# Allergy and Asthma

## NEW TRENDS AND APPROACHES TO THERAPY

EDITED BY

A.B. KAY

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# Preface

The development of new forms of therapy for bronchial asthma and other diseases which have a substantial allergic component is one of the most active areas of medical research today. Traditionally the approach to treatment has been allergen avoidance, antiallergic drugs and, where indicated, immunotherapy. Some recent advances in these broad areas of asthma and allergy treatment are covered in the present volume. New ideas on the prevention of allergy are discussed with particular reference to neonatal sensitization. There are many new and novel approaches to pharmacotherapy. Historically, lipid mediators, particularly 5-lipoxygenase products and platelet-activating factor, are thought to play an important role in the asthma process. The future promise of agents which inhibit the formation and action of leukotrienes and platelet-activating factor is addressed and a number of new and novel agents which inhibit the interaction of agonists with specific receptors or prevent their formation are described. The prospect of altering membrane phospholipids by dietary manipulation with fish oil is another intriguing approach. It is recognized that in bronchial asthma and a number of allergic diseases, a specialized inflammatory component is prominent and therefore, not surprisingly, compounds have been developed which affect, for instance, eosinophil accumulation, the mucosal mast cell and infiltration of other cell types. Compounds such as nedocromil sodium, an antiasthma drug with steroid-sparing effects; cetirizine, a selective $H_1$-antagonist which affects infiltrating inflammatory cells, particularly eosinophils; azelastine, a novel oral antiasthma compound with several modes of action, are all described. Intriguingly, the prospect of modulating the allergic response by biotechnology is closely becoming a reality. This is discussed in a general sense and more specifically in the chapter which deals with factors identified and characterized which suppress the IgE response. Immunotherapy by traditional hyposensitization using improved and safer vaccines is also considered. These contributions are based on a meeting held at the Royal Society of Medicine in November 1987. The presentations provoked a lively discussion and an edited version of these interchanges is included to reflect, apart from anything else, the enthusiasm and buoyancy of the field. This volume would not have been possible without the skill and dedication of Miss Jennifer Mitchell, the editorial assistant and technical editor.

A.B. KAY

# Contents

# I

# Lipid Mediators and their Antagonists

# 1

# The future promise of agents which inhibit the formation and action of lipid mediators

M.K. BACH

## Summary

A number of inhibitors of 5-lipoxygenase (5-LO) and antagonists of leukotriene (LT) $D_4$ have been tested for efficacy in various provocation-testing models of asthma. While some beneficial activity was seen in some of these trials, on the whole the tests have failed to reveal the profound beneficial effects which had been hoped for. These findings raise questions about the direction which should be followed in future trials. On the one hand, it may be premature to conclude that, in fact, the products of the 5-LO pathway of arachidonate metabolism are not the key aetiological agents in asthma which they have been postulated to be. While it may be true that $LTD_4$ is not responsible for causing these symptoms by itself, the combined activity of $LTC_4$ and $LTD_4$ or the combination of all the sulphidopeptide leukotrienes and $LTB_4$ may still be responsible for causing a major part of the symptoms. To definitively exclude this possibility it will be necessary to combine carefully designed clinical challenge protocols of a potent and selective 5-LO inhibitor with objective and quantitative measurement of the effects of this inhibitor on the levels of the products of the 5-LO pathway in the target organ (presumably employing bronchoalveolar lavage) at time points which are critically related to the times for the development of symptoms. In this context, it may be more meaningful to consider the development of the so-called 'late-phase' response than to concentrate primarily on the prevention of acute bronchospasm. On the other hand, recent findings of the pro-inflammatory properties of eicosanoids from other branches of the cascade, such as the products of the 15-LO or some of the metabolites of prostaglandin (PG) $H_2$ (9$\alpha$, 11$\beta$-$PGF_2$), suggest that it may be appropriate to consider developing inhibitors to modulate these pathways. Finally, it may prove advantageous to focus on antagonists of platelet-activating factor (PAF-acether) since some of the more recent data suggest that this mediator may play a more central role as the initiator of the inflammatory or chronic component of the asthmatic syndrome. It seems unlikely, however, that a single agent can be found which will effectively control all the lipid mediator-dependent symptoms. Thus it may

become necessary to consider developing dual or multiple-specificity inhibitors, or, more practically, combinations of inhibitors each of which is selective for a certain pathway.

