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PREDNISONE INHIBITS THE APPEARANCE OF INFLAMMATORY MEDIATORS AND THE INFLUX OF EOSINOPHILS AND BASOPHILS ASSOCIATED WITH THE CUTANEOUS LATE-PHASE RESPONSE TO ALLERGEN[1]

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To better define the effect of systemic glucocorticoids on the cutaneous early and late phase response (LPR), nine atopic subjects were examined in a double-blind cross-over study using skin chambers fixed over denuded skin blisters. A challenge was carried out by placing allergen in the chamber for 60 min in subjects who received either a 3-day pretreatment with 60 mg/day of prednisone or placebo. Skin chamber cell counts and inflammatory mediators (histamine, PGD_2, and leukotriene C_4 (LTC_4)) were measured at hourly intervals for 12 h. Prednisone pretreatment did not alter the immediate skin erythema or release of histamine but ablated the late secondary erythema and rise in histamine. The median histamine values during h 10, 11, and 12 in the placebo and prednisone pretreatment visits were 3.73 and 0.22 ng/ml, respectively ($p \leq 0.02$). Prednisone did not alter PGD_2 production; however, LTC_4 production was suppressed during the LPR. The cumulative median LTC_4 values during h 7, 8, and 9 were 5.6-fold ($p \leq 0.05$) more after placebo than after prednisone pretreatment. Prednisone altered cellular traffic more dramatically than it did inflammatory mediators. The influx of eosinophils, which peaked during the 9th and 10th h in placebo-treated patients, was completely blocked by prednisone ($p \leq 0.02$) for every h from 6 through 12. The influx of basophils, which started during the 9th h and peaked during the 12th h in placebo-treated patients, was suppressed at all time points ($p \leq 0.02$) in prednisone-treated patients. There was no significant alteration in neutrophil transit into the skin chambers induced by prednisone. We suggest that the selective blockade of eosinophil and basophil influx by prednisone and the associated decrease in inflammatory mediators may contribute to the blockade of the clinical expression of the cutaneous LPR.

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Allergic individuals exposed to allergens to which they are sensitive mount a complex response that, although usually expressed by cutaneous or respiratory symptoms, may have protean manifestations (1-4). As in other human disease processes, investigators have developed a number of models with which to explore the pathogenesis of allergic responses (5-8). For many years these models focused on the acute response after allergen challenge in various organs, such as the nose, lung, and skin (5-8). More recently, it has been recognized that in addition to an early response to Ag there is also a LPR[3] (9-11). This LPR is more complex than the acute response in that it involves recruitment of inflammatory cells from the circulation as well as inflammatory mediators, such as histamine and leukotrienes, which are common to both the early and LPR (12, 13). The late response to allergen has elicited considerable interest among researchers because it seems more relevant to the clinical states we wish to study, which clearly involve the influx and activation of inflammatory cells (14-16).

Although glucocorticoids are among the most potent and effective agents used in the treatment of serious allergic diseases, their precise mechanisms of action are incompletely characterized (17, 18). Many studies have demonstrated little or no effect of glucocorticoids on the acute wheal and flare response to cutaneous Ag challenge in humans (19-23). In contrast, the LPR, characterized by induration, edema, and erythema, is inhibited by glucocorticoids (23-25). Several investigators have studied the effect of glucocorticoid treatment on the leukocytic infiltrate that occurs during the LPR (25-27). Oral and topical glucocorticoids have been shown to inhibit the emigration of neutrophils and eosinophils into blister sites and biopsy tissues during the LPR to cutaneous Ag challenge in humans (25-27). Results of these studies of Ag-driven reactions are supported by similar studies in which the Rebuck skin window was used to analyze the emigration of leukocytes (28, 29). The cutaneous blister chamber model has several advantages over biopsy or skin window studies (16, 30). The fluid bathing the skin chamber can be sampled often and can be analyzed both for cellular elements and chemical mediators. The present study was initiated to explore the effects of glucocorticoids on both acute and late cutaneous responses to Ag, using the skin chamber model, with the hope of gaining further insight into the pathogenesis of experimental

[3] Abbreviations used in this paper: LPR, late-phase response; RW, ragweed; OCD, optimal challenge dose; PNU, protein nitrogen unit; LT, leukotriene.

allergic responses to Ag challenge and expanding our knowledge of the mechanisms involved in the clinical efficacy of glucocorticoids.

MATERIALS AND METHODS

The study group consisted of nine atopic subjects (three male, six female) with a median age of 38 y. Written informed consent was obtained before entry into the study, and approval was given by the day before the study. Heat/suction-induced blisters were placed on the volar aspect of each forearm, as described previously (16, 30). The blisters were aseptically unroofed the next day and sterile chambers, with entry and exit ports, were adfixed to the skin to be challenged with either Ag or buffer (16). Previously, we have shown that neither non-allergic subjects challenged with Ag nor allergic subjects challenged with an irrelevant Ag responded with erythema, mediator release or cellular influx into the blisters (31). Before challenge, the chambers were rinsed thoroughly with saline to reduce mediator levels to zero (32).

