

The role of basophils and mast cells in acute and late reactions in the skin

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Allergy 1997; 52(suppl 34): 31-43. © Munksgaard 1997.

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Key words: basophils; mast cells; allergic
diseases; antigen.

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The skin was first used as a model for the study and diagnosis of allergic diseases in 1865 when Blackley created an immediate reaction on his own forearm with crude rye grass pollen that resulted in a late exacerbation of his asthma (1). Subsequently, it was established that the cutaneous late-phase response (LPR) is mediated through the triggering, either by specific antigens or by anti-IgE, of skin mast cells. Nearly half a century later, Cooke reported immediate wheal and flare reactions followed by a late reaction (2). Cooke also found that repeated local injections of the same antigen resulted in attenuation of both the immediate and late reactions in the skin (2). Pepys' interest in the late-phase response (LPR) was prompted by his interest in allergic bronchopulmonary aspergillosis. When he observed a bi-phasic response to skin testing, he hypothesized that this late-phase response was a Type III reaction (Gell and Combs) and observed some similarity between cutaneous LPR biopsies and biopsies of experimental Arthus reactions (3). Dolovich et al. were the first to describe the events leading to cutaneous LPR with specific IgE (4). In 1976 Solley and coworkers demonstrated that the skin's ability to react to antigen, both the immediate response and late response, could be passively transferred to non-allergic subjects (5). When they removed the serum IgE, they found the serum had lost the ability to passively transfer the skin LPR. Thus, it was firmly established that the cutaneous LPR was mediated through the triggering, either by specific antigens or anti-IgE, of cutaneous mast cells.

For the purposes of this chapter, I will define the LPR as a delayed inflammatory process involving the skin and/or the airways and consisting of a polymorphous inflammatory infiltrate occurring in response to mast cell activation by either antigen or non-antigen mast cell activators. This LPR may be biphasic or it may represent a continuum of the immediate response. Lastly, it is important to note that multiple mechanisms may be involved in what today is described as an LPR, be it in the skin or in the airways.

The skin as a model with which to study allergic disease received impetus from the finding that a LPR is often observed in patients with both reactive airway disease and allergic rhinitis (6, 7). The relevance of the LPR was confirmed when Warner et al. showed that immunotherapy in a group of children affected by house dust mite allergic asthma resulted in a marked attenuation of the LPR following a bronchoprovocation challenge (8). It was later found that nonspecific bronchial hyperreactivity increased after antigen bronchoprovocation depending on the intensity of LPR (9).

Agents that can induce an LPR in the skin

There are several non-antigenic substances and stimuli which have been reported to cause late-cutaneous reactions similar to that described with antigens. Although histamine alone does not induce an LPR in the skin, compound 48/80, a skin mast cell degranulator, can induce a LPR by non-

immunologically causing mast cell degranulation (10). Products of the lipoxygenase pathway of arachidonic acid metabolism can cause clinical and histologic skin lesions akin to the LPR (11). Interestingly, prostaglandin D₂ (PGD₂), a product of the mast cell cyclo-oxygenase pathway, does not cause a cutaneous LPR. Furthermore, intradermal injection of platelet-activating factor (PAF) results in a dermal picture identical to that of the cutaneous LPR (12, 13). This may be clinically significant because PAF selectively induces an eosinophilic dermal infiltrate in allergic, but not in non-allergic, individuals (14). In fact, PAF is the only mediator in the lung known to cause a prolonged increase in bronchial hyper-responsiveness (15). The degree of bronchial hyper-responsiveness appears to be related to the intensity of the late-phase, therefore this association may be mediated in part by PAF. Weiss and associates reported that a histamine releasing factor (HRF) derived from platelets can also induce a LPR (16). It is not known whether all of these agents reported to cause late-phase cutaneous reactions do so by the same mechanisms. It is certainly possible that there may be several operative mechanisms by which both the skin and the airways exhibit bi-phasic responses, which may be why it has been difficult to identify a single inflammatory cell or a single mediator as the critical factor for LPR development.

The skin mast cell and the peripheral blood basophil

Mast cells, like their basophilic peripheral blood counterparts, have high affinity receptors for IgE (Fc_ε RI) and are involved in IgE dependent allergic reactions. Mast cells and basophils are similar in that they both contain histamine and proteoglycan in their granules and both cells stain metachromatically with aniline dyes. Basophil might share a common precursor with the eosinophil and other leukocytes (17, 18). Unlike basophils, mast cells do not circulate in the blood as mature cells, instead they mature in the dermis following the deposition of blood-born precursors in the dermal tissue. Using a mast cell-deficient mouse model that genetically lacked the c-kit receptor, Kitamura's group demonstrated that the mast cell phenotype was dependent on local tissue factors (19). For instance, if mast cells were transplanted into the skin of one of these mast cell-deficient mice, the mast cells assumed the phenotype characteristic for that organ (e.g. skin, lung, or gut). Thus, local cytokine and growth factors produced by dermal stromal cells could cause the functional heterogeneity of mast cells

observed when comparing skin mast cells with mast cells of the lung and the gut.

Although both the lung and the skin mast cell respond to anti-IgE, only skin mast cells respond to opioids (codeine and morphine) and neuropeptides (substance P, vasoactive intestinal peptide, and somatostatin). The response of the skin mast cell to these neuropeptides is probably a major non-immunological stimulus in the skin. Indeed, the triple response of Lewis is neurally mediated because it can be blocked by local infiltration with lidocaine anesthetic resulting in a blockade of the antidromic transmission of impulses from the dermal unmyelinated C fibers. This unique effect of neuropeptides on the skin mast cell is reciprocal in that the neurotransmitter substance P, by inducing histamine release from mast cells, is also a potent stimulus for the wheal and flare response. This coincides with the finding that the skin mast cell is located in the dermis where it can interface with neurohumoral factors (perineuronally distributed) and where it can respond to allergens (perivascular superficial blood vessels). Support for a link between the skin mast cell and neuropeptides comes from the observation that depletion of cutaneous neuropeptides with topical capsaicin attenuates both heat and cold induced urticaria which are disorders of the mast cell (20). The ability of the skin mast cell to release mediators in response to neuropeptide stimulation favors a neuroimmune interaction within the skin. A two-way communication has been postulated to exist between skin mast and the sensory nerves. The ability of neuropeptides to activate a rapid release of histamine from the skin mast cell provides a mechanism by which neuropeptides might mediate a direct influence on the skin mast cell resulting in a variety of functions including cutaneous blood flow, angiogenesis, and fibroblast activation (21). The skin mast cell is uniquely positioned to respond to locally invading antigens, whereas the peripheral blood basophil is a circulating cell which has the potential to target the distant site of allergic inflammation.

The neutral proteases tryptase and chymase are present in skin mast cells, whereas basophils contain very little, if any, tryptase or chymase activity. All mast cells contain tryptase, but the skin mast cell is unique in that it contains both neutral proteases, whereas the lung and gut mast cells lack chymase activity (22). Consequently, human mast cells are divided into two groups: those containing only tryptase (T mast cells) and those containing both tryptase and chymase (TC mast cells). Like histamine, tryptase and chymase are stored preformed in the mast cell granule and their release can be triggered by both immuno-

logic and non-immunologic stimuli. Neutral proteases have been identified in the peripheral blood several hours after an anaphylactic episode, and thus, unlike histamine, which is rapidly degraded by a first pass through the body, they can be clinically useful.

