

Interleukin-6 is released in the cutaneous response to allergen challenge in atopic individuals

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The mechanisms responsible for cutaneous response to antigen are complex. Interleukin-1 (IL-1) and interleukin-6 (IL-6) are proinflammatory cytokines that share many properties. Previous studies with a blister-chamber model have demonstrated IL-1 to be produced in the cutaneous response to antigen. Since IL-2 production by activated T cells and IL-6 production by macrophages are both stimulated by IL-1, we hypothesized that IL-2 and IL-6 may be involved in the cutaneous late-phase response (LPR) to antigen. We examined antigen-challenged sites for IL-2 immunoreactivity (ELISA) but found no difference between antigen- and diluent-challenged skin sites ($N = 4$). Since IL-2 has been demonstrated to be produced in response to IL-1 and IL-1 activity has been demonstrated to be greatest between hours 10 and 12, we speculated that IL-2 might not be detected until after hour 12. We were unable to demonstrate any increase in IL-2 production, even by extending our studies to 24 hours in two subjects. Antigen-challenged, skin blister-chamber fluids from atopic subjects demonstrated the appearance of IL-6 (ELISA) in pooled samples representing hours 1½, 3½, 5½, and 7, but not at diluent control sites ($p < 0.05$; $N = 6$). IL-6 reached a median peak of 0.66 ng/ml at 3½ hours. Median levels of IL-6 fell to baseline at 8 hours, followed by a second peak of 0.25 ng/ml at hour 10. Three distinct patterns of IL-6 release were noted: early release of IL-6 followed by a sustained slower rise that peaked at hour 9 before declining to baseline levels at 12 hours, early release of IL-6 followed by a fall to baseline levels at hours 7 to 9 with a second smaller peak at hours 9 to 11, and isolated early release of IL-6. Early IL-6 production correlated with late histamine production ($R = 0.801$; $p = 0.06$), and late IL-6 production correlated with eosinophil influx ($R = 0.813$; $p = 0.05$). The area of the LPR at skin test sites correlated with early IL-6 peak levels ($R = 0.977$; $p = 0.004$) and with total early IL-6 production ($R = 0.885$; $p = 0.05$), but not with late IL-6 production. We conclude that IL-6 is released locally in antigen-challenged sites in atopic subjects, that an early monophasic or biphasic release may be observed in individual subjects, and that its degree of early release may play an important role in the pathogenesis of the cutaneous LPR. (J ALLERGY CLIN IMMUNOL 1992;89:1010-20.)

Key words: IL-6, IL-2, interleukins, late-phase response, blister chamber, allergic cutaneous response

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Cytokines have been an area of interest in the study of the human immune response, and recently, there has been increasing interest in defining their role in allergy.¹⁻⁴ The inflammatory events initiated by the cross-linking of IgE on the surface of mast cells are complex, and cytokines that may play important roles in allergic events, include IL-3, a potent mast cell growth factor,⁵ IL-4, which induces IgE production and low-affinity IgE receptor (Fc_ε receptor II) production by macrophages and B cells,⁶ and IL-5, demonstrated to induce maturation and activation of eosinophils⁷ and to generate hypodense eosinophils.⁸

Other cytokines may play roles in the allergic response as well. IL-2 has many functions. It is secreted by activated T cells and promotes the growth of activated T cells and B cells,¹ activates large granular lymphocytes,⁹ cytotoxic T cells, and natural killer cells,¹⁰ and promotes the autocrine growth of T cells.⁵ IL-2 receptors are up regulated by IL-1,¹¹ and IL-2 is optimally produced in the presence of antigen, the major histocompatibility II receptor, and IL-1.¹ IL-6 shares many properties with IL-1, including the synthesis of acute-phase reactants¹¹ and activation of neutrophils.¹² Like IL-2, IL-6 production is induced by IL-1.¹¹

Using the skin blister-chamber model, Bochner et al.³ demonstrated the appearance of IL-1 in skin-chamber fluids of atopic subjects challenged with antigen. This model has the advantages of simplicity and accessibility for studying mediator production and cellular influx in response to antigen stimulation. These authors demonstrated IL-1 bioactivity in blister-chamber fluids during the LPR and IL-1 immunoreactivity, early and late in the allergic response, suggesting that this proinflammatory cytokine may play a role in IgE-mediated inflammatory events. Since both IL-2 and IL-6 are up regulated by IL-1,^{9, 11} we hypothesized that they may be important in the allergic response. In the current study, we examined skin blister-chamber fluids of atopic subjects challenged with antigen for the appearance of IL-2, IL-6, histamine, and inflammatory cells, using the skin blister-chamber model. Additionally, serum from antigen-challenged atopic subjects was examined for the detection of systemic IL-6 release.

MATERIAL AND METHODS

Subjects

Seven adult atopic subjects, with histories of allergic rhinitis or allergic rhinitis with asthma, were studied in blister-chamber experiments (Table I). Two subjects were not able to complete the study. One subject had the blister chamber slip from position during the study, and one subject

Abbreviations used

IL:	Interleukin
LPR:	Late-phase response
RLH:	Ringer's lactate with heparin
TNF- α :	Tumor necrosis factor- α
PNU:	Protein nitrogen unit

developed anaphylaxis, necessitating cessation of the study. All subjects had 4+ positive intradermal skin tests to either cat, mountain cedar, or dust mite (*Dermatophagoides pteronyssinus*) extracts. The study was approved by the Wilford Hall United States Air Force Medical Center Institutional Review Committee under Air Force Regulation 169-6 Exempt Protocols. All subjects were coinvestigators.

