# Cutaneous Late-Phase Response to Allergen

## Mediator Release and Inflammatory Cell Infiltration

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## Abstract

**To better define the inflammatory infiltrates and kinetics of mediator release during the cutaneous late-phase reaction (LPR), we examined skin biopsies at 8 h, and skin chamber cell counts and mediator release for 12 h after antigen challenge. Compared with the control sites, the antigen-stimulated biopsy sites contained 14 times as many basophils ($P < 0.01$) and six times as many eosinophils ($P < 0.001$) with one to two fold more mononuclear cells ($P < 0.03$) and neutrophils ($P \leq 0.01$). Similar changes were found in the skin chambers. Although there were neutrophils in the control chamber, they were only twice as numerous in the antigen challenged site ($P < 0.01$). Eosinophils were 35-fold ($P \leq 0.03$) more prevalent in the antigen chamber than the control chamber for hours 8–12 and basophils were noted starting in the eighth hour and were 20-fold ($P \leq 0.03$) more concentrated in the antigen chamber during the next 4 h. The mononuclear cells were not significantly different between antigen and control blisters. With respect to inflammatory mediators, there was an initial peak of histamine (13.2±2.9 ng/ml) in the blister fluid at 1 h. The level then fell to ~ 2 ng/ml, followed by a secondary rise starting at the eighth hour and increasing to 9.8±2.8 ng/ml by the twelfth hour. This secondary increase in histamine correlated significantly ($r = 0.81$, $P < 0.05$) with the observed influx of basophils. $PGD_2$ in the blister fluid rose to 371±25 pg/ml during the first 4 h and then slowly decreased to half this level during the last 4 h. Thus, the cutaneous LPR has been shown to manifest a secondary increase in histamine levels and a markedly specific increase in eosinophils and basophils with mediator release apparently being derived from the latter cells.**

## Introduction

Allergic individuals, when exposed to an appropriate antigen challenge, suffer an acute response followed several hours later by a late-phase reaction (LPR).[1] In recent years investigation has focused on the latter, because it is felt that this inflammatory response, which involves not only mediators but also mobilization of a number of types of cells, more accurately reflects clinical disease than does the less complex acute response (1–6). This paper focuses on the late-phase response in the skin, an appropriate place to execute such studies because the initial work defining the LPR in man used a cutaneous model (7–11).

The biphasic response to antigen was originally felt to involve a succession of IgE and IgG antibody-mediated events, with the latter causing tissue damage due to dermal complement and fibrin deposition (12, 13). 15 yr ago Dolovich and Gleich and their associates demonstrated, in separate studies, that both the acute and the late response to antigen could be transferred by serum in which IgE antibody was found to be the critical element (7, 8). These and other investigators demonstrated that nonspecific mast cell–degranulating agents, such as compound 48/80, could also induce both early and late responses (14, 15). Biopsies 6–8 h after antigen administration showed dermal edema associated with neutrophil accumulation and scattered eosinophils. These acute inflammatory cells were then replaced 24 h later by a predominantly mononuclear cell infiltration.

This early work was not significantly extended until recently, but in the last several years there have been renewed efforts to elucidate the pathogenesis of the LPR. It has now been established that antigen challenge of the upper and lower airways leads to a biphasic clinical response in which both phases are associated with the release of putative mast cell mediators (16–19). After antigen bronchoprovocation, histamine and neutrophil chemotactic factors are recovered from peripheral blood; in the nose, histamine, kinin, and leukotrienes $C_4$, $D_4$, and $E_4$ are observed in both the early and late-phase response (16, 20–24). $PGD_2$, a product of the human mast cell but not of the basophil, is present in the early phase but not the late phase, a finding that has led us to suggest that the late-phase mediator release was a result of infiltrating basophils (16). As noted above, earlier histological studies have provided no evidence of basophil involvement in the skin, although Platts-Mills and his colleagues have shown that antigen applied to dermal abrasions led to an accumulation of basophils within 24–48 h (24).

Although the basophil has not been observed to be involved in the cutaneous LPR, there is considerable evidence in the skin and other organs for other inflammatory cells, especially eosinophils and neutrophils. Eosinophils and their toxic products have been observed in many types of allergic foci including late-phase bronchial washings (25–27). More re-

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1. *Abbreviations used in this paper:* HRF, histamine-releasing factor; LPR, late-phase reaction; OCD, optimal challenge dose; PAF, platelet-activating factor; RLH, Ringer's lactate with 250 μg/ml of heparin; RW, ragweed.

cently, work by Lieferman et al. has shown the tissue deposition of eosinophil major basic protein during chronic cutaneous reactions (28). There is little doubt that neutrophils are present in the LPR, and there is evidence that these cells may contribute to the increased bronchial reactivity observed after bronchoprovocation-induced late-phase reaction (23).

