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The skin as a model to study the pathogenesis of IgE-mediated acute and late-phase responses

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The skin has been used as a model for the study and diagnosis of allergic diseases as long ago as 1865, when Blackley¹ created an immediate reaction on his own forearm with crude ryegrass pollen that culminated in a late exacerbation of his

asthma. Forty-nine years later Cooke² reported immediate wheal-and-flare reactions, which were followed by a late reaction. Cooke further noted that repeated local injections of the same antigen resulted in attenuation of both the immediate and late reactions in the skin. Pepys³ was the first of the classically trained allergists to become interested in the late-phase response (LPR) as a result of his interest in allergic bronchopulmonary aspergilliosis. When he observed a biphasic response to skin testing, he hypothesized that this LPR was a type III reaction and observed some similarity between the findings of cutaneous LPR biopsies and those of experimental Arthus reactions. Dolovich et al.⁴ first related the events leading to cutaneous LPR with specific IgE. In 1976 Solley et al.⁵ demon-

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Abbreviations used

GM-CSF:	Granulocyte-macrophage colony-stimulating factor
IL:	Interleukin
LPR:	Late-phase reaction
LTC ₄ :	Leukotriene C ₄
MBP:	Major basic protein
mRNA:	Messenger RNA
PAF:	Platelet-activating factor
PGD ₂ :	Prostaglandin D ₂
TNF:	Tumor necrosis factor

strated that the skin's ability to react to antigen, both the immediate response and late response, could be passively transferred to nonallergic volunteers. Furthermore, when they removed the serum IgE by passing it over an anti-IgE solid-phase immunosorbent, they also removed the ability of the serum to passively transfer the cutaneous LPR. Thus it was firmly established that the cutaneous LPR was mediated through the triggering, either by specific IgE or anti-IgE, of cutaneous mast cells. For the purposes of this article I will define the LPR as a delayed inflammatory process involving the skin and/or the airways consisting of a polymorphous inflammatory infiltrate occurring in response to mast cell activation by either antigen or nonantigen mast cell activators. This LPR may be biphasic, or it may represent a continuum of the immediate response. Last, the reader must bear in mind that this is an area of rapidly evolving knowledge and it is possible that what we broadly describe today as a LPR, either in the skin or the airway, may involve multiple mechanisms.

The importance of the skin as a model to study allergic disease has gained momentum with the clinical recognition that patients with both reactive airway disease and allergic rhinitis frequently demonstrate a LPR.^{6, 7} The significance of the LPR was underscored by Warner et al.,⁸ who showed that immunotherapy in a group of children with asthma who were allergic to house dust mite resulted in a marked attenuation of the LPR after a bronchoprovocation challenge. A few years later, Hargreave et al. reported an increase in nonspecific bronchial hyperreactivity after antigen bronchoprovocation and showed this to be proportional to the intensity of LPR.⁹ If the pathophysiologic mechanisms that are responsible for the observed late reactions in the skin are analogous to what has been observed clinically in the airways, then the use of the skin as a model to

study the cutaneous LPR assumes greater importance in the study of allergic diseases.

AGENTS CAUSING A CUTANEOUS LPR

Several nonantigenic substances and stimuli have been reported to cause late cutaneous reactions similar to that described with antigens. Although histamine alone is incapable of inducing a LPR in the skin, it has been shown that compound 48/80, a skin mast cell degranulator, can induce a LPR by nonimmunologically causing mast cell degranulation.¹⁰ Leukotriene C₄ (LTC₄) and other products of the lipoxygenase pathway of arachidonic acid metabolism have also been shown to induce both clinical and histologic skin lesions that resemble the LPR.¹¹ Interestingly, prostaglandin D₂ (PGD₂), a product of the mast cell cyclooxygenase pathway, is not capable of producing a cutaneous LPR. In addition, intradermal injection of platelet-activating factor (PAF) produces a dermal picture identical to that of the cutaneous LPR.^{12, 13} This may be of particular clinical relevance because PAF selectively induces an eosinophilic dermal infiltrate in allergic but not in nonallergic persons.¹⁴ In fact, PAF is the only known mediator in the lung to result in a prolonged increase in bronchial hyperresponsiveness.¹⁵ Hargreave and others have noted an association between the degree of bronchial hyperresponsiveness and the intensity of the LPR. Therefore it is possible that this association may be mediated in part by PAF. Weiss et al.¹⁶ reported that a histamine-releasing factor derived from platelets is also capable of inducing a LPR. It remains to be seen whether all these agents reported to cause cutaneous LPR 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 responses exhibit biphasic responses. This may explain why it has been difficult to identify a single inflammatory cell or a single mediator as the critical factor for LPR development.

