

Cytokine levels and inflammatory responses in developing late-phase allergic reactions in the skin

Burton Zweiman, MD,^a Allen P. Kaplan, MD,^b Lijuan Tong, PhD,^b and Anne R. Moskovitz, BS^a Philadelphia, Pa., and Stony Brook, N.Y.

Background: Cytokines could play roles in the attraction of leukocytes into sites of IgE-mediated late-phase reactions (LPR) or in the activation of such cells at the sites. Previous studies found increased release of IL-6, IL-1, and granulocyte-macrophage colony-stimulating factor into skin chambers overlying allergen-challenged sites, mainly after 6 hours when LPR are already well-developed.

Objective: To compare levels of several cytokines with inflammatory responses in skin chambers overlying developing LPRs.

Methods: Skin chambers were appended to denuded blister bases in 15 sensitive subjects, then challenged over a 5-hour period with pollen allergens (Ag) or buffer control (B). Levels of several chemotactic cytokines, eosinophil cationic product (released from eosinophils), and lactoferrin (released from neutrophils) were measured and leukocyte accumulation was assessed.

Results: Levels of the chemokines IL-8, RANTES, and monocyte chemotactic protein-1 (but not IL-1, IL-6, or granulocyte-macrophage colony-stimulating factor) were significantly higher at Ag-challenge sites than at B-challenge sites. IL-5 was not detected. In individual chamber fluids at Ag-challenge sites, (1) IL-8 levels correlated strongly with levels of lactoferrin but not with eosinophil cationic protein levels; (2) RANTES levels correlated with numbers of eosinophils but not with eosinophil cationic protein levels; and (3) levels of monocyte chemotactic protein-1 correlated weakly with histamine released after the first hour.

Conclusions: During LPRs developing during the first 5 hours of Ag challenge, there is increased local release of several chemokines, which could play roles in the observed attraction and subsequent activation of leukocytes and continued histamine release observed at such sites. (*J Allergy Clin Immunol* 1997;100:104-9.)

Key words: Late phase, skin chamber, allergic, cytokines, chemokines, inflammation

Despite intensive investigation, the pathogenesis of late phase IgE-mediated reactions (LPR) is still not well

Abbreviations used

Ag:	Antigen
B:	Buffered saline diluent
ECP:	Eosinophil cationic protein
GM-CSF:	Granulocyte-macrophage colony-stimulating factor
LPR:	Late-phase reaction
MCP:	Monocyte chemotactic protein
PBS:	Phosphate-buffered saline
RANTES:	Regulated upon activation normal T-cell expressed and presumably secreted (chemokine)

defined. Studies by us and others, which used a skin chamber model, have delineated a number of humoral and cellular inflammatory events occurring during the development and persistence of skin LPR over a 24-hour period. It is now recognized that locally released cytokines could be a major proinflammatory stimulus in LPRs.¹ Increased levels of IL-1 and granulocyte-macrophage colony-stimulating factor (GM-CSF) have been found in overlying chamber fluids, starting more than 6 hours after allergen skin challenge of sensitive humans.^{2,3} Lee et al.⁴ found increased chamber-fluid levels of IL-6, but the temporal pattern varied among subjects. However, LPR are already macroscopically well expressed by 6 to 8 hours in sensitive subjects.⁵ We hypothesized that cytokines may also play proinflammatory roles during the development of LPR in the first several hours after antigen challenge. However, there has been no extensive side-by-side comparison of the release of a variety of cytokines into skin chamber fluids with the inflammatory events occurring in developing LPRs during the first 5 hours after antigen challenge. In this study we report the results of such an analysis, with findings that suggest a significantly increased release of chemokines, but not of the other cytokines assessed, at sites of developing LPR. The levels of such released chemokines correlated with certain inflammatory events.

METHODS

Subjects

Fifteen subjects were selected who had developed both immediate and late-phase responses after intradermal injections of 10 and 100 protein nitrogen units/ml ragweed or grass extract (Greer Labs., Lenoir, N.C.). All studies were performed

From ^athe Department of Medicine, Allergy and Immunology Division, University of Pennsylvania School of Medicine and ^bthe Department of Medicine, State University of New York, Stony Brook Health Science Center.