This study is a further effort to understand the pathogenesis of the late-phase reaction in the skin using the skin blister technique previously described by Dunsky and Zweiman (29). We measured histamine release and $PGD_2$ production in skin chambers extending over 12 h after antigen challenge. During the same time period, we studied the influx of three potentially involved cells: basophils, eosinophils, and neutrophils. We correlated the appearance of these cells in blister fluid with the dermal inflammatory cell infiltrate in skin biopsies taken from beneath the blister base at 8 h. We demonstrate, for the first time, a biphasic appearance of histamine in the cutaneous LPR. We also describe the arrival of basophils both in the dermis beneath the blister and, even more markedly, into the tissue fluid itself. This appearance of basophils correlates with the secondary appearance of histamine in the blister fluid. There is a marked eosinophil infiltration into both the dermis and the skin chamber whereas neutrophils, although representing the predominant cell type, are less prominently increased with antigen challenge as compared with the control sites of both dermal biopsies and skin chamber exudates.

## Methods

*Skin chamber antigen challenge.* The study protocol was approved by the Institutional Review Committee and all subjects had given written informed consent. All subjects had been previously skin tested with ragweed (RW) extract (in Hollister-Steir saline diluent with 0.3% human albumin) in concentrations ranging from 0.1 to 1,000 PNU/ml and were shown to have a clinical cutaneous LPR consisting of erythema, soft tissue swelling, and induration. The concentration of RW extract required to elicit a wheal > 10 mm with pseudopods or > 15 mm without pseudopods, and accompanied by > 45 mm of erythema, was defined as the optimal challenge dose (OCD). The skin was then challenged in all instances with 10 times the OCD of RW antigen that remained in the skin chamber for 60 min before removal. The same dose and route of antigen challenge was followed for both the 8-h biopsy studies and the 12-h skin chamber studies.

12 h before the placement of the skin chamber, a heat/suction-induced blister was induced on the volar aspect of each arm of seven atopic subjects (three male, four female), 18–50 yr of age. A hollow plate warmed to 40°C was placed over the precleaned skin on the volar aspect of the arm. Continuous suction (60 mmHg) was applied for 45–60 min until a complete 5-mm blister was formed. On the morning of the study, the blisters formed 12 h previously were aseptically unroofed. Sterile aluminum chambers, with entry and exit ports, were placed over the unroofed blister and taped in place. A cutaneous adhesive (Skin Bond, Largo, FL) was then used to secure an adequate seal with the skin. 500 μl of RW extract, in Ringer's lactate with 250 μg/ml of heparin (RLH), representing 10 times the OCD, was placed into the test chamber. On the opposite arm an equal amount of RLH only was placed into the control chamber. The chamber contents were removed hourly for 12 h and replaced with 500 μl of RLH. The blister fluid from each sample was spun at 1,000 rpm in microcentrifuge tubes for 2 min and the supernatant was saved for measurement of $PGD_2$ and histamine. The pellet was resuspended in 500 μl of RLH and gently agitated, after which 10 μl were placed on a hemocytometer (Neubauer; American Optical, Buffalo, NY) for cell counting.

Cell counts were done on at least 100 nucleated cells in nine large squares. The cell suspension was then placed in a centrifuge (Cytospin; Shandon Southern Instruments, Inc., Sewickley, PA) and spun at 600 rpm for 6 min. Paired slides were stained with DIFF-QUIK (a modified Wright-Giemsa stain, American Scientific Products, McGaw Park, IL) and 0.5% alcian blue (pH of 1.0) for 60 min. After rinsing with distilled water, the alcian blue–stained cells were counter-stained with 0.1% Safranin in 1% acetic acid. For the Wright-Giemsa-stained cells, a total of 200–300 cells were counted. For alcian blue–stained cells, either the total number of cells on the slide or 1,000 cells were counted, whichever was less (30).

*Skin testing.* Simultaneous with the introduction of RW antigen into the skin chamber, each subject was skin tested with 0.02 ml of the RW concentration that had previously been determined to cause a +4 reaction followed by a cutaneous LPR. The skin test was placed on the upper portion of the same arm that was challenged with antigen in the chamber. The surface area of the LPR was calculated using a composite diameter derived by averaging the long and short axis of the cutaneous erythema and edema.

*Cutaneous biopsy.* Bilateral biopsies from five atopic subjects (three male, two female) were made at the base of a heat/suction-induced blister 8 h after either challenge with RW antigen or a sterile PBS solution. The blister base was cleaned with Betadine (Baker, Ft. Lauderdale, FL) and allowed to dry for 5 min, followed by circumferential infiltration with 1% lidocaine. 4-mm punch biopsies, using disposable punches, were made over the denuded blister bases. The specimens were placed in a modified Karnovsky's (glutaraldehyde/paraformaldehyde) fixative for 5 h, and then transferred to a cacodylate buffer (pH 7.4) (31). After fixation, the specimens were embedded in methacrylate, cut to 1-μm thickness and stained with the following: hemotoxylin and eosin, Giemsa, toluidine blue, and chloroacetate esterase (α-napthol-*AS*-D-chloroacetate esterase).

