Early and late asthmatic reaction after allergen challenge

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Introduction

In the early 1950s Herxheimer (1) clearly showed that two distinct components in the obstructive response to inhaled allergens could be discerned, which he named the immediate and late reaction. But it was not until the late 1960s before Pepys (2) and de Vries and coworkers (3) started to investigate the mechanisms of the immediate and late response to inhaled allergen by drug protection studies. Based on different effects of several drugs on the immediate and late reaction they concluded that different mechanisms must underlie these response patterns. It was suggested that the immediate reaction was due to constriction of the smooth airway muscles and the late reaction the result of inflammation. Gökemeijer (4) showed that allergen challenge also leads to an increase in airway reactivity (AR). However, he found no relationship between the increase in AR and the immediate or late response. Then, Cockcroft et al. (5) demonstrated an increase in AR in association with the late allergic response (LAR). Later studies showed that subjects with an isolated EAR also develop an increase in AR, but to a much lesser extent than patients with a LAR (6). It has now generally been accepted that the increase in AR is usually associated with increased mucosal inflammation of the airways (7). Therefore, it is intriguing that AR is already increased 3 h after allergen challenge, when the FEV₁ has returned to prechallenge values, thus even preceding the LAR.

With the introduction of fibreoptic bronchoscopy, in recent years, broncho-alveolar lavage (BAL) and biopsies have become new research tools to study the cellular events in asthmatic airways after allergen challenge which can be investigated directly by microscopic studies including sophisticated immuno-histochemistry.

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The present review includes a summary of recent developments in knowledge on the pathogenesis of the EAR and LAR. The focus is on the pathophysiological mechanisms of the EAR, LAR and associated AR. We will also discuss the different drugs that modulate the early and late reaction and the associated AR.

Allergen inhalation

Bronchial allergen challenge in sensitized atopic asthmatics results in bronchoconstriction that develops within 10 min, reaches a maximum between 15 and 30 min, and generally resolves within 1–3 h. This period of bronchoconstriction is referred to as the early allergic response, which is defined as a fall in FEV₁ of 15–20% or more, or a fall of 40% or more in SGaw (8). Three to four or more hours after the onset of the EAR, a second period of bronchoconstriction may occur constituting the late allergic response (8). The duration of the LAR as measured with spirometry may be 12 h or even longer. Although the LAR need not be preceded by a clinically evident early response, isolated late reactions are rare in adults. This is in contrast with children where isolated late reactions are a frequent occurrence.

Diurnal variation in airway tone and the effect of medication withdrawal may interfere with the detection of the LAR, so this can be missed or not so pronounced (8). Hence, a control day, with saline challenge must be performed in that corrections for these confounders can be made [Fig. 1(a) and (b)].

Pathophysiology of the asthmatic airways after allergen challenge

INFLAMMATORY PROCESSES DURING THE EAR

The exact mechanisms leading to immediate airway narrowing after allergen challenge are still
unclear. Traditionally, the EAR has been associated with the activation of pulmonary mast cells with subsequent release of preformed histamine, tryptase and de novo synthesis of spasmogenic products of the cyclooxygenase pathway of arachidonic acid metabolism (PGD$_2$, TxA$_2$, along with products of the 5-lipoxygenase pathway (leukotrienes) (9). Mast cells may be activated to secrete their mediators by a wide variety of mechanisms, the most familiar being the IgE-dependent process [high-affinity IgE (Fc$_{RI}$) receptor] (10).