Mast cells, including skin mast cells, can also be distinguished from basophils according to prostaglandin D_2 (PGD_2) production (23, 24). The human mast cell releases approximately $60 \text{ ng}/10^6$ of PGD_2 , whereas the basophil does not synthesize this prostanoid. This observation prompted Naclerio to implicate the basophil as the source of late-phase histamine release because while both histamine and PGD_2 are released during the acute response to antigen challenge, in the late phase only histamine is released (25). Studies using purified skin mast cells incubated with radiolabelled arachidonic acid and stimulated with either calcium ionophore A23187 or anti-human IgE demonstrated that PGD_2 is the major cyclo-oxygenase product and that LTC_4 is the major lipoxygenase product, albeit in small amounts (25). Similar results were obtained with vasoactive intestinal peptide (VIP), somatostatin, morphine and compound 48/80 as the mast cell stimulus (26). When the skin mast cell was compared with the lung mast cell, the latter produced nine times more leukotrienes that did the skin mast cell, suggesting heterogeneity of eicosanoid production between different populations of mast cells (25). Thus, the major product of arachidonic acid metabolism from the skin mast cell is PGD_2 , and this mediator further distinguishes the mast cell from the basophil. Lawrence and his colleagues compared the responses of enzymatically dispersed mast cells from adult human skin and neonatal foreskin to both temperature and secretagogues (24). The adult skin mast cell exhibited maximal release at 23°C to 30°C , whereas neonatal foreskin responded best at 37°C . This is further evidence that the microenvironment plays a major role in determining how the mast cell responds. It is noteworthy that the adult skin mast cell was 10-fold less sensitive to anti-human IgE than was the neonatal mast cell, however, the two cell types responded equally to morphine sulfate, substance P, compound 48/80, f-Met peptide, and C5a (24).

As indicated above, neuropeptides cause a wheal and flare response in the skin, in part by the release of histamine from the skin mast cell. These peptides do not cause histamine release from basophils. The phospholipid mediator, PAF, causes neutrophilic leukocytes, including basophils, to undergo an oxidative burst and secrete their primary and secondary granules. After intradermal injection, PAF causes a wheal and flare response very similar

to that induced in the skin by neuropeptides such as substance P. Platelet activating factor neither activates mast cells *in vitro* nor potentiates anti-IgE-, substance P-, nor A 23187-induced histamine release (27). Thus, it appears that the basophilic leukocyte but not the human skin mast cell is activated by PAF. Nevertheless, PAF is a potent chemotactic factor for eosinophils and contributes to the late inflammatory events following antigen challenge.

The foregoing discussion clearly shows that skin mast cells and peripheral blood basophils have distinct differences (see Table 1). The release of chemical mediators from these cells is mediated by activation of high affinity IgE receptor; however there are non-specific factors that control histamine release and this is known as "releasability". Furthermore, basophil releasability and mast cell releasability are independently controlled (28, 29). Marone and his colleagues have shown that basophil releasability to IgE-mediated stimuli is increased in asthmatic subjects when compared to subjects with allergic rhinitis; however, this *in vitro* basophil releasability did not correlate with *in vivo* skin mast cell releasability (29). This same group of investigators demonstrated that basophils from atopic dermatitis patients released significantly more histamine, both spontaneously and in response to anti-IgE or D_2O , than did controls (30). Therefore, releasability of basophils to antigen, anti-IgE, and D_2O appear to be markers for atopy and this phenomenon does not appear to extend to the skin mast cell.

The above discussion indicates that, although both the skin mast cell and the basophil contain histamine as a preformed mediator and both have Fc_ϵ RI, these two cell types are clearly distinct (see Table 1). The mast cell releases PGD_2 , whereas the basophil releases LTC_4 in response to anti-IgE *in vitro*. Glucocorticoids exert a specific inhibitory effect on the basophil when cultured overnight with dexamethazone, whereas the mast cell appears to be refractory to this effect (31). Unlike the mast cell, the basophil has H_2 receptors on its cell surface and, when exposed to a histamine H_2 agonist such as dimaprit, basophil histamine release is inhibited whereas the mast cell does not appear to have H_2 receptors (32). This has certain practical considerations since there is a theoretical possibility that H_2 antagonists alone might actually promote histamine release from the basophil unless the patient is also H_1 -receptor blocked. The skin mast cell is a resident fixed tissue cell and the basophil is a circulating leukocyte which must be attracted to the site of an allergic reaction. Despite their remarkable similarities, mast cells and basophils

Table 1. Properties of the basophil and the mast cell

	High affinity IgE receptor	Major product of Arachidonic acid	Major basic protein	Charcot-Leyden crystal	H ₁ receptor	H ₂ receptor
BASOPHIL	Positive	LTC ₄	Positive	Positive	Positive	Positive
MAST CELL	Positive	PGD ₂	Negative	Negative	Positive	Negative

are distinct cells and the basophil has more in common with the eosinophil than it does with the mast cell. In fact, the eosinophil is characterized by the presence of major basic protein (MBP) and Charcot-Leyden crystal protein (CLC), both of which can be found in basophils in diminished amounts but not in the cutaneous mast cell (33). The early immediate response of the allergic reaction is mainly mast cell-mediated, whereas the basophil, eosinophil, neutrophil, and lymphocyte all combine to cause the late-phase response.

The skin as a model to study allergic disease

Because of its easy accessibility, the skin is widely used to study the events surrounding the late-phase response to antigen. This accessibility prompted early investigators to perform routine biopsies of LPR skin test sites to learn more about the pathogenesis of the LPR. In early studies investigators interested in cutaneous inflammatory events used the Rebuck skin window, but this does not provide a means with which to monitor cells and mediators over time. Recently, investigators have been applying newer methods to study the LPR: lymphocyte subset analysis, specific monoclonal antibodies, and, more recently, the use of messenger RNA probes to identify the production of minute quantities of directed proteins.

The skin chamber model affords the opportunity to study both cutaneous mediators and cellular traffic in a dynamic and controlled fashion. This simple model consists of adfixing a chamber to the base of an unroofed heat/suction induced blister. This method was first described in the dermatology literature by Blank in 1950 but it was not reported to have been used by others until 18 years later when another dermatologist, Kiistala, used this method to study dermal inflammatory events (34, 35). Zweiman's group deserves the credit for modifying this skin model in their study of pathophysiologic mechanisms of allergic disease and our group has used the skin chamber to study the late-phase events following a single high dose antigen challenge (37).

The preceding discussion has focused primarily on the mast cell and the basophil as the two cell

types responsible for the release of histamine in the early and late-phase response to antigen, respectively. However, the late events following antigen challenge are complex and one cannot define the LPR in the context of a single cell type. The events that take place after antigen activation of the mast cell are complex and include a panoply of cells, cytokines, and mediators (Fig. 1). Attention has focused on the role of the endothelial cell as the door by which inflammatory cells enter the site of the allergic reaction and much has been learned regarding the upregulation of adhesion molecules on the endothelial cell surface and their respective counter ligands on the infiltrating inflammatory cells. The remainder of this chapter will be devoted to these aspects of the cutaneous LPR in greater detail.

