

Patterns of inflammatory responses following rechallenge of skin late-phase allergic reaction sites

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Background: Previous studies have suggested altered responses to repeat skin tests in the sites of IgE-mediated late-phase reactions (LPRs) induced within the previous 48 hours. To explore the possible modulation of LPRs in such rechallenge sites, we compared inflammatory responses in skin chambers induced over previous LPR and control sites.

Methods: Skin blisters were induced and unroofed in 12 human subjects over two sites of previous LPRs induced by intradermal injection of pollen antigens 24 hours or 48 hours earlier and two sites previously injected with buffer diluent (B). Skin chambers containing the same antigens were appended to one intradermal antigen site (called Ag/Ag) and one intradermal B site (B/Ag), and B-containing chambers were placed over antigen (Ag/B) and B (B/B) intradermal sites. Fluids were collected after the first and the second through fifth hours of challenge.

Results: In skin chamber challenges 24 hours after the intradermal injection, there was no significant difference after the first hours between the Ag/Ag or B/Ag sites in either histamine or tryptase levels; both were significantly higher than at Ag/B or B/B sites ($p < 0.01$). The same pattern of events was seen in fluids obtained from the second through fifth hours. The same pattern of findings was seen in examination of levels of the total leukocyte accumulation, total eosinophil accumulation, and frequency of activated (EG₂⁺) eosinophils. Levels of lactoferrin, released from activated neutrophils, and eosinophil cationic protein, released from activated eosinophils, were also similar at Ag/Ag and B/Ag sites; both were significantly higher than at B/B sites, whereas levels at Ag/B sites were intermediate between those found at B/Ag and B/B sites. The pattern of events in skin chamber challenges 48 hours after intradermal injection was similar to that seen at 24 hours, except that levels of inflammatory mediators/cells in Ag/B sites were more intermediate between the B/Ag and B/B sites.

Conclusion: There is no significant alteration of mediator or inflammatory cell responses after antigen rechallenge of previous LPR sites when compared with those found in antigen challenge of non-LPR sites. (*J Allergy Clin Immunol* 1998;101:94-8.)

Key words: Late-phase reactions, rechallenge, allergic

Abbreviations used

Ag/Ag: Skin chamber antigen challenge of previous LPR (Ag-injected) site
Ag/B: Skin chamber buffer challenge of previous LPR (Ag-injected) site
B: Buffer diluent
B/Ag: Skin chamber challenge of previous non-LPR (buffer injected) site
B/B: Skin chamber buffer challenge of previous non-LPR (B-injected) site
ECP: Eosinophil cationic protein
LPR: Late-phase reaction

For many years, it has been postulated that repeated inhalational exposures to seasonally occurring aeroallergens may lead to a degree of *priming*. Such priming is thought to lead to increased nasal reactions to subsequent environmental exposures to similar airborne levels of the same allergen.¹ Also, the threshold airborne allergen levels to induce any nasal symptoms may be decreased, possibly explaining persistence of allergic rhinitis symptoms in the later part of the pollen season when airborne pollen levels are greatly diminished.²

These clinical impressions of a priming effect were supported by findings in experimental nasal challenge studies in which sensitive human subjects were challenged with increasing allergen concentrations on two occasions.^{2,3} It was found that clinical symptoms were often induced at lower allergen challenge doses, and local mediator release was greater during the second challenge, which was carried out 24 hours after the first challenge.³ Also, levels of selected inflammatory mediators measured in nasal secretions were higher on the second than on the first challenge after similar allergen challenge doses.