Supported by National Institutes of Health grant RO1 AI14332.

Received for publication Nov. 26, 1996; revised Jan. 24, 1997; accepted for publication Jan. 27, 1997.

Reprint requests: Burton Zweiman, MD, Allergy and Immunology Division, University of Pennsylvania School of Medicine, 512 Johnson Pavilion, Philadelphia, PA 19104-6057.

Copyright © 1997 by Mosby-Year Book, Inc.

0091-6749/97 \$5.00 + 0 1/1/80635

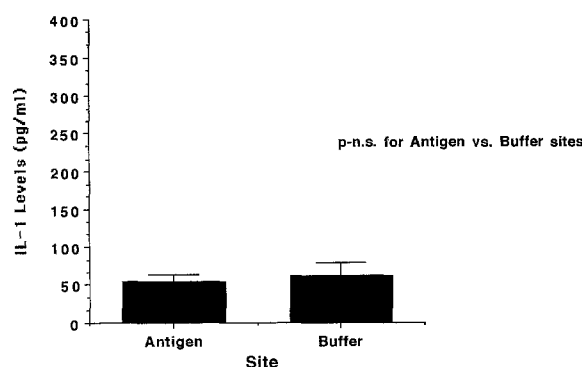


FIG. 1. IL-1 levels in skin chamber after 2- to 5-hour challenge ($n = 10$).

outside the grass and ragweed pollinating seasons after informed consent was obtained.

Skin chamber studies

Skin chamber studies were performed as previously described.⁶ In brief, four blisters were induced on the volar surface of the forearms by gentle heat and suction. The epidermal blister roofs were removed from the blister bases at the dermal-epidermal junction, and collection chambers were appended. After irrigating the blister bases with buffer diluent (phosphate-buffered saline [PBS]) twice for 5 minutes each, 0.3 ml of grass or ragweed pollen antigens (Ag) (Greer Labs.) diluted to 100 PNU/ml in PBS containing 10 U/ml of heparin was placed in chambers at two sites. As a control, PBS containing 10 U/ml heparin (B) was placed in chambers at the other two sites. After 1 hour, the fluids were removed and the fluids from the two antigen-challenge site chambers were pooled for each subject. In a similar manner, the chamber fluids from the two buffer-challenge sites were also pooled. These pooled fluids from each subject were then assayed for the following:

- (1) Histamine levels by ELISA (Immunotech; assay sensitive to 0.1 ng/ml)
- (2) Tumor necrosis factor- α by ELISA (R&D Systems, Minneapolis, Minn.; assay sensitive to 15 pg/ml)
- (3) IL-1 β by ELISA (R&D Systems; assay sensitive to 4 pg/ml)
- (4) IL-8 by ELISA (R&D Systems; assay sensitive to 31 pg/ml)
- (5) Monocyte chemoattractant protein-1 (MCP-1). ELISAs were performed according to standard procedures with 96-well microtiter plates (Immulon-4).⁷ The wells were coated with 5 μ g/ml of the rabbit anti-MCP-1 IgG in 100 μ l of 0.05 mmol/L sodium bicarbonate buffer (pH 9.6) for 24 hours at 4°C. The wells were washed, and unbound sites were blocked by incubation for 60 minutes at 37°C with 1% nonfat milk in PBS. After washing, triplicate samples of 100 μ l MCAF/MCP-1 standards or skin-chamber fluids (at dilutions of 1:1 and 1:10 in PBS containing 0.05% Tween) were added and incubated for 2 hours at 37°C. The plates were washed again with PBS/Tween, filled with 100 μ l of anti-MCAF 40-2 monoclonal antibody at a concentration of 10 μ g/ml, and incubated for 2 hours at 37°C. The wells were washed, and alkaline phosphatase-conjugated goat anti-mouse IgG, diluted 1:2000 in PBS/Tween, was added and incubated for 2 hours at 37°C. After washing, 100 μ l of p-nitrophenyl phosphate (1 mg/ml) in 10% diethanol-