Cellular composition of the LPR

There is general agreement that the reaction underlying the cutaneous LPR is a polymorphic inflammatory infiltrate consisting of neutrophils, eosinophils, lymphocytes and, in some reports, basophils (4, 5, 10, 37). As shown in Fig. 1, antigen cross linking of mast cell IgE is ultimately responsible for the involvement of eosinophils, neutrophils, basophils, and lymphocytes during the LPR. We have demonstrated the presence of basophils in the cutaneous LPR with both skin biopsies and skin chamber cytology (38). Neutrophils were numerically the most prominent cells but they were also noted in control biopsies in response to non-specific trauma. Wershil et al. showed that recruitment of neutrophils during the IgE dependent cutaneous LPR in the mouse is mast cell dependent and is partially inhibited with anti-TNF- α (39). Eosinophils were six-fold greater in the antigen biopsies than in the control biopsies and appeared to be critical to the development of the LPR in our study and an important component of the LPR. Leiferman and associates have studied the time course for the deposition of extracellular major basic protein (MBP) after antigen and found that by the third hour there is extensive deposition of both MBP and neutrophil elastase in the dermis (40). Their data suggest that eosinophils come in early and that the deposition of cationic proteins

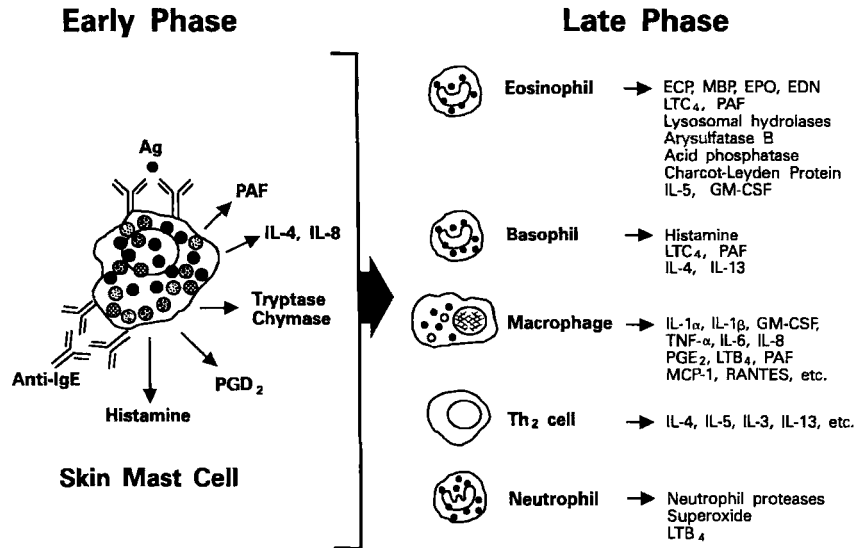


Fig. 1. Diagram of events surrounding antigen induced hypersensitivity reactions which culminate in the late-phase response. The mast cell is activated by antigen cross-linkage of the IgE receptor resulting in the release of preformed histamine and tryptase. Factors which are not completely understood ultimately result in the release of various cytokines culminating in an inflammatory cell infiltration of eosinophils, neutrophils, basophils, and lymphocytes.

persist for up to 56 hours. In collaboration with Leiferman's group, we measured MBP in skin chamber fluids. Patients with the greatest number of eosinophils appeared to have the most cationic protein in the chamber fluids (Fig. 2). We were unable to consistently detect cationic proteins in the chamber fluids of all patients, probably because of binding of the cationic proteins with the heparin placed in the chamber fluids to avoid the deposition of a fibrin clot at the blister base. Zweiman et al. examined chamber fluids for MBP and eosinophil-derived neurotoxin (EDN) in similar chambers which were challenged with a continuous pollen and they observed elevation of both MBP and EDN from the second to the fifth hour and that the levels of these cationic proteins correlated with the histamine release during the first hours (41).

Many of the previous histologic studies have been divided on whether or not basophils were present in the skin during the LPR. Basophils were quantitatively less than the eosinophils but they were clearly increased in our antigen challenged chambers (38). A variety of factors hinder the identification of basophils in biopsy specimens, among which an abundance of degenerating cells that obscure the cellular field and the lack of a specific stain that reliably discriminates between the two metachromatic cell types. In addition, the number of infiltrating basophils in the LPR may depend upon the antigen concentration and/or the duration of challenge. There appears to be no question that basophils do indeed infiltrate the cutaneous LPR and we have shown that the

number of basophils influxing into skin chambers during the LPR significantly correlates with the amount of histamine released into the skin chambers during the same time (38). Using quantitative immunohistology, Frew and his colleagues have shown an increased number of CD4⁺ T-cells of the memory T-cell phenotype (CD45RO⁺) in cutaneous LPR biopsies which correlate with the number of activated eosinophils (42). The lymphocyte could be the main cell type that orchestrates the events leading to the LPR by the production of cytokines.

It is significant that only the eosinophil and the basophil are selective for the antigen chamber, suggesting that these two cell types are preferentially recruited to the late-phase (38). In addition, we frequently note eosinophils in cytologic preparations that displayed a vacuolated appearance consistent with "activated" hypodense eosinophils. It can be concluded that these activated eosinophils may significantly contribute to the pathology observed in the clinical LPR since these cells have been shown to have increased metabolic activity leading to enhanced production of LTC₄ and release of cationic proteins (43). In addition to their well known cytotoxic effects, the eosinophil cationic proteins (particularly the major basic protein) may play a direct role in enhancing basophil and mast cell activation.

Inflammatory mediators of the LPR

Zweiman and associates demonstrated a rapid rise of histamine into the skin chamber, this peaks by

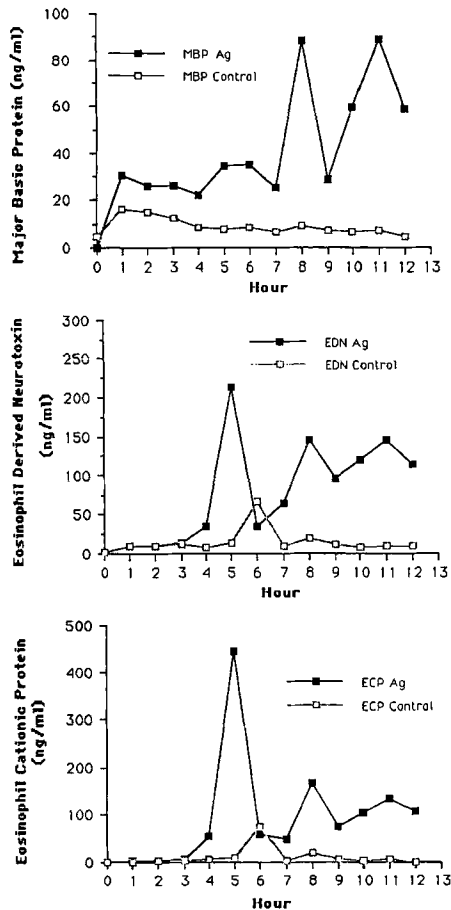


Fig. 2. Major basic protein recovered from the skin chamber following a single high dose antigen challenge to the skin chambers.