A double-blind placebo controlled cross-over study was performed. The subjects received either a 3-day pretreatment with 60 mg/day of prednisone or placebo in three divided daily doses. The subjects were instructed not to take any other medications for the duration of the study with the exception of topical nasal medications. At least 7 days separated the cross-over visits.

All subjects had been skin tested previously with 0.1 to 1000 PNU/ml of RW extract (in Hollister-Stier normal saline with 0.3% human albumin) and had clinical cutaneous LPR consisting of erythema, soft tissue swelling, and induration. The concentration of RW required to elicit a wheal of more than 10 mm with pseudopods (or more than 15 mm without pseudopods) and accompanied by more than 45 mm of erythema was defined as the OCD (16). In these subjects, 10 or 100 PNU was the OCD. Simultaneous with the introduction of RW Ag or diluent 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 surface area of erythema was calculated at 15 min and then hourly for 12 h with a composite diameter derived by averaging the long and short axis of cutaneous erythema. The opposite arm was consistently used for placing both skin tests and introducing Ag into the chamber during the subsequent visit to avoid interference induced by a local refractoriness. The skin chamber was challenged in all instances with 10× the OCD of RW (100 to 1000 PNU) or diluent in the chamber on the other arm as control that remained in the skin chamber for 60 min before removal and replacement hourly with Ringer's lactate with 250 μg/ml of heparin. Thus, 1000 PNU was the highest Ag dose used for challenge. The same dose and route of Ag challenge was used for the prednisone and placebo pretreatment visits. To avoid interference induced by local refractoriness, different arms were used on the 2 study days.

Blister fluid was centrifuged (3000 rpm for 5 min) and the pellet was resuspended in Ringer's lactate with 250 μg/ml of heparin. At least 100 nucleated cells within 9 large squares were counted in a Neubauer hemocytometer (Reichert Scientific Instruments, Division of Warner-Lambert Technologies Inc., Buffalo, NY). A portion of the cell suspension was cytospin centrifuged onto slides (Shandon Southern Instruments, Inc., Sewichley, PA) at 600 rpm for 6 min. Paired slides were stained with Diff-Quik (Baxter Health Care Corp., McGraw Park, IL) (a modified Wright-Giemsa stain) or 0.5% Alcian blue, pH = 1.0, for 60 min. The latter were counterstained with 0.1% Safranin in 1% acetic acid. A total of 100 Wright-Giemsa stained cells was counted. For alcian blue-stained cells, either the total number on the slide or 500 cells was counted, which ever was least (33).

After removal of the cells by centrifugation, skin chamber fluids to be used for measurement of LTC_4 and PGD_2 were diluted 1/4 with ethyl alcohol and stored at −70°C until assayed as previously described (34–36). The histamine levels from the drug and placebo visits were assayed together, using a previously described radioenzymatic assay with a sensitivity of 0.05 ng/ml (37). Unless otherwise stated, all values reported represent the net mediator release or cellular influx for each hour, with the control value being subtracted from the corresponding Ag challenged value at each time-point.

After comparison of the data sets by analysis of variance that showed significant difference between the curves, non-parametric analysis, using the Wilcoxon signed-rank test, was used to compare prednisone vs placebo data because the values did not necessarily follow a Gaussian distribution. A Z value was determined and the two-tailed probability was obtained.

RESULTS

Three days of treatment with prednisone had, as expected, no significant effect on the acute response to intradermal skin testing with Ag. The mean area of erythema 15 min after Ag challenge was 35.1 ± 4.4 cm^2 during the placebo pretreatment and 29.5 ± 2.5 cm^2 after prednisone pretreatment (p = NS). On both days, the acute erythema and induration subsided by >50% within 2 h and remained at this level until the 7th h at which time a LPR to Ag began in the placebo-treated patients. The cutaneous LPR to allergen peaked during the 8th h and was significantly suppressed by prednisone pretreatment for every h from the 7th through 12th (Fig. 1).

The effects of prednisone pretreatment on both the preformed (histamine) and newly synthesized mediators (LTC_4 and PGD_2) are shown in Figure 2. The pattern of histamine release in response to Ag challenge is similar to our previously reported results in which histamine peaked sharply during the 1st h and then fell to a nadir by the 6th or 7th h before showing a slow secondary increase that peaked during the 11th or 12th h (16). The secondary rise in histamine in the Ag-stimulated chambers was abolished by prednisone pretreatment. The histamine levels at the Ag challenged site never exceeded those of the control site and were significantly lower than those in the Ag challenged site on the placebo day for h 10 through 12. The median histamine values during h 10, 11, and 12 during placebo and prednisone pretreatment were 3.73 and 0.22 ng/ml, respectively ($p < 0.02$). Immunoreactive leukotrienes have shown considerable intersubject variability when measured in the skin blister model (38). Similar to the results of Zweiman and associates (38), we find that LTC_4 is variably elevated during the first 4 h after Ag challenge, followed by further increase from the 8th through the 12th h (Fig. 2). Although prednisone had no significant effect on the production of LTC_4 during the first several hours after Ag challenge, there was significant suppression of LTC_4 for h 8 through 10 ($p < 0.05$), with the levels during placebo visit being five- to sixfold more than those during prednisone. LTC_4 appeared to rise during the 11th h, however, the values were not different from control. Although significant differences in this mediator were found during prednisone and placebo, due to the variability of these data, we doubt that these differences are biologically significant.