It is not clear what pattern of inflammatory events occur during antigen rechallenge in the skin. Two previous reports^{4,5} described a reduction of immediate or late-phase reactivity in sites of repeated antigen challenge 1 day or 1 week later. No investigation of mediator or inflammatory cell responses was carried out in such studies. Using a skin chamber approach, we previously reported that continued antigen challenge of skin sites in sensitive subjects for up to 9 hours induced an

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TABLE I. Skin chamber protocol

Site type	Injection type	24/48 hours	First-hour chamber incubation	Second through fifth-hour chamber incubation
Ag/Ag	Antigen (100 PNU)	Blister	Antigen (100 PNU)	Antigen (100 PNU)
B/Ag	Buffer	Blister	Antigen (100 PNU)	Antigen (100 PNU)
Ag/B	Antigen (100 PNU)	Blister	Buffer	Buffer
B/B	Buffer	Blister	Buffer	Buffer

initial peak followed by a lower level plateau of histamine release into overlying skin chambers.^{6,7} A degree of local partial desensitization to the challenge antigen was suggested by the observation that this plateau in histamine release was not greater even if the antigen concentration used in continuous challenge was increased fivefold, a concentration that would ordinarily induce a much more prominent histamine release in initial challenge. This local *desensitization* appeared to be antigen specific in that challenge with a second, unrelated pollen antigen after 5 hours of continuous challenge with the first antigen induced a second peak of local histamine release in the skin of subjects strongly allergic to both pollen antigens. Therefore it would be of great interest to know whether the local desensitization to a particular antigen observed after 6 hours of continuous challenge would persist for longer time periods. This report describes the findings in skin chamber studies carried out in sensitive subjects rechallenged at either 24 or 48 hours after the initial challenge.

METHODS

Subjects

A total of 12 volunteer human subjects were studied after informed consent was obtained. All subjects had previously documented strong immediate wheal-and-flare and late-phase reactions (LPRs) (>8 mm diameter of induration 6 hours after intradermal injection of grass or ragweed extract [100 PNU/ml; Greer Labs, Lenoir, N.C.]). All subjects were studied outside the relevant pollinating seasons. Subjects were not receiving any antiallergy medications or allergy injection treatment.

Skin chamber protocol

In previous skin chamber experiments we have found a prominent, tightly adherent fibrin layer in the blister base 24 hours after pollen antigen challenge in such sensitive subjects. Therefore it was not feasible to rechallenge such subjects in sites of skin chamber reactions induced 24 hours earlier because the fibrin layer interferes with absorption of the antigen from the overlying chamber fluid into the blister base. Therefore an alternative protocol was adopted (Table I). Intradermal injections were made in four marked sites on the forearms. In sites 1 and 3 on one arm (chosen at random), 0.05 ml of grass or ragweed pollen extract (100 PNU/ml of antigen, depending on the subject's sensitivity) was injected. In sites 2 and 4 on the other forearm, 0.1 ml of the buffer diluent (B) containing 10 units heparin per milliliter was injected. Measurements of wheal diameters at 20 minutes confirmed the strong immediate reactivity to the antigen solution and absence of reactions to the B.

In six of the subjects, intradermal injection sites were measured at 24 hours, and skin blisters were induced over each site by a combination of gentle heat and suction. The epidermal blister roofs were removed, leaving blister bases at the dermal-epidermal junction. Collection chambers were appended as reported previously by us.^{6,8}

After irrigating the blister bases with B twice for 5 minutes each, 0.3 ml of the same grass or ragweed pollen antigen solutions used for the intradermal injection (diluted to 100 PNU/ml in B solution containing 10 U/ml heparin) were placed in the chambers at sites 1 and 2, hereafter referred to as the Ag/Ag and B/Ag, respectively (Table I). B solution, instead of antigen, was placed in skin chambers at sites 3 and 4, hereafter called Ag/B and B/B, respectively. After 1 hour, the fluids were centrifuged, and the supernatants were removed. Afterwards, they were stored at -70° for later assays of inflammatory mediator levels.