Cells were counted to characterize the inflammatory infiltrate (32, 33). Morphometric counts were made by using a square grid that is divided into 100 smaller squares etched onto a 10× eyepiece (American Optical, Buffalo, NY). Each of the small squares measures 0.22 mm × 0.022 mm ×400 μm. A total of 40 1-μm squares were counted by moving the microscope stage laterally across the reticular dermis. Observations were made by one investigator (E. N. Charlesworth) and confirmed independently by a second investigator (A. Hood). A third observer (N. Soter) confirmed that the metachromatic cells being called basophils were indeed consistent with basophils and not mast cells. An original effort to read these slides blinded was abandoned because the differences between antigen challenge and control site were immediately obvious. Basophils were identified as cells having a definite multilobular nucleus with dispersed chromatin, and large cytoplasmic granules that stained metachromatically with toluidine and Giemsa. Any metachromatically staining cell that was clearly within the lumen of a dermal blood vessel was considered a basophil. Cells that were clearly metachromatic but that could not be clearly identified as either a mast cell or basophil were labeled as "indeterminate."

*Measurement of mediators.* For measurement of $PGD_2$, 200 μl of blister fluid were precipitated in 1,600 μl of HPLC grade ethyl alcohol. The tubes were centrifuged at 5,000 rpm for 5 min and the supernatants, which contained the prostanoids, were transferred to separate tubes and placed in an evaporating centrifuge for 3 h. The specimens were then stored at −70°C until assayed. A competitive RIA with a sensitivity of 40 pg/ml, as described previously, was used for $PGD_2$ measurements (34, 35). The identity of the prostanoid measured by the RIA has been confirmed by negative ion gas chromatography mass spectrometry. Blister fluid for the histamine assay was frozen immediately after collection and stored at −70°C. The radioenzymatic assay for histamine has been previously described and has a sensitivity of 0.05 ng/ml (36). This assay is based on the conversion of histamine to [$^3$H]methylhistamine in the presence of histamine-*N*-methyltransferase and [$^3$H]*S*-adenosyl-L-methionine (36).

*Statistical analysis.* Nonparametric analysis was used as there is no evidence that the inflammatory cell influx or mediator release necessarily follows a Gaussian distribution. The Wilcoxon signed-rank test was applied using a commercial statistics program (Statview). A *Z*

value was determined and the two-tailed probability was obtained from a table of probabilities.

## Results

*Biopsy data.* Five atopic individuals were biopsied 8 h after challenge with RW antigen in a dose previously shown to elicit a cutaneous LPR. The antigen was added to the skin blister chamber and the biopsies were taken from the base of the unroofed blister to compare the dermal histology of the antigen challenged and the control blister sites. Two metachromatic stains were used, Giemsa and toluidine blue, in addition to a chloroacetate esterase stain that stains mast cells and neutrophils but not basophils. Although subject to minor technical differences among the histologic sections, we found the Giemsa stain to be superior to toluidine blue, showing good cellular detail for both mast cells and basophils in addition to contrasting eosinophils with neutrophils. Therefore, cell counts are all reported from Giemsa-stained specimens. Because the chloroacetate esterase stain also stains neutrophils, in many instances the overwhelming number of these cells in various stages of degranulation obscured the mast cells sufficiently to prevent an accurate count (37).

There were a moderate number of neutrophils and mononuclear cells present in the biopsy specimens taken from the control blisters at 8 h, but very few eosinophils or basophils were found. There was a significant infiltration of neutrophils ($P \leq 0.01$), eosinophils ($P \leq 0.001$), mononuclear cells ($P \leq 0.05$), and basophils ($P \leq 0.02$) in the antigen-stimulated blister base when compared with the control side (Fig. 1). Neutrophils and eosinophils dominated the cutaneous LPR cellular infiltrate at 8 h, each representing about one-third of the total cell number. Note, however, that the relative number of eosinophils in the antigen challenge site was > sixfold higher than in the control site ($P < 0.01$), whereas the neutrophil accumulation, although significant ($P < 0.01$), was only twofold greater. The number of mononuclear cells in the antigen-stimulated blister base was approximately the same as the neutrophils and eosinophils, but mononuclear cells were increased by only 1.4-fold ($P \leq 0.03$) over the control side; the latter appear to infiltrate the control site as a nonspecific response to the trauma of blister formation. As shown in Fig. 1 *C*, basophils accounted for < 4% of the infiltrating cells. The intense inflammatory infiltrate, cellular debris, and the karyorrhexis of neutrophils made an exact quantification of basophil number difficult as illustrated by the increased number of indeterminate metachromatic cells observed after antigen stimulation. There was, however, a clear increase in the number of basophils seen at 8 h after antigen challenge. Moreover, the relative increase in this cell type in the antigen-stimulated site as compared with the control site, was greater than that observed with the eosinophil, more than 14-fold ($P < 0.01$). Fig. 2 shows a photomicrograph of a longitudinally cut dermal blood vessel in which two metachromatic cells, together with neutrophils and eosinophils, are seen within the vessel lumen.

