors in the control of cell proliferation.³⁶⁻³⁹

Airway remodeling is also associated with an increased deposition of the extracellular matrix components, probably induced by enhanced production of TGF- β by the airway epithelium.¹⁵ Intriguingly, TGF- β signaling is, at least in part, mediated by C/EBPs.⁵⁰ Taken together, imbalanced expression of C/EBPs might readily contribute to increased proliferation and airway wall remodeling.

INTERACTIONS OF C/EBPs WITH OTHER TRANSCRIPTION FACTORS

The differential effects of C/EBP isoforms on gene transcription, either positive or negative, are considered to reflect differences with respect to (1) the promoter architecture, (2) the relative affinity of specific sites for C/EBP isoforms, and (3) the interactions with other transcriptional activators. As a consequence, genes containing similar regulatory elements in their promoter might undergo completely divergent regulation. The specific promoter composition and the participation of different non-C/EBP transcriptional modulators can partially explain this apparent paradox.

Dimerization of C/EBPs is a prerequisite for DNA binding. Several combinations of either homodimers or heterodimers can be formed in vivo, giving rise to multiple polypeptides with distinct physiologic properties, whose differential occupancy of promoters can be determined by their relative abundance and affinity and by steric interactions with other transcription factors. A number of different transcription factors are able to physically and functionally interact with C/EBP members. The interactions of C/EBPs with members of the nuclear factor (NF) κ B family^{51,52} are of particular interest because they link the pathways of 2 prominent mediators of inflammation: IL-1 and IL-6. Adjacent C/EBP and NF- κ B binding sites are observed in many genes that require IL-1 and IL-6 for optimal expression, including several proinflammatory cytokine genes involved in asthma,^{40,41} suggesting that cooperative interaction between the C/EBP and NF- κ B transcription factor families represents a general mechanism coordinating transcriptional regulation. Synergistic activation by C/EBP and NF- κ B members has indeed been observed for the genes encoding IL-6, IL-8, IL-12, and G-CSF.⁵³⁻⁵⁷ However, C/EBP and NF- κ B interactions can also have antagonistic effects.^{51,58} Therefore promoter architecture and timing of activated transcription factors ultimately play the decisive role. Increased affinity of C/EBP and NF- κ B for their respective consensus sequences has been reported,^{51,59} and DNA-binding protein complexes can be demonstrated to be composed of both members of the C/EBP family and NF- κ B.^{57,59,60} Considering these interactions of C/EBP and NF- κ B, it is not surprising that NF- κ B has been implicated in asthma-associated inflammation.⁶¹

Histamine, prostaglandins, and β -adrenoceptor agonists induce cyclic adenosine monophosphate (AMP) as

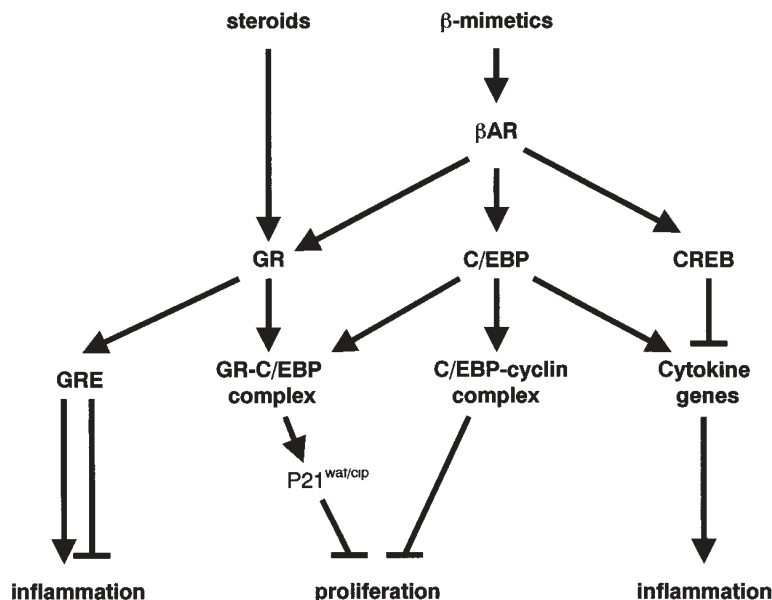


FIG 1. The C/EBP transcription factors fulfill pivotal roles in cross-talk of signaling pathways. Inflammation and proliferation is strongly modulated by the presence of C/EBPs. The ultimate outcome of antiasthma drugs might depend on the cell type because it is determined on the basis of differential occupancy of promoters by C/EBP isoforms and steric interactions with other transcription factors. Arrows indicate activation; blunt arrows indicate inhibition. For details, see text. βAR , β -adrenoceptor.

a second messenger. Cyclic AMP triggers protein kinase A, which activates the cyclic AMP response element binding (CREB) protein. Several studies have implicated this signaling pathway in asthma.⁶² Again, the C/EBPs could be involved because C/EBP α and C/EBP β compete for DNA binding with CREB.⁶³

An overview of possible interactions of C/EBPs and their role in inflammation and proliferation is depicted in Fig 1.