30 minutes and is followed by a decline over the subsequent four hours (37). They also showed that the total amount of histamine released during this initial phase correlated significantly with the intensity of the subsequent LPR as measured by skin testing (44). Shalit et al. extended these studies to examine mediator release after a continuous antigen challenge, the rationale being that natural exposure to antigen in allergic subjects occurs over a protracted period of hours or even days (45). In response to a continuous antigen challenge, they found that both histamine and mast cell-derived tryptase were abruptly released, followed by a complete return to baseline values of tryptase but not of the histamine. Interestingly, they were able to show a continuous release of histamine over the six hour observation period. The source of this continued release of histamine remains obscure; however, since tryptase declined, it is most likely the basophil is the source of the histamine since the basophil contains no tryptase. Alternately, there might be more than one mechanism for the release of these two pre-formed mediators; how-

ever, as yet there is no evidence to support this hypothesis.

By using the skin chamber model to study the LPR for an extended twelve hour period, we demonstrated a second rise of histamine which is analogous to that reported as occurring in the airways (38). We found an initial peak of histamine (13.2 ± 2.9 ng/ml) into the chamber during the first hour. The histamine then declined to approximately 2 ng/ml during the next six hours. This was followed by a slow, but persistent, rise which culminated in almost 10 ng/ml of histamine being released during the twelfth hour. Subsequent experiments have shown that this second rise in histamine usually peaks at hour eleven or twelve and is followed by a slow decline to baseline. The difference in time kinetics between the early and the late histamine release suggests that the basophil, rather than the mast cell, may be the source of this late-phase histamine. The early pattern of PGD_2 production, without late production, would tend to support the thesis that the late-phase histamine originates from the basophil since this cell does not produce PGD_2 . This second rise in histamine, previously unobserved, was not evident in earlier studies because the studies were not extended long enough. It is also likely that LPR histamine may be dependent upon the antigen dose of the challenge and the Johns Hopkins group consistently uses a high challenge dose. Furthermore, this late rise in histamine contributes to the pruritus and tissue swelling observed in the skin, and in the lung this late rise in histamine contributes to bronchoconstriction.

Mediators other than histamine and prostanoids may also play a role in orchestrating the events in the late-phase. Using the skin chamber model, Talbot et al. have shown that LTC_4 is released in response to an antigen challenge, peaking between second and fourth hours (46). The kinetics of LTC_4 production in their study revealed considerable variability among their subjects without the predictable peaks or release patterns of either histamine or PGD_2 . We have examined LTC_4 production in response to antigen and have noted it to be elevated both early and late; however, there was marked intrasubject variability. Furthermore, when our subjects were treated with 60 mg of prednisone for the three days preceding the study, there was significant suppression of LTC_4 that correlated with the suppression of eosinophils. These data suggest that the majority of the leukotrienes recovered from late-phase blister fluid emanates from the eosinophil. Undoubtedly, this group of mediators may play a role in the LPR in the airways given their ability to cause bronchocon-

striction in very small amounts. It is likely that production of LTC₄ by activated eosinophils, in concert with other mediators, significantly contributes to clinical late-phase. LTB₄ has not been shown to be released into the skin chambers in response to a single high dose antigen challenge. However, LTB₄ can be detected in skin chambers which have been challenged with a continuous low dose of antigen, again suggesting that there may be different mechanisms involved in these two challenge models (47). One will recall that the pattern of mediator release detected in the skin chamber may not fully reflect the kinetics of local tissue production and that very small concentrations of a given mediator may have a profound effect on the local tissue milieu immediately surrounding their production.

One of the more interesting mediators and one which may play a critical role in the LPR response is PAF. Shalit et al. recently recorded the presence of PAF in skin chambers in 74% of the allergic subjects challenged with a continuous low dose antigen (47). PAF was first detected during the third hour and production continued for nine hours following antigen challenge. It has been demonstrated that PAF can cause release of LTC₄ from eosinophils and it has been proven to be one of the most potent chemotactic factors known for eosinophils (48). Since eosinophils have low-affinity receptors for IgE, it is conceivable that antigen may activate eosinophils through this low affinity receptor and cause the production and release of PAF into the surrounding tissues. Fibrin has been described as a component of eosinophils, in concert with other mediators, and it significantly contributes to clinical late-phase. LTB₄ has not been shown to be released into the skin chambers in response to a single high dose antigen challenge. However, LTB₄ can be detected in skin chambers which have been challenged with a continuous low dose of antigen, again suggesting that there may be different mechanisms involved in these two challenge models (47). One will recall that the pattern of mediator release detected in the skin chamber may not fully reflect the kinetics of local tissue production and that very small concentrations of a given mediator may have a profound effect on the local tissue milieu immediately surrounding their production.

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Numerous other mediators may play a role in the LPR in both the skin and the airway. One exciting area which may prove pivotal in our understanding of the LPR is the role played by cytokines. Bochner and Charlesworth et al. have shown that IL-1_β is increased in late-phase skin blister fluids (50). While the exact role which IL-1 might play in the cutaneous LPR is uncertain; IL-1 has many biological properties which could up-regulate and magnify the intensity of the LPR. Certainly IL-1 has been shown to stimulate the adherence of leukocytes to vascular endothelium by the induction of specific surface proteins such as ELAM-1, endothelial leukocyte adherence molecule 1 (51, 52). IL-1 stimulation of endothelial cells promotes the adherence of eosinophils, basophils, and neutrophils, all of these cells having been associated with the LPR. Although IL-1 is produced primarily by mononuclear cells, many other cell types, including the mast cell, can produce this cytokine. There is evidence from several groups that IL-1 might enhance basophil histamine release. This may help explain our observations of increased histamine, increased basophils, and increased IL-1 in our late-phase skin chamber studies (53, 54). Corticosteroids are known to be specific for the late-phase and they have no effect on the early events. Since corticosteroids inhibit both the LPR and IL-1 production, it is tempting to speculate that they might act in part by blocking IL-1 production.

Several cytokines contribute to the complex events in the LPR and this area of ongoing research will probably yield further information about the LPR. Our group next looked for the presence of IL-6 using the skin chamber technique because this cytokine shares many of the proinflammatory properties of IL-1 (37). Fig. 3 shows the pattern of

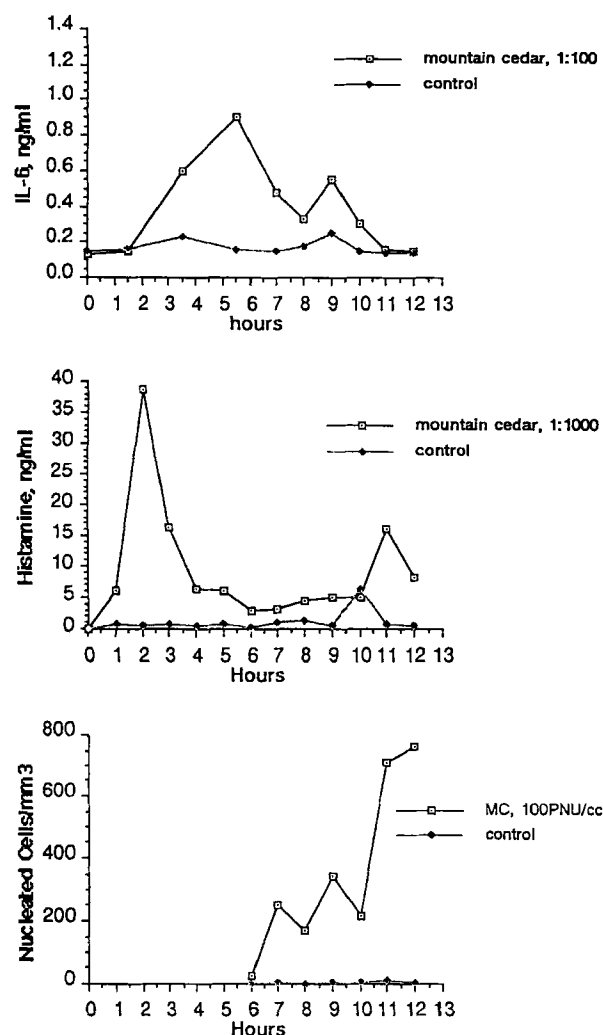


Fig. 3. Results of a single experiment in which IL-6 was measured using a radioimmunoassay, showing a biphasic release of IL-6 (top) and histamine (middle). The late IL-6 and histamine occurred during the inflammatory phase of the LPR in which there was an influx of nucleated cells into the skin chambers (bottom).