The appearance of PGD_2 follows a different pattern after Ag challenge than does LTC_4 (Fig. 2). There is a clear increase in PGD_2 before the LPR that peaks during the 2nd through 4th h. The increase in PGD_2 has slower kinetics than previously found for the acute release of histamine. PGD_2 levels fall to baseline by h 5 or 6 and show no secondary rise as was found for histamine. Consistent with the selective late-phase effect of prednisone, the drug had no significant effect on the production of this inflammatory mediator.

Analysis of the appearance of eosinophils, neutrophils, and basophils into the cutaneous blister chamber after control or Ag challenge is displayed in Figure 3. Ag challenge increased the numbers of eosinophils, basophils, and neutrophils in the chamber starting at 4, 6, or 8 h for neutrophils, eosinophils, and basophils, respectively. Neutrophil influx was also seen in saline control-chal-

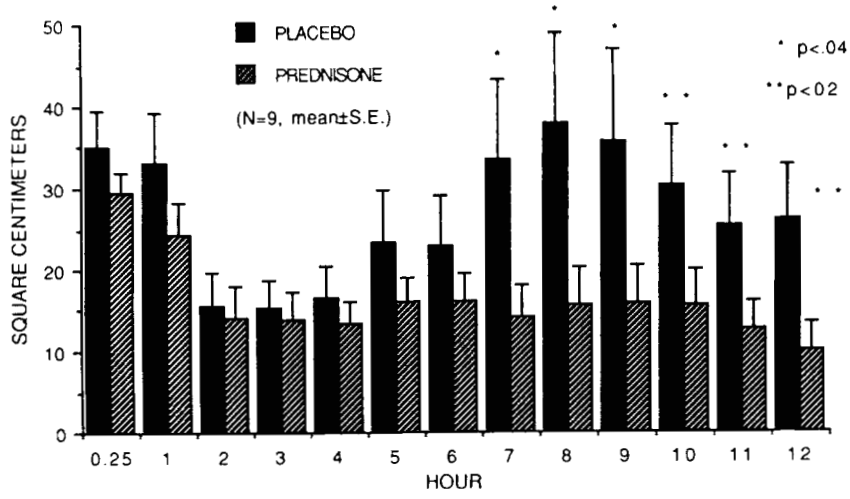

Figure 1. Skin test erythema in response to cutaneous ragweed Ag challenge with their OCD, in allergic subjects on placebo or prednisone. The mean ± SEM are shown for all times.

lenged blister chambers, but eosinophil and basophil influx was not seen in the absence of Ag challenge. Peak levels of neutrophils, eosinophils, and basophils were observed at approximately 10 to 12 h after Ag challenge. Prednisone pretreatment caused a complete abolition of the influx of eosinophils and basophils into the chamber but failed to modify the influx of neutrophils.

DISCUSSION

Many studies have demonstrated that oral glucocorticoids inhibit the late but not the acute response to experimental allergen challenge of the lungs, the upper airways, and the skin in humans (21, 23–27, 39–41). These observations have been confirmed by the present study in which 2-day treatment with oral prednisone (60 mg/day in three divided doses) caused marked inhibition of late phase erythema and induration with little or no influence on the acute response to intracutaneous allergen challenge (Fig. 1). The failure of glucocorticoids to inhibit the acute phase response has been attributed to an insensitivity of mast cells to steroids (17, 42, 43). Along with the failure of prednisone treatment to inhibit the acute phase physiologic response, prednisone treatment had no significant inhibitory effect on the appearance of mast cell mediators including histamine, PGD_2, and LTC_4 during the first 3 h of the Ag challenge protocol. The appearance of mast cell mediators in the acute phase nasal response is also unaffected by oral prednisone treatment (41). Inasmuch as the acute phase cutaneous responses of erythema and edema were not inhibited by oral prednisone, we can speculate that the steroid failed to alter mediator-induced changes in the vascular dilation and permeability that are responsible for these responses.