The same five subjects, plus four nonatopic subjects, were studied in the subsequent experiment for detection of systemic IL-6 release (Table I).

Reagents

Cat, mountain cedar, standardized *D. pteronyssinus* extracts, and sterile saline diluent with 0.5% human serum albumin were purchased from Hollister-Stier Laboratories (Spokane, Wash.). RLH was prepared from sterile, pyrogen-free Ringer's lactate (Travenol Laboratories, Deerfield, Ill.) and heparin (Steris Laboratories, Phoenix, Ariz.), which was added for a final heparin concentration of 250 μ g/ml. ³H-S-adenosyl-L-methionine was purchased from DuPont Medical Products (Boston, Mass.).

Skin testing

Titrated intradermal skin tests were performed to establish the appropriate challenge dose of antigen. The intradermal skin test dose required to elicit a 4+ immediate reaction (>15 mm wheal and >45 mm flare) was determined. The skin chamber challenge dose was defined as 10 times that producing a 4+ intradermal response. The late cutaneous response was assessed at 6, 9, or 12 hours after challenge. Records of the size of the immediate wheal, flare, and LPR induration were taken by tracing the margins onto transparent adhesive tape. The area of the wheal, flare, and LPR indurations were calculated with a computer planimeter (ZIDAS, Carl Zeiss, Oberkochen, Germany).

Blister-chamber challenge

Two skin blisters were created at least 12 hours before challenge on the volar surface of each forearm with gentle heat and suction, as previously described.^{3, 14} Immediately before challenge, the blisters were unroofed, and sterile aluminum chambers were applied. Aliquots of 0.5 ml of RLH were added to each chamber. The chamber was washed twice with RLH before the introduction of the challenge dose of antigen or RLH into contralateral chambers. The antigen and RLH were removed from either chamber after 1 hour and replaced with 0.5 ml of RLH. Fluids were re-

TABLE I

Subject	Atopic	AR, A	Clinically sensitive*	Antigen	Antigen dose	Wheal (mm ²)	Flare (mm ²)	LPR (mm ²)	Study
E. N. C.	Yes	AR, A	Yes	<i>D. pteronyssinus</i>	100 AU/ml	82.8	2276	1098	C, S
C. E. L.	Yes	AR	Yes	Cat	2800 PNU/ml	142	1542	1245	C, S
C. E. L.	Yes	AR	Yes	Cat	2800 PNU/ml	91	2546	1734	C, S
M. E. N.	Yes	AR	Yes	Mountain cedar	20 PNU/ml	123	941	1265	C, S
D. C. F.	Yes	AR	Yes	Mountain cedar	1000 PNU/ml	ND	ND	ND	C†
H. G. T.	Yes	AR, A	No	Cat	28000 PNU/ml	130	1851	779	C
B. F. V.	Yes	AR	No	Cat	28000 PNU/ml	147	1933	488	C
C. H. Y.	Yes	No	No	<i>D. pteronyssinus</i>	1000 AU/ml	ND	ND	ND	C‡
T. M. F.	No	No	No	Cat	280 PNU/ml	None	None	None	S
J. G. D.	No	No	No	Cat	280 PNU/ml	None	None	None	S
W. A. N.	No	No	No	N/A	N/A	N/A	N/A	N/A	S
A. F. B.	No	No	No	N/A	N/A	N/A	N/A	N/A	S

AR, Allergic rhinitis; A, asthma; C, subjects in blister chamber; S, systemic IL-6-release studies; ND, not done; N/A, not applicable.

*Clinically sensitive to challenge antigen.

†Excluded from study because of slippage of blister chamber.

‡Study stopped because of anaphylaxis and excluded from results.

moved hourly and replaced with fresh RLH. Samples were immediately frozen for the first 6 hours of the experiment. For samples beyond 6 hours, the fluid was centrifuged (high-speed centrifuge, HSC10K, Savant Instruments, Inc., Farmingdale, N.Y.) before immediately freezing the supernatant at -70°C . Frozen supernatants were thawed before being assayed for cytokines and histamine. The cellular pellet was resuspended in 0.5 ml of RLH for immediate quantitation of cellular influx.

Quantitation of leukocytes

Cell pellets resuspended in RLH were counted manually with an improved Neubauer hemocytometer (American Optical, Buffalo, N.Y.). Differential cell counts were performed after cytocentrifugation (Cytotek, Miles, Inc., Diagnostics Division, Elkhart, Ind.) and staining with a modified Hansel's stain (Lide Laboratories, Florissant, Mo.).

Systemic IL-6 release

Blood was obtained from both arms before antigen challenge. The previously determined concentration of antigen producing a 4+ intradermal skin test response was administered intradermally (0.02 ml) on one arm and saline diluent was administered on the contralateral arm. Two nonatopic subjects received a 1:1000 dilution (280 PNU/ml) of cat-pelt extract and saline diluent. Blood was obtained from each arm at 3, 6, 9, and 12 hours after challenge. Two nonatopic subjects were unchallenged, and blood was drawn at these times to observe for diurnal variation in IL-6 levels. All samples were allowed to clot for 15 minutes and were centrifuged; serum samples were frozen at -70°C until they were assayed for IL-6.