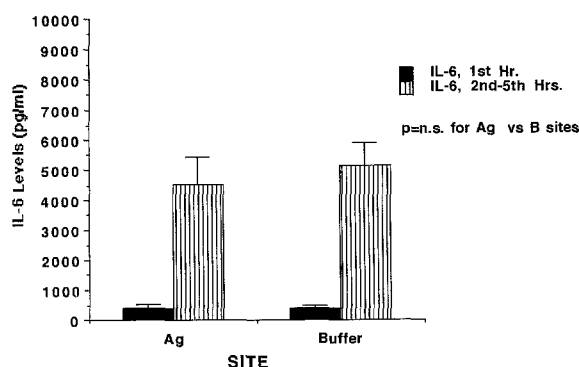


FIG. 2. IL-6 levels (mean $\pm$ SEM) in skin chambers after 1-hour (solid columns) and after 2- to 5-hour (striped columns) challenge ($n = 10$).

amine-HCL buffer (pH 9.6) was added to the wells. The absorbance was read after 30 minutes at 405 nm on a Thermomax microplate reader (Molecular Devices, Menlo Park, Calif.). This assay was sensitive to 200 pg/ml of MCP-1.

- (6) RANTES. ELISA was performed analogous to that described above for MCP-1.^{7,8} The assay uses two anti-RANTES monoclonal antibodies that recognize different, noncompeting determinants. The specificity of the ELISA was tested with other chemokines of the C-X-C and C-C families, none of which were shown to cross-react in the assay. All experiments were repeated twice. In selected experiments, low levels of RANTES were confirmed with an ELISA kit (R&D Systems) with sensitivity to 2-5 pg/ml.

The chamber bases were then irrigated with PBS; fresh Ag or B solutions containing heparin (10 U/ml) were placed in the chambers previously containing Ag and B solutions, respectively, and allowed to remain for an additional 4 hours. Fluids from the four challenge chambers were removed, fluids from the duplicate challenge sites were pooled, and total cell counts were obtained. The two pooled fluids from each subject were then centrifuged and the supernatants were analyzed for levels of (1) histamine; (2) TNF- α ; (3) GM-CSF by ELISA (Genzyme; assay sensitive to 4 pg/ml); (4) IL-5 by ELISA (R&D; assay sensitive to 8 pg/ml); (5) IL-8; (6) IL-6 by ELISA (R&D; assay sensitive to 3.1 pg/ml); (7) RANTES; (8) MCP-1; (9) eosinophil cationic protein (ECP) by radioimmunoassay (Pharmacia; assay sensitive to 2 ng/ml); and (10) lactoferrin by ELISA (assay sensitive to 2 μ g/ml).⁶ The cell pellets were resuspended in PBS/heparin; aliquots were centrifuged (Shandon) and subsequently stained with Wright's stain (Dif-Quick) to obtain differential counts. Because of the limited amount of chamber fluid available, not every assay could be carried out in every fluid, even when employing quite sensitive assays.

Statistical analysis

Levels of cytokines and inflammatory mediators in skin chamber fluids at Ag sites were compared to levels in concomitant B challenge sites in the same individuals by paired student's t test. Correlations between levels of individual cytokines and individual inflammatory responses in the same challenge sites were investigated by using the Spearman ranking analysis.

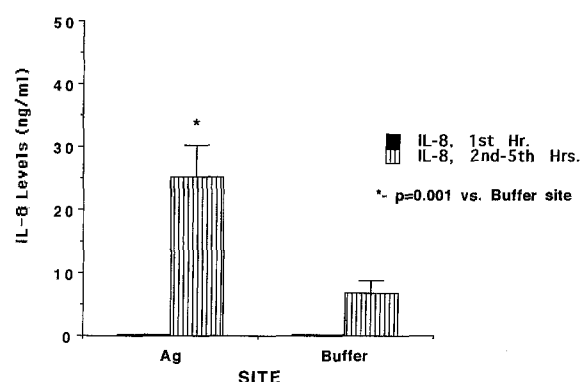


FIG. 3. IL-8 levels (mean $\pm$ SEM) in skin chambers after 1-hour (solid columns) and 2- to 5-hour (striped columns) challenge ($n = 12$).