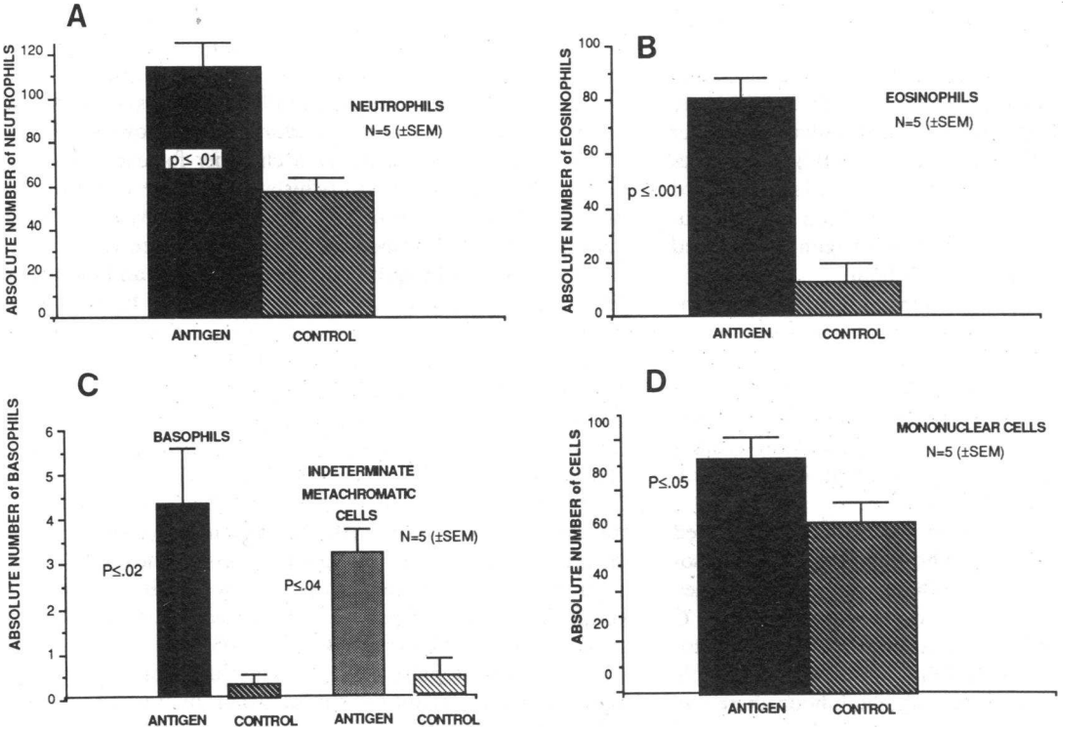


*Figure 1.* Skin biopsy at 8 h after cutaneous antigen challenge. Results of morphometric cell counts from 1-μm-thick histologic sections at the site of antigen challenge 8 h previously. Histologic sections were stained with hemoyoxylin and eosin and Giemsa. (*A*) 8 h after antigen challenge a neutrophilic infiltrate appeared in the dermis which, although statistically significant ($P \leq 0.01$), was also seen in the control blister base as a result of the trauma of blister induction. (*B*) Eosinophils are shown to be a predominant histologic component of the cutaneous LPR. This appears to be highly specific ($P \leq 0.001$) for the antigen-challenged site. (*C*) Although basophils are numerically a minor component of the late-phase biopsy in the skin, these cells appear to be specific ($P \leq 0.02$) for the antigen-challenged skin site and are most likely the source of the late-phase histamine release seen in the skin. (*D*) Mononuclear cells were significantly ($P < 0.05$) increased in response to antigen challenge, but they were also seen in the control site.

