Effects of Dexamethasone on Mediator Release from Human Lung Fragments and Purified Human Lung Mast Cells

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ABSTRACT Purified human lung mast cells released histamine, leukotrienes, prostaglandin (PG) D_2, thromboxane B_2 (TxB_2), and $PGF_{2\alpha}$ in response to anti-IgE stimulation. Incubation of the cells for 24 h with 10^{-6} M dexamethasone, a treatment that inhibits mediator release from human basophils, had no effect on the release of these mediators from mast cells. Dexamethasone treatment of human lung fragments led to little or no inhibition of anti-IgE-induced release of the mast cell-derived mediator, histamine, but produced a significant inhibition of the release of PGE_2, $PGF_{2\alpha}$, and 6-keto-$PGF_{1\alpha}$. As was the case with purified mast cells, the steroid did not inhibit the release of PGD_2 or TxB_2 from human lung fragments. Comparison of the quantities of PGD_2 and TxB_2 produced by purified cells and human lung fragments reveals that the mast cells produce quantities of these metabolites sufficient to account for the entire amount produced by challenged lung fragments. Dexamethasone inhibited spontaneous release from lung fragments of all cyclooxygenase products measured. These results suggest that the human lung parenchymal mast cell phospholipase is not inhibited by dexamethasone, whereas other phospholipase(s) in the lung are inhibited by the steroid. These results may be useful in explaining the resistance of acute allergic reactions, including anaphylaxis, to steroids, despite the potent antiinflammatory activity of steroids on subacute and chronic inflammation, such as in bronchial asthma, which may be initiated by IgE-dependent mechanisms.

INTRODUCTION

The glucocorticosteroids are among the most effective therapeutic agents available for a variety of acute and chronic inflammatory diseases, including those with an immunological etiology. Although these steroids are effective in the treatment of some chronic immunoglobulin (IgE)-dependent allergic diseases such as allergic asthma and allergic rhinitis, acute IgE-dependent reactions, such as the skin test response, systemic anaphylaxis, and antigen-induced bronchospasm appear to be resistant to steroid treatment (1, 2). We have recently demonstrated that IgE-mediated histamine release from human basophils is inhibited by exposure to low concentrations of glucocorticoid for 24 h (3). This action of steroids, which has also been observed in mouse and rat mast cells (4, 5), is not due to a steroid-induced shedding of cell surface IgE Fc receptors and also appears not to be due to an inhibition of phospholipase A_2 by the steroids (3, 6).

The present study was undertaken for two reasons: to determine the spectrum of arachidonic acid metabolites released from human lung mast cells, and to evaluate the effect of the potent glucocorticoid, dexamethasone, on mediator release from human lung mast cells. We have used a recently developed technique by which human lung mast cells can be purified to near homogeneity (7). After exposure for 24 h to dexamethasone, the cells were challenged with goat anti-

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human IgE, and the release of mediators including histamine, leukotrienes, and a series of arachidonic acid cyclooxygenase metabolites (AACM: prostaglandin (PG) D_2, 6-keto-$PGF_{1\alpha}$, PGE_2, $PGF_{2\alpha}$, and thromboxane (Tx) B_2)[1] was monitored. The results of this study reveal a basic difference between human mast cells and human basophils in their sensitivity to inhibition of mediator secretion by glucocorticosteroids.

METHODS

Preparation of lung fragments and purification of mast cells

The procedure for preparation of human lung tissue and purification of mast cells has been described elsewhere (7). Briefly, lung tissue obtained after thoracotomy for cancer was dissected free of tumor, large airways, pleura, and visible blood vessels. For chopped lung experiments, the tissue was cut into 100-mg fragments. For purification of mast cells, the tissue was minced into smaller fragments (~10 mg) and then dispersed by a series of enzymatic digestions with pronase, chymopapain, elastase, and collagenase into a single cell suspension. After filtration through nytex cloth the cell suspensions were washed in calcium-free Tyrode's buffer (NaCl, 8 g/liter; KCl, 0.2 g/liter; Na_2HPO_4, 0.05 g/liter; $CaCl_2$-$2H_2O$, 0.26 g/liter; $MgCl_2$-$6H_2O$, 0.25 g/liter, and glucose, 1.0 g/liter, pH 7.2) containing gelatin (1 g/liter) and deoxyribonuclease type 1 (10 mg/liter). Mast cells were partially purified by elutriation using a Beckman J2-21 centrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, CA) fitted with an elutriation apparatus. Elutriation fractions having the highest purity mast cells were pooled, passively sensitized with a purified human anti-benzyl penicilloyl (BPO) IgE antibody, and subjected to affinity chromatography using a BPO affinity column as described (7, 8). Mast cells of high purity (in these studies, ~90%) were eluted with the monovalent hapten BPO-formyllysine, washed, and then suspended in medium. Mast cells were enumerated using the alcian blue staining technique (9). Cells obtained by this technique were viable as assessed by vital dye staining, electron microscopic examination (by Dr. A. Dvorak, Harvard Medical School), histamine release, and responsiveness to pharmacological agonists (7).