The mechanism by which glucocorticoids inhibit the LPR is still open for question. The present study has addressed this question by analyzing the appearance of cells and mediators in the cutaneous blister chamber after Ag challenge in placebo and steroid-treated individuals. Prednisone treatment caused virtual abolition of the appearance of eosinophils and basophils into the blister chamber after Ag challenge. In marked contrast, the neutrophil influx in both control and Ag-challenged blister chambers was unaffected by pretreatment with prednisone. Corresponding with inhibition of eosinophil and basophil influx was a significant inhibition of the appearance of histamine and LTC_4 by prednisone during the LPR. Inhibition of the emigration of eosinophilic and basophilic leukocytes and concomitant reduction of the release of these mediators may partially explain the ability of prednisone to diminish the LPR.

This study and a previous study demonstrate a greater proportional increase in the numbers of eosinophils and basophils than neutrophils after Ag challenge compared to saline control challenge of the blister chamber (16). The mechanism underlying this relative selectivity for basophils and eosinophils in these IgE-mediated reactions is unclear but may rely on the generation of selective chemoattractants or selective leukocyte "priming" factors.[4] Intracutaneous injection of platelet-activating factor has been shown to selectively recruit eosinophils in allergic but not normal subjects (44). Recent demonstrations that Ag challenge in the skin is associated with increased appearance of lymphocytes and lymphocyte activation leave open the possibility that lymphocyte-derived cytokines may be important for selective recruitment of eosinophils and basophils (45). For example, IL-5 has been demonstrated to induce eosinophil-selective chemotaxis in vitro (46). However, IL-3 stimulates increased basophil adherence and up-regulation of adherence molecules but fails to stimulate the same responses in eosinophils or neutrophils (47). Recent studies have demonstrated the appearance of IL-1 in the cutaneous blister chamber after Ag challenge.[5] Inasmuch as IL-1 can activate endothelial cells to express ELAM-1 and acquire adhesive properties for neutrophils, eosinophils, and basophils, local generation of IL-1 may also participate in the recruitment of inflammatory cells during the late phase (see footnote 5) (48–52).

In control blister chambers, neutrophils, but not eosinophils or basophils, increase in numbers steadily throughout the test period. The factors that lead to selective recruitment of neutrophils in control challenges are not known. It is possible that chemoattractants with selectivity for neutrophils are being generated. Injection of C5a into the skin of human volunteers induces a neutrophilic infiltrate (53). Thus, a small amount of

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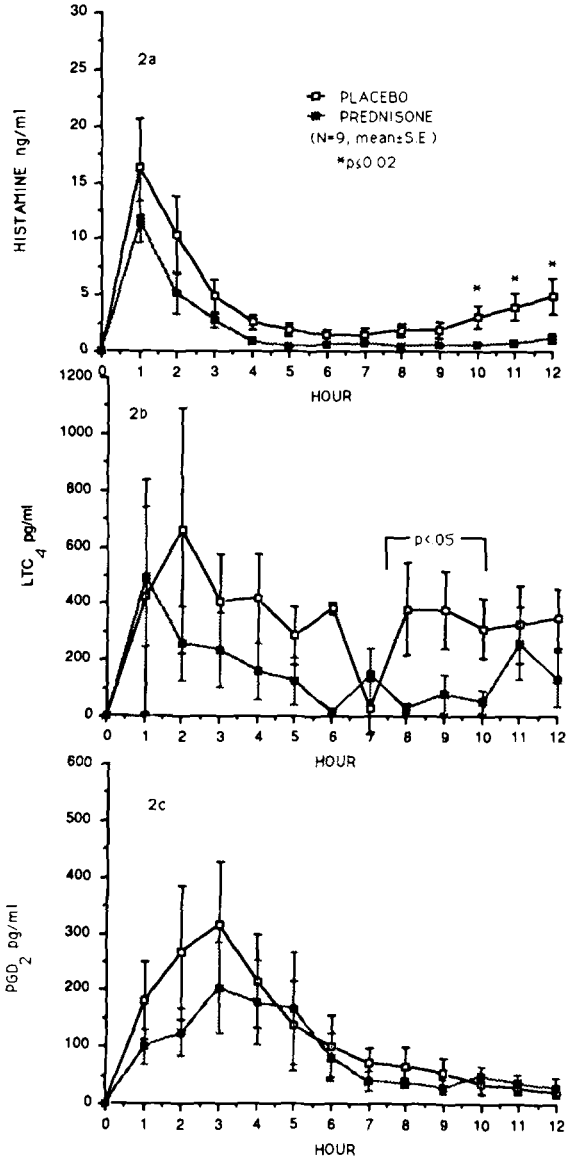

Figure 2. Time course of the appearance of histamine, LTC_4, and PGD_2 in skin chamber fluids during the early and late-phase response to antigen challenge with 10 times the OCD of Ag in the chambers. All values reported represent mediator production with the control value being subtracted from the respective antigen for each time point. Mean ± SEM are shown.