Cytokine assays

Commercial ELISA kits with monoclonal antihuman cytokines were used for IL-2 and IL-6 assays of blister-cham-

ber fluids (Genzyme Corp., Cambridge, Mass.). The standard curves were constructed with dilutions of recombinant human IL-2 or IL-6 supplied with the kits. The sample sizes were 100 μl , and each sample was run in duplicate. The lower limit of sensitivity of the assay was 100 pg/ml.

Commercial ELISA kits with monoclonal antihuman IL-6 were used for systemic IL-6 release experiments (Genzyme Corp. and R&D Systems, Inc., Minneapolis, Minn.). The standard curves were constructed with dilutions of recombinant human IL-6 supplied with the kits. Sample sizes were 100 or 200 μl for the kits from Genzyme and R&D Systems, respectively. Each sample was again run in duplicate. The sensitivity of the assays was 100 and 30 pg/ml, respectively. In addition, serum samples were assayed with and without heat treatment in a 56°C water bath (Precision Scientific, Chicago, Ill.) for 30 minutes to inactivate any IL-6 binding activity.

Histamine assay

Histamine levels of blister-fluid supernatants were assayed with a histamine *N*-methyltransferase radioenzymatic assay with a sensitivity of 0.5 ng/ml.¹³

Statistical analysis

The results were analyzed with a computer-statistics program (StatView 512+, BrainPower, Inc., Calabasas, Calif.). Median values are reported unless it is otherwise noted. Nonparametric analysis was used for comparison of groups (Wilcoxon's signed-rank test, two-tailed). Student's *t* test (two-tailed) was used for groups of less than six subjects. Regression analysis was used to provide correlations.

RESULTS

IL-2 assay of blister-chamber fluids

We were unable to detect the presence of IL-2 immunoreactivity in blister-chamber fluids in studies ex-

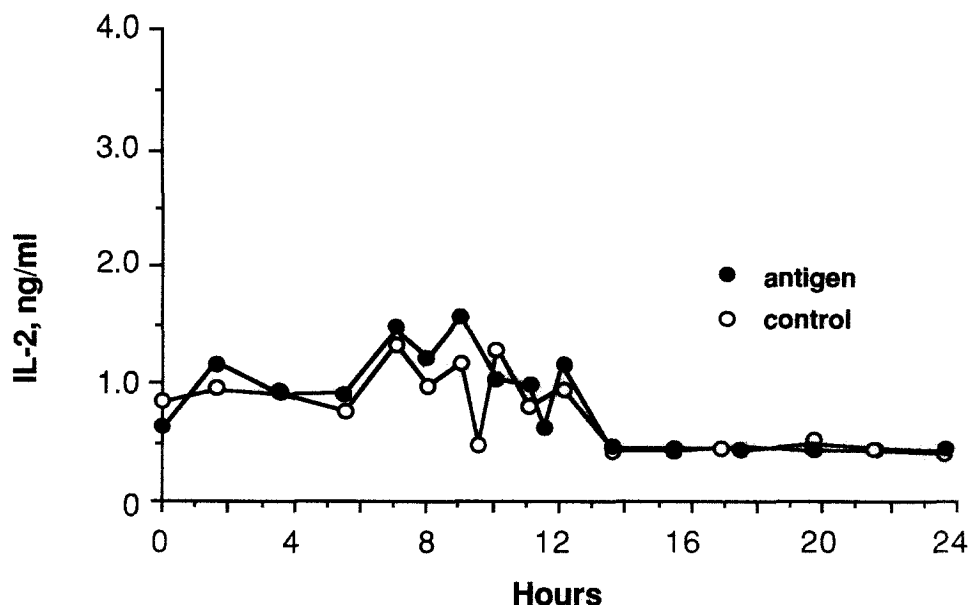


FIG. 1. IL-2 immunoreactivity in blister fluid, as determined by ELISA. Values are medians; $n = 4$, hours 0 to 12; $n = 2$, hours 12½ to 17; $n = 1$, hours 17½ to 23½.

tending to 12 hours (Fig. 1). Since IL-2 is up regulated by IL-1 and bioactive IL-1 was demonstrated by Bochner et al.³ to be produced late in the response to antigen, we believed that 12 hours might not be sufficient time to detect IL-2 production. We therefore extended our studies to 24 hours, but we still were unable to detect significant appearance of IL-2 in chamber fluids.

IL-6 assay of blister-chamber fluids

In contrast to our IL-2 studies, we were able to detect significant amounts of IL-6 immunoreactivity in skin-chamber fluids in response to antigen challenge. Antigen-challenged chamber fluids from atopic subjects demonstrated significant amounts of IL-6 in pooled samples representing hours 1½, 3½, 5½, and 7 ($p < 0.05$), but not diluent control sites (Fig. 2, A). IL-6 reached a median peak of 0.66 ng/ml at the 3½-hour sample. Median levels of IL-6 fell toward the baseline by the eighth hour and were followed by a second peak of 0.25 ng/ml at hour 10. The size of the average late IL-6 peak was relatively small because two subjects demonstrated no late IL-6. In the four subjects that demonstrated late IL-6 production, the late IL-6 represented 46% of the early IL-6 production.