Alveolar macrophages generally have a suppressive effect, but this may be impaired in asthma after allergen exposure (11,12). The alveolar macrophage has even been incriminated in the EAR, being activated by allergens via an IgE-dependent process [low-affinity IgE (Fc$_{RII}$) receptor] and releasing both acute phase and chemotactic mediators. These
Table I. Effect of released inflammatory mediators after allergen inhalation

<table>
<thead>
<tr>
<th>Mediator</th>
<th>Bronchoconstriction</th>
<th>Mucus secretion</th>
<th>Microvascular leakage</th>
<th>Chemotaxis</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histamine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Mast cell, Basophil</td>
</tr>
<tr>
<td>PGD₂, PGF₂α</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>?</td>
<td>Mast cell, Eosinophil</td>
</tr>
<tr>
<td>PGE₂</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>Epithelial cell</td>
</tr>
<tr>
<td>LTC₄, D₄, E₄</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>Mast cell, Epithelial cell</td>
</tr>
<tr>
<td>PAF</td>
<td></td>
<td>+</td>
<td></td>
<td>+</td>
<td>Alv. macro, Eosinophil</td>
</tr>
<tr>
<td>Kinins</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>Mast cell</td>
</tr>
</tbody>
</table>

Acute phase products include the spasmogenetic TxA₂, TxB₂, LTB₄ and PAF, as well as reactive oxygen species and lysosomal enzymes, which may lead to local tissue damage (13,14). In addition to alveolar macrophages, dendritic cells are also very effective antigen-presenting cells and may therefore play a very important role in the initiation of an allergen-induced response (11). Additionally, airway epithelium may also play a role in this process. After antigenic stimulation, epithelial cells are able to release LTC₄, 12-HETE, PGE₂, and PGF₂α and chemotactic mediators such as LTB₄, 15-HETE and possibly PAF (15).

The above mentioned inflammatory mediators have important bronchospasmogenic effects, next to their stimulatory role in mucus secretion, vasodilation, microvascular leakage and chemotaxis (Table 1). These mediators may be at least responsible for the immediate clinical reaction after allergen challenge. Moreover, release of chemotactic mediators by mast cells, alveolar macrophages, dendritic cells and epithelial cells may also be involved in the development of the EAR. More disputable is the role of T cells during the EAR. But after local allergen challenge a direct increase in CD4+ cells was shown in BAL fluid. This provides evidence to support the role of T cells in this early state of the asthmatic response (16).

**Inflammatory Process Between the EAR and LAR**

The role of mast-cell-dependent products in the development of the late-phase inflammatory events after allergen challenge in atopic asthmatics is still controversial. Some studies have reported that release of mast-cell-associated high-molecular-weight neutrophil chemotactic factor (NCF) as well as eosinophil chemotactic factor (ECF) occurs during the EAR with a close relationship of NCF to the development of the ensuing LAR (17,18). The close relationship of NCF or ECF to the LAR may be relevant to the control of the mechanisms that govern the accumulation of eosinophils in the lung (19). More recently, it has become apparent that mast cells release, in an IgE-dependent fashion, an array of cytokines such as IL-3, IL-4, IL-5, GM-CSF. These cytokines have also been implicated in the pathogenesis of late-phase inflammatory events, especially in relation to eosinophils (20-22). Although it has been suggested that one or more of the cytokines released by mast cells are in fact NCF or ECF, it should be pointed out that cytokine release does not start until 2 h after allergen challenge while NCF or ECF release occurs much earlier (17). Additionally, it should be recognized that most of the available data are derived from *in vitro* studies of murine mast cell lines. These results, therefore, can not be automatically applied to the human lung mast cell, but generally indicate that release of cytokines (IL-3, IL-4, IL-5, GM-CSF) or NCF and ECF by mast cells is relevant for the development of the LAR. It is known that IL-3, IL-5 and GM-CSF are involved in the control of eosinophil degranulation, activation of mature eosinophils in terms of increased cytotoxicity and oxidative metabolism and in prolonging eosinophil survival *in vitro* (23). In bronchial lavage fluid and biopsies of the submucosa of asthmatics an increase of activated eosinophils is seen 3-4 h after allergen challenge, well before the late phase reaction (24,25). However, no release of cytotoxic eosinophil mediator ECP is detected in BAL fluid at this point in time. Mast cells also release IL-4 which is involved in the survival and proliferation of T-helper cells, especially the T₄₂ phenotype (26).
Not only mast cells, but also alveolar macrophages release a diversity of cytokines. Human alveolar macrophages produce predominantly IL-1β. IL-1 expression reaches its maximum 2–3 h after in vitro stimulation. Alveolar macrophages therefore, have the capacity to initiate an inflammatory response via the release of IL-1, which may activate T cells and dendritic cells are able to release IL-1 as well (11).