THE SKIN AS A MODEL TO STUDY ALLERGIC DISEASE

The simple accessibility of the skin enhanced the use of this model to study the events surrounding the LPR 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 the past, investigators interested in cutaneous inflammatory events have used the Rebuck skin window, but

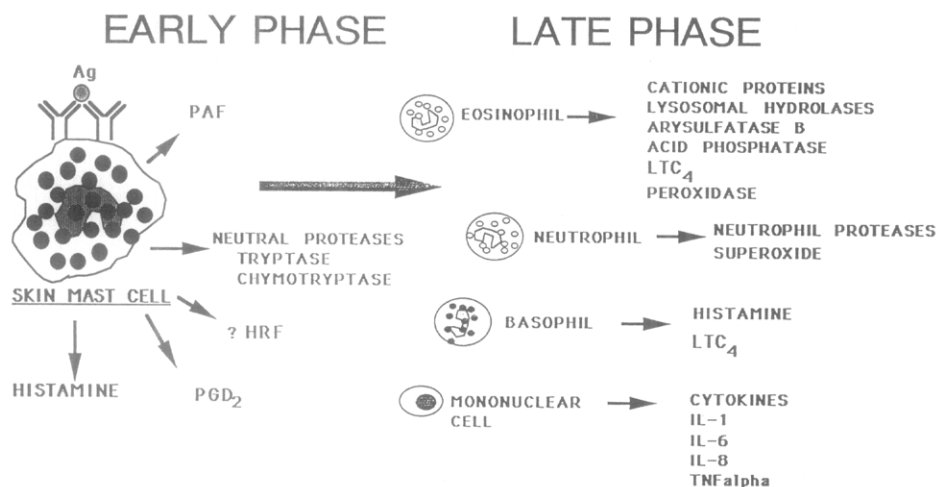


FIG. 1. Diagram of events surrounding antigen-induced hypersensitivity reactions that culminate in LPR. The mast cell is activated by antigen crosslinkage of the IgE receptor, resulting in the release of preformed histamine and tryptase. Factors that are not completely understood ultimately result in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

this had the disadvantage of not providing a means to monitor the cells and mediators over a temporal course. More recently investigators have been applying newer methods to study the LPR, including lymphocyte subset analysis, specific monoclonal antibodies, and more recently the use of messenger RNA probes to identify the production of minute quantities of ribosomally directed proteins.

The skin chamber model affords an investigator the opportunity to study both cutaneous mediators and cellular traffic in a dynamic and controlled fashion. This simple model consists of affixing a chamber to the base of an unroofed heat-suction-induced blister. This method was first described in the dermatology literature by Blank and Miller¹⁷ in 1950, but it was not reported to have been used by others until 18 years later when other dermatologists, Kiistala and Mustakallio,¹⁸ incorporated this method to study dermal inflammatory events. In the past decade Zweiman et al. of the University of Pennsylvania have been primarily responsible for modifying this skin model in their study of pathophysiologic mechanisms of allergic disease¹⁹ and our group has used the skin chamber to study the late-phase effects of a single high dose antigen challenge.