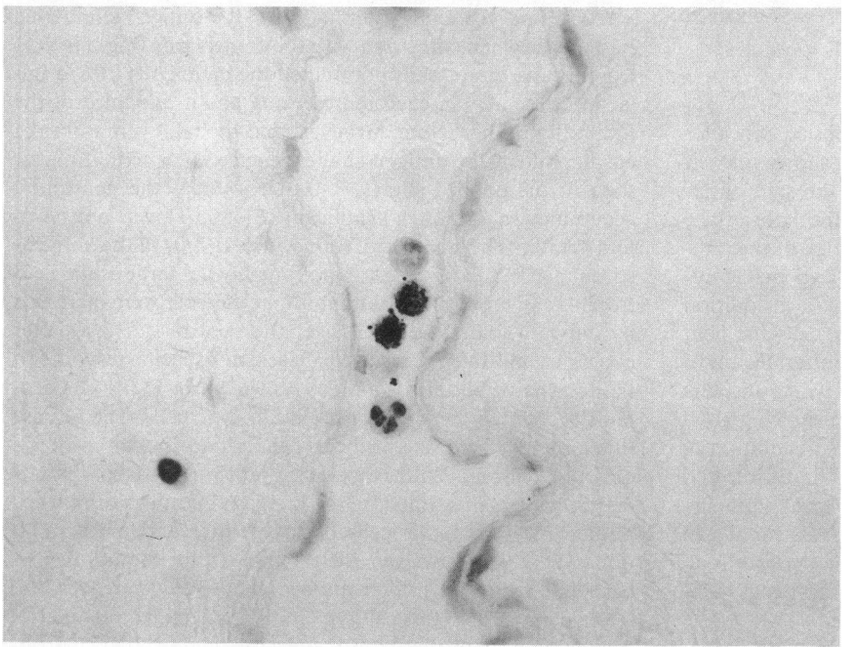

*Figure 2.* Photomicrograph of cutaneous LPR in the skin. Photomicrograph demonstrating two metachromatically staining cells within the lumen of a longitudinally cut 1-$\mu$m-thick section of skin obtained from the antigen challenged site at 8 h and stained with Giemsa. Frequently these cells show a more condensed chromatin pattern, making it difficult to perceive the multilobed nucleus which is characteristic of basophils migrating into the site of antigen challenge. The granules are more coarse and larger than those seen within mast cells. ×1,550.

*Skin chamber studies: cells.* We examined the cells entering the skin chamber on an hourly basis for 12 h. Fig. 3 illustrates the influx of neutrophils, eosinophils, basophils, and mononuclear cells into the skin chambers with all values representing the net increase of cells during the respective hour, by subtracting the cells in the control chamber from those in the antigen-stimulated chamber. Although neutrophils appeared in the control chamber, there were two- to three-fold more neutrophils recovered from the antigen-stimulated chamber for hours 7–12. This was significant ($P < 0.02$) at all times except hours 8 and 12. Note that in Fig. 3 *A*, the large standard errors indicate considerable individual variation in the number of neutrophils recovered in the antigen stimulated chamber, particularly after the seventh hour.

The number of eosinophils migrating into the antigen-stimulated chamber was only 30% as great as the number of neutrophils found during the corresponding hour. The eosinophil infiltration appeared, however, to be more specific for the antigen-challenged site with very few eosinophils being noted in the control chamber. An average of 35-fold more eosinophils were found in the antigen chambers than in the control chambers at hours 6–12 ($P \leq 0.03$).

No basophils were observed in the antigen challenged chamber until the sixth or seventh hour; the number of basophils then increased until the eleventh hour at which time they appeared to reach their peak. Cell counts shown in Fig. 3 *C* represent the net number of cells counted in the antigen-stimulated chamber and, although there were a few basophils found in the control chambers after the eighth hour, there was an average of 20 times ($P \leq 0.03$) more basophils in the antigen challenged chambers. The standard errors are rather large, indicating individual variation especially during the last 2 h of the study.

Consistent with the skin biopsy data (Fig. 1 *D*), the number of mononuclear cells counted in the skin chamber 6–12 h after antigen challenge was only 1.4-fold greater than that observed in the control chamber, and none of the comparisons reached statistical significance. Fig. 3 *D* indicates that there was a small net increase in mononuclear cells in the antigen-stimulated blister chamber, but this cell was clearly less well represented in the chamber than it was in the skin biopsy. It appears that the neutrophils infiltrate 1 or 2 h before the entry of eosinophils and that the basophils appear promptly thereafter (Fig. 3, *A–D*). The mononuclear cell accumulation appeared to be coincident with the neutrophilic influx into the skin chamber.

*Skin chamber studies: mediators.* Fig. 4 shows the histamine concentrations in the skin chamber for each hour; all values represent the net hourly histamine release with the trivial values from the control site (< 1 ng/ml in any hour) being subtracted from the respective antigen-stimulated values. Histamine peaked at 13 ng/ml during the first hour and thereafter fell during the subsequent 6 h. After reaching a baseline level of ~ 2 ng/ml during hours five to seven, the histamine levels measured in the chamber then steadily rose during the remaining hours of the study. The late-phase rate of increase in histamine levels maintains the same slope suggesting continuing histamine release rather than the brief single peak that is characteristic of mast cell activation seen in the first hour. During the twelfth hour the histamine level was 9.8 ng/ml, representing 74% of the first-hour histamine value. It is not clear from this study at what point the histamine levels would begin to taper off in the late phase. Note that the basophil count significantly correlated with the secondary peak in histamine concentration ($r = 0.81$, $P \leq 0.05$).