Human tracheal and bronchial epithelial cells are capable of synthesizing IL-1 and TNF after allergen stimulation. IL-1 and TNF may in turn induce the production of GM-CSF, IL-6 and IL-8 from epithelial cells (27).

In addition to these cytokines, chemotactic factors (LTB₄, 15-HETE, PAF) are liberated from mast cells, alveolar macrophages and epithelial cells. It is known that all of the above mentioned cytokines and chemotactic mediators can recruit, prime, and activate alveolar macrophages, T cells, neutrophils, eosinophils and basophils, and can also increase immunoglobulin secretion (IgE of B cells) and regulate the proliferation of mast cells (Table 2). However, the exact mechanisms responsible for selective recruitment of inflammatory cells into the airway (sub)mucosa in allergic inflammation are not fully known. Cytokines are thought to play a role in this recruitment by upregulation of adhesion molecules for cells involved in the LAR (28,29). Three types of adhesion molecules have been observed on endothelial cells: ELAM-1 (endothelial leukocyte adhesion molecule-1), ICAM-1 (intracellular adhesion molecule-1) and VCAM-1 (vascular cell adhesion molecule-1). ELAM-1 is expressed on endothelial cells after stimulation with IL-1, TNFα and IFNγ, and may be expressed at sites of allergic inflammation (30). ICAM-1 is constitutionally expressed on endothelial cells and is markedly up-regulated in allergically inflamed tissue and after IL-1β, TNFα and IFNγ incubation (31). Endothelial expression of VCAM-1 may be upregulated by IL-4 (32). It is intriguing that these cytokines, TNFα, IL-1 and IL-4, which stimulate the expression of the appropriate adhesion molecules, are released by allergen triggered mast cells and other cells. This provides an important link between mast cell activation, upregulation of adhesion molecules, and subsequent T cell and eosinophil recruitment.

After appropriate stimulation, ELAM-1 is maximally expressed after 4–6 h, returning to baseline at 24 h (30). ICAM-1 and VCAM-1 are expressed later, reaching a maximum at 24–48 h (31,32). It could be hypothesized that ELAM-1, which is maximally upregulated at the time of the LAR, is involved in the development of the LAR, whereas ICAM-1 and VCAM-1 are not, but rather support the allergen-induced inflammatory process for a longer period of time. Furthermore, it is known that VCAM-1 binds preferentially to specific adhesion molecules of eosinophils, whereas ELAM-1 and ICAM-1 bind to specific adhesion molecules of both eosinophils and neutrophils (25).