Analysis of chamber fluids for histamine demonstrated an early peak at 2 hours, followed by a decline toward baseline at 10 hours. All points except the 10-hour samples reached statistical significance at $p < 0.05$ compared with points of control sites (Fig.

2, B). Since two of five subjects failed to demonstrate late-phase histamine release, the median results failed to demonstrate a late rise, although this finding was demonstrable when mean values were plotted. Differential cell counts demonstrated an influx of nucleated cells and eosinophils appearing at antigen-challenged sites (Fig. 2, C).

When individual subjects were examined, three patterns of IL-6 release were evident: a single early peak of IL-6 in the first 2 to 4 hours without either sustained rise or a second peak, early release of IL-6 followed by a fall to near baseline by hours 7 to 9 accompanied by a smaller peak at hours 9 to 11, or early release of IL-6 followed by a slower sustained release of IL-6, peaking at hour 9 before declining to baseline.

Monophasic early IL-6 release by a representative subject is illustrated in Fig. 3, A. A single early peak of IL-6 was noted at 1½ hours, followed by a rapid decline to baseline levels by 5½ hours. Histamine production also demonstrated only an early peak, with maximum levels at 2 hours and a decline to baseline by 6 hours (Fig. 3, B). Cellular influx in this subject was also small with few nucleated cells and eosinophils detected (Fig. 3, C). There were two subjects with this pattern of IL-6 release, and both subjects displaying this pattern were skin test positive, but not clinically sensitive to their respective antigens.

A representative example of biphasic IL-6 release is illustrated in Fig. 4, A. An early rise in IL-6 peaking at 4½ hours was followed by a fall to near baseline at hour 8 before the detection of a smaller second peak