Fig. 4 also depicts $PGD_2$ production; again the $PGD_2$ values represent net antigen stimulated production less control values. $PGD_2$ rose to 300 pg/ml during the first hour but unlike the early histamine release, this level of $PGD_2$ was maintained for the first 4 h of the experiment. The $PGD_2$ levels then slowly decreased to an average of 180 pg/ml during the last 4 h of the study. During the time period corresponding to the secondary rise in histamine, there was a corresponding decrease in $PGD_2$ production. Thus, the histamine level in the blister fluid at 12 h is five times the 6-h level while, with respect

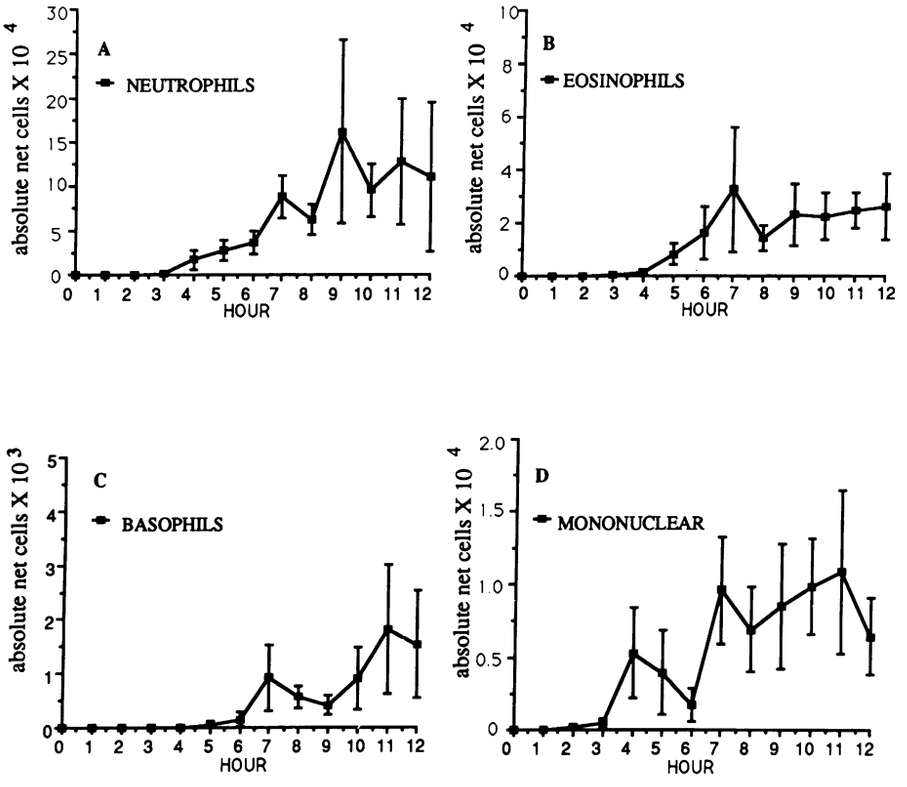


*Figure 3.* Hourly cellular influx into skin chambers after antigen challenge. All cell counts represent a net influx with the number of cells migrating into the control chamber being subtracted from the antigen-stimulated skin chamber. (*A*) Neutrophils are quantitatively the dominant cell type. The neutrophilic infiltration begins at 5 h and appears to be greatest during the 10th hour. (*B*) Eosinophils begin to migrate into the skin chamber several hours after the neutrophils and remain elevated from hour 7–12. (*C*) Basophils show much later time kinetics than either the neutrophil or the eosinophil. They do not begin to migrate into the skin chamber until the seventh hour and are greatest at hours 11 and 12. (*D*) Mononuclear cells have similar time kinetics to the neutrophils and are not a predominant cell type.

to the $PGD_2$, the twelfth-hour level is one half that of 6 h. Unlike the results with histamine, there was evidence of spontaneous prostaglandin production in the control chamber with the $PGD_2$ levels in the control chamber showing, after the first several hours, the same kinetics as those observed in the chamber challenged with antigen, although quantitatively only one-third as great. $PGD_2$ levels in the antigen challenged chamber were significantly above control at all times measured ($P < 0.01$). The observed levels of $PGD_2$ in the buffer-challenged blisters were not surprising as cells other than the skin mast cell have a capability to produce prostanoids, including the Langerhans cell (38–40).