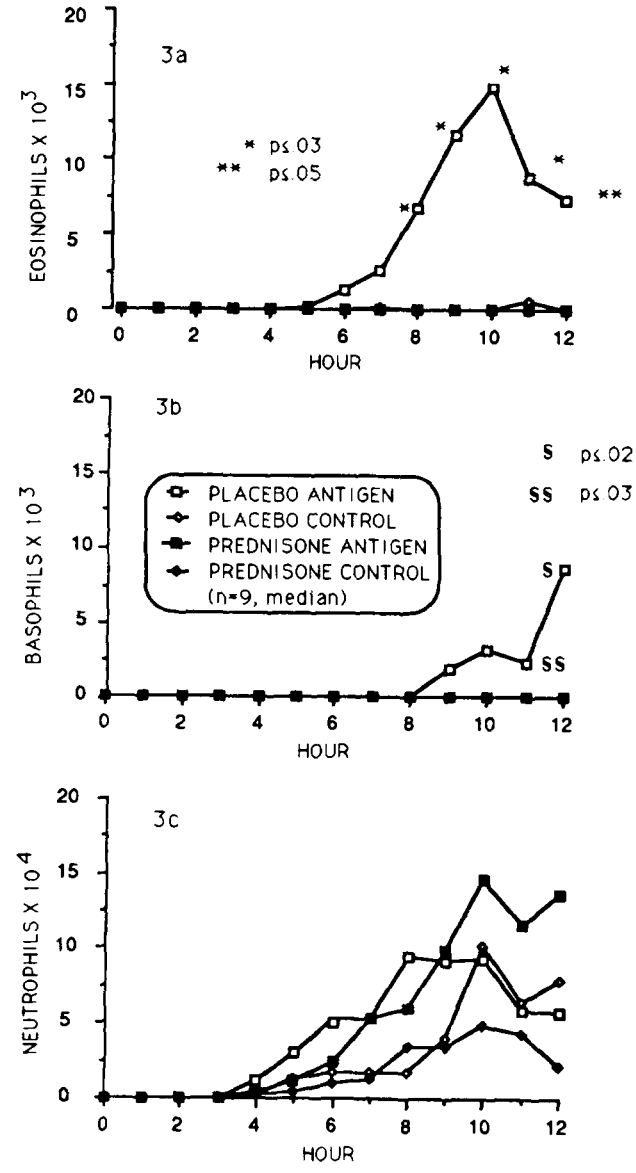

Figure 3. The effect of prednisone pretreatment on the median hourly cellular migration into skin chambers in response to Ag challenge with 10 times the OCD of Ag in the chambers. No cells were found until 4 to 6 h. Thereafter, eosinophils and basophils increased specifically in response to the Ag; neutrophils entered under all conditions. The influx of eosinophils and basophils, but not neutrophils, was ablated by prednisone pretreatment. All four conditions were evaluated for each cell type. Due to very low numbers, only the placebo Ag curve is visible for eosinophils and basophils.

plasma exudation, with local formation of anaphylatoxin, may explain the appearance of neutrophils in unchallenged blister chambers.

The mechanism by which prednisone selectively inhibits its eosinophil and basophil migration, without influencing neutrophil migration to the blister chamber, is open to question. One possible contributing factor is steroid-induced changes in the number of these cells in the circulation. However, in a similar study, using 60 mg prednisone/day, we have observed the following numerical changes in leukocyte number in the circulation: neutrophils (137 ± 46% of control, $p \leq 0.05$); eosinophils (88 ± 88% of control, p = NS); and basophils (49.9 ± 24% of control, $p \leq 0.05$, $n = 12$) (41). Although we did not monitor changes in circulating leukocyte number in the present study, if similar proportional changes in neutrophil, basophil, and eosinophil numbers in the circulation occurred, these changes would be unlikely to explain the dramatic selective effect of prednisone on emigration of eosinophils and basophils.

Various in vitro and in vivo studies in animals and humans have addressed the possible mechanisms by which glucocorticoids inhibit leukocyte emigration. Steroids do not inhibit the chemotactic response of purified human neutrophils or eosinophils in vitro, and do not block the binding of human neutrophils, basophils, or eosinophils to cultured endothelial cells (52, 54). Glucocorticoids inhibit the formation and release of chemotactic factors, endothelial activators, and leukocyte priming cytokines in many in vivo and in vitro models, effects that could explain steroid-induced diminution of the appearance of leukocytes (55). The factors that recruit leu-

kocytes into the cutaneous blister chamber are unknown. However, in contrast to most cell-derived chemoattractants, such as hydroxyeicosatetraenoic acids, LTB_4, platelet-activating factor, IL-8 (macrophage-derived neutrophil chemotactic factor) etc., C5a is a plasma-derived chemoattractant. The formation of C5a is, therefore, unlikely to be directly influenced by glucocorticoids, the actions of which are cell directed. Glucocorticoids fail to inhibit neutrophil emigration induced by C5a in vivo in humans (53). It is plausible that neutrophil emigration in the blister chamber may in part be the result of local plasma exudation and formation of chemotactic anaphylatoxins, a process relatively undisturbed by prednisone.