## Introduction

Slow-reacting substance of anaphylaxis (SRS-A) has long been believed to play a central role in the elicitation of the symptoms of asthma. The elucidation of the structure of the leukotrienes, the demonstration that these materials are derived from arachidonic acid and the demonstration that SRS-A is in reality a mixture of the leukotrienes, $LTC_4$, $LTD_4$ and $LTE_4$, has resulted in great commitment of effort to the discovery of potent and selective antagonists of these substances or inhibitors of their formation. A number of potent inhibitors and antagonists have been found, and in the last year or two the results of initial clinical trials with some of these substances have been reported. On the whole, these trials have shown lack of activity or, at best, modest activity.

It is my task to present a framework within which we can view the results and, hopefully, also address future approaches. I propose to do this by first briefly reviewing the products of the 5-LO pathway in the context of the biochemistry of their formation and interconversions, pointing out where there might be further opportunities for looking for pharmacological inhibitors. I shall then expand my discussion to a consideration of other eicosanoids which have more recently become candidate aetiological agents for inflammation and asthma and, from there, to PAF-acether which, although a lipid, is not directly related to the eicosanoids. As I conclude the chapter, I hope that I shall have left you with the impression that the complex interactions firstly among the lipid mediators themselves and secondly among the many other inflammatory signals to which the lungs are exposed with the lipid mediators or the cells producing them make it highly unlikely that a single 'magic bullet' can be found which will control this condition. I would rather hope that attention in the future will shift to a more careful consideration of the rational design of drug trials in which combinations of drugs are used, each of which is capable of effectively blocking one or a limited number of mediators.

## Products of 5-lipoxygenase as mediators of anaphylaxis and inflammation

I have listed the lipid mediators with which we shall be concerned in Table 1.1. The belief that the sulphidopeptide leukotrienes are responsible for some of the symptomatology of asthma goes back to the early 1960s [1]. More

**Table 1.1** Lipid mediators of anaphylaxis and inflammation

| | 5-Lipoxygenase | 15-Lipoxygenase | Cyclooxygenase |
|---|---|---|---|
| PAF | $LTB_4$ | 15-HETE | $PGF_{2\alpha}$ |
| | $LTC_4$ | LXA (?) | $PGD_2$ |
| | $LTD_4$ SRS-A | LXB (?) | $9\alpha,11\beta\text{-}PGF_2$ |
| | $LTE_4$ | | $TxA_2$ |

recently it has been shown by several groups that inhalation of aerosols of these substances causes changes in lung function which are characteristic of the bronchoconstriction of asthma; furthermore, asthmatic volunteers appear to be somewhat more susceptible to these substances than are normal individuals [2–5]. To satisfy the third of Dale's criteria in the proof of the role of these mediators in asthma, efforts have been made to find receptor antagonists for the leukotrienes or inhibitors of their synthesis. The search for antagonists is complicated by the fact that, even though $LTC_4$ is converted to $LTD_4$ which in turn is metabolized to $LTE_4$, there are distinct receptors for at least two of these substances [6, 7] and there is indirect evidence suggesting the existence of a distinct receptor for the third [8]. Furthermore, the search for receptors for $LTC_4$ is complicated by the fact that glutathione-S-transferases, which are ubiquitous enzymes, mimic receptors, i.e. $LTC_4$ can bind to them in a saturable manner [9]. Thus, it may be disappointing but perhaps not completely unexpected that even potent receptor antagonists of $LTD_4$ such as LY-171 883 and L-649 923 only had marginal effects in allergen-challenge protocols in human asthmatic volunteers, even though it could be shown that at least one of these compounds was able to antagonize the effects induced by inhalation challenge with $LTD_4$ [10, 11].