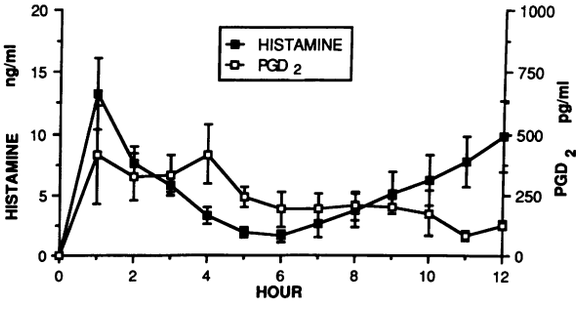


*Figure 4.* Time kinetics for histamine release and $PGD_2$ production in antigen-stimulated skin chambers. Histamine release occurs immediately after antigen challenge and can be seen to peak during the first hour. Histamine then reaches a nadir at hour 6 followed by a slow, steady increase up to hour 12. $PGD_2$ production peaks during the first 4 h. In contrast to the kinetics of histamine release, $PGD_2$ does not show an elevation during the later time periods that is consistent with the biochemical profile of the basophil.

Fig. 5 shows the temporal relationship between histamine release and the skin test reactivity over the 12-h observation period. The peak of the cutaneous LPR as determined by skin test reactivity occurs at 8 h, whereas the histamine released into the skin chamber is still rising during the twelfth hour. This may represent a delay of histamine recovery in the chamber secondary to the time required for passive diffusion into the chamber or, less likely, that the peak of skin test reactivity precedes the secondary rise in histamine release.

## Discussion

The studies that documented the role of IgE in the late-phase reaction reconfirmed that antigen challenged skin was subject to late-phase erythema and subcutaneous swelling (41). It has been difficult, however, to study the pathogenesis of this response. This study reveals the basis for some of this difficulty: the LPR in the skin has different kinetics from that observed in the airway. In the lungs, the late-phase response often begins as early as 3 h and rarely starts later than 6 h after antigen challenge (5). In the skin, mediator release reaches a nadir at 6 h by which time inflammatory cell infiltration has barely begun to be manifest in the skin chamber (42, 43). Thus, extending our studies for a full 12 h after antigen challenge has revealed a number of elements in the cutaneous LPR that were not heretofore clear.

It becomes apparent that, in the skin, as in the upper and lower airways, there is late-phase release of mediators. The secondary release of histamine, which had not been observed in earlier studies, begins ~ 7 h after antigen stimulation and is still increasing at 12 h, when it reaches levels approximately three-quarters as great as the histamine release observed in the

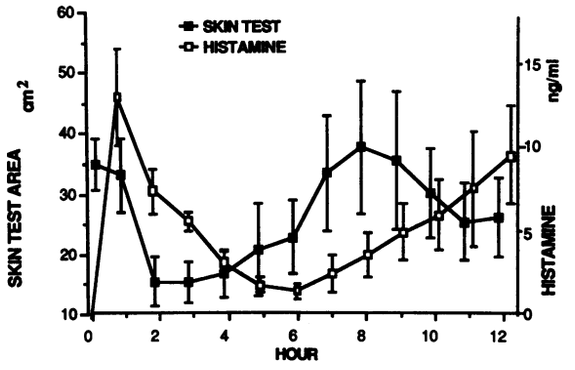


*Figure 5.* Skin test reactivity in response to an epicutaneous RW antigen challenge and skin chamber histamine levels in subjects previously shown to manifest a cutaneous LPR. The peak skin test reactivity occurs during the eighth hour and slowly declines over the subsequent 4 h. The histamine release into the skin chamber slowly rises during the same time period and appears to reach maximum concentration during the 12 h.

acute reaction. It is noteworthy that histamine release in the acute-phase peaks within the first hour and thereafter promptly falls toward baseline, whereas the late-phase increase of this mediator is far slower and more persistent. Pienkowski and associates have demonstrated a correlation between the size of the cutaneous LPR and the level of $PGD_2$ in skin blister fluid after antigen challenge at 30 min, 5.5 h, 8.5 h, and 24 h (44). It is noteworthy that this study also demonstrated higher histamine release at both the 8.5- and the 24-h time points than was noted in the group that did not have a cutaneous LPR during the same time points. That Pienkowski was not able to demonstrate a second rise in histamine is most likely because he did not sample frequently enough and because he sampled by a direct transcutaneous aspiration rather using an adfixed chamber as we did.

The pattern of $PGD_2$ production in the skin is also quite different from that found in our studies of the upper airways. In the latter model, the pattern of prostaglandin generation and release is not significantly different from that of histamine (i.e., peaking within 20 min after antigen challenge and promptly falling to baseline levels). In the skin, $PGD_2$ levels are elevated by the first hour and this level is maintained for several hours. As in the upper airways model, however, prostaglandin levels thereafter fall and during the late phase, whereas the histamine levels are increasing, the prostaglandin levels are returning to baseline.