The hypothesis that inhibition of cell recruitment by steroids is the result of a local inhibitory effect on tissue cells that release chemoattractants, endothelial activators, and priming cytokines rather than an effect on circulating leukocytes, is further supported by studies in the nasal challenge model in which topical steroids had an equal or greater efficacy in inhibiting leukocyte recruitment to the upper airways compared to oral steroids (56). Similar studies with topical application of steroids in the blister chamber model should provide further insight into this important question.

REFERENCES

1. **Pepys, J.** 1973. Immunopathology of allergic lung disease. *Clin. Allergy 3:1.*
2. **Gleich, G. J.** 1982. The late phase of the immunoglobulin E mediated reaction: a link between anaphylaxis and common allergic disease? *J. Allergy Clin. Immunol. 70:160.*
3. **Cooke, R. A.** 1922. Studies in specific hypersensitiveness. IX. On the phenomenon of hypersensitization (the clinically lessened sensitiveness of allergy). *J. Immunol. 7:219.*
4. **Umemoto, L., J. Poothullil, J. Dolovich, and F. Hargreave.** 1976. Factors which influence the cutaneous allergic responses. *J. Allergy Clin. Immunol. 58:60.*
5. **Ting, S., E. H. Dunsky, R. M. Lavker, and B. Zweiman.** 1980. Patterns of mast cell alterations and *in vivo* mediator release in human allergic skin reactions. *J. Allergy Clin. Immunol. 66:417.*
6. **Dorsch, W., J. Ring, H. J. Reiman, and R. Geiger.** 1982. Mediator studies of skin blister fluid from patients with dual skin reaction after intradermal allergen injection. *J. Allergy Clin. Immunol. 70:236.*
7. **Tannenbaum, S., H. Oertel, W. R. Henderson, and M. Kaliner.** 1980. The biologic activity of mast cell granules. I. Elicitation of inflammatory responses in rat skin. *J. Immunol. 125:325.*
8. **Shampain, M. P., B. L. Behrens, G. L. Larsen, and P. M. Henson.** 1982. An animal model of the late pulmonary responses to *Alternaria* challenge. *Am. Rev. Respir. Dis. 126:493.*
9. **Robertson, O. G., A. T. Kerigan, F. E. Hargreave, R. Chalmers, and J. Dolovich.** 1974. Late asthmatic responses induced by ragweed pollen allergen. *J. Allergy Clin. Immunol. 54:244.*
10. **Solley, G. O., G. J. Gleich, R. E. Jordon, and A. L. Schoeter.** 1976. The late phase of the immediate wheal and flare skin reaction: its dependence on IgE antibodies. *J. Clin. Invest. 58:408.*
11. **Dolovich, J., F. E. Hargreave, R. Chalmers, K. J. Shier, J. Gauldie, and J. Bienenstock.** 1973. Late cutaneous allergic response in isolated IgE dependent reactions. *J. Allergy Clin. Immunol. 52:38.*
12. **Kaliner, M.** 1984. Hypothesis on the contribution of the late-phase allergic responses to the understanding and treatment of allergic diseases. *J. Allergy Clin. Immunol. 73:311.*
13. **deShazo, R. D., A. I. Levinson, and H. F. Dvorak.** 1983. The late-phase skin reaction: paradigm or epiphenomen? *Ann. Allergy 51:166.*
14. **Warner, J. O.** 1976. Significance of late reactions after bronchial challenge with house dust mite. *Arch. Dis. Child. 51:905.*
15. **Naclerio, R. M., D. Proud, A. G. Togias, N. F. Adkinson, Jr., D. A. Meyers, A. Kagey-Sobotka, M. Plaut, P. S. Norman, and L. M. Lichtenstein.** 1985. Inflammatory mediators in late antigen-induced rhinitis. *N. Engl. J. Med. 313:65.*
16. **Charlesworth, E. N., A. F. Hood, N. A. Soter, A. Kagey-Sobotka, P. S. Norman, and L. M. Lichtenstein.** 1989. The cutaneous late phase response to allergen: Mediator release and inflammatory cell infiltration. *J. Clin. Invest. 83:1519.*
17. **Schleimer, R. P., H. M. Claman, and A. Oronsky.** 1989. *Antiinflammatory Steroid Action: Basic and Clinical Aspects.* Academic Press, New York.
18. **Schleimer, R. P.** 1988. Glucocorticosteroids: their mechanisms of action and use in allergic disease. In *Allergy: Principals and Practice, 3rd ed.* E. Middleton, C. E. Reed, E. F. Ellis, N. F. Adkinson, and J. W. Yunginger, eds. C.V. Mosby Company, St. Louis, MO, p. 739.
19. **Cooke, R. A., W. B. Sherman, A. E. O. Menzel, H. B. Chapin, C. M. Howell, R. B. Scott, P. A. Myers, and L. M. Downing.** 1951. ACTH and cortisone in allergic diseases. *J. Allergy 22:211.*
20. **Stollerman, G. H., S. J. Rubin, and C. M. Plotz.** 1951. Effect of cortisone on passively induced skin hypersensitivity in man. *Proc. Soc. Exp. Biol. Med. 76:261.*
21. **Mancini, R. E., P. A. Colombi, H. Galli, and L. Orcivoli.** 1961. Effect of glucocorticoid hormones on experimentally induced allergic reactions on human skin. *J. Allergy 32:471.*