Recognizing these difficulties and, further, the possible contributions to the syndrome by $LTB_4$, there may be more appeal to finding inhibitors of the biosynthesis of the 5-LO products than to relying on what would necessarily have to be a series of specific antagonists. A considerable number of inhibitors have been described [reviewed in 10] and some clinical trials have been reported [12–14]. One of these [14] was more in the nature of a safety trial than an efficacy trial in that only the effect on a cholinergic stimulus was monitored, and even though hyperreactivity to acetylcholine is a hallmark of asthma, one would not expect this symptom to abate over a 4-day treatment course. The other two trials also failed to reveal benefits in exercise [12] or antigen challenge protocols [12, 13]. In the case of piriprost [12], treatment was by inhalation which should have caused optimal inhibition in the lungs at the site where this would be the most desirable. Unfortunately, bronchoalveolar lavage was not included in the clinical protocols and we thus do not know if the administered dose of drug was sufficient to cause inhibition of the 5-LO

at the target site. A possible substitute for such information, although somewhat less desirable, would have been to monitor inhibition of the 5-LO in blood *ex vivo* after drug administration. However, the known reversibility of the inhibition by piriprost [15] and the rapid clearance of this drug from blood precluded performing any meaningful *ex vivo* tests. In the second trial, the plasma levels of nafazatrom, which had been given orally, were reported as less than 10% of those which would have been required to achieve a 50% inhibition of the 5-LO [13]. Thus, the question of the possible therapeutic benefit to be derived from the inhibition of the 5-LO remains open.

Studies of the biosynthesis of the leukotrienes over the last few years have helped pinpoint a number of new approaches in the search for potential inhibitors (Fig. 1.1). It was felt at one time that inhibitors of the LTC synthase, the enzyme responsible for the conjugation of glutathione with $LTA_4$, might offer unique possibilities of selectivity since it was found that this enzyme differs in many critical aspects from the other known glutathione-S-transferases [16]. However, considerations such as the desirability of including the

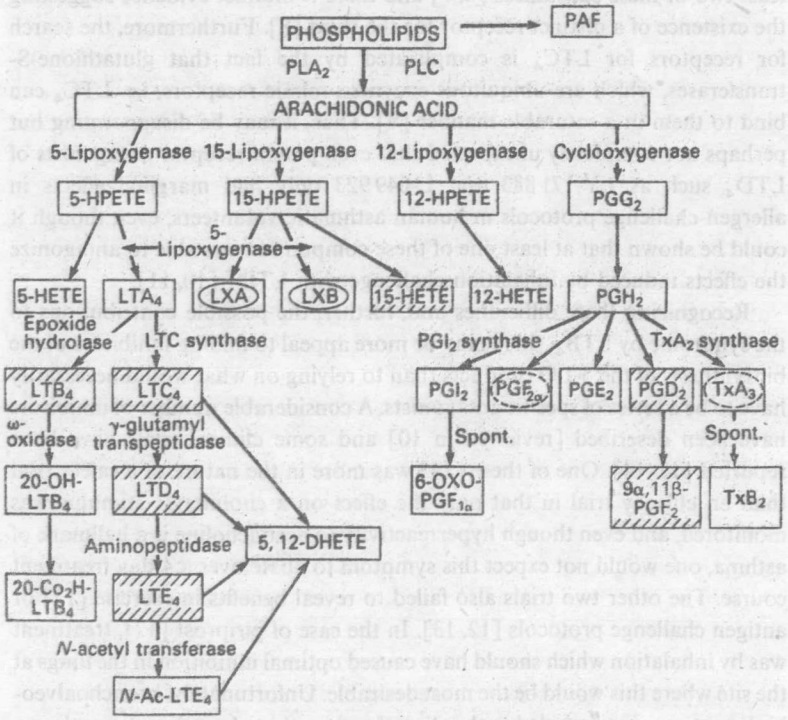


**Fig. 1.1** Biosynthesis and metabolism of lipid mediators of anaphylaxis.

inhibition of the formation of $LTB_4$ in the spectrum of activities of a potential inhibitor make this enzyme a less desirable target than is the 5-LO or the mobilization of arachidonate from phospholipid pools.