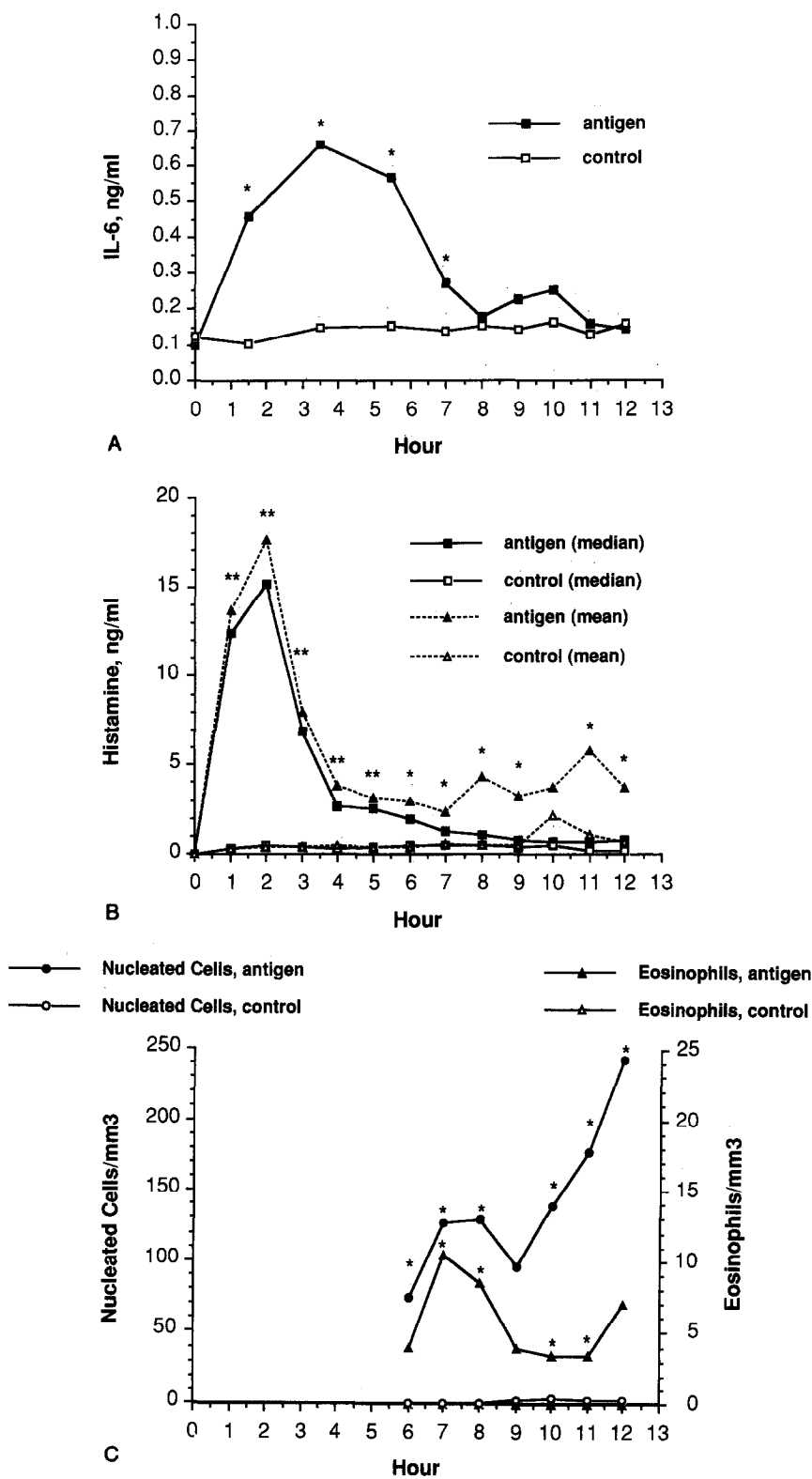


FIG. 2. Blister-fluid analyses, all subjects. **A**, IL-6 results; $n = 6$; $*p < 0.05$, Wilcoxon's signed-rank test, two-tailed. **B**, Histamine results; median values (—); mean values (---); $n = 6$; $*p < 0.05$, Wilcoxon's signed-rank test, two-tailed; $**p < 0.05$, Wilcoxon's signed-rank test, two-tailed and $p < 0.05$, Student's t test, two-tailed. **C**, Cell count results; $n = 6$; $*p < 0.05$, Wilcoxon's signed-rank test, two-tailed.

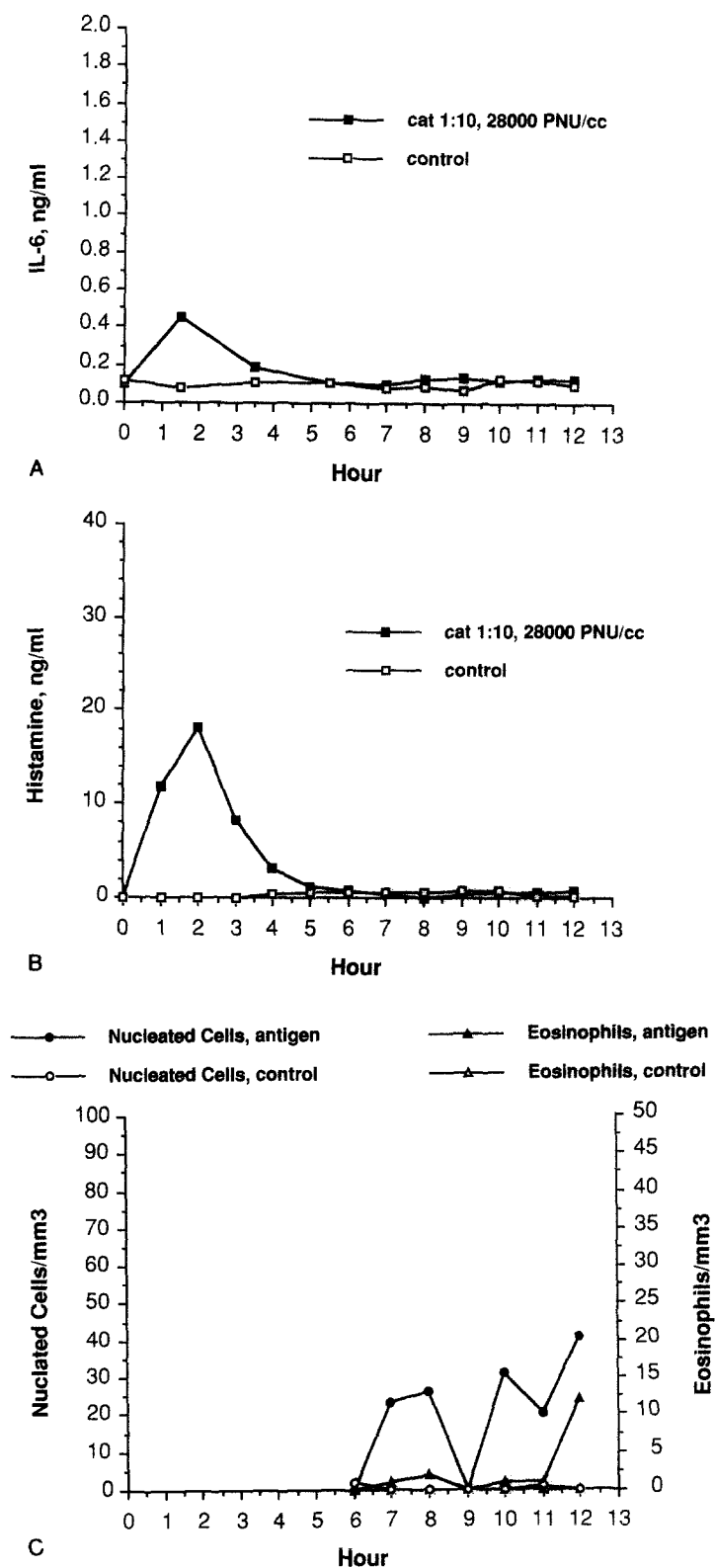


FIG. 3. Blister-fluid analyses, subject H. G. T. Pattern: single, early IL-6 peak. **A**, IL-6, subject H. G. T. **B**, Histamine, subject H. G. T. **C**, Cell counts, subject H. G. T.