22. **Slott, R. I., and B. Zweiman.** 1974. A controlled study of the affect of corticosteroids on immediate skin test reactivity. *J. Allergy Clin. Immunol. 54:229.*
23. **Poothullil, J., L. Umemoto, J. Dolovich, F. E. Hargreave, and R. P. Day.** 1976. Inhibition by prednisone of late cutaneous allergic responses induced by antiserum to human IgE. *J. Allergy Clin. Immunol. 57:164.*
24. **Gronneberg, R., K. Strandberg, G. Stalenheim, and O. Zetterstrom.** 1981. Effect in man of anti-allergic drugs on the immediate and late phase cutaneous allergic reactions induced by anti-IgE. *Allergy 36:201.*
25. **Felarca, A. B., and F. C. Lowell.** 1968. Local effects of cortisol in the time course of eosinophiliotaxis with the use of an improved technique. *J. Allergy 43:114.*
26. **Eidinger, D., R. Wilkinson R, and B. Grose.** 1964. A study of cellular responses in immune reactions utilizing the skin window technique. I. Immediate hypersensitivity reactions. *J. Allergy 35:77.*
27. **Zweiman, B., R. I. Slott, and P. C. Atkins.** 1976. Histologic studies of human skin test responses to ragweed and compound 48/80. *J. Allergy Clin. Immunol. 58:657.*
28. **Rebuck, J. W., and R. C. Mellinger.** 1953. Interruption by topical cortisone of leukocytic cycles in acute inflammation in man. *Ann. N.Y. Acad. Sci. 56:715.*
29. **Dale, D. C., A. S. Fauci, and S. M. Wolff.** 1974. Alternate-day prednisone. Leukocyte kinetics and susceptibility of infections. *N. Engl. J. Med. 291:1154.*
30. **Talbot, S. F., P. C. Atkins, M. Valenzano, and B. Zweiman.** 1984. Correlations of in vivo mediator release with late cutaneous allergic responses in humans. I. Kinetics of histamine release. *J. Allergy Clin. Immunol. 74:819.*
31. **Pienkowski, M. M., N. F. Adkinson, Jr., M. Plaut, P. S. Norman, and L. M. Lichtenstein.** Prostaglandin D_2 and histamine during the immediate and the late-phase components of allergic cutaneous responses. *J. Allergy Clin. Immunol. 82:95.*
32. **Reshef A, A. Kagey-Sobotka, N. F. Adkinson, Jr., L. M. Lichtenstein, and P. S. Norman.** 1989. The pattern and kinetics in human skin of erythema and mediators during the acute and late-phase response (LPR). *J. Allergy Clin. Immunol. 84:678.*
33. **Gilbert, H. S., and L. Ornstein.** 1975. Basophil counting with a new staining method using alcian blue. *Blood 46:279.*
34. **Adkinson, N. F., Jr.** 1977. Prostaglandin production by human peripheral blood *in vitro*. *J. Lab. Clin. Med. 90:1043.*
35. **Hayes, E. C., D. L. Lombardo, Y. Girard, A. L. Maycock, J. Rokach, A. S. Rosenthal, R. N. Young, R. W. Egan, and H. J. Zweerink.** 1983. Measuring leukotrienes of slow reacting substance of anaphylaxis: development of a specific radioimmunoassay. *J. Immunol. 131:429.*
36. **Schulman, E. S., H. H. Newball, L. M. Demers, F. A. Fitzpatrick, and N. F. Adkinson, Jr.** 1981. Anaphylactic release of thromboxane A_2, prostaglandin D_2 and prostacylin from human lung parenchyma. *Am. Rev. Respir. Dis. 124:402.*
37. **Brown, M. J., P. W. Ind, R. Causon, and T. H. Lee.** 1982. A novel double-isotope technique for the enzymatic assay of plasma histamine: application to estimation of mast cell activation assessed by antigen challenge in asthmatics. *J. Allergy Clin. Immunol. 69:20.*
38. **Talbot, S., P. Atkins, E. J. Goetzl, and B. Zweiman.** 1985. Accumulation of leukotriene C_4 and histamine in human allergic skin reactions. *J. Clin. Invest. 76:650.*
39. **Booij-Noord, N., N. G. M. Orie, and K. deVries.** 1971. Immediate and late bronchial obstructive reactions to inhalation of house dust and protective effects of disodium cromoglycate and prednisolone. *J. Allergy Clin. Immunol. 48:344.*
40. **Nakazawa, T., T. Yoyoda, M. Furukawa, T. Taya, and S. Kobayashi.** 1976. Inhibitory effects of various drugs on dual asthmatic responses in wheat flour-sensitive subjects. *J. Allergy Clin. Immunol. 58:1.*
41. **Pipkorn, U., D. Proud, L. M. Lichtenstein, R. P. Schleimer, S. P. Peters, N. F. Adkinson, Jr., A. Kagey-Sobotka, G. K. Adams III, P. S. Norman, and R. M. Naclerio.** 1987. Effect of short-term systemic glucocorticoid treatment on human nasal mediator release after antigen challenge. *J. Clin. Invest. 80:957.*
42. **Schleimer, R. P., E. S. Schulman, D. W. MacGlashan, Jr., S. P. Peters, G. K. Adams III, L. M. Lichtenstein, and N. F. Adkinson, Jr.** 1983. Effects of dexamethasone on mediator release from human lung fragments and purified human lung mast cells. *J. Clin. Invest.*