CELLULAR COMPOSITION OF THE LPR

Several investigators have examined the histopathology of the cutaneous LPR, and there is general

agreement that the reaction is a polymorphic inflammatory infiltrate consisting of neutrophils, eosinophils, lymphocytes, and in some reports basophils.^{4, 5, 10, 20} Fig. 1 illustrates that antigen crosslinkage of mast cell IgE is ultimately responsible for calling in eosinophils, neutrophils, basophils, and lymphocytes during the LPR. We have published data documenting the presence of basophils in the cutaneous LPR with both skin biopsies and skin chamber cytology.²¹ Neutrophils were numerically the most prominent cells, but they were also noted in control biopsies in response to non-specific trauma. Wershil, working with Galli and his group²² in Boston, showed that recruitment of neutrophils during the IgE-dependent cutaneous LPR in the mouse is mast cell dependent and is partially inhibited with anti-tumor necrosis factor- α . Eosinophils were six times greater in the antigen biopsy specimens than in the control specimens and appeared to be critical to the development of the LPR in our study; they are clearly an important component of the LPR. Leiferman et al.²³ studied the time course for the deposition of extracellular major basic protein (MBP) after antigen challenge in the skin and found that by the third hour there is extensive deposition of both MBP and neutrophil elastase in the dermis. Their data suggest that eosinophils come in early and that the deposition of cationic proteins persists for up to 56 hours. Many of the previous histologic studies have been divided on whether basophils

were present in the skin during the LPR. Basophils were fewer than eosinophils, but they were clearly increased in our antigen-challenged chambers.²¹ Factors that make identification of basophils in biopsy specimens difficult include an abundance of degenerating cells, which 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 on the antigen concentration and/or the duration of challenge. Recent studies have focused on the role of the lymphocyte in the pathogenesis of the LPR. Using quantitative immunohistologic methods, Frew et al.²⁴ showed an increased number of CD4⁺ T cells of the memory T-cell phenotype (CD45RO⁺) in cutaneous LPR biopsy specimens that correlates with the number of activated eosinophils. It may be that the lymphocyte is the chief 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 were selective for the antigen challenged side in chamber studies, suggesting that these two cell types are preferentially recruited to the late phase.²¹ In addition, we frequently noted 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 pathologic findings observed in the clinical LPR because these cells have been shown to have increased metabolic activity leading to enhanced production of LTC₄ and release of cationic proteins.²⁵ In addition to their well-known cytopathic effects, the eosinophil cationic proteins (particularly MBP) may play a direct role in enhancing basophil and mast cell activation.

INFLAMMATORY MEDIATORS OF THE LPR

Ting working in Zweiman's laboratory¹⁹ demonstrated a rapid rise in the skin chamber of histamine, which peaks by 30 minutes and which is followed by a decline during the subsequent 4 hours. They were also able to show 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.²⁶ Shalit et al.²⁷ extended these studies to examine mediator release after a continuous antigen challenge and reasoned that natural exposure to antigen in allergic subjects occurs during a protracted period of hours or even days. In re-

sponse 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 histamine. Interestingly, they were able to show a continuous release of histamine during the 6-hour observation period. The source of this continued release of histamine remains obscure; however, because the tryptase level declined, it is most likely the basophil that is the source of the histamine because it contains no tryptase. Alternatively it is possible that more than one mechanism might exist for the release of these two preformed mediators; however, at this time there is no evidence to support this hypothesis.

By using the skin chamber model to study the LPR for an extended 12-hour period, we have been able to demonstrate a second rise of histamine that is analogous to that which has been reported to occur in the airways.²¹ As noted previously, an initial peak of histamine (13.2 ± 2.9 ng/ml) was released into the chamber during the first hour. The histamine level then declined to approximately 2 ng/ml during the next 6 hours. This was followed by a slow but persistent rise that culminated in the release of almost 10 ng/ml of histamine during hour 12. Subsequent experiments have shown that this second rise in histamine usually peaks at 11 or 12 hours 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 release. The early pattern of PGD₂ production, without late production, tends to support the thesis that the late-phase histamine release originates from the basophil because this cell does not produce PGD₂. This second rise in histamine, previously unobserved, was not evident in earlier studies because they were not extended long enough. It is also likely that LPR histamine levels may be dependent on 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 LPR. Slow-reacting substance of anaphylaxis has been of interest to allergists for a number of years and is now recognized as leukotrienes. Using the skin chamber model, Talbot et al.²⁸ showed that