The chamber bases were then irrigated with phosphate-buffered saline. Aliquots (0.3 ml) of the antigen and B solutions containing heparin (10 U/ml) were then placed in the chambers previously containing like solutions and allowed to remain for an additional 4 hours. Fluids from the four challenge chambers were then removed, and total cell counts were assessed in aliquots of these fluids. The remainder of the chamber fluid specimens were centrifuged, and the supernates were removed and aliquoted for determination of mediator levels. The cell pellets were resuspended, and aliquots were cytocentrifuged for different leukocyte counts (Dif-Quick) and the frequency of cells binding the EG₂ antibody, a putative marker of activated eosinophils as described previously by us.⁸

In the other six subjects a similar skin chamber protocol to that described above was used except that the skin blisters were induced at the injection sites 48 hours rather than 24 hours after intradermal injection of the antigen or B solutions.

Mediators

Histamine levels were assayed by ELISA (Immunotech, Westbrook, Me.) sensitive to 0.1 ng/ml⁹; tryptase levels were assayed by the B12 monoclonal antibody ELISA sensitive to 2 ng/ml, as previously reported⁹; levels of lactoferrin released from neutrophils were assayed as described previously by us¹⁰; and levels of eosinophil cationic protein were assayed by RIA (Pharmacia, Piscataway, N.J.) as described by us.¹¹

Statistical analysis

The degree of individual inflammatory responses was compared at the Ag/Ag, B/Ag, Ag/B, and B/B sites for each subgroup of six subjects by analysis of variance. If statistically significant differences were detected, then findings in individual sites were compared by paired *t* tests for normally distributed findings and by Wilcoxon rank-sum analysis for nonparametric analysis when findings were not normally distributed.

RESULTS

Rechallenge at 24 hours

Gross skin test reactions. Prominent immediate wheal reactions (mean $\pm$ SEM diameter, 18 ± 3 mm) were seen at 20 minutes after intradermal injection of the pollen antigen. These were followed by strong LPRs at 6 hours in all subjects (mean $\pm$ SEM diameter of induration, 12 ± 4 mm). At 24 hours, some gross induration was present in the sites of three subjects (3, 8, and 15 mm, respectively) and not apparent in the other three subjects. In comparison, there was a very small immediate wheal reaction (2 ± 1 mm in

TABLE II. Histamine and tryptase levels in skin chamber challenge sites

	Ag/Ag	B/Ag	Ag/B	B/B
24-hour rechallenge				
First-hour histamine	37 ± 11*†	36 ± 8†	15 ± 0.5‡	0.7 ± 0.2
First-hour tryptase	109 ± 43†	124 ± 38†	2 ± 2	2 ± 1
Second through fifth-hour histamine	21 ± 5†	22 ± 4†	6 ± 3	4 ± 1
48-hour rechallenge				
First-hour histamine	43 ± 4†	45 ± 9‡	12 ± 5	3 ± 1
Second through fifth-hour histamine	29 ± 8§	31 ± 12§	10 ± 4	3 ± 2

*Mean ± SEM (ng/ml).

†Significantly higher than corresponding Ag/B and B/B sites; $p < 0.01$.‡Significantly greater than corresponding Ag/B and B/B sites; $p < 0.03$.§Significantly greater than corresponding B/B site; $p = 0.04$.

||There was not sufficient first-hour chamber fluid available from all subjects to also assay for tryptase levels.

diameter) and no gross LPR at 6 or 24 hours at the B injection sites.

Skin chamber histamine and tryptase levels. Histamine levels after 1 hour of skin chamber antigen challenge were similar at sites of previous LPRs to intradermal antigen (Ag/Ag, 37 ± 11 ng/ml) and at sites of previous intradermal B injection without induced LPR (B/Ag, 36 ± 8 ng/ml) (Table II). These levels were significantly greater than those seen after 1 hour of B-chamber challenges in the B/B sites (0.7 ± 0.2 ng/ml, $p < 0.01$). Histamine levels after 1 hour of B challenge at sites of former LPRs to intradermal antigen (Ag/B, 15 ± 0.5 ng/ml) were significantly less than those seen at Ag/Ag and B/Ag sites ($p < 0.01$ for each) but greater than those seen at B/B sites ($p < 0.01$). Tryptase levels were not significantly different after the first hour of challenge at Ag/Ag and B/Ag sites (109 ± 43 and 124 ± 38 ng/ml, respectively) and were significantly higher than those at Ag/B and B/B sites (2.3 ± 2 and 2 ± 1 ng/ml, respectively; $p < 0.01$) (Table II).