IL-6 release in a single patient. There is a biphasic release of both histamine and IL-6; however, some subjects may show a single early release, whereas a continuous monomorphous release is observed in others. We extended these studies to look for IL-8 and IL-8 was clearly elevated in the antigen-stimulated chambers and to a lesser degree in the control chambers (unpublished data). Interestingly, human mast cells have been shown to produce IL-8 and it may be that antigen stimulation of mast cells account for the IL-8 in the antigen stimulated chambers (56). Others have shown that human mast cells may also be a source of IL-4, IL-5, and IL-6, thus contributing to the thesis that the mast cell releases products other than histamine and arachidonic acid metabolites (57). Fig. 4 shows the presence of TNF- α , GM-

CSF, and IL-4 in an allergic subject who was unique in demonstrating a rather marked biphasic skin test reactivity and biphasic histamine release (58). TNF- α was elevated in four of seven subjects and IL-4 was marginally elevated in all of the subjects. Interestingly, total TNF- α was significantly correlated with the early IL-4 ($r_2=0.88$, $P=0.001$) (54). Our inability to consistently show the presence of GM-CSF may be the result of the sensitivity of the ELISA assay used since Massey and associates clearly demonstrated the presence of GM-CSF mRNA at allergen-challenged cutaneous LPR (59). The importance of these cytokines in the orchestration of the allergic inflammatory response is further being characterized by the detection of mRNA for specific cytokines (60). There is compelling evidence for ribosomal message for Th₂ lymphocytes and it appears that immunotherapy may result in the upregulation of Th₁ cytokines such as IFN- γ and IL-2 (60). More recently, it has been shown that the predominant lymphocyte found in the bronchoalveolar lavage fluid of asthmatic patients is also of the Th₂ phenotype favoring the production of IL-4, IL-5, and IL-6 (61). This is an area of research that will continue to unfold and it offers tremendous possibilities for the understanding and treatment of allergic disease.

We have already alluded to the fact that basophils appear to track to the site of late-phase inflammation in conjunction with the eosinophil. Many of the above cytokines have significant biological effects on eosinophils that may contribute to disease activity. Adhesion and migration of eosinophils and basophils are integral to cell recruitment and activation in the inflammatory response. Activated endothelial cells express several adhesion molecules that belong to different adhesion families, including ELAM-1 (selectins), ICAM-1 and VCAM-1 (immunoglobulin superfamily). Cytokines, including IL-1 and TNF- α can induce the synthesis and expression of specific adhesion molecules (e.g. ELAM-1) on the endothelial cell surface. Leung and coworkers studied the inducibility of ELAM-1 in ragweed- or dust mite-challenged skin using serial biopsies taken between 20 minutes and 24 hours after an intra-dermal allergen challenge (62). These investigators found that ELAM-1 was expressed concurrently with the inflammatory infiltrate and that the expression of ELAM-1 could be blocked with antibodies to IL-1 and TNF- α (62). To address the question as to whether the cells infiltrating the site of antigen challenge from the peripheral blood were responsible for the production of IL-1 and TNF- α , these investigators used *ex vivo* skin explants and demonstrated convincingly that these

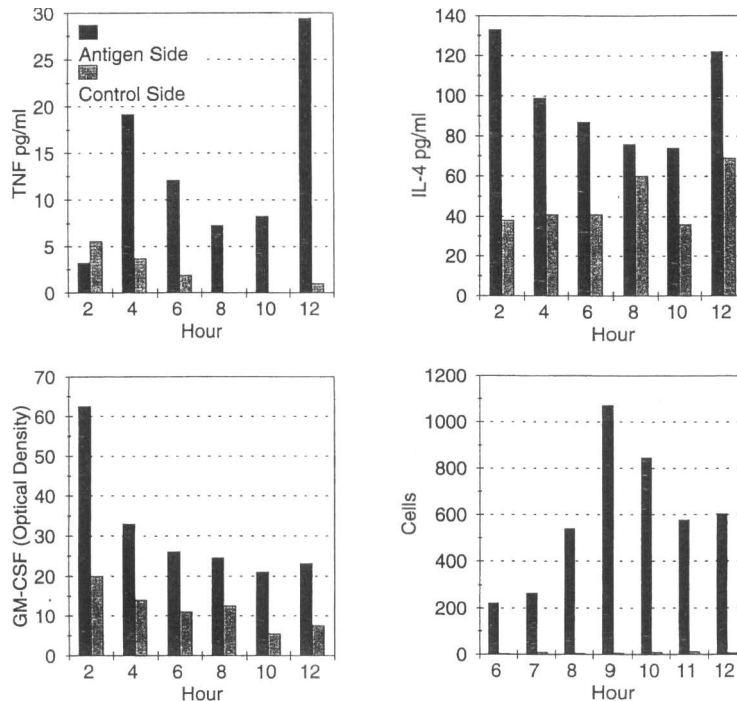


Fig. 4. The same subject as in Fig. 3 was studied in a similar but separate experiment for the presence of TNF- α , GM-CSF, and IL-4 using a commercial ELISA kit. All three cytokines were elevated in a biphasic pattern.

two cytokines were being produced by resident skin cells. It is becoming clear that the mast cell is capable of producing several cytokines including IL-1, TNF- α , IL-3, IL-4, IL-5, IL-6, and GM-CSF (63). It is also very likely that the mast cell is responsible for the cytokines released after antigen activation of the skin mast cell and this is consistent with work by Klein et al. who reported ELAM-1 within two hours of anti-IgE of skin organ cultures (63). It has been further shown in a primate asthma model that antibodies to ELAM-1, but not to ICAM-1, markedly reduce the neutrophil influx and late-phase bronchoconstriction (64). Other *in vitro* studies have demonstrated a preferential selectivity of eosinophil and basophil binding to the VCAM-1 receptor on endothelial cells by the VLA-4 counter receptors (65–67). It is becoming increasingly clear that events take place following activation of the Fc ϵ on the mast cell which result in a multiplicity of cytokines thus favoring the Th $_2$ lymphocyte phenotype. This ultimately results in a differential upregulation of endothelial cell adhesion molecules and their respective counter ligands on the infiltrating inflammatory cells. Future treatment modalities using monoclonal antibodies or specific receptor blockers may prevent or attenuate the inflammatory aspects of allergic disease and the next section of this chapter will briefly focus on pharmacologic modulation of the LPR.