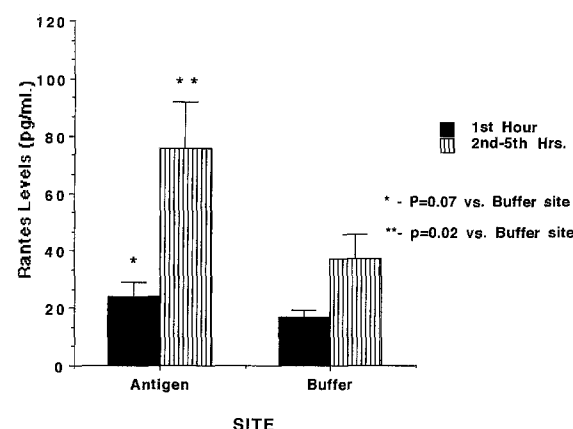


FIG. 4. RANTES levels (mean $\pm$ SEM) in skin chambers after 1-hour (solid columns) and 2- to 5-hour (striped columns) challenge ($n = 12$).

RESULTS

Cytokine levels (after first-hour challenge)

After the first hour, there was a markedly higher level of histamine in the skin chamber fluids overlying sites challenged with Ag than overlying sites challenged with B (110 ± 16 ng/ml versus 4 ± 2 ng/ml; $p = 0.001$). However, there was very modest, if any, release of any cytokine into such fluids; the levels were not significantly more at Ag sites than at B sites. In the next section, these levels are compared with the cytokine levels obtained after further challenge.

Cytokine levels (after second- to fifth-hour challenge)

- (1) IL-1 β was detectable in low levels in skin-chamber fluids, but levels were not significantly different between the Ag and the B challenge sites (55 ± 11 pg/ml versus 65 ± 20 pg/ml) (Fig. 1).
- (2) TNF α levels were detectable in chamber fluids at Ag sites in only 3 of 15 subjects and at B sites in only 1 of 15 subjects studied. There was no difference in the

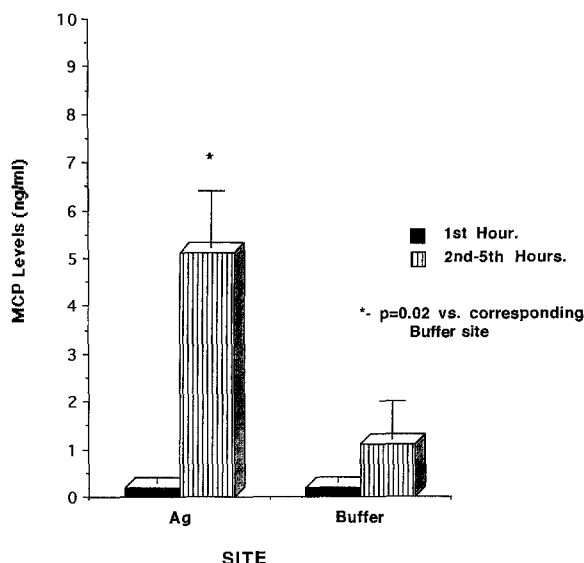


FIG. 5. MCP-1 levels (mean $\pm$ SEM) in skin chambers after 1-hour (solid columns) and 2- to 5-hour (striped columns) challenge ($n = 8$).

pattern of inflammatory manifestations in this minority of subjects with evidence of locally released TNF α .

- (3) IL-6 levels were high in fluids at both Ag and B sites, with no significant difference in concentration between the two sites (4500 ± 1000 pg/ml vs 5000 ± 800 pg/ml; Fig. 2).
- (4) GM-CSF was detected in only occasional chamber fluids at Ag sites and not at B sites.
- (5) IL-8 was released in considerable quantities (in the nanogram per milliliter range) into the overlying chamber fluids with significantly higher levels at the Ag- than at the B-challenge sites (26.5 ± 16.1 ng/ml versus 7.7 ± 1.9 ng/ml; $p = 0.01$) (Fig. 3).
- (6) RANTES levels in chamber fluids were significantly higher at Ag-challenge sites than at B-challenge sites (75.6 ± 16.1 pg/ml versus 37.2 ± 8.7 pg/ml; $p = 0.02$) (Fig. 4).
- (7) MCP-1 levels were also significantly higher at Ag than at B sites (5.1 ± 1.2 ng/ml versus 1.1 ± 0.8 ng/ml; $p = 0.02$) (Fig. 5).