Culture of lung fragments and purified mast cells

Human lung fragments and purified mast cells were cultured in RPMI 1640 medium containing 25 mM Hepes, 2 mM glutamine, gentamicin, and either 10% heat-inactivated fetal calf serum or 10% normal, type-matched (usually AB+) human serum. Lung fragments were cultured in multi-well tissue culture plates in replicate wells containing five 100-mg fragments in 3 ml of medium. Purified human lung mast cells were cultured at a cell density of $\sim 5 \times 10^5$/ml of medium in replicate wells containing 1 ml of medium. During the 24-h culture period, the purity of the mast cell preparations improved, apparently due to adherence to the plate of extraneous cells. In the experiments shown in Table I, the purity before culture was 81±4% (n = 5) and purity following culture was 91±2.7% (SEM) for the controls and 92±2% for the dexamethasone-treated cells. Recovery of mast cells following culture was always >80% and usually almost 100%.

Anti-IgE challenge of lung fragments and purified mast cells

After culture of lung fragments in medium containing 10^{-6} M dexamethasone, or ethanol control (0.005%), the fragments were transferred to test tubes, washed in Tyrode's buffer containing the same concentrations of dexamethasone and/or ethanol, resuspended in 3 ml of Tyrode's buffer (five fragments per test tube), and challenged with either buffer or anti-IgE (at concentrations ranging from 0.3 to 3 μg/ml) for 30 min at 37°C. Anti-IgE was a generous gift of Dr. K. Ishizaka (Johns Hopkins Medical School). After challenge, the supernatants were harvested for assay of mediators, and the tissue was boiled in 3 ml of 2% $HCLO_4$ for 10 min to determine residual tissue histamine content. Purified mast cells were washed after culture in Pipes-buffered saline (Pipes, 7.6 g/liter; NaCl, 6.4 g/liter; KCl, 0.37 g/liter; 10 N NaOH, 4.2 ml/liter; human serum albumin, 30 mg/liter) and resuspended in Pipes-buffered saline containing calcium and magnesium (both at 1 mM). Control and dexamethasone-treated cells were counted, and each were divided into two tubes; one for challenge with normal goat serum, the other for challenge with an optimal concentration of goat anti-IgE (usually 1 or 3 μg AbN/ml). Each tube contained from 3 to 6×10^5 mast cells. When the total number of mast cells was sufficient, more tubes were added for challenge with several concentrations of anti-IgE or control serum. The total volume of the challenge mixture was 500 μl. After this challenge period (45 min at 37°C), four 10-μl aliquots were removed from each challenge tube to determine total histamine content, and control and anti-IgE mediated histamine release. For total histamine content, cells were lysed in 1 ml of Pipes-buffered saline containing 2% $HClO_4$. After samples for histamine release were taken, the remaining mast cell suspension was centrifuged and the supernatants were harvested and stored frozen for later assay of prostaglandin and leukotriene content.

Determination of mediators

Histamine. Histamine released from challenged lung fragments and purified mast cells was expressed as a percentage of the total histamine content, after subtraction of control ("spontaneous") release (2–5%) from both values. Histamine contents in all supernatants were determined with an automated fluorometric technique (10).

Prostaglandins. Quantities of PGD_2, 6-keto-$PGF_{1\alpha}$, PGE_2, $PGF_{2\alpha}$, and TxB_2 were measured by radioimmunoassay (RIA) (11). All antibodies were highly specific and displayed <4% cross-reactivity to heterologous ligands as previously detailed (11).