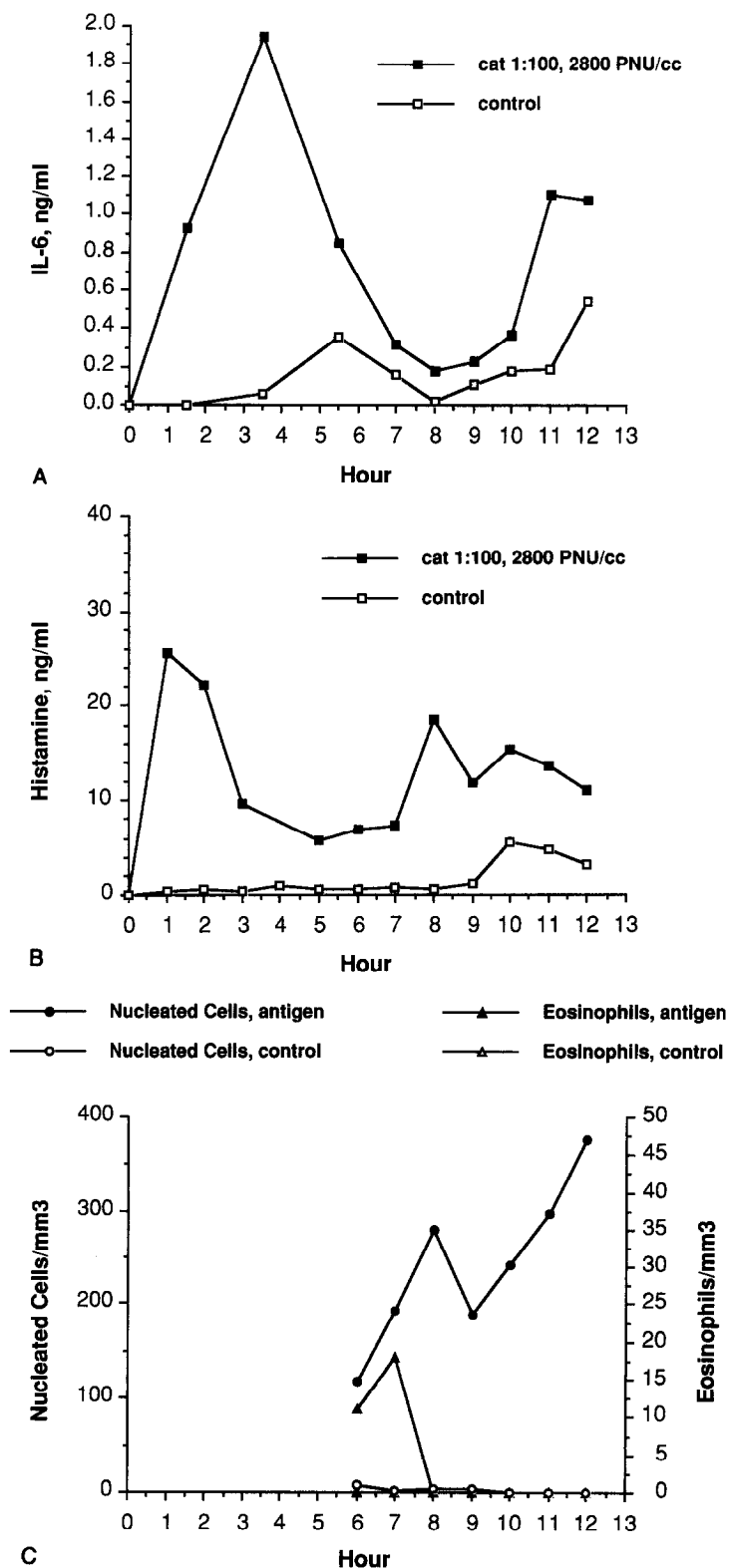


FIG. 4. Blister-fluid analyses, subject C. E. L. Pattern: biphasic IL-6 release. **A**, IL-6, subject C. E. L. **B**, Histamine, subject C. E. L. **C**, Cell counts, subject C. E. L.

at hour 11. This subject also demonstrated smaller peaks at control sites 1 to 2 hours after the appearance of peaks at antigen sites, suggesting systemic IL-6 release. Histamine assays of chamber fluid also demonstrated a biphasic pattern, as illustrated in Fig. 4, *B*. Influx of nucleated cells was more prominent in this subject, but few eosinophils were detected (Fig. 4, *C*). There were two subjects that demonstrated this biphasic pattern of IL-6 release, and both subjects were clinically sensitive to their respective antigens.

A final subject demonstrated a single prolonged IL-6 peak (not illustrated). IL-6 levels progressively increased until hour 9 and then fell toward baseline by hour 12. Like the subjects with biphasic IL-6 release, this subject was also clinically sensitive to the challenge antigen.

Systemic release of IL-6

Because of the suggestion of systemic IL-6 release in one subject, we next challenged four atopic subjects and two nonatopic subjects with antigen and diluent on opposite arms, assaying for IL-6 in serum drawn from each arm (Table I). Two subjects were unchallenged and had serum IL-6 measured to detect any diurnal variation. All atopic subjects, including the subject that had the blister-chamber experiment suggestive of systemic IL-6 release, developed immediate wheal-and-flare responses followed by cutaneous LPRs.