Inflammatory responses (after second- to fifth-hour challenge)

As expected from our previous experience with skin chamber approaches,^{1,6,9} several inflammatory manifestations were significantly greater at sites after 2- to 5-hour Ag challenge compared to B challenge sites (Table I).

- (1) Histamine levels at Ag sites were significantly higher than at B sites (36 ± 8 ng/ml versus 1.4 ± 0.8 ng/ml; $p = 0.001$)
- (2) There was a significantly greater exudation of leukocytes into the overlying chamber fluids at Ag-than

TABLE I. Inflammatory responses in skin chambers appended to skin challenge sites

Mediator	Antigen challenge site	Buffer challenge site
Histamine (ng/ml) (after first-hour challenge)	110 ± 16*†	4 ± 2
Histamine (ng/ml) (after second- to fifth-hour challenge)	36 ± 8‡	2 ± 1
Lactoferrin (μg/ml)	14 ± 3‡	5 ± 1
Eosinophil cationic protein (ng/ml)	100 ± 17‡	18 ± 3
Granulocytes (× 10 ⁵)	20 ± 4‡	4 ± 1

*Mean ± SEM values for 15 subjects (for all these measurements).

†*p* < 0.0001, antigen challenge site versus corresponding buffer sites.

‡*p* < 0.001, antigen challenge site versus corresponding buffer sites.

at B-challenged sites ($20 \pm 4 \times 10^5$ cells versus $4 \pm 1 \times 10^5$ cells; *p* < 0.001). Approximately 90% of the exuding leukocytes at Ag sites were neutrophils, 8% were eosinophils, and 2% were mononuclear cells. At the B sites, 83% of leukocytes were neutrophils, 2% were eosinophils, and 15% were mononuclear cells.

- (3) There was evidence of increased activity of these leukocytes at Ag sites, with significantly greater release into the cell-free chamber fluids of lactoferrin, a component of neutrophil-specific granules, at Ag than at B sites (14 ± 3 μg/ml versus 5 ± 1 μg/ml; *p* < 0.001). Levels of ECP, a granule component in eosinophils, were also greater in the cell-free chamber fluids at Ag sites than at B sites (100 ± 17 ng/ml versus 18 ± 3 ng/ml; *p* < 0.001).

Correlations

The IL-8 levels in individual chamber fluids from Ag sites correlated moderately by ranking analysis with the number of neutrophils (*r* = 0.62; *p* < 0.05) and correlated strongly with the lactoferrin levels (*r* = 0.81; *p* = 0.002) (Table II). However, IL-8 levels did not correlate with ECP levels in such fluids. There was no correlation between IL-8 levels and any inflammatory response at B-challenged sites.

RANTES levels in chamber fluids from Ag sites correlated strongly with the number of eosinophils (*r* = 0.79; *p* < 0.01) and the total number of granulocytes in such fluids (*r* = 0.71; *p* = 0.02). However, there was no significant correlation between RANTES levels and either ECP levels (*r* = 0.3; *p* = 0.4) or lactoferrin levels (*r* = 0.1; *p* = 0.7) in these fluids. There was no association of RANTES levels and histamine levels in the chamber fluids after 2- to 5-hour Ag challenge (*r* = 0.3; *p* = 0.24). There was no correlation between RANTES levels and any inflammatory parameter in chamber fluids at B sites.

MCP-1 levels in chamber fluids at Ag sites did not correlate with numbers of total granulocytes (*r* = 0.15; *p* = 0.7) or eosinophils (*r* = 0.25; *p* = 0.4) or levels of

TABLE II. Correlations between levels of chemokines and inflammatory responses in skin chambers at antigen sites

Chemokines	Inflammatory response	<i>r</i> value*	<i>p</i> value
IL-8	No. of granulocytes	0.62	<0.05
IL-8	Lactoferrin level	0.81	0.002
RANTES	No. of eosinophils	0.79	<0.01
RANTES	No. of granulocytes	0.71	0.02
RANTES	ECP level	0.30	0.40
RANTES	Histamine	0.30	0.24
MCP-1	No. of granulocytes	0.15	0.70
MCP-1	ECP level	0.10	0.80
MCP-1	Histamine level	0.60	0.07