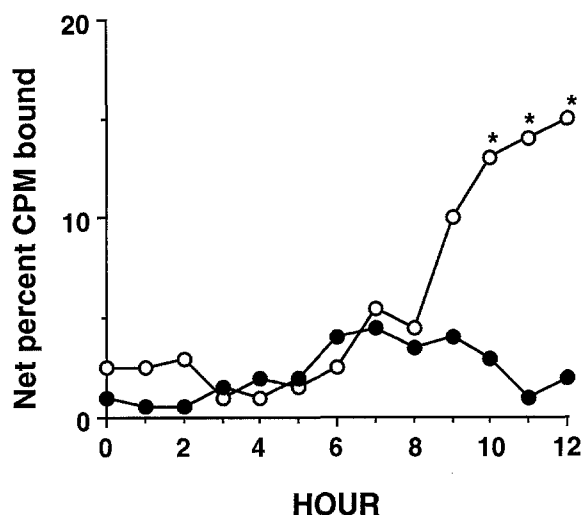


FIG. 2. IL-1 bioactivity was detected in skin chamber fluids for hours 10, 11, and 12 ($p < 0.05$). This bioactivity in chamber fluids was significantly neutralized by anti-IL-1 β antiserum and confirmed with a radioimmunoassay. (From Bochner BS, et al. *J ALLERGY CLIN IMMUNOL* 1990;86:830-9.)

LTC₄ is released in response to an antigen challenge, peaking between the hours 2 and 4. The time kinetics of LTC₄ production in their study revealed considerable variability among their subjects without the predictable peaks or release patterns of either histamine or PGD₂. We examined LTC₄ production in response to antigen and have noted it to be elevated both early and late; however, intrasubject variability was marked. Furthermore, when our subjects were treated with 60 mg of prednisone for the 3 days preceding the study, significant suppression of LTC₄ 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. Certainly this group of mediators may play a role in the LPR in the airways via their ability to cause bronchoconstriction in very small amounts. It is likely that production of LTC₄ by activated eosinophils, in concert with other mediators, significantly contributes to the clinical LPR. Leukotriene B₄ has not been shown to be released into the skin chambers in response to a single high-dose antigen challenge. However, leukotriene B₄ can be detected in skin chambers that have been challenged with a continuous low dose of antigen, again suggesting that different mechanisms may be involved in these two challenge models.²⁹ 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.²⁹ recently recorded the presence of PAF in skin chambers in 74% of the allergic subjects challenged with continuous low-dose antigen. PAF was first detected during hour 3 and production continued for 9 hours after antigen challenge. It has been demonstrated that PAF is capable of causing release of LTC₄ from eosinophils, and it has been proven to be one of the most potent chemotactic factors known for eosinophils.³⁰ Because 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 the cutaneous LPR, and it has been shown that both thrombin and collagen are capable of releasing PAF from platelets. Human lung mast cells are capable of producing PAF, but the role of PAF production by the skin mast cell is still being studied. Chilton and Connell³¹ recently demonstrated that the metabolism of PAF is intricately involved with the arachidonic acid pathway, providing further evidence for linkage of PAF metabolism with the cyclooxygenase and the lipoxygenase pathways. The conclusions from these studies clearly dictate a major role for PAF in the LPR, which results in increased vascular permeability, increased tissue edema, and enhanced eosinophil diapedesis.

A myriad of other mediators may play a role in the LPR in both the skin and the airway. One exciting area that may prove pivotal in our understanding of the LPR is the role played by the various cytokines. Bochner et al.³² have shown that interleukin (IL)-1 β is increased in late-phase skin blister fluids as noted in Fig. 2. Although the exact role that IL-1 might play in the cutaneous LPR is uncertain, IL-1 has many biologic properties that could upregulate 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 endothelial leukocyte adherence molecule-1.^{33, 34} It has been shown that IL-1 stimulation of endothelial cells promotes the adherence of eosinophils, basophils, and neutrophils, all of which have been associated with the LPR. Although IL-1 is produced primarily by