71:1830.

43. **Cohan, V. L., B. J. Undem, C. C. Fox, N. F. Adkinson, Jr, L. M. Lichtenstein, and R. P. Schleimer.** 1989. Dexamethasone does not inhibit the release of mediators from human mast cells residing in airway, intestine or skin. *Am. Rev. Respir. Dis. 140:951.*
44. **Henocq, E., and B. B. Vargaftig.** 1986. Accumulation of eosinophils in response to intracutaneous PAF-acether and allergen in man. *Lancet 2:1378.*
45. **Kay, A. B., A. J. Frew, J. Varley, R. Moqbel, M. Azzawi, A. Hartnell, M. K. Church, and S. T. Holgate.** 1989. Bronchial infiltration by T lymphocytes in the late asthmatic reaction in the guinea pig. *J. Allergy Clin. Immunol. 83:44*(Abstr.).
46. **Yamaguchi, Y., Y. Hawashi, Y. Sugama, Y. Miura, T. Kasahara, S. Kitamura, M. Torisu, S. Mita, A. Tominaga, K. Takatsu, and T. Suda.** 1988. Highly purified murine interleukin 5 (IL-5) stimulates eosinophil function and prolongs in vitro survival. *J. Exp. Med. 167:1737.*
47. **Bochner, B. S., A. A. McKelvey, S. A. Sterbinsky, J. E. K. Hildreth, C. P. Derse, D. A. Klunk, L. M. Lichtenstein, and R. P. Schleimer.** 1990. Interleukin-3 augments adhesiveness for endothelium and CD11b expression in human basophils but not neutrophils. *J. Immunol. 145:1832.*
48. **Fleming, W. E., and C. J. Dunn.** 1985. Interleukin-1 and lipopolysaccharide stimulate delayed PMN-leukocyte adhesion via direct interaction with vascular endothelial cells. *Fed. Proc. 44:1260.*
49. **Bevilaqua, M. P., J. S. Pober, M. E. Wheeler, R. S. Cotra, and M. A. Gimbrone, Jr.** 1985. Interleukin 1 acts on cultured human vascular endothelium to increase the adhesion of polymorphonuclear leukocytes, monocytes, and related leukocytic cell lines. *J. Clin. Invest. 76:2003.*
50. **Schleimer, R. P., and B. K. Rutledge.** 1986. Cultured human vascular endothelial cells acquire adhesiveness for leukocytes following stimulation with interleukin-1, endotoxin, and tumor-promoting phorbol esters. *J. Immunol. 136:649.*
51. **Lamas, A. M., C. R. Mulroney, and R. P. Schleimer.** 1988. Studies on the adhesive interaction between human eosinophils and cultured vascular endothelial cells. *J. Immunol. 140:1500.*
52. **Bochner, B. S., P. T. Peachell, K. E. Brown, and R. P. Schleimer.** 1988. Adherence of human basophils to cultured umbilical vein vascular endothelial cells. *J. Clin. Invest. 81:1355.*
53. **Yancey, K. B., C. H. Hammer, L. Harvath, L. Renfer, M. M. Frank, and T. J. Lawley.** 1985. Studies of human C5a as a mediator of inflammation in normal human skin. *J. Clin. Invest. 75:486.*
54. **Schleimer, R. P., H. S. Freeland, S. P. Peters, K. E. Brown, and C. P. Derse.** 1989. An assessment of the effects of glucocorticoids on degranulation, chemotaxis, binding to vascular endothelial and formation of leukotriene B4 by purified human neutrophils. *J. Pharmacol. Exp. Ther. 250:598.*
55. **Schleimer, R. P.** 1990. Effects of glucocorticoids on inflammatory cells relevant to their therapeutic applications in asthma. *Am. Rev. Respir. Dis. 141:S59.*
56. **Pipkorn, U., D. Proud, L. M. Lichtenstein, A. Kagey-Sobotka, P. S. Norman, and R. M. Naclerio.** 1987. Inhibition of mediator release in allergic rhinitis by pretreatment with topical glucocorticosteroids. *N. Engl. J. Med. 316:1506.*