*Spearman ranking correlation analysis.

lactoferrin (*r* = 0.1; *p* = 0.9) or ECP (*r* = 0.1; *p* = 0.8). However, there was a modest association of MCP-1 levels and histamine levels in chamber fluids after 2- to 5-hour Ag challenge (*r* = 0.6; *p* = 0.07). There was no such association between MCP-1 and histamine levels in chamber fluids after 2- to 5-hour B challenge (*r* = 0.05; *p* = 0.9). There was also no correlation between MCP-1 levels and other inflammatory manifestations at these B challenge sites.

There were no significant correlations between levels of IL-8, RANTES, and MCP-1 in individual Ag site chamber fluids, suggesting that there was not a quantitatively similar release of these three chemokines in individual Ag-challenge sites.

DISCUSSION

The findings reported here may help enhance our understanding of the pathogenesis of inflammatory responses that occur in the sites of developing IgE-mediated LPRs in the skin.^{4,9} We and others have found several proinflammatory mediators released into skin chambers appended to such sites, including leukotriene B₄ and platelet activating factor.¹⁰ Recently, there has been considerable interest in the proinflammatory effects of cytokines. For example, in experimental models, intradermal injection of IL-1 and TNFα leads to a nonspecific local accumulation of granulocytes, likely by increasing expression of adhesion molecules on endothelial cells and possibly on the granulocytes themselves.¹ IL-5 is a chemoattractant and activator of eosinophils, as is the chemokine RANTES.^{11,12} GM-CSF and IL-5 have been incriminated in prominent accumulation of eosinophils in the airways.^{11,13,14} Furthermore, GM-CSF, and to a lesser extent RANTES, IL-5, TNFα, and IL-3, can degranulate eosinophils.¹¹ The chemokine MCP-1 is also a potent attractant and activator of basophils, leading to prominent histamine release.¹⁵ IL-3 does not induce histamine release from basophils except in very high concentrations but enhances the histamine release stimulated by other agonists.¹⁶ In contrast, the several activities of IL-8 that have been

described include potent attraction and activation of neutrophils (with attraction of basophils, eosinophils, and lymphocytes under certain conditions) and inhibition of basophil histamine release.^{15, 17, 18} Therefore, it could be very appropriate to determine whether there is increased local secretion or release of these cytokines in developing skin LPRs characterized by accumulation of activated neutrophils, eosinophils, and basophils. Basophils are the likely source of the continued local histamine release we have observed in such reactions.¹⁹

In this study, we found prominently increased levels of the chemokines IL-8, RANTES, and MCP-1 in skin chamber fluids overlying developing LPRs, whereas levels of IL- β and IL-6 were not higher than at sites challenged with buffer control. TNF α levels were increased in only 3 of 15 subjects, and GM-CSF was found only occasionally. IL-5 was not detected with an immunoassay sensitive to 8 pg/ml.

After 2 to 5 hours of antigen challenge in our study, skin-chamber RANTES levels correlated strongly with the degree of total granulocyte and eosinophil accumulation but, surprisingly, did not correlate with the levels of locally released ECP (a marker of eosinophil activation). The impressive correlation between RANTES and total granulocyte levels in individual chamber fluids is puzzling since RANTES is not a chemoattractant for neutrophils, which constitute the majority of the cells in the chamber fluids. It is possible that RANTES levels parallel that of another cytokine (not yet identified) that plays a more direct role in neutrophil attraction.

In contrast to the situation with RANTES release, IL-8 levels in such fluids showed only a borderline correlation with the number of neutrophils that had emigrated into those chambers; however, IL-8 levels did correlate very strongly with the levels of lactoferrin released from locally accumulated (and presumably activated) neutrophils. In analyzing the possible role of cytokines in stimulating the persistent histamine release seen in sites of such developing LPRs, we found a modest correlation between levels of MCP-1 and histamine in individual chamber fluids. Levels of IL-8, MCP-1, and RANTES did not correlate with each other in individual chamber fluids, suggesting absence of quantitatively similar patterns of secretion of these cytokines in individual Ag-challenge sites.