This failure to find increased levels of $PGD_2$ during the upper airways LPR suggested that the mediator release was caused by the basophil, which can not generate $PGD_2$, rather than the mast cell, which can. This study provides additional, strong evidence that this is also the case in the skin. Infiltration of basophils into the skin chamber begins at 7 h, the same time that the secondary increase in histamine levels is first noted. Basophil infiltration continues to increase until 11 or 12 h after antigen challenge, at which point the highest levels of histamine are observed. The correlation between the number of infiltrating basophils and the increase in histamine concentration in the antigen challenged skin site is highly significant.

We have previously shown that early in the cutaneous LPR (at 8 h), the blister fluid contains a histamine-releasing factor (HRF) (45). HRF causes basophil histamine release only from atopic individuals, and more recent studies have demonstrated that it does so by interacting with IgE (46, 47). These studies also showed, by using specific blocking antibodies, that antigen does not persist in the blisters at 8 h and this would probably be the case in this 12-h study. It is possible that some residual antigen might be present during the first several hours; however, this would be unlikely to cause the significant secondary release of histamine noted in our study. It is our hypothesis that the mediator release observed during the LPR is a result of basophil stimulation by HRF.

In addition to basophils, other inflammatory cells participate in the LPR. This study adds a dimension to previous observations by quantifying inflammatory cell infiltration in biopsies of antigen-stimulated sites as well as in skin blister chambers. Previous biopsy studies suggested that the late phase is attended by a predominantly polymorphonuclear cell infiltrate, with some eosinophils (11). Using a skin chamber model, Shalit et al. showed increased oxidative metabolism of neutrophils at 5 h after antigen challenge and Fleekop et al. showed that the neutrophil remained the predominant cell in the skin chamber up to 6 h (42, 48). Whereas previous studies did not document an increase in basophils during the cutaneous LPR, we were able to demonstrate the presence of basophils not only in the chambers but also in biopsied tissue. This study also documents increased neutrophils and eosinophils in both the dermis and the skin chamber. There are, however, important differences between the infiltration by basophils and eosinophils and that of neutrophils and mononuclear cells in both the skin biopsies and the skin chamber counts. The skin biopsy study shows eightfold more eosinophils appearing in the antigen challenged than in control tissue. The basophils, which can be clearly counted, are 14-fold higher and if all metachromatically stained cells are included, this ratio is increased. In contrast, the neutrophil infiltrate into the antigen challenged biopsy site increases only twofold above the control and the ratio with respect to mononuclear cell infiltration is even less impressive. These markedly different ratios are similarly observed in the blister chamber. The antigen challenged skin chambers demonstrate a > 20-fold increase in eosinophils and basophils as compared to controls. As in the biopsied tissue, the increase in neutrophils and mononuclear cells in the antigen-challenged blister chambers is twofold or less.

These cellular differentials suggest that the basophil and eosinophil are specifically attracted to the site of antigen-mast cell interaction, whereas the neutrophils and the mononuclear cells appear secondary to a nonspecific inflammatory stimulus, be it allergic or not. Our data would also suggest that the cells most involved in the pathogenesis of the LPR are those specifically attracted to the site, primarily the basophil and the eosinophil. The basophil is probably the cell responsible for the secondary histamine release and the induration and tissue destruction characteristic of the late phase reaction is likely the result of the eosinophil infiltration, with the attendant release of its toxic proteins, well described by Gleich and his colleagues (25, 49). Despite the small number of basophils recovered from the skin chambers relative to other cells, there is a significant correlation ($r = 0.81$, $P < 0.05$) between the numerical basophil influx and the secondary rise in histamine. The cumulative histamine recovered in the chambers from the 7th–12th h represents 57% of the available histamine content of the total cumulative basophils recovered during the same

time period. Our studies of the upper airways are consistent with the hypothesis supporting the basophil and eosinophil as playing a major pathogenetic role in the LPRs. We have observed that treatment with corticosteroids completely blocks the clinical aspects of the airways LPR and ablates the influx of eosinophils and basophils, without affecting the infiltration of neutrophils (50).

Studies of the pathogenesis of the late phase have just begun. We have certainly not examined all of the potential inflammatory mediators and we are proceeding to measure levels of leukotrienes, platelet-activating factor (PAF), kinins, and other mediators. We are confident that we can modify the nature of the cellular infiltrate with several different pharmacologic agents, such as steroids and new antihistamines, such as cetirizine (51). It is also clear that we can either modify the release of mediators (i.e., aspirin decreases $PGD_2$) or block their effects with newly defined antagonists, i.e., leukotriene or PAF antagonists. An ability to alter the type of mediators released, influence their interaction with tissue receptors and to modify the nature of the cellular infiltrate, together with an evaluation of the effects of these interventions on the clinical evolution of the cutaneous LPR should lead to far more insight into what elements are important in the generation of this response in man.

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