After the second through fifth hours of skin chamber antigen challenge, histamine levels were very similar at the Ag/Ag and B/Ag sites (21 ± 5 vs 22 ± 4 ng/ml, respectively); both were significantly greater than levels found at Ag/B and B/B sites (6 ± 3 vs 4 ± 1 ng/ml, respectively; $p < 0.01$) (Table II). Tryptase levels were not assessed in these second through fifth-hour challenged chamber fluids because we had previously found greater tryptase levels at antigen than at B skin chamber sites after the first hour but not after the second through fifth hours of challenge.⁷

Inflammatory cell responses. As we had observed previously, very few inflammatory cells were seen in any of the skin chamber fluids after the first hour of challenge at any of the four sites.¹² After the second through fifth hours of challenge, the mean ± SEM inflammatory cell counts were not significantly different in chamber fluids at Ag/Ag and B/Ag sites (2.9 ± 0.9 vs 4.3 ± 0.9 × 10⁵ cells, $p = 0.25$) (Table III). Both of these responses were significantly greater than those at Ag/B

TABLE III. Inflammatory cell responses in skin chamber challenge sites

	Ag/Ag	B/Ag	Ag/B	B/B
24-hour rechallenge				
Total leukocytes*	2.9 ± 0.7	4.3 ± 0.9	0.7 ± 0.2	0.3 ± 0.1
Eosinophils†	2.9 ± 0.7	3.9 ± 0.4	0.2 ± 0.02	0.03 ± 0.01
EG ₂ ⁺ cells†	1.2 ± 0.2¶	1.2 ± 0.1¶	0.1 ± 0.05	0.01 ± 0.01
Lactoferrin‡	13 ± 5.3	17 ± 9.7	10 ± 3.1	7 ± 2.3
ECP§	111 ± 12¶	97 ± 20#	35 ± 15	22 ± 10
48-hour rechallenge				
Total leukocytes*	3.4 ± 0.5#	4.2 ± 1.2	2.9 ± 0.9	0.9 ± 0.4
Eosinophils†	3.6 ± 1.2#	3.5 ± 1.3#	1.6 ± 0.5#	0.2 ± 0.2
EG ₂ ⁺ cells†	1.8 ± 1.0#	1.8 ± 1.0#	0.9 ± 0.7	0.1 ± 0.1
Lactoferrin‡	10 ± 3.6#	14 ± 1.2#	16 ± 6.3	4 ± 2.4
ECP§	112 ± 41	35 ± 45#	89 ± 17	50 ± 15

*Mean ± SEM × 10⁵.†Mean ± SEM × 10⁴.

‡Mean ± SEM micrograms per milliliter.

§Mean ± SEM nanograms per milliliter.

|| $p \leq 0.03$ versus corresponding Ag/B and B/B sites.¶ $p \leq 0.01$ versus corresponding Ag/B and B/B sites.# $p = 0.04$ versus corresponding B/B sites.

sites (0.6 ± 0.2 × 10⁵ cells, $p = 0.03$) and at B/B sites (0.3 ± 0.1 × 10⁵ cells, $p = 0.02$). Most of these cells were neutrophils. Levels of lactoferrin (micrograms per milliliter) released from activated neutrophils were also not significantly different at Ag/Ag and B/Ag sites (13 ± 5.3 vs 17 ± 9.7 × 10⁵ cells, respectively). Both were greater than those at Ag/B and at B/B sites (10 ± 3.1 and 7 ± 2.3 × 10⁵, respectively), but not significantly so (Table III).