Leukotrienes. Release of leukotrienes by anti-IgE-challenged purified human lung mast cells was quantified in all experiments using the guinea pig ileum bioassay, and confirmed in one experiment with a newly developed RIA (12) and by high performance liquid chromatography (Peters et al., unpublished observations). Ileal strips were suspended in organ baths containing Tyrode's solution equilibrated with 95% O_2/5% CO_2, containing atropine (5×10^{-7} M) and diphenhydramine (10^{-6} M). Identification of leukotriene was

[1] *Abbreviations used in this paper:* AACM, arachidonic acid cyclooxygenase metabolites; BPO, benzylpenicilloyl; LT, leukotriene, PG, prostaglandin; Tx, thromboxane; AbN, antibody nitrogen.

confirmed using the specific antagonist, FPL-55712. A complete description of the validation of the assay is provided elsewhere (13). Results were expressed in LTD_4 equivalents compared with authentic LTD_4 (a gift of Dr. Joshua Rokach, Merck-Frosst). No leukotriene activity was detected in control supernatants from cells challenged with normal goat serum.

Statistics. Statistical analysis of the data was performed using a Student's paired *t* test.

RESULTS

Mast cells purified from five different lung specimens were cultured for 24 h in the presence or absence of 10^{-6} M dexamethasone. Following culture, the purities ranged from 85 to 97% mast cells. The cells were challenged with anti-IgE at an optimal concentration (1 or 3 μg/ml; predetermined using cells of lower purity) and the mediators listed in Table I were measured. The major arachidonic acid metabolites released from anti-IgE challenged purified mast cells were PGD_2 and leukotriene, in roughly equimolar amounts (13, 14). Cells challenged with normal goat serum at an equivalent concentration (~1:3,000 or 1:1,000 for 1 or 3 μg/ml, respectively) produced <1 ng/10^6 cells of each of the prostaglandins and undetectable amounts of leukotriene ($<0.8 \times 10^{-11}$ LTD mol-equivalent/10^6 mast cells). The purified mast cells also released TxB_2 and $PGF_{2\alpha}$ following anti-IgE challenge: Challenged cells released 5–15 times more of these metabolites than controls. This was true in all experiments, including the two experiments in which mast cell purity was 97%, suggesting that these metabolites may be mast cell derived. This result is probably not due to specificity of the antibodies since cross-reactivity of the anti-TxB_2 or anti-$PGF_{2\alpha}$ for PGD_2 does not exceed 1%. Prostacyclin (PGI_2) metabolite (6-keto-$PGF_{1\alpha}$) and PGE_2 were in most cases not detected in supernatants of either control or stimulated cells. Histamine release (36% in controls) was similar to that seen in fresh human lung fragments or lung cell suspensions (15).

Incubation for 24 h with 10^{-6} M dexamethasone had no consistent inhibitory effect on the release of arachidonic acid metabolites (PGD_2, TxB_2, $PGF_{2\alpha}$, and leukotrienes) from the purified cells. This was true whether the drug was present during both the culture and challenge incubations, or only during the culture incubation. Unlike observations with the human basophil (3), and murine mast cells (4, 5), 10^{-6} M dexamethasone did not inhibit the release of histamine from the purified human lung mast cells (Table I). Using mast cells of 44% purity, incubation with 10^{-6} M dexamethasone for 48 h did not significantly inhibit histamine release (30 vs. 33% in control). Furthermore, it was noted that when the cells were cultured for 24 h in 10% human serum, but not fetal calf serum, dexamethasone produced a significant enhancement of 78±25% ($n = 3$; $P < 0.05$) of subsequent histamine release (data not shown).