Pharmacologic modulation of the cutaneous LPR

Pipkorn et al. examined the effect of short-term corticosteroids on the pattern of nasal mediator release and found that steroids block the late-phase release of histamine but had no effect on the early release of histamine (68). Similar studies using the skin chamber model showed the same selective late-phase suppression of histamine as previously observed by Pipkorn in the nose (69). Not only was the late release of histamine ablated, but the influx of eosinophils and basophils into the skin chambers was also blocked. This ablation of late-phase histamine release and basophil influx into the skin chambers correlates with *in vitro* studies demonstrating that dexamethasone inhibits anti-IgE-induced histamine release from the basophil but not from the mast cell. Again, this suggests a role for the basophil in the LPR and that systemic steroids are capable of selectively blocking both the release of histamine and the accumulation of basophils (70). These studies have also shown a selective blockade of eosinophil infiltration into the site of the LPR and part of the clinical effects of corticosteroids may relate to their attenuating cytokine production. These observations are not surprising as clinicians have long known that systemic steroids are capable of attenuating the late symptoms of both asthma and allergic rhinitis while

having little effect on the immediate reaction to antigen. With the use of topical steroids delivered to the airways, clinical allergists are now able to minimize the side-effects associated with systemic steroids and still attain relief of symptoms during the late-phase.

Recently, certain of the newer antihistamines have been shown to inhibit late-phase eosinophil migration in the skin and this may have important implications in the treatment of airway allergies. Fadel et al. demonstrated that cetirizine blocks the migration of eosinophils onto a skin window twenty-four hours after antigen challenge, a phenomenon not explained by H_1 blockage (71). These studies were enlarged upon by our group and we were able to show suppression of LPR cellular traffic using the skin chamber model as observed in Fig. 5 (73). In these studies we showed that eosinophil infiltration was decreased by 68%, while the number of neutrophils were reduced by a similar magnitude. Basophils were also decreased by 64% for hours six through eight. It appears that cetirizine influences the pathogenesis of the LPR by causing a significant reduction in the inflammatory cell infiltrate and this drug may prove clinically efficacious in treating late-phase symptoms. Although we were not able to confirm that cetirizine exerted an effect unique to the eosinophil, these studies confirm that the suppression of late-phase inflammatory cells may be unique to cetirizine since this effect was not observed with either promethazine or chlorpheniramine. It therefore appears that some antihistamines may have effects which cannot be explained solely by their H_1 receptor antagonism and some of these selected drugs may have properties which make them useful for treating the symptoms of the late-phase. The pharmacologic modulation of the cutaneous LPR continues to be an active part of research and it is predicted that the skin model will lead to a greater understanding of the complex events surrounding the late-phase response.

A report by Alam and his colleagues suggests that misoprostol might have a selective inhibitory effect on the cutaneous LPR (73). Like cetirizine, misoprostol inhibited the dermal infiltration of eosinophils and to a lesser extent neutrophils in skin biopsies obtained at 5 hours. This group then showed that this prostaglandin E analog inhibited *in vitro* eosinophil chemotaxis while inhibiting the production of GM-CSF by lymphocytes, but without having an effect on the immediate wheal and flare response to antigen. If these results are confirmed, it might suggest that a prostaglandin E analog should be counted among drugs such as cetirizine and corticosteroids as drugs that have antiallergic properties.

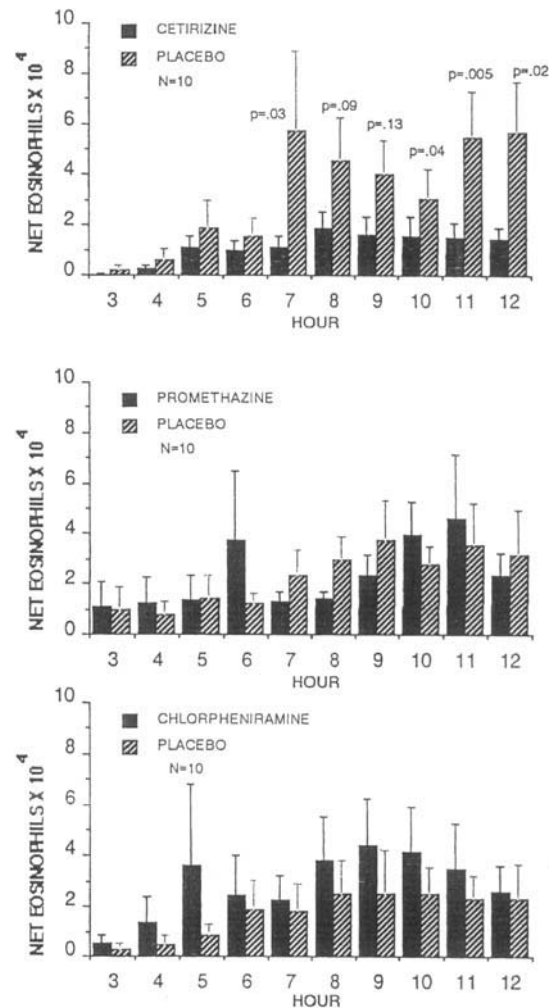


Fig. 5. Ten different allergic groups were studied using the skin chamber technique to examine the effect of three antihistamines (cetirizine, promethazine, and chlorpheniramine) on cells and mediators in the LPR to antigen. Although there was no alteration of either early or late histamine release, there was a decrease in the inflammatory infiltrate into the skin chambers. This figure only shows the effect on eosinophils for cetirizine (top), promethazine (middle), and chlorpheniramine (bottom).

Concluding remarks

Undoubtedly the study of early and late phase skin reactions in response to antigen challenge *in vivo* has helped to unravel some basic aspects of the pathophysiology of allergic reactions. In particular, this model has markedly contributed to a better understanding of the complex immunological and biochemical interactions between chemical mediators and inflammatory cells during cutaneous late phase reactions. Some of this information has been of paramount importance in understanding similar late phase reactions occurring in less accessible models such as lung parenchyma. The skin model has produced a tremendous amount of novel infor-

mation concerning the role played by adhesion molecules in inflammatory cell trafficking from peripheral blood into the inflamed skin site. It has also shaped our understanding of the chemical mediators produced during early- and late-phase allergic reactions. The pattern and kinetics of preformed and *de novo* synthesized mediators released by activated skin mast cells have been elucidated. More recent studies are starting to provide insights into the complex pattern of cytokine production during skin allergic reactions. A final, intriguing aspect that is attracting a lot of attention is the possibility of pharmacologically controlling the early- and late-phase reactions. For instance, studies along these lines have revealed unexpected antiinflammatory properties of some newer antihistamines such as cetirizine. It appears that the pharmacologic modulation of the cutaneous LPR will continue to be a promising area of investigation.