Recent studies have shown that the 5-LO is an exquisitely regulated enzyme. In addition to the fact that the action of the enzyme appears to be 'suicidal' in that the enzyme appears to be destroyed in the course of the reaction, it is now clear that the 5-LO has requirements for calcium, ATP, a hydroperoxide and at least three larger cofactors. One of these cofactors, which is found in the high-speed pellet fraction from leucocyte homogenates, appears to serve as an anchor to which the enzyme can attach reversibly in a calcium-dependent reaction [17]. The other cofactors are present in the high-speed supernatant fraction from these cells and can be separated from the bulk of the 5-LO activity by ammonium sulphate precipitation and ion-exchange chromatography. Preliminary results have suggested [Bach, unpublished observations] that the action of one cytosolic cofactor is more strongly dependent on the ATP concentration than is the activity of the 5-LO itself. Furthermore, demonstration of the activity of this cofactor in *in vitro* enzyme incubations is only possible under conditions where the amount of enzyme used is limiting the reaction. Failure to find evidence for such a co-factor in purified enzyme preparations from other cells in reports from two other groups [18, 19] may be due to their use of larger amounts of enzyme in their incubations. It is clear from the above that there is at least a formal resemblance between the regulation of the 5-LO and our present understanding of the regulation of protein kinase C. It is interesting, therefore, that a recent paper [20] suggested that the antiallergic drug, sodium cromoglycate, may be acting by inhibiting protein kinase C.

It has become increasingly apparent in recent years that the 'mobilization' of arachidonate from phospholipid pools is a complex event (see Fig. 1.1). Early results [21, 22] had shown that the 5-LO prefers to utilize arachidonate from endogenous phospholipid pools over exogenously supplied, free arachidonate. Arachidonate can be mobilized by at least two quite distinct pathways in the cell: one depends on the phosphoinositide cycle in which arachidonate mobilization occurs via a phospholipase C which is coupled to a diacylglycerol lipase. In the other, which appears to predominate in more protracted arachidonate-dependent reactions, mobilization depends on a phospholipase $A_2$. It has also been recognized that the extent of arachidonate mobilization and the utilization of the mobilized arachidonate depend considerably on the stimulus employed even when the same cell population is studied [23–25]. Clark and his associates have described fascinating experiments which have led them to the conclusion that there is a positive-feedback loop which results in the mobilization of arachidonate in the presence of sulphidopeptide leukotrienes [26–31]. They have been able to show that:

1 This stimulation is a time-dependent reaction which requires both RNA and protein synthesis and involves phospholipase $A_2$ [26–28].
2 The effect is expressed preferentially on the arachidonate which is then converted to products of the cyclooxygenase pathway [27–29].
3 The effect can be preferentially inhibited by dexamethasone but not by aspirin [30].
4 It can be explained by the induced formation of a phospholipase $A_2$-stimulatory protein which this group have successfully isolated, characterized and cloned [31].

Finally, there is, of course, considerable evidence for the existence of multiple forms of phospholipase $A_2$ even in homogeneous cell populations [32]. These enzymes can differ in their substrate specificity (for head groups on the phospholipid or for the nature of the fatty acid to be cleaved), their requirement for calcium ion, their pH optimum and their soluble or membrane-bound character. I will not consider the voluminous literature dealing with the endogenous inhibitor(s) of the phospholipases which can be induced by corticosteroids, the macrocortins [33], although they too obviously offer opportunities for the regulation of this reaction.