To compare the profile of AACM produced by purified human lung mast cells with that produced by anti-IgE-challenged human lung fragments, and to establish that the lack of effect of dexamethasone was not the result of damage to mast cells during the purification procedure, analogous experiments with human lung fragments were carried out. In three experiments, human lung fragments (three replicates of five fragments per condition) were cultured with or with-

TABLE I

Effect of Dexamethasone on Mediator Release from Purified Human Lung Mast Cells

Culture condition	Anti-IgE*	Mediator						
		PGD_2	TxB_2	$PGF_{2\alpha}$	PGE_2‡	6-Keto-$PGF_{1\alpha}$‡	LT	Histamine
Control§	–	0.6±0.2	0.7±0.3	0.3±0.1	0.3	0.3	<0.8	36.9±12
	+	58.2±24.8	4.8±1.3	3.3±1.2	1.1	0.5	46±4.7	
Dexamethasone	–	0.5±0.1	0.4±0.1	0.8±0.5	0.5	0.4	<0.8	43.7±10.6
	+	48.2±16.2	6.1±1.8‖	3.8±2.1	0.9	0.4	40±7.6	

Values shown for LT are LTD_4 mol-equivalents ($\times 10^{11}$) per 10^6 mast cells. Values shown for histamine are net percent of total histamine released. Values shown for prostaglandins are nanograms per 10^6 mast cells. SEM from $n = 5$ is shown. Purities of mast cells were: control, 91±2.7; dexamethasone, 92±2.

* Data shown are those derived from stimulation with an optimal concentration of anti-IgE, usually 1 or 3 μg/ml. Results from other concentrations of anti-IgE were comparable.

‡ In most experiments these prostaglandins were undetectable.

§ Control cultures contained 0.005% ethanol, dexamethasone cultures contained 10^{-6} M dexamethasone and 0.005% ethanol.

‖ $P < 0.05$ vs. control: paired *t* test. All other comparisons of anti-IgE-stimulated mediator production by dexamethasone vs. control cells were nonsignificant ($P > 0.10$).

TABLE II

*Effect of Dexamethasone on Cyclooxygenase Products from Human Lung Fragments**

Culture condition	Anti-IgE‡	PGD_2	TxB_2	$PGF_{2\alpha}$§	PGE_2§	6-Keto-$PGF_{1\alpha}$
Control	−	4.5±1.3	3.4±0.5	15.8±2.3	27.8±3	96±15.6
	+	127±45	11.1±1	33.5±3	55.6±24	253±73.5
Dexamethasone	−	1.8±0.9‖	1±0.2‖	3±1‖	3.3±0.8‖	20.8±7.2‖
	+	121±48	7.3±0.7	11.1±4‖	8±2‖	94±32‖

* Values shown are mean±SEM of data, expressed as nanograms of AACM per gram (wet weight) of tissue, derived from three separate experiments.

‡ Data shown are those derived from stimulation with an optimal concentration of anti-IgE, either 1 or 3 μg/ml. Results from other concentrations of anti-IgE were comparable.

§ These metabolites were measured in two of the three experiments.

‖ $P < 0.05$ vs. control, Student's paired t test.

out 10^{-6} M dexamethasone for 24 h. After culture, the fragments were washed and challenged with buffer or an optimal concentration of anti-IgE, and histamine release and AACM release were determined. Histamine release was 10.5±0.3% and 13.5±3%, from control (no drug) and dexamethasone-treated fragments, respectively. Shown in Table II is AACM production from control and dexamethasone-treated lung fragments after challenge with either buffer or an optimal concentration of anti-IgE. As has been demonstrated previously, the predominant metabolite from this tissue was the PGI_2 metabolite, 6-keto-$PGF_{1\alpha}$ (11). Anti-IgE induced a significant increase in the production of all metabolites. Interestingly, based on the data in Tables I and II, and assuming a mast cell content of $\sim 2 \times 10^{-6}$ per gram of tissue (7), the PGD_2 and TxB_2 formed after anti-IgE challenge of lung fragments can be accounted for by the expected production of these metabolites by the tissue mast cells.

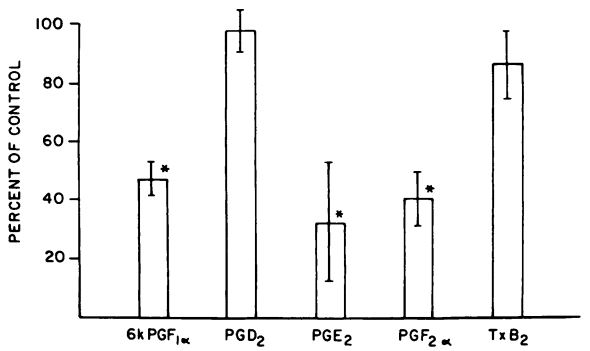

FIGURE 1 Effect of dexamethasone on net AACM release from anti-IgE-challenged human lung fragments. Data shown are mean±SEM from three experiments (except in the case of PGE_2 and $PGF_{2\alpha}$, which are derived from two experiments). Data are the net (anti-IgE stimulated) AACM generation from dexamethasone-treated fragments expressed as a percentage of net AACM generation from control fragments. Data are derived from the same experiments shown in Table II. *$P < 0.05$