Surprisingly, no subject demonstrated significant increase in serum IL-6 from either arm during these experiments. There was no diurnal variation detected in any of the unchallenged subjects.

Correlations

There was a trend for early IL-6 to correlate with late histamine, but this trend fell short of statistical significance at $p = 0.06$ and $R = 0.801$ (Fig. 5, *A*). Early production of IL-6 failed to correlate with either early histamine production, total neutrophil influx, or eosinophil influx. Total production of IL-6 in the last 6 hours of the experiment did significantly correlate with the total influx of eosinophils. Late IL-6 production was not correlated with either early or late histamine production or total neutrophil influx.

We found that late histamine production correlated with the total influx of neutrophils, but not eosinophils (Fig. 5, *B*). Early histamine had no correlation with cellular influx.

Interestingly, we found the size of the cutaneous LPR correlated with the total amount of IL-6 produced in the first 6 hours of the experiment (Fig. 5, *C*), suggesting that IL-6 production may be necessary to maximize the cutaneous LPR. There was a highly

significant correlation of the cutaneous LPR with peak levels of IL-6 achieved in the first 6 hours of the experiment. The size of the cutaneous LPR was not correlated with late IL-6 production or with total IL-6 produced.

DISCUSSION

In this study, we were unable to detect a significant difference in IL-2 production between antigen- and control-challenged sites in our subjects. Reasons for this observation may include that there is in fact no IL-2 produced, that 24 hours may not be sufficient for production of detectable levels of IL-2, or that IL-2 may be produced, but may not be detectable. Failure to detect this cytokine with our immunoassay could be due to binding to cellular IL-2 receptors, thus removing it from the supernatants assayed, or simply because there may be few activated lymphocytes present at challenge sites at sample times. It is clear from studies of biopsy specimens that lymphocyte infiltration of allergen-challenged sites occurs late in the allergic cutaneous response and that lymphocytes comprise the minority of the cell types found in chamber fluids, even at later hours.¹⁴

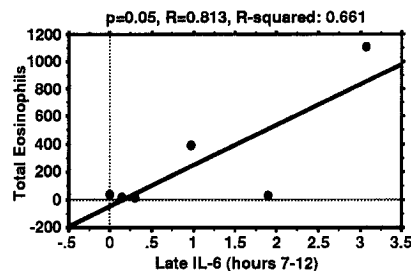
We were, however, able to detect the presence of immunoreactive IL-6 at antigen-challenged sites, both early and late in the response. Individual subjects demonstrated three patterns of IL-6 release: early monophasic production, biphasic production, and prolonged IL-6 production. It is interesting that the subjects demonstrating a single early peak of IL-6 were skin test positive, but not clinically sensitive when they were naturally exposed to the challenge antigen. This finding is in contrast to subjects who demonstrated biphasic or prolonged IL-6 release, all of whom were clinically sensitive to environmental exposure, and suggests that late IL-6 may be important in the production of clinical symptoms in atopic subjects.

It should be noted that the assays for IL-2 and IL-6 used in this study were immunoassays. They do not distinguish between biologically active and biologically inactive cytokine or degradation fragments of cytokine, and they are dependent on the quality of the antibody used. A bioassay is necessary to determine that the IL-6 detected is biologically active.

The correlation of late IL-6 with total eosinophil influx is also interesting, since IL-6 has not been demonstrated to be chemotactic for eosinophils. This finding may be explained by virtue of a mutual association with other mediators chemotactic for eosinophils, such as platelet-activating factor.^{15, 16} Platelet-activating factor has been demonstrated to be newly produced by mast cells after degranulation^{17, 18} and to augment IL-6 production by monocytes and endothelial cells.¹⁹

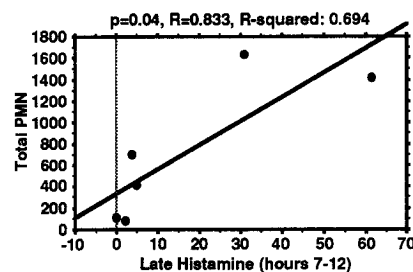
	Early Histamine	Late Histamine	Total PMN	Total Eosinophils
Early IL-6	p=0.33 R=0.483	p=0.06 R=0.801	p=0.21 R=0.599	p=0.99 R=0.003
Late IL-6	p=0.686 R=0.212	p=0.525 R=0.328	p=0.36 R=0.456	<i>p=0.05</i> <i>R=0.813</i>

A



	Total PMN	Total Eosinophils
Early Histamine	p=0.11 R=0.719	p=0.31 R=0.506
Late Histamine	<i>p=0.04</i> <i>R=0.833</i>	p=0.74 R=0.174

B



	Early IL-6	Late IL-6	Total IL-6	Peak Early IL-6	Peak Late IL-6
LPR	<i>p=0.05</i> <i>R=0.885</i>	p=0.96 R=0.031	p=0.70 R=0.235	<i>p=0.004</i> <i>R=0.977</i>	p=0.97 R=0.026