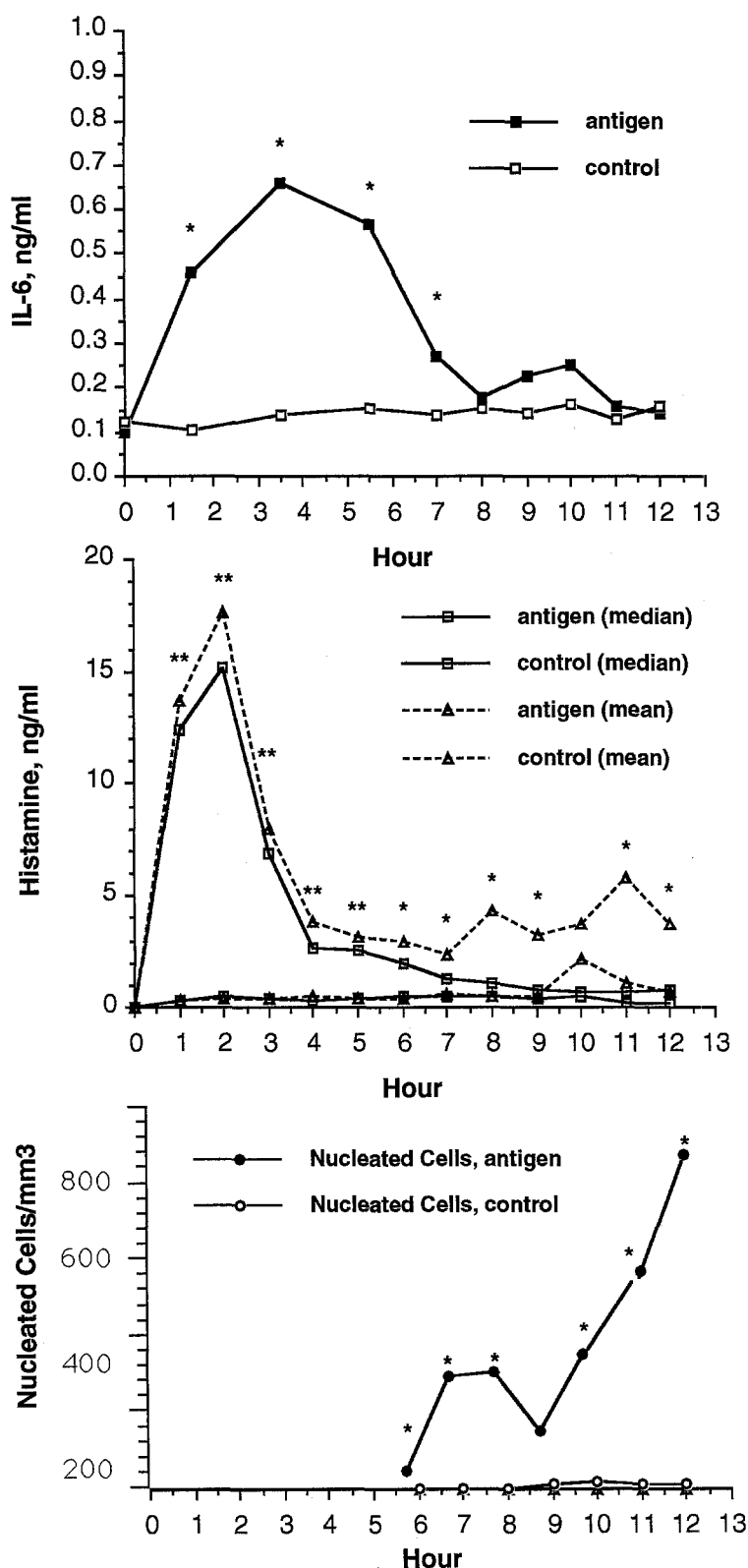


FIG. 3. Results of single experiment in which IL-6 was measured with a radioimmunoassay, showing a biphasic release of IL-6 (*top*) and histamine (*center*). Late IL-6 and histamine release occurred during the inflammatory phase of the LPR in which there was an influx of nucleated cells into skin chambers (*bottom*). (From Lee CE, Neuland ME, Teaford HG, et al. J ALLERGY CLIN IMMUNOL 1992;89:1010-20.)