Adhesion molecules are also expressed on leukocytes where they may act as specific ligands for the molecules described above. Three heterodimers have been described, consisting of a common β-chain (CD18) and different α-chains LFA-1 (CD11a), MAC-1 (CD11b), and P150,95 (CD11c). CD11a,b,c and CD18 act as specific ligands for ICAM-1 on endothelial cells. LFA-1 has been involved in T cell adhesion. All three heterodimers are involved to varying degrees in neutrophil, eosinophil, and monocellular cell adhesion. More recently, it has been shown in vitro that recombinant IL-3 and recombinant GM-CSF selectively enhance basophil adhesiveness to endothelial cells and expression of CD11b on this cell (33,34). Very late antigens (VLA) 1–6 are yet another type of adhesion molecule on leukocytes. VLA-1 is expressed on activated T cells while VLA-4 has only been detected on eosinophils adherent to VCAM-1 on endothelium (32). Expression of these VLA adhesion molecules on leukocytes is observed 6 h after allergen challenge. Although many of these findings were mainly obtained in in vitro studies, Wegner et al. (31) have shown that in Ascaris sensitized monkeys repeated allergen challenge caused an increase in ICAM-1 expression on both airway epithelium and submucosal endothelium, accompanied by eosinophilic infiltration of the mucosa and increased AR to methacholine. The latter two events were attenuated by intravenous infusion or inhalation of a blocking anti-ICAM-1 monoclonal antibody. These observations support the view that ICAM-1 plays an important role in the specific eosinophil inflammatory response after allergen inhalation. Although the kinetics of its upregulation in tissue suggest that this may be especially the case in chronic allergic inflammation. In addition to chemotactic signals released from allergically inflamed tissue, these adhesion mechanisms may be important in the generation of selective recruitment of circulating leukocytes into the airways (Fig. 2).

INFLAMMATORY PROCESS DURING THE LAR

The role of the T cell in the regulation and expression of airway inflammation in atopic asthma has been given considerable attention (35). IL-1, released by alveolar macrophages, dendritic and epithelial cells, regulates maturation and activation of T cells (CD3+). Such cells can be divided into two
<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Cell activation</th>
<th>Cell proliferation</th>
<th>Cell survival</th>
<th>Cell recruitment</th>
<th>Expression of adhesion molecules</th>
<th>Effect on IgE production</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL1</td>
<td>T-cell</td>
<td></td>
<td></td>
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<td>Mast cell, Alv. macro. Epithelial cell Alv. macro. T&lt;sub&gt;H&lt;/sub&gt; cell Mast cell, T cell</td>
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<tr>
<td>IL1β</td>
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<td>T&lt;sub&gt;H&lt;/sub&gt;1-cell</td>
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<tr>
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<td>Alv. macro.</td>
<td>Mast cell</td>
<td>Eosinophil</td>
<td>Basophil</td>
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<td>Mast cell, T&lt;sub&gt;H&lt;/sub&gt; cell</td>
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<tr>
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<td>Eosinophil</td>
<td>Eosinophil</td>
<td>Basophil</td>
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<td>T, B cell, Mast cell Epithelial cell Endothelial cell Alv. macro. Alv. macro. T cell, Fibroblast Epithelial cell Endothelial cell Alv. macro., T cell Endothelial cell Epithelial cell</td>
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<tr>
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<td>T-cell</td>
<td></td>
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<td></td>
<td>+</td>
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<tr>
<td>IL8</td>
<td>Neutrophil</td>
<td></td>
<td></td>
<td>Neutrophil</td>
<td></td>
<td></td>
<td>Alv. macro.</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>Alv. macro.</td>
<td>Granulcyt</td>
<td>Eosinophil</td>
<td>Basophil</td>
<td></td>
<td></td>
<td>Alv. macro., Mast cell Epithelial cell</td>
</tr>
<tr>
<td>TNFα</td>
<td>Epithelial cell</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>Mast cell, T cell</td>
</tr>
<tr>
<td>INFγ</td>
<td>T-cell</td>
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<td></td>
<td>Alv. macro.</td>
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<td>+</td>
<td>Mast cell, T cell</td>
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major immunocytochemical and functional subgroups, i.e. CD4 and CD8 cells. A decrease of CD4+ T cells and an increase in CD8+ T cells was shown in BAL fluid 6 h after allergen challenge of early responders. This is in contrast with dual responders, who showed an increase of CD4+ T cells and a much less pronounced increased in CD8+ T cells in BAL fluid (36). Recently, another study has reported that submucosal CD8+ T cells increased at 3 h and 24 h after allergen challenge by dual responders, and that appearance of these cells in biopsies correlated inversely with the severity of the LAR (37). The results of both studies provide evidence to support the hypothesis that CD8+ T cells may play a role in suppressing the LAR.