The chemokines reported here can be synthesized by a variety of cells.^{7, 15} IL-8 could be synthesized by several different types of cells in the skin after stimulation. It is conceivable that much of the IL-8 we found in the skin chamber fluid was released from the activated neutrophils accumulating in the LPR site themselves, with subsequent autocrine effects.

These potentially important findings can be considered with regard to previous observations in this area of study. As noted above, studies by another group have found elevated levels of IL-6 in skin chambers overlying developing skin LPRs in some, but not all, subjects; increased IL-6 levels were again seen at 8 to 12 hours when LPR would be expected to be well-established.⁵

The reasons for the difference between those findings and those reported in this study (sizable IL-6 levels, without significant difference, at both antigen and control challenge sites) are not known. Although IL-1 can stimulate IL-6 production systemically, it is unlikely that different levels of IL-1 released during developing LPR explains these differences, because we and others have not found impressively increased skin chamber IL-1 levels during the first 5 hours of antigen challenge.²² Likewise, impressive increases in skin chamber GM-CSF levels have been reported only after more than 7 hours of antigen challenge,³ compatible with our finding of no increased local GM-CSF release after the first 5 hours of antigen challenge. Nevertheless, GM-CSF released later in antigen-challenged sites could be partially responsible for a later attraction of eosinophils into the site, as noted in the airways.^{13, 14}

Our failure to detect increased skin chamber IL-5 levels at antigen-challenged sites may reflect insufficient sensitivity of the ELISA that we used, with a suggestion that increased IL-5 levels could be detected by a more sensitive bioassay.²⁰ The potential for local IL-5 production is suggested by the detection of messenger RNA for IL-5 in developing LPR sites by both polymerase chain reaction and in situ hybridization techniques, as employed by our group and others.^{21, 22} Locally secreted IL-5 and RANTES could both play roles on the prominent accumulation and activation of eosinophils at different time periods in the expression of LPR.²³ IL-5 may play a more prominent role in the inflammatory reactions seen 24 hours after allergen challenge of the skin and airways.^{1, 20} However, eosinophil recruitment and activation appear to be complex processes that are just beginning to be understood. Other β chemokines such as MCP-3 and eotaxin could play roles in eosinophil responses,²⁴ and warrant future investigation.

In summary, our findings point to the possible participation of chemokines in the accumulation and activation of inflammatory cells in developing LPRs. Chemokines could also play roles in the activation of such leukocytes, with release of potentially toxic components and stimulation of basophils, responsible for the continued local release of histamine we have observed in such developing LPR.¹⁹ Eosinophil recruitment is a complex process but is just beginning to be understood. Other β chemokines such as MCP-3 and eotaxin could play roles²⁴ and warrant future investigation. Other cytokines could play a more prominent role in the enhancement and perpetuation of LPR inflammatory responses.

REFERENCES

1. Zweiman B. The late-phase reaction: role of IgE, its receptor and cytokine. *Curr OP Immunol* 1993;5:950-5.
2. Bochner BS, Charlesworth EN, Lichtenstein LM, Deese CP, Gilles S, Dinarello CA, Schleimer RP. Interleukin-1 is released at sites of human cutaneous allergic reactions. *J Allergy Clin Immunol* 1990; 830-9.