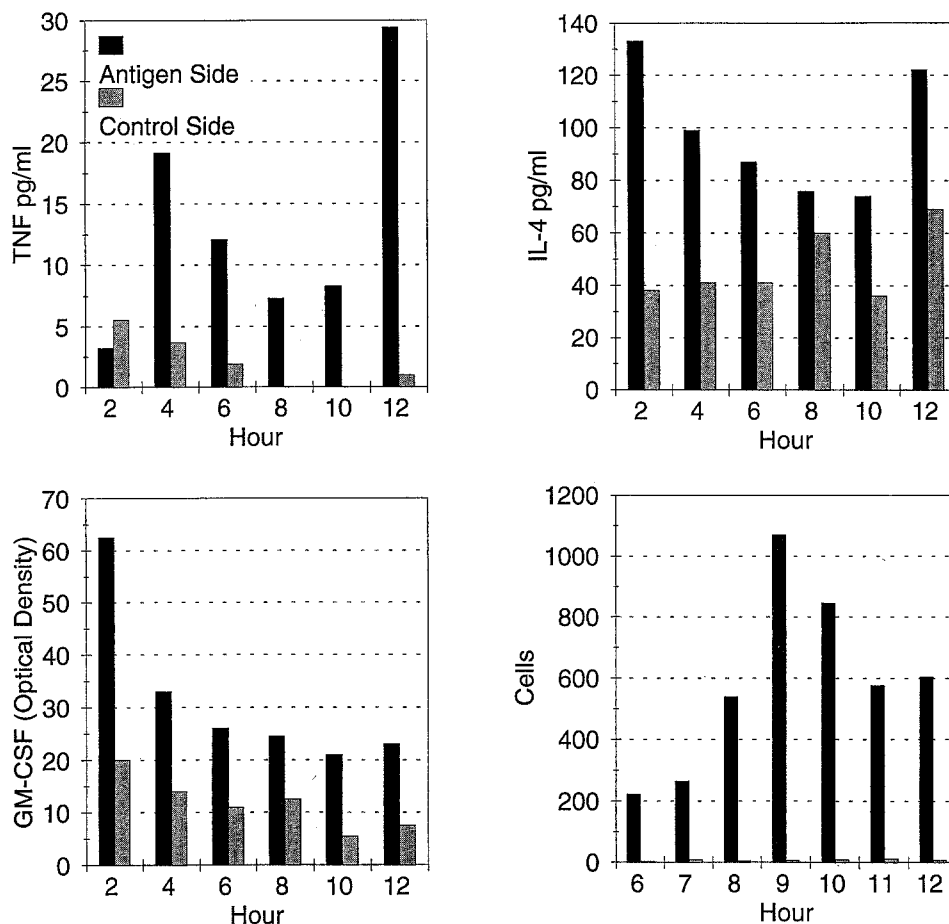


FIG. 4. The same subject as in Fig. 3 was studied in similar but separate experiment for presence of TNF- α , GM-CSF, and IL-4 with commercial ELISA kits. All three cytokines were elevated in a biphasic pattern. (From Morris FM, et al. *J ALLERGY CLIN IMMUNOL* 1994;93:261.)

mononuclear cells, many other cell types, including the mast cell, are capable of producing this cytokine. Evidence is emerging from several different groups that IL-1 might enhance basophil histamine release. This may help explain our observations of increased histamine, increased basophil count, and increased IL-1 in our late-phase skin chamber studies.^{35, 36} Corticosteroids are known to be specific inhibitors of the LPR, and they have no effect on the early events. Because corticosteroids inhibit both the LPR and IL-1 production, it is tempting to speculate that they might act clinically in part by blocking IL-1 production.

In reality, probably several cytokines actually contribute to the complex events in the LPR, and this area of ongoing research promises further information about the LPR. Our group next looked for the presence of IL-6 with the skin chamber technique because this cytokine has many of the same proinflammatory properties as

IL-1.³⁷ Fig. 3 shows the pattern of 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, which 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 accounts for the IL-8 in the antigen-stimulated chambers.³⁸ 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.³⁹ Fig. 4 shows the presence of TNF- α , granulocyte-macrophage colony-stimulating factor (GM-CSF), and IL-4 in an allergic subject who we studied and