CD4+ T cells (Th1 cells) are now recognized as important in allergic inflammatory responses. Antigen-activated Th1 cells can be divided into two functionally distinct subsets referred to as Th1 and Th2, based on the pattern of cytokines secretion. Th1 cells secrete only IL-2, INFγ, TNF-β. Th2 cells secrete only IL-4, IL-5 and IL-6, which are thought to be involved in the cellular response during the LAR. In several studies (38-40), a selective increase in Th1 cells in BAL fluid was observed 24 and 48 h after allergen challenge in those subjects who had previously been shown to develop a late-phase reaction. As these CD4+ T cells express positive hybridization signals specifically for IL-4, IL-5, and GM-CSF, but not for IL-2, IL-3 and INF, after 24 h, this indicates a cytokine secretion profile of the Th1 cell subtype upon allergen exposition (40,41). The same profile has been found in airway wall biopsies, showing an increase in mRNA expression for IL-5 and GM-CSF (42). Th2-cell-derived IL-4 is intimately involved in the regulation of IgE production, as it stimulates and switches the immunoglobulin production of B cells to IgE in response to airborne allergens. Also, the survival and proliferation of Th2 cells is dependent on IL-4. Other cytokines derived from Th2 such as IL-3, IL-5, GM-CSF, play a pivotal role in the activation, maturation and survival of eosinophils. It is well known that eosinophils in BAL fluid increase during the LAR in asthmatic patients (43). Subsequently, biopsies of the inflamed submucosa of the airway 24 h after allergen challenge has demonstrated an association between activated (EG2+) eosinophils and the number of IL-5 mRNA positive cells (42) and this coincides with an increase in ECP (24). It is known that preformed eosinophils products, such as ECP, MBP, EDN, and EPO are cytotoxic and neurotoxic and induce degranulation of mast cells and basophils (39,43,44). MBP and ECP are cytotoxic to epithelial cells of the airway in vitro and give rise to desquamation of the epithelial layer (45). As a consequence detachment of ciliated cells and destruction of individual cells may occur, leaving behind only basal cells at the epithelium lining (46). By others, it has been shown that this destruction leads to an increased incidence of tight junction openings of bronchial epithelium or widening in the intercellular space epithelium. This again was associated with eosinophil infiltration (47). In addition to the release of basic proteins by activated eosinophils, other spasmodogenic mediators such as LTC4, PAF, PGE1, 15-HETE, TxB2 and toxic oxygen-derived radicals are also released, which may induce an involvement in the allergic inflammatory process during the LAR (44). All these data support the role of the eosinophil as an important effector cell in this
process while $\text{T}_{h2}$-cells are possibly important for the regulation of the allergic inflammation in the airways during the LAR.

In addition to eosinophils, neutrophils have also been implicated in the LAR (43,44). The neutrophil may be recruited earlier to sites of inflammation in the lung, but this transient, soon giving way to an eosinophilic infiltration.

- Basophils may be also important during the late phase reaction, since tissue basophilia is found 6 h after allergen challenge (45). IL-3 and GM-CSF activate basophils leading to release of histamine.

In conclusion, T cells become activated during, and perhaps even before, the late phase reaction possibly as a consequence of IL-1 and IL-4 release by mast cells and other cells. Activated T cells also release cytokines (IL-3, IL-5, GM-CSF) which are involved in the activation and survival of eosinophils, possibly also activating basophils and mast cells. The (pro)inflammatory mediators liberated by these activated inflammatory cells have numerous activities, including induction of smooth muscle contraction, increased mucous secretion, vasodilation and increased vascular leakage resulting in tissue edema, all of which are histological features during the LAR.