References

- Blackley CH. Hay fever: its causes, treatment, and effective prevention. In: Experimental Researches. 2nd ed. London: Tindall and Cox, Ltd, 1873;77-89.
- Cooke RA. Studies in specific hypersensitiveness. IX. On the phenomenon of hypersensitization (the clinically lessened sensitiveness of allergy). *J Immunol* 1922;6:219-42.
- Pepys J. Hypersensitivity diseases of the lung due to fungi and organic dust. In: Kallos P, Inderbitzin TM, Miescher PA, Waksman BH, eds. Monographs in Allergy. Vol IV. Basel: S. Kauger, 1969:22-68.
- Dolovich J, Hargreave FE, Chalmers R, et al. Late cutaneous allergic responses in isolated IgE-dependent reactions. *J Allergy Clin Immunol* 1973;52:38-46.
- Solley GO, Gleich GJ, Jordon RE, et al. Late phase of the immediate wheal and flare skin reactions. Its dependence on IgE antibodies. *J Clin Invest* 1976;58:408-20.
- Hexheimer H. The late bronchial reaction in induced asthma. *Int Arch Allergy Appl Immunol* 1952;3:323-7.
- Robertson DG, Kerigan AT, Hargreave FE, et al. Late asthmatic responses induced by ragweed pollen allergen. *J Allergy* 1974;54:244-54.
- Warner JO, Soothill JF, Price JF, et al. Controlled trial of hyposensitization to *Dermatophagoides pteronyssinus* in children with asthma. *Lancet* 1978;912-7.
- Cartier A, Thompson NC, Frith PA, et al. Allergen induced increase in bronchial responsiveness to histamine: relationship to the late asthmatic response and change in airway caliber. *J Allergy Clin Immunol* 1982;70:170-7.
- Atkins P, Green GR, Zweiman B. Histologic studies of human skin test responses to ragweed, compound 48/80 and histamine. *J Allergy Clin Immunol* 1973;51:263-73.
- Soter NA, Lewis RA, Corey EJ, et al. Local effects of synthetic leukotrienes (LTC₄, LTD₄, LTE₄ and LTB₄) in human skin. *J Invest Dermatol* 1983;80:115-9.
- Basran GS, Page CP, Paul W, et al. Platelet-activating factor: a possible mediator of the dual response to allergen? *Clin Allergy* 1984;14:75-9.
- Michel L, Mencia-Huerta J, Benveniste J, et al. Biologic properties of LTB₄ and PAF-acether *in vivo* in human skin. *J Invest Dermatol* 1987;88:675-81.
- Henocq E, Vargaftig BB. Skin eosinophilia in atopic subjects. *J Allergy Clin Immunol* 1988;81:691-5.
- Barnes PJ. New concepts in the pathogenesis of bronchial hyperresponsiveness and asthma. *J Allergy Clin Immunol* 1989;83:1013-26.
- Weiss M, Fisher RH, Kagey-Sobotka A, et al. *In vivo* response to platelet derived histamine releasing factor. *J Allergy Clin Immunol* 1988;81:224.
- Dvorak AM, Ishizaka T, Galli SJ. Ultrastructure of human basophils developing *in vitro*. Evidence for the acquisition of peroxidase by basophils and for different effects of human and murine growth factors on basophil and eosinophil maturation. *Lab Invest* 1985;53:57-71.
- Denburg JA, Richardson M, Telizyn S, Bienenstock J. Basophil/lung mast cell precursors in human peripheral blood. *Blood* 1983;61:775-80.
- Kitamura Y, Nakayama H, Fujita J. Mechanism of mast cell deficiency in mutant mice of the W/W^v and SI/SId genotype. In: Galli SJ, Austen KF, eds. Mast cell and basophil differentiation and function in health and disease. New York: Raven Press, 1989:15-25.
- Toth-Kasa I, Jancso G, Obal F, Husz S, Simon N. Involvement of sensory nerve endings in cold and heat urticaria. *J Invest Dermatol* 1983;80:34-6.
- Benyon RC. Review: the human skin mast cell. *Clin Exper Allergy* 1989;19:375-87.
- Schwartz LB, Irani AM, Roller K, Castells M, Schechter NM. Quantitation of histamine, tryptase, and chymase in dispersed human T and CT mast cells. *J Immunol* 1987;138:2611-5.
- Holgate ST, Burns GB, Robinson C, et al. Anaphylactic and calcium dependent generation of prostaglandin D₂ (PGD₂), thromboxane B₂ and other cyclooxygenase products of arachidonic acid by dispersed human lung cells and relationship to histamine release. *J Immunol* 1984;133:2138.
- Lawrence ID, Warner JA, Cohan VL, et al. Purification and characterization of human skin mast cells: evidence of human mast cell heterogeneity. *J Immunol* 1987;139:3062-9.
- Naclerio RM, Proud D, Togias AG, et al. Inflammatory mediators in late antigen-induced rhinitis. *N Engl J Med* 1985;313:65-70.
- Robinson C, Benyon C, Holgate St, et al. The IgE and calcium dependent release of eicosanoids and histamine from human purified cutaneous mast cells. *J Invest Dermatol* 1989;93:397-404.
- Thomas G, Church MK. Platelet activating factor does not release histamine from human dispersed cutaneous mast cells. *Clin Exp Allergy* 1990;90:377-82.
- Casolaro V, Galeone D, Giacommo A, et al. Human basophil/mast cell releasability. V. Functional comparisons of cells obtained from peripheral blood, lung parenchyma and from bronchoalveolar lavage in asthmatic. *Am Rev Respir Dis* 1989;139:1375-82.
- Casolaro V, Spadaro G, Marone G. Human basophil releasability VI. Changes in basophil releasability in patients with allergic rhinitis or bronchial asthma. *Am Rev Respir Dis* 1990;142:1108-11.
- Marone G, Giugliano R, Lembo G, Ayala F. Human basophil releasability. II. Changes in basophil releasability in patients with atopic dermatitis. *J Invest Dermatol* 1986;87:19-23.
- Schleimer RP, Schulman ES, MacGlashan DW, et al. Effects of dexamethasone on mediator release from human lung fragments and purified human lung mast cells. *J Clin Invest* 1983;71:1830-5.
- Peters SP, Naclerio RM, Schleimer RP, et al. The pharmacologic control of mediator release from human basophils and mast cells. *Respiration* 1986;50:116-22.