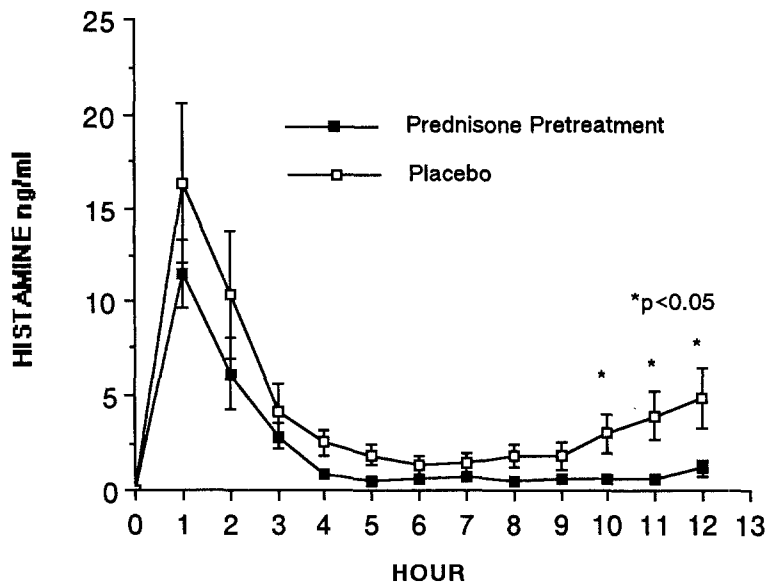


FIG. 5. Nine allergic subjects were pretreated with 60 mg/day of prednisone for 3 days and challenged with antigen in the skin chamber. Prednisone pretreatment did not significantly alter the immediate release of histamine; however, it totally ablated late-phase histamine released into the skin chambers. (From Charlesworth EN, et al. *J ALLERGY CLIN IMMUNOL* 1991;146:671-6.)

who was unique in demonstrating a rather marked biphasic skin test reactivity and biphasic histamine release.⁴⁰ When the total group of seven subjects was examined, TNF- α was elevated in four subjects and IL-4 was marginally elevated in all the subjects. Interestingly, the total TNF- α level was significantly correlated with the early IL-4 ($R^2 = 0.88$, $p = 0.001$).³⁶ Our inability to show consistently the presence of GM-CSF may be the result of the sensitivity of the ELISA used because Massey et al.⁴¹ were clearly able to demonstrate the presence of GM-CSF mRNA at allergen-challenged cutaneous LPR. 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. There is compelling evidence for ribosomal message for Th2 lymphocytes, and it appears that immunotherapy may result in the up-regulation of Th1 cytokines such as interferon- γ and IL-2.⁴² This is an area of research that will continue to unfold, and it offers tremendous possibilities for the understanding and treatment of allergic disease.

PHARMACOLOGIC MODULATION OF THE CUTANEOUS LPR

Pipkorn et al.⁴³ 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. Similar studies with the skin chamber model⁴⁴ showed the same selective late-phase suppression of histamine as previously observed by Pipkorn in the nose. Not only was the late release of histamine ablated as shown in Fig. 5, 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.⁴⁵ 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, because 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 LPR.