Allergen-induced dysfunction of the neural pathways

Allergen-induced biphasic response may lead to impaired $\beta$-adrenoreceptor function (48). Twenty-four hours after allergen challenge in asthmatics, $\beta$-adrenoreceptor numbers were reduced by 20% while their function (as measured by c-AMP production) was diminished by 40%. These changes, measured in lymphocyte membranes, are the result of uncoupling and downregulation of the $\beta$-adrenoreceptor. An in vitro study showed that preincubation of peripheral blood lymphocytes with IL-1, IL-2, IFN$\gamma$ and GM-CSF for 20 h diminished c-AMP production (49). These findings suggest that a defect in $\beta$-adrenoreceptor function and a decrease in numbers of peripheral blood lymphocytes may be the consequence of allergen challenge, possibly caused by cytokine release, from allergen-triggered cells in the airways.

There are indications that parasympathetic inhibitory muscarinic$\_3$-receptors dysfunction may occur in asthmatics, resulting in exaggerated cholinergic reflex response. This has been observed after allergen challenge and may contribute to the severity of the airway obstruction and increase in AR after the LAR, by increased release of acetylcholine (50,51).

In addition to these neural systems human airways are also innervated by a noncholinergic nonadrenergic-system (NANC) of which the inhibitory-NANC system releases neuropeptides such as vasoactive intestinal peptide (VIP) and nitrous-oxide (NO) which are bronchodilatory. I-NANC activities can be impaired in animals after allergen challenge and this dysfunction may be due to associated protease release by mast cells (52).

Overall these data suggest that allergen challenge results in a dysfunction of several neural regulating systems of the airways which contributes to bronchoconstriction and increased AR. Their exact role in humans is as yet however not fully clear.

Allergen-induced airway reactivity

Allergen-induced increase in non-specific AR has recently been shown to be present even before the LAR and is sustained for many days (5,44,53). Histamine and methacholine are normally used to measure allergen-induced AR and are more or less direct stimuli for smooth muscle contraction. This increase of non-specific AR, as measured by direct stimuli, is possibly the result of allergen induced decrease in the geometric diameter of the airways which could be caused by the airway wall oedema, vasodilation resulting in thickening of the wall and smooth muscle contraction. Indirect stimuli are also used to measure increases in AR, such as 5'-adenosine-monophosphate (AMP) and bradykinin (BK). One study compared methacholine with AMP and showed only an increase in AR to AMP 3 h after the allergen provocation, but not after 24 h, in contrast with AR to methacholine which is increased at both time-points (6). This finding with AMP may be due to either mast cell depletion after allergen challenge or desensitization of prejunctional $\text{A}_2$-receptors on cholinergic nerve endings during the LAR (54–56). Airway reactivity to BK was higher than for methacholine at 8 h and 3 weeks after allergen provocation (57). This increase at 8 h after the provocation is thought to be due to allergen-induced irritability of sensory nerves endings, whereas the sustained increase in AR to BK could be a consequence of slow epithelial repair.

The mechanisms underlying allergen-induced increase in non-specific AR are not fully known, but it is likely that activated eosinophils, mast cells and their mediators may play an important role. Allergen-triggered mast cells give a release of leukotrienes and PGD$_2$. Inhalation of LTC$_4$, LTD$_4$, and LTE$_4$ has been shown to enhance histamine reactivity of human asthmatic airways 4 h after leukotriene inhalation (58). This is also the case for PGD$_2$, which gives an increase in AR to inhaled
instance, neurokinine A (NKA) is a very potent
excitatory or E-NANC system may produce many
of the pathophysiologic features of asthma. For
example, sensory nerves by inflammatory mediators such as
histamine, prostaglandins, PAF, leukotrienes and
bradykinin, known to be liberated during the EAR
and LAR, can result in the release of many neuro-
peptides via a neural reflex. These neuropeptides of
excitatory or E-NANC system may produce many
of the pathophysiologic features of asthma. For
instance, neurokinine A (NKA) is a very potent
constrictor of human airways and enhances cholin-
ergic neurotransmission. Substance P (SP) is a
vasodilator, causes microvascular leakage and stimu-
lates mucus secretion from submucosal glands and goblet cells. Calcitonin-gene related peptide (CGRP)
is a potent and long-lasting vasodilator (61). These
effectors may act on mast cells and other migratory
cells. These neuropeptides are catabolized by neuro-
endopeptidase (derived from airway epithelial cells)
and this counter-regulation is therefore decreased after
shedding of the epithelium. I-NANC system
neuropeptides such as VIP and NO are catabolized
by tryptase released from mast cells in allergically
inflamed tissue (62). It can thus be hypothesized that
release of neuropeptides and increase in irritability of
sensory nerves after shedding of airway epithelium
is the underlying pathophysiology mechanism to
increase AR when it is sustained for days.