A minority of the cells in the inflammatory exudate were eosinophils, with a mean ± SEM of 2.9 ± 0.7 × 10⁴ cells in the Ag/Ag sites and 3.9 ± 0.4 × 10⁴ cells in the B/Ag sites. These were not significantly different from one another; however, both were significantly more than the 0.03 ± 0.01 × 10⁴ eosinophils in the exudate at the B/B challenge sites ($p = 0.03$). The number of eosinophils in the Ag/B sites (0.24 ± 0.02 × 10⁴ cells) was intermediate between that found in the B/Ag and B/B sites (Table III).

The number of EG₂⁺ cells (presumably activated eosinophils) was very similar at the Ag/Ag sites (1.2 ± 0.2 × 10⁴ cells) and the B/Ag sites (1.2 ± 0.1 × 10⁴ cells). Both were significantly greater than the EG₂⁺ cells found at the B/B sites (0.01 ± 0.01 × 10⁴ cells; $p = 0.01$) (Table III). The number of EG₂⁺ cells in the Ag/B sites (0.1 ± 0.05 × 10⁴ cells) was intermediate between that found in the Ag/Ag and B/Ag sites and that found in the B/B sites (Table III). The levels of eosinophil cationic protein (ECP) released into the skin chamber fluid from activated eosinophils was not significantly different at the Ag/Ag sites (111 ± 12 ng/ml) than at the B/Ag sites (97 ± 20 ng/ml); both were significantly higher than those found at the B/B sites (22 ± 10 ng/ml; $p \leq 0.01$). The ECP levels at the Ag/B sites (35 ± 15 ng/ml) were intermediate between those found at the Ag/Ag and B/Ag sites and those found in the B/B sites (Table III).

Rechallenge at 48 hours

Gross skin test responses. In the six subjects studied there was a prominent immediate wheal response to intradermal injection of 100 PNU/ml of the relevant pollen antigen (mean diameter at 20 minutes, 17 ± 4 mm). There was also a prominent LPR in these sites at 6 hours (mean diameter of induration, 13 ± 2 mm). However, there was no gross induration seen at 48 hours in any subject. As noted previously, there was no immediate wheal reactions or LPRs in the site of intradermal injection of B.

Skin chamber histamine levels. After the first hour of skin chamber challenge, histamine levels were not significantly different at the Ag/Ag (43 ± 4 ng/ml) and B/Ag (45 ± 9 ng/ml) sites (Table II). The histamine levels at both the Ag/Ag and B/Ag sites were significantly greater than those found at the Ag/B sites (12 ± 5 ng/ml) or at the B/B sites (3 ± 1 ng/ml) ($p < 0.01$ and $p < 0.03$, respectively).

A similar pattern in histamine levels was seen after the second through fifth hours of skin chamber challenge. Levels in the Ag/Ag site (29 ± 8 ng/ml) were not significantly different than those found at the B/Ag sites (31 ± 12 ng/ml, $p = 0.7$). These levels were greater than those found at the Ag/B sites (10 ± 4 ng/ml), although not significantly so ($p = 0.09$). The histamine levels at the Ag/Ag and B/Ag sites were significantly greater than those found at the B/B sites (3 ± 2 ng/ml, $p = 0.04$).

Inflammatory cell responses. The total inflammatory cell counts in skin chamber at the Ag/Ag sites ($2.4 \pm 0.5 \times 10^5$ cells) were lower than those found in the B/Ag sites ($4.1 \pm 1.2 \times 10^5$ cells), but not significantly so ($p = 0.08$) (Table III). Cell counts in the Ag/B sites ($2.9 \pm 0.9 \times 10^5$ cells) were similar to those found in the Ag/Ag and B/Ag sites. However, cell counts in the B/B sites ($0.9 \pm 0.4 \times 10^5$ cells) were significantly lower than those found in Ag/Ag sites ($p = 0.04$) and B/Ag sites ($p = 0.03$).