Treatment with dexamethasone led to a significant reduction in the spontaneous production of all AACM products displayed in Table II (spontaneous release after culture with dexamethasone ranged from 5 to 60% of non-drug-treated control; 23±4% of control for all metabolites combined, $P < 0.05$). When the spontaneous release was subtracted, and the net, or anti-IgE-induced, AACM release was analyzed for each experiment, the results displayed in Fig. 1 were obtained. Synthesis of each metabolite by dexamethasone-treated tissue is shown expressed as a percent of non-drug-treated control. Dexamethasone treatment led to a 50% or greater reduction in the anti-IgE-induced formation of each AACM except PGD_2 and TxB_2. In two additional experiments (not shown), 10^{-8} M dexamethasone produced a half maximal (~30%) inhibition of spontaneous and net (anti-IgE-induced) 6-keto-$PGF_{1\alpha}$ synthesis.

DISCUSSION

Previous reports have described the production of PGD_2 by partially purified human lung mast cells and the generation of leukotrienes by highly purified human lung mast cells (13, 14). We report here that anti-IgE stimulation of high purity human lung mast cells led to the generation of significant quantities of leukotrienes, PGD_2, and TxA_2 (detected as its stable metabolite, TxB_2). The results with PGD_2 confirm previous studies, which show it to be the major cyclooxygenase metabolite released by both rat and human mast cells (14). In contrast to the rat mast cell (14), the human mast cell releases TxA_2 but not PGI_2 following stimulation with anti-IgE. The release of the potent

platelet-activating substance, TxA_2, may be important in light of recent findings suggesting that platelet activation occurs during antigen-induced bronchospasm (16). Lewis et al. (14) reported that human mast cell suspensions of 70% purity prepared by gradient centrifugation techniques do not produce TxB_2 in response to anti-IgE stimulation. Although the reason for the difference between those and the present results is not clear, it is useful to note that mast cells purified by gradient centrifugation have a significantly reduced capacity to produce leukotrienes compared with those prepared by elutriation and affinity chromatography (13, 17).

Incubation of purified human lung mast cells with 10^{-6} M dexamethasone did not inhibit the release of any of the mediators tested. This result contrasts with the findings in human basophils and murine mast cells where consistent inhibition is observed (3–6). The unresponsiveness to dexamethasone of purified human lung mast cells was not the result of exposure to the enzymatic digestion, elutriation, or affinity chromatographic procedures, since the response of mast cells in lung fragments, as assessed by histamine release, was also not affected by the steroid treatment. It is possible that exposure to elevated steroids before and during surgery lead to a reduction in mast cell activity and apparent insensitivity to steroids; this possibility has not been ruled out and is difficult to test. It is interesting to note that the net release of PGD_2, and for the most part TxB_2, from anti-IgE-challenged lung fragments was unaffected by dexamethasone; this finding is in keeping with the hypothesis that these metabolites are principally mast cell derived under these conditions. In further support of this hypothesis is the demonstration that purified lung mast cells can produce quantities of these metabolites sufficient to account for all that is derived from lung fragments.

Glucocorticoids inhibit phospholipase activity and the subsequent release of arachidonate in many different cell types (18, 19). It is likely that this effect occurs via induction, by the steroids, of an inhibitor of phospholipase termed macrocortin or lipomodulin (20, 21). In sensitized guinea pig lung, glucocorticoids inhibit activation of phospholipase and the release of arachidonate metabolites by a variety of stimuli including antigen and rabbit aorta-contracting substance-releasing factor (an antigen-induced factor that stimulates the release of endoperoxides and TxA_2 in guinea pig lung) (22). However, dexamethasone has no effect on the release of rabbit aorta-contracting substance-releasing factor, indicating that the drug is not abrogating the primary response to antigen (22, 23).