In considering parameters of airway reactivity, it is
important to distinguish between the position of the
dose-response curve to a non-specific stimulus and
the presence of a plateau in the curve, both which
may be multicausally determined. It has been shown
that allergen challenge, causing a dual response but
not a single response, not only shifts the methachol-
ine dose-response curve to the left but also increases
the maximal airway narrowing or even causes a loss
of the plateau of the curve 24 h after the exposure
(63). This suggests that airway inflammation also is
important for increase in maximal airway narrowing.
It can be concluded that the release of inflammatory
mediators gives submucosal oedema, increases in
mucus secretion and shedding of the epithelium,
which may directly influence neural reflex-pathways,
resulting in an increase in more AR as well as
enhance of maximal airway narrowing. Loss of the
plateau in the dose-response curve for methacholine
or histamine is associated with excessive airway
narrowing, which is a characteristic feature in
asthma (64).

Effect of drugs on the EAR and LAR

In order to better understand the mechanisms
underlying the EAR and LAR, several studies with
drugs modulating inflammatory mediators or neural
pathways have been performed. As histamine is
thought to be a prime candidate bronchoconstrictor
during the EAR, antihistamines have been investi-
gated. In fact only terfenadine partially protected the
EAR whereas azelastine and cetirizine had no effect
(65,66). This does, however, not exclude a role for
histamine in the EAR, as it might well be that these
oral drugs do not give adequate tissue levels in the
airways. This notion is supported by the discrepancy
between inhaled β-adrenoceptor drugs that provide
protection against histamine provocation and oral
β-adrenoceptor drugs that do not. As the cyclo-
oxigenase inhibitor flurbiprofen is capable of reduc-
ing the LAR, but not the EAR, prostaglandins may
have a prominent role during the LAR (65). A role
for leukotrienes during the EAR is supported by a
protective study with leukotriene-antagonists and a
leukotriene synthetase inhibitor (67,68). Moreover,
the reduction of urinary LTE₄ production was
associated with a decrease of the EAR. An animal
study showed that microvascular leakage was even
more reduced than smooth muscle contraction by
a leukotriene-antagonist (69). Effects of PAF-
antagonists have been studied as well. While in
animal studies, an inhaled PAF-antagonist reduced
the LAR and the associated AR, no effect of an oral
antagonist has been shown in humans (70,71). These
results suggest a role for PAF during the LAR and in
the induction of AR, nevertheless no effect was found
by an oral antagonist, so a role in humans is till open for question.

In addition to drugs which target one mediator, there are drugs with a more general mechanism, such as glucocorticosteroids, sodium cromoglycate, nedocromil sodium, and xanthine derivatives. A single dose of oral or inhaled corticosteroids before allergen challenge inhibits the LAR and the induced AR, but has no effect on the EAR. However, after a continuous treatment for several weeks, a reduction of the EAR, LAR and associated AR was observed (3,72,73). Protection studies with single dose sodium cromoglycate and nedocromil sodium show a significant reduction of the allergen-induced early-, and late reaction and increase in AR (3,74). There are indications of inhibitory effects of theophylline, especially the slow-release form, on the EAR, LAR and induced AR suggesting some anti-inflammatory properties in addition to their bronchodilating effects (75,76).