STERIODS, β -MIMETICS, AND C/EBP PROTEINS

Because steroids and β -mimetics have been recognized for their anti-inflammatory and antiproliferative action, it is worthwhile investigating whether C/EBPs are involved in the mechanism of these antiasthma drugs. In regard to T_H2-like inflammation, both *IL4* and *IL5* gene expression are downregulated by steroids.^{64,65} In contrast, *IL4* gene expression is less sensitive to β -mimetics.⁶⁶ The ultimate effect of β -adrenoceptor stimulation, however, largely depends on the microenvironment of the cells. T lymphocytes are less susceptible to cyclic AMP increases when additional signaling pathways are activated simultaneously (eg, those induced by the cytokines that signal through the common chain of the IL-2 receptor).^{67,68} Likewise, the effect of β -mimetics, which enhance intracellular cyclic AMP levels, on *IL5* gene expression might also depend on the microenvironment. Despite the stimulatory effects of C/EBPs on *IL4* and *IL5* gene transcription,^{40,41} the role of these transcription factors in steroid- and β_2 -mimetic-mediated gene repression in lymphocytes is not known.

For resident lung-derived cells, the picture is more transparent. The proliferation of nonasthmatic primary HASM cells is decreased in response to both steroids and β -mimetics.^{69,70} Furthermore, β -mimetics activate the glucocorticoid receptor (GR) in human pulmonary vascular smooth muscle cells,⁷¹ lung-derived fibroblasts,⁷¹ and HASM cells¹⁴ independently of the presence of glucocorticoids, a mechanism that has also been observed in vivo.⁷² The antiproliferative effect of both steroids and β -mimetics is mediated by a DNA-binding GR-C/EBP α complex that activates the promoter of the cell-cycle inhibitor p21^{waf1/cip1}.⁷¹ The combination of both drugs induced an even faster and persistent synthesis of this cell-cycle inhibitor.¹⁴ More recently, we found that proliferation of HASM cells from asthmatic patients is less susceptible to steroids than nonasthmatic control cells, a phenomenon associated with a diminished expression of C/EBP α (unpublished observations, 2002). Apparently, adequate cell-cycle repression by these drugs requires simultaneous activation of the GR and C/EBP α to form a transactivating complex that induces p21^{waf1/cip1}. The simultaneous activation of GR and C/EBP α might explain a number of antiasthmatic effects of steroids and β -mimetics, such as the reduced inflammatory cytokine levels reported in clinical studies (Fig 1).

ASTHMA: AN IMBALANCE OF C/EBP ISOFORMS?

From the evidence presented above, it is clear that the C/EBPs are prominent supermodulators of inflammatory and proliferative responses. In asthma cytokine gene expression and proliferation seem to be deregulated,

resulting in T_H2 -like inflammation and increased production of HASM cells. It is remarkable that these apparently distinct asthmatic pathogenic aberrations and anti-asthma drugs share a common regulatory component, namely C/EBPs. Therefore we hypothesize that increased proliferation and inflammation observed in asthma is the result of a deficiency with respect to expression, activation, or both of C/EBP isoforms. Even though C/EBPs are present in most cell types, the physiologic outcome after their activation might be cell type-specific because of 2 basic mechanisms. First, C/EBPs compete in protein-protein interactions (eg, with NF- κ B, cyclins, and/or GR; Fig 1). The effects of the antiasthma drugs on proliferation are possibly mediated at this level. Second, C/EBP isoforms compete with other transcription factors for DNA binding, notably CREB and NF- κ B, and modulate the expression of genes involved in inflammation. It should also be realized that the effects of C/EBPs on proliferation and gene expression can be mediated at the same time because they are spatially separated. For instance, C/EBP α can inhibit cell-cycle progression through binding to cyclins, but at the same time, it can affect gene expression by means of protein-protein interactions in the cytosol or by means of DNA-protein interactions in the nucleus.

Our hypothesis implies that differential expression of C/EBP isoforms in distinct cell types leads to differential effects on proliferation and gene expression caused by competition and cell type-specific promoter occupancy. Hence an imbalance of C/EBP isoforms in HASM cells might lead to an increased proliferation rate, whereas in T lymphocytes it might enhance cytokine gene expression, in particular *IL4* and *IL5* expression, which subsequently contributes to the observed T_H2 -like inflammatory response. We thus postulate a unifying hypothesis that could explain the enhanced activity and aberrant proliferation of cells in asthma. Our hypothesis opens a window to an unexploited field of research previously not implicated in the still elusive pathogenesis of asthma.

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