3. Massey W, Friedman B, Kaio M, Cooper P, Kagey-Sobotka A, Lichtenstein LM, et al. Appearance of GM-CSF activities at allergen-challenged cutaneous late-phase reaction. *J Immunol* 1993;150:1084-92.
4. Lee CE, Newland MW, Teaford HG, Villacis BF, Dixon PS, Valtheer S, Yeh CH, et al. Interleukin-6 is released with the cutaneous response to allergen challenge in atopic individuals. *J Allergy Clin Immunol* 1992;89:1010-20.
5. Lemanske RF, Jr., Kaliner MA. Late phase reactions. In: Middleton E, Jr., Reed CE, Ellis EF, Adkinson NF, Jr., Yunginger JW, Busse WW, editors. *Allergy: principles and practice*. 4th ed., St. Louis: Mosby, 1993:320-61.
6. Zweiman B, Kucich U, Shalit M, von Allmen C, Moskovitz A, Weinbaum G, et al. Release of lactoferrin and elastase in human allergic skin reactions. *J Immunol* 1990;144:3953-60.
7. Kuna P, Lazarovich M, Kaplan AP. Chemokines in seasonal allergic rhinitis. *J Allergy Clin Immunol* 1996;97:104-12.
8. Kuna P, Reddigari SR, Schall TJ, Rucinski D, Sadick M, Kaplan AP, et al. Characterization of the human basophil response to cytokines, growth factors and histamine releasing factors of the intercrine/chemokine family. *J Immunol* 1993;150:1932-43.
9. Zweiman B. Mediators of allergic inflammation in the skin. *Clin Allergy* 1988;18:419-33.
10. Shalit M, Valone FH, Atkins PC, Ratnoff WD, Goetzl EJ, Zweiman B. Late appearance of phospholipid platelet activating factor and leukotriene B₄ in human skin after repeated antigen challenge. *J Allergy Clin Immunol* 1989;83:691-6.
11. Horie S, Gleich GJ, Kita H. Cytokines directly induce degranulation and superoxide production from human eosinophils. *J Allergy Clin Immunol* 1996;98:371-81.
12. Zhang L, Redington AE, Holgate ST. RANTES: a novel mediator of allergic inflammation. *Clin Exp Allergy* 1994;24:899-904.
13. Broide DH, Lotz M, Cuomo AJ, Cobura DA, Federman EC, Wasserman SI, et al. Cytokines in symptomatic asthma airways. *J Allergy Clin Immunol* 1992;89:958-67.
14. Sim TC, Grant JA, Hilsmeier KA, Fukuda Y, Alam R. Pro-inflammatory cytokines in nasal secretions of allergic subjects after antigen challenge. *Am J Respir Crit Care Med* 1994;150:1038-48.
15. Kaplan AP, Kuna P, Reddigari SR. Chemokines as allergic mediators: relationship to histamine-releasing factors. *Allergy* 1994;49:495-501.
16. Brunner T, Heusser CH, Dahinden CA. Human peripheral blood basophils primed by interleukin-3 (IL-3) in response to IgE-receptor stimulation. *J Exp Med* 1993;177:605-11.
17. Swensson O, Schubert C, Christophers E, Schroeder JM. Inflammatory properties of NPA-1/IL-8 in human skin. *J Invest Dermatol* 1991;96:682-9.
18. Shute J. Interleukin-8 is a potent eosinophil chemoattractant. *Clin Exp Allergy* 1994;24:203-6.
19. Shalit M, Schwartz LB, von Allmen C, Atkins PC, Lavker RM, Zweiman B. Release of histamine and tryptase during continuous and interrupted cutaneous challenge with allergen in humans. *J Allergy Clin Immunol* 1989;50:41-51.
20. Ohnishi T, Kita H, Werle D, Sun S, Sedgewick JB, Calhoun WJ, et al. IL-5 is the predominant eosinophil-active cytokine in the antigen-induced pulmonary late-phase reaction. *Am Rev Respir Dis* 1993;147:901-7.
21. Kay AB, Ying S, Varney V, Gaga M, Durham SR, Moqbel R, et al. Messenger RNA expression of the cytokine cluster IL-3, IL-4, IL-5, and GM-CSF in allergen-induced late phase reactions in atopic subjects. *J Exp Med* 1991;173:775-8.
22. Vowels BR, Rook AL, Cassin M, Zweiman B. Expressions of IL-4 and IL-5 mRNA in developing cutaneous late phase reactions. *J Allergy Clin Immunol* 1995;96:92-6.
23. Zweiman B, Atkins PC, von Allmen C, Gleich GJ. Release of eosinophil granule proteins during IgE-mediated allergic skin reactions. *J Allergy Clin Immunol* 1991;87:984-91.
24. Martin LB, Kita H, Leiferman K, Gleich GJ. Eosinophils in allergy: role in disease, degranulation, and cytokines. *Int Arch Allergy Immunol* 1996;109:207-15.