## Products of 15-lipoxygenase as mediators of anaphylaxis and inflammation

The first demonstration that 15-hydroxy-5,8,11,13-eicosatetraenoic acid (15-HETE) is the single major eicosanoid which is produced by specimens of asthmatic but not normal lung dates back to 1980 [34]. Several studies over the past few years have shown that granulocytes as well as primary isolates of epithelial cells from human and dog tracheas can generate large amounts of 15-HETE when they are incubated in the presence of relatively high concentrations of exogenous arachidonate or other toxic stimuli [35–40].

A variety of actions have been reported for 15-hydroperoxy-5,8,11,13-eicosatetraenoic acid (15-HPETE) and 15-HETE when these are used at $\mu$M concentrations but on the whole these actions are general reactions of lipid hydroperoxides and are not unique to the product of the 15-LO. More recently, several more specific actions have been reported, for which 15-HETE concentrations of only 0.01–1 $\mu$M were required. Among these are an inhibition of the expression of C3b complement receptors on B-cells [41], suppression of $\beta$-adrenergic receptor expression and potentiation of the response to histamine in guinea pig airways [42, 43] and a potent stimulation of mucus production in dog tracheas *in vivo* when only nanogram amounts of 15-HETE are instilled into the cranial thyroid artery [44]. Given the extremely low concentrations of 15-HETE which cause these responses and the fact that 15-HETE is apparently more active than is 15-HPETE in the dog model, it seems

very unlikely that these responses require the further metabolism of the 15-LO products.

To the extent that the responses of 15-HETE which have just been described mimic or can explain the development of some of the symptoms of asthma, these observations satisfy the first two criteria of Dale's postulates in establishing a role for 15-HETE in the aetiology of asthma even though this role may not primarily involve bronchoconstriction. To test the possibility further, selective inhibitors of the 15-LO will be required. No such inhibitors have been described thus far, although some of the known 5-LO inhibitors do inhibit this enzyme as well.

## Products of cyclooxygenase as mediators of anaphylaxis and inflammation

It was recognized in the early days of research with prostaglandins that $PGF_{2\alpha}$ was a potent bronchoconstrictor. In fact, it was proposed that the several thousand-fold increased susceptibility of asthmatics to the effects of this eicosanoid could explain the hyperreactive airways of asthmatics [45]. Thromboxane (Tx) $A_2$, which is also a smooth muscle contractant, was similarly considered as a possible contributor to bronchoconstriction. The bronchoconstrictive effect of synthetic $TxA_2$ in animals has been recently reported [46].

The potential contribution of $PGD_2$ (Fig. 1.2) to bronchoconstriction has been considered in the last few years. This has been encouraged by the observation that $PGD_2$ appears to be a major eicosanoid metabolite produced by human mast cells during anaphylactic challenge [47]. Indeed, careful analysis has shown that, of all the cyclooxygenase products which have been measured in nasal secretions, only $PGD_2$ levels appear to rise and fall *in vivo* in a manner which is temporally coincident with the rise and fall of allergic symptoms in volunteers who have been challenged with antigen

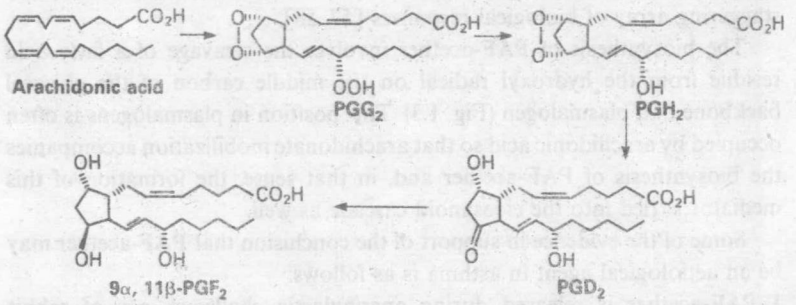


Fig. 1.2 Biosynthesis of $PGD_2$ and $9\alpha,11\beta$-$PGF_2$.