Our results suggest that glucocorticoids do not inhibit mediator release from human lung parenchymal mast cells. Because neither PGD_2, TxB_2, nor leukotriene release from these cells was inhibited by dexamethasone, there is no indication that the steroid was inhibiting mast cell phospholipase. In human lung fragments however, there was a clear inhibitory effect of dexamethasone on the release of arachidonate metabolites that are apparently not mast cell derived: namely, 6-keto-$PGF_{1\alpha}$, PGE_2, and $PGF_{2\alpha}$. In addition, the spontaneous release of all metabolites was inhibited by treatment with dexamethasone. It thus appears that while the mast cell phospholipase is not sensitive to steroids, the phospholipase(s) that provide arachidonate in the unchallenged tissue as well as that which is stimulated secondary to mast cell activation are steroid sensitive. Since spontaneous AACM production by purified mast cells was unaffected by dexamethasone, the steroid-sensitive phospholipase responsible for spontaneous AACM generation in lung fragments is probably not of mast cell origin. Now that a human prostaglandin releasing factor has been identified (24), it will be of interest to determine whether in the human, as is the case in the guinea pig with rabbit aorta-contracting substance-releasing factor, the steroid effect is limited to the cells responsive to the factor rather than the cell that releases it (presumably the mast cell).

In light of the results of others showing that murine peritoneal mast cell histamine release is inhibited by 24-h incubation with steroids, it will be necessary to determine whether human mast cells derived from other tissue sources such as skin, gut, or peritoneum are also sensitive to steroids (4, 5). Prednisolone has been reported to inhibit antigen-induced histamine release from human skin (25); since only brief incubations with high concentrations of drug ($\sim 10^{-4}$ M) were used, it is not clear that this was a specific glucocorticoid effect, however.

We note that certain chronic IgE-dependent immediate hypersensitivity reactions (e.g., allergic rhinitis, some forms of allergic asthma) are responsive to steroid therapy while many acute reactions (e.g., wheal and flare, skin test reactions, systemic anaphylaxis, and antigen-induced bronchospasm) are not (1, 2, 26). These clinical observations are in concordance with the present finding that dexamethasone does not alter the acute release of mediators by human lung mast cells. Further, our results suggest that an important component of the antiinflammatory action of steroids in chronic immediate hypersensitivity reactions may be an inhibition of the release of proinflammatory arachidonate metabolites from tissues that can respond to mast cell products. Finally, the observation that the release of mediators by human basophils is inhibited by steroids, while that from human mast cells is not, also raises the possibility that basophils may be an im-

portant IgE-bearing cell in chronic immediate hypersensitivity reactions.