The majority of these cells were neutrophils. Levels of lactoferrin released from activated neutrophils during the second through fifth hours of skin chamber challenge were not significantly different at Ag/Ag sites (10.2 ± 3.6 μ g/ml) and B/Ag sites (14.1 ± 1.2 μ g/ml, $p = 0.08$). Lactoferrin levels were actually higher at Ag/B sites (18 ± 7.7 μ g/ml), but not significantly so ($p = 0.35$). However, the lactoferrin levels at Ag/Ag and B/Ag sites were both significantly higher than those found at B/B sites (4 ± 2.4 μ g/ml, $p = 0.04$).

A minority of inflammatory cells in the skin chambers were eosinophils. The total eosinophil counts were very similar at Ag/Ag sites ($3.6 \pm 1.2 \times 10^4$ cells) and B/Ag sites ($3.5 \pm 1.3 \times 10^4$ cells) (Table III). These cell counts were greater than those found at the Ag/B sites ($1.6 \pm 0.5 \times 10^4$), but not significantly so. However, eosinophil counts at Ag/Ag and B/Ag sites were significantly greater than seen at B/B sites ($0.2 \pm 0.2 \times 10^4$ cells, $p = 0.03$). The numbers of EG₂⁺ cells at Ag/Ag ($1.8 \pm 1 \times 10^4$ cells) and B/Ag ($1.8 \pm 1 \times 10^4$ cells) sites were virtually identical and significantly greater than those found at B/B sites ($0.1 \pm 0.1 \times 10^4$ cells, $p = 0.04$) (Table III). Levels of ECP released from activated eosinophils were not significantly different at Ag/Ag sites (112 ± 41 ng/ml) than at B/Ag sites (148 ± 46 ng/ml, $p = 0.45$)

(Table III). These were higher than levels found at Ag/B sites (79 ± 25 ng/ml), but not significantly so ($p = 0.25$). ECP levels at B/Ag sites were significantly greater than those found at B/B sites (50 ± 15 ng/ml, $p = 0.04$).

DISCUSSION

The findings reported here add to our knowledge about repeated pollen antigen challenges in the skin of sensitive subjects. Previous studies suggested that a degree of desensitization may occur in such sites. Andersson and Pipkorn⁵ found decreased immediate wheal reactions to skin prick tests in sites of skin prick test reactions induced 24 hours earlier when compared with the original responses. Histamine skin prick test reactivity was unchanged, suggesting no alteration in end organ responsiveness in the skin. In an earlier report Shaikh et al.⁴ described unaltered immediate wheal responses but decreased LPRs to pollen antigens injected in grossly normal skin sites of previous LPRs induced 1 to 2 weeks earlier. The decreased LPR in such cases was not immunologically specific in that it was observed when the rechallenge was carried out with an antigen antigenically unrelated to the antigen used for the initial skin test (in subjects sensitive to both antigens). Thus varying responses to antigen rechallenge in the skin have been reported. In these skin studies no measurements of local mediator release or inflammatory cell responses were carried out.

As described above, in our earlier skin chamber studies, prolonged pollen antigen challenge resulted in an initial peak of histamine release (from local mast cells) followed by a low level histamine release for at least 9 hours, even though the local mast cells were capable of releasing much more histamine.^{6,7} Several subsequent lines of evidence suggested that the source of the later low-level histamine release were basophils accumulating in the challenge sites, including the observation that tryptase was not released during the period of low-level histamine release.^{6,7} We have recently found an increased number of basophils accumulating in challenge sites during this period of low-level histamine release.¹³ On the basis of these previous observations, it is certainly conceivable that there would be even less mediator release if the site were rechallenged at even later time periods. However, in this study we have found no evidence of decreased mediator release or inflammatory cell responses when sites of previous prominent skin LPRs were rechallenged with the same pollen antigens 24 or 48 hours later. To control for any alteration in skin responsiveness caused by the minimal trauma that might occur with any prior intradermal injection, we compared skin chamber responses at sites of previous immediate and late LPRs and sites injected previously with B, which we have repeatedly found to induce no significant gross or histologic inflammatory responses.