C

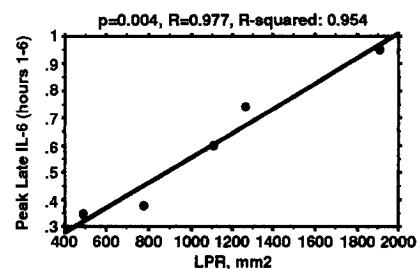
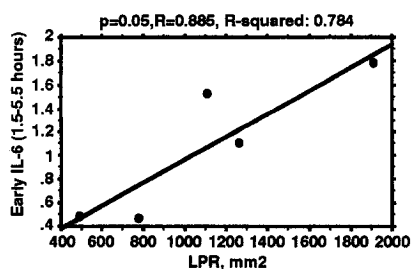


FIG. 5. Correlations of IL-6, histamine, and late-phase skin test reactivity. Significant correlations in **bold italic**. **A**, Early and late IL-6 correlations. Early IL-6, total produced from hours 1 to 6; late IL-6, produced from hours 7 through 12. **B**, Early and late histamine correlations. Early histamine, total produced from hours 1 to 6; late histamine, produced from hours 7 through 12. **C**, Late cutaneous response (LPR) correlations. Area of late cutaneous response in square millimeters.

Previous studies by one of the authors has clearly demonstrated that late-phase histamine released into the skin chambers correlates with the numerical influx of basophils into the chamber and that basophils will be underrepresented in differential cell counts with routine stains unless metachromatic stains, such as alcian blue, are used.¹⁴ We believe that our current data demonstrating a significant correlation ($R = 0.833$; $p = 0.04$) between the Hansel's-stained neutrophils and the late-phase histamine actually represent activated basophil influx, and we suspect that some basophils were counted as neutrophils in differentials. Unfortunately, we did not consistently have enough cells for both routine and metachromatic staining in this study. Although we presume that this late-phase histamine is of basophil origin, we can not absolutely exclude a role for the mast cell. However, recent work by Shalit et al.,^{20, 21} in which serial histamine and tryptase levels were examined, also suggest that the basophil may be the primary cell responsible for late-phase histamine.

The apparent presence of histamine production beyond the first hourly sample may have been from residual histamine left in the chamber, since chambers were not washed between samples.

Although our inability to detect immunoreactive IL-6 in the systemic circulation, despite rather large cutaneous reactions, suggests only local IL-6 production, it should be noted that this finding may also be a result of insufficient assay sensitivity or serum factors inducing alterations in the IL-6 immunoassay. Adelman et al.²² found twofold to sevenfold increases in serum IL-6 bioactivity in the serum of both normal subjects and subjects with common variable immunodeficiency when samples were heat-treated before assay. We were unable to detect immunoreactive IL-6 in our samples. This finding may be a result of the limitations of the immunoassay technique, as noted above. A more sensitive bioassay will be necessary to exclude systemic release of IL-6 in response to cutaneous antigen challenge.

The correlation of the size of the late cutaneous response with early IL-6 production and with peak early IL-6 levels suggests that early IL-6 production may play a role in determining the magnitude of clinical response. These results appear to suggest a possible direct role of IL-6 with the cellular and inflammatory events of the LPR.

It is of interest that it is early IL-6 production that correlates with the LPR and not late IL-6 production. Since early IL-6 production is closely correlated with late events, it is tempting to speculate its source might, in fact, be the mast cell. The mast cell has recently been demonstrated as a source of cytokine production,²³⁻²⁸ lending support for this suspicion. Burd et al.,²³ Hültner et al.,²⁶ Plaut et al.,²⁷ and Costa et al.²⁸

have demonstrated IL-6 bioactivity and/or IL-6 mRNA production by murine mast cell lines in response to calcium ionophores and Fc ϵ receptor I cross-linking. Mican et al.²⁹ have recently demonstrated IL-6 mRNA to be produced by passively sensitized mouse skin in response to antigen stimulation. Additionally, TNF- α has now also been demonstrated to be produced by mast cells.³⁰ Production of cytokines in response to mast cell degranulation may be an important link between early events and the later inflammatory and cellular events that follow. Alternatively, surrounding tissue cells, such as macrophages, fibroblasts, activated T cells, endothelial cells, and/or epithelial cells, might be a source of this IL-6.^{31, 32} Late-phase IL-6 recently has been demonstrated to be produced by bronchoalveolar lavage-derived alveolar macrophages from antigen-challenged atopic subjects with asthma.³³ It is tempting to speculate that the family of proinflammatory cytokines, IL-1, IL-6, and TNF- α , may be very important in amplifying the inflammatory response to antigen and thus may be relevant factors in determining the degree of clinical response to allergen challenge. If this is so, IL-6 may be an important modulating factor in the allergic response, since its production is up regulated by IL-1, TNF- α , and itself^{11, 31, 32, 34} and since it also down regulates TNF- α production.³⁵

In conclusion, we have demonstrated that IL-6 is produced in the cutaneous response to antigen challenge in atopic individuals and that IL-6 production may occur in three distinct patterns: monophasic early release, biphasic release with early and late peaks, or prolonged release. Additionally, there was a trend for early IL-6 production to correlate with late histamine, and late IL-6 production correlated significantly with eosinophil influx. Late-phase skin test reactivity correlated with early IL-6 release and was highly correlated with early peak levels of IL-6.

The emerging role of the proinflammatory cytokines in the pathogenesis of the LPR clearly needs further investigation, and the skin blister-chamber model offers the advantages of ease of use and accessibility.

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