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REFERENCES

1. Plaut, M., and L. M. Lichtenstein. 1982. Cellular and chemical basis of the allergic inflammatory response. Component parts and control mechanisms. *In* Allergy: Principles and Practice. E. Middleton, C. E. Reed, E. F. Ellis, editors. C. V. Mosby Co., St. Louis, MO. 2nd edition. In press.
2. 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:** 164–169.
3. Schleimer, R. P., L. M. Lichtenstein, and E. Gillespie. 1981. Inhibition of basophil histamine release by anti-inflammatory steroids. *Nature (Lond.).* **292:** 454–455.
4. Daeron, M., A. R. Sterk, F. Hirata, and T. Ishizaka. 1982. Biochemical analysis of glucocorticoid-induced inhibition of IgE-mediated histamine release from mouse mast cells. *J. Immunol.* **129:** 1212–1218.
5. Heiman, A., and F. T. Crews. 1982. Inhibition of histamine release from rat peritoneal mast cells by hydrocortisone. *Fed. Proc.* **41:** 1042.
6. Schleimer, R. P., D. W. MacGlashan, Jr., E. Gillespie, and L. M. Lichtenstein. 1982. Inhibition of basophil histamine release by anti-inflammatory steroids. II. Studies on the mechanism of action. *J. Immunol.* **129:** 1632–1636.
7. Schulman, E. S., D. W. MacGlashan, Jr., S. P. Peters, R. P. Schleimer, H. H. Newball, and L. M. Lichtenstein. 1982. Human lung mast cells: purification and characterization. *J. Immunol.* **129:** 2662–2667.
8. MacGlashan, D. W., Jr., and L. M. Lichtenstein. 1980. The purification of human basophils. *J. Immunol.* **124:** 2519–2521.
9. Gilbert, H. S., and L. Ornstein. 1975. Basophil counting with a new staining method using alcian blue. *Blood.* **46:** 279–285.
10. Siraganian, R. P. 1974. An automated continuous-flow system for the extraction and fluorometric analysis of histamine. *Anal. Biochem.* **57:** 383–391.
11. 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 prostacyclin from human lung parenchyma. *Am. Rev. Respir. Dis.* **124:** 402–407.
12. 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. Zwerink. 1983. Measuring leukotrienes of slow reacting substance of anaphylaxis: development of a specific radiimmunoassay. *J. Immunol.* In press.
13. MacGlashan, D. W. Jr., R. P. Schleimer, S. P. Peters, E. S. Schulman, G. K. Adams III, H. H. Newball, and L. M. Lichtenstein. 1982. Generation of leukotrienes by purified human lung mast cells. *J. Clin. Invest.* **70:** 747–751.
14. Lewis, R. A., S. T. Holgate, L. J. Roberts II, J. A. Oates, and K. F. Austen. 1981. Preferential generation of prostaglandin D_2 in rat and human mast cells. *In* Biochemistry of the Acute Allergic Reactions. E. L. Becker, A. S. Simon, and K. C. Austen, editors. A. R. Liss, Inc., New York. 239–254.
15. Peters, S. P., E. S. Schulman, R. P. Schleimer, D. W. MacGlashan, Jr., H. H. Newball, and L. M. Lichtenstein. 1982. Dispersed human lung mast cells: pharmacologic aspects and comparison with human lung tissue fragments. *Am. Rev. Respir. Dis.* **126:** 1034–1039.
16. Knauer, K. A., L. M. Lichtenstein, N. F. Adkinson, Jr., and J. E. Fish. 1981. Platelet activation during antigen-induced airway reactions in asthmatic subjects. *N. Engl. J. Med.* **34:** 1404–1407.
17. Lewis, R. A., J. M. Drazen, E. J. Corey, and K. F. Austen. 1981. In SRS-A and Leukotrienes. P. J. Piper, editor. John Wiley and Sons, Ltd., Chichester. 101–117.
18. Gryglewski, R. J., B. Panczenko, R. Korbut, L. Grodzinska, and A. Ocetkiewicz. 1975. Corticosteroids inhibit prostaglandin release from perfused lungs of sensitized guinea pig. *Prostaglandins.* **10:** 343–355.
19. Hong, S. L., and L. Levine. 1976. Inhibition of arachidonic acid release from cells as the biochemical action of anti-inflammatory corticosteroids. *Proc. Natl. Acad. Sci. USA.* **73:** 1730–1736.
20. Blackwell, G. J., R. Carnuccio, M. DiRosa, R. J. Flower, L. Parente, and P. Persico. 1980. Macrocortin: a polypeptide causing the anti-phospholipase effect of glucocorticoids. *Nature (Lond.).* **287:** 147–149.
21. Hirata, F., E. Schiffmann, K. Venkatasubramanian, D. Salomon, and J. Axelrod. 1980. A phospholipase A_2 inhibitory protein in rabbit neutrophils induced by glucocorticoids. *Proc. Natl. Acad. Sci. USA.* **77:** 2533–2536.
22. Blackwell, G. J., R. J. Flower, F. P. Nijkamp, and J. R. Vane. 1978. Phospholipase A_2 activity of guinea pig isolated perfused lungs: stimulation, and inhibition by anti-inflammatory steroids. *Br. J. Pharmacol.* **62:** 79–89.
23. Nijkamp, F. P., R. J. Flower, S. Moncada, and J. R. Vane. 1976. Partial purification of rabbit aorta contracting substance-releasing factor and inhibition of its activity by anti-inflammatory steroids. *Nature (Lond.).* **263:**479–482.
24. Steel, L., and M. Kaliner. 1981. Prostaglandin-generating factor of anaphylaxis. Identification and isolation. *J. Biol. Chem.* **256:** 12692–12697.
25. Greaves, M. W., and V. M. Plummer. 1974. Glucocorticoid inhibition of antigen-evoked histamine release from human skin. *Immunology.* **27:** 359–363.
26. 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:** 160–169.