33. Leiferman KM, Gleich GJ, Kephart GM, et al. Differences between basophils and mast cells: Failure to detect Charcot-Leyden crystal protein (lysophospholipase) and eosinophil granule major basic protein in human mast cells. *J Immunol* 1986;136:852-5.
34. Blank IH, Miller OG. A method for the separation of the epidermis from the dermis. *J Invest Dermatol* 1950;15:9-10.
35. Kiistala U, Mustakallio KK. Dermato-epidermal separation with suction. Electron microscopy and histochemical study of initial events of blistering on human skin. *J Invest Dermatol* 1967;48:466-77.
36. Ting S, Dunskey EH, Lavker RM, et al. Patterns of mast cell alterations and *in vivo* mediator release in human allergic skin reactions. *J Allergy Clin Immunol* 1980;66:417-23.
37. Zweiman B, Kornstein M, Atkins P. Immunohistologic studies of late cutaneous allergic reactions. *J Allergy Clin Immunol* 1987;79:169 (abstract).
38. Charlesworth EN, Hood AF, Soter NA, et al. Cutaneous late-phase response to allergen: mediator release and inflammatory cell infiltration. *J Clin Invest* 1989;83:1519-26.
39. Wershil BK, Wang Zhen-Sheng, Gordon JT, Galli SJ. Recruitment of neutrophils during IgE-dependent cutaneous late phase reactions in the mouse is mast cell-dependent. *J Clin Invest* 1991;87:446-53.
40. Leiferman KM, Fujisawa T, Gray BH, Gleich GJ. Extracellular deposition of eosinophil and neutrophil granule proteins in the IgE-mediated cutaneous late phase reaction. *Lab Invest* 1990;62:579-89.
41. Zweiman B, Atkins PC, von Almen C, Gleich GJ. Release of eosinophil granule proteins during IgE-mediated allergic skin reactions. *J Clin Invest* 1991;87:984-92.
42. Frew AJ, Varney VA, Gaga M, Kay AB. Immunohistology of human allergic late-phase skin reactions. *Skin Pharmacology* 1991;(4 suppl) 1:71-8.
43. Weller PF, Lee CW, Fost DW, et al. Generation and metabolism of 5-lipoxygenase pathway leukotriene by human eosinophils: predominant production of leukotriene C₄. *Proc Natl Acad Sci USA* 1983;80:7626-30.
44. Weiman B. Immunohistologic studies of late cutaneous allergic response in humans. I. Kinetics of histamine release. *J Allergy Clin Immunol* 1984;74:819-26.
45. Shalit M, Schwartz LB, Golzar N, et al. Release of histamine and tryptase *in vivo* after prolonged cutaneous challenge with allergen in humans. *J Immunol* 1988;141:821-6.
46. Talbot SF, Atkins PC, Goetzl EJ, et al. Accumulation of leukotriene C₄ and histamine in human allergic skin reactions. *J Clin Invest* 1985;76:650-6.
47. Shalit M, Valone FH, Atkins PC, et al. Late appearance of phospholipid plate-activating factor and leukotriene B₄ in human skin after repeated antigen challenge. *J Allergy Clin Immunol* 1989;85:691-6.
48. Tamura N, Agrawal DK, Suliaman, et al. Effects of platelet activating factor on the chemotaxis of normodense eosinophils from normal subjects. *Biochem Biophys Res Commun* 1987;142:638-44.
49. Chilton FH, Connell TR. 1-0-alkyl-2-arachidonoyl-sn-glycero-3-phosphocholine. A common source of platelet-activating factor and arachidonate in human polymorphonuclear leukocytes. *J Biol Chem* 1984;259:12014-9.
50. Bochner BS, Charlesworth EN, Kagey-Sobotka, et al. Interleukin-1 is released at sites of human cutaneous allergic reactions. *J Allergy Clin Immunol* 1990;86:830-9.
51. Schleimer RP, Rutledge BK. Cultured human vascular endothelial cells acquire adhesiveness for neutrophils after stimulation with interleukin 1, endotoxin, and tumor-promoting phorbol esters. *J Immunol* 1986;135:649-54.
52. Bevilacqua MP, Pober DL, Mendrick, et al. Identification of an inducible endothelial-leukocyte adhesion molecule. *Proc Natl Acad Sci USA* 1987;92:38-42.
53. Subramanian N, Bray MA. Interleukin 1 releases histamine from human basophils and mast cells *in vitro*. *J Immunol* 1987;138:271-5.
54. Haak-Frendscho M, Dinarello CA, Kaplin AP, et al. Recombinant human interleukin 1 beta causes histamine release from human basophils. *J Allergy Clin Immunol* 1988;82:218-23.
55. Lee CE, Neuland ME, Teaford HG, et al. Interleukin-6 is released in the cutaneous response to allergen challenge in atopic individuals. *J Allergy Clin Immunol* 1992;89:1010-20.
56. Moller A, Lippert U, Lessmann D, et al. Human mast cells produce IL-8. *J Immunol* 1993;151:3261-6.
57. Bradding P, Feather IH, Wilson S, et al. Immunolocalization of cytokines in the nasal mucosa of normal and perennial rhinitic subjects: The mast cell as a source of IL-4, IL-5, and IL-6 in human allergic mucosal inflammation. *J Immunol* 1993;151:3853-65.
58. Morris FM, Whisman BA, Charlesworth EN. Measurement of TNF- α , GM-CSF, & IL-4 in the cutaneous late-phase response to antigen. *J Allergy Clin Immunol* 1994;93:261 (abstract#593).
59. Massey W, Friedman B, Kato M, et al. Appearance of granulocytes-macrophage colony stimulating factor activity at allergen-challenged cutaneous late-phase reaction sites. *J Immunol* 1993;150:1084-92.
60. Varney VA, Hamid QA, Gaga M, et al. Influence of grass pollen immunotherapy on cellular infiltration and cytokine mRNA expression during allergen-induced late-phase cutaneous responses. *J Clin Invest* 1993;92:644-51.
61. Robinson DS, Hamid Q, Ying S, et al. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992;326:298.
62. Leung DYM, Pober JA, Cotran RS. Expression of endothelial-leukocytes adhesion molecule-1 in elicited late phase allergic reactions. *J Clin Invest* 1991;87:1805-9.
63. Klein LM, Lavker RM, Matis WL, et al. Degranulation of human mast cells induces an endothelial antigen central to leukocytes adhesion. *Proc Natl Acad Sci USA* 1989;86:8972-6.
64. Gundel RH, Letts LG, Gleich GJ. Human eosinophil major basic protein induces airway constriction and airway hyperresponsiveness in primates. *J Clin Invest* 1991;87:1470.
65. Bochner BS, Luscinskas FW, Gimbrone MA, et al. Adhesion of human basophils, eosinophils and neutrophils to interleukin-1 activated human vascular endothelial cells: contributions of endothelial cell adhesion molecules. *J Exp Med* 1991;173:1553.
66. Weller PF, Rand TH, Goetzl SE, et al. Human eosinophil adherence to vascular endothelium mediated by binding to vascular cell adhesion molecule 1 and endothelial leukocyte adhesion molecule 1. *Proc Natl Acad Sci USA* 1991;88:7430.
67. Walsh GM, Mermoud JJ, Hartnell A, Kay AB, et al. Human eosinophil, but not neutrophil, adherence to IL-1 stimulated human umbilical vascular endothelial cells is α 4 β 1 (very late antigen-4) dependent. *J Immunol* 1991;146:3419.
68. Pipkorn U, Proud D, Lichtenstein LM, et al. Effect of short-term glucocorticoid treatment on human nasal mediator release after antigen challenge. *J Clin Invest* 1987;80:957-61.
69. Charlesworth EN, Kagey-Sobotka A, Schleimer RP, et al. Prednisone inhibits the appearance of inflammatory mediators and the influx of eosinophils and basophils associated with the cutaneous late-phase response to allergen. *J Immunol* 1991;146:671-6.

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70. Schleimer RP, Lichtenstein LM, Gillespie E. Inhibition of basophil histamine release by anti-inflammatory steroids. *Nature* 19;292:454-55.
71. Fadel R, Herpin-Richard N, Rihoux JP, Henolig E. Inhibitory effect of cetirizine 2 HCL on eosinophilia *in vivo*. *Clin Allergy* 1987;17:373-6.
72. Charlesworth EN, Massey WA, Kagey-Sobotka A, et al. Effect of H1 receptor blockade on the early and late response to cutaneous allergen challenge. *J Pharmacol Exp Ther* 1992;262:964-70.
73. Alam R, Dejarnatt A, Stafford S, et al. Selective inhibition of the cutaneous late, but not immediate, allergic response to antigens by misoprostol, a PGE analog. *Am Rev Respir Dis* 1993;148:1066-70.