Conventional doses of bronchodilators such as terbutaline and salbutamol totally protect the EAR but not the LAR. However, a single high dose of salbutamol (2.5 mg) inhibits the EAR and LAR up to 7 h after the allergen challenge as well as the allergen-induced AR (77). More recently, it has been shown that a single dose of the new long-acting $\beta_2$-agonist, salmeterol, ablates the EAR and LAR and inhibits the associated AR for 34 h (78). The authors suggested that salmeterol has pharmacological effects on allergen-induced inflammatory responses in addition to its bronchodilator action. These data are in contrast with a protection study with salmeterol we recently performed (79). After corrections for the bronchodilator action of salmeterol, incomplete inhibition was found for both the EAR and LAR. While no protection from allergen-induced increase of AR after 24 h was observed. It might be argued that these effects can be explained by a sustained bronchodilator action and functional antagonism of $\beta_2$-agonist rather than inhibiting of the allergen-induced late phase reaction. Formoterol also has an inhibitory effect on the EAR, LAR and induced AR for at least 12–24 h (80). However, only a reduction of the LAR and induced AR occurred and not a total ablation. A methodologically correct analysis of lung function following bronchodilation is necessary in order to separate the bronchodilatory and anti-inflammatory effect of these drugs.

**Conclusion**

Bronchial allergen challenge is a useful research tool for unravelling of the pathophysiology of bronchial asthma. The dynamics and mechanisms of the allergen-induced airway response can be studied by measuring physiologic parameters, variations in serum constituents and by the effect of pretreatment with various drugs. Since the introduction of the fibreoptic bronchoscope direct information on the local allergic inflammatory response can be obtained using broncho-alveolar lavage and (sub)mucosal biopsies. Airway resident and infiltrating cells involved in the EAR and IAR can be studied as well as their activation state and released mediators. Available data suggest that the fall in FEV$_1$ during the EAR is not only a consequence of bronchospasm, but also results from increased vascular permeability and oedema. In addition, the release of cytokines and chemo-attractive mediators from mast cells, alveolar macrophages, dendritic cells and epithelial cells may be important for the recruitment (via expression of adhesion molecules on endothelial cells and on circulating leukocytes) and activation of eosinophils and T-cells into the lung. The release of these cytokines may be essential for the development of the LAR, especially release of IL-1 which activates $T_H$ cells. Additionally, evidence is accumulating that activation of the eosinophil is also very important for the development of the LAR and the associated AR. This increased eosinophil activation may be initiated by the release of IL-3, IL-5 and GM-CSF by mast cells and $T_H$ cells, likely the $T_H$-2 subtypes.

The release of many (pro)-inflammatory mediators by activated inflammatory cells and their effects on the neural system in the airways may account for the allergen-induced increase in AR.

The well-known anti-inflammatory drugs, sodium cromoglycate, nedocromil sodium and glucocorticosteroids, reduce or prevent both EAR and LAR and the allergen-induced AR. Conventional doses of short-acting $\beta_2$-agonists protect the EAR, whereas high doses affect both EAR and LAR. However, long-acting $\beta_2$-agonists inhibit both the EAR and LAR. It is not yet clear whether this is due to the sustained bronchodilating effect and functional antagonism or to a 'real' anti-inflammatory effect.

Since specific antagonists and specific synthesis inhibitors of inflammatory mediators, have shown only partial inhibition of the EAR and/or LAR it is likely that no single mediator can account for the early and/or late phase reaction. The allergic asthmatic reaction and allergen-induced increase in AR, is orchestrated by many (pro-) inflammatory mediators released by allergen-triggered resident cells, airway epithelium and circulating cells selectively infiltrating the airways. Therefore, it may be more realistic to consider the EAR, LAR and
increase in AR as clinically measurable aspects of one
and the same ongoing inflammatory process.

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