In such comparisons we found that levels of histamine and tryptase released during the first challenge hour (from mast cells) were very similar in sites of skin chamber antigen challenge of previous immediate/late responses (Ag/Ag sites) and in sites injected with diluent (B/Ag sites) 24 hours earlier. Histamine release during the second

through fifth hours (presumably from basophils), inflammatory cell exudation, eosinophil exudation, and levels of lactoferrin and ECP (released from activated neutrophils and eosinophils, respectively) were also not significantly different in the Ag/Ag and B/Ag sites. However, they were all significantly greater than levels found in skin chamber challenges with B carried out in sites of previous intradermal injections with the diluent (B/B).

We have also found no evidence of primed (enhanced) responses in antigen-rechallenged sites. It is conceivable that such priming would have been observed if lower antigen concentrations had been also used in these skin chamber challenges. However, several logistic considerations made this approach unfeasible. Our skin chamber format permits concomitant measurements at just four sites, all of which are used for challenges with the single concentration of antigen and appropriate controls reported here. The antigen concentration used here (100 PNU/ml) was that found by us to induce prominent skin chamber mediator and inflammatory responses in almost all of the large number of subjects we have studied over many years. Responses to lower antigen concentrations would have been more variable in this study group, making evaluation of altered responses during rechallenge more difficult.

The lack of different responses to skin chamber antigen challenge we observed at sites with or without a previous LPR occurred even though there was evidence of a residual inflammatory reaction from the LPR itself. The mean skin chamber levels of histamine and inflammatory cell responses in sites of previous LPR challenged with B (Ag/B sites) were consistently higher than when B skin chamber challenge was carried out in sites of previous intradermal buffer injection (B/B sites). The responses at Ag/B sites were intermediate between those at the Ag/Ag and B/Ag sites on the one hand and at the B/B sites on the other hand. These Ag/B responses were, if anything, greater at 48 hours than at 24 hours after intradermal injection, even though we no longer saw any gross induration at any intradermal injection sites by 48 hours. The fact that the increased histamine levels in the Ag/B sites at 24 hours was not accompanied by increased tryptase levels suggests that the source of this residual histamine release was ingressing basophils.

The findings reported here differ considerably from those described for antigen rechallenge in the nose of sensitive individuals. Wachs et al.³ found that nasal rechallenge 24 hours after a previous early nasal reaction to challenge with the same antigen resulted in a *primed* response characterized by threshold symptomatic reactivity to a lower antigen challenge dose than that required the day before. There was also increased mediator release and cellular inflammatory responses in the nose. Such hyperreactivity was not observed when the second challenge was carried out 2 weeks instead of 24 hours after the initial challenge. Iliopoulos et al.² extended these studies, reporting that 60% of their subjects exhibited enhanced symptomatic responses during nasal challenges 24 hours after the initial challenge. There were also increased mediator levels in the nasal secretions. These authors postulated that the enhanced response to nasal antigen rechallenge observed by them was due to

the residual inflammatory reaction from the first challenge. However, they found no correlation between the intensity of the LPR to the first challenge and the degree of priming observed during rechallenge of the same individuals.

It is conceivable that the different patterns of mediator/inflammatory cell responses seen during rechallenge of the nose versus those found in the skin may reflect differences in the way in which the tissue was exposed to the antigen during the initial challenge. In the nose the antigen was applied topically, possibly leading to increased migration of mast cells and, subsequently, basophils superficially toward the epithelial layer during the expression of the initial LPR. The intradermal injection of antigen during the first challenge in our study may not have led to such cellular migration and therefore to no enhanced response during the rechallenge as observed in the nose.

Further studies will be required to compare the control mechanisms during initial and subsequent antigen challenges in the same sites. These findings are of considerable potential importance in our understanding of the pathogenesis of symptomatic responses during continued daily environmental exposures to allergens.

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