Recently, certain of the newer antihistamines

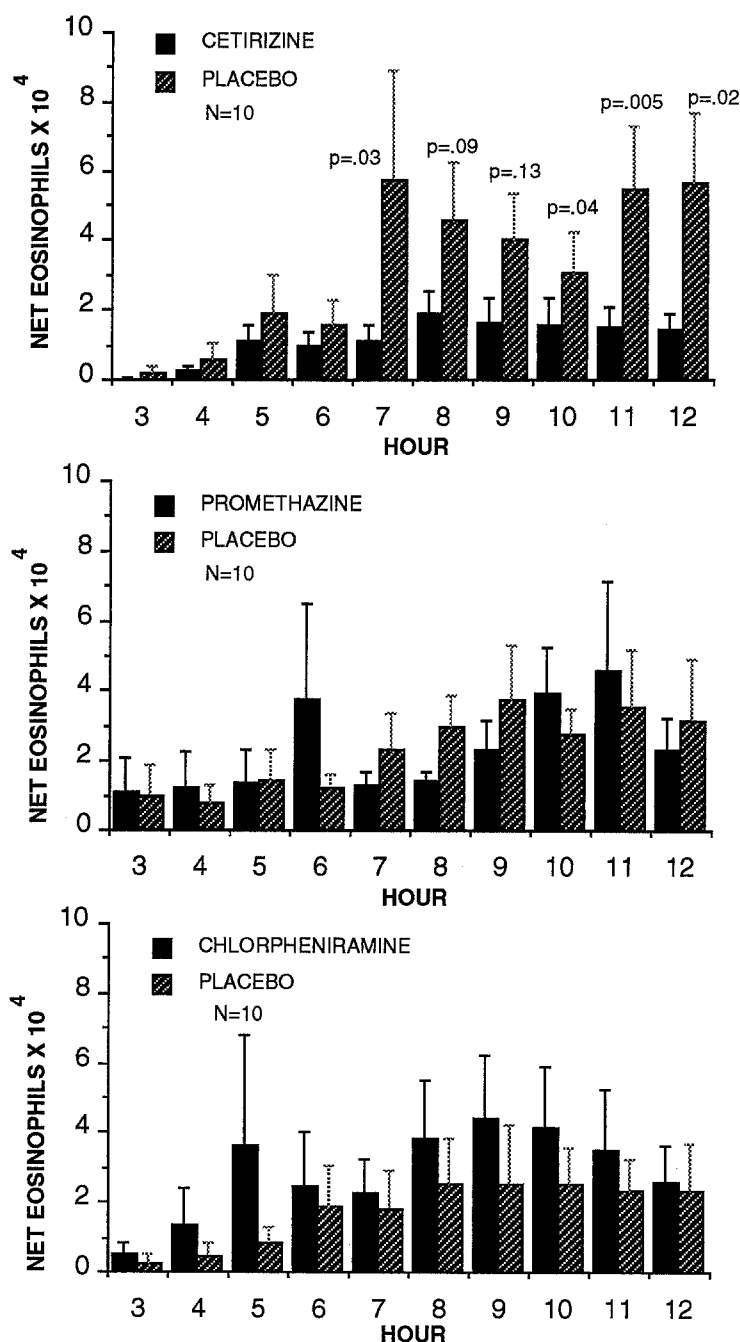


FIG. 6. Ten different allergic groups were studied with the skin chamber technique to examine the effect of three antihistamines (cetirizine, promethazine, and chlorpheniramine) on cells and mediators in the LPR to antigen. Although there was no alteration of either early or late histamine release, the inflammatory infiltrate into skin chambers was decreased. Only effects on eosinophils for cetirizine (*top*), promethazine (*middle*), and chlorpheniramine (*bottom*) are shown. (From Charlesworth EN, et al. *J Pharmacol Exp Ther* 1992;262:964-70, by permission.)

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.,⁴⁶ demonstrated that cetirizine blocks

the migration of eosinophils onto a skin window 24 hours after antigen challenge, a phenomenon not explained by H₁ blockade. These studies were enlarged on by our group, and we were able to

show suppression of LPR cellular traffic with the skin chamber model as observed in Fig. 6.⁴⁷ In these studies we showed that eosinophil infiltration was decreased by 68%, whereas the number of neutrophils was reduced by a similar magnitude. Basophils were also decreased by 64% for hours 6 through 8. Cetirizine appears to influence 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 because this effect was not observed with either promethazine or chlorpheniramine.

A recent report by Alam et al.⁴⁸ suggested that misoprostal might have a selective inhibitory effect on the cutaneous LPR. Like cetirizine, misoprostol inhibited the dermal infiltration of eosinophils and, to a lesser extent, neutrophils in skin biopsy specimens obtained at 5 hours. They then showed that this prostaglandin E analog inhibited eosinophil chemotaxis in vitro and inhibited the production of GM-CSF by lymphocytes but had no effect on the immediate wheal-and-flare response to antigen. If these results are confirmed by other groups, then it might suggest that this prostaglandin E analog should be included with cetirizine and corticosteroids as drugs having antiallergic properties. The pharmacologic modulation of the cutaneous LPR continues to be an active area of research, and it is predicted that the skin model will continue to contribute to our greater understanding of the complex events surrounding the LPR.

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Eosinophils and basophils in allergic airway inflammation

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Airway inflammation has been accepted as a feature of asthma and other allergic inflammatory diseases. The many cells participating in this process include mast cells, basophils, eosinophils, and lymphocytes. Of these cells